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Investigations on the Light and Scanning Electron Microscopic Structure of Tongue Papillae of Kangal Sheep: Taste and Mechanical

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ABSTRACT This study was conducted to examine the morphological, histological, and ultrastructural structure of the tongue papillae in Kangal sheep, which is the largest local type of the Akkaraman sheep breed, raised in Central Anatolia and especially in the Sivas region. After the tissues were prepared according to the necessary tissue processing protocols, they were examined using light (LM) and scanning electron microscopy (SEM). Histological examination revealed that the dorsal surface of the tongue was covered with stratified squamous keratinized epithelium, and the underlying layers contained lamina propria, submucosa, well-developed skeletal muscle bundles running in various directions, and serous/seromucous glands. SEM examinations revealed that the filiform papilla consisted of a main body and a pair of secondary papillae located on either side. Two types of lentiform papilla, pyramidal and irregularly shaped, were distinguished. Two types of taste papillae were identified: fungiform papilla and circumvallate papilla. It was observed that the fungiform papillae were typically mushroom-shaped and contained taste pores on their surfaces. It was determined that the circumvallate papillae were surrounded by a trench and contained taste pores. Although the tongue papilla structure of the Kangal sheep generally shows similarities with other ruminant species, specific morphological differences were identified, such as the consistent presence of a single pair of secondary extensions in the filiform papilla. These findings provide descriptive data for Kangal sheep and may serve as a basis for future comparative and functional studies.

Keywords: *Tongue, Light microscopy, Kangal sheep, Papilla, Scanning electron microscopy.*

ÖZ

Kangal Koyununun Dil Papillalarının Işık ve Taramalı Elektron Mikroskobik Yapısı Üzerine Araştırmalar: Tat ve Mekanik Papillalar

Bu çalışma, Orta Anadolu'da ve özellikle Sivas bölgesinde yetiştirilen Akkaraman koyun ırkının en büyük yerel tipi olan Kangal koyunlarında dil papillalarının morfolojik, histolojik ve ultrastrüktürel yapısını incelemek amacıyla yapılmıştır. Doku örnekleri gerekli doku işleme protokollerine göre hazırlandıktan sonra ışık mikroskobu (LM) ve taramalı elektron mikroskobu (SEM) ile incelenmiştir. Histolojik olarak, dilin dorsal yüzeyinin çok katlı yassı keratinize epitel ile kaplı olduğu ve altta yatan tabakaların lamina propria, submukoza, çeşitli yönlerde uzanan iyi gelişmiş iskelet kas demetleri ve seröz/seromuköz bezler içerdiği belirlenmiştir. SEM incelemeleri, papilla filiformisin bir ana gövde ve her iki yanında bulunan bir çift sekonder papilladan oluştuğunu ortaya koymuştur. Piramidal ve düzensiz şekilli olmak üzere iki tip papilla lentiformis ayırt edilmiştir. İki tip tat papillası tanımlanmıştır: papilla fungiformis ve papilla vallata. Papilla fungiformis'in tipik olarak mantar şeklinde olduğu ve yüzeylerinde tat alma gözenekleri içerdiği gözlemlenmiştir. Papilla vallata'nın ise bir hendekle çevrili olduğu ve tat alma tomurcukları içerdiği belirlenmiştir. Kangal koyununun dil papilla yapısı genel olarak diğer geviş getiren türlerle benzerlik gösterse de, papilla filiformis'te tek bir çift ikincil uzantının sürekli varlığı gibi belirli morfolojik farklılıklar tespit edilmiştir. Bu bulgular, Kangal koyunları için tanımlayıcı veriler sağlamakta olup, gelecekteki karşılaştırmalı ve fonksiyonel çalışmalar için bir temel oluşturabilir.

Anahtar Kelimeler: *Dil, Işık mikroskobu, Kangal koyunu, Papilla, Taramalı elektron mikroskobu.*

INTRODUCTION

Kangal sheep, which is the largest local type of the Akkaraman sheep breed in terms of size, is a sheep breed raised in Central Anatolia, especially in the Sivas Region (Aydin et al. 2024).

How food is masticated into a bolus in domestic mammals, whether the taste of the food is perceived, where the

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receptors for taste sensation are located, and the effect of feeding conditions on taste sensation have been subjects of scientific research from the past to the present. It is reported that feeding type and diet are two important factors determining an organism's adaptation to its environment (Erdoğan and Sağsöz 2018). The tongue is the organ that enables the perception of taste sensation and the grinding of food taken into the mouth to form a bolus (Dyce et al. 2009; König and Liebich 2020). Specialized papillae are located on the surface of the tongue to perform these functions. Those that enable the mechanical grinding of food are called mechanical papillae (papilla mechanica), and those that enable the perception of taste sensation are called taste papillae (papilla gustatori). The number, shape, and presence of these papillae vary among species in domestic mammals. Ruminants have three mechanical papillae, namely filiform papilla, lentiform papilla, and conic papilla, and two taste papillae called fungiform papilla and circumvallate papilla. The foliate papilla, which is present in other domestic mammals, is absent in ruminants (Dyce et al. 2009; König and Liebich 2020). Numerous studies have been conducted to reveal the morphological and ultrastructural organization of the lingual papillae in sheep and goats. While surface scanning was performed using scanning electron microscopy, the histology of these structures was revealed using light microscopy. These studies have even been reduced to the breed level, attempting to determine the relationships between the environment in which sheep and goat breeds live and the structure of their lingual papillae. Karacabey Merino (Can et al. 2016), Morkaraman (Demircioğlu et al. 2024), Hamdani (Güzel et al. 2025), Norduz (Delibaş et al. 2023), Rahmani (Madkour and Mohammed 2021; Madkour et al. 2023a; Madkour et al. 2023b), and Egyptian Ossimi sheep (Abumandour et al. 2023) are some of the sheep breeds currently studied. Egyptian goat (Mahdy et al. 2021), Saanen (Kurtul and Atalgın 2008), and Angora goat (Toprak 2023) are the goat breeds where such studies have been conducted. Furthermore, these studies have also been performed on lambs to investigate the development of lingual papillae (Tadjalli and Pazhoomand 2004; Güzel et al. 2025). A literature search revealed no such study on the Kangal sheep. This study aims to examine the lingual papillae in Kangal sheep using light and scanning electron microscopy and to identify their morphological characteristics in comparison with those reported for other sheep breeds and ruminant species.

MATERIAL AND METHODS

Tongue samples belonging to 10 adult Kangal sheep slaughtered in abattoirs in the Sivas region were washed with physiological saline, and then the necessary procedures were applied to the samples taken from determined regions of the dorsal surface of the tongue for examination under light and electron microscopes. This study was approved by the Sivas Cumhuriyet University Local Ethics Committee for Animal Experiments with decision number 65202830-050.04.04-126 dated 26/12/2025.

Light Microscopy

Histological evaluation was performed at the Department of Histology and Embryology, Faculty of Veterinary Medicine, Cumhuriyet University. Tissues determined from 6 regions of the tongue in sheep were taken in 1 cm^3 size, and the tissues were fixed in 10% buffered formalin for 24-48 hours. Following washing under running water, routine histological tissue processing was applied (dehydration in 70%, 80%, 96%, 100% alcohols). After clearing in xylene, the tissues were blocked in paraplast. Sections 4-5 µm thick were cut and mounted on slides using a microtome (Leica RM2125RTS) from the resulting blocks. The sections mounted were stained with trichrome staining and evaluated under a microscope (Zeiss Axio 500), and photographs of the necessary areas were taken (Bancroft and Cook 1984).

Scanning Electron Microscopy (SEM)

Samples taken for scanning electron microscopy were kept in 10% formaldehyde solution for 24 hours. After washing twice for ten minutes in Phosphate Buffered Saline (0.1M PBS) solution, they were kept in glutaraldehyde (2.5%) for 5-6 hours. Following 5 repeated washes with buffer solution, they were kept in 70%, 80%, 96%, and 100% alcohol series for dehydration. The resulting samples were dried in a critical point dryer at the Erciyes University Nanotechnology Application and Research Center in Kayseri, then gold-coated and examined under a Zeiss Evo LS10 scanning electron microscope.

RESULTS

Light Microscopy

Tissues taken from the 6 determined regions of the tongue were examined. Generally, without regional distinction, the dorsal surface of the tongue was determined to be covered with stratified squamous keratinized epithelium. The epithelium was distinguished as covering the upper surface of the papillae located on the dorsal surface, with varying thicknesses depending on the regions taken from the tongue. Filiform papilla, conic papilla, lentiform papilla (Figure 1 A, B, C), fungiform papilla, and circumvallate papilla (Figure 2 A, B) were distinguished by their shapes and locations.

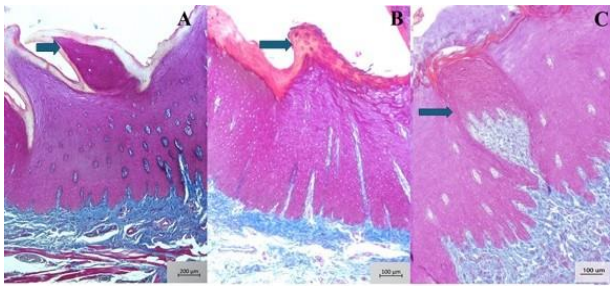

Figure 1: Light microscopic appearance of the mechanical lingual papillae in Kangal sheep. (A) Filiform papilla. (B) Conical papilla. (C) Lentiform papilla. Arrows indicate the papillae. Masson's trichrome staining. Scale bars: A = 200 µm; B = 100 µm; C = 100 µm.

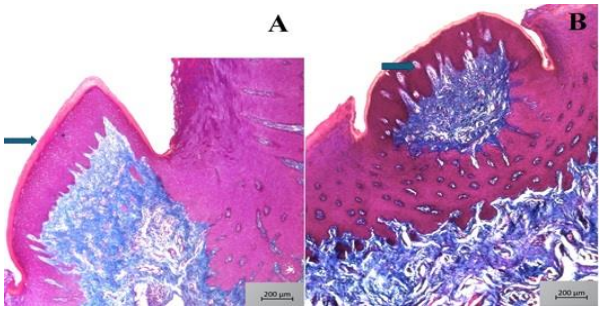

Figure 2: Light microscopic appearance of the gustatory lingual papillae in Kangal sheep. (A) Fungiform papilla. (B) Circumvallate papilla. Arrows indicate the papillae. Masson's trichrome staining. Scale bars: A = 200 µm; B = 200 µm.

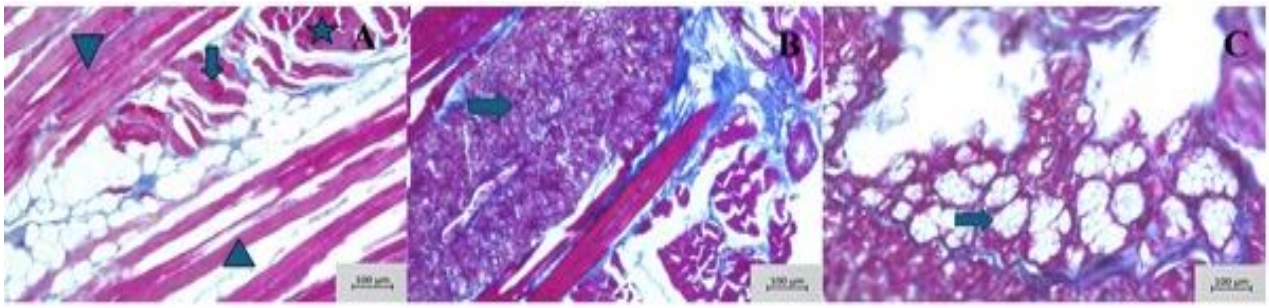

Figure 3: Light microscopic appearance of the deeper structures of the tongue in Kangal sheep. (A) Skeletal muscle bundles running in different directions beneath the papillae (arrowhead, longitudinal; arrow, vertical; star, transverse). (B) Serous glands between muscle bundles (arrow). (C) Seromucous glands (arrow). Masson's trichrome staining. Scale bars: A = 100 µm; B = 100 µm; C = 100 µm.

Taste pores were determined to be located on the vallate papillae. The standard layers of this epithelium covering the entire surface of the tongue and the upper parts of the papillae, showing stratified keratinization-stratum basale, stratum spinosum, stratum granulosum, and stratum corneum-were observed separately. It was determined that the stratum basale had a single layer of prismatic cell structure, the stratum spinosum was formed by polygonal cells, the stratum granulosum consisted of several layers of flattened cells containing keratohyalin granules, and the keratin layer was distinct and varied in thickness in places. Below the lamina epithelialis, the lamina propria and the submucosa, composed of looser connective tissue, were observed. It was determined that the lamina propria sent extensions into the epithelial layer, forming microscopic papillae, and these microscopic papillae were distinguished as clusters within the epithelium. Skeletal muscles running in various directions, sometimes intertwined, were detected beneath the submucosa (Figure 3 A). These muscles were identified as skeletal muscles responsible for voluntary movement in the tongue, running longitudinally, vertically, and horizontally, and forming bundles of varying thicknesses. Lingual glands were photographed among the muscles (Figure 3 B, C).

Scanning Electron Microscopy

In photographs examined by scanning electron microscopy of the Kangal sheep tongue, it was determined to possess three different mechanical papillae: filiform papilla, lentiform papilla, and conic papillae. Furthermore, two different types of taste papillae, named fungiform papilla and circumvallate papilla, were distinguished.

Filiform papillae were found densely on the dorsal surface of the tongue, especially in regions close to the apex, other regions of the dorsum although not as densely as at the apex. Furthermore, it was determined that the lengths of these papillae were longer and sharper at the apex lingua, but shorter and blunter in the corpus lingua. The filiform papillae were observed to be arranged as a main papilla (Mfp) emerging from the center and smaller secondary papillae (Sfp) emerging from both sides of this papilla. It was determined that the secondary papillae did not reach the main papilla in terms of either length or width. It was determined that both lentiform papilla and fungiform papillae were irregularly distributed among these papillae (Figure 4).

The pyramidal shaped lentiform papillae were generally observed leaning on one another. It was determined that the irregularly shaped ones were more independent than the others (Figure 5). It was determined that fungiform papillae were distributed among these papillae.

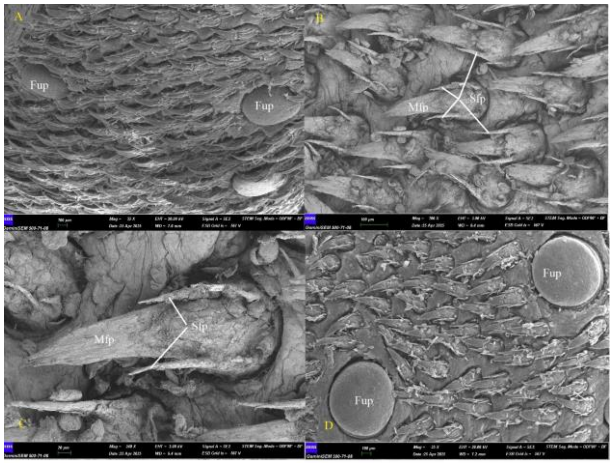

Figure 4: Scanning electron microscopic appearance of filiform and fungiform papillae on the dorsal surface of the tongue in Kangal sheep. (A) Fungiform papillae (Fup) interspersed among densely arranged filiform papillae at the apex linguae. (B) Filiform papillae showing a main filiform papilla (Mfp) and paired secondary papillae (Sfp). (C) Higher-magnification view of a filiform papilla showing the main papilla (Mfp) and secondary papillae (Sfp). (D) Fungiform papillae (Fup) distributed among filiform papillae. Scale bars: A = 100 µm; B = 100 µm; C = 20 µm; D = 100 µm.

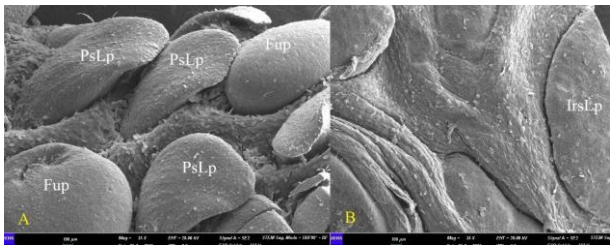

Figure 5: Scanning electron microscopic appearance of lentiform papillae in Kangal sheep. (A) Pyramidal-shaped lentiform papillae (PsLp) located among fungiform papillae (Fup). (B) Irregular-shaped lentiform papilla (IrsLp). Scale bars: A = 100 µm; B = 100 µm.

Papillae conicae were primarily found scattered in the lateral parts of the torus linguae and on the caudal surface of the radix linguae. It was determined that the distribution of Conic papillae on the tongue surface was not as dense as that of filiform papilla, and they were found more sparsely. It was determined that their shape resembled a blunt cone at the apex (Figure 6).

Circumvallate papillae were observed to exhibit a round structure surrounded by a trench and their surfaces were smooth. They were generally located on both sides of the radix lingua, behind the torus lingua (Figure 6).

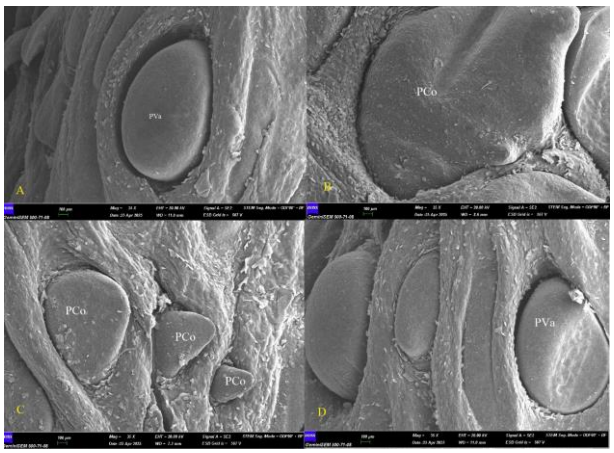

Figure 6: Scanning electron microscopic appearance of circumvallate and conical papillae in Kangal sheep. (A, D) Circumvallate papillae (PVa) surrounded by a trench. (B, C) Conical papillae (PCo). Scale bars: A = 100 µm; B = 100 µm; C = 100 µm; D = 100 µm.

Fungiform papilla typically exhibited a mushroom shape, a structure connected to the tongue by a round or oval head and a narrow neck. Taste pores (openings), where taste pores open, were observed on the surface of their heads (Figure 7).

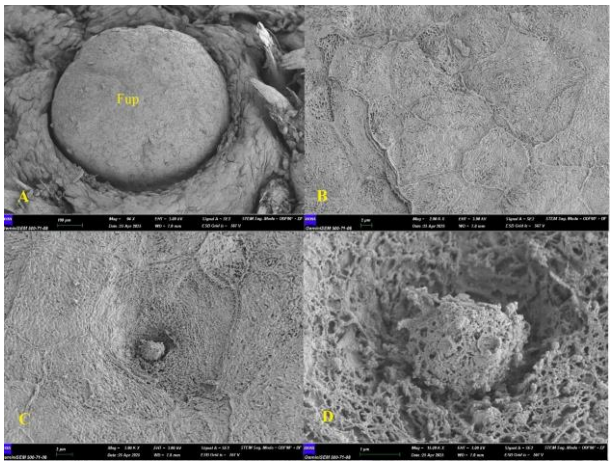

Figure 7: Scanning electron microscopic appearance of a fungiform papilla and its surface features in Kangal sheep. (A) Fungiform papilla (Fup). (B) Surface micromorphology of the fungiform papilla. (C) Taste pore on the surface of the fungiform papilla. (D) Higher-magnification view of the taste pore. Scale bars: A = 100 µm; B = 2 µm; C = 2 µm; D = 1 µm.

DISCUSSION AND CONCLUSION

This study provides descriptive morphological and ultrastructural data on the lingual papillae of Kangal sheep and allows comparison with previous reports in other ruminant breeds. Previous studies have suggested that pasture characteristics, diet, and climatic conditions may influence papillary morphology; however, the present study was not designed to test these relationships directly (Erdoğan and Sağsöz 2018).

Filiform papilla: It was stated that in Morkaraman sheep, they are densely located throughout the tongue, and secondary papillae are also present alongside the main papilla (Demircioğlu et al. 2024). In Karacabey Merino, it was determined that two secondary papillae emerge from the right and left sides of every main filiform papilla, and 3 or 4 smaller secondary papillae branch off from these secondary papillae, that secondary papillae are not found on the lateral surface of the tongue, that the lengths of the secondary papillae do not reach the length of the main papilla, and that the surface layer of the epithelium is keratinized, especially in the anterior parts of the papillae (Can et al. 2016). In Norduz sheep, primary papillae originating from a single stem and secondary papillae emerging from their right and left sides were observed. It was determined that smaller papilla extensions, varying in number between 1-2, were present on the sides of the secondary papillae (Delibaş et al. 2023). In another study, it was stated that 2-6 secondary extensions emerge from the sides of the main papillae, that the number of secondary papillae increases at the back of the tongue, that these projections exhibit a longer and fringed appearance on the sides of the corpus lingua, and that secondary papillae are absent in front of the torus lingua and surrounding the fossa lingua (Erdoğan and Sağsöz 2018). In the study conducted on Ossimi sheep, it was reported that the directions of the filiform papilla vary according to the region they are in, that they can have different shapes, that there are 5 subtypes, and that the subtypes show shape differences according to their location (Abumandour et al. 2023). In our study, the filiform papilla also exhibited the characteristics demonstrated in these studies. However, the number of secondary papillae did not change in our study and was observed as a single pair everywhere (Figure 4B, C, D). This aspect can be seen as a difference between our findings and the sheep species mentioned above. These differences may reflect breed-related or population-level morphological variation; however, direct associations with feeding habits, climate, environment, or pasture conditions cannot be established on the basis of the present descriptive findings alone (Erdoğan and Sağsöz 2018).

Lentiform papilla: It has been reported that in Morkaraman sheep, two types of lentiform papilla are located on the torus of the tongue: pyramid-shaped and irregularly rounded (Demircioğlu et al. 2024). In Karacabey Merino, it was determined that they were found only on the torus lingua, having two types-pyramidal and rounded-where the pyramid-shaped ones had pointed tips and the rounded ones had a blunt apex (Can et al. 2016). In Norduz sheep, they were found densely on the torus of the tongue, and the presence of pores at their tips was detected under high magnification (Delibaş et al. 2023). In another study conducted on sheep, it was found that these papillae were both larger and terminated in bifurcated tips, especially in the anterior half of the torus lingua compared to those located laterally on the corpus (Erdoğan and Sağsöz 2018). Different shapes of these papillae were not mentioned in Ossimi sheep (Abumandour et al. 2023). In our study, two types of lentiform papilla, pyramidal and irregular, were distinguished, similar to those in Morkaraman sheep (Figure 5).

Conic papilla: It has been stated that they are located in the lateral region of the tongue in Morkaraman sheep (Demircioğlu et al. 2024); in Karacabey Merino, they have a rounded base and a blunt tip on the torus and its lateral side, and their surface areas are covered with squamous epithelium (Can et al. 2016); in Norduz sheep, they resemble a typical cone (Delibaş et al. 2023); and in Ossimi sheep, those on the tongue are smaller and wider than the paralingual ones (Abumandour et al. 2023). In our study, conic papillae were observed on the torus lingua consistent with the literature (Figure 6 B, C). The findings

in the studies mentioned above are consistent with the findings in our study.

Fungiform papilla: In a similar study conducted on Morkaraman sheep, it was stated that the fungiform papilla appeared like shriveled peas in some regions (Demircioğlu et al. 2024). The sparse presence of fungiform papilla on the dorsal part of the tongue and their rare presence on the radix in Morkaraman sheep is consistent with the findings of our study (Demircioğlu et al. 2024). In Karacabey Merino, it was determined that they were scattered among the filiform papillae on the apex, corpus, and radix of the tongue, that they were rounded, mushroom-like structures, that their number per cm2 area decreased from the apex towards the radix, and that crater-like pores were present under high magnification (Can et al. 2016). In Norduz sheep, it was detected that they were distributed starting from the apex of the tongue along its body, that their number per cm^2 area decreased from the apex towards the radix, and that Taste pores were present on them (Delibaş et al. 2023). In another study, it was reported that the convex surfaces of these papillae were quite prominent, some were spherical, and others were outwardly conical, that a shallow groove was present on their surfaces, and that taste pores could be distinguished under high magnification (Erdoğan and Sağsöz 2018). In the study conducted on Ossimi sheep, it was found that these papillae had two subtypes, oval and rounded, that they were surrounded by a circular trench, and that they possessed taste pores (Abumandour et al. 2023). In our study, the fungiform papilla did not present a shriveled pea appearance in any of the scanned regions (Figure 5 A, 7). In all SEM photographs, this papillae were determined to have a very regular rounded mushroom appearance (Figure 5 A, 7). Furthermore, the trench surrounding this papilla and the taste pores located on it were also detected in our study (Figure 7).

Circumvallate papilla: In Morkaraman Sheep, they were large and small papillae of round-oval shape arranged in two rows behind the torus of the tongue (Demircioğlu et al. 2024); in Karacabey Merino, they were arranged in varying sizes on the sides of the torus (Can et al. 2016); in Norduz Sheep, they were shown to be arranged in two rows on the radix of the tongue and composed of three parts: the mucosal vallum, the parietal trench, and the ring-shaped cushion (Delibaş et al. 2023). Similar findings were also detected in the study conducted on Ossimi sheep (Abumandour et al. 2023). In another study, it was found that 6-10 circumvallate papillae were arranged laterally in the caudal half of the torus lingua, that the pedicle surrounding the papillae were not continuous, that some lacked a surrounding pedicle, and that the taste pores and the rough appearance of the epithelium were noticeable under high magnification (Erdoğan and Sağsöz 2018). In our study, the circumvallate papilla exhibited the mucosal vallum, parietal trench, and ring-shaped cushion, similar to those in Norduz and Ossimi sheep (Figure 6 A, D).

When the light microscopic findings in the examined mechanical and taste papillae were reviewed based on general structural characteristics, the epithelial feature being stratified squamous epithelium and the variation in thickness according to the papillae and their locations, observed in cattle (Ding et al. 2016), camels (Nabipour 2011), goats (Kurtul and Atalgın 2008), and sheep (Murad 2011), were similarly observed in the Kangal sheep breed. Muscles, connective tissue vascular arrangement, Ebner's glands, and excretory duct structures in the lamina propria were determined to be parallel in cattle (Ding et al. 2016), buffaloes (Prakash and Rao 1980), camels (Nabipour 2011), goats (Kurtul and Atalgın 2008), and many sheep breeds (Murad 2011; Delibaş et al. 2023; Demircioğlu et al. 2024; Güzel et al. 2025) and in Kangal sheep.

With the introduction of the electron microscope into the scientific world, numerous studies have been conducted and are still ongoing to reveal the surface anatomy of organs and tissues at macro and micro levels. Many studies have also been carried out to reveal the lingual papillae at macro and micro levels. In our study, the lingual papillae of the Kangal Sheep, an endemic subspecies in our country, were revealed both histologically and using an electron microscope. In the present study, the lingual papillae of Kangal sheep were described histologically and by scanning electron microscopy, and some minor morphological differences were noted in comparison with previous reports on other sheep breeds. These findings provide baseline descriptive data on the lingual papillae of Kangal sheep and may be useful for future comparative and functional studies. Future studies should evaluate whether variations such as the number of secondary extensions of the filiform papillae or the continuity of the cushion surrounding the circumvallate papillae are associated with functional features of the tongue. In conclusion, the present findings may serve as a reference for future studies in this field.

CONFLICTS OF INTEREST

The authors report no conflicts of interest.

AUTHOR CONTRIBUTIONS

Idea/Concept: LT, SU, İO
Supervision/Consultancy: LT, SU
Data Collecting and/or Processing: LT, SA, SU
Analysis and/or Interpretation: LT, SU, İO, SA, EK
Writing the Article: LT, SU
Critical Review: LT, SU, İO

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