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Research Article

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

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ABSTRACT

In this study, the structure of the papillae in the tongue of Morkaraman sheep was investigated by light (LM) and scanning electron microscopy (SEM). The aim of this study was to investigate the morphology and topographic distribution of the lingual papillae of the tongues of Morkaraman sheep breed. Adult male Morkaraman sheep tongues were used in the study. Tongue samples for light and scanning electron microscopy were taken from the apex, corpus, radix and torus lingua regions of the upper surface of the tongue and the lower surface of the tongue tip. Two types of taste papillae, papillae fungiformis and vallata, were observed on the tongue. Papillae fungiformis were found under the tip of the tongue, on the upper surface and sides of the tip and body of the tongue and on torus lingua. Papillae vallata were observed on both posterior sides of the torus lingua of the tongue. SEM examination showed that the surface of the papillae was smooth and there were no taste pores opening to the surface. On LM examination, filiform papillae at the lingual apex were common in materials. These papillae had secondary keratin extensions on the main body. The light and scanning electron microscopic structures of the taste papillae on the tongue of Morkaraman sheep were examined and their similarities and differences with other ruminant species were determined.

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INTRODUCTION

Morkaraman sheep breeds are intensively bred in Turkey, especially in Central Anatolia and they constitute almost half of the existing sheep population. These sheep breeds are very well adapted to the regional conditions and are raised in the regions close to Anatolia, especially in Eastern and Southeastern Anatolia, Black Sea and Mediterranean regions (Akçapınar 2000; Türkyılmaz 2014).

In vertebrate organisms, diet is one of the most important factors in adaptation to the environment (Roth and Wake 1989). The tongue has a great importance in nutrition like other anatomical structures in the oral cavity. The structural differences of the tongue in all living organisms actually show the changes in the life sphere of the organism. Therefore, the morphological differences of the tongue among mammals show that there is a correlation between the adaptation between lifestyles (Erdogan and Sagsoz, 2018). In mammals, there are mechanically effective tongue papillae and taste receptors on the tongue. Mechanically acting papillae are involved in the intake, disintegration and swallowing of food. Taste-sensing papillae play a role in food selection by providing sweet, bitter, sour, salty, etc. flavours (Toprak et al. 2020). Simultaneously, an important part of the tongue gets covered with variable papillae, which exhibit regional variations in the mucous membrane and primarily focus on the upper surface responsible for mechanical or gustatory tasks. Each species exhibits variations in the distribution, size, quantity, and shape of the papillae. (König and Liebich 2014).

Many studies have been carried out on ruminant tongues by gross, scanning electron microscopy and light microscopy, revealing the anatomical structures of the tongue (Kurtul and Atalgın 2008; Shao et al. 2010; Emura et al. 2011; Kokubun et al. 2012).

For this purpose, the morphology and topographical distribution of the lingual papillae of the tongues of Morkaraman sheep breed, anatomical differences on the surface area and mucosal structures of the lingual papillae, taking into account the similarities and differences of the species-specific features.

MATERIAL AND METHODS

Material

Morkaraman sheep tongues used in the study were collected from slaughterhouses in Diyarbakır province. Tongues were washed with 0.9% saline immediately and freshly after slaughtering in a local slaughterhouse. Tongue samples were collected from certain sections of the tongue, including the apex, corpus, root, and lingual torus regions on the upper surface of the tongue, as well as the ventral surface of the lingual apex. These samples were then examined using light and scanning electron microscopy.

Light microscopy (LM)

Morkaraman sheep tongue samples were fixed in 10% formaldehyde for light microscopic examination. Then, washing, dehydration, paraffin and embedding procedures were performed for these tissues. Sections of 5 µm thickness were taken from these paraffin blocks. These sections were stained with Mallory's trichrome and Hematoksilen-eosin (H&E) staining. The sections were examined and photographed with an Olympus CX21 light microscope.

Scanning electron microscopy (SEM)

The parts taken from the tongue apex, dorsum, torus and radix blot were kept in 10% formaldehyde for 24 hours. After washing with 0.1 M PBS (Phosphate buffer solution) 2 times for 10 minutes each, they were kept in 2.5% glutaraldehyde for 6 hours and washed 5 times in 0.1 M buffer solution. It was kept in appropriately in four different ethyl alcohol series in ratios appropriate to the literature (Baygeldi et al. 2023). After drying and coating in the incubator, images were taken with SEM (JEOL JSM 5600 LV) at Dicle University Science and Technology Application and Research Centre.

RESULTS

The histological results

The specimens taken from various regions of the tongue including apex, dorsum, torus and radix were found to have a cutaneous mucosa. The outermost mucosa layer consisted of multilayered keratine epithelial cells, the submucosa, which is also characterised as stroma consisting of connective tissue, and the muscular structure, which constituted most of the tongue, was located at the bottom. There were papillae (filiformis, fungiformis, lenticularis, circumvallata) with different histological structures on the surface of the tongue. Filiform papillae at the lingual apex were common in the specimens examined. These papillae were seen to have secondary keratin extensions on a main body. It was observed that papillae fungiformis were rare in Morkaraman sheep. Papillae filiformis were more common. Filiform papillae were also common in the lingual torus. Fungiform and especially lenticular papillae were more common in the lingual torus. The submucosa was mostly composed of connective tissue, but there were serous and mucous glands, lymphoid and adipose tissues in the stroma. In the profund tissue, skeletal muscle fibrils extending transversally and longitudinally, as well as nerve endings, lymph follicles, adipose tissue and blood vessels were observed (Figure 1 and Figure 2).

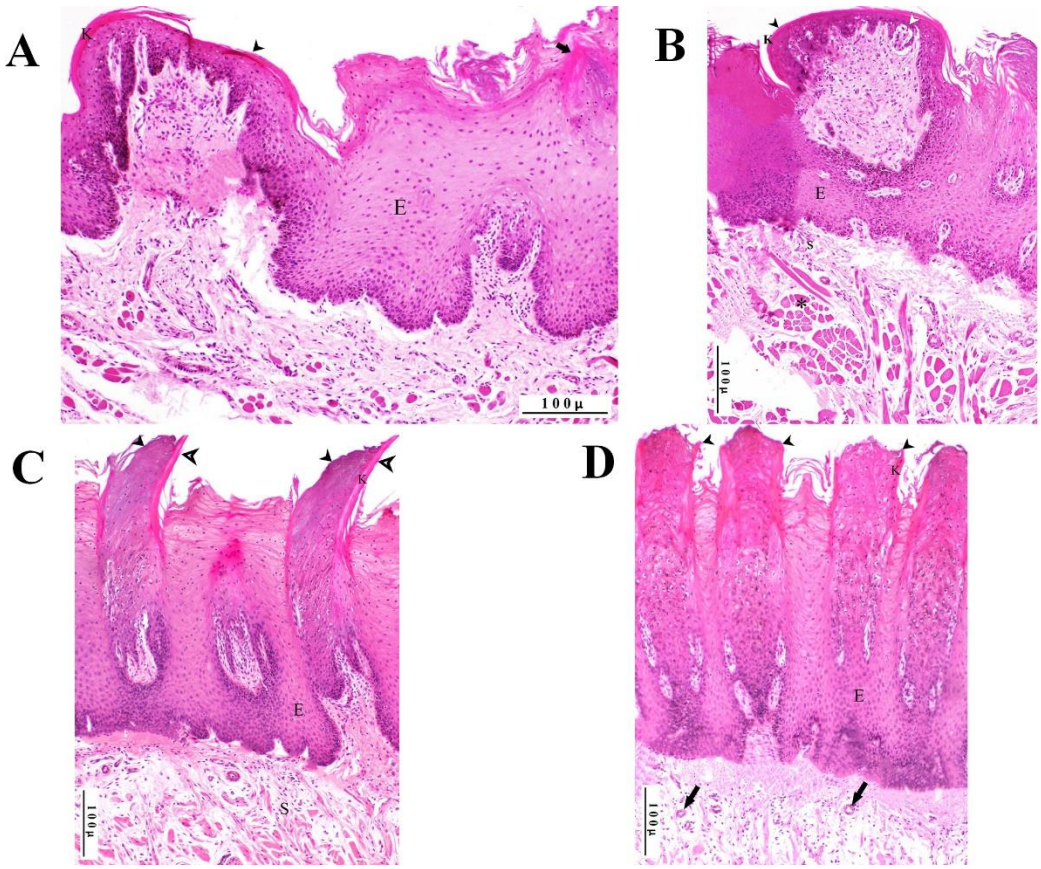

Figure 1. *Histological image of the tongue of Morkaraman sheep (H&E)*

A: Papillae filiformis (arrow), papillae fungiformis linguinal (arrowhead), lingual apex, Morkaraman, HE, X100. C; Papillae filiformis (arrowheads), keratin spines (hollow arrowheads), lingual dorsum, Morkaraman, HE, X200. B: Papillae circumvallata (arrowhead), taste buds (hollow arrowhead), muscular tissue (star), lingual torus, Morkaraman, HE, X200. D; Papillae lenticularis (arrowheads), ductus glandularis (arrows), lingual radix, HE, X200. K; Keratin, E; Epithelium, S; Stroma

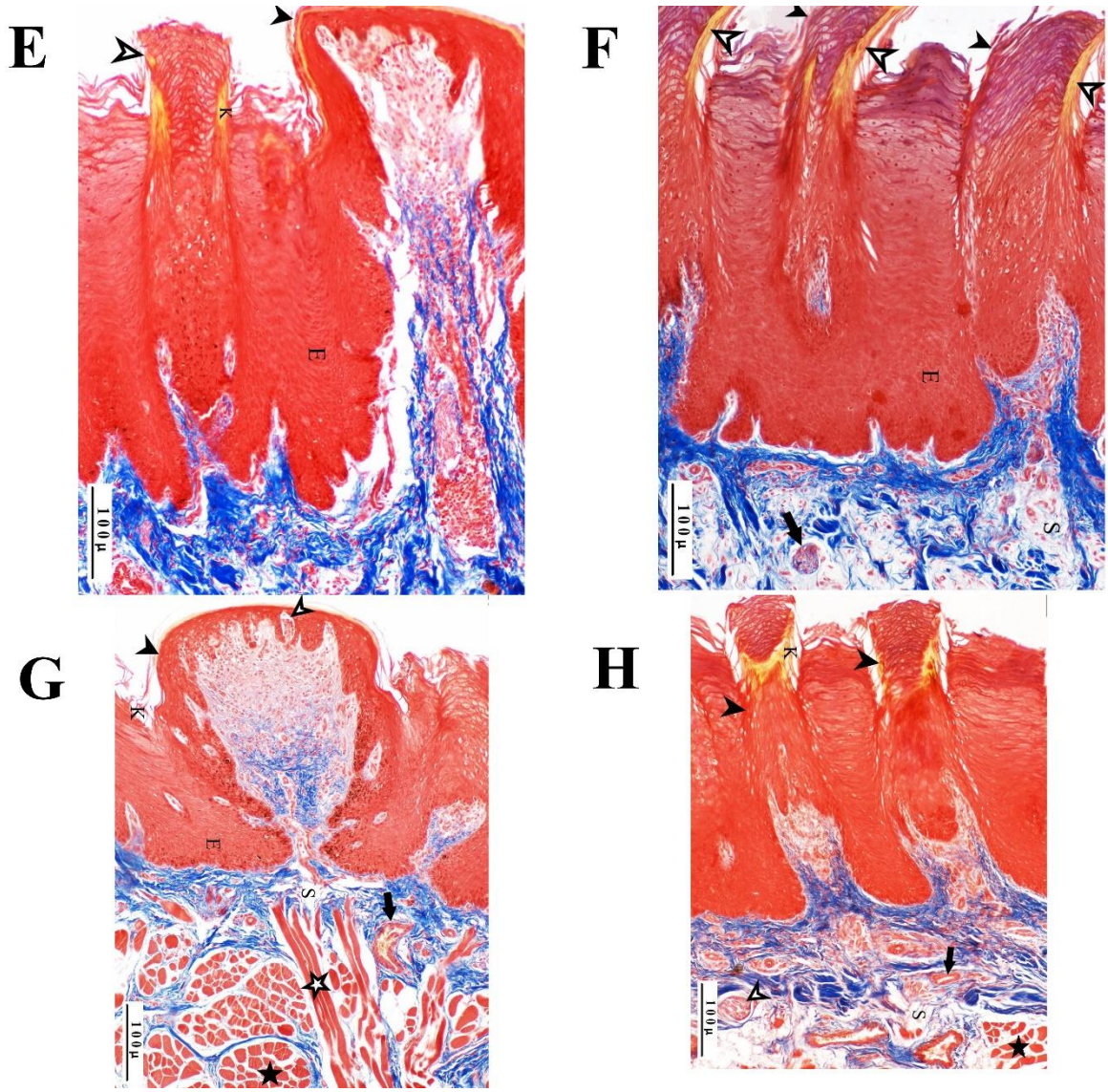

Figure 2. *Histological image of the tongue of Morkaraman sheep (Mallory's trichrome)*

E: Papillae filiformis (hollow arrowhead), papillae fungiformis (arrowhead), lingual apex, Morkaraman, MTC, X200. F: Papillae filiformis (arrowheads), keratin spines (hollow arrowheads), mucous glandula (arrow), lingual dorsum, Morkaraman, MTC, X200. G: Papillae circumvallata (arrowhead), taste buds (hollow arrowhead), transversal muscle fibrils (asterisk), longitudinal muscle fibrils (hollow asterisk), blood vessel (arrow), lingual torus, Morkaraman, HE, X200. H: Papillae lenticularis (arrowheads), ductus glandularis (arrow), mucous glandula (hollow arrowhead), muscular tissue (asterisk), lingual radix, HE, X200. K: Keratin, E: Epithelium, S: Stroma

Scanning electron microscope results

Papillae fungiformis were seen to be quite abundant at the apex of the sheep tongue. Papillae fungiformis had the golden mushroom appearance and sometimes had the appearance of deflated peas (Figure 3). Papillae fungiformis were sparse in the dorsal part of the tongue (Figure 4). In addition, papillae fungiformis were observed very rarely in the torus part of the tongue (Figure 5). When the papillae filiformis were examined, it was observed that they were widespread throughout the tongue and were mostly seen in the apex and dorsal part of the tongue as main papilla filiformis and secondary papilla filiformis (Figure 3,4). Papillae lentiformis were located in the torus region of the tongue and were seen as two types that differed from each other in shape. There were pyramid-shaped (type-I) lenticular papillae and irregular round-shaped (type-II) lenticular papillae (Figure 5). Papillae vallata, bordered by a ditch, were observed as round or oval shaped large and

small papillae in two rows postero-lateral to the lingual torus of the tongue. Papillae conica of different sizes were observed in the lateral part of the tongue (Figure 6).

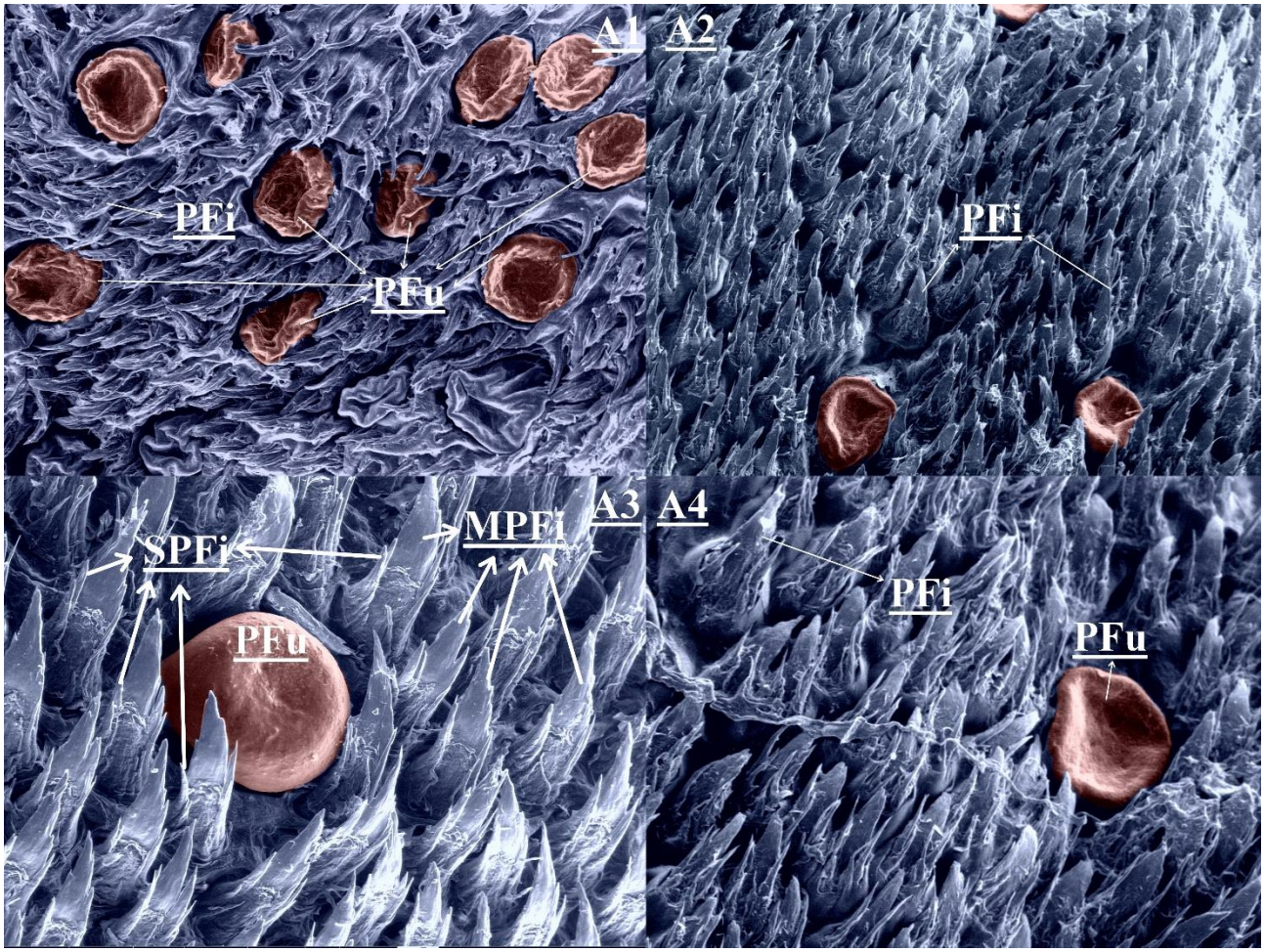

Figure 3. *Scanning electron microscope image of the apex of the tongue of Morkaraman sheep*

A1: PFi: Papillae filiformis, PFu: Papillae fungiformis; A2: PFi: Papillae filiformis; A3: PFu: Papillae fungiformis, MPFi: Main Papillae filiformis, SPFi: Seconder Papillae filiformis; A4: PFi: Papillae filiformis, PFu: Papillae fungiformis

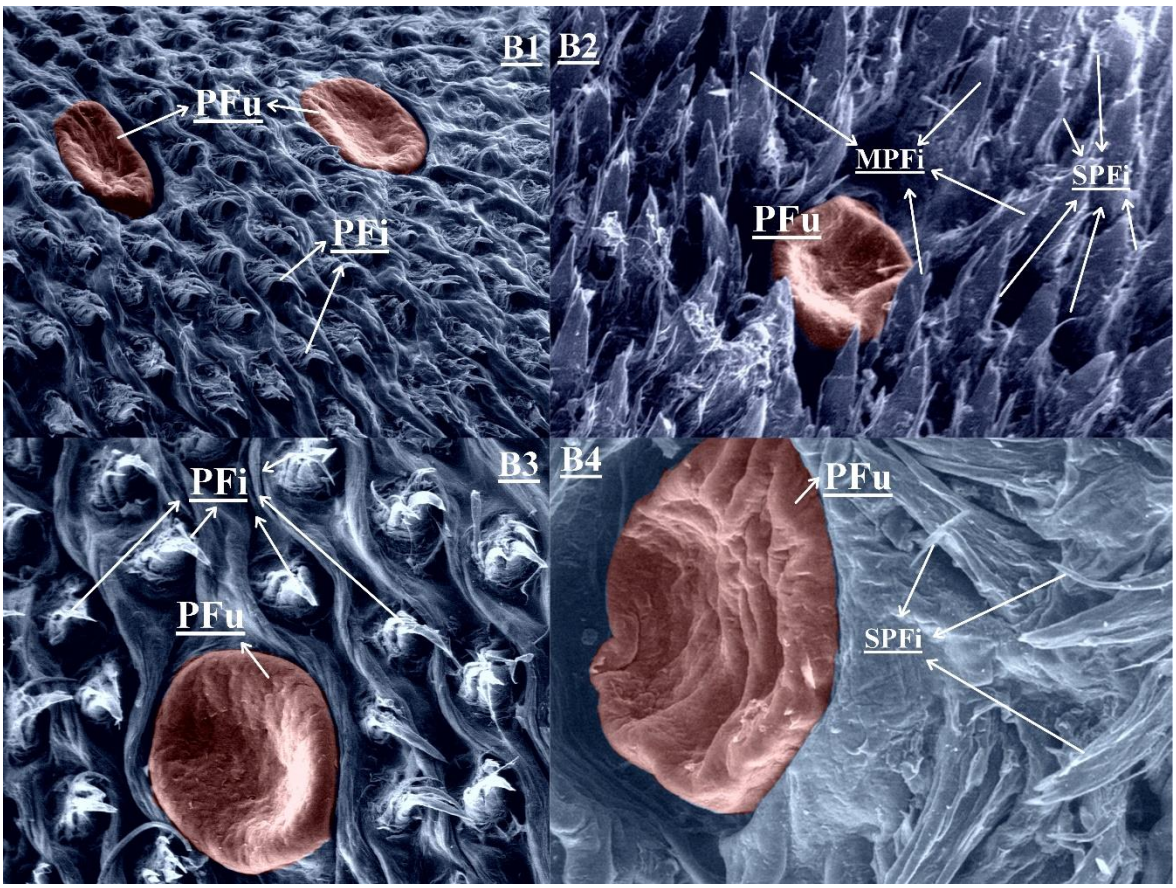

Figure 4. *Scanning electron microscope image of the dorsal part of the tongue of Morkaraman sheep*

B1: PFi: Papillae filiformis, PFu: Papilae fungiformis; B2: PFu: Papilae fungiformis, MPFi: Main Papillae filiformis, SPFi: Seconder Papillae filiformis; B3: PFi: Papillae filiformis, PFu: Papilae fungiformis; B4: PFu: Papilae fungiformis, SPFi: Seconder Papillae filiformis

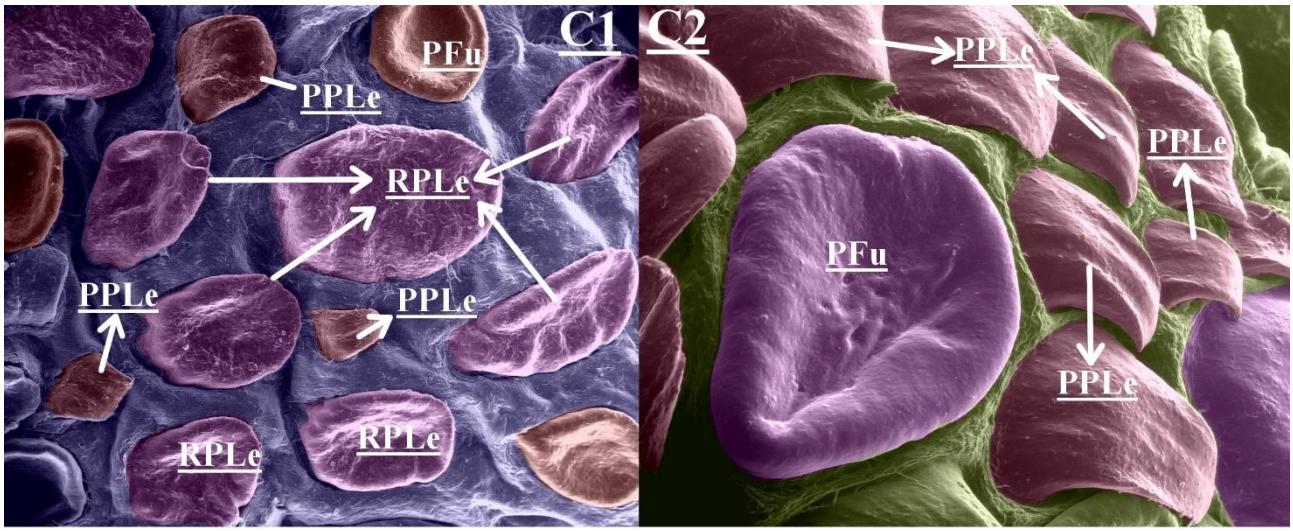

Figure 5. Scanning electron microscope image of the torus section of the tongue of Morkaraman sheep

C1: PFu: Papilae fungiformis, PPLe: Pyramid-shaped (type-I)lenticular papillae, RPLe: Irregular round-shaped (type II) lenticular papillae; C2: Papilae fungiformis, PPLe: Pyramid-shaped (type-I)lenticular papillae

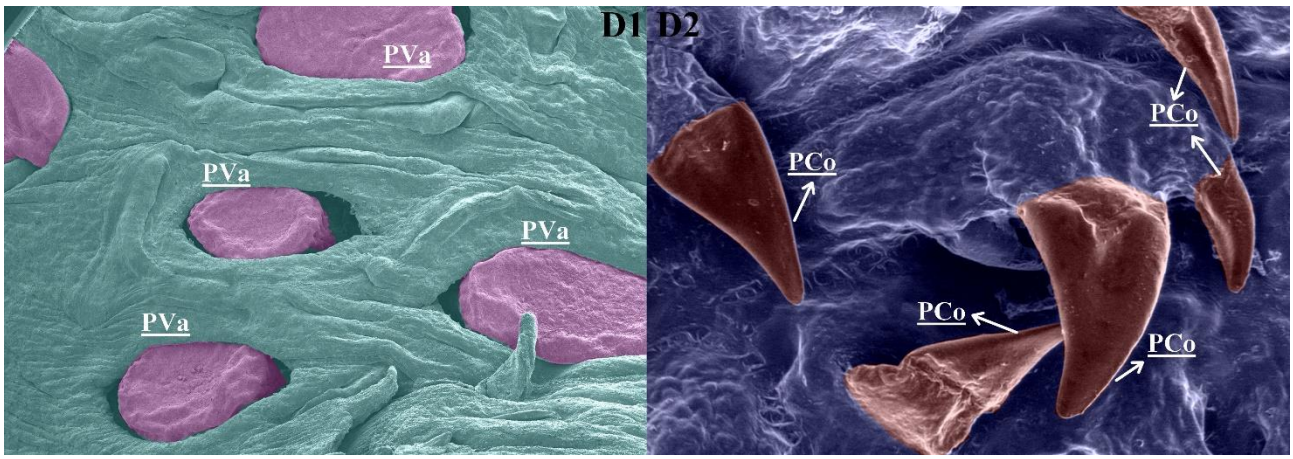

Figure 6. *Scanning electron microscope image of the radix of the tongue and lateral aspect of the tongue of Morkaraman sheep*

D1: PVa: Papillae vallatae; D2: PCo: Papillae conica

DISCUSSION

In this study, histological and scanning electron microscopic investigations were carried out on the tongue papillae of Morkaraman sheep. Contrary to the reports that cow (Chamorro et al. 1986) has 3 types of taste papillae as papilla fungiformis, papilla vallata and papilla foliata and hippopotamus (Yoshimura et al. 2009) has two types of taste papillae as papilla fungiformis and papilla foliata, two types of taste papillae as papilla fungiformis and papilla vallata were found in the tongue of Morkaraman sheep.

According to Erdoğan and Sağsöz (2018), the filiform papillae on the borders of the ventral surfaces of the tip of the tongue consist of one main papilla and 2-6 subordinate papillae. Kumar et al. (1998) observed that in Jamunapari goats, the filiform papillae on the ventro-lateral side of the tongue tip are composed of a primary papilla and 2-4 subsidiary papillae, which agrees with previous findings. The findings presented in this investigation were found to be consistent with the findings of previous studies.

Jamunapari goat (Kumar et al. 1998), Zavot cattle (Sarı et al. 2010), Dwarf deer (Agungpriyono et al. 1995), Saanen goat (Kurtul and Atalgın 2008), gazelle (Harem et al. 2011), Angora goat papillae vallata were surrounded by a deep moat, similar to those reported in the Markhoz goat (Goodarzi and Hoseini 2015), alpaca (Erdoğan et al. 2016; Gozdziewska-Harłajczuk et al. 2015) and Karacabey merino (Can et al. 2016). The papillae trenches had an annular pads. In Akkaraman sheep (Harem et al. 2009), the papilla vallata are surrounded by a moat, either individually or sometimes in groups of 2-3, and do not have a distinct annular pad. In sheep (Erdoğan and Sağsöz 2018), some papilla vallata do not have an annular pad. In this study these findings were not found.

In sheep (Erdoğan and Sağsöz 2018), it was reported that the papilla vallata had a multilayered squamous epithelial layer in light microscopic examination and this was also observed in this study. It has been reported that there is a weak keratinisation on the surface of papilla vallata in alpaca (Gozdziewska-Harłajczuk et al. 2015) Akkaraman sheep (Harem et al. 2009), dwarf deer (Agungpriyono et al. 1995), and sheep (Erdoğan and Sağsöz, 2018). Harem et al. (2011) reported that the keratin layer of the deer was of medium thickness. A weak keratin layer was observed in dwarf deer (Agungpriyono et al. 1995), Akkaraman sheep (Harem et al. 2009), alpaca (Gozdziewska-Harłajczuk et al. 2015) and sheep (Erdoğan and Sağsöz 2018). In dwarf deer (Agungpriyono et al. 1995), cattle (Tabata et al. 2003), Akkaraman sheep (Harem et al. 2009), Zavot cow (Sarı et al. 2010), gazelle (Harem et al. 2011), dromedary camel (El Sharaby et al. 2012), buffalo (El Sharaby et al., 2014), alpaca (Gozdziewska-Harłajczuk et al, 2015), and sheep (Erdoğan and Sağsöz 2018), taste buds in the papillae vallata are found intraepithelially on the medial walls of the papillae facing the ditch and these taste buds open into the papillae ditches with taste pores. Tabata et al. (2003) reported that there were two types of taste bud cells, type 1 and type 2, in the taste buds of papilla vallata in cattle. This study found that the number of papilla vallata was limited and the structure contained secondary keratin.

Gozdziewska-Harłajczuk et al. (2015) documented the existence of flavor pores on the outer layer of fake papillae within the papilla vallata of alpacas. Chamorro et al. (1986) documented the existence of a papilla vallata trench and flavor pores on the surface of papillae in cows, Goodarzi and Hoseini (2015) in Markhoz goats, Yanping et al. (2016) in cattle-goats, and Erdoğan and Sağsöz (2018) in sheep. Nevertheless, this study did not observe any flavor holes on the surface of the papilla vallata. Emura et al. (1999) reported the absence of irregular round and pyramid-like lenticular papillae in deer. In this study has the opposite findings of these findings.

CONCLUSION

The light and scanning electron microscopic findings of the taste papillae in the tongue of Morkaraman sheep were compared with the findings obtained from the taste papillae in the tongue of other domestic and wild ruminants. As a result, similarities and differences were observed. It is thought that these differences may be due to the type of food and diet of Morkaraman sheep. It is predicted that the data obtained from this study will contribute to the knowledge in the field.

CONFLICT OF INTEREST

The authors do not declare any conflicts of interest in the study.

AUTHOR CONTRIBUTION

All authors have contributed equally.

ETHICAL APPROVAL

The relevant article of law in our country states "**Procedures with dead animals or tissues, slaughterhouse materials, waste fetuses are not subject to the permission of the Local Ethics Committee for Animal Experiments**" in accordance with Article 8, paragraph eight (k) of the Regulation No. 28914 on the Working Procedures and Principles of Animal Experimentation Ethics Committees, the study materials are not subject to permission because they are taken from the slaughterhouse.

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