



## Morphological Examination of Tongue Papillae in Norduz Sheep: A Scanning Electron Microscopic Study

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### ABSTRACT

The tongue is a mobile organ consisting of apex, corpus and radix parts with a distinctive mucosa in the digestive system. In the case of digestion; it should not be forgotten that the macroscopic structure of all digestive organs, especially the tongue mucosa, is of great importance. Norduz sheep is known as a variety of Akkaraman sheep bred in the Norduz region located within the borders of the Gurpinar district of Van province. In addition, the fact that the Norduz sheep are only raised in the Norduz region and exhibit unique yield performance makes this breed different from other breeds. For this reason, in our study, it was aimed to examine the tongue papillae of Norduz sheep, which is a different breed, by a scanning electron microscope. In our study, the tongue tissues of 10 Norduz sheep were used. In scanning electron microscopy papillae filiformes; was observed as spiny in the region from the apex of the tongue to the torus linguae. The ends of papillae conicae were typically cone-shaped. Papillae lentiformes were found to be the clearest and most voluminous mechanical tongue papillae on the torus linguae. Papillae fungiformes which is a taste papillae were found to be mushroom-like and scattered among papillae filiformes. Papillae vallatae were composed of mucosal valium, parietal trench, and annular pad located on either side of the radix linguae. As a result, in Norduz sheep; many similarities and differences in terms of tongue papillae between species and races were determined.

**Keywords:** Norduz sheep, Papillae, Scanning electron microscopies, Tongue.

### öz

## Norduz Koyununda Dil Papillalarının Morfolojik Olarak İncelenmesi: Bir Taramalı Elektron Mikroskop Çalışması

Dil sindirim sistemi içerisinde kendine özgü bir mukozaya sahip apex, corpus ve radix kısımlarından meydana gelen hareketli bir organdır. Sindirim olayında; dil mukozası başta olmak üzere tüm sindirim organlarının makroskopik yapısının büyük bir öneme sahip olduğu unutulmamalıdır. Norduz koyunu: Van ili Gürpınar ilçesi sınırları içerisinde yer alan Norduz bölgesinde yetiştirilen Akkaraman koyununun bir varyetesi olarak bilinmektedir. Ayrıca Norduz koyununun yalnızca Norduz bölgesinde yetişmesi ve kendine özgü verim performansı sergilemesi, bu ırkı diğer ırklardan farklı kılmaktadır. Bu sebeple çalışmamızda farklı bir ırk olan Norduz koyununda dil papillalarının taramalı elektron mikroskop ile incelenmesi amaçlanmıştır. Yaptığımız çalışmada 10 adet Norduz koyununun dil dokusu kullanılmıştır. Taramalı elektron mikroskop incelemesinde papillae filiformes; dilin apexinden torus linguae'ye kadar olan bölgede dikensi şekilde gözlemlendi. Papillae conicae; uçları sivri tipik koni şeklindeydi. Papillae lentiformes; torus linguae'nin üzerinde en net ve hacimli görülen mekanik dil papillaları olarak tespit edildi. Tat papillalarından papillae fungiformes; mantara benzer şekilde ve papillae filiformes arasında dağınık halde gözlemlendi. Papillae vallatae radix linguae'nin her iki yanında konumlanmış mukozal valium, parietal hendek ve halkasal ped'ten meydana gelmişti. Sonuç olarak Norduz koyununun; türler ve ırklar arasında dil papillaları yönünden birçok benzerlikler ve farklılıklar gösterdiği tespit edilmiştir.

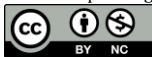
**Anahtar Kelimeler:** Dil, Norduz koyunu, Papillae, Taramalı elektron mikroskop.

### INTRODUCTION

The tongue is a mobile organ consisting of apical, corpus and radix parts with a characteristic mucous membrane in the digestive system (Dursun 2008). Mucous membrane of the tongue; together with other structures of the oral

cavity, especially in the process of digestion; provides the breakdown of incoming nutrients (Scala et al. 1995). In case of digestion; it should not be forgotten that the macroscopic structure of all digestive organs, especially the mucous membrane of the tongue, plays an important role (Shao et al. 2010).

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Considering the nutritional characteristics of ruminants, it is an indisputable fact that they consume food in very different patterns. While most of this situation is related to environmental adaptation, a small part of it results from genetic factors (Shao et al. 2010). For this reason, the difference in the shape of papillae on the mucous membrane of the tongue and the features of their distribution on the tongue in all ruminants is important in classifying ruminants in terms of comparative anatomy.

Norduz sheep is known as a variety of Akkaraman sheep, bred in the Norduz region located within the borders of the Gurpinar district of Van province (Mis et al. 2018; Dalga et al. 2021). In fact, there are many varieties of Akkaraman sheep living in different environmental conditions and having different morphological characteristics, such as Karakas, Southern, Karaman and Kangal (Bingöl 1998; Aysan Dayan and Bingöl 2008). However, Norduz sheep are bred only in the Norduz region, adapting to the harsh climate of the Eastern Anatolia region and showing unique performance indicators that distinguish this breed from other breeds.

When research is being done in this area; Jamonopari goat (Kumar et al. 1998), European mole (Jackowiak 2006), Bear (Pastor et al. 2011), Bison (Plewa and Jackowiak 2020), Wolf (Halgöl et al. 2018), lamb (Tadjalli and Pazhoomand 2004), Nile fox (Mahdy 2021), Saanen goat (Kurtul and Atalgın 2008), Karacabey merino sheep (Can et al. 2016), Antilope Cervicapra (Emura et al. 1999), Barbary sheep (Emura et al. 2000) and Akkaraman sheep (Harem et al. 2009), the similarity or morphological differences of tongue papillae between species have been demonstrated using scanning electron microscopic.

Similarly, in our study, the goal was to study the tongue structure of Norduz sheep, which are a specific breed, morphologically using a scanning electron microscopic.

## MATERIAL AND METHODS

### Ethical Declaration

The study protocol was approved by the Ethics Committee (Van Yuzuncu Yil University Animal Experiments Local Ethics Committee, Van, Türkiye, Decision No: 2022/08-02).

### Animals

Tongue samples were obtained from 10 Norduz sheep, aged 4, with an average weight of 65 kg, brought from the Norduz campus of Gurpinar Van district and slaughtered in the city slaughterhouse under the supervision of a livestock specialist. 10 taken tissues of the tongue were washed with 0.1 M phosphate buffer solution (pH-7.4) completely and one at a time, after which they were left in containers containing 10% formaldehyde solution and delivered to the anatomical laboratory. A total of four small pieces of mucosa (approximately 60 mm<sup>2</sup>) were taken from the apex, body, and dorsolateral region of each tongue (Figure 1).

### Scanning Electron Microscopic

Fragments taken from different parts of the tongue tissue to study the papillae of the tongue were preliminarily thoroughly washed with a 0.1 M phosphate buffer solution (pH-7.4) in accordance with the procedure for preparing biological samples for scanning electron microscopy. Immediately after washing, the first fixation was performed on the samples and kept in 2.5% glutaraldehyde at +4 °C for 2 days. After this process, washing was again carried out in 0.1 M phosphate buffer

solution (pH-7.4). Then 1% osmium tetroxide was kept at room temperature for 1 hour for the 2nd fixation process. After the fixation process was completed, it was passed through a series of acetone at 60%, 70%, 80%, 90% and 100% for dehydration. Finally, tissues were dried in a critical point dryer (CPD) and transferred to a scanning electron microscopic unit. Pre-dried tongue samples were coated with gold palladium for visualization and photography. In the process of visualization; a scanning electron microscopic (Sigma 300 Zeiss Gemini FE-SEM, Germany) was used.

## RESULTS

The entire tongue taken from the Norduz sheep was about 15 cm averaged long. Structurally, the apical, corpus, and radix parts are clearly distinguished. A prominent torus lingua was observed in the caudodorsal position of the body. The indentation (fossa linguae) was very prominent at the anterior end of the torus linguae and in the middle position of the linguae corpus. In addition, an irregularly shaped median sulcus was found on the back of the tongue.

Tongue papillae: On the mucosa of the tongue, three mechanical papillae of the tongue (papillae filiformes, papillae conicae and papillae lentiformes), two taste papillae (papillae fungiformes and papillae vallatae) were observed in a total of five different ways.

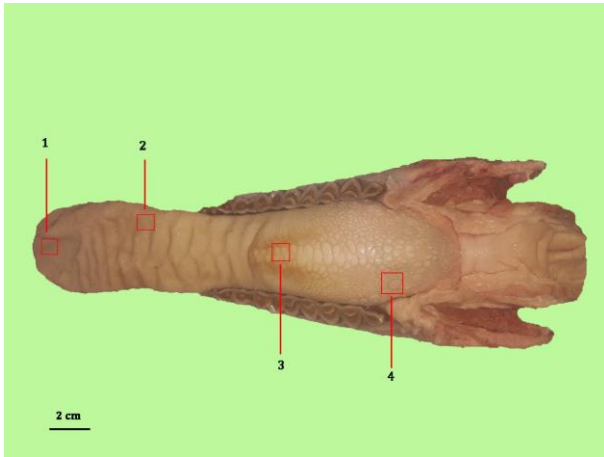
Papillae filiformes: defined as the densest papillae of the tongue on the lingual mucosa. It shows spread, especially from the region of the apex of the tongue and extending from the lateral regions of the body of the tongue to the base of the torus of the tongue. On scanning electron microscopic of specimens taken from apex linguae, the papillae filiformes originate from one broad body and consist of primary papillae tapering towards the ends and secondary papillae located on the right and left margins of the root of these primary papillae (Figure 2).

In addition, small and thin extensions of the papilla (small secondary papilla) originating from both the caudal part of the secondary papilla on the right and the caudal part of the secondary papilla on the left, the number of which varies from 1 to 2, are also among the visible structures (Figure 3). After detailed examination, it was found that the primary filiform papillae were approximately 285 to 440 µm in height at 32X magnification, and their width varied from 65 to 120 µm. When the morphological structures of the small secondary filiform papillae were examined at the same magnification, it was noted that their height varied from 110 µm to 235 µm and their width from 8 µm to 40 µm (Figure 2).

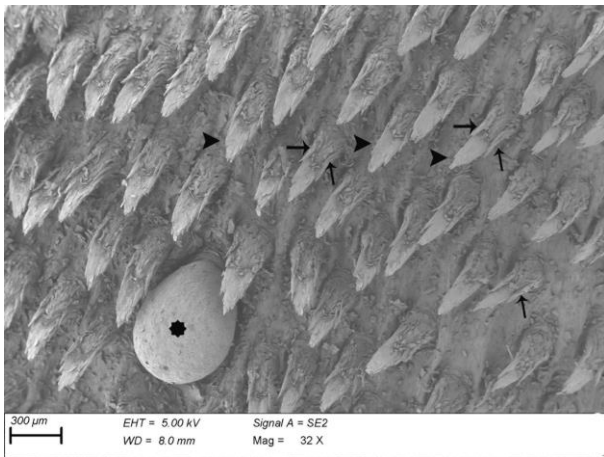
Papillae conicae: the conical papilla, which is the most prominent papilla of the papillae of the tongue; As the name suggests, these are mechanical lingual papillae covering the lateral inner surface of the lips and especially the inner surface of the cheeks, which resemble a typical cone shape (Figure 4).

Papillae lentiformes: Another of the mechanical papillae of the tongue, the lentiform papillae are located on the torus of the tongue. The length of these papillae, the number of which varies from 35 to 42, was 1200-1400 µm at 40X magnification, and the width was determined as 650 µm and 850 µm (Figure 5). In addition, at a magnification of 1.00 KX, the presence of pores with an average diameter of 8 µm at the ends of the lentiform papillae was determined (Figure 6). Both the small secondary extensions present in Papillae filiformes and any protrusions similar to this

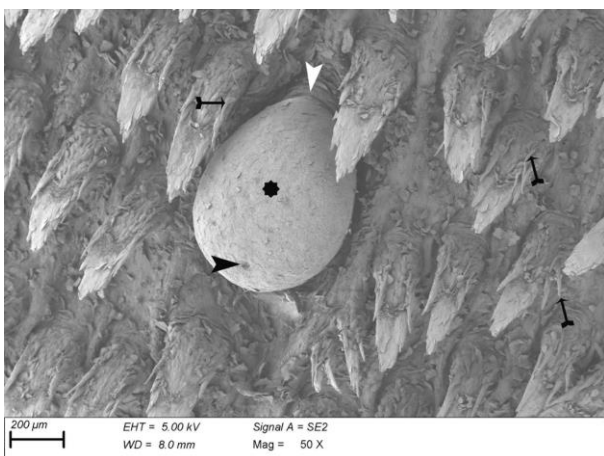
morphological structure were not observed in these papillae.



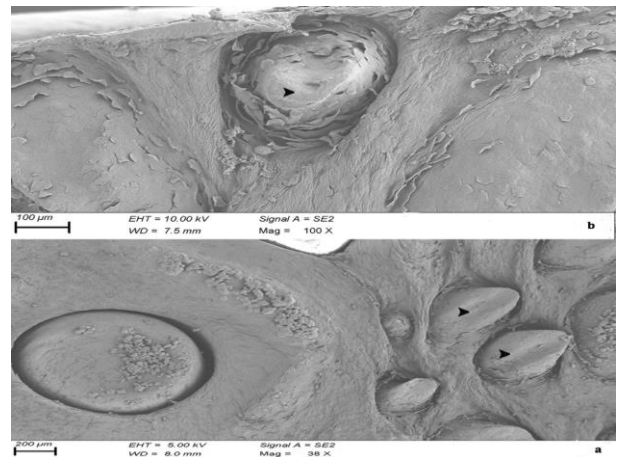
**Figure 1.** Overview of the dorsal surface of the tongue (1, 2, 3, 4 numbers displaying sample acquiring pattern).



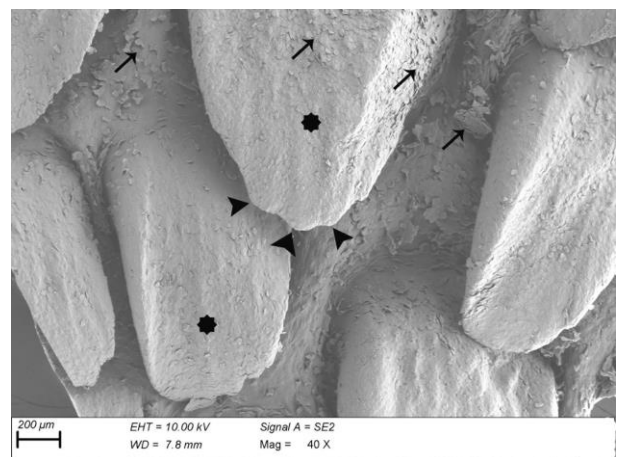
**Figure 2.** Image of papillae filiformes and a papillae fungiformes (arrowheads: papillae filiformes and papillae fungiformes appendages, black arrows: Secondary papillae filiformes appendages, black asterisk: a papillae fungiformes).



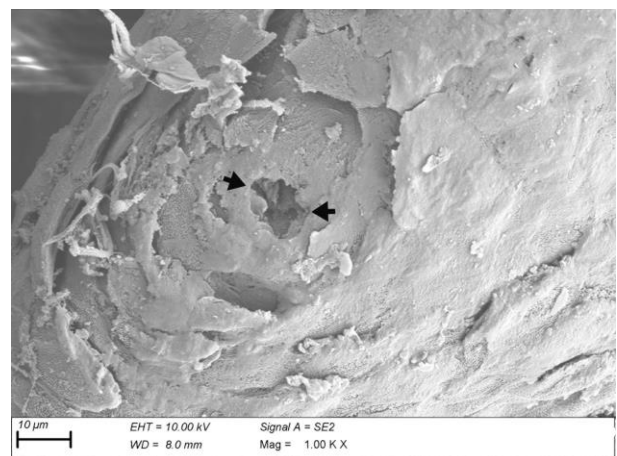
**Figure 3.** Papillae filiformes and one papillae fungiformes at 50X magnification (black arrows: small secondary papillae filiformes, black asterisk: one papillae fungiformes, black arrowhead: taste pore of papillae fungiformes, white arrowhead: annular groove in the root portion of papillae fungiformes).



**Figure 4.** a) arrowheads: papillae conicae; b) arrowhead: papillae conicae and layer of keratinized epithelium.

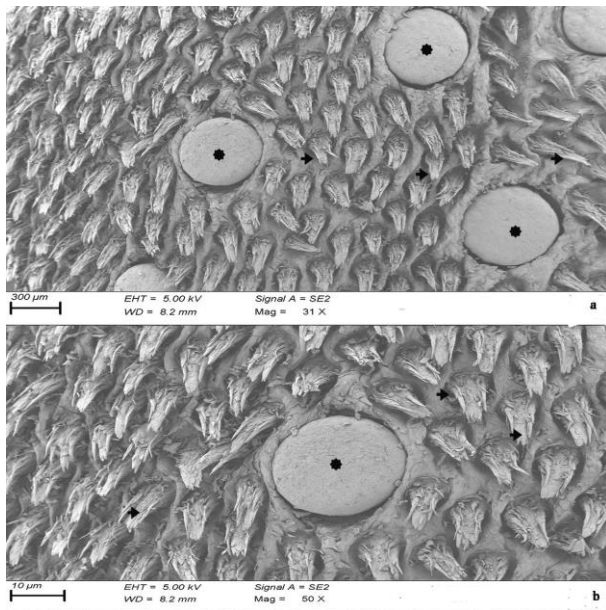


**Figure 5.** Papillae lentiformes (black asterisk: body of papillae lentiformes, black arrows: keratinized epithelial layer, black arrowheads: protrusions at the ends of papillae lentiformes).

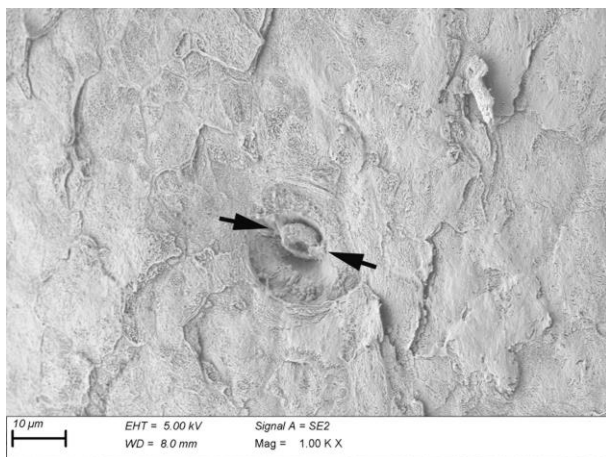


**Figure 6.** Papillae lentiformes image at 1.00 KX magnification (black arrows are the pore located at the tip of the papillae lentiformes).

**Papillae fungiformes:** These papillae involved in the perception of the sense of taste are papillae that appear as white dots, especially from the apex of the tongue, scattered throughout the body of the tongue (Figure 7). If on 1 cm<sup>2</sup> of the area of the corpus linguae their number averaged 26, then in the apex of the linguae this number was determined as 34. In our studies, it was found that the head of these papillae was approximately 300 µm at 50X magnification. In addition, the head parts of Papilla fungiformes; they form a constriction before joining with the tongue (Figure 3). This situation looks as if a ring has formed in the root parts of the papilla fungiformes (Figure 3). On closer examination, it was observed that both mucosal cells were separated by interseptal lines, and these cells had pores approximately 4 µm in diameter at 1.00 KX magnification (Figure 8).

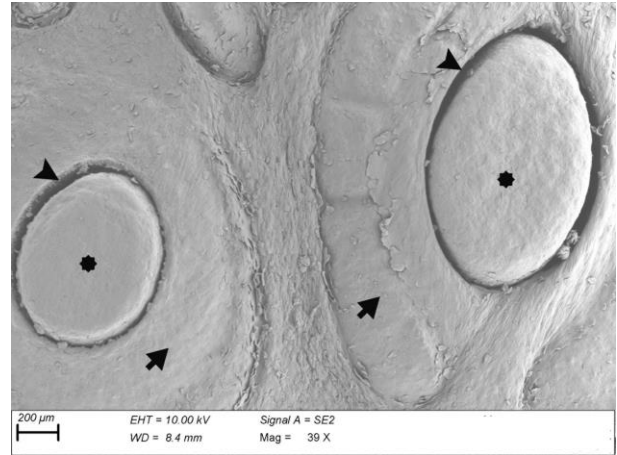


**Figure 7.** Papillae fungiformes scattered between papillae filiformes and papillae filiformes (black arrows (a,b): papillae filiformes, black asterisk (a,b): papillae fungiformes).



**Figure 8.** Image of papillae fungiformes at 1.00 KX magnification (black arrows: taste pores on papillae fungiformes).

**Papillae vallatae:** Taste papillae, which are located in two rows symmetrically on either side of the radix linguae and macroscopically resemble suction cups of octopuses. These papillae basically consist of 3 parts in the centro-lateral position. these; It consists of 1. Mucosal valium (round and flat mucosal area forming the center of the papillae), 2. Parietal trench, 3. Annular pad (Figure 9).



**Figure 9.** Image of papillae vallatae (black asterisks: mucosal valium, arrowheads: parietal ditch, black arrows: annular pad).

Their average number was determined as 26. In the scanning electron microscopic examination process, it was observed that the rostro-caudal lengths of the papillae vallata at 39X magnification ranged from 620 to 1800 µm from the smallest to the largest. Again, at the same magnification, Mucosal valium sizes were found to vary between 400 and 1000 µm. The parietal ditch was deep and clearly observed. It was observed that the annular pad was not flat but divided by 2-3 small notches. When the horizontal diameter lengths of the papillae vallata were examined from the smallest to the largest, it was observed that they varied between 520-1400 µm.

## DISCUSSION AND CONCLUSION

Nutrition process is a physiological state necessary for all living beings to ensure the continuation of their kind and survival. Living beings reach and receive nutrients only within the possibilities offered by their environment (Iwasaki 2002; Meyer et al. 2016). In this regard, it is known that the structure of nutrients consumed by ruminant species in different age periods and under different feeding conditions (closed, semi-open and pasture cultivation) is different.

Digestive system organs of ruminants; in particular tongue differs morphologically according to nutrition and food types. Norduz campus, located within the borders of Gürpınar district of Van; It contains pastures with different nutritional products in terms of sheep feeding. For this reason; The morphological character of the tongue structure of the Norduz sheep, which is an endemic breed and bred only in the Norduz region, attracts our attention.

When our study was examined, it was seen that Filiformes papillae were concentrated in the apex part of the tongue and the head parts ended long and sharply. This is the case, *Capra hircus* (Mahdy et al. 2021), Mehreban sheep (Tadjalli and Pazhoomand 2004), Karacabey merino sheep (Can et al. 2016), Angora goat (Toprak et al. 2020), Akkaraman sheep (Harem et al. 2009) similar to the results of such studies. However, unlike the results of our

study, the shape of the papillae filiformes; Anatolian water buffalo (Can and Atalgın 2015) and Bison banasus (Plewa and Jackowiak 2020) were also cone-shaped. Also the tip has taken the shape of a round rod in papillae filiformes in Chinese yellow cattle (Fu et al. 2016), South American Camelids (Erdoğan et al. 2016), These cases showed that the papillae filiformes can take different forms in animal species.

Secondary small filiformes papillae extensions were commonly seen in Norduz sheep. While this situation is one each on the right and left of the primary papilla protrusions. It can sometimes be found in smaller protrusions originating from the sides of these secondary protrusions. Therefore, papillae filiformes can contain 3-4 secondary small papillae filiformes projections in Norduz sheep. While this number is between 6 and 9 in Saanen goats (Kurtul and Atalgın 2008). It is 3-6 in Jamunapari goat (Kumar et al. 1988), and in Bighorn sheep (Takayuki et al. 2002), Karacabey merino (Can et al. 2016). Reeves muntjac deer (Zheng and Kobayashi 2006) was determined as two. Our findings in this study are similar to those of the Jamunapari goat (Kumar et al. 1988).

Papillae conicae are conical shaped papillae located near the root of the tongue and on the inner surface mucosa of the cheeks. When these papillae were examined at high magnifications with a scanning electron microscopic, they were found to be covered with keratinized tissue that aids in mechanical digestion. Also, this papillae no taste pore was found. While this situation is in line with the studies of Chinese manch (Zheng and Kobayashi 2006) and Anatolian water buffalo (Can et al. 2015), it differs by the any absence of papillae conicae in dromedary camel (Quayyum et al. 1988).

Papillae lentiformes are morphologically large tongue papillae that take part in the mechanical digestion process by being scattered in the upper part of the torus linguae of the tongue. In our study, it was observed that the tip of these papillae ended with a few blunt projections. Which was similar to the Chital deer (Erdoğan and Perez 2014), the South American camel (Erdoğan et al. 2016), and Sitatunga (Emura et al. 2011). In addition, as a result of our research, the presence of a pore was determined, unlike other studies, in the high magnification of the ends of these papillae with a scanning electron microscopic.

Papillae fungiformes is one of the taste papillae located scattered among the papilla filiformes mentioned earlier. These papillae are located in the region between the tip of the tongue (apex linguae) and the torus linguae. When examined in terms of shape, it is seen that they exhibit 2 different morphologies. In one of them, the papillae fungiformes are flattened and embedded in the papillae filiformes, while the other papillae fungiformes are more voluminous and protrude above the tongue mucosa. Again, this papillae in our study resembles a typical mushroom as it is separated from the tongue mucosa by an annular groove. Nonaka et al. (2008), Adnyane et al. (2010) mentioned the presence of the groove in the papilla fungiformes in their studies. However, this situation gray cattle (Tütüncü 2020) formocene goat antelope (Takayuki et al. 2002) were not found in studies.

Papillae vallatae are observed in two parallel rows on both sides of the radix part of the tongue, and it was determined that there were 23 in total. In some studies, it has been reported that this number varies between species and races. Deer with 10-30 papillae valalta (Adnyane et al. 2010) can be given as an example for the difference in species, while Barbary sheep (Emura et al. 2000) and

Bighorn sheep (Takayuki et al. 2002) with 26 papillae vallata can be given as examples. This situation differed from our study. In addition, the morphological structures of papillae vallatae also differ in animal species and breeds. This is due to the fact that the annular pad part has a developed possible or small weak structure. In our study, the annular pad was well developed and had 2-3 notched surfaces. This situation; similarly one humped camel (Quayyum ve ark. 1988) and ox (Chamorro et al. 1986). Our study; It differed with formosan serow (Atoji et al. 1998), in which the annular pad was weak. In our study, the largest rostro-caudal length of Papillae vallatae was found to be 1800 µm on average. In this respect, it has been revealed that the papillae vallatae in Norduz sheep has a longer morphological structure than Karacabey merino (Can et al. 2016).

As a result in Norduz sheep; it has been found that there are many similarities and differences between species and races in terms of tongue papillae. In this study, it was observed that the mechanical papillae of the tongue in Norduz sheep are in many ways similar to other breeds and species. However, the presence of a pore at the tip of the lentiform papillae in Norduz sheep is a special case. Papillae fungiformes of taste buds; contrary to many studies was observed in 2 different morphological structures with a flat and elongated appearance, and an annular notch was found in the root parts of the long papillae fungiformes. Another taste bud, papillae vallatae, was found to have an average of 26 and is located on either side of the radix linguae, similar to a typical octopus's sucker. This number remained below the mean value of 28 for papillae vallatae in sheep. However, it has been found that the papillae vallatae of Norduz sheep are averaged longer (1800 µm) than those of other breeds and breeds. With the results of this study, we believe that scanning electron microscopic can give good results in morphological studies and support future research in the same direction.

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## CONFLICTS OF INTEREST

The authors report no conflicts of interest.

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## AUTHOR CONTRIBUTIONS

Idea / Concept: VD, ZS  
 Supervision / Consultancy: VD, GÇ, MSG  
 Data Collection and / or Processing: VD, GÇ  
 Analysis and / or Interpretation: VD, MSG  
 Writing the Article: VD  
 Critical Review: VD, ZS, GÇ

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