

Light Microscopic Investigations on the Circumvallate Papillae of the Young and Aged Akkaraman Sheep

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Summary

In this light microscopic study, morphometric parameters of the circumvallate papillae and the number of their taste buds in tongues of young (6-9 month-old) and aged (7 year-old) Akkaraman sheep were compared in order to determine general morphology and whether there were age-related differences in general morphology and numbers of the taste buds between young and aged animals. The tongues were obtained from a local slaughterhouse. Tissue samples containing dorsal lingual papillae of 32 tongues (16 young and 16 aged) from both sexes (equal numbers of the males and females) were used for histological investigation. The mean number and diameter of the circumvallate papilla, mean number and diameter of the taste buds, and the taste bud number per circumvallate papilla were determined under the light microscope. There were no significant differences between the young and aged animals in the mean number and diameter of circumvallate papillae, the taste bud number and the diameter, taste bud number per papilla. Nevertheless, the aged animals had larger circumvallate papillae. These results showed that number of the taste buds and circumvallate papilla did not change with aging in Akkaraman sheep. Because that general morphology of the circumvallate papillae and their taste bud numbers did not change even in older animals, any age-related differences in the sense of taste can not be attributed to gross degenerative changes in taste buds of circumvallate papillae.

Keywords: Akkaraman sheep, Circumvallate papilla, Taste bud, Aging

Genç ve Yaşlı Akkaraman Koyunlarının Papilla Sirkumvallataları Üzerinde Işık Mikroskopik Araştırmalar

Özet

Bu ışık mikroskopik çalışmada, dildeki papilla sirkumvallataların ve bunların tat tomurcuklarının morfolojik parametreleri ile tat tomurcuklarının sayısında yaşa bağlı değişikliklerin meydana gelip gelmediğinin belirlenmesi amacıyla genç (6-9 aylık) ve yaşlı (7 yaş), Akkaraman koyunu dilindeki papilla sirkumvallataların genel morfolojileri ve morfolojik parametreleri ile tat tomurcukları sayıları karşılaştırıldı. Diller yerel bir mezbahadan temin edildi. Histolojik inceleme için dilin üst yüz papillalarını içeren, her iki cinsiyetten ve her iki yaş grubundan eşit sayıda (16 adet genç ve 16 adet yaşlı) olmak üzere toplam 32 dilden alınan doku örneği kullanıldı. Işık mikroskopunda yapılan incelemelerde ortalama papilla sirkumvallata sayısı ve çapı, papilla sirkumvallatalardaki tat tomurcuklarının ortalama çap ve sayılarıyla papilla başına düşen tat tomurcuğu sayısı belirlendi. Genç ve yaşlı hayvanların ortalama papilla sirkumvallata sayıları ve çapları, tat tomurcuğu sayıları ve çaplarıyla papilla başına düşen tat tomurcuğu sayıları arasında önemli fark bulunmadı. Bununla birlikte, yaşlı hayvanlar daha büyük çaplı papilla sirkumvallatalara sahipti. Bu sonuçlar, Akkaraman koyununda yaşlanmayla birlikte papilla sirkumvallata ve tat tomurcuğu sayılarının değişmediğini gösterdi. Papilla sirkumvallataların genel morfolojilerinin ve bunların tat tomurcuğu sayılarının yaşlı hayvanlarda değişmemesi nedeniyle, tat duyusu alımında yaşla ilgili değişikliklerin, papilla sirkumvallatadaki tat tomurcuklarında oluşan dejeneratif değişikliklere bağlanamayacağı sonucuna varıldı.

Anahtar sözcükler: Akkaraman koyunu, Papilla sirkumvallata, Tat tomurcuğu, Yaşlılık

INTRODUCTION

Two of the sensory systems, the taste and smell play crucial roles in the awareness and either acceptance or refusal

of the food by chemical stimulation¹. The sense of smell has a special importance in food intake. In sensing of the smell,



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food temperature and sense of quality based on visual effects also play significant roles. Quality of the chemical stimulus is directly effective on the sufficient enjoyment and pleasure with the selected foods¹⁻³. The importance of the taste sense in food preference is possibly related with giving priority to the foods obtaining special needs of the animal.

Taste sensing receptor cells together with supportive cells and reserve cells form special histological structures, known as taste buds. The taste buds are distributed in the gustatory papillae. There are 3 different types of the gustatory papillae, as circumvallate, foliate and fungiform papillae, which their distribution depends on the species of the animal. Because that the taste sense is perceived through interaction between the receptor cells and stimulating ions in the milieu, quality of the sense directly correlated to the number of the taste buds in the tongue⁴. Individual differences in the taste perception in human beings and animal species possibly arise from the differences in number and distribution of the gustatory papillae and their taste buds⁵.

Generally, it is believed that the sensory organs lose their functional capacity with aging⁶. In some of the previous studies⁷⁻¹² on age-related differences in gustatory papillae, it was claimed that the taste quality significantly or partially decreased with the decrease of the taste bud numbers. Conversely, the other researchers¹³⁻¹⁸ did not observe any age-related changes in both the number and structure of the gustatory papillae, and any loss in the function of sensory nerves.

Because of the lack of any consensus among the researchers, further studies are necessary to elucidate the relation between the taste perception and aging. In this study, the number and diameter of the lingual circumvallate papillae and their taste buds were compared in the tongue of young and aged Akkaraman sheep.

MATERIAL and METHODS

In this study, the tongues of 16 young and 16 aged Akkaraman sheep, a total of 32 tongues from both sexes were used. The tongues were obtained from a local (Konya Metropolitan Municipality) slaughterhouse. Age of each animal was determined according to the degree of wear of front incisive teeth¹⁹, and 6-9 month-old animals were classified as young whereas, 7 year-old animals were classified as aged groups.

Following decapitation of the animals, the tongues were excised and divided into two halves, each half was washed thoroughly with tap water and grossly examined for abnormal structures and lesions, fixed in 0.1M buffered formal-saline (pH 7.4) for 6 days²⁰. In order to facilitate the identification of circumvallate papillae, the tongues were stained with 0.1% methylene blue solution²¹ and the papillae were counted. The circumvallate papillae in right

half of each tongue were dissected; tissue samples containing the papillae were processed by means of routine histological methods and immersed in paraffin blocks. The blocks were transversally sectioned in 6 µm thick sections in order to do measurements of the taste buds and circumvallate papillae. The sections were stained with hematoxylin and eosin.

Counting the Circumvallate Papillae and Taste Buds and Measuring the Diameter of the Taste Buds and Circumvallate Papillae

The circumvallate papillae were counted on methylene blue stained lingual samples under a stereo microscope. These papillae located in a V-shaped line which, its bottom facing to the root of tongue. The papilla diameter was determined on serial cross sections and mean diameter was calculated.

The number of taste buds was determined with Bradley's formula²¹, which was given below. Briefly; at first step, the taste bud number of each section was determined and it was multiplied by the section thickness. The result was divided by mean diameter of the taste bud.

$$\text{Taste bud number per papilla} = \frac{\text{Taste bud number in each section} \times \text{section thickness}}{\text{Mean taste bud number}}$$

The mean taste bud number per papilla of each individual was multiplied with total papilla number and total taste bud number of each individual was found. The mean taste bud diameter was calculated as follows: the diameter of 10 taste buds in a given section was measured from beginning to the end, and the result was divided by the measured taste bud number. The data was analyzed by ANOVA and the mean values of the groups were compared and P<0.05 was considered statistically significant.

RESULTS

Results of the Gross Morphological and Stereo Microscopic Observations

Two types of the dorsal lingual papillae with taste buds (gustatory papillae) were observed in Akkaraman tongue. Circumvallate papillae located close to oro-pharyngeal line and in a V-shaped line (Fig. 1).

In cross sections, a circumvallate papilla was seen as a mucosal prominence typically surrounded by a papillary groove (Fig. 2). At low magnification, the taste buds were seen as pale structures in the stratified squamous epithelial layer of the circumvallate papillae (Fig. 3).

At higher magnifications, nuclei of the sensory cells in the taste bud cells located 2/3 basal region of the cell. Reserve cells were also seen in basal regions of the taste buds (Fig. 4).

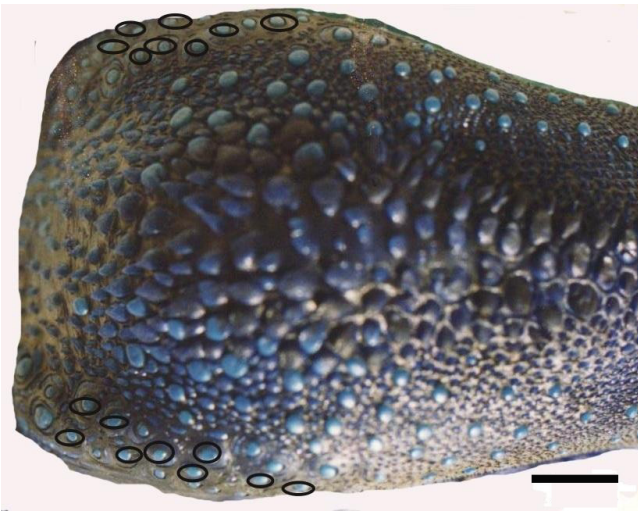


Fig 1. Circumvallate papillae (○) are seen on dorsal posterior surface of a young Akkaraman sheep tongue, which was stained with 0.1% methylene blue. Bar: 2 cm

Şekil 1. %0.1'lik metilen mavisi ile boyanmış genç bir Akkaraman koyun dilinin arka bölgesi üst yüzündeki papilla sirkumvalatolar görülmektedir. Bar: 2 cm

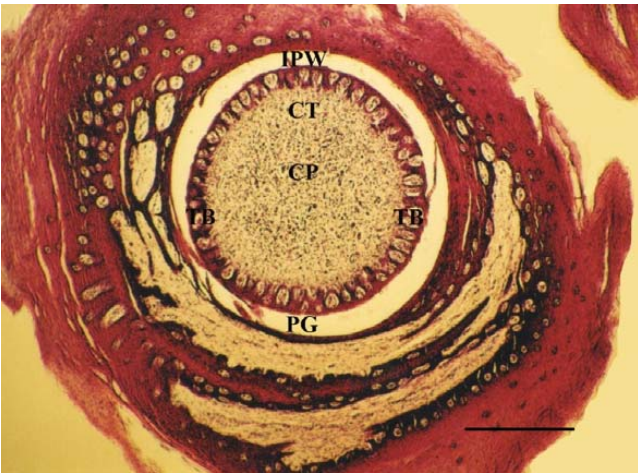


Fig 2. Taste buds are seen in a section of circumvallate papilla from a young animal. The section was taken through parallel to papillar surface. CT: Connective tissue, IPW: Inner papillar wall, PG: Primary papillar groove, CP: Circumvallate papilla, TB: Taste buds. H&E, Bar: 50 µm

Şekil 2. Genç bir hayvanın papilla sirkumvalatasından alınan bir kesitte tat tomurcukları görülmektedir. Kesit papilla yüzeyine paralel alınmıştır. CT: Bağ doku, IPW: Papilla iç duvarı, PG: Papilla çukuru boşluğu, CP: Papilla sirkumvalat, TB: Tat tomurcukları. H&E, Bar: 50 µm

Results of Morphometric Investigations of Circumvallate Papillae and Taste Buds

Mean papillae numbers were between 30-40 per tongue. The number and location of circumvallate papillae were quite uniform and individual differences were very low. Age- and sex-linked differences were insignificant ($P < 0.05$, Table 1). Individual differences were relatively high. The papilla diameter values of the young animals were between 829-1018 µm, whereas those of the aged group arranged between 1211-1478 µm. Significant ($P < 0.05$) differences were observed between mean papilla diameter of the young and aged groups.

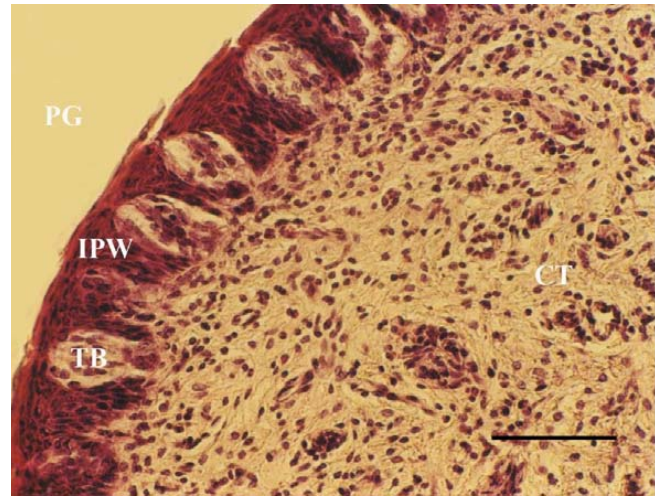


Fig 3. Taste buds are seen as pale regions in the mucosal epithelium of the papilla in a section through parallel to papillar surface. CT: Connective tissue, IPW: Inner papillar wall, PG: Primary papillar groove, TB: Taste bud. H&E, Bar: 500 µm

Şekil 3. Papilla yüzeyine paralel bir kesitte papillanın mukozal epitelinde tat tomurcukları soluk bölgeler olarak görülmektedir. CT: Bağ doku, IPW: Papilla iç duvarı, PG: Papilla çukuru boşluğu, TB: Tat tomurcukları. H&E, Bar: 500 µm

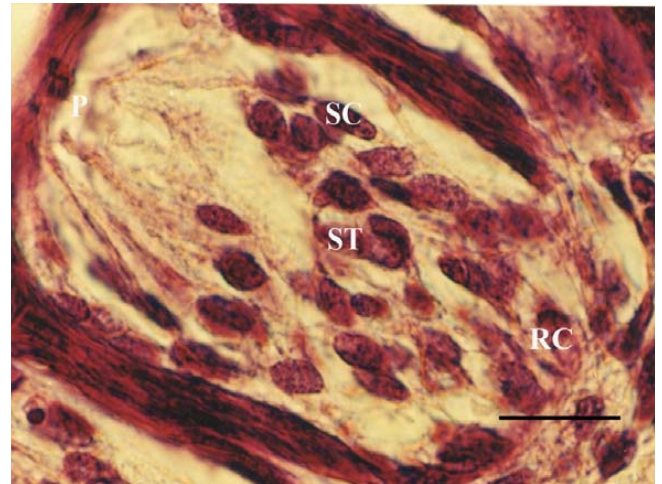


Fig 4. Higher magnification of a taste bud. SC: Sensory cell, ST: Sustentacular cell, RC: Reserve cell, P: Bud's pore. H&E, Bar: 1250 µm.

Şekil 4. Bir tat tomurcuğunun yüksek büyütmedeki görünümü. SC: Duyu hücresi, ST: Destek hücresi, RC: Rezerv hücre, P: Tat tomurcuğu poru. H&E, Bar: 1250 µm

Total taste bud number of the circumvallate papillae of each individual was 18830-21462 for the young and 19623-21971 and for the aged animals and the difference between age groups was not significant ($P > 0.05$). Mean taste bud numbers in circumvallate papillae of individuals arranged between 527-678 and 577-650 in the young and aged animals, respectively. Taste bud number per papilla was similar in both sex age groups and there was no significant difference ($P > 0.05$) between the groups. Taste bud number distribution of the aged animals arranged at the limits those of the young animals. The taste bud diameters were measured between 39.5-43.4 µm in the young animals and 37.5-43.3 µm in the aged animals. Neither individual nor age differences were not significant ($P > 0.05$).

Table 1. Results of the measurements of circumvallate papillae and their taste buds of young and aged Akkaraman tongues**Tablo 1.** Genç ve yaşlı Akkaraman koyun dillerinde sirkumvallat papilla ve tat tomurcuklarının ölçüm sonuçları

Groups	Number of the papillae	Taste bud number per papilla	Total number of taste buds	Papilla diameter	Taste bud diameter
Young	35.62±1.5 ^a	605.51±10.5 ^a	21435±558 ^a	929.4±14 ^b	39.25±0.9 ^a
Aged	34.93±0.5 ^a	603.54±8.3 ^a	21237±631 ^a	1308.5±31 ^a	40.05±8.0 ^a

* Values reported are (X±SE), ^{a-b}: The difference between values in the same column with different superscripts are statistically significant (P<0.05)

DISCUSSION

Two types of the gustatory lingual papillae, fungiform and the circumvallate types were seen on dorsal surface of in Akkaraman sheep. The circumvallate papillae primarily located at radix lingua, close to the oropharyngeal border in a V-shaped line fashion. The papillae surrounded by a hollow primary groove.

Individual mean circumvallate papilla number was 35 per tongue in Akkaraman sheep and it is very close to that of the cattle^{22,23}. Rhesus monkey²¹, human¹⁵, rat¹. Mistretta¹⁵ reported that the circumvallate papillae located more closely to the lingual root, also function as fungiform papillae, which are not found the sheep. The in previous researchers^{4,13,14,21} mainly investigated the number of the papillae and taste buds. Nevertheless, little is known about morphological features of both papillae and taste buds.

Mistretta and Baum¹ observed close similarities in both diameter and morphological features of young and aged rats. Davies et al.²², reported the diameter of the circumvallate papillae for the cattle 1.5-4.5 mm. In this study, mean diameter of the papillae arranged between 0.9-1.5 mm and it was smaller than that of the cattle.

Davies et al.²² reported the taste bud diameter as 30 µm, for the cattle circumvallate papillae and Mistretta and Baum¹ as 46 µm, for both the young and aged rats. Bradley et al.²¹ investigated taste buds in five different age group of Rhesus monkeys and identified the mean diameter as 48 µm, There was no statistically significant (P>0.05) differences between the taste bud diameters of the groups.

In this study, the diameter of taste buds of the circumvallate papillae was 39.0-40.5 µm and averagely 39.8 µm in the young and aged Akkaraman sheep tongue and the values were higher than those of the cattle²², whereas smaller than those of the rat¹. There was not statistically significant difference (P>0.05) between the mean diameters of the young and aged animals. Sex-linked difference was not also observed.

The taste bud number per papilla was 600.8 for the young and 594.5 for aged animals. Total numbers were 21487 for the young and 21277 for the aged animals. The values were higher than that of humans²⁴, cattle²², rats¹. Nevertheless, the values were smaller than that of Rhesus monkey²¹.

There are great contradictions between the results of the previous and recent studies on the age-dependent numerical changes of the gustatory papillae and taste buds. Although, in previous studies^{7,8} significant reductions in both gustatory papillae and their taste buds of the human tongue have been observed in aged individuals. However, results of the more recent studies¹⁴⁻¹⁸ have revealed that there was no significant decrease in both parameters with aging. In the previous studies^{7,8}, the experiment material have been obtained from cadavers, which their detailed health status was unknown. Although the researchers^{7,8} have suggested that 50% decline in the circumvallate papilla number was found, their statistical method is not clear. In a previous study⁹ claiming 30% decrease occurred with aging, only limited amount of tissue material have been investigated. Their investigations⁹ are also lack of statistical analyses.

In a previous study²⁵, on the histology of taste buds, leukocytes were observed in the taste buds. Beidler et al.²⁶, Beidler^{27,28} reported relatively high cell growth and destruction rate in the taste bud. Beidler and Smallman²⁹ determined life span of the bud cell as 250±50 h¹. These finding show that a continuous regeneration event occurs in the bud cell population. Mc Bride and Mistretta³⁰ electro physiologically studied on the taste buds of Fisher's rats with 24 months lifespan. The researchers³⁰ recorded strong signals in response to salt, acid, sucrose, quinine solutions, and concluded that there was not any significant loss in the taste sense with aging in the rat.

Results of the recent experiments show that the number of gustatory papilla and their taste buds did not change with aging. In accordance with previous researchers, Davies et al.²² in gustatory papillae of the 4-6 old cattle, Mistretta and Baum¹ in 5-7 and 23-24 month old Wistar rats, Bradley et al.²¹ in 4-31 years-old Rhesus monkeys, Ünsal et al.¹⁸ 6-9 month-old and 5-6 years-old Akkaraman sheep, Arvidson¹³ in 2-90 years-old humans, Cheng and Robinson¹⁶ and Miller³¹ in 27-80 years-old humans Mavi and Ceyhan¹⁷ that there were not any significant changes in the number both of the gustatory papillae and taste buds in 17-25 and 65-85 years-old humans showed. These studies, which performed on healthy subjects, have confirmed that there was no significant differences between young and aged populations of the number of gustatory papilla and the taste bud number did not change in the aged individuals.

Based on the results it was concluded that the aged Akkaraman sheep had larger circumvallate papillae. However,

there was not any significant difference both in the number of circumvallate papilla and their taste buds of the tongue between the young and aged groups.

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