

**MORPHOLOGICAL FEATURES OF THE LINGUAL
PAPILLAE IN EGYPTIAN WATER BUFFALO
(BUBALUS BUBALUS)**

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ABSTRACT

Morphology and distribution of the lingual papillae of water buffalo was investigated using light and scanning electron microscope. The caudal part of the tongue had a rounded prominence (torus linguae), in front of which deep fossa (fossa linguae). Although, the buffalo' tongue carried a variety of papillae; filiform, conical, lentiform, fungiform and vallate the foliate papillae were not observed. The filiform papillae were distributed all over the dorsum of tongue, the lateral border and the marginal part of the ventral surface of tongue. The conical and lentiform papillae were restricted to the torus linguae. The fungiform papillae were distributed all over the dorsum and the lateral borders of the tongue. There were two types of fungiform papillae; pigmented type which was restricted to the apex linguae and none pigmented one, restricted to the periphery of the tongue. The vallate papillae were arranged in 2:3 rows. Their number ranged from 18:24 on each side.

INTRODUCTION

Understanding how the diet selection and food intake are controlled occupies an important place this. In case of ruminant animals, the special reasons for our interest in the subject are the complexities of the digestive system and consequent metabolic peculiarities (Fisher, 1996). The tongue is musculo-membranous highly mobile and versatile organ essential for prehension, sorting of solid food in some animals and intake of liquid in others. In new born animals the tongue plays an important role in suckling as well as plays an important role in chemical selection of food by means of taste buds (Nickel et al., 1979). Several studies had been carried out on the tongue of different animal species as; buffalo (Sengar and Singh 1970; Dhingra and Barnwal 1979; Dhingra and Barnwal 1980; Prakash and Rao 1980; Gupta, et al. 1989; Scala, et al. 1993; and Kadam, et al. 1995); bovine (Davies, et al. 1979; Steflik, et al. 1983; Yamada, et al. 1983; De paz Cabello, et al. 1988 and Gargiulo, et al. 1995); goat (Qayyum and Beg 1975; Kumar, et al. 1998; Kobayashi et al. 2005 and Igbokwe and Pkolie 2009); barbary sheep (Emura, et al. (2000); newborn lambs (Tadjalli, 2004); dromedary camel (Qayyum, et al. 1988; Ibrahim, 1983; Qayyum, et al. 1991 and EL-Sharaby, 2006); bacterian camel (Eerdunchaolu, et al. 2001); lesser mouse deer (Agungpriyono et al. 1995); horse (Pfeiffer, et al. 2000 and Chamoro, et al. (1986); donkey (Abd-Elnaeim, et al. 2002; and Salem, 2002); pig (Kumar and Bate 2004; Balah, 1979 and Schneider, et al. 1996); dog (Soliman and Attia 1974); cat (Ojima, et al. 1997); silver fox (Jackowiak and Godynicki 2004); black rhinoceros (Emura, et al. 2000); European mole (Jackowiak, 2006); common shrew (Jackowiak, et al. 2004); Japanese macaque and savanna monkeys Emura et al. (2002) and of human (Azzali, et al. 1989; Riva, et al. 1999 and Junquera, et al. (1971). This study aimed to determine the morphological-functional relationship of the lingual papillae.

MATERIALS AND METHODS

This study was carried out on ten buffalo's tongues of both sexes collected directly after slaughtering from Kafer EL-Sheikh abattoir and subjected for gross, light microscopic and scanning electron microscopical studies.

I- macroscopical study

Six buffalo's tongues were used for this purpose. The tongues were immediately fixed by immersion in 10% formaldehyde solution. The macroscopic features and the distribution of the lingual papillae were describe the by naked eye, using photographed by digital camera.

II- Light microscopical study

Samples for light microscope study were collected from two tongues. Specimens were taken from the apex l, fossa, torus, radix, corpus linguae and the mucous membrane of the ventral surface of tongue. These specimens were immediately fixed in Bouin solution for 24 h., dehydrated in gradual ethanol series, cleared in xylene then embedded in paraffin. Sections of 4 μ m thickness were made by Leica RM microtome and stained either by Harris hematoxylin and Eosin (H&E) for general histological examination or periodic Acid Schiff (PAS) technique for demonstration of neutral mucopolysacchardes, Alcian blue at pH 2.5 (AB2.5) for demonstration of acidic mucopolysacchardes, Crossman's trichrome for differentiation between connective tissue fibers and muscles, Van Gieson and Verhoff stain for differentiation between connective tissue fibers. The staining methods were adopted according to Bancroft and Stevens (1997).

III- Scanning electron microscopical study (SEM)

Samples for SEM were collected immediately after slaughtering, rapidly removed and pieces of small blocks, fixed in 3% gluteraldehyde for 24 hours then dehydrated in a gradual acetone series. After complete dehydration was, the blocks were transformed into the critical point drier, where a liquid CO₂ gradually substitute the acetone within the tissue blocks. The dried samples were mounted on metal stubs with conducting carbon paint and sputter coated with gold by sputtering device (Teoljfc-1100E). The specimens were examined with a scanning electron microscope (Jeol jsm 5300) at 25 KV. The nomenclature was adopted according to the electronic version of Nomina Anatomica Veterinaria (2005) whenever possible.

RESULTS

1. Lingua (Tongue)

The tongue of buffalo fills the oral cavity proper when the upper and lower teeth are in contact with each other. It was situated on the floor of mouth cavity between the two horizontal rami of mandible. The length of tongue of buffalo was ranging from 36-38 cm from the root of tongue to the lingual apex and about 32 cm from the palatoglossal fold to apex. The free part was measured about 10cm in length so the free part of the tongue constitutes about 25% of the total length of the tongue. The width of the tongue varies along the whole length. It was found that the rostral part (apex) was wider than the caudal one. The tongue was divided into 3 main parts; apex, body and root. The apex was dorsoventrally flattened,

had dorsal and ventral surfaces and two borders. The caudal part presented three surfaces; dorsal and two lateral surfaces (Fig. 1a, 1b, 1d). The dorsal surface of the tongue contain large rounded prominence called torus linguae, in front of it deep fossa called fossa linguae (Fig. 1a, 1b, 1c).

II Papillae Linguales (Lingual papillae)

The tongue of buffalo carried a variety of mechanical and gustatory papillae. The mechanical papillae included the filiform, conical and lentiform papillae, while the gustatory papillae included the fungiform and vallate papillae (Fig. 1b, 1c, 1f).

i Papillae Filiformis

The filiform papillae were the most numerous lingual papillae, distributed all over the dorsum up to the torus linguae, on the lateral borders and the marginal part of the ventral surface of the apex and also founded in the area of the fossa linguae. It also found on the lateral surface of the body of the tongue (Fig. 1b, 1c, 1f). The filiform papillae was short, thick, caudally directed, gave the tongue it a raspy appearance. With the aid of S.E.M., The dorsal surface of this was long band-like, directed caudally, with blunt apex. The surface of this papilla was covered by scales of keratinized epithelium (Fig. 1g). These papillae were lack of secondary papillae. The site of emergence of the filiform papillae was depressed from the surface. The inter-papillary spaces were studded with exfoliated keratinized epithelium like the surface of the filiform papillae. The filiform papillae of the marginal part of the ventral

surface of the tongue were less numerous, shorter than that of the dorsum. It has pointed tips and longitudinal groove. It also lacks the secondary papillae (Fig. 1h). The filiform papillae were cornified, thread like contain two surfaces; caudal concave and rostral convex surfaces. The keratinized layer could be differentiated into two parts according to the degree of keratinization; the rostral part was lightly keratinized, while the caudal part was highly keratinized. The connective tissue core was under the level of epithelial surface (Fig. 4d, 4e).

ii Papillae Conicae

The distribution of the conical papillae was restricted to the torus linguae especially in its central parts. They varied in size, larger on the center of the torus and began to diminish towards the periphery and some of them were directed laterally (Fig. 1c). With the aid of S.E.M. the conical papillae appear large in size, broad and blunt and directed caudally toward the root of the tongue. The rostral surface was sloped and steeper caudally. Inter and papillary space surface of the papillae was rough and carried exfoliated epithelium (Fig. 2e). The higher magnification revealed the presence of longitudinal groove on its rostral surface. The conical papillae were formed of highly keratinized stratified squamous epithelium and connective tissue core which not reach the level of the surface epithelium and carry secondary papillae (Fig. 4 h).

iii Papillae Lentiformes

The lentiform papillae were lentil-like papillae of various sizes. They were present on the dorsal surface of the tongue being limited to the area of torus linguae. They were more concentrated caudally and medial to the conical papillae.

iv Papillae Fungiformes

The fungiform papillae of buffalo were widely distributed all over the dorsum and lateral borders of the tongue. Two types of fungiform papillae could be distinguished on the buffalo tongue. The first one was pigmented and restricted mainly to the apex linguae and the lateral borders of the tongue. The second type was non pigmented and present on the caudal part of the apex and over the torus linguae especially on the periphery than the central part of the dorsum. There were different sizes of fungiform papillae present within the same animal (Fig. 1 b, 1 c). By the aid of S.E.M. the fungiform papillae of buffalo appeared circular, rounded or dome shaped in outline. They were scattered among the filiform papillae on the dorsum of the tongue and the marginal part of the ventral surface of the lingual apex. The dorsal surface of the fungiform papillae was convex, appeared uneven and contains exfoliated keratinized epithelium with higher magnification. The surface contained about three circular openings (taste pores) each one surrounded by higher area and raised above the surface of the tongue (Fig. 1h, 2c, 2d) and demarcated from the surface epithelium by a shallow furrow (Fig. 4f, 4g). It was covered with stratified squamous keratinized epithelium. its basal cell layer contained pigment granules in some papillae. We could not found taste buds in the apical surface of the papillae with the light microscope level. The connective tissue core was highly vascular dense irregular connective tissue from which originates secondary papillae (Fig. 3h, 4a).

v *Papillae Vallatae*

The vallate papillae of buffalo were present on the caudal part of the tongue in front of the palatoglossal fold (Fig. 1d), arranged in 2-3 irregular rows. The size of the vallate papillae varies from one papilla to another (Fig. 1e). They were rounded in shape surrounded by groove (moat) which in turn was surrounded by an elevation called vallum. Two vallate papillae seen surrounded with one groove and vallum. The number of vallate papillae varies from one animal to another. They were ranged from 18-24 in number on each side of the tongue. It was observed that, there were some difference in number between the right and left side of the tongue ranged from 1-2 vallate papillae (Fig. 1d, 1e). By using the S.E.M. the vallate papillae appear rounded in shape, surrounded by deep groove (moat). The moat was surrounded by an elevated edge (vallum). The vallum carries secondary grooves which end into the primary groove. The surface of the vallate papillae appear flattened, not raised above the surface of the tongue. The free surface of the papillae was carried numerous small and rounded openings (Fig. 2h, 3a). The circumvallate papillae were quadrilateral in shape surrounded by furrow (moat). It was covered by stratified squamous epithelium which was keratinized at the dorsal surface of the papilla and less keratinized at the lateral surface. Taste buds could be observed as lightly stained small areas in the lateral lining epithelium facing to the moat (furrow). The core of the circumvallate papilla was formed of highly vascular and innervated dense irregular connective tissue carrying secondary papillae

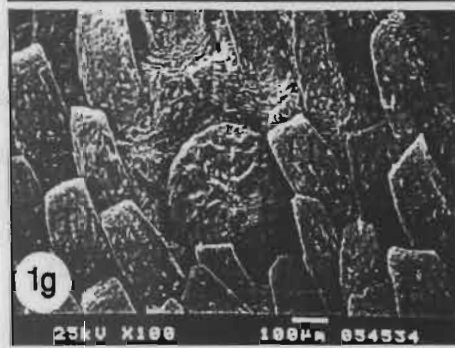
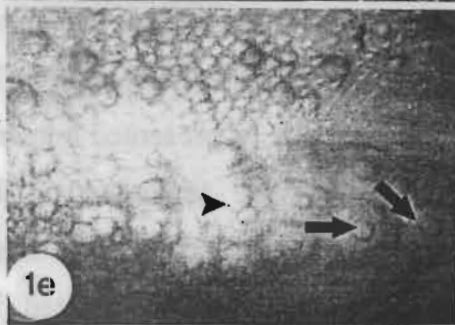
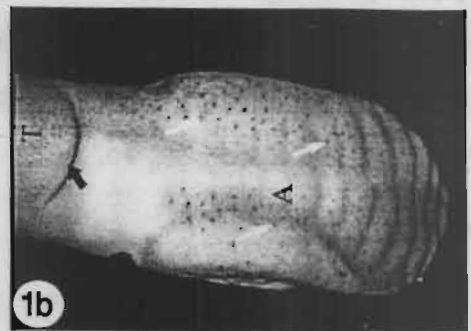
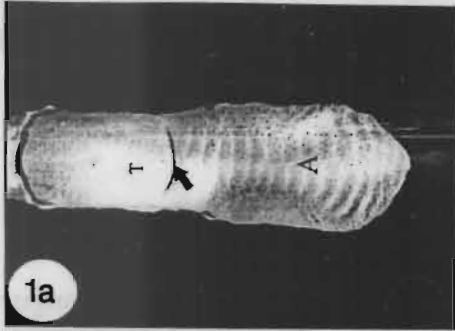
which penetrate the epithelial lining of the papillae. Lobules of seromucoid glands were found in the connective tissue under the papillae especially toward the moat. The ducts of these glands opened into the base of the moat (Fig 5a, 5b, 5c).

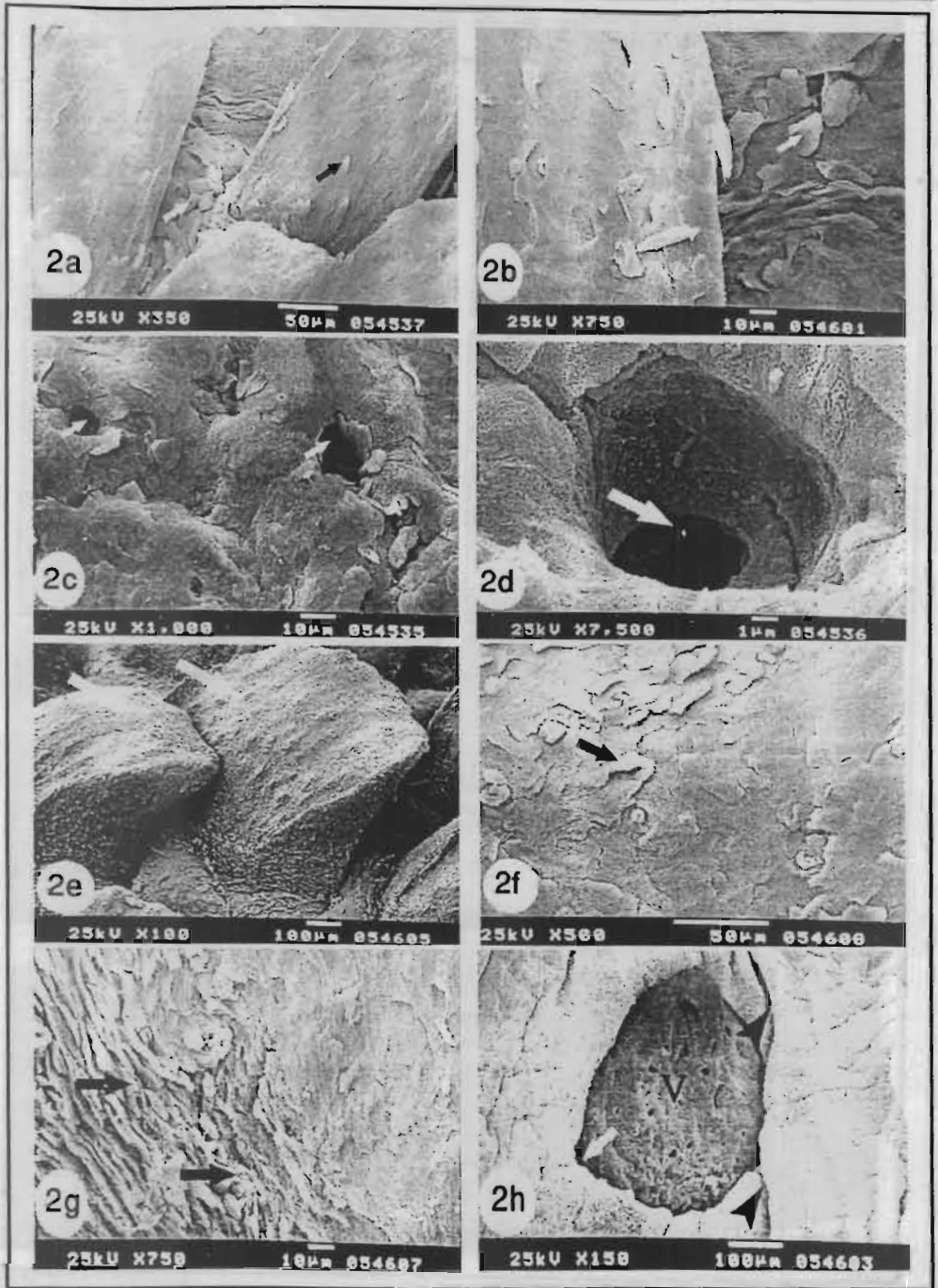
vi Papillae Foliatae

Microscopically, there were no foliate papillae on the tongue of buffalo. But there were low mucosal folds of tongue in front of glossopalatine fold.

III Root of Tongue

The root of the tongue was smooth, free from any type of lingual papillae but contain openings of the posterior lingual salivary glands which cover the surface of the root with its mucous secretion. The root of the tongue slope caudally and downwards to the base of epiglottis (Fig. 1d). By S.E.M. the root of the tongue was appear to presented numerous folds and depressions (Fig. 3b). The folds were separated by deep grooves, by higher magnification the root was revealed irregular shaped openings of posterior lingual glands. The edges of it was elevated (Fig. 3c).The surface of the cells of the root carries numerous micro ridges (Fig. 3e). In sections taken at the root of the tongue, there were seromucoid and mucous glands were found between the skeletal muscle bundles. Their ducts open in the dorsal surface of the tongue. The seromucoid glands were numerous than the mucous ones (Fig. 4c).







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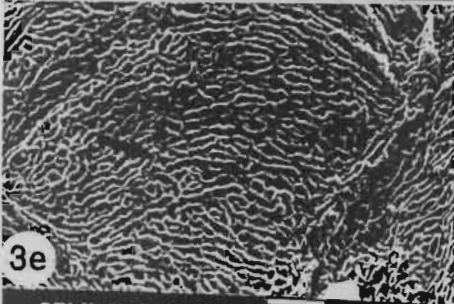
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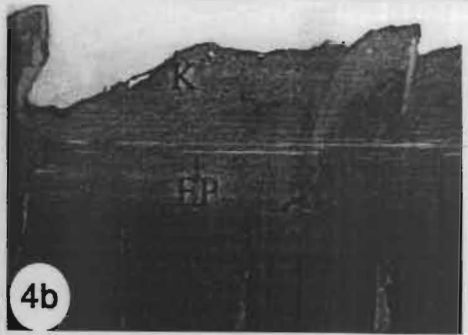
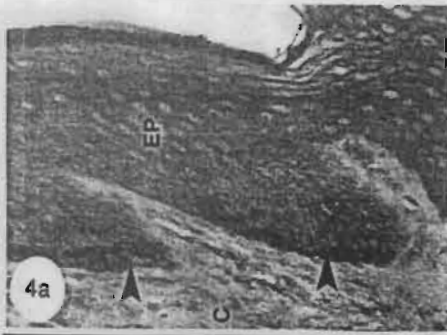


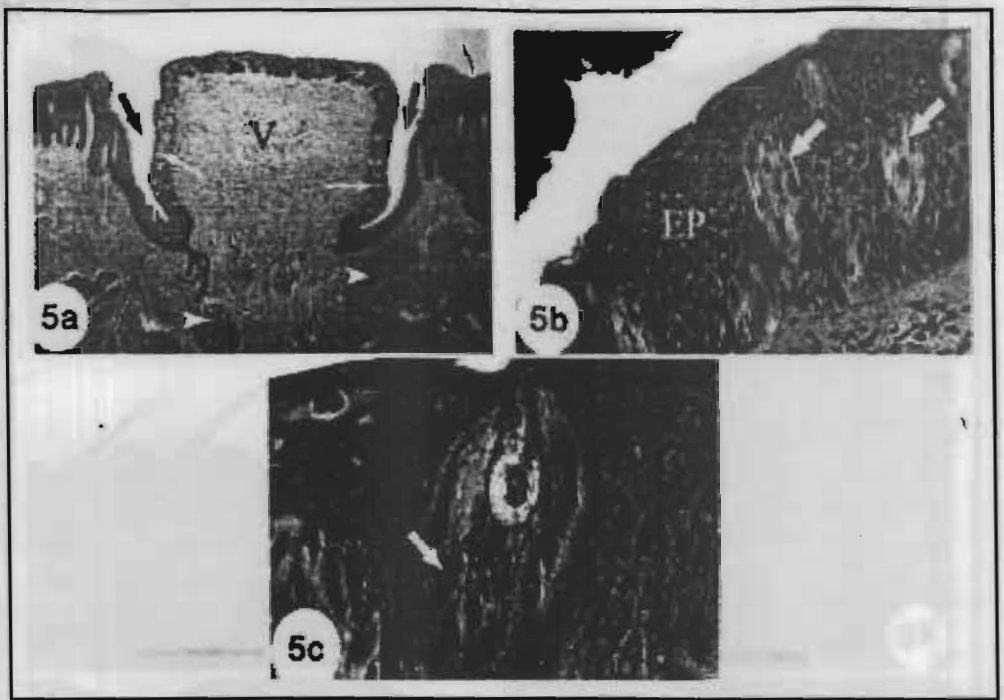
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LEGENDS

- Fig. (1a):** Photograph of isolated tongue showing, apex linguae (A), torus linguae (T) and fossa linguae (black arrow).
- Fig. (1b):** Photograph of isolated tongue showing, torus linguae (T), fossa linguae (black arrow), apex of tongue (A) and fungiform papillae (white arrow).
- Fig. (1c):** Photograph of isolated tongue showing, conical papillae (black arrow) and fungiform papillae (white arrow).
- Fig. (1d):** Photograph of isolated tongue showing, root of tongue (R) and vallate papillae (black arrow).
- Fig. (1e):** Photograph of the caudal part of isolated tongue showing, vallate papillae (black arrow), vallum (white arrow) and gustatory furrow (black arrow head).

- Fig. (1f):** Photograph of the lateral surface of isolated tongue showing, filiform papillae (FI) and fungiform papillae (black arrow).
- Fig. (1g):** A scanning electron micrograph of filiform papillae (FI) and fungiform papillae (FU) of dorsal surface of tongue showing a groove surround the fungiform papilla (black arrow). X. 100.
- Fig. (1h):** A scanning electron micrograph of filiform papillae (FI) and fungiform papillae (FU) on the marginal part of ventral surface of tongue showing a longitudinal groove on the surface of filiform papillae (white arrow). X. 100.
- Fig. (2a):** A scanning electron micrograph of filiform papillae showing, scales of keratinized epithelium on the surface of filiform papillae (black arrow) and scales of keratinized epithelium on the inter papillary space (white arrow). X. 350.
- Fig. (2b):** A scanning electron micrograph of filiform papillae showing, scales of keratinized epithelium on the surface of filiform papillae and inter papillary space. X. 750.
- Fig. (2c):** A scanning electron micrograph of apical surface of fungiform papilla showing, taste pores of fungiform papilla (white arrow). X. 1000.
- Fig. (2d):** A scanning electron micrograph of fungiform papillae showing, deep and round taste pore (white arrow). X. 7500.
- Fig. (2e):** A scanning electron micrograph of the conical papillae (white arrow).
- Fig. (2f):** A scanning electron micrograph of the conical papillae showing scales of keratinized epithelium on the anterior surface of the papilla (black arrows). X. 500.
- Fig. (2g):** A scanning electron micrograph of the inter-papillary space of conical papillae showing scales of highly keratinized epithelium of the torus linguae. X. 750.

- Fig. (2h):** A scanning electron micrograph of the vallate papillae (V) showing, gustatory furrow (white arrow), vallum (white arrow heads) and secondary grooves on the vallum (black arrow). X. 150.
- Fig. (3a):** A scanning electron micrograph of the vallate papillae showing, numerous pores (black arrows). X. 500.
- Fig. (3b):** A scanning electron micrograph of the root of tongue showing, mucosal folds (black arrow) and deep furrows (white arrow). X. 1000.
- Fig. (3c):** A scanning electron micrograph of the root of tongue showing, crater- like openings of the posterior lingual glands (black arrow). X. 500.
- Fig. (3d):** A scanning electron micrograph of the root of tongue showing, elevations (black arrows) and depressions (white arrows) on the surface of the root of tongue. X. 200.
- Fig. (3e):** A scanning electron micrograph of the root of tongue showing, numerous micro ridges (black arrows) on the cell surface of the root of tongue. X. 15000.
- Fig. (3f):** Photomicrograph of the dorsal surface of the tongue showing, filiform papillae (black arrow), stratified squamous keratinized epithelium (EP), lamina propria (LP) and skeletal muscle fibers (SK). Stain H&E., X. 40.
- Fig. (3g):** Photomicrograph of the ventral surface of the tongue showing, skeletal muscle fibers (SK) and stratified squamous keratinized epithelium (EP). Stain H&E., X. 40.
- Fig. (3h):** Photomicrograph of the fungiform papillae showing, stratified squamous keratinized epithelium (EP), connective tissue papillae (C), groove around fungiform papilla (black arrow) and pigment granules (black rectangle). Stain Verhoff. X. 100.
- Fig. (4a):** Photomicrograph of the fungiform papillae showing, stratified squamous keratinized epithelium (EP), connective tissue papillae (C) and pigment granules (black arrow heads). Stain Verhoff. X. 400.

- Fig. (4b):** Photomicrograph of torus linguae showing, stratified squamous epithelium (EP) and thick keratin layer (K). Stain H&E., X. 40.
- Fig. (4c):** Photomicrograph of the root of tongue showing, stratified squamous epithelium (EP), lamina propria (LP), skeletal muscle fibers (SK), serous glands (SG), mucous glands (MG) and duct of glands (black arrow head). Stain H&E., X. 40.
- Fig. (4d):** Photomicrograph of the filiform papillae (FI) showing, stratified squamous keratinized epithelium (EP). Stain H&E., X. 40.
- Fig. (4e):** Photomicrograph of the filiform papillae (FI) showing, anterior highly keratinized part (white arrow) and posterior lightly stained part (black arrow). Stain H&E., X. 100.
- Fig. (4f):** Photomicrograph of the fungiform papilla (FU) showing, stratified squamous keratinized epithelium (EP), connective tissue papillae (C) and secondary connective tissue papillae (white arrow). Stain H&E., X. 100.
- Fig. (4g):** Photomicrograph of the fungiform papilla showing, stratified squamous keratinized epithelium (EP). Stain H&E., X. 400.
- Fig. (4h):** Photomicrograph of the conical papillae (black arrows) showing, stratified squamous keratinized epithelium (EP) and connective tissue papilla (white arrow). Stain H&E., X. 40.
- Fig. (5a):** Photomicrograph of the vallate papilla (V) showing, gustatory furrow (black arrow), taste buds (white arrows), secondary connective tissue papillae (black arrow heads) and Von Ebner's gland (white arrow head). Stain H&E., X. 40.
- Fig. (5b):** Photomicrograph of the vallate papilla showing, stratified squamous epithelium with keratinized lateral wall (EP) and taste buds (white arrows). Stain H&E., X. 400.
- Fig. (5c):** Photomicrograph of taste bud of vallate papillae showing, neuroepithelial cells (white arrow) and supporting cell (black arrow). Stain H&E., X. 1000.

DISCUSSION

The present study has demonstrated the existence of filiform, fungiform, conical, and lenticular and vallate papillae as reported in buffalo (*Scala et al., 1993*), ox (*Chamorro et al., 1986; De paz cabello et al., 1988*), dromedary camel (*Ibrahim, 1983*) and goat (*Kumar et al., 1998*). The apex linguae of buffalo tongue represented about 25% of the total length of the tongue. This result is in accordance with that of *Gupta et al. (1989)* who mentioned that the ratio between free and fixed part of buffalo tongue is 1: 4.1 and with the results of *Sengar and Singh (1970)* and *Dhingra and Barnwal (1979)*, who mentioned that the free part of the tongue not exceed one third of the length of the tongue. This large proportion of free part of buffalo tongue could be attributed to the role of tongue in prehension of food. In agreement with *Sengar and Singh (1970)* in buffalo, *Ibrahim (1983)* in dromedary camel as well as *Dyce and Wensing (1971)* in ox, the tongue of buffalo contain large rounded prominence on the dorsal surface of tongue called torus linguae. This structure play an important role during the mastication process as it presses the food against the hard palate. This structure is characteristic for mammals eating fibrous vegetations such as grass (*Kobayashi et al., 2003*). The arrangement of the filiform papillae provide the tongue with rough surface to be suited for grinding and food movement, a result that in line with that mentioned by *Agungpriyono et al., (1995)*. Regarding the caudal direction of the filiform papillae, they are commonly inclined toward the radix linguae and their keratinization is harder than that in the inter papillary area in the anterior regions of the tongue. These papillae are softer than in the posterior regions. Therefore, the papillae are easily bended in the direction of the radix linguae but not in the opposite direction. This property facilitate the retention of food on the dorsal

surface of the tongue, a result which agreed with that mentioned by; *Krause and Cutts (1982)*, *Rentrop et al. (1986)*, *Mackenzie and Dabelsteen (1987)*, *Iwasaki and Miyata (1989)*, *Iwasaki et al. (1992)* and *Iwasaki (1992)*.

In accordance with the results of *Kumar et al. (1998)* in goat; *Eerdunchaolu et al. (2001)* in bacterian camel and *Agungpriyono et al. (1995)* in lesser mouse deer this study revealed that the filiform papillae are surrounded by depression at the site of its emergence. On contrast with these results, in lambs *Tadjali and Pazhoomand (2004)* mentioned that, a bulb like structure formed the basal portion of the papilla. This difference could be attributed to either species variation or age variation. More over *Kullaa- Mikkonen et al. (1987)* classify the filiform papillae according to presence or absence of hairy- like projections emerged from the primary papilla into simple type (contain no projections) and compound type (contain hairy –like projections), according to this classification the filiform papillae in buffalo belong to the simple type. This study revealed that the caudal aspect of the filiform papillae was more keratinized (cornified) than the rostral one. This result is opposite to that obtained by *Agungpriyono et al. (1995)* in lesser mouse deer and *Zheng and Kobayashi (2006)* in reeve’s muntjac deer. They observed that the sagittal section of the filiform papillae revealed that, the wall of the anterior aspect of the papilla contain more keratohyalin granules than those of the caudal aspect.

The distribution of the conical papillae on the torus linguae could be explained on the basis of it compensate the absence of the filiform papillae on this part of the tongue and the absence of rugae palatine on

the caudal part of hard palate which lies opposite to the torus linguae. So, the presence of the conical papillae on the torus linguae plays an important role in mastication of food and direction of it toward the pharynx. These results are in accordance with *Zheng and Kobayashi (2006)*.

In agreement with the result obtained in this study, *Nahed and Maha (2004)* mentioned that, the fungiform papillae showed morphological variations. The existence of non taste bud fungiform papillae was observed in camel and goat. Some fungiform papillae appeared pigmented especially those found on the rostral part of the tongue. This result confirmed by histological findings, which revealed the presence of pigmented granules in the basal cell layer of the covering epithelium. This result is in agreement with that of *Gupta et al. (1989)*.

In agreement with *Kadam et al. (1995)* in buffalo, *Eerdunchaolu et al. (2001)* in bacterian camel and *El-Sharaby (2006)* in dromedary camel, the present study revealed that the covering epithelium of vallate papillae is stratified squamous keratinized at the apical surface of the papilla and stratified squamous less keratinized at the lateral surface facing to the furrow, this is due to the localization of the taste buds at the lateral surface in which the taste pore open. The annular pad regulates the access and retention of the saliva in the groove by means of their smooth muscle fibers in new born lambs (*Tadjalli, M.2004*). *Andres et al.(1977) and Horstmann(1955)* attributed the high density of connective tissue papillae to the functional adaptation against the external mechanical stimuli. The present study suggested that the connective tissue papillae play a role as a supporting structure against mechanical stress.

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