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Light and scanning electron microscopic studies on the tongue of adult pig. 1. Gustatory papillae

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ABSTRACT

The vallate, foliate and fungiform papillae were collected from adult pigs (9), of local mixed breed immediately after their slaughter. All these papillae were lined by stratified-squamous-epithelium with varying number of cell layers. Large number of taste buds was observed in the foliate and vallate papillae especially, towards the basal portion of the medial wall of the trench. However, only a few taste buds were observed in the fungiform papillae present on the dorso-lateral surface of the tongue. The lamina propria submucosa of these papillae had loose irregular connective tissue with predominance of collagen fibres. The deeper portion of the lamina propria submucosa had bundles of nerve fibres, large clusters of serous (von Ebner's) and mucous glandular acini being separated by the fasciculi of striated skeletal muscles oriented in different directions. The serous glands showed predominance of neutral whereas the mucous acini presented mainly the acidic mucopolysaccharides. Electron microscopy revealed a large central papilla with pseudopapillae but without an annular pad in the vallate papilla. The foliate papillae presented parallel folds of lingual mucosa separated by gustatory furrows which were pronounced in the cut surface. Fungiform papillae were oval to round with flat surface having shallow transversely oriented grooves. Higher magnification of the surface of all the types of papillae revealed flat squamous cells with microridges having pitted appearance and the taste pores.

Key words: Gustatory papillae, Histology, Pig, SEM

The tongue surface is modified into specialized structures called papillae which are involved in gustatory and mechanical functions. Fungiform, vallate and foliate papillae constitute the gustatory papillae of the pig (Chamorro *et al.* 1994, Kumar and Bate 2004) and play important role in selection of food material to be consumed by the animal. Four types of cells were identified in taste buds on the basis of electron density (Suzuki 2007). Type-II cells and the sensory afferent nerve were in contact with type-III cells but no ultrastructural evidences indicating a synaptic presence between type-II cells and gustatory afferents were found (Clapp *et al.* 2004). Flavoring agents including sugar were incorporated among the solid feed articles to make them interesting in weanling pigs (Forbes 1995). Thus, these papillae possessing large number of taste buds may serve as a model for carrying out nutrition-related problems in humans and also because of other similarities between the 2 species. The lingual papillae of wild boar and pig were compared (Chamorro *et al.* 1994) which created an interest to envisage the present study in the non-descript local mixed breed of domestic pig.

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MATERIALS AND METHODS

The present study was conducted on gustatory papillae of 9 adult pigs of either sex of local non-descript mixed breed. The mouth cavity was exposed to collect tongue tissues containing fungiform, vallate and foliate papillae from six animals, fixed in 10% neutral buffered formalin and processed for paraffin technique of light microscopy. Paraffin sections (5–6 μ) were stained with routine Harris' hematoxylin and eosin stain, Gomori's method for reticulum, Weigert's for elastic fibres, Bielschowsky's for axis cylinders and dendrites, Holme's method for nerve cells and fibres, McManus' for glycogen (PAS), PAS-Alcian blue for mucosubstances (pH 2.5), diastase digestion method (Luna 1968) and Crossman's trichrome stain for collagen fibres (Crossman 1937). The tissues collected from remaining 3 tongues were washed thoroughly in chilled 0.1 M phosphate buffer (pH 7.4) and fixed in 2% glutaraldehyde for 8 h. The tissues were rewashed in the buffer followed by dehydration in ascending grades of ethanol, critical point drying and sputter coating with gold. Scanning electron microscopy

(SEM) was performed at EM Laboratory, AIIMS, New Delhi, India using Leo 435–VP (Japan).

RESULTS AND DISCUSSION

Vallate papilla: The vallate papillae present towards root of the tongue were flat and characterized by presence of trench/moat and lined by stratified-squamous-keratinised epithelium towards the outer as well as surfaces lining the trench (Fig 1). The thickness and degree of keratinization of the epithelium varied at different places as reported in goat, cattle and buffalo (Qayyum and Beg 1975, Dhingra and Barnwal 1980 and Lahkar *et al.* 1989). The latter authors established an inversely proportional phenomenon with regard to epithelial and keratin thickness towards base of the tongue in goat indicating lesser conversion of superficial epithelial layers into keratin. Stratum basal layer was comprised of single row of vertically oriented oval-elongated nuclei having irregular distribution of fine chromatin material. Their darkly stained nucleoli were eccentric in position. Stratum spinosum constituted by varying number of cell layers had similar type of nuclei except their larger dimensions. The nuclei of stratum granulosum were still larger and less basophilic due to presence of a few but larger clumps of chromatin. The nuclei of superficial layers were oriented horizontally. Cytoplasm of all these cell types was eosinophilic and finely granular. The free surfaces of the cells of stratum spinosum and granulosum presented large sized microvilli which inter-digitated with those of others. Stratum corneum was constituted by a few layers having flat elongated cells. Their nuclei were deeply basophilic and showed degenerative changes however their cytoplasm was strongly eosinophilic with more concentration of keratohyaline granules. Most superficially placed keratin layer was desquamating at different places. The keratinization may be helpful to prevent mechanical injury to the mucosa due to ingestion of coarse feed particles.

The stratified-squamous-epithelium showed ovoid shaped taste buds towards the mid-ventral portion of the medial wall of trench (Figs 1, 2) which were comprised of different cell types. Type-I nuclei were elongated being oriented parallel to longitudinal axis of taste buds and densely basophilic masking the presence of nucleoli. Large sized round to oval nuclei of type-II cells appeared vacuolated due to distantly placed fine clumps of chromatin and possessed centric/eccentric nucleoli. Cytoplasm of both the cell types was finely granular and eosinophilic as reported in rat (Pumplin *et al.* 1997). The apical end of type-II cells extended in to a thin cilium like structure, taste hairlet, which projected into taste pore were gustatory cells and their concentration was more as compared to type-I supporting cells. Taste buds were also reported oval in the goat, buffalo (Qayyum and Beg 1975, Dhingra and Barnwal 1980, Ramayya *et al.* 2000, Prakash and Rao 1980) and camel (Eerdunchaolu *et al.* 2001). The nerve endings within the taste buds were widely distributed and the fibres appeared to end in close association with the gustatory cells and thus playing role in gustatory mechanism (Murray and Murray 1960). The body and basal ends of gustatory cells were surrounded by a plexus of sensory nerve endings in goat (Qayyum and Beg 1975). The number of taste buds not only varied in different vallate papillae but also towards right and left halves of the same papilla. The quantification of taste buds was not carried out during the present study however, a greater number of taste buds (732.67 per papilla) in vallate than lateral fungiform papilla were attributed due to greater volume of the first one (Irving *et al.* 1988, Mack *et al.* 1997). Similarly, a maximum of 1786 and 1094 taste buds were reported in a single papilla in bovine, respectively by Davies *et al.* (1979) and Dasgupta *et al*. (1990). The authors also reported that the number of papillae and the taste buds were essentially equal towards both sides of the tongue.

The lamina propria submucosa having loose irregular connective tissue was constituted by fine reticular, elastic and bundles of collagen fibres. The reticular fibres formed the basement membrane which was interrupted in the region of taste buds. Fine reticular fibres along with collagen and a few elastic fibres also surrounded the glandular acini, nerve bundles and striated muscles. Isolated elastic fibres were vertically oriented in between the pegs of surface epithelium and their concentration increased towards the base of trench. Collagen bundles were oriented vertically in superficial portion whereas these were horizontally and obliquely arranged in the deeper part. Lymphoid aggregation without any definite arrangement was also noticed in the superficial portion. The connective tissue core of these papillae was branched and showed numerous blood vessels in the goat (Ramayya *et al.* 2000). Two types of glandular acini occupied the majority of the portion and extended up to tunica muscularis where these were separated by bundles of striated muscles. Serous type von Ebners' glands in superficial portion (Fig 2) as well as mucous glandular acini towards deeper portion were strongly PAS positive for glycogen however, the former had strong affinity for neutral and the latter for acidic mucopolysaccharides. Both the types of glands were reported in goat (Ramayya *et al.* 2000).

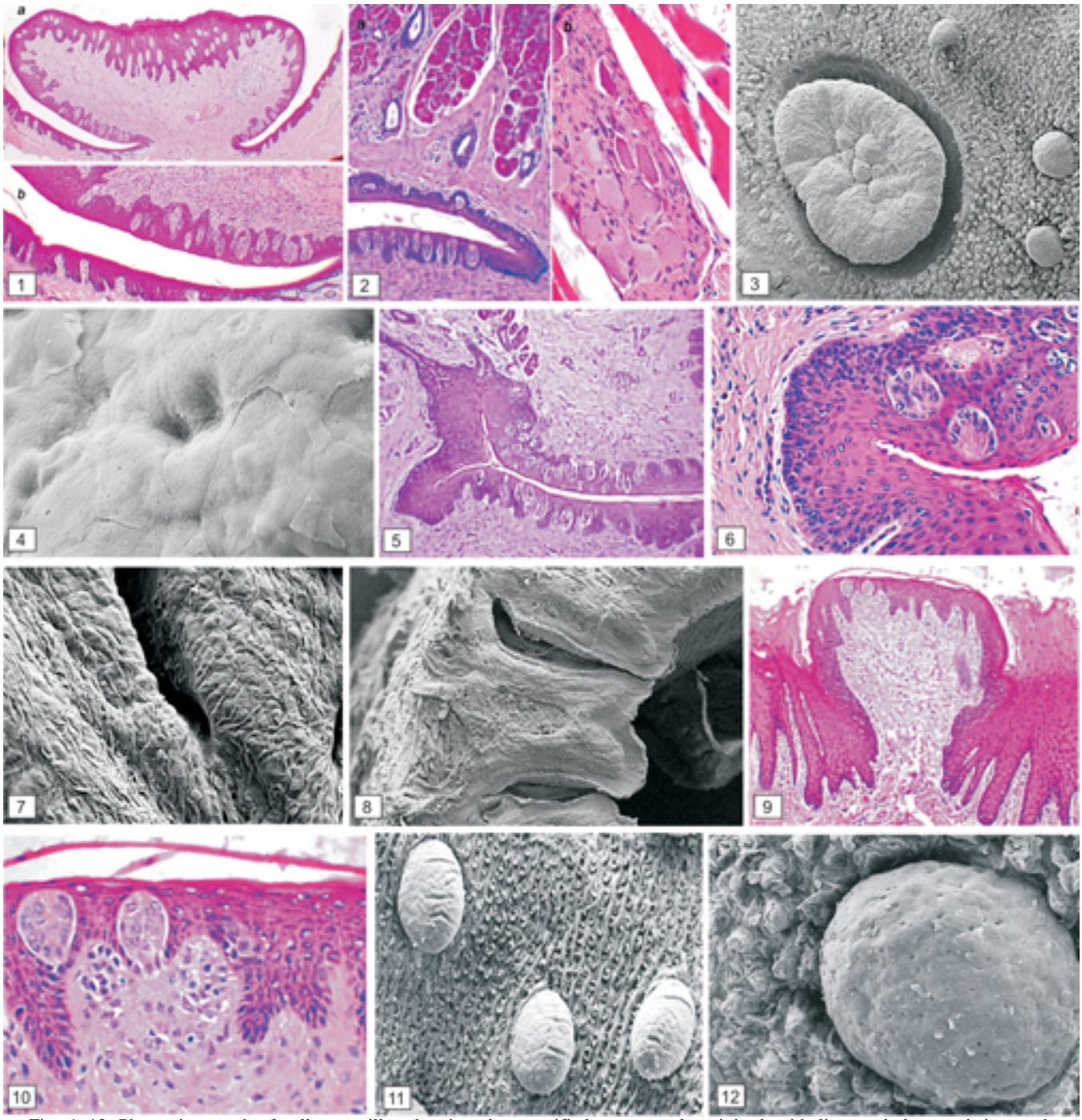
Some of the glandular ducts opening into the trench epithelium and duct associated lymphoid tissue were also visible. Wider dilatation of ducts seemed to be related with a constant flow of the secretion that kept the mucous membrane of the tongue moist and protected from injury due to coarse feed (Prakash and Rao 1980). Bundles of myelinated and non-myelinated nerve fibres along with encapsulated ganglion were interspersed in between the muscles and glandular acini (Fig 2). The ganglion containing argyrophilic multipolar neurons was surrounded by amphicytes and had tendency to occur towards the base of the trench. This ganglion was shown to be connected to the nerve fibre plexus present under the epithelium forming inner wall of trench,

adjacent salivary glands and a special accessory epithelial papilla situated in the apical part of the organ's epithelium (Reutter 1981). Special neurons of this intrinsic ganglion were involved in chemoreceptive, mechanoreceptive and secretory activity of the papillae. Similar observations were reported in the goat (Qayyum and Beg 1975) and buffalo (Prakash and Rao 1980). In addition, authors reported small trunks of nerve fibres passing in a circle along the borders of the serous cells and constituted a periacinar plexus, from which the fibres terminated in the free endings on the gland cells. Some of the nerve fibres terminated in the form of knob-shaped enlargements in between the epithelial cells. Similar types of structures were not recognized during present study. The ganglion cells appeared to be migrated from the ganglion of facial and glossopharyngeal nerves and may be related with secretion of lingual gland (Romanoff 1966). Striated muscles oriented in longitudinal, transverse and oblique planes were responsible to provide maximum mobility to the tongue. A few isolated nerve bundles were observed in between the muscle fasciculi in contrast to an exceptionally rich innervation in buffalo (Prakash and Rao 1980). Large blood vessels were observed towards deeper part of propria submucosa.

SEM revealed oval shaped vallate papilla having rough irregular surface with elevated areas of irregular shapes as pseudopapillae (6–7) towards the centre (Fig 3). These pseudopapillae were further categorized into spicule like and the fungiform type (4–5) with circular outline in wild boar and conical shaped in pig (Chamorro *et al.* 1994). Shallow peripheral secondary grooves observed during present study were reported in pig but absent in wild boar. The papilla lacking the annular pad was surrounded by a deep trench as reported in the horse (Kumar and Timoney 2006). In contrast, a well developed annular pad with serrated margins facing the moat was reported in the pig (Chamorro *et al.* 1994, Kumar and Bate 2004). These papillae possessed a small central vallum papilla and a thick, wide annular pad being separated by a shallow sulcus papilla in ruminants (Chamorro *et al.* 1986, Kumar *et al*. 1998 a, 2003) and camel (Kumar *et al.* 1998 b, Eerdunchaolu *et al.* 2001). This pad functionally regulated the access and retention of saliva in the groove by means of smooth muscles (Bargmann 1981). Higher magnification of the surface presented polygonal cells with microridges or pitted appearance (Fig 5) instead of various patterns of branching microplicae as reported in wild boar (Chamorro *et al.* 1994), goat, sheep and horse (Kumar *et al*. 1998 a, 2003, Kumar and Timoney 2006). The surface areas with microplicae and pitted appearance were attributed due to non-keratinised and keratinised epithelium, respectively (Kullaa-Mikkonen *et al.* 1987). The mechanical stress of food on the surface epithelium was able to modify it partly into keratinised form in the wild boar (Chamorro *et al.* 1994). The microplicae/microridges were mainly associated with mucous secreting epithelial cells for efficient mucous retention mechanism (Sperry and Wassersug 1976) and also facilitated an intimate contact between saliva soluble food particles and taste buds in bovines (Scala *et al.* 1995). Microplicae may be involved in protection of epithelium either by holding the mucous or reduce the surface area available for environment contact. The taste pores (Fig 4) were present in between the polygonal cells especially towards the periphery of the papilla facing the moat as reported in bovine (Chamorro *et al.* 1986), goat, camel and sheep (Kumar *et al.* 1998 a, b, 2003). Secretory end products, food particles and desquamated cells were also observed in the moat.

Foliate papillae: Foliate papillae present on caudo-lateral part of the tongue as linearly arranged lingual mucosa were characterized by presence of gustatory furrows. These papillae were lined by stratified squamous non-keratinised epithelium towards the outer surface and presented keratinization towards the gustatory furrows (Figs 5, 6). The foliate papillae of mammals were considered as simplest form if they occurred glandular duct type or complicated when furrow with taste buds were observed (Qayyum *et al.* 1974). Vertically-oriented nuclei of stratum basal cells having fine chromatin material being arranged in irregular clumps had one or more centric/eccentric nucleoli (Fig 6). The chromatin of nuclei of stratum spinosum cells tended to accumulate towards the periphery and these cells generally presented two nucleoli. Round to oval to elongated nuclei of stratum granulosum were larger in dimensions. A cell layer sandwiched in-between stratum granulosum and corneum was characterized by a sparse distribution of irregular shaped and very small sized nuclei with degenerative changes. The cytoplasm of these cells was less eosinophilic with a transparent appearance. These cells possessed very large sized processes which intermingled with those of adjacent ones. This layer was comparable to that of stratum-lucidum seen in other places of body. Stratum corneum had few layers of cells showing strong eosinophilic cytoplasm and nuclei with degenerating changes. The strata except stratum basale showed varying number of cell layers. Large number of taste buds of varying dimensions opening towards both the sides of furrow was observed (Figs 5, 6). The structure of taste buds was similar as described earlier in the vallate papilla. The lamina propria submucosa constituted by loose irregular connective tissue, glandular acini, striated muscles, ganglion and bundles of nerve fibres were almost similar to those of vallate papillae.

Foliate papillae in the SEM presented linearly oriented folds of lingual mucosa which were separated by deep crypts/ gustatory furrows (Figs 7, 8) as reported in the horse (Kumar and Timoney 2006). The outer surface was rough, irregular having small folds being separated by shallow grooves and small elevated structures comparable to papillae (Fig 7). Pseudopapillae of varying shapes and dimensions reported towards free margin of foliate papillae in pig (Chamorro *et*



Figs 1–12. Photomicrograph of vallate papillae showing: **1.a.** stratified squamous keratinised epithelium and characteristic trench on both the sides. H & E. × 40. **b.** Note presence of large number of taste buds opening towards medial wall of the trench. H & E. × 100. **2. a.** taste buds and strongly PAS positive von Ebner's glands in the lamina propria submucosa. McManus' PAS method. × 100. **b.** presence of a ganglion in between glands and striated muscles. H & E × 400. **3.** SEM showing a central papilla with pseudopapillae surrounded by a well developed trench. × 23. **4.** SEM at higher magnification showing flat squamous cells with microridges and a taste pore. ×1910. Photomicrograph of foliate papilla showing: **5.** stratified squamous keratinised epithelium towards gustatory furrow and large number of taste buds. H & E. ×100. **6.** different cell layers of epithelium and the taste buds. H & E. ×400. **7.** SEM showing gustatory furrow and folded mucosa. × 108. **8.** cut surface with well developed gustatory furrows. × 47. Photomicrograph of fungiform papilla present at dorso-lateral surface of tongue showing: **9.** flat surface lined by stratified squamous keratinised epithelium and taste buds. H & E. ×100. **10.** taste buds opening towards the free surface. Note vacuolated areas towards free surface of the epithelium. H & E. ×400. **11.** SEM showing oval shaped papillae with characteristic transversely oriented shallow grooves. ×42. **12.** round fungiform papilla without presence of groove. Note: large number of taste pores like structures present towards the surface. ×109.

al. 1994, Kumar and Bate 2004) could not be observed during present study. The gustatory furrows were distinct in the cut surface of the foliate papillae (Fig 8). The surface having polygonal cells with microridges and the taste pores located towards deeper part of furrows were similar to those of vallate papillae. However, microplicae was reported in foliate papillae of wild boar and pig because of non-keratinised epithelium. In addition the taste pores were present at the surface of pseudo-papillae having pitted appearance and towards internal faces of the leaves of papillae in the pig (Chamorro *et al*. 1994).

Fungiform papillae: The round to oval fungiform papillae were located on the dorsal surface extending from tip to mid portion, close to vallate papilla and dorso-lateral surface. These papillae showing transition from gustatory to mechanical were mainly gustatory which were present on the dorso-lateral surface of the tongue and their free surfaces were flat rather than mushroom shaped (Fig 9). A shallow groove or trench separated the base of papilla from adjacent lingual mucosa similar to buffaloes (Dhingra and Barnwal 1980) and camel (Eerdunchaolu *et al.* 2001) which were reported absent in other domestic animals. The fungiform papillae were line by stratified squamous keratinised epithelium (Figs 9, 10). Elongated nuclei of stratum basale were vertically oriented having fine chromatin and one or more eccentric nucleoli. A few cells having dark nuclei were also incorporated among these cells. The stratum spinosum was constituted by varying number of cell layers having oval to elongated nuclei with tapering ends. The nuclei of stratum granulosum were larger in dimensions and comparatively less basophilic. Only a few deeply basophilic nuclei showing pyknotic changes were observed in the stratum corneum. This was interesting to note that large number of nuclei of stratum granulosum along with a few from stratum spinosum showed shrinkage and vacuolated appearance (Fig. 10). Cytoplasm of all the cell types was finely granular and eosinophilic and the eosinophilia accentuated towards the surface layers. Taste buds were concentrated only towards the dorsal surface of the papillae located on dorso-lateral surface of tongue (Figs 9, 10). Similar observations were reported in goat (Qayyum and Beg 1975), buffaloes (Dhingra and Barnwal 1980) and camel (Eerdunchaolu *et al.* 2001). The number of taste buds per fungiform papilla was considerably less in the papillae present on dorsal surface as compared to lateral side of the tongue (Mack *et al.* 1997). The core of the papilla was supported by loose irregular connective tissue mainly by collagen and a few reticular and elastic fibres along with large number of small sized blood capillaries. Some of the papillae showed large number of nerve bundles and nerve endings as reported in goat (Qayyum and Beg 1975).

The fungiform papillae under SEM were observed round to oval with the predominance of latter towards dorso-lateral surface of the tongue (Figs 11, 12). These papillae were separated from adjacent filiform papillae or lingual mucosa by a shallow groove as reported in ox, horse (Chamorro *et al.* 1986), camel, goat and sheep (Qayyum *et al.* 1988, Kumar *et al.* 1998 a, b; 2003). The free surface of the oval shaped papillae was slightly convex to flat, rough, irregular due to presence of a few desquamating cells and possessed transversely oriented shallow grooves (Fig 11). Higher magnification revealed flat squamous cells having microridges with pitted appearance and a few taste pores in the form of crater like depressions. It was interesting to observe large number of taste pores like structures on the surface (Fig 12) but higher magnification revealed that majority of these structures were shallow and closed. These were not actually the taste pores but may be corresponded to vacuolated structures in the epithelium as seen by the light microscopy during present study. This was confirmed again as large number of taste pores like structures were observed in the fungiform papillae present on the dorsal surface of the tongue by SEM but no taste buds were observed by light microscopy (self communication). Only on the basis of SEM, taste pores were reported earlier in the pigs (Chamorro *et al*. 1993, 1994, Kumar and Bate 2004). Approximately 637 and 818 fungiform papillae were observed on the tongue of wild boar and pig, respectively with more number of taste pores in the papillae present towards the lateral surface (Chamorro *et al*. 1993, Mack *et al.* 1997).

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