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Scanning electron-microscopic studies on the tongue of adult chicken

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Abstract
The present study was conducted on tongue of 04 adult broilers. The tissues collected from the apex, body and root of the tongue including the caudal lingual papillae were processed for scanning electron microscopy. The apex had a large number of leaf-like papillae, some of which were exfoliated. Their free tips were pointed to blunt with sharp lateral borders. These papillae had longitudinal folds and the higher magnification presented micro ridges of different types. The density of these papillae was comparatively reduced towards a caudal portion of the body of the tongue. A transverse row of large-sized papillae was observed at the junction of the body and root. These papillae towards centre or medial part were pointed conical shaped and more fluffy and blunt type towards caudolateral portion. The size of these papillae also increased from the medial to the lateral side. The surface of these papillae had squamous cells which further showed microridges/microplicae of different patterns.

Keywords: tongue, filiform, conical papillae, SEM

Introduction
The tongue in fowl serves important functions related to feeding and swallowing, taste sensation, vocalization, hydration, grooming, and thermal regulation. It is an essential anatomical structure that enables fowl to engage in vital behaviours, maintain proper nutrition, communicate, and adapt to their environment. The composition of nutrients in a bird's diet and the process of digestion have been found to have an impact on the morphological changes observed in the tongue structures of birds, as supported by studies conducted by Jackowiak *et al.* (2011) [18]. These changes are influenced by various factors, including dietary habits and living conditions of the birds, as highlighted by Erdogan and Perez (2015) [10]. It is through these influences that the tongue adapts to the specific dietary needs and environmental conditions of different bird species. Previous studies have conducted detailed histological and histochemical examinations of the tongue and anterior larynx in broiler chicks (Bansal *et al.*, 2018, 2019) [4, 3]. Additionally, research using light and electron microscopy has been conducted on the tongues of various bird species, including the golden eagle (Parchami *et al.*, 2010b) [23], woodpecker (Emura *et al.*, 2009) [7], ostrich (Jackowiak and Ludwig, 2008) [17], owl (Emura and Chen, 2008) [6], and penguin (Kobayashi *et al.*, 1998) [19]. The scanning electron-microscopy has also been conducted on chicks of fowl of different age groups (Bansal and Kumar, 2020) [2]. Building upon this existing knowledge, the objective of this study is to investigate the surface features of the tongue in adult broiler chickens and compare them with those of other species.

Materials and Methods
For the present study, a total of four healthy adult broiler chickens of 6 weeks of age were included. Fresh tissues were collected from different regions of the tongue, including the apex, body, caudal lingual papillae, and root. To prepare the tissues for scanning electron microscopy (SEM), the tissues were thoroughly washed with chilled 0.1M phosphate buffer (pH 7.4) and subsequently, fixed in a 2% glutaraldehyde solution for a period of 6-8 hours. After fixation, the tissues underwent two additional washes with chilled 0.1M phosphate buffer. The fixed tissues were then transferred to the EM Lab., AIRF, JNU, New Delhi, India, for further processing. The tissue samples were dehydrated in ascending grades of ethanol, critical point dried and coated with a layer of gold using a sputter-coating technique. This gold coating enhances the conductivity of the tissues, enabling better visualization under the scanning electron microscope (SEM). The processed tissue samples were then examined and photographed using a scanning electron microscope to document and analyze the observed features and structures.

Results and Discussion

The findings of the present study regarding the shape and morphology of the tongue in domestic fowl were consistent with previous research. The triangular shape of the tongue observed in chickens aligns with earlier studies conducted by Ertas and Erdogan (2019) [11] and Bansal and Kumar (2020) [2]. However, other bird species, such as quails, eagles, hawks, and owls, have been reported to exhibit different tongue shapes, including spade-like with an oval tip, bifid with an oval tip, and triangular with a slightly rounded apex (Parchami *et al.*, 2010b; Jackowiak and Godynicki, 2005; Emura *et al.*, 2008; Abou-Zaid, 2008; Pourlis, 2014) [23, 16, 8, 1, 26]. These variations in tongue shape can be attributed to differences in feeding habits among the birds. The division of the chicken tongue into an apex, body, and root, as well as the presence of a concave depression on the dorsal surface of the apex, were consistent with previous reports in fowl, quails, European magpies, common ravens, and owls (Bansal *et al.*, 2018; Bansal and Kumar, 2020; Ertas and Erdogan, 2019; Abou-Zaid, 2008; Erdogan and Alan, 2012; Emura and Chen, 2008) [4, 2, 11, 1, 9, 6]. However, the absence of the groove on the dorsal surface of the apex reported in previous studies on fowl (Iwasaki and Kobayashi, 1986) contrasts with the present findings [14].

The filiform papillae (Fig. 1, 2, 3) were densely arranged except towards the anterior part of the tongue's apex similar to the findings in chickens aged 18 days onwards, owls and quails (Bansal and Kumar, 2020; Ertas and Erdogan, 2019; Abou-Zaid, 2008; Pourlis, 2014) [2, 11, 1, 26]. However, these papillae were densely arranged towards the lateral border of the tongue's apex in chickens upto 14 days of age, seagulls, and laughing doves (Bansal and Kumar, 2020; Ertas and Erdogan, 2019; Abou-Zaid, 2008; Onuk *et al.*, 2015; Farouk and Hassan, 2015) [2, 11, 1, 21, 12]. The presence of scales-like structures with microplicae on the antero-lateral portion of the apex of the tongue in chickens was consistent with earlier findings (Ertas and Erdogan, 2019; Pourlis, 2014; Parchami *et al.*, 2010a; Jackowiak and Godynicki, 2005) [11, 26, 22, 16]. In contrast, golden eagles exhibit densely distributed processes on the dorsal surface of the lingual apex, which resemble leaves (Parchami *et al.*, 2010b) [23]. These papillae (Fig. 1, 2), which vary in shape and size, have also been observed in quails and golden eagles, while thread-like papillae were seen in owls (Parchami *et al.*, 2010a, b; Abou-Zaid, 2008) [22, 23, 1]. The surface of the papillae exhibited longitudinal folds, which, under higher magnification, revealed various arrangements of microplicae (Fig. 3, 4). The parallel arrangement of microridges on the cellular surface near the openings of glandular ducts were observed in quails and emu, suggesting their association with friction and their potential role in increasing surface area, mucus adherence, and spreading to maintain moisture (Pourlis, 2014; Crole and Soley, 2010) [26, 5].

In addition to the oblique grooves on the dorsal surface of the tongue's body in chickens, small longitudinal folds irregularly distributed towards the lateral portion on either side of the caudal region were also observed. In contrast, previous studies reported only one medial groove in domestic pigeons, quails, and laughing doves (Parchami and Dehkordi, 2011; Pourlis, 2014; Farouk and Hassan, 2015) [25, 26, 12]. Regarding the distribution of filiform papillae, while they were found throughout the surface of the tongue's body in chickens, in quails, they were mainly localized between the wings of the body (Pourlis, 2014) [26]. The high density of papillae masked the appearance of small openings of the glandular ducts of lingual glands which have been reported in previous reports in chickens and quails (Abou-Zaid, 2008; Pourlis, 2014) [1, 26].

A fold of the lingual mucosa, the papillary crest, was oriented transversely, separated the body by a shallow transverse groove, forming a "V" shape (Fig. 5). Similar observations were reported by (Ertas and Erdogan (2019), Jackowiak *et al.* (2011), and Parchami *et al.* 2010b) [11, 18, 23]. In geese, the papillary crest was adorned with two transverse rows of papillae, as reported by Jackowiak *et al.* (2011) [18]. The posterior border of the crest displayed conical-shaped caudal lingual papillae (Fig. 5), as reported in fowl, quails, white-eared bulbuls, and laughing doves by (Bansal and Kumar 2020, Abou-Zaid 2008, Ertas and Erdogan 2019, Pourlis 2014, Parchami and Dehkordi 2013, Madkour 2018, Farouk and Hassan 2015) [2, 1, 11, 26, 24, 20, 12]. In Japanese quails, these papillae were described as cactus leaf-like by Madkour (2018) [20].

The number of caudal lingual papillae varied across different bird species. In the previous study, these papillae were classified into three categories: 12-14 medial papillae, 5-6 lateral papillae, and 3-4 giant papillae (Bansal and Kumar, 2020) [2]. Similarly, in another previous research conducted by Ertas and Erdogan (2019) [11], chickens were found to have 25 medial conical papillae and 2-6 very large-sized papillae located caudo-laterally to the large conical papillae [11]. Quails, on the other hand, exhibited a papillary crest with a row of 24-26 main conical papillae, accompanied by two sets of 2-3 giant papillae towards the lateral ends on each side, as reported by Pourlis (2014) [26]. However, in the present study 14-16 medial papillae, 6-7 lateral papillae, and 5-6 giant papillae were observed (Fig. 5, 6). The size of the conical papillae (Fig. 6-8) increased gradually from the central to the lateral portion, and their surface showed exfoliated cells, which aligns with previous findings in fowl reported by (Abou-Zaid 2008; Bansal and Kumar 2020) [1, 2]. Additional small papillae, similar to secondary papillae, were observed at the base of the primary papillae. These papillae had scales (Fig. 8), which were likely a result of their interaction with feed particles during ingestion, indicating their mechanical nature. Under higher magnification, the surface of these papillae exhibited microridges/ microplicae of varying shapes and sizes, consistent with previous reports (Abou-Zaid, 2008; Bansal and Kumar, 2020) [1, 2]. The giant papillae present on the lateral sides showed bifid to trifid modifications towards their free surfaces (Fig. 9). Their dorsal surface was having small scales similar to those of the conical papillae which showed flat squamous cell-like arrangement with varying patterns of microridges (Fig. 10).

In European magpies and common ravens, the papillae resembling giant papillae were identified as the second row consisting of two large papillae, as documented by Erdogan and Alan (2012) [9]. The papillae exhibited a smooth surface with minimal shedding of cells and microplicae, similar to the caudal lingual papillae. Each cell had a polygonal outline with slightly thickened margins between adjacent cells. Additionally, the cell surfaces displayed subtle elevated ridges, and no secondary papillae were detected. Relatively smooth cellular surfaces were predominantly observed on and around the conical papillae. Similar variations in the surface characteristics of epithelial lingual cells have been noted in chickens, likely representing adaptations of the tongue to feeding mechanisms and environmental habitats as reported by Iwasaki and Kobayashi (1986) [14], and Iwasaki (2002) [15].

The conical papillae found in owls have been reported to play a role in swallowing ingested food and preventing regurgitation, according to Abou-Zaid (2008) [1].

The root of the tongue exhibited an irregular structure characterized by transverse folds and lacked any papillae (Fig. 5). Within this region, a few small openings of the glandular ducts were observed, which aligns with previous findings in chickens, quails, and Japanese quails reported by Ertas and Erdogan (2019) [11], Pourlis (2014) [26], and Madkour (2018) [20]. In the case of the white-tailed eagle, the openings of the anterior lingual glands were localized towards the lateral surface of the posterior part of the lingual body, while the posterior lingual glands were reported towards the root, as described by Jackowiak and Godynicki (2005) [16]. Similarly, Emura *et al.* (2008) [8] reported the presence of similar types of openings with varying shapes throughout the surface of the body and root of the tongue in falcons. The glandular secretions from these openings likely serve a protective role by acting as a barrier to prevent the entry of pathogens into the oral cavity, as suggested by previous studies conducted by Gargiulo *et al.*(1991) [13] and Sagsoz *et al.*(2013) [27].

Regarding taste buds, they could not be specifically located in any particular region of the tongue during the present study or in previous detailed studies utilizing light and electron-microscopy (Bansal *et al.*, 2018; Bansal and Kumar, 2020) [4, 2]. However, more taste buds have been reported in the stratified squamous epithelium of the anterior part of the body compared to the root of the tongue in chickens (Abou-Zaid, 2008) [1].

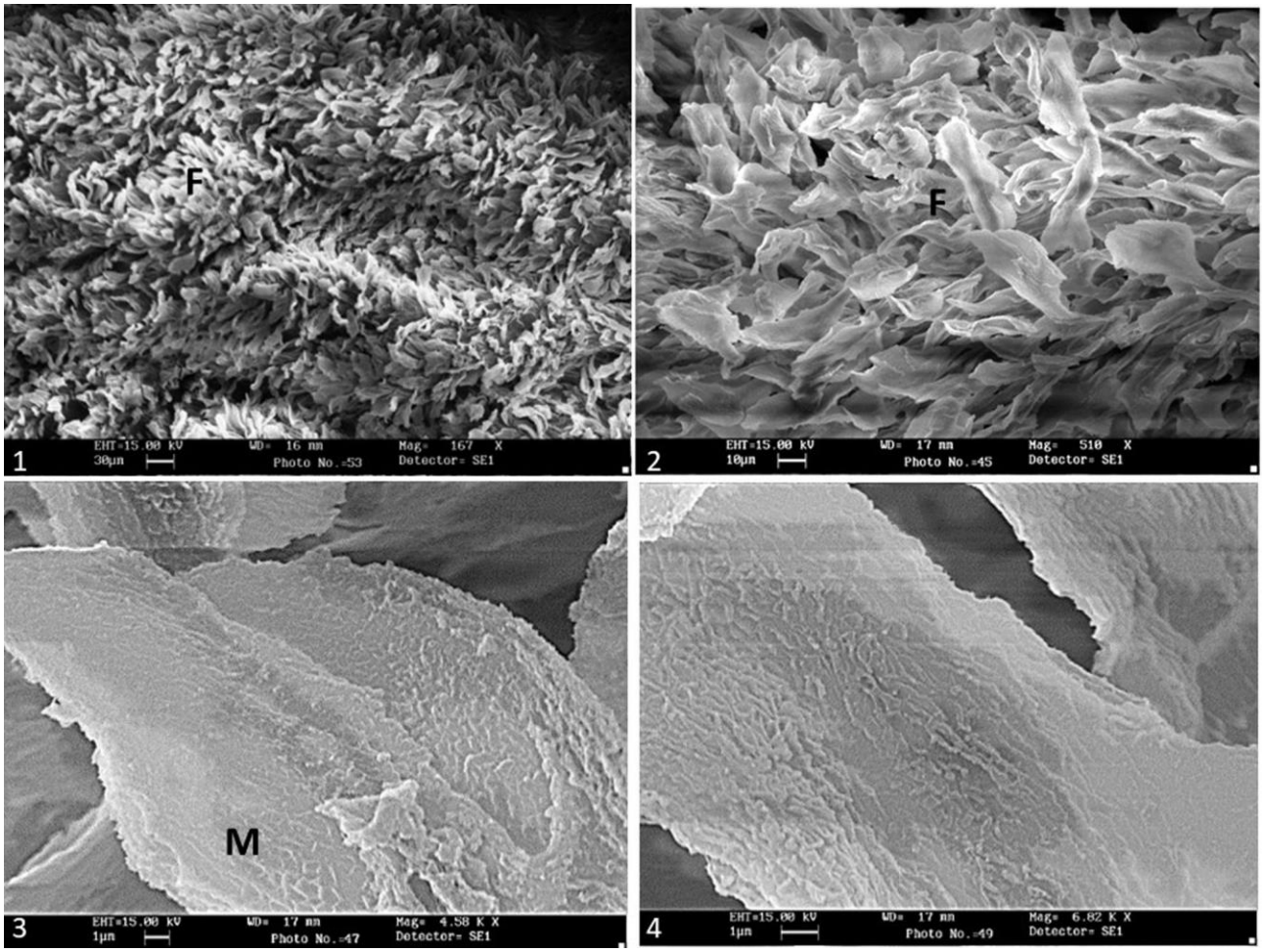

Fig 1-4: SEM of the dorsal surface of the tongue of an adult chicken showing 1. A dense arrangement of filiform papillae (F) separated by grooves. X 167. 2. An irregular arrangement of filiform papillae (F). X 510. 3. Higher magnification of the surface of the filiform papillae showing varying patterns of micro ridges (M). x 4580. 4. Further higher magnification of filiform papilla of Figure 3 to show the arrangement of micro ridges. x 6820.

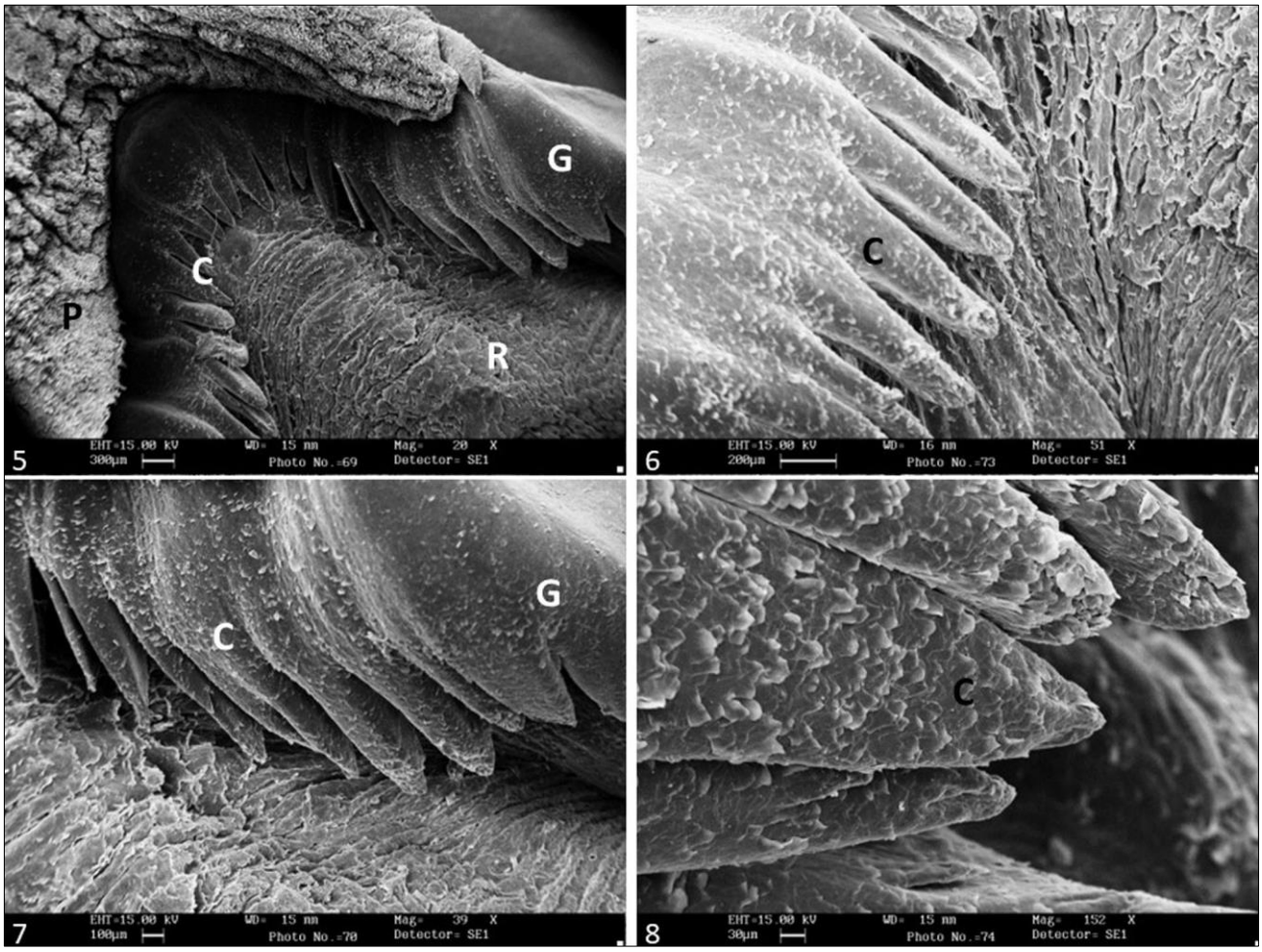

Fig 5-8: SEM of the dorsal surface of the caudal portion of the tongue of adult chicken showing 5. Papillary crest (P), conical papillae (C), giant papillae (G) and root (R). x 20. 6. Medially placed conical papillae (C). x 51. 7. Laterally place conical (C) and giant (G) papillae. X 39. 8. Higher magnification of conical papillae (C) showing scales-like arrangement. x 152.

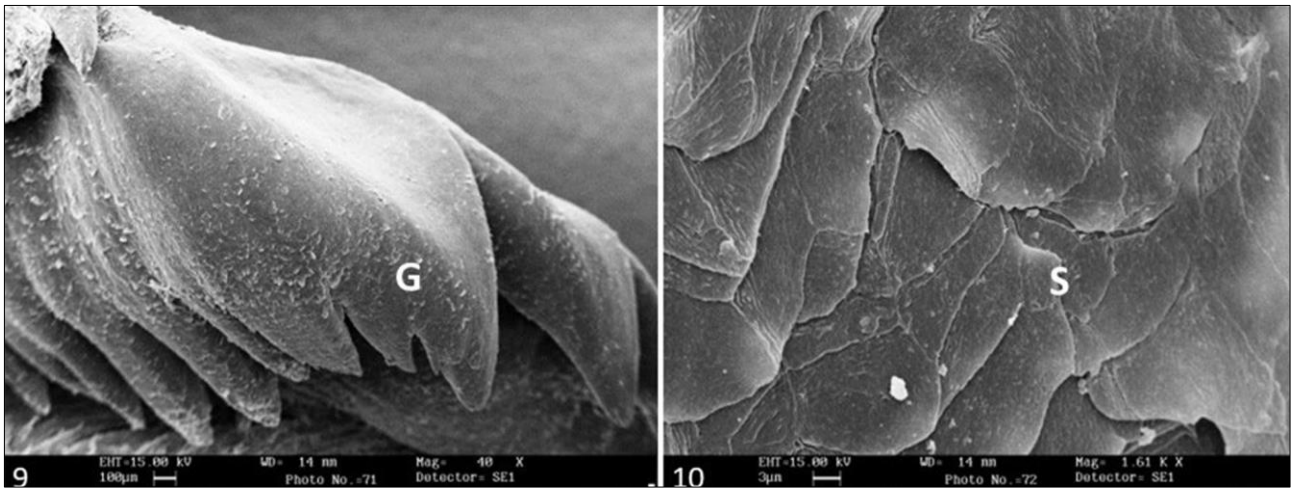

Fig 9-10: SEM of the dorsal surface of the caudal portion of the tongue of adult chicken showing 9. Giant papillae (G). x 40. 10. Higher magnification of the surface of giant papilla showing squamous cells-like arrangement (S) with micro ridges. x 1610.

Conclusion

The present study was conducted in a systematic manner on the tongue of 4 adult broilers. The tongue exhibited a triangular outline with apex having a large number of leaf-like papillae. Their free tips were pointed to blunt with sharp lateral borders. At higher magnification, these papillae presented microridges of different types. A transverse row of gaint papillae was observed at the junction of the body and root. These papillae towards centre or medial part were pointed conical shaped and blunt type towards caudolateral portion. The surface of these papillae had squamous cells with microplicae of different patterns. These surface features of the tongue can provide insights into the feeding mechanism and nutritional requirements of broiler chicks.

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