



Comparative Histological and Ultrastructural Studies on the Tongue of Two Avian Species (*Lonchura oryzivora* and *Upupa epops*)

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ABSTRACT

Background: Avian tongue has a significant role in the feeding process. The current investigation was planned to study the histological and ultrastructural features of the tongue in two avians. Java sparrow (*Lonchura oryzivora*) and Hoopoe (*Upupa epops*).

Methods: Fourteen birds were used including seven Java sparrows and seven Hoopoes during the current investigation. Careful procedures were used in the dissection process of the two avian species. Their heads were separated and four tongue samples (from each species) were taken to fixed in 10% buffered formalin for histological observations.

Result: Histologically, the lingual glands in *Lonchura oryzivora* were simple-branched acinar and compound acinar. Whereas, the lingual glands in *Upupa epops* were simple acinar and simple-branched acinar. Three tongue samples (from each species) were taken to fixed in 2.5% of glutaraldehyde for the ultrastructural observations. The SEM findings in both species showed main three parts of the tongue; apex (anterior part), corpus (middle part) and radix (posterior part). *Lonchura oryzivora*'s tongue has an elongated dart form with anterior terminal curved pointy conical papillae. Mid-dorsal trench (*Sulcus medianus*) extended from the apex to the anterior region of the radix. While the tongue of *Upupa epops* was triangular form with a convex tip and it has a V-form papillary crest. The observed findings of the histological and ultrastructural dissimilarities of Java Sparrow and Hoopoe emphasized more insight into the feeding behaviors and adaptation of the two avians.

Key words: Bird, Histological, *Lonchura oryzivora*, Ultra-structural, Tongue, *Upupa epops*.

INTRODUCTION

Avian lifestyle is different according to feeding habits and habitats (Emura *et al.*, 2009). *Lonchura oryzivora* (Java sparrow or Java rice sparrow) belongs to order Passeriformes, family Estrildidae and feeds mainly on seeds (especially rice). *Upupa epops* (Hoopoe) belongs to the order Bucerotiformes, family Upupidae and feeds mainly on insects. So, Java Sparrow (*Lonchura oryzivora*) is considered as a granivorous bird while Hoopoe (*Upupa epops*) is considered as an insectivorous bird. Their different feeding habits and habitats may have an economic and environmental importance.

Many researchers studied the structure of the tongue in avian species to understand the relationship between the structure of the tongue and its function. Kobayashi *et al.*, (1998), Jackowiak *et al.*, (2006), Crole and Soley (2010), Uppal *et al.*, (2019), Ilgun *et al.*, (2020) and Franzo *et al.*, (2021) were studied the tongue of Penguin, Cormorant, emu, Guinea Fowl and partridge respectively. Histologically, the bird's tongue was structured from three layers. The mucosal layer was distinguished by stratiform epithelium (Franzo *et al.*, 2021). Submucosa was characterized by the existence of loose connective tissue and lingual glands (Taki-El-Deen, 2017). The lingual glands were tubule-alveolar in black-winged kite (Mohammed, 2017) and branched tubule-alveolar in cattle egret (Al-Zahaby, 2016). Muscularis layer has aggregations of striated muscle fibers and supported internally by hyaline cartilage which was extended

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throughout the tongue (Rossi *et al.*, 2005). Al-Nefei and Alahmary (2015) stated that the mucopolysaccharides are associated with the lubrication of food particles and facilitation their passage to the esophagus in the avian species. Lingual secretions protect the tongue from the roughness effect of food particles (Tabak *et al.*, 1982) and they have a protective role against the dangerous effect of bacteria (Gargiulo *et al.*, 1991). Previous SEM investigations showed variation in the shapes of the tongues in different birds. For instance, a spear form was in pigeon (Alm-Eldeen, 2005), mushroom form was in cormorant (Jackowiak *et al.*, 2006) and spatula form was in black-winged kite (Mohammed, 2017). Also, the lingual papillae may be found as in domestic pigeon (Parchami and Dehkordi, 2011) or

completely absent as in ostrich (Jackowiak and Ludwing, 2008). The lingual papillae were directed backward to speed up the passage of food particles to the entrance of the alimentary canal (Moussa and Hassan, 2013). Therefore, this research was planned to study the histological and ultrastructural features of the tongues in two different birds (*Lonchura oryzivora* and *Upupa epops*) and compare them to provide more insight into the feeding behaviors and adaptation in the two avian.

MATERIALS AND METHODS

Animals

During 2019 AD, seven healthy java sparrow (*Lonchura oryzivora*) were obtained from ornamental bird shops in Cairo. While seven healthy hoopoe (*Upupa epops*) were obtained from "Abou-Rawash" constituency. According to Frances, (2009), the adult weight of *Lonchura oryzivora* was about 25 g and its length was about 17 cm. Whereas, the adult *Upupa epops* weight was between 50 g to 90 g and its length was between 26 to 32 cm.

Histological preparations

Four tongue samples of *Lonchura oryzivora* and *Upupa epops* were fixed by using 10% buffered formalin in the biological lab of faculty of education, Ain Shams University, Egypt. The dehydration was performed via ascending concentrations of ethanol. Then, the tissue samples were cleared in terpineol and followed by the embedding process in paraffin wax. Sections of 4-6 µm thickness were stained with hematoxylin and eosin (Bancroft and Gamble, 2002). Masson's trichrome staining method was used to demonstrate the connective tissue and muscles in the tongue specimen of the bird species (Mahoney, 1973). Whereas, demonstration of the mucopolysaccharides was done by using the Alcian PAS staining method (Hotchkiss, 1948).

Electron microscopic preparations

The preparation was done for scanning electron microscopy in Cairo at "Regional Center of Mycology and Biotechnology", Egypt. Three tongue samples of *Lonchura oryzivora* and *Upupa epops* tongues are fixed by using 2.5% of glutaraldehyde. Then, they were dried by sequential degrees of dilution of ethanol (Russell and Daghljan, 1985). After that, the tongues were dried by using a carbon dioxide critical point dryer "Model: Audosamdri-815, Tousimis; Rockville, Maryland, USA" and covered by gold "SPI-Module". The ultrastructural studies of the tongues were achieved via scanning electron microscope Model: JSM-5500 LV; JEOL Ltd-Japan".

RESULTS AND DISCUSSION

Histological findings

The tongue in both birds has three layers. The mucosal layer consists of stratiform squamous epithelial cells (Uppal *et al.*, 2019). the basal row of the epithelial cells has cuboidal nuclei and located on the basement membrane (Fig 1 and 2). The

stratiform epithelial cells protect the bird's tongue during food manipulation (Igwebuike *et al.*, 2013). The mucosal layer is constructed from stratified epithelium covered with moderate keratinization thickness in *Lonchura oryzivora* in comparison to its thickness in *Upupa epops* which have thicker keratinization. Similar findings were in southern caracara (Franzo *et al.*, 2021). The keratinization in white quail was found only in the tongue's apex (Uppal *et al.*, 2014). The submucosal layer was characterized by loose connective tissue rich in blood vessels and lingual glands (Fig 3 and 4). The muscularis layer in the two birds was formed from bundles of striated muscle fibers aggregated inside a sheath of connective tissue and supported by hyaline cartilage (Ismail, 2000). Two types of lingual glands were found in *Lonchura oryzivora*'s tongue; simple-branched acinar and compound acinar. Also, their secretions were acid mucopolysaccharides (Fig 5). The lingual glands in *Upupa epops* were differentiated into simple acinar and simple-branched acinar. Their secretions were acid and neutral mucopolysaccharide (Fig 6). The lingual glands were simple branched tubular in muscovy duck (Igwebuike and Anagor, 2013) and simple branched tubule-alveolar in emu's tongue (Uppal *et al.*, 2019). Lingual glands have neutral

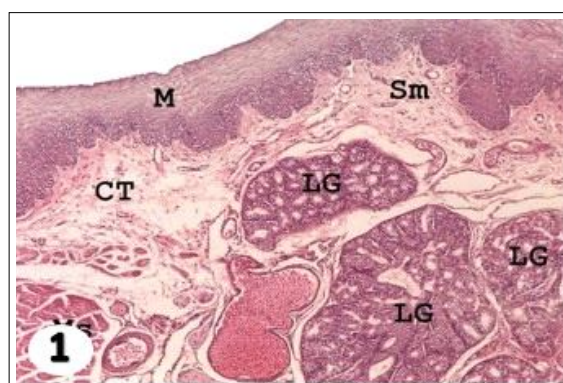


Fig 1: Light photomicrograph of *Lonchura oryzivora*'s tongue showing: mucosa (M), submucosa (Sm), connective tissue (CT), lingual gland (LG) and Muscularis (Ms). (H&E. stain X. 100)

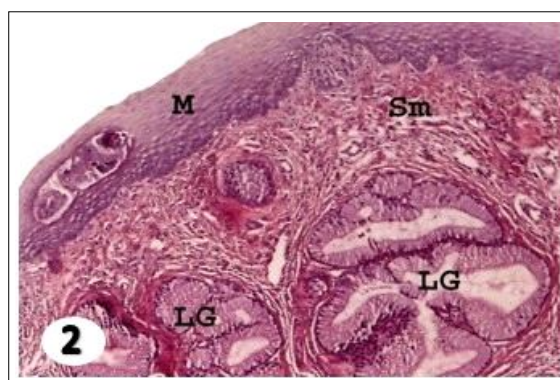


Fig 2: Light photomicrograph of a transverse section of *Upupa epops*'s tongue showing: mucosa (M), submucosa (Sm) and lingual gland (LG). (H&E. stain X. 100).

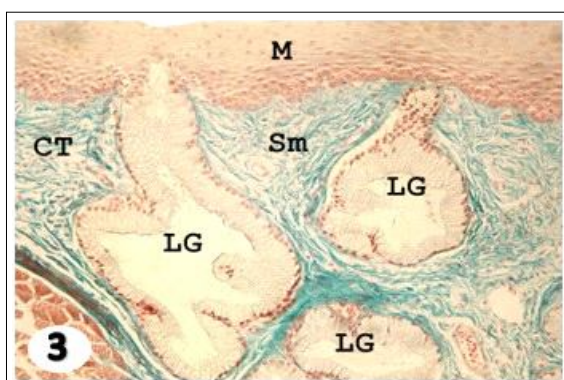


Fig 3: Light photomicrograph of *Lonchura oryzivora*'s tongue showing: mucosa (M), submucosa (Sm), connective tissue (CT), lingual glands (LG) and Muscularis (MS). (Masson's trichrome stain X.200).

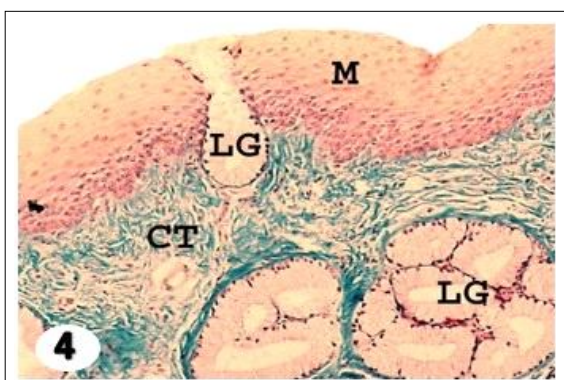


Fig 4: Light photomicrograph of a transverse section of *Upupa epops* 's tongue showing: mucosa (M), connective tissue (CT) and lingual glands (LG). (Masson's trichrome stain X.200).



Fig 5: Light photomicrograph of *Lonchura oryzivora*'s tongue showing: lingual gland (LG). (Alcian PAS. stain X.100).

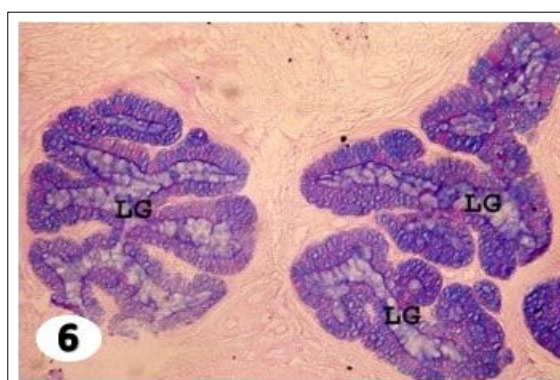


Fig 6: Light photomicrograph of a transverse section of *Upupa epops* 's tongue showing: lingual gland (LG). (Alcian PAS. stain X.100).

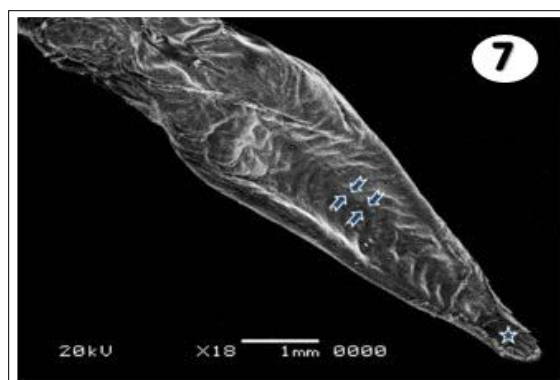


Fig 7: Scanning electron micrograph of *Lonchura oryzivora*'s tongue showing: sulcus medianus (arrows) and Spoon form anterior part (star).

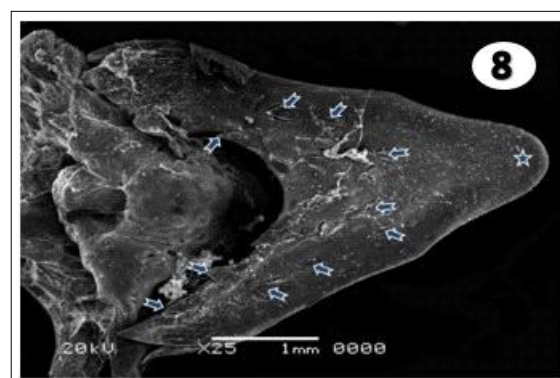


Fig 8: Scanning electron micrograph of the dorsal surface of the *Upupa epops* 's tongue showing: convex tip (star) and papillary crest (arrows).

mucosecretion in Common Myna (Kadhim *et al.*, 2013). While their secretions in *Struthio camelus* were neutral and acid mucopolysaccharides (Guimarães *et al.*, 2009).

Ultrastructural findings

The current SEM study demonstrated that the tongue was elongated dart form with spoon-like tip in *Lonchura oryzivora* as indicated in Fig 7. On the other hand, in *Upupa epops*,

tongue was a triangular form with a smooth convex tip as indicated in Fig 8. Emura *et al.*, (2009) noticed that the tongue of a woodpecker was a tube form and Mohammed (2017) stated a spatula tongue form in the black-winged kite. The available literature demonstrated variation in the shape of the tongue's tip in different bird's species. *Lonchura oryzivora* 's tongue has elongated arrow form with many

curved pointed conical papillae at its terminal part. While, the tongue of *Upupa epops* is triangular with convex tip. Whereas, the partridge's tongue has a sharp tip (Rossi *et al.*, 2005). The current SEM study demonstrated three recognized parts in the tongue of the two birds as the anterior part (apex), the middle part (corpus) and the posterior part (radix). Similar findings were observed in duck (Mohamed, 2019). The total length of *Lonchura oryzivora*'s tongue was about 8.5 mm. whereas, the total length of *Upupa epops*'s tongue was about 4.3 mm. There was a longitudinal mid-dorsal trench (sulcus medianus) extended from the apex to the anterior region of the radix of *Lonchura oryzivora*'s tongue (Fig 7). A similar observation was noticed in the tongue of quail (Uppal *et al.*, 2014). Whereas, this sulcus was absent in white-throated kingfisher (El-Beltagy, 2013). Longitudinal mid-dorsal trench (sulcus medianus) extending in the *Lonchura oryzivora*'s tongue from apex to the beginning of its radix. Sulcus medianus was found in *Lonchura oryzivora*'s tongue whereas it was absent in the tongue of *Upupa epops*. Many superficial mucosal concavities and elevations were observed in the *Lonchura oryzivora*'s tongue (Fig 9). The dorsal surface of *Upupa epops*'s tongue has desquamated superficial cells (Fig 10

and 11). A similar result was revealed in Emu's tongue (Crole and Soley, 2009). The tongue's shape helps the bird in the manipulation of food particles (Erdoğan *et al.*, 2012). *Lonchura oryzivora*'s tongue has many curved and pointy conical papillae surrounding the U-shaped apex. These curved papillae are directed internally towards the central region of apex (Fig 12). Conical papillae with big dimensions were noticed in the dorsal surface of the radix part and they caudally directed. Many scattered conical papillae was located in the dorso-lateral regions of the radix (Fig 13 and 14). In *Upupa epops*'s tongue, conical papillae with different sizes were produced a V-shaped crest. Most papillae in the lingual crest were medium in size and few number were large or small (Fig 15). The lingual papillae in turkey were arranged in one row (Abd El-Fatah *et al.*, 2000). Whereas, their arrangement in pati duck was in two rows (Sarma and Deka, 2015). The papillary crest was recorded in the form of V-shaped in White-tailed eagle (Jackowiak and Godynicki, 2005) and in the form of U-shaped in domestic pigeon (Parchami and Dehkordi, 2011). The arrangement of the conical papillae was important in the feeding process as they pouch food particles to the alimentary canal (El-Beltagy, 2013). Guimarães *et al.*, (2009) were noticed the absence of the lingual papillae on

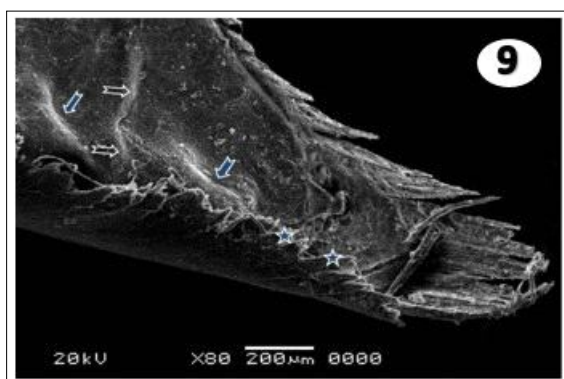


Fig 9: Scanning electron micrograph of the apex of *Lonchura oryzivora*'s tongue showing: curved pointed conical papillae (stars), mucosal concavities (solid arrows) and mucosal elevations (open arrows).

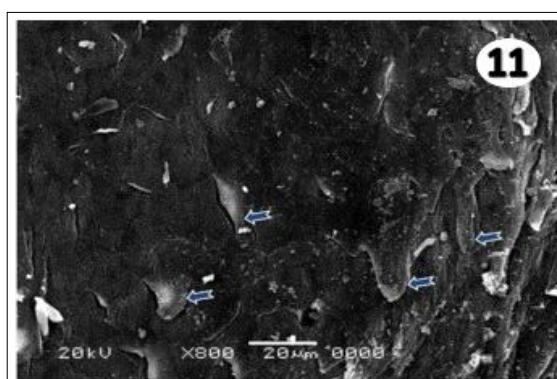


Fig 11: Scanning electron micrograph of the dorsal surface of the body of *Upupa epops*'s tongue showing: desquamated superficial cells (arrows).

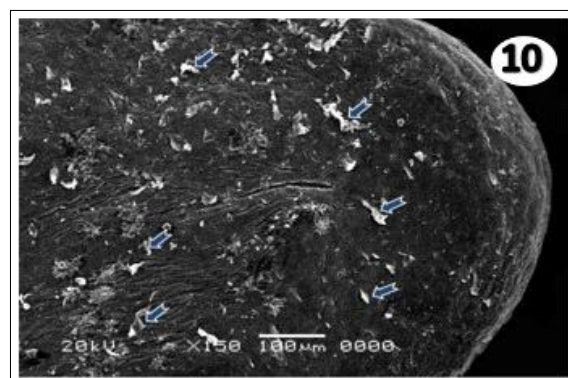


Fig 10: Scanning electron micrograph of the apex of *Upupa epops*'s tongue showing: desquamated superficial cells (arrows).

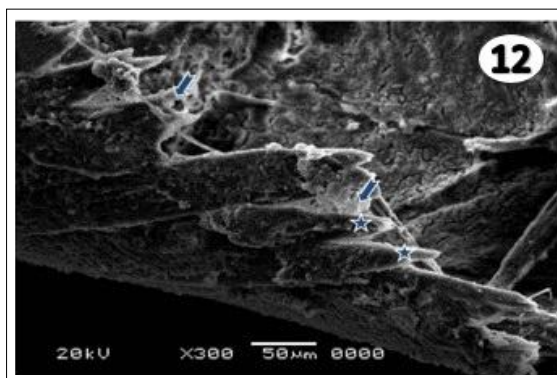


Fig 12: Scanning electron micrograph of the apex of *Lonchura oryzivora*'s tongue showing: curved pointed conical papillae (stars) and secretions of lingual glands (arrows).

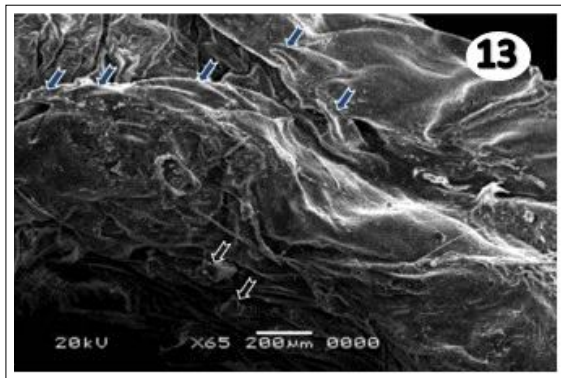


Fig 13: Scanning electron micrograph of the radix of *Lonchura oryzivora*'s tongue showing: dorsal large conical papillae (solid arrows) and dorso-laterally conical papillae (open arrows).

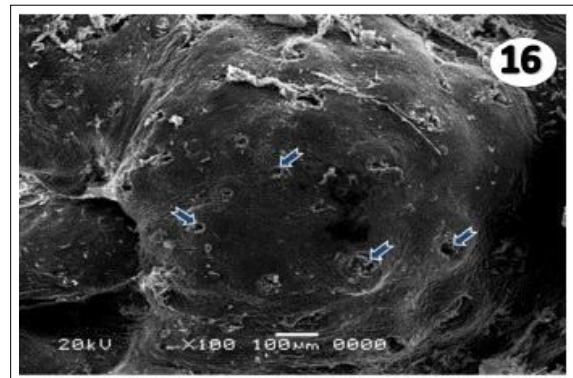


Fig 16: Scanning electron micrograph of the dorsal surface of the *Upupa epops*'s tongue radix showing: orifices of lingual glands (arrows).

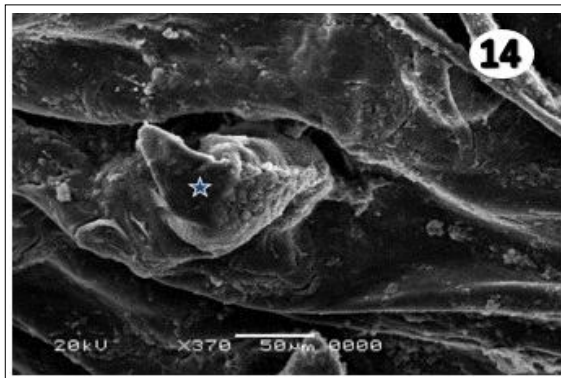


Fig 14: Scanning electron micrograph of the radix of *Lonchura oryzivora*'s tongue showing: dorso-laterally conical papilla (star).

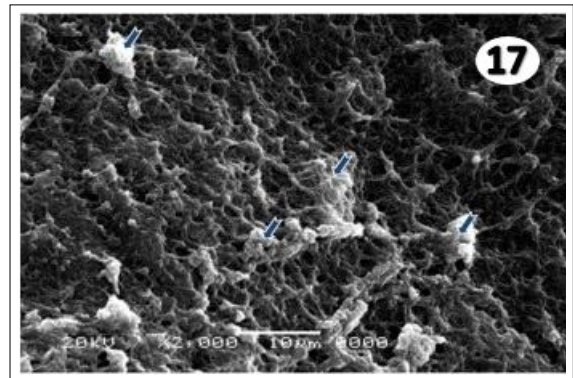


Fig 17: Scanning electron micrograph of the body of *Lonchura oryzivora*'s tongue showing: mucous secretions of the lingual glands (arrows).

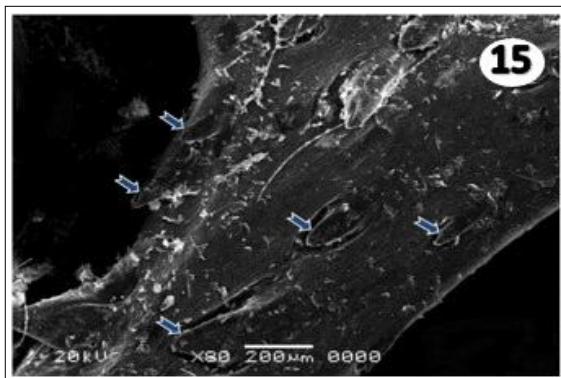


Fig 15: Scanning electron micrograph of the dorsal surface of the radix of *Upupa epops*'s tongue showing: posteriorly directed conical papillae with different sizes (arrows).

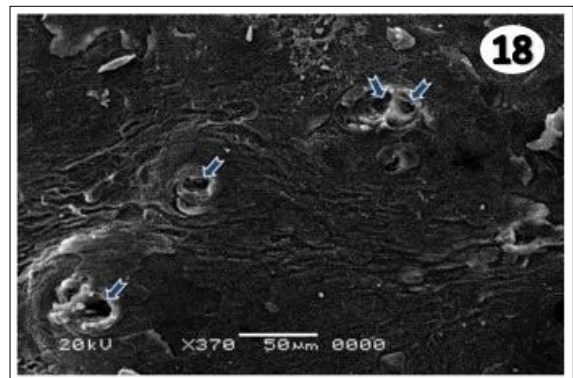


Fig 18: Scanning electron micrograph of the dorsal surface of the radix of *Upupa epops*'s tongue showing: Orifices of the lingual glands with secretions (arrows).

the tongue of *Struthio camelus*. In the two birds (*Lonchura oryzivora* and *Upupa epops*), the lingual glands were distributed along with the tongue. The mucous secretions of lingual glands exit superficially by many orifices which located on the dorsal surface of the tongue (Fig 16). The lingual secretions are noticed on the dorsal surfaces of the tongues (Fig 17 and 18).

CONCLUSION

Main basic similarities in the structure of the tongues of the two birds were recorded. However, some differences were observed in the tongue among different avian species. Sulcus medianus was observed in *Lonchura oryzivora*'s tongue whereas it was absent in the tongue of *Upupa epops*. The obtained findings illustrated various shapes of papillae

which located in different parts of the tongue in *Lonchura oryzivora* and *Upupa epops* V-form crest of papillae was recorded in *Upupa epops* 's tongue while this feature was absent in the tongue of *Lonchura oryzivora*. As for the lingual glands, they appeared in the tongue of the two birds, but they differed in their types from simple-branched acinar and compound acinar in *Lonchura oryzivora* 's tongue to simple acinar and simple-branched acinar in *Upupa epops* 's tongue. The nature of muco-secretions was varied, as it was acid mucopolysaccharides in *Lonchura oryzivora* 's tongue while it was acid and neutral mucopolysaccharide in *Upupa epops* 's tongue. Results in this investigation confirm the previous works which studied the structure of the avian tongue. Therefore, the similarities and dissimilarities between *Lonchura oryzivora* 's tongue and *Upupa epops* 's tongue were owing to the varieties in the feeding manipulation, the type of food and the environmental habitat.

Conflict of Interest: None.

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