Histo-Morphological Comparison of the Tongue between Grainivorous and Insectivorous Birds

Comparación Histo-Morfológica de la Lengua entre Aves Granívoras e Insectívoras

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SUMMARY: The study aimed to illustrate the influence of feeding habits in the anatomical feature and histological structure as well as some histochemical observations on the tongue of two species of birds which differ in their classification, activity and habitat as; The domestic pigeon, Grainivorous bird, and cattle egret, Insectivorous bird, using light and SEM studies. Results showed that the tongue of two species was differing in size, shape and structure. The tongue of pigeon appeared short and triangular; while in cattle egret was long lanceolate in shape with narrow tapering apex. Dorsal large conical lingual papillae presented between the body and the root of both tongue of the studied birds. They were arranged in form of U-letter in pigeon and in form of wide V-letter in cattle egret. Histologically, both dorsal and ventral lingual surfaces lined with keratinized stratified squamous epithelium, which decreased in the thickness and the degree of keratinization toward the base. The dorsum of the apex of pigeon tongue showed desquamated epithelial cells with filiform papillae, these papillae not observed in the egret's tongue. The tongue of cattle egret contained longitudinal tendinous tissue as intra-lingual ligament appeared parallel and accompanied with skeletal muscle bundles and attached with entoglossal cartilage. It extended longitudinally from the root to the body of the egret's tongue. The salivary glands presented in the propria submucosal layer of the dorsal surface that extended laterally from apex to the root, while the ventral surface devoid from any glandular structures. The nature of lingual salivary glands showed variations in their histochemical observation to Alcian blue stain and PAS technique. It has been summarized that the morphological and histological variations of both tongues may be correlated to their feeding habits.

KEY WORDS: Tongue; Birds; SEM; Histology; feeding habits; Salivary glands.

INTRODUCTION

The pigeon considered the oldest world’s domesticated bird from about ten thousand years ago. They have an importance to humanity, particularly in the times of war also considered important source of meat for people (Blechman, 2007). The cattle egret spread in the hot areas with long expansions. Most people especially the farmers called it Abo-quirdan, meaning “father of ticks”, and this name originate from the large number of ticks of birds which spreads in their breeding colonies. The egrets appeared mostly in Egypt, in Delta and Nile Valley, making nests colony close to water bodies on trees and shrubs. They mostly appear together with animals to pick up insects as ticks and flies. The tongue has an important role in mechanism of food in vertebrates especially in birds. Therefore, this mechanism is very important to detect the adaptation and perseverance of vertebrates to their environmental habits (Darwish, 2012).

Variations in morphology in shape and size of the tongue allowing its functions as a special tool for obtaining, manipulation, swallowing and processing food. Many authors has been studied the morphology of the tongue in vertebrates (Zweers, 1982). According to their lifestyle, birds have different feeding habits, with differences corresponding to the shape of their beak and tongues. Most bird’s lives in different environment such as the air, the land and the water, many authors illustrated that the shape and structure of the tongue differs according to the type of food and method of food intake (Jackowiak et al., 2011; Al-Zahaby & Elsheikh, 2012).
The tongues of birds are adapted for manipulation, collection, and swallowing of foods. Studies of morphological and functional aspects of different species of birds indicated a close relation to the histological structure of the tongue with their feeding habits (Emura et al., 2009a; Guimares et al., 2009; Abou-Zaid & Al-Jalaud, 2010; Mahmoud et al., 2017).

The structure of mucosa of the tongue, type and distribution of lingual papillae and the degrees of keratinization of the lingual epithelium in relation to feeding habits were described by many authors as white tailed eagle (Jackowiak & Godynicki, 2005), cormorant (Jackowiak et al., 2006), ostrich (Jackowiak & Ludwig, 2008), peregrine falcon and common kestrel (Emura et al., 2008), spot-billed duck (Emura, 2009a), three species of herons (Emura, 2009b), woodpecker (Emura et al.), common quail (Parchami et al., 2010), domestic pigeon (Parchami & Dehkordi, 2011), red jungle fowl (Kadhim et al., 2014), chukar partridge (Erdogan et al., 2012), Muscovy duck (Igwebuike & Anagor, 2013), white-throated kingfisher and common buzzard (El-Beltagy, 2013), Black Francolin (Kadhim et al.), the common kingfisher (Al-Zahaby & Elsheikh), southern lapwing (Erdogan & Perez, 2015). From the previous observations, there are a correlation between shape, structure of the tongue and nature of food, also the mechanism of food intake and bird’s habits.

This study aimed to illustrate the influence of the nature of food and the methods of feeding intake on the macroscopic and microscopical observations of the tongue between two different birds in feeding habits and type of food as the domestic pigeon (Grainivorous) and the egret (Insectivorous), using light microscopy and scanning electron microscope studies, to interpret the present result to other previous studies in relation to avian feeding habits.

MATERIAL AND METHOD

Eight tongues of both pigeons and egrets were investigated. The birds were captured from El-Menofiya Governorate, Egypt. The birds were observed prior to being sacrificed, to ensure their health condition and for any clinical signs indicating infection. Only apparently healthy birds were selected for this study; the tongues were quickly dissected from the mouth cavity and processed as follows: For scanning electron microscopy (SEM), different parts of tongue were rapidly fixed overnight in modified glutaraldehyde solutions (2 % paraformaldehyde and 2.5 % glutaraldehyde containing 0.1 M phosphate-buffered solution, pH 7.4) at 4 °C. After rinsing in 0.1 M phosphate buffer (pH 7.4) the samples were post fixed in phosphate-buffered solutions (pH 7.4) of 1 % osmium tetroxide at 4 °C for 2 hours. These specimens were then washed in 0.1 M phosphate buffer solution several times before treating with 3 N hydrochloric acid for 20 min at 60 °C to remove extra cellular mucus from the lingual surface. The specimens were then washed in phosphate buffer solution and dehydrated in ascending graded ethanol series to the critical-point of drying and gold coated. The specimens were then examined in a JEOL-JSM 5300 Scanning Electron Microscope at the faculty of Medicine, Tanta University (Hayat, 2000).

For light microscopy, different parts of the tongues were rapidly set in 10 % neutral-buffered formalin solution for at least 48 hours, then dehydrated in ascending ethanol-xylene and embedded in paraffin wax. 5 µm serial sections were cut transversely and stained with haematoxylin and eosin, Masson's trichrome stain for detection of the demonstration of collagen fibers, Weigert's elastic tissue stain for demonstration of elastic fibers and some special histochemical stains as Alcian blue stain and Periodic acid Schiff techniques for detection the nature of lingual salivary glands secretion (Bancroft & Gamble, 2002).

RESULTS

Gross morphology. Neither the shape nor the dimensions of the tongue of the birds showed specific-sex differences. The dorsum of the tongue of Pigeon and Egret were distinguished into three parts: apex, body and root. It is distinguished dorsally from its anterior half by a clear median groove, except on its apical region and some parts along its posterior part. This groove was dividing the lingual apex and the body of the tongue into two symmetrical halves. The tongue of adult domestic pigeon appeared triangular, small with narrow anterior part and wide fleshy posterior part (Fig. 1). While it was very long, lanceolate with narrow and tapering apex in cattle egret (Fig. 2). Conical papillae were arranged symmetrically in the marginal region between the body and the root, these papillae take the form of the letter (U) in pigeon while take (V) letter in egret. The lateral papillae were larger and thicker than the middle one in both pigeon and egret (Figs. 1 and 2).

Scanning Electron Microscope Studies (SEM): SEM in the domestic pigeon revealed that, deep sulcus dividing the apex and body of the tongue into two symmetrical halves (Fig. 3A). The surface of the dorsum of the anterior part of the tongue was covered by a large number of irregular scaley protrusions of the deciduous epithelial growths. (Fig. 3B). Filliform papillae were compactly distributed along the
lingual body. They were long, slender with broad tip. The filiform papillae were backwardly directed and situated around the median sulcus (Fig. 3C), in the margin between the body and the base of the tongue, small sized conical papillae were observed and they were numerous arranged asymmetrically in the form of a letter (U). The lateral papillae were larger and thicker than the middle one. The dorsum of the base of tongue showed smooth appearance with no densely packed desquamated cells and no lingual papillae are observed (Fig. 3D).

While in the cattle egret, SEM revealed that, the dorsum of the anterior lingual part from the apex to the end of the body of the tongue was somewhat appeared smooth, devoid of any lingual papillae. The epithelium
of this region was slightly undulated. Scanty number of irregular scales was observed on the dorsal surface of the lingual apex with characteristic median groove dividing the tongue into two symmetrical halves (Fig. 4A). Longitudinal pellicae were observed at both sides of the anterior part of the lingual body. These pellicae arranged at almost regular intervals, in addition few scales were frequently arranged over the surface of the pellicae (Fig. 4B). On the caudal part of the lingual body, longitudinal and transverse ridges or pellicae also noticed at both sides of the tongue around the median sulcus with widely distributed tiny scales on their surfaces (Fig. 4C). Giant papillae were located in a transverse row at the end of the lingual body which completed by caudal continuation of giant conical papillae, larger and thicker than the middle one. (Fig. 4D). The middle papillae were dome-shaped in outline and some scales frequently observed on their dorsal surface. Well-developed micro ridges were widely distributed on the cell surface of the dorsal surface of the giant conical papillae (Fig. 4E). The lateral lingual papillae was larger inclined backwardly toward the pharynx (Fig. 4G). The surface of root also devoid of lingual papillae showing widely distributed rounded openings of the caudal lingual salivary glands; the openings of the salivary glands were surrounded by capsule (Fig. 4F).

**Light Microscope Studies.** Histologically, the lining epithelium of the dorsal surface of the tongue in both pigeon and egret consisted of a stratified squamous keratinized epithelium. The keratinized layer was thicker in the ventral and lateral surfaces especially in the tip of the lingual apex (Figs. 5A and 6A). The dorsal surface was thicker than the ventral surface. The base of this epithelium was uneven, being thrown into shallow and narrow folds. The cells of both the basal and the deep intermediate layers were rounded or elliptical in shape and have large, central and spherical nuclei. From the deep intermediate layer to the superficial layer, the cells and the nuclei gradually flattened and desquamating cells were detectable on the upper surface (Figs. 5B and 6B). On the superficial layer of the dorsal surface of the body of the pigeon tongue, there were distinct

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**Fig. 4.** Scanning electron micrograph of the dorsal surface of the cattle egret tongue showing. A, longitudinal groove on the anterior part of the lingual body (dark arrow) and ridges on the lateral lingual surface (yellow arrow). B, Longitudinal pellicae arranged at almost regular intervals, with few scales on their surface (black arrow) on the lingual body. C, longitudinal and transverse ridges (pellicae) (black arrow) with compactly distributed scales and the central lingual sulcus. D, the widely distributed openings of the caudal lingual salivary glands. Note, the giant conical papillae. (GP), on the root of the tongue. E, Higher magnification of Fig.(D) showing the giant papillae (GP) of the egret tongue. Note that the giant papillae are dome –shape in outline with characteristic scales on their surface. (Black arrow). F, opening of salivary glands on the root of egret tongue. G, the giant conical lateral papillae directed caudally toward the pharynx (black arrows) and opening of salivary glands (Gl) on the root.
protrusions of the desquamated epithelial cells which may represented as filliform papillae (Fig. 5C). The keratinization was also highly developed at the giant conical papilla which separating the body of the tongue from the base fixed part which inclined backwardly toward the pharynx (Figs. 5D and 6D). Gustatory lingual papillae were not found in the epithelium covering the tongue in both studied birds. The lamina propria consisted of loose connective tissue containing collagen fibers, thin elastic fibers and numerous blood vessels (Fig. 6C). There were muscles in the lamina propria of the dorsal surface of the tongue. The muscles were arranged thin and striated in the form of circular in the lingual apex, but oriented in the form of circular and longitudinal in different direction in the body and root of the tongue.

The skeleton of the tongue in pigeon was supported by cartilage hyoid apparatus revealed on entoglossal bone as skeletal element of the tongue which extending from the lingual root to lingual apex (Figs. 5C, D, E). While in cattle egret, an intra-lingual tendon was longitudinal fibrous structure parallel and accompanied with skeletal muscle fibers and attached with entoglossal cartilage (Figs. 6E and F). The lingual salivary glands of pigeons and egrets were located in the lamina propria of the second half of the free part of the tongue till the root of the tongue. Therefore, the lingual salivary glands of both studied birds could be divided into two types: Anterior lingual glands at the anterior part of the tongue and posterior salivary glands at the root of the tongue, both of them were typically mucous gland in the pigeon. The mucous secretory units composed of tall columnar cells with extensive vesicular cytoplasm. These glands were surrounded by connective tissue capsule with septa dividing the gland into lobules) (Figs. 5F and 6G). They open on the epithelial surface through minute pores (Fig. 6F).

The secretory cells of the lingual salivary glands of the pigeons, contained large amount of acid mucopolysaccharide substances that showed strong positive reaction to alcaion blue stain while give negative reaction to PAS techniques (Fig. 5G). The ventral surface of the tongue is devoid of any glandular structure. while in the egret, the anterior one appeared serous adenomere while mixed (sero mucoid) in the posterior one. The alcianophilic substances appeared to be increased in amount in the cytoplasm of secretory cells of the posterior salivary lingual glands and showed strong positive reaction to alcaion blue stain (Fig. 6H).
DISCUSSION

**Gross morphology.** The present study was carried out to clarify the structural features of the tongue between two birds living in different environments feeding habits and food sources. The structural characteristics of components of the avian digestive tract are largely determined by the kind of diet consumed by the particular species. There are different types of adaptation of the bird's tongue, such as for accumulating food, handling food and swallowing (Jackowiak & Godynicki).

Previous studies in the avian tongue revealed that the morphology, structure of the epithelium linguae, supportive components also papillae localization are thoroughly related with the nature of food, mode of feeding also the different habitats (Whittow, 2000). Results achieved from the current study revealed that the tongue of domestic pigeon is a characteristic triangular organ with three distinct anatomical parts: apex, body and root. These morphological features resemble those of common quail (Parchami et al.), domestic chicken (Homberger & Meyers, 1989) and chukar partridge (Erdogan, 2012). While the long cattle egret’s tongue of the current study prolongs to fill almost the cavity of the lower beak and is terminating with sharp, tapered apex. This offers suitable eating possibilities for searching for small food items such as insects in rubbish dumps and bodies of animals (Al-Zahaby, 2016). These description of the tongue is same similar to that of Chukar partridge (Erdogan et al.), which also feeds on insects in the ground pastures.

Data found from this study also showed that definite median sulcus divides the apex and body of the tongue into two similar halves. These results resemble of those described on the tongue of white tailed eagle, grey heron (Abou-Zaid & Al-Jalaud) and domestic goose (Jackowiak et al., 2011), however it is lacking on the tongue of chickens and ostrich, besides these features show the adaptation of the tongue to aid swallowing grains as whole pieces in the esophagus (Iwasaki, 2002). Also the current study showed that a main row of great conical papillae are situated symmetrically in the form of the letter U in the marginal region between the
lingual body and the lingual root. These results are similar
to those the documented by Parchami et al. and Parchami &
Dehkordi (2011), in common quail and domestic pigeon. In
the chucker partridge and common quail, the root of the
tongue have conical papillae with the pointed apex directed
caudally arranged in the letter V; behind this row there is an
additional row composed of laterally sited large papillae
(Erdogan et al.). While in the Middendroff’s bean goose and
domestic goose there are giant conical papillae located
between the body and basal region also on the lateral sides
of the anterior region of the tongue. There are lingual hairy
papillae densely distributed and small numbers of large
cylindrical papillae are arranged between these lingual hairs
(Iwasaki et al., 1997), that is not noted in the present study.
On the other hand, Pasand et al. (2010) reported the giant
conical papillae in ostrich were not detected between the
lingual body and root. Occurrence of these lingual papillae
has been reflected to be related to specific feeding habits of
birds, the conical papillae found in the lingual body was
helping in the transfer of swallowed food towards the
esophagus and preventing its regurgitation.

**Scanning Electron Microscope.** The ultrastructural
observations in the present study revealed that the pigeon
tongue has large numbers of backwardly directed scales on
the lingual apex and slender filiform papillae with a broad
tip on the rest of the lingual body. Moreover, no fungiform
papillae presents between the filiform papillae as mentioned
in grey heron (Abou-Zaid & Al-Jalaud), domestic chick (El-
Beltagy) also King fisher (Al-Zahaby & Elsheikh). This
result was similar to some species of birds but the shape of
the processes differing from each other, they were needle –
shaped processes in woodpecker (Emura et al.), lamellar
shaped in pigeon (Parchami & Dehkordi, 2011), carpet-
shaped in the peregrine falcon and common kestrel (Emura
et al.), thread-shaped in the owl species (Emura et al., 2009a),
spine-like in the penguins (Kobayashi et al., 1998), acicular
processes in the European Magpie and the Common Raven
(Erdogan & Alan, 2012), also many processes can be
observed in the black kite (Emura).

The microridges present on the root in the pigeon
and egret may develop the transport of food otherwise, seed
or insects through the surface of the tongue and perform as
sites for preservation of the mucous produced by the lingual
salivary glands located on the base of tongue. The distribution
of the apical scales and in the present investigation, giant
papillae were arranged at the final part of the lingual body
in both specimens, being big and conical in shape in the
pigeon and small with rounded profile in the egret. These
much larger giant papillae in the pigeon may help in pushing
the dry seeds into the pharynx. However, these papillae are
smaller in the egret because the food consists of worms and
insects, which are soft enough for smooth swallowing. These
giant papillae so-called “lingual spikes” by Kooloos (1986).
The distribution pattern of these papillae was the same in
the Mallard (Kooloos), in the chicken (Iwasaki & Kobayashi,
1986), in the little tern (Iwasaki), in duck (Abdalla, 1994), in
Middendroff’s Bean Goose (Iwasaki et al.). The presence
of these giant papillae in these different species of birds may
be related to their phylogenetic origin.

**Light microscope observation.** In this current study, we
observed that mucosa of the dorsum of the lingual apex (tip)
is lined with a thick keratinized stratified squamous
epithelium only on the lateral and ventral surface, whereas
the lingual body and root are lined with non- keratinized
stratified squamous epithelium, this result is similar to that
described through (Jackowiak & Godynicki) in white tailed
eagle and by Parchami & Dehkordi (2011) in domestic
pigeon. Contrary to reports in chucker partridge, the dorsal
surface of its tongue was lined with thick keratinized
stratified squamous epithelium Erdogan et al., whereas in
the ostrich, the dorsal and ventral surfaces of the tongue were
lined by non-keratinized stratified squamous epithelium
(Jackowiak & Ludwig).

The changes in the degree of keratinization of the
lingual epithelium between different species look to be
related to the differences in habitat. These differences clearly
appear in chickens’ live in habitat, being much drier than
that of the water fowls like Middendröff’s bean goose,
domestic ducks and the little tern. In birds, the amount of
keratinization of lingual epithelium looks to be a certain
degree, to reflect differences in their life style (Erdogan &
Perez). In most birds, anterior tip of the tongue is directly
contact with food and may avert injuries during feeding. In
this study, the lingual epithelium of pigeon is thick
keratinized and has solid plates, as it is exposed to solid
grains and seeds during feeding. Lateral edges and ventral
surface of the tongue may be exposed to hard grains and
seeds, during handling and when stored in the buccal cavity.
Therefore, epithelium covered with a thick keratinized layer.
This interpretation agreed with Al-Nefely (2015) in laughing
dove. Observations of the present study showed that the la-
mina propria is dense irregular connective tissue, which
contains adipose cells and numerous blood vessels. This
connective tissue supported by the strong layer of striated
muscle fibers, which are oriented in longitudinal and circu-
lar direction in the body and base of the tongue. In addition,
the tongue contains hyaline cartilage, which extends from
the lingual apex to the lingual root and surrounded by lingual
muscle fibers. These observations are similar to that of
Pasand et al. in male ostrich and Parcham & Dehkordi (2011)
in domestic pigeon. Homberger and Meyers stated that in
birds, the tongue maintained by unpaired paraglossae.
extending through the lingual tip and articulates caudally with the basihyoid apparatus.

In the present study, the tongue of domestic pigeon and cattle egret supported by elements of the hyoid apparatus. It spreads ventrally to the apex of the tongue as a narrow point, paraglossal apex, then becomes flat and thicker in the tongue’s body called the corpus paraglossale. This last cartilage remains caudally and bifurcates in the tongue’s root as two paraglossalis caudalis ending on the each side of the trachea as a hyoid bone. In cattle egret showing different structure not observed in the pigeon hyoid apparatus, which is the paraglossal apparatus enveloped with a definite perichondrium consisted of fibrous connective tissue and striated muscle fibers, assisting for moving of the tongue out of the mouth cavity. Likewise, Erdogan et al. stated that the hyaline paraglossal cartilage supporting the chukar partridge’s tongue, which extends in the lingual root, body and apex. Igwebuike & Anagor also accepted the presence of the paraglossal skeleton and associated striated muscle fibers of the tongue in Muscovy duck. In brief, the tongue apparatus of the egret displays certain anatomical and microscopical structures that are distinctive to this bird species. This may be an adjustment to the method of food intake, the type of food, lifestyles and bird’s habitat (Al- Zahaby).

In this study, we showed that the lingual glands are simply branched tubulo alveolar glands; they did not appear in the lingual apex but appeared in the lingual body, which open on the epithelial surface through minute pores. It increased in numbers through the basal region of the tongue. The lingual salivary glands prolonged from the apex of the tongue to both sides of the laryngeal cleft in the white-eared bulbul (Parchami & Dehkordi, 2013). The lingual salivary glands are absent in the fundamental tongue of cormorants (Jackowiak et al., 2006). In this study, the lingual salivary gland in both pigeon and egret take positive reaction to alcian blue stain while negative to PAS technique as observed on the tongue of the little egret. In contrast, in red jungle fowl, Zebra finch, Black Francolin and common kestrel which their salivary gland take strong positive to PAS reaction which indicated to their content of neutral mucin (El-Beltagy; Kadhim et al.). Saliva aids in softening ingested food to facilitate swallowing, keep the mucous membrane of the upper digestive tract protected from injures of hard grains (Parchami & Dehkordi, 2011).

In conclusion, results of the present study revealed that the tongue of both studied birds were different from each other in their shape; electron microscope observations and histological analysis identified their differences in feeding habit and habitat.

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