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Abstract

This study was mainly designed to discriminate the comparative aspects on the tongue of ***Gallinula cholorpus*** (omnivorous bird) and ***Coturnix coturnix*** (grainivorous bird) using light and SEM investigations. ***G. cholorpus*** was obtained from the Mediterranean area of Gamasa city while the ***C. coturnix*** was purchased from the local market in Mansoura city (four individuals for each species). Two tongues from each species were processed for SEM while the others were processed for histological and histochemical investigation. The obtained gross morphological anatomy results revealed that, the tongue of ***G. cholorpus*** is apparently long and narrower with slightly tapered apex while that of ***C. coturnix*** appeared short, broad and triangular with obviously tapered apex. Histological results displayed that, the tongue dorsum of ***G. cholorpus*** is covered with highly keratinized stratified epithelium if compared with that of ***C. coturnix*** tongue. Also, the tongue of ***C. coturnix*** appeared rich with high density of branched lingual glands as well as numerous filiform and fungiform papillae while that of ***G. cholorpus*** appeared with rarely distributed lingual glands however the papillae represented by low density of conical ones. The SEM investigation indicated that the tongue of ***G. cholorpus*** is bilaterally provided with compact skeletal muscles and foliated centrally located keratinized epithelium. On the other hand, the SEM investigation of ***C. coturnix*** tongue showed numerous papillae on either side especially around the tongue body with multiple pointed desquamated epithelial cells scattered all-over the tongue surface. As revealed by histochemical study, the intensity of acid and neutral mucin appeared strongly expressed in the lingual glands of ***C. coturnix*** while the lingual glands of ***G. cholorpus*** displayed moderate to weak expression.

1. Introduction

Ca Among vertebrates, the method of food intake, type of food and habitat are fundamentally depends on the structure of tongues (**Jackowiak *et al*., 2011; Al-Zahaby & Elsheikh, 2014**). Anatomically the tongue of non-mammalian vertebrates is differentiated into three main parts; the apex, the body and the root (radix) (**Dehkordi *et al*., 2010).** It has been documented that there were single or double papillary crest composed of mechanical conical papillae between lingual body and root (**Vollmerhaus and Sinowatz, 1992**). Among birds, the dorsum of the tongue is supported with various types of lingual papillae to enhance capturing, picking up, swallowing and in taking of food particles. Furthermore, the type and localization of lingual papillae, structure of tongue mucosa , 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**), ostrich (**Jackowiak & Ludwig, 2008**), peregrine falcon (**Emura *et al*., 2008**), spot-billed duck (**Emura, 2009a**), woodpecker (**Emura *et al*., 2009b**), common quail (**Parchami *et al*., 2010**), chukar partridge (**Erdogan *et al*., 2012**), red jungle fowl (**Kadhim *et al*., 2011**), Muscovy duck (**Igwebuike & Anagor,2013**), white-throated kingfisher and common buzzard (**El-Beltagy, 2013**), Black Francolin (**Kadhim *et al*., 2014**), the common kingfisher (**Al-Zahaby & Elsheikh, 2014**), southern lapwing (**Erdogan & Perez, 2015**). Additionally, among avian tongue, there are high variation in number and distribution of salivary gland which correlated with feeding habits. Studies has been showed that salivary glands are of two types , anteriorly serous gland and posteriorly mucous gland .Salivary glands are well developed in granivorous, insectivorous and woodpeckers species (**King & McLelland, 1984; Blanks, 1993**).

The common Quail, *C. coturnix*, is classified as a member of the phasianidae family and coturnix genus. It widely distributed in the Palaearctic, winters in the Sahel and, after migration, it reaches its breeding grounds in northern Africa and Eurasia (**Guyomarc'h *et al.*, 1998**). Mostly *C. coturnix* feeds on grains like wheat and barley and scarcely feeds on other types of grains (**Parchami *et al.*, 2010**). While Common Moorhen *Gallinula chloropus* belongs to family Rallidae and genus Gallinula. Not only distributed in North and South America, tropical Africa, and the cold and temperate zones of Asia and Europe (**Sauer 1984**), but also found in the Arab countries including Egypt (**Walker, 2009**). It named the water hen or swamp chicken because it inhabiting channels, around the boons, vegetable lands, and other wetlands. *G. chloropus* prefers robust, tall, vegetated grasses within water pools (**Bannor & Kiviat, 2002**). In addition, it sometimes feeds on small fishes or crustacean species, Thus it classified as an omnivorous bird (**Abumandour & El-Bakary, 2017; Cramp & Simmons, 1980**).

Accordingly, the current study aimed to evaluate the comparative aspects of the tongue of *C. coturnix* and *G.cholorpus* and its correlation with the nature of feeding.

2. Materials and Methods

1. Experimental animals:

This study was applied on two avian species with different feeding habit. The two selected species are *C. coturnix* (gainivorous bird) and *G. cholorpus (*omnivorous bird*)*. Quails were purchased from the local market in Mansoura city, while *G. cholorpus* was obtained from the Mediterranean area of Gamasa city, Egypt. The studied species were checked for any gross morphological abnormalities, and transferred to the lab in separate cages. After 2weeks of acclimatization, the animals were sacrificed; the tongue was removed from the oral cavity, cleaned, and photographed by using digital camera. Anatomical terms follow the Avian Tongue (Johnston, 2014). For the current study two tongues of each species were used for histological and histochemical investigation. On the other hand, the other two tongues were prepared for investigation by the scanning electron microscope.

2. Investigated parameters

A. Histological and Histochemical Studies:

The tongues were washed with saline solution to remove any food debris and immediately fixed in 10% neutral formalin. Each tongue was longitudinally cut into two halves, then dehydrated in ascending series of alcohols, cleaned in xylene and finally embedded in paraffin wax at 60°C. The longitudinal and transverse paraffin sections at 5-6μm in thick were prepared. The prepared slides were deparaffinized and hydrated in descending grades of alcohol. For routine histological investigation some of these sections were stained with Haematoxylin and Eosin according to Carleton (1980). Other sections of the tongue were stained with combined alcian blue (Ph 2.5) – PAS stain to detect the histochemical activity of acid mucin and neutral mucin in the lingual glands (Mowry, 1956; Schumacher *et al*., 2004)**.**

B. Scanning Electron Microscopic Studies:

The tongues from two selected species were washed in 0.1 M chilled phosphate buffer (pH 7.4), fixed in 2% formaldehyde, 1.25% glutaraldehyde in 0.1 M sodium cacodylate buffer, pH 7.2 at 4 °C for about 4days. Following fixation, the tongues were washed in 0.1 M sodium cacodylate containing 5% sucrose; post fixed in 1% buffered osmium tetroxide for 24 h at 4 °C, and then dehydrated in ascending grades of alcohol. Subsequently the tongues were dried in liquid CO2, mounted and coated them with gold palladium in a sputtering device (Pelco model 3 sputter coater 91000) (Yoshimura *et al*., 2008). SEM analysis was performed using a JEOL 100CXl at the Unit of Electron Microscopy, Faculty of Agriculture, Mansoura University.

3. Results

1. Gross morphology

Morphologically, the tongue of ***C.coturnix*** is short, broad and triangular with obviously tapered apex, while that of ***G.cholorpus*** is relatively long and narrower with slightly tapered apex. On the dorsal surface of the tongue of both ***C.coturnix*** and ***G.cholorpus***, three distinctive parts are distinguished: apex, body and root. Furthermore, dorsal surface of lingual body and apex were divided into two symmetrical halves by a specific median groove which have been observed in the two studied species. Another notable morphological feature found in both ***C.coturnix*** and ***G.cholorpus*** is the papillary crest, which separates lingual body from lingual root and represented by transverse row of conical papillae directed backward toward pharynx (Figure 1).

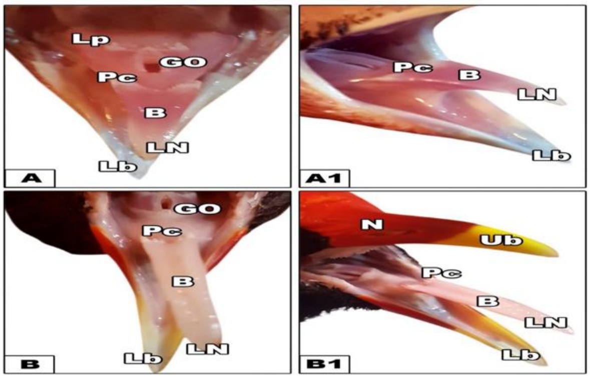

Fig. 1: Illustrating the gross anatomy of tongue of *C.coturnix* (A&A1) and *G.cholorpus* (B&B1). Note: The tongue of *C.coturnix* is shor, broad and triangular with distinct tapered apex while that of *G.cholorpus* is longer and narrower slightly tapered apex. In both species, the papillary crest lies at median line between lingual body and root carrying caudally directed conical papillae followed by median glottis. Comparatively, papillary crest *of G.cholorpus* is thinner than that of *C.coturnix.*
Abbreviations; Lb, lower beak; Ub, upper beak; Ln, lingual nail (apex); B, body; N, nose; Pc, papillary crest; Go, glottis, Lp, laryngeal papillae

2. Scanning electron microscopy (SEM):

SEM imagery revealed that the dorsal surface of the tongue apex of ***C.coturnix*** covered with stratified squamous keratinized epithelium forming lingual nail, while the lingual body covered by stratified squamous non- keratinized epithelium. Additionally, the dorsal surface of lingual body bears numerous irregular desquamated epithelial cells. Furthermore, numerous mechanical lingual papillae, filiform papillae, have been observed laterally and alongside of apex and body of the tongue (Figure2 A &B). The junction between lingual body and lingual is supported by transverse row of back warded conical papillae, papillary crest, which arranged as median and lateral conical papillae. On the lateral sides of papillary crest, giant conical papillae are present. Just behind lingual body, lingual root appeared with smooth dorsal epithelial surface. The floor of tongue root bearing many scattered openings of posterior salivary glands. Also, the tongue root showing obvious mucus secretion and glandular orifice. Posteriorly to tongue root, glottis appeared circular followed by two transverse rows of pharyngeal papillae; anterior and posterior papillae. Anterior pharyngeal papillae have been found to be larger and duplicated while posterior pharyngeal papillae appeared small and single (Figure 3A-D).

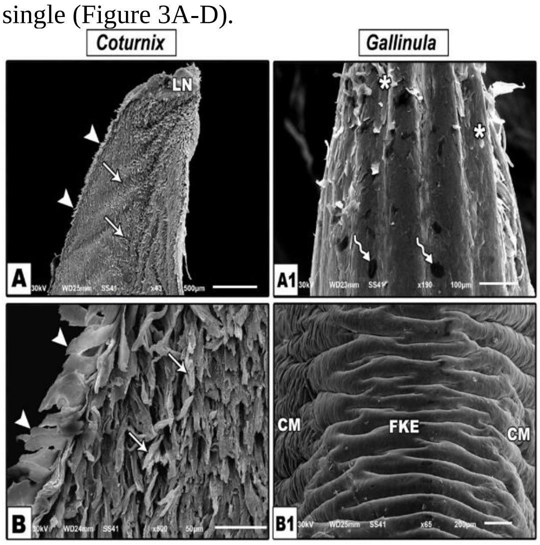

Fig 2. Scanning electron micrograph (SEM) of the tongue of C.coturnix (A&B), and G.cholorpus (A1&B1). Note: the tongue of C.coturnix showing (lingual nail with numerous dorsal fine processes (Arrow), lateral fine process (filiform papillae) arranged laterally alongside lingual body (arrow head) however, the tongue of G.cholorpus showing few numbers of desquamated epithelial cells (asterisks), glandular orifices (zigzag arrow), foliated keratinized epithelium and compacted muscles on both sides of lingual body.

Abbreviations; Cm, compacted muscle; FkE, foliated keratinized epithelium

On the other hand, SEM of the tongue of ***G.cholorpus*** showed many differences from that of ***C.coturnix*** despite it displayed the same three distinctive parts of the tongue: apex, body and root. Tongue of ***G.cholorpus*** appeared compacted with muscles. Lingual apex and body devoid of any processes, except little desquamated epithelial cells are noticed. The dorsal surface of lingual apex and body covered with highly keratinized epithelium which forming numerous epithelial folds on the dorsum of tongue. Further, dorsal surface of lingual apex and body devoid from lingual papillae but supported with compacted muscle. Additionally, many glandular orifices of the posterior lingual salivary glands are distributed at tongue body (Figure2 A1&B1). Presence of papillary crest is, a characteristic feature in avian tongue, has been confirmed from gross morphology.

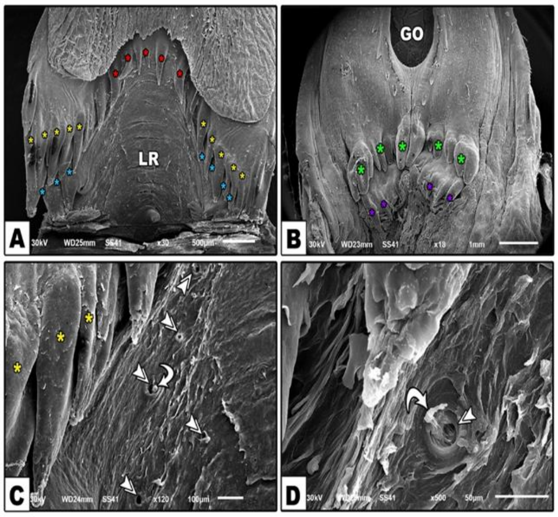

Fig 3. SEM of the tongue body and root of *C.coturnix* showing papillary crest, papillary crest made up of transverse raw of back warded conical papillae, divided into small median conical papillae (red star) and lateral giant conical papillae (yellow star), followed by an additional raw made up of two giant lateral conical papillae (blue star) (image A). Posterior to lingual root, a circular median glottis followed by two rows of transverse pharyngeal papillae, anterior giant papillae (green star) and posterior giant papillae (purple star) (image B). Images C&D illustrating magnified papillary crest and floor of lingual root with smooth surface containing numerous small circular orifices of posterior lingual glands (double arrow head) and secretory mucus (curved arrow). ***Abbreviation:*** LR, lingual root; Go, glottis

3. Light microscopy

3.1 Histological observations

The obtained histological results showed that the tongue of two selected species has the same histological layers; outer epithelium, middle lamina properia with dispersed connective tissues among them and inner muscular layer in which tongue epithelium made up of four successive layers; the stratum basale, stratum granulosum, stratum spinosum, and stratum corneum. The tongue of *C.coturnix* is covered by stratified squamous epithelium which appeared keratinized at the tongue apex, giving lingual nail however this keratinization is lost on both tongue body and root. On the other hand, tongue of G.cholorpus appeared with complete keratinization allover tongue parts. Furthermore, the lingual epithelium of both species is supported internally with numerous fine papillae which appeared numerous in the tongue of *C.coturnix* if compared with those of *G.cholorpus*. Additionally, the tongue epithelium for both studied species displayed filiform papillae which appeared thicker and more elevated in *G.cholorpus* than those of *C.coturnix* (Figure 4A-B1).

The lamina properia of *C.coturnix* tongue appeared rich with high density of simple tubular lingual glands in the apex however the tongue body revealed branched tubule-alveolar ones. On the other hand, the lamina propria of *G.cholorpus* tongue, the lingual glands were completely absent in the tongue apex however, the tongue body appeared with low density of simple tubular lingual glands (Figure A-B1).

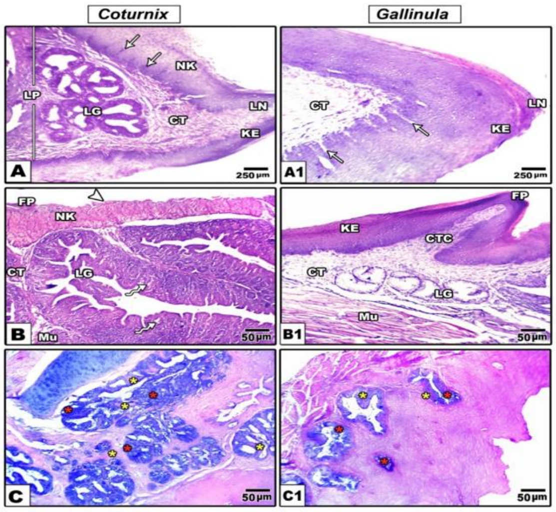

Fig .4: Photomicrograph of histological sections through the tongues of *C.coturnix (A, B&C) and G.cholorpus (A1, B1&C1). A-B1 stained with H&E, C&C1 stained with Alcian blue- PAS.*

Note: In images A-B1: the tongue of *C.coturnix* showing stratified keratinized (on apex) and non-keratinized (on body), high density of lingual glands, numerous and small filiform papillae and prominent interspersed with connective tissue as well as little muscle fibers. In contrast, the tongue of *G.cholorpus* showing stratified keratinized epithelium only, low density of lingual glands, little and large papillae and little inter-glandular connective tissue as well as prominent muscle fibers.

In images C&C1: showing strong histochemical activity of acid mucin (red star) but low activity of neutral mucin (yellow star) in the tongue sections of *C.coturnix* however the tongue section of *G.cholorpus* displaying the reverse.

Abbreviation: NK, non keratinized epithelium; KE, keratinized epithelium; LG, lingual gland ; LN, lingual nail; CT, connective tissue; Mu, muscle; FP, filiform papillae; CTC, connective tissue core; LP, lamina propria

Comparatively, the tongue of *G.cholorpus* appeared supported with highly striated muscle fibers with obvious distribution of connective tissues fibers among the lingual glands. The connective tissue fibers appear relatively long and oriented vertically beneath the keratinized epithelium. (Fig4, B1).

In a comparative account, the tongue of *G.cholorpus i*s highly muscular and less glandular if compared with the tongue of *C.coturnix*

3.2 Histochemical observation:

Histochemical observations using combined PAS & alcian blue stain displayed strong histochemical activity of acid mucin but low activity of neutral mucin in the tongue sections of *C.coturnix* however the tongue section of *G.cholorpus* the activity of neutral mucin appeared prominent while the activity of acid mucin appeared less active. (Figure 4C&C1, Table 1).

Table 1. Illustrating the degree of histochemical activity of neutral mucin (PAS stain) and acid mucin activity (Alcian blue stain).

Stain	***C.coturnix***	***G.cholorpus***
Neutral mucin (by PAS)= Pink color	+	+++
Acid mucin (by Alcian blue)= blue color	+++	+

+= ***Weak reaction*** **+++=** ***Strong reaction***

4. Discussion

Generally, the tongue of birds is morphologically differentiated into three distinct parts; apex, body and root. Such differentiation is noticed and confirmed through the present obtained results. Previous studies emphasized that avian tongue showing considerable variations in the morphology, structure of the epithelium linguae as well as papillae number and their distribution. Such variations are closely related to the nature of diet, feeding habits, bird lifestyle in addition to different habitats (**Whittow, 2000**)

The current results revealed that, the tongue of *C. coturnix* appeared triangular in shape which represents most common lingual shape among birds (**Parchami** ***et al*****., 2010**). Such observation agrees with studies recorded in domestic chicken (**Homberger & Meyers, 1989)** and chuker partridge **(Erdogan** ***et al*****., 2012b)**. **Iwasaki (2002**) explained that, this lingual shape offer suitable adaptation of the tongue for collecting and swallowing grains as whole pieces in the esophagus. On the other hand, tongue of *G. cholopus* appeared elongated flattened with rounded apex. Similar observations were recorded in the tongue of the aquatic birds like waterfowl and ducks (**Vollmerhaus & Sinowatz, 1992 ; Iwasaki et al., 1997**) and Domestic goose (**Jackowiak** ***et al*****., 2011**). The authors reported that these features of tongue were involved in holding and manipulating large food particles such as fishes as well as broad herbs.

In the present work, SEM investigation elucidated that the tongue of *C. coturnix* has numerous microridges, irregular oriented superficial desquamated epithelial cells, on the lingual apex and plentiful filiform papillae on lateral sides of lingual body. The recorded findings are consistent with the results of **Iwasaki (1992)**, **Iwasaki** ***et al*****. (1997**) and **Parchami** ***et al.*** **(2010**). In grainivorus birds like pigeon and hens the microridges and superficial desquamated epithelial cells help in adhesion of mucus to the epithelial surface of tongue to facilitate transferring food especially in birds through the tongue (**Iwasaki, 1992)**. Comparatively to the tongue of *C .coturnix*, SEM study on the tongue of *G.cholorpus* revealed little number of processes and many glandular orifices of deep salivary glands distributed on the lingual apex and body of the tongue. The presence of little processes on the tongue of *G. cholorpus* may be related to its omnivorous feeding.

In a unique study on the tongue root of *C. coturnix,* SEM investigation recorded the presence of the papillary crest at the border between lingual body and root of the tongue, which composed of main transverse row of back-warded conical papillae followed by an additional row of two giant conical papillae on both lateral sides of main row. Depending upon the size, main row is divided into small median conical papillae and large lateral conical papillae. Similar results were observed in chucker partridge **(Erdogan *et al*., 2012b**) and chicken (**Iwasaki & Kobayashi, 1986**). Whereas, papillary crest represented by only single transverse row of conical papillae have been reported by **Parchami &Dehkordi (2011)** and **Iwasaki (1992)** in pigeon and little tern respectively.

In addition, it is absent in ostrich (**Jackowiak & Ludwig, 2008; Pasand *et al*., 2010**), Japanese Pygmy Woodpecker (**Emura *et al*., 2009b**) and penguin (**Kobayashi *et al*., 1998**). **Jackowiak & Godynicki (2005**) revealed that, the papillary crest considered as a common specific structure among most birds which play an essential role in feeding process as it facilitate passage of food toward esophagus besides it prevent regurgitation. Moreover, lingual root displayed smooth appearance dispersed with numerous circular opening of posterior salivary glands. These results are in agreement with the recorded findings of **Emura *et al*. (2008)**, **Erdogan & Alan (2012)** and **Jackowiak *et al*. (2010)** on different avian species.

Furthermore, a median glottis of *C.coturnix* was detected just behind the lingual root. Such pattern is represented as a common feature in all avian species (**Crole & soley, 2010b; Erdogan & Alan, 2012)**. In the present work , glottis appeared circular, while in Egyptian laughing dove was conical or pear shaped (**Abumandour & El-Bakary, 2019**) and in Eurasian coot was elongated and triangular (**Abumandour & El-Bakary, 2017**). In addition, the observed circular glottic opening appeared without papilla. These findings agree with the recorded observations of **El-Mansi *et al*. (2020b**) in Egyptian nightjar and **Crole & Soley (2010a**) in the Emu. While papillated glottis was observed in the European magpie and common raven (**Erdogan & Alan, 2012**), the southern lapwing (**Erdoğan &Pérez, 2015**), the house sparrow (**Abumandour, 2018**), and the long-legged buzzard (**Kabak *et al*., 2007**).

The data concerning with pharyngeal papillae revealed that they were arranged transversely in two successive rows, caudally oriented as well as it classified into large duplicated papillae in anterior row and small single papillae in posterior row. These results go parallel with the findings of **Erdogan & Alan (2012**) in European magpie and common raven, **Sağsöz *et al*. (2013**) in Chukar partridge and **Erdogan *et al*. (2012a**) in long-legged buzzard. However, in both Eurasian hoopoe (**Abumandour & Gewaily, 2019)** and cattle egret (**Al-Ahmady Al-Zahaby, 2016**) only one pharyngeal papillae row was observed.

The different avian species exhibit variations in shape and arrangement of pharyngeal papillae, such variation were correlated with feeding habit since these pharyngeal papillae are involved in directing food particles toward esophagus (**Jackowiak & Godynicki, 2005; Emura *et al*., 2008 and Erdogan & Alan, 2012)**.

Keratinization of lingual epithelium is a common feature among most vertebrates and its degree closely depends on the nature of grains (soft or dry) (**Iwasaki, 2002**). However, non-keratinized epithelium is compensated by abundant secretions from salivary glands on both the dorsal and ventral parts of the tongue (**Crole & Soley, 2011**). Notable that the dorsal lingual epithelium in avian tongue, in particular, anterior tip is well developed and thicker referring to the lingual nail to achieve nutritional needs.

The histological results of the present work showed that dorsal surface of lingual apex of *C. coturnix* is covered with keratinized stratified squamous epithelium, while lingual body and root covered with non-keratinized stratified squamous epithelium. Such observations resemble those of white tailed eagle (**Jackowiak & Godynicki, 2005**) and domestic pigeon (**Parchami & Dehkordi, 2011**). While, the dorsal epithelium of the tongue of *G. cholorpus* is covered with thick keratinized stratified squamous epithelium. Similar findings have been reported in chucker partridge (**Erdogan *et al*., 2012b**), little tern (**Iwasaki, 1992**) and common buzzard (**El-Beltagy, 2013**). In addition keratinization is completely absent on both the dorsal and the ventral surfaces of the tongue of ratites (**Jackowiak & Ludwig, 2008; Crole & Soley, 2009b; Guimarães *et al*., 2009; Pasand *et al*., 2010 ; Santos *et al*., 2011**).

The observations deal with the lingual glands of *C. coturnix* showed that there are many lingual glands distributed mainly within the lamina propria beneath the dorsal epithelium. They oriented along both the lingual apex and body, and represented by two types of lingual salivary glands; simple tubular salivary glands and branched tubule-alveolar salivary glands. According to secretory units, anterior salivary glands are seromucous while posterior ones are mucous only (**Liman et al., 2001 ; Capacchietti et al., 2009**). On the other hand, tongue of *G. cholorpus* showed only simple tubular glands which appeared in lamina propria of lingual body only. These results agree with **Farner & Ziswiller (1972)** and **Crole & Soley (2009)** who reported that grainivorous birds fed on dry food had high density of salivary glands as compared with species fed on naturally lubricated food since the degree of the development of the salivary glands is closely related with the nature of food. Although salivary glands in avian species are commonly distributed within the dorsal epithelium of the tongue, they distributed on both dorsal and the ventral surfaces of tongue in the ratites (**Crole & Soley, 2009, 2010b; Guimarães *et al*., 2009 ; Pasand *et al*., 2010**). However, lingual salivary glands are absent in the cormorant (**Jackowiak *et al*., 2006**).

In the current study, the lingual salivary glands of the two selected species showed positive reactivity to combined alcian blue - PAS stain with variable degree of reaction intensity. In *C. coturnix*, the salivary glands showed strong reaction for alcian blue stain comparing with those of G. cholorpus which indicate their high content of acid mucosubstances. These observations are consistent with the results of **Erdogan *et al*. (2012b**) in Chukar partridge and **Gargiulo *et al*. (1991**) in chicken. In contrast, salivary glands of *G. cholorpus* revealed high reactivity for PAS stain comparing with those of *C. coturnix*, reflecting their neutral mucin content. These salivary secretions display multidisciplinary functions in lubricating and moistening ingested food to facilitate swallowing (**Liman *et al*., 2001**; **Jackowiak & Godynicki, 2005; Onuk *et al*., 2015),** protecting the mucosa against bacterial activity (**Montreil, 1980; Gargiulo *et al*., 1991; Samar *et al*., 2002**) and protecting lingual mucosa against injures of hard grains (**Parchami & Dehkordi, 2011**).

In conclusion, based on the recording findings of the current study the morphological, histological and histochemical investigations revealed that the tongue of C. coturnix is more complicated than that of G. cholorpu

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