

## Histomorphological and Histochemical Study on the Tongue of Black Francolin (*Francolinus francolinus*)

<sup>1</sup>Khalid K. Kadhim, <sup>1</sup>Mahdi Abdul Kareem Atia and <sup>2</sup>AL-Timmemi Hameed

<sup>1</sup>Department of Anatomy and Histology,

<sup>2</sup>Department of Surgery and Obstetrics, Faculty of Veterinary Medicine, University of Baghdad,  
Baghdad, Iraq

**Abstract:** Histomorphological and histochemical observations of black Francolin tongue. Ten tongues (five males and five females) of adult birds were carried out macroscopically and microscopically. The length and width of tongues were measured. Routine histological staining and special stains for mucin were done. The tongue width but not length showed significantly ( $p \leq 0.05$ ) higher in male compared with female. The dorsal surface of the tongue had median groove. At the tongue body-base junction there was single row of backward directed papillae. Double transverse rows of pharyngeal papillae located behind the laryngeal cleft. Hard keratinized plates are covered the ventro-lateral surfaces of the tongue. The dorsal surface of the tongue is covered by parakeratinized stratified squamous epithelium except the ventro-lateral surface and the lingual papillae. Simple branched tubulo-alveolar lingual salivary glands were occupied the lateral and the base of the tongue. The secretory cells of the Medial group (Mg) of the Anterior lingual glands (Alg) and the posterior lingual glands (Plg) contained large amount of mucin compared with the Lateral group (Lg). The secretions had neutral reaction. However, the weak carboxylated acid mucin is detected in Lg. Meanwhile, both carboxylated and sulphated acid mucin reactions were observed in the Mg of the Alg and the Plg. The histomorphological observation of the male and female black francolin tongue showed no difference.

**Keywords:** Black francolin, lingual gland, mucin reaction, tongue

### INTRODUCTION

The Black Francolin (*Francolinus francolinus*) in the pheasant family (Phasianidae) of the order Galliformes, gallinaceous birds. It was formerly known as the Black Partridge, bird native to Asia (Bashir, 1960). Studies on the structure of the tongue in birds have been conducted on number of avian species, i.e., goose (Iwasaki *et al.*, 1997), partridge (Rossi *et al.*, 2005), eagle (Jackowiak and Godynicki, 2005), ostrich (Jackowiak and Ludwing, 2008; Pasand *et al.*, 2010) and in red jungle fowl (Khalid *et al.*, 2011). These studies proved that the tongue was modified according to the method of food intake, type of food and habitat. The tongue has provided by conical papillae that arrange in transverse row (Emura *et al.*, 2008) in common kastral, (Khalid *et al.*, 2011) in red jungle fowl. However, in goose tongue, these conical papillae are located in the midline between the lingual body and radix (Iwasaki *et al.*, 1997). The pharyngeal papillae of the red jungle fowl are arranged in one transverse row (Khalid *et al.*, 2011) and double rows in chicken (Iwasaki and Kobayashi, 1986). The root and dorsum tongue are cover by par keratinized epithelium

(Jackowiak and Godynicki, 2005). The tongue is covered with thick keratinize mucosa in the herbivorous and grainivorous birds (Jackowiak and Ludwing, 2008). And with par keratinized mucousa in the water habitat birds (Iwasaki, 2002; Jackowiak *et al.*, 2006). Some literature that were conducted on the lingual salivary glands of different types of birds (Rossi *et al.*, 2005; Al-Mansor and Jarrar, 2007). No information is available concerning morphological study of the tongue in the black francolin, thus this study was undertaken to describe it in both genders.

### MATERIALS AND METHODS

Ten adult (five males and five females) birds were used; mean weighted 451 gm and 4130 gm for male and female respectively. The birds were euthanized by intraperitoneal administration of sodium pentobarbitone (80 mg/kg) (REILLY, 2001). All the heads of birds were dissected immediately. The study was approved by the Local Ethical Commity at Faculty of Veterinary Medicine/Baghdad University.

The tongues were washed with normal saline solution and then kept in 10% neutral buffered

formalin. The shape of the tongue and dimensions include the length from the tip to the body base junction and the width at this point of the tongues was studied in details under stereomicroscope image analysis (SMZ 1500 digital camera). The tongues were cut off transversally. Paraffin sections (6  $\mu$ m) were obtained from the tongue. Staining with routine haematoxylin eosin for studying of general microstructures of the tongue, Periodic acid Schiff (PAS) for neutral mucine (Humason, 1972); alcian blue (pH 1) for acid mucin, alcian blue-PAS for acid and neutral mucin, Aldehyde fuchsin-alcian blue stain for sulphate and carboxylated acid mucin (Totty, 2002). The sections were documented in Olympus microscope; model BX 50, provided by digital camera (MEM 1300).

## RESULTS

**Dissecting microscopically observations:** The tongue of the black francolin had triangular shaped and occupied the cavity of the lower beak. Figure 1 showing no significantly difference ( $p>0.05$ ) between the male and female tongue length, but there was difference in its width, the male tongue showed significantly ( $p>0.05$ ) wider tongue at its body- base junction. In both genders, the dorsal surface was wide with shallow median groove extended from the tip to the mid of the tongue body (Fig. 2). Hard conical papillae were directed backward and arrange transversely between tongue body and its base. The larger one located at each side of the body-base junction. However, there were additional one or two papillae just behind this transverse row on each side (Fig. 3). Numerous lingual glands were opened on the dorsal surface of the tongue base. The ventrolateral surfaces of the tongue had hard plate (Fig. 4). One row of pharyngeal conical papillae were directed backward and arrange transversely behind the laryngeal cleft, however, there were another rudimentary conical papillae that arranged transversally behind the first row (Fig. 5).

**Histological and histochemical observations:** The dorsal of the tongue is covered by thick mucous membrane which composed of no keratinized stratified squamous epithelium. The dorsal surface, however presented desquamated cells (Fig. 6), but with a thin keratinized layer of the stratum corneum at the ventrolateral surface of the tongue and the lingual papillae (Fig. 6 and 7). The lamina propria and submucosa contained dense connective tissue with many blood vessels. The tongue was supported by basihyal bone and the extrinsic muscles.

The tip and the ventral surface of the tongue were devoid of any glandular structure. The tongue contained anterior and posterior lingual salivary glands, located ventrolaterally and at the base of the tongue respectively. The mucous glands were simple tubulo-alveolar. The

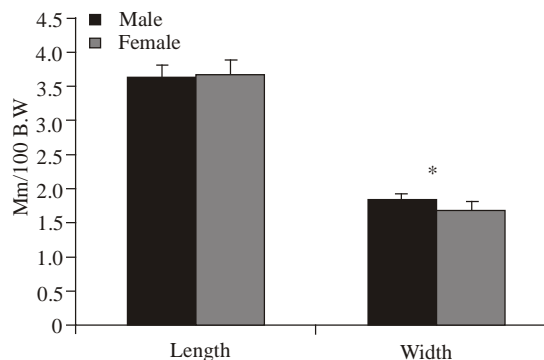


Fig. 1: The tongue length and width for male and female black francolin. Values are means  $\pm$  SD, N = 5. (\*) Star indicating significant differences ( $p<0.05$ )

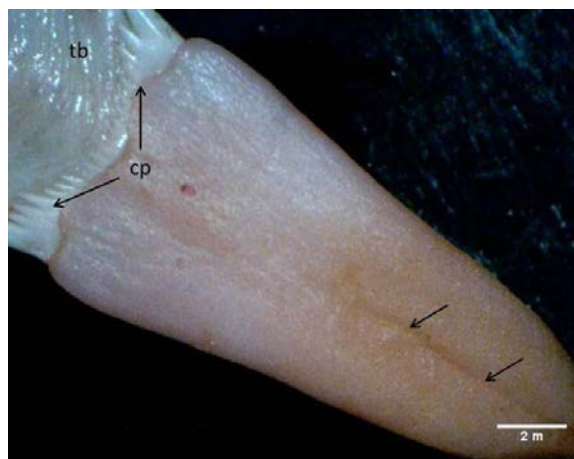


Fig. 2: Macrophotographs of the black francolin tongue (Dorsal view). Dorsal surface of the tongue with median groove (arrows), lingual conical papillae (cp), tongue base (tb)

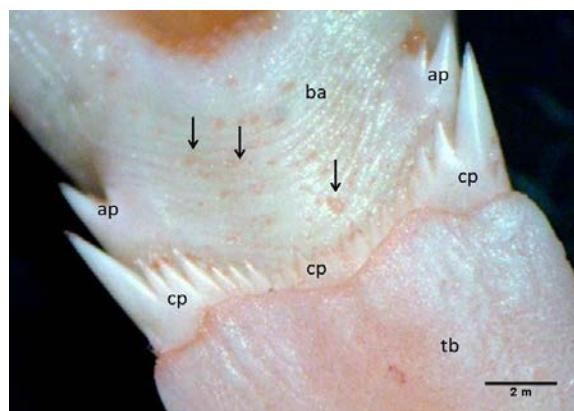


Fig. 3: Macrophotographs of the black francolin tongue (Dorsal view) showing the transverse line of the conical papillae (cp) between the body (tb) and the base (ba) of tongue, the additional conical papillae (ap) and the openings of the lingual salivary glands (arrows)

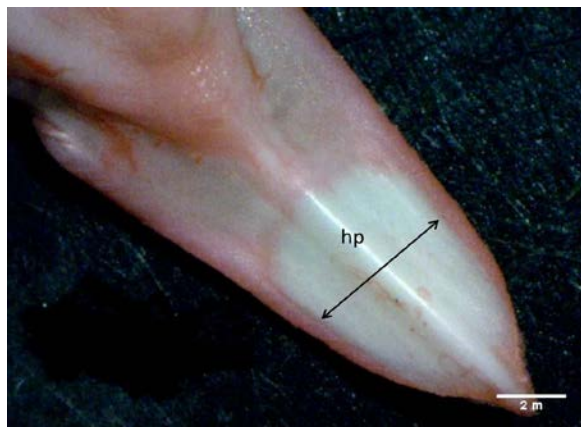


Fig. 4: Macrophotographs of the black francolin tongue (Ventral view) showing the hard keratinized plate (hp) at the ventrolateral surface of the tongue

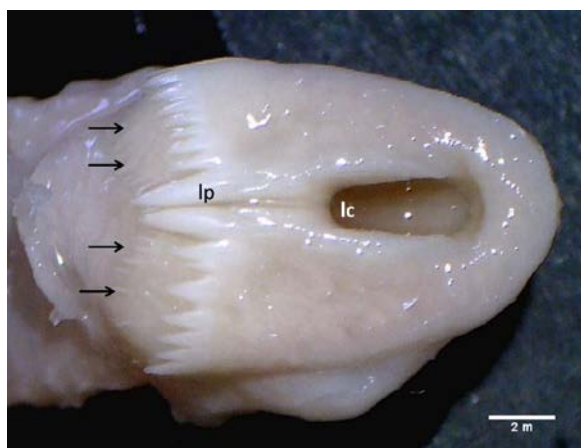


Fig. 5: Macrophotographs of the black francolin tongue (Dorsal view) showing the laryngeal conical papillae (lp) and the rudimentary papillae (arrows) behind the laryngeal cleft (lc)

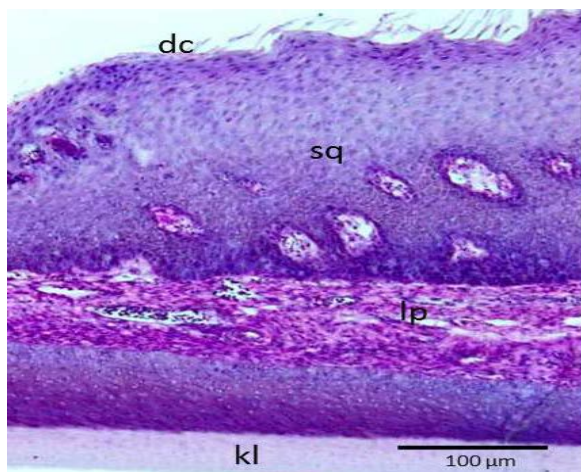


Fig. 6: Microphotograph of the tongue tip (longitudinal section) showing thick stratified squamous epithelium (Sq), desquamated cells (dc), lamina propria (lp), keratinized layer (kl). H&E

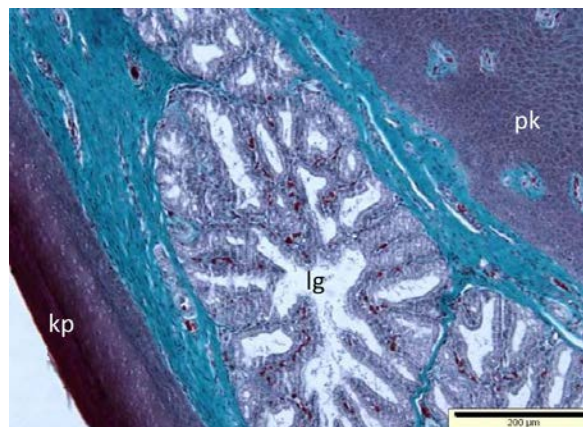


Fig. 7: Microphotograph of the tongue (longitudinal section) showing parakeratinized stratified squamous epithelium (pk), keratinized mucosa (kp), anterior lingual salivary glands (lg) Masson's Trichrome stain

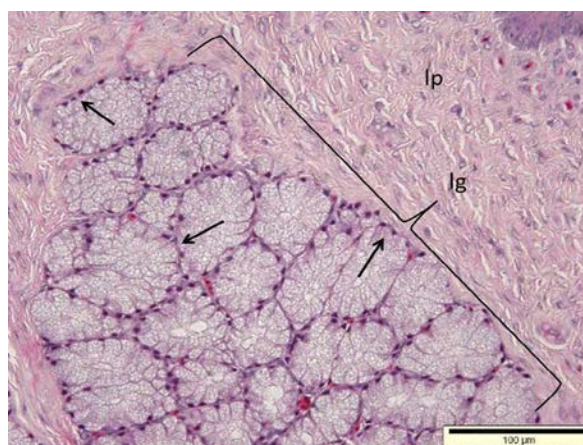


Fig. 8: Microphotograph at the tongue base showing simple branched tubule-alveolar mucous glands (lg), the acini lined by columnar cells with basal located nuclei (arrows), connective tissue of the lamina propria (lp). H&E

columnar cells with basal located nuclei were represents the secretory cells of these secretory units (Fig. 8).

The anterior group of the lingual salivary glands was smaller than that at the base of the tongue. However, after the histochemical stain, the amount of mucin in the anterior salivary glands showed differences; the medial group had more mucin quantity than the lateral group as appeared after PAS stain (Fig. 9). The section at the base of the tongue showed similar appearance of the posterior lingual glands with that of the medial group (Fig. 10). It has been observed that the lateral group of the anterior lingual glands had weak acid mucin reaction compared with the medial group or with the posterior lingual glands after alcian blue stain (Fig. 11) and (Fig. 12), respectively. Meanwhile, the granules of the mucin in the cytoplasm



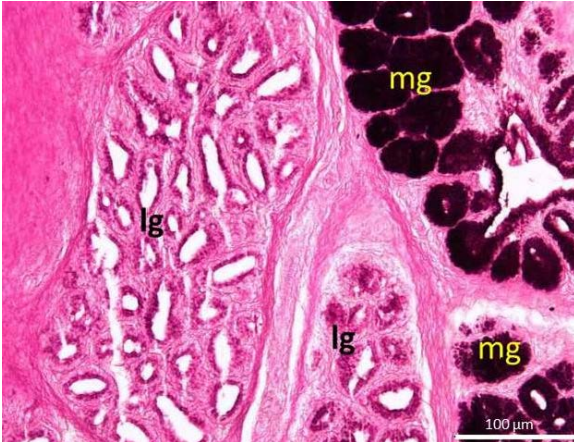


Fig. 9: The interior lingual glands (cross section), showing different amount of mucin between the lateral group (lg) and the medial group (mg). PAS stain

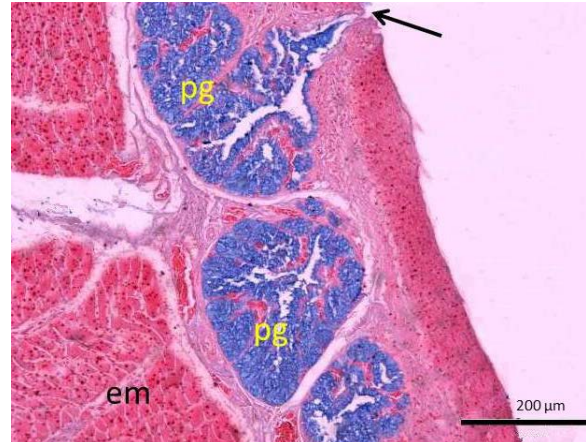


Fig. 12: The tongue baseshowing; the posterior lingual glands (pg) with strong acid mucin reaction, the opening of the gland (arrow). Alcian blue (pH 1)



Fig. 10: The tongue base, showing the amount of mucin in the posterior lingual glands (pg) after PAS stain

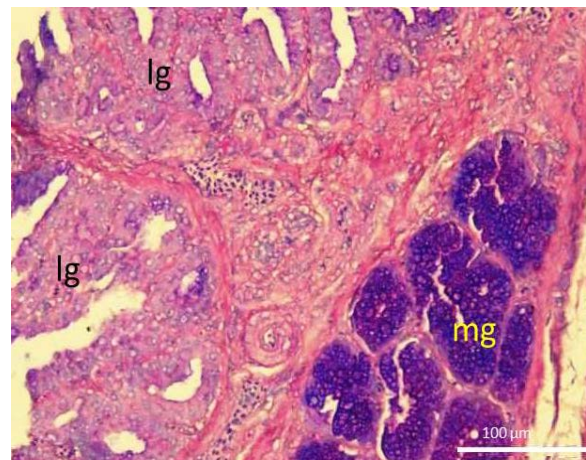


Fig. 13: The interior lingual glands showing PAS positive (neutral mucin) reaction in the lateral group (lg) and both neutral and acid mucin reaction in the medial group (mg). Alcian blue-PAS stain

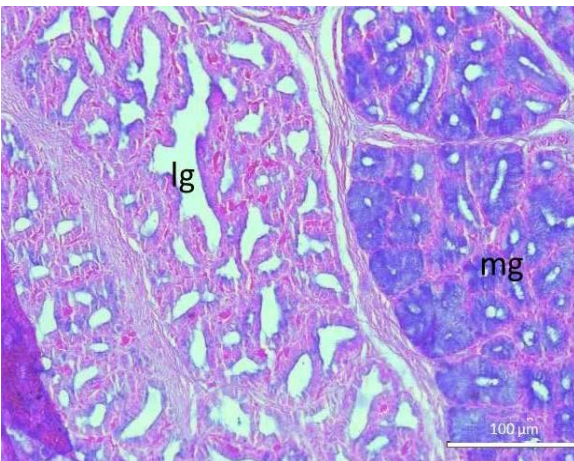


Fig. 11: The interior lingual glands, showing weak acid mucin reaction in the lateral group (lg) and strong acid mucin reaction in the medial group (MG). Alcian blue (pH 1)

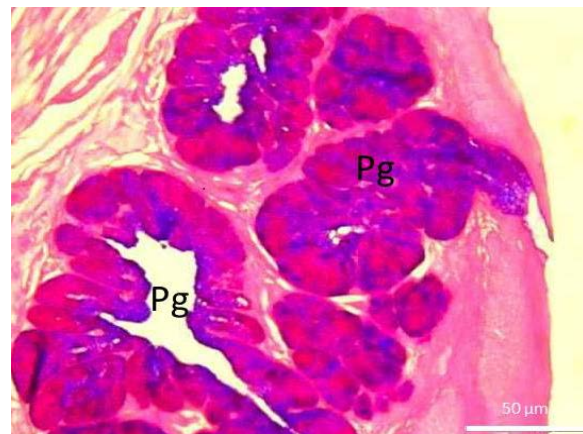


Fig. 14: The posterior lingual glands (pg) showing PAS positive (neutral mucin) reaction and alcian positive (acid mucin) reaction. Alcian blue-PAS stain



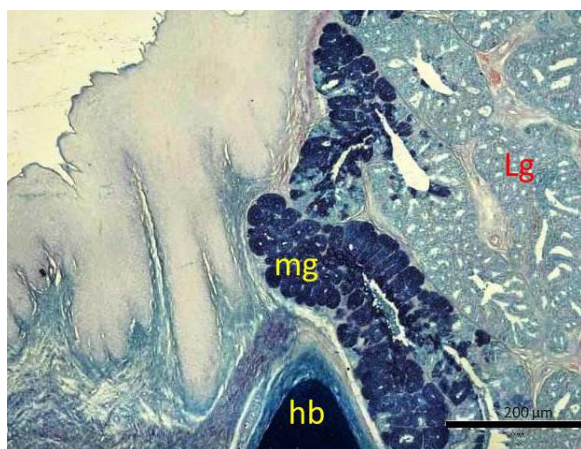


Fig. 15: The interior lingual glands, showing carboxylated type of mucin in the lateral group (lg) and both sulphated and carboxylated mucin in the medial group (mg). base hyoid bone (hb). Aldehyde fuchsin-Alcian blue stain

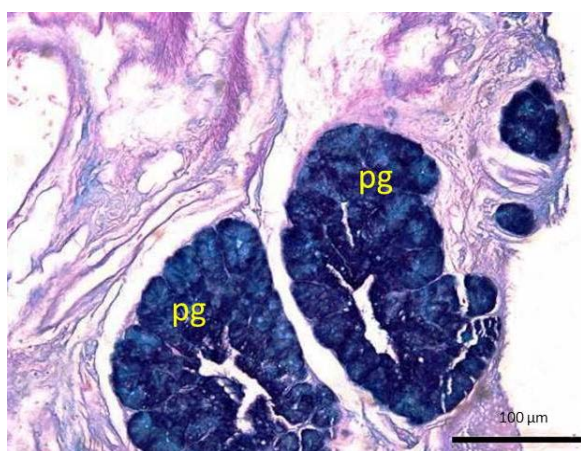


Fig. 16: The posterior lingual glands (pg), showing both sulphated and carboxylated mucin reaction after Aldehyde fuchsin-Alcian blue stain

of the secretory cells contained both neutral and acid mucin in the medial group of the anterior lingual glands and the posterior lingual glands, but it seemed mostly neutral reaction in the lateral group (Fig. 13 and 14). When the sections of tongue has treated with aldehyde fuchsin- alcian blue stain, most of the mucin of the lateral group of the anterior lingual glands has reacted positively with alcian blue (carboxylated mucin). Meanwhile, both sulphated and carboxylated mucin reaction were occurred in the medial group as well as in the posterior lingual glands (Fig. 15 and 16).

## DISCUSSION

It has been shown that the tongue of birds is adapted to the route and type of food intake (Pasand *et al.*, 2010). The lingual papillae of the black francolin

tongue appeared no different in arrangement with that reported by Khalid *et al.* (2011) in red jungle fowl tongue and in the common kestrel (Emura *et al.*, 2008), in these species, the conical papillae arrange in a transverse row. However, these papillae are restricted in the midline between the body and the base of the tongue in goose (Iwasaki *et al.*, 1997; Hassan *et al.*, 2010), in the eagle tongue these papillae are arrange as a single crest from the body to the root (Jackowiak and Godynicki, 2005). Meanwhile, there are no papillae in the ostrich tongue (Pasand *et al.*, 2010). Emura *et al.* (2008) has been reported that the caudal direction of papillae is to facilitate the prehension and swallowing of food. Similar to the present study, the pharyngeal papillae of the chicken appear as double rows (Iwasaki and Kobayashi, 1986). But it is a single row in red jungle fowl tongue (Khalid *et al.*, 2011).

The tongue of the black francolin was covered by parakeratinized stratified squamous epithelium, except the hard keratinization of the conical papillae and on the ventrolateral surface of the tongue. Similar to these results were detected in some species of birds. However, the keratinization of the tongue epithelium depends mainly on the type of food intake, Jackowiak and Ludwig (2008) had reported that it's appeared heavily cornified in herbivorous and granivorous birds. But in water habitats birds, lesser degree of keratinization occur (Iwasaki, 2002; Jackowiak *et al.*, 2006). The location of the lingual gland of the black francolin tongue seems different than that mention in ostrich, that the lingual salivary glands occupied most of the dorsal and ventral surfaces of the tongue (Pasand *et al.*, 2010). According to our results, the salivary glands of the tongue had exclusively mucous secretion. This result was in line with Rossi *et al.* (2005) in partridge. In contrast, no lingual salivary glands are found in cormorants tongue (Jackowiak *et al.*, 2006). In present study, we did not find any explanation to the histological differences that found between the medial and lateral groups of the anterior lingual salivary gland, despite these differences were mention previously in some species of birds too. Like in red jungle fowl (Khalid *et al.*, 2011) that shown that the anterior and the posterior lingual glands have some difference after histological stain. However, our suggestion that the lateral group may be exposed to more external pressure than that for the medial group as its located more superficially, thus the former group had changed according to this influential factor. For this reason, the mucous granules that detected by PAS stain (glycoconjugates containing vicinal diol group) in the cytoplasm of the secretory cells of the medial group were less than that in the lateral group or in the posterior lingual glands. In contrast, the tongue of the little egret is free of neutral mucin (Al-Mansour and Jarrar, 2007).

The lateral group of the anterior lingual glands in black francolin contained mostly neutral mucin, while the medial group and the posterior lingual gland had both neutral and acid mucin. These results were similar to that founded in chicken (Suprasert and Fujioka, 1987). However, Olmedo *et al.* (2000) reported that differences in substructure were found even within the same acinus and the same cell of the avian tongue.

The medial group of the anterior lingual gland and the posterior gland tend to the strong acid mucin reaction after alcian blue (pH1) and containing both sulphated and corboxylated group. In contrast, the lateral group which showed weak acid reaction with mainly sulphated type. However, these data were in line with Gargiulo *et al.* (1991) in chicken, Al-Mansour and Jarrar (2007) in the little egret. It is known that neutral mucin act as lubricant of food to facilitate swallowing and preserves hydration by providing a hydrophilic environment. In addition, the acid mucin play a role in the modulation of the oral calcium channel activity (Slomiany *et al.*, 1996).

It was concluded that the unique features of the tongue in black francolin were the arrangement of the lingual conical papillae and the presence of double rows of the laryngeal papillae. In addition there is different histochemical reaction in salivary glands component. However, there is no difference between genders.

## REFERENCES

- Al-Mansour, M.I. and B.M. Jarrar, 2007. Morphological, histological and histochemical study of the lingual salivary glands of the little egret, *Egretta garzetta*. Saudi J. Biol. Sci., 14: 75-81.
- Bashir, A., 1960. Iraqi Birds. Alribat Press, Baghdad, Iraq.
- Emura, S., T. Okumura and H. Chen, 2008. Scanning electron microscopic study of the tongue in the peregrine falcon and common kestrel. Okajimas Folia Anatomica Japonica, 85(1): 11-15.
- Gargiulo, A.M., S. Lørvik, P. Ceccarelli and V. Pedini, 1991. Histological and histochemical studies on the chicken lingual glands. Br. Poult. Sci., 32: 693-702.
- Hassan, S.M., E.A. Moussa and A.L. Cartwright, 2010. Variations by sex in anatomical and morphological features of the tongue of Egyptian goose (*Alopochen aegyptiaca*). Cells Tissues Organs, 191: 161-165.
- Humason, G.L., 1972. Animal Tissue Techniques. 3rd Edn., W.H. Freeman and Co., San Francisco, pp: 180-182.
- Iwasaki, S., 2002. Evolution of the structure and function of the vertebrate tongue. J. Anat., 201: 1-13.
- Iwasaki, S. and K. Kobayashi, 1986. Scanning and transmission electron microscopical studies on the lingual dorsal epithelium of chickens. Acta Anat., 61: 83-96.
- Iwasaki, S., A. Tomoichiro and A. Chiba, 1997. Ultrastructural study of the keratinization of the dorsal epithelium of the tongue of middendorff's bean goose, *Anser fabalis middendorffii* (*Anseres, Anseridae*). Anat. Rec., 247: 149-163.
- Jackowiak, H. and S. Godynicki, 2005. Light and scanning electron microscopic study of the tongue in the white tailed eagle (*Haliaeetus albicilla*, *Accipitridae, Aves*). Anat. Anzeiger, 187: 251-259.
- Jackowiak, H., W. Andrzejewski and S. Godynicki, 2006. Light and scanning electron microscopic study of the tongue in the cormorant *Phalacrocorax carbo* (*Phalacrocoracidae, Aves*). Zool. Sci., 23(2): 161-167.
- Jackowiak, H. and M. Ludwig, 2008. Light and scanning electron microscopic study of the structure of the Ostrich (*Strutio camelus*) tongue. Zool. Sci., 25: 188-194.
- Khalid, K.K., A.B.Z. Zuki, S.M.A. Babjee, M.M. Noordin and M. Zamri-Saad, 2011. Morphological and histochemical observations of the red jungle fowl tongue *Gallus gallus*. Afr. J. Biotechnol., 10(48): 9969-9977.
- Olmedo, L.A., M.E. Samar, R.E. Avila, M.G. de Crosa and L. Dettin, 2000. Avian minor salivary glands: An ultra structural study of the secretory granules in mucous and seromucous cells. Acta Odontol. Latinoam, 13: 87-99.
- Pasand, A.P., M. Tadjalli and H. Mansouri, 2010. Microscopic study on the tongue of male ostrich. Eur. J. Biol. Sci., 2(2): 24-31.
- Rossi, J.R., S.M. Baraldi-Artoni, D. Oliveira, C. Cruz, V.S. Franzo and A. Sagula, 2005. Morphology of beak and tongue of partridge *Rhynchotus rufescens*. Cienc. Rural, 35: 1-7.
- Slomiany, B.L., V.L. Murty, J. Piotrowski and A. Slomiany, 1996. Salivary mucins in oral mucosal defense. Gen. Pharmacol-Vasc. S., 27(5): 761-771.
- Suprasert, A. and I. Fujioka, 1987. Lectin histochemistry of glycoconjugates in esophageal mucous gland of the chicken. Jpn. J. Vet. Sci., 49: 555-557.
- Totty, B.A., 2002. Mucins. In: Bancroft, J.D. and M. Gamble (Eds.), Theory and Practice of Histological Techniques. 5th Edn., Churchill Livingstone, New York, pp: 163-200.