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Full Length Research Paper

Morphological and histochemical observations of the red jungle fowl tongue *Gallus gallus*

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Morphological and histochemical study of the tongue of ten adult red jungle fowl (RJF), *Gallus gallus* were carried out at macroscopic and microscopic levels. The tongue was triangular with a wide dorsal and ventrolateral surface with median groove at the rostral part. Between the body and the roots appears a transverse row of the lingual conical papillae which was directed backwards. Behind the laryngeal cleft, there was a single row of pharyngeal papillae. The lingual mucosa showed parakeratinization, while there was a clearly recognizable keratinized band on the ventrolateral surface and the conical papillae. The cell cytoplasm of the medial group (MG) of the anterior lingual glands and the posterior glands contained large amounts of mucin compared with the lateral group (LG). The mucin of the lingual glands contained vicinal diol groups. Moreover, the sulphate containing glycoconjugates indicated in the MG and the posterior glands with a strong acid mucin reaction. Meanwhile, the LG of the anterior lingual glands exhibited carboxylated mucin with weak acid mucin reaction. In conclusion, the differences in the arrangement of the lingual and pharyngeal papillae in the RJF than that in other birds particularly domestic chicken may reflects the changes which occurred for the latter during domestication. The contents of mucins in the medial and lateral groups of the anterior lingual gland were varied, however, no differences histochemistry between the medial group and the posterior lingual gland were observed.

Key words: Lingual salivary glands, mucin reaction, red jungle, tongue.

INTRODUCTION

The red jungle fowl (***Gallus gallus***) is a tropical member of the pheasant family and the direct ancestor of the domestic chicken (Collias and Saichuae, 1967). The tongue of the ***Gallus domesticus*** has been studied by McLelland (1975), Iwasaki and Kobayashi (1986) and Homberger and Meyers (1989). Some literatures have reported different species of bird with emphasis on parrot (Homberger and Brush, 1986), little tern (Iwasaki, 1992), goose (Iwasaki et al., 1997), eagle (Jackowiak and Goynicki, 2005), kestrel (Emura et al**.,** 2008), cormorant (Jackowiak et al., 2006) and ostrich (Jackowiak and Ludwing, 2008). The results of these morphological studies conducted so far indicate that the shape of the tongue has a close correlation with the method of food intake, type of food and habitat. Hill (1971) and Iwasaki and Kobayashi (1986) have shown that there is transverse row of giant conical papillae between the anterior and posterior part of the tongue in the chicken, in little tern (Iwasaki, 1992) and in common kestrel (Emura et al., 2008). However, Iwasaki et al. (1997) and Hassan et al. (2010) have shown that similar lingual papillae are restricted midline between the lingual body and radix of the goose tongue. Thick mucosal fold exists over the bases of the lingual papillae of the ostrich tongue

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Abbreviations: RJF, Red jungle fowl; **MG**, medial group; **LG**, lateral group; **PAS,** periodic acid-schiff.

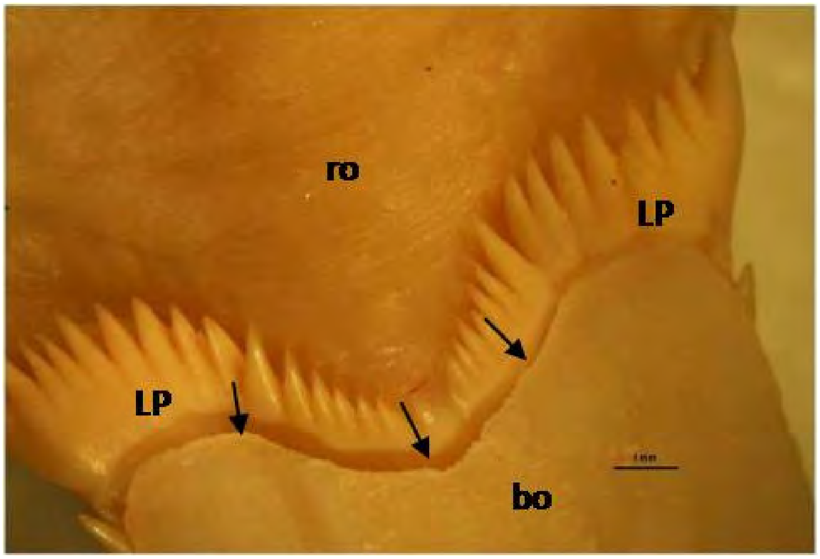

Figure 1. Photograph of the dorsal surface of the tongue of the RJF. The arrangement of the lingual papillae (LP) in concave transverse row and a short flat plate-like fold (arrows) between the body (bo) and the root (ro) of the tongue are shown.

(Jackowiak and Ludwig, 2008). However, McLelland (1975) stated that in the domestic fowl the pharyngeal papillae are arranged in two transverse rows. In the herbivorous and granivorous birds, the lingual mucosa is strongly keratinized (Susi, 1969; Iwasaki, 1992; Jackowiak and Ludwing, 2008). On the other hand, the tongue of water habitat birds exhibit less keratinization (Iwasaki, 2002; Jackowiak et al., 2006). The stratified parakeratinized epithelium covers the root and dorsum tongue (Iwasaki et al., 1997; Jackowiak and Godynicki, 2005). The lingual salivary glands studied extensively in different types of birds (Toryu et al., 1960; Duke, 1986; Rossi et al., 2005; Al-Mansour and Jarrar, 2007). The secretory cells of the chicken salivary gland contain both neutral and sulphate mucin (Suprasert et al., 1986; Suprasert and Fujioka, 1987; Gargiulo et al., 1991).

Scarcity of information on the red jungle fowl especially with regard to morphological study of the tongue has made the subject at hand. Therefore, in this study, the macroscopic and microscopic observation on the morphology of RJF tongue were made in addition to characterize histochemically the secretion of the lingual salivary glands and determine if there is RJF tongue modification during domestication of chicken.

MATERIALS AND METHODS

Animals

A total of 10 adult male RJF were used in this study. The RJF were reared in University Putra Malaysia farm, Selangor, Malaysia. The RJF is descended from stock, which agrees with wild RJF and differ from domestic chickens in all eight characters that differentiate most sensitively between wild RJF and domestic chicken (Jackson and Diamond, 1996). The birds euthanized by intravenous (cutaneous ulnar vein) administration of 80 mg/kg sodium pentobarbitone (Mitchell and Smith, 1991).

Samples

The tongue was washed with saline solution and fixed in 10% neutral buffered formalin.

The external surface of the tongue was examined by Nikon stereomicroscope image analysis (SMZ 1500 digital camera). Longitudinal and cross sections (5 µm) were cut from tongue for histological and histochemical examinations. Samples were processed using histological procedures. Staining methods were employed as follows: Harris haematoxylin-eosin; PAS technique for presence of mucin (Humason, 1972); PAS technique after amylase digestion (Drury et al., 1973); combined alcian blue-PAS technique for acid and neutral mucins; alcian blue (pH 2.5) and (pH 1) for weak and strong acid mucins respectively; combined aldehyde fuchsin-alcian blue methods for sulphated and carboxylated acid mucin (Totty, 2002).

RESULTS

Macroscopic findings

Observations showed that RJF tongue was triangular and situated in the lower part of the beak cavity which did not extend to the full limit of the lower beak. It is divided into the apex, body and root. The free part had a wide dorsal and ventrolateral surface. The rostral part of the dorsal surface had a median groove. Between the body and the root of the tongue lies a transverse row of backward directed lingual conical papillae. In addition, a short flat plate-like fold, extending for a short distance over the base of these papillae (Figure 1). The papillae vary in

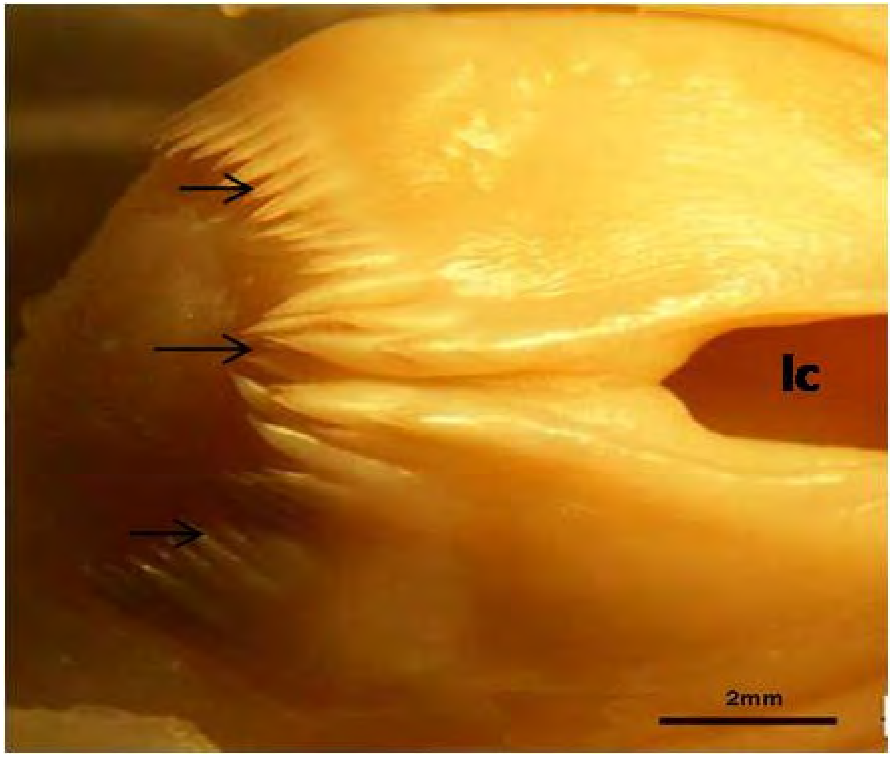

Figure 2. Photograph of the pharyngeal papillae of the tongue RJF. The arrangement of single row of pharyngeal papillae (arrows) and laryngeal cleft (LC) are shown.

size, the smallest being closer the midline. A short row with three to four large conical papillae extended caudally from each end of the transverse row. The transverse row of the lingual papillae showed a marked concavity in the middle line of the tongue. The openings of the anterior and posterior lingual salivary glands were seen in the lateral surface and at the base of the tongue respectively. The posterior lingual glands extended to the laryngeal cleft. Posterior to the lingual root was the laryngeal cleft which is provided with backward conical papillae. Figure 2 illustrated a single row of pharyngeal papillae behind the laryngeal cleft.

Histological and histochemical findings

The tongue is covered by a stratified squamous epithelium. The mucosa on the dorsal surface of the tongue was thicker than in the ventrolateral surface, and had the stratum basale, stratum spinosum, stratum granulosum and a thin parakeratinized layer represented by the stratum corneum (Figure 3). However, on the ventrolateral surface, the latter layer appeared as a strong keratinized band in the first third of the tongue (Figure 4). While there was no keratinized band found on the dorsal surface of the tongue except for the conical papillae. The connective tissues of the lamina propria were rich with blood vessels and mucous glands which are found with ducts that project into the epithelium surface. The lingual salivary glands were located in the lamina propria of the second half of the free part of the tongue (anterior lingual glands) and in the dorsal part of its base (posterior lingual glands). The anterior part of the tongue was devoid of any glandular structure. After staining, the cellular features enabled to identification of lateral and medial groups of the anterior lingual glands. The posterior glands located between the dorsal epithelium and the extrinsic muscles bundles dorsal and dorsolateral to the basihyal bone. The secretory units of these glands consisted of tall columnar mucous cells with basal located nuclei. In the LG of the anterior glands, the cells have only little mucin and the cytoplasm appeared somewhat darker in the hematoxylin and eosin (HE) staining (Figure 5A). The round nuclei of these cells were larger than in the MG and the posterior glands. However, the cell cytoplasm of the MG of the anterior glands and the posterior glands was extensively vesicular due to containing large amounts of the mucin and appeared lighter with dark, small and flattened nuclei (Figure 5B). The results of the histochemical reaction of the glycoconjugates of the lingual salivary glands are shown in the Table 1. The secretory cells were PAS positive. An intense reaction at the MG of the anterior and posterior lingual glands was greater than in the LG of the anterior lingual glands (Figure 6). There were no changes in the intensity of the PAS reaction after digestion with α

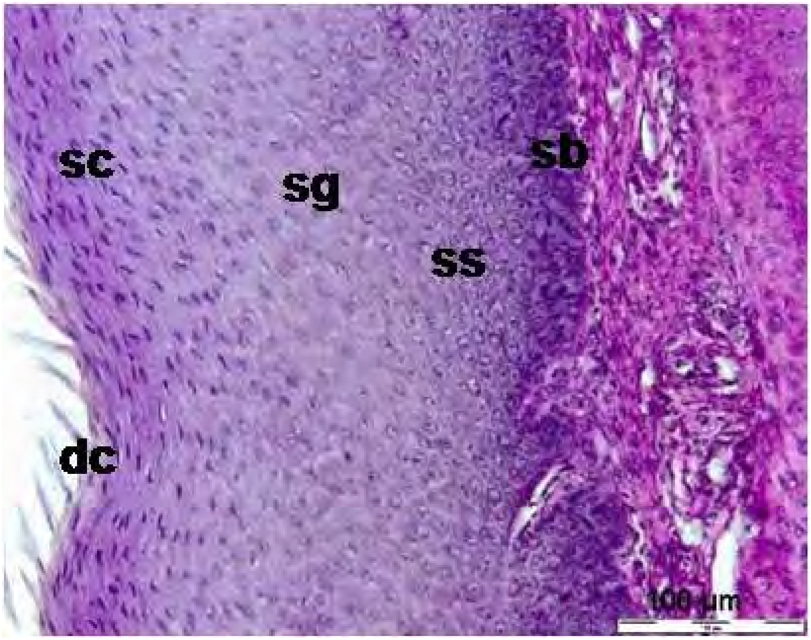

Figure 3. Microphotographs of the anterior part of the tongue of the RJF shows the dorsum surface with thick stratified squamous epithelium, desquamating cell (dc), stratum corneum (sc), stratum granulosum (sg), stratum spinosum (ss), stratum basale (sb). Hematoxylin and eosin (HE) stain.

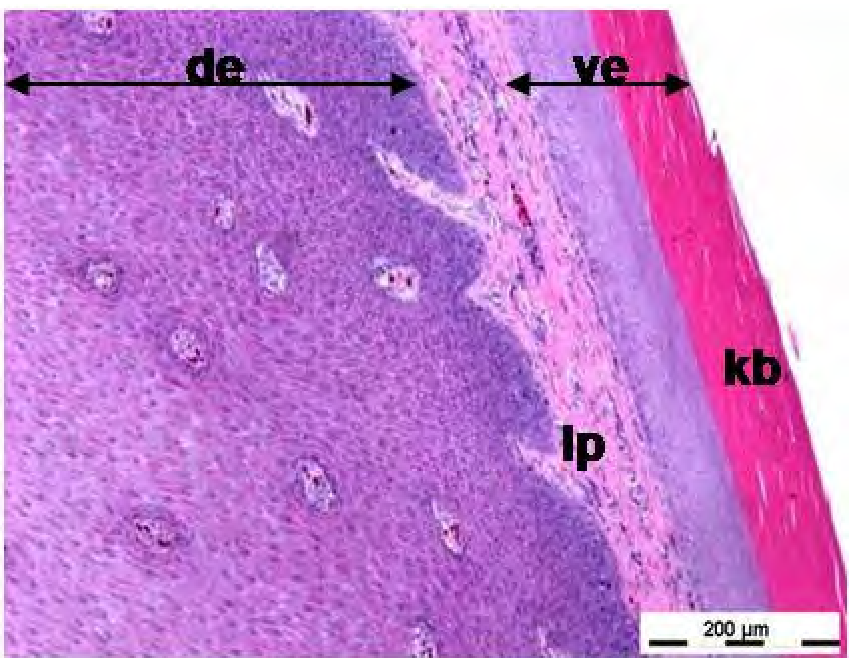

Figure 4. Microphotograph of the longitudinal sections through the anterior part of the tongue of the RJF shows the dorsum surface with thick epithelium (de) and the ventrolateral surface with thin epithelium (ve), keratinized band (kb), lamina propria (lp). Hematoxylin and eosin (HE) stain.

amylase. The MG of the anterior and the posterior lingual glands were moderately stained with alcian blue pH 1, whereas the LG of the anterior lingual glands was weakly stained (Figure 7). The alcian blue (pH 2.5) gave rise to a

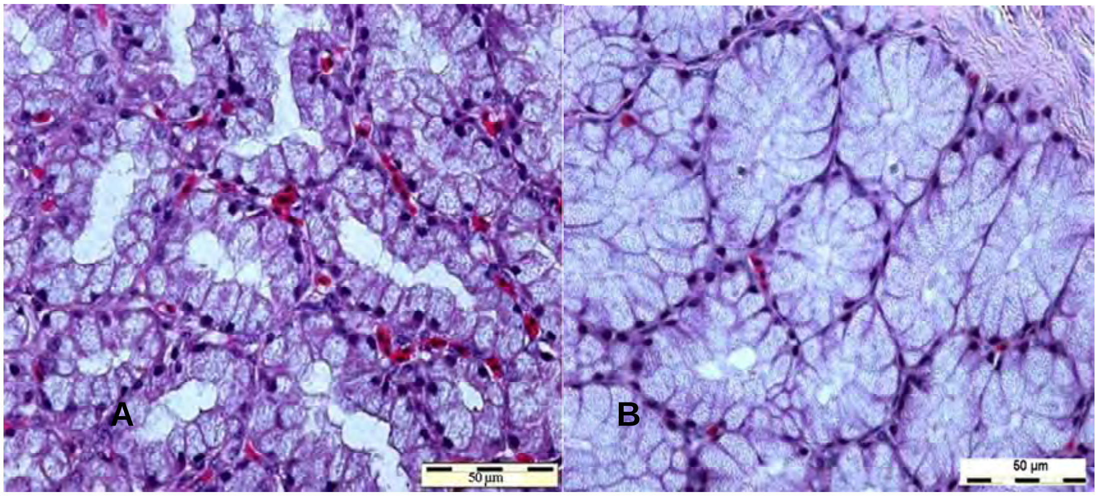

Figure 5. Microphotographs of the lingual glands of the RJF show: A) the lateral group of the anterior lingual glands with darker stained secretory cells, and B) the posterior group with lighter stained secretory cells. Hematoxylin and eosin (HE) stain.

Table 1. Histochemical reaction of the glycoconjugates in the lingual mucous glands of the red jungle fowl.

Staining	Anterior lateral (gp)	Anterior medial (gp)	Posterior (gp)
PAS	2p	3p	3p
α amylase-PAS	2p	3p	3p
AB (pH1)	1b	2b	2b
AB (pH 2.5 - 3.2)	2b	3b	3b
AB (pH 1)-PAS	m	p	p
AB (pH 2.5)-PAS	1p	2p	2p
A F-AB	b	bm	bm

b = blue, m = magenta, p = purple. Results are given in arbitrary units on a 3-point scale, with 0 representing absence of staining and 3 indicating darkest staining. Number indicates intensity of staining reaction. PAS (periodic acid Schiff); AB (Alcan blue) and AF (aldehyde fuchsin).

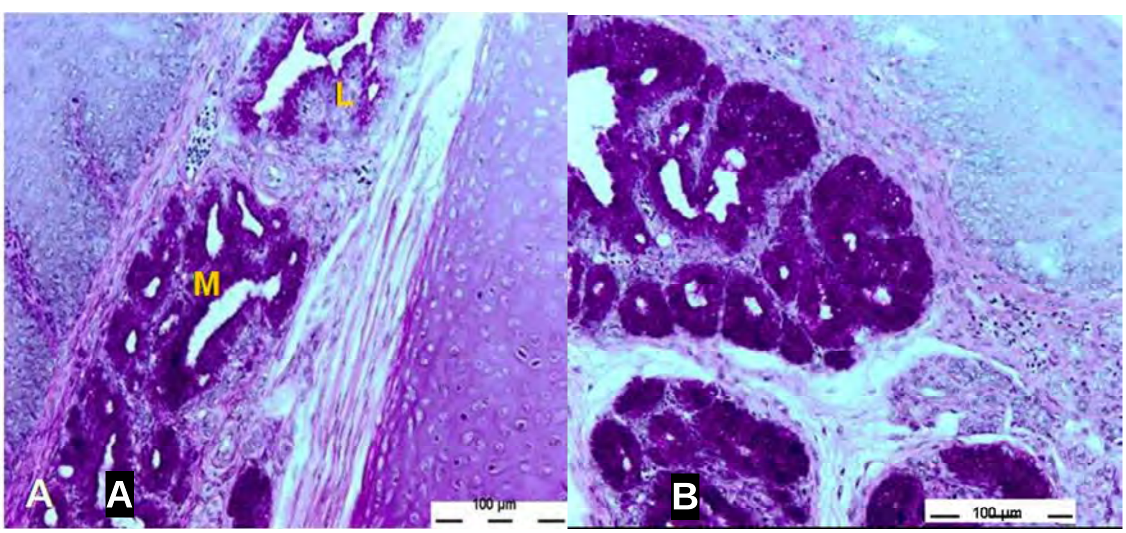

Figure 6. Microphotographs of the lingual salivary glands of the RJF show: A) the anterior lingual glands with low density of stain for lateral group (L), high density of stain in medial group (M). B) Posterior lingual glands with high density of stain. Periodic acid-schiff (PAS) stain.

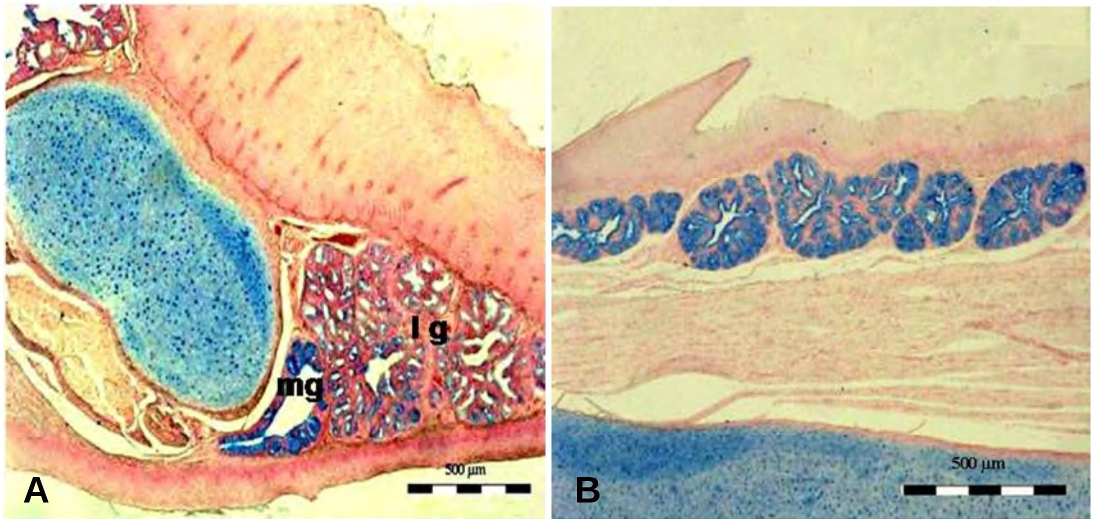

Figure 7. Microphotographs of the tongue of the RJF shows: A) the anterior lingual salivary glands with weak acid mucin reaction in the lateral group (lg) moderate acid mucin reaction in the medial group (mg). B) Posterior lingual salivary glands with moderate mucin reaction. Alcian blue (pH 1).

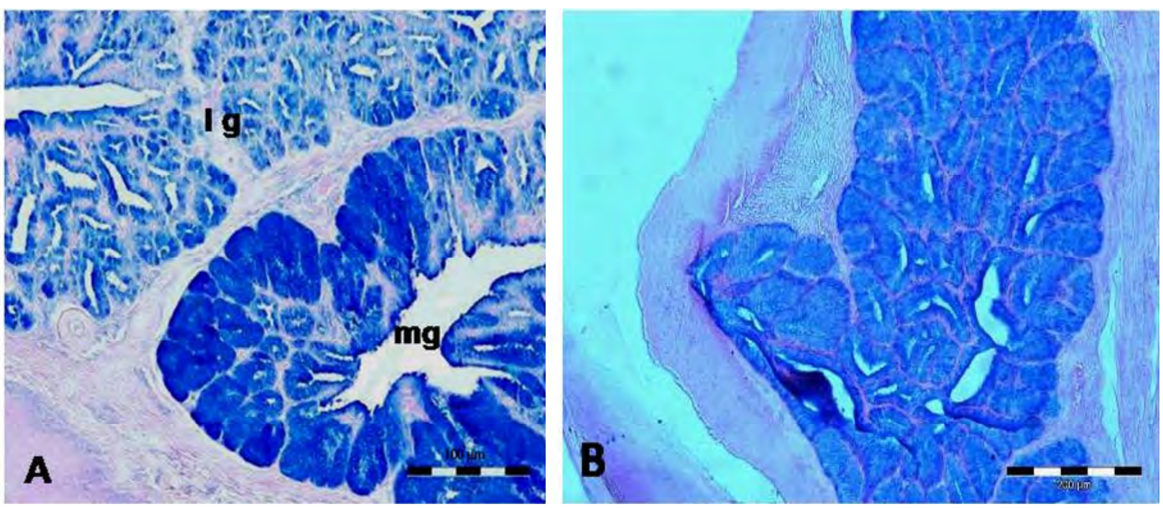

Figure 8. Microphotographs of the tongue of the RJF shows: A) the anterior lingual salivary glands with moderate acid mucin reaction in the lateral group (lg) strong acid mucin reaction in the medial group (mg). B) Posterior lingual salivary glands with strong mucin reaction. Alcian blue (pH 2.5).

strong positive reaction in the secretory granules of the MG of anterior and the posterior lingual glands with moderate positive reaction in the LG of the anterior lingual glands (Figure 8). After alcian blue-PAS stain, the lingual gland cells reacted positively with both stains, although, the LG of the anterior lingual glands showed acid and neutral mucin reactions at pH 2.5 (Figure 9), however, at pH 1, very weak acid mucin reaction was exhibited in contrast to the MG and the posterior glands which showed strong reaction.

Dual staining with aldehyde fuchsin-alcian blue resulted in staining the MG of the anterior lingual glands and the posterior lingual glands with both stains, while the LG of the anterior lingual glands reacted positively only to alcian blue (Figure 10). The lingual salivary glands were surrounded by a connective tissue capsule rich in blood vessels, parts from capsule were extended inwards and separated the glandular tubules. Openings of these ducts of the gland into the oral cavity were lined with stratified squammous epithelium for a short distance and change into the low columnar toward the cavity of the glands. The skeleton of the tongue is formed by hyaline cartilage (entoglossal) crossing the entire length and joining the basihyal at the base of the tongue with skeletal muscles

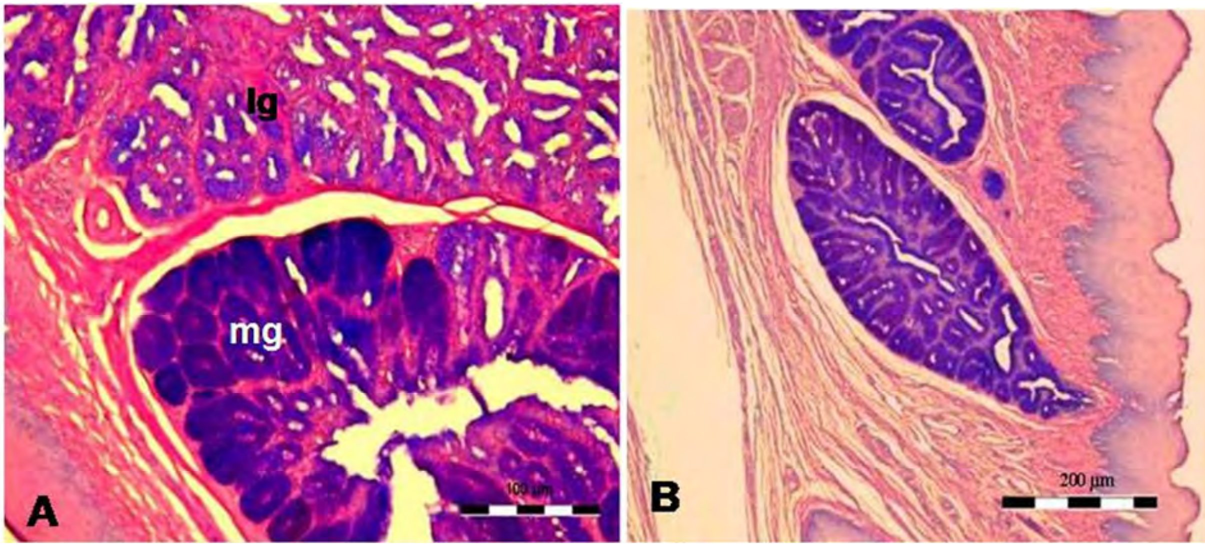

Figure 9. Microphotographs of the lingual salivary glands of the RJF shows: A) the anterior lingual glands tended to neutral mucin reactin (magenta) in the lateral group (lg), both acid and neutral mucin reaction (purple) in the medial group (mg). B) Posterior lingual glands tended to both acid and neutral mucin reaction. Alcian blue (pH 2.5) - Periodic acid-schiff (PAS) stain.

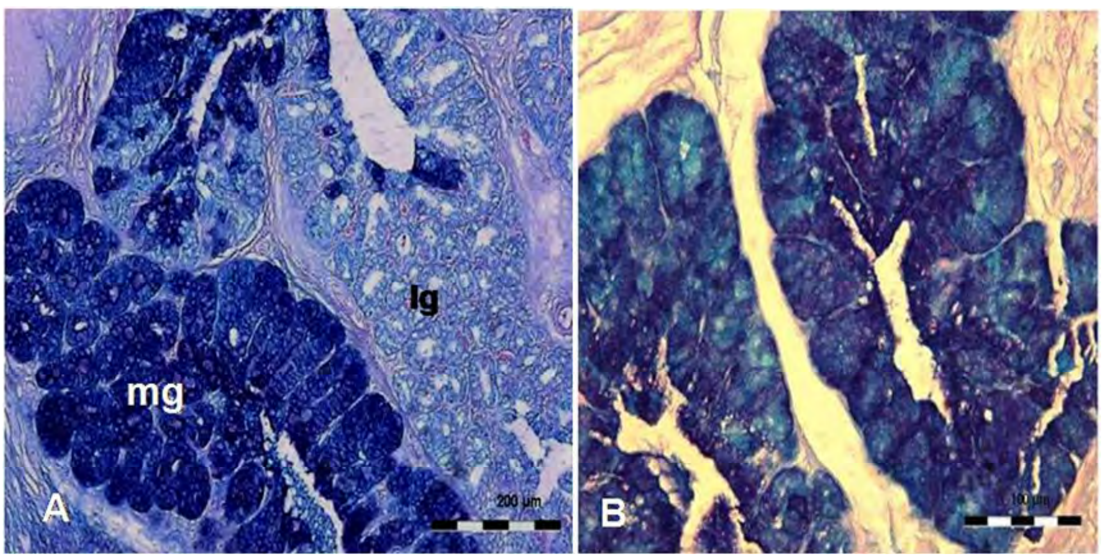

Figure 10. Microphotographs of the lingual salivary glands of the RJF. A) The anterior lingual glands shows carboxylated mucin in the lateral group (lg), carboxylated and sulphated mucin reactions in the medial group (mg). B) The posterior lingual glands show carboxylated and sulphated mucin reactions. Aldehyde fuchsin-alcian blue stains.

fixed between them. However, ossification of the cartilages was observed at the anterior extremity of the basihyal and posterior end of entoglossum.

DISCUSSION

Several parts of the digestive system including the beak and the tongue are considerably modified and adapted according to the diet birds receive. In present study, the arrangement of the lingual papillae is agreed with the findings obtained on the chicken (Hill, 1971; Iwasaki and Kobayashi, 1986). However, the tongue of goose has only giant conical papillae in the midline between the body of the tongue and radix (Iwasaki et al., 1997; Hassan et al., 2010). While in the common kestrel, several conical papillae are located between the body and the base of the tongue (Emura et al**.,** 2008). The lingual conical papillae in the eagle are restricted to a single crest at the posterior end of the lingual body which extends to the tongue root (Jackowiak and Godynicki, 2005). Emura et al. (2008) have demonstrated the importance of the conical papilla which includes assisting the transfer of food towards the esophagus as well as prevent regurgitation. Ostrich tongue possesses a well developed mucosal fold which is directed backwards between the body and the base of the tongue (Jackowiak and Ludwig, 2008). However, in present study, a short plate-like fold extension over the base of the lingual papillae was observed which was not mentioned in the findings of Nickel et al. (1977) and Hodges (1974). Our results agreed with the finding of Nickel et al**.** (1977) in fowl and Emura et al. (2008) in common kestrel that these lingual papillae arranged in concave transverse row. But seem different to that reported by Iwasaki (1992) in respect to the straight transverse line of the lingual papillae in the little tern. McLelland (1975) have stated that the pharyngeal papillae in domestic fowl is represented by two rows of giant conical papillae was however different from this report. This may be due to the changes and modification that occurred in the gastrointestinal tract of the domestic fowl during selection programs.

Our results also showed that the surface of the tongue is covered by a thick stratified squamous epithelium which was heavily cornified on the ventrolateral surface and the lingual papillae; this however, was supported by the findings of Hill (1971). Similar results were observed in different species of birds. However, the keratinized layer of the epithelium usually differs in thickness depending on the type of food. A strongly keratinized epithelium is seen mainly in herbivorous and granivorous birds (Susi, 1969; Iwasaki**,** 1992; Jackowiak and Ludwing, 2008). A lesser degree of keratinization of the epithelium is found in birds living in water habitats (Iwasaki, 2002; Jackowiak et al**.,** 2006). Similar to our observation, Iwasaki et al. (1997) and Jackowiak and Godynicki (2005) reported that the parakeratinized epithelium cover the dorsum and the lingual root. In this study, the areas with high degree of keratinization showed four distinguishable layers of stratified squamous epithelium which therefore agrees with the result of Homberger and Brush (1986) in the parrot tongue. While the stratum granulosum is absent from these area of the chicken tongue (Susi, 1969). Our results are in line with the report of Nickel et al**.** (1977) and Homberger and Meyers (1989) that the anterior third of the tongue is entirely free of musculature while the extralingual muscles spread into the remaining parts of the tongue. The salivary gland in this report was similar to other birds. Same results reported by Toryu et al**.** (1960) that there are differences between the anterior and posterior lingual glands in chicken after histological stain. Gargiulo et al. (1991) and Rossi et al. (2005) have demonstrated that the secretory cells consist exclusively of mucus. Toryu et al. (1960) and Duke (1986) have shown similar results in chickens and turkeys while Jerrett and Goodge (1973) however believed that chickens and turkeys produce very little amylase compared to the songbirds. Surprisingly, the lingual glands are absent in cormorants (Jackowiak et al**.**, 2006). However, these results are in contrast to that of various mammalian submucous glands (Testa et al., 1985; Estecondo et al., 2005). The histochemical reactions in this study revealed to the presence of glycoconjugates containing vicinal diol groups in the secretory granules subsequent to PAS stain. The MG of the anterior and the posterior lingual glands showed positive reaction in the whole cell cytoplasm indicating that they contain large amount of mucin.

A lowest amount of mucin was demonstrated in the cytoplasm of the LG of the anterior lingual gland cells. These secretory granules contained mostly mucin because there was no detectable change in the intensity to the PAS reaction after digestion with α- amylase. However, there are slight changes in the stain according to the results reported by Toryu et al**.** (1960). Whereas, the lingual salivary glands of the little egret show PAS negative reaction and hence considered to be free of glycogen or neutral mucosubstances (Al-Mansour and Jarrar, 2007). The MG of the anterior and the posterior lingual glands contained the vicinal diol and sulphate containing glycoconjugates after alcian blue-PAS stain. Meanwhile, the LG of the anterior lingual glands contained vicinal diol. These data agree with that of Suprasert et al. (1986), Suprasert and Fujioka (1987) and Gargiulo et al**.** (1991). However, our data agreed with the report of Gargiulo et al**.** (1991) that the LG of the anterior lingual glands contained weak acid mucin while the MG of the anterior and the posterior lingual glands showed strongly acid mucin reaction after alcian blue stain. Furthermore, from these results observation of MG of the anterior and posterior lingual glands after aldehyde fuchsin-alcian blue stain showed that the glycoconjugates may contain carboxylated groups in addition to sulphated groups which however agreed with the report of Gargiulo et al. (1991) in chicken and in egret (Al-Mansour and Jarrar, 2007). The results of the present study showed that the tongue of the RJF is similar to that of other birds particularly with domestic fowl although, differences were observed in arrangement of the conical papillae that may reflect changes occurred during domestication of chickens. The lingual salivary glands form a blend of mucinous saliva that may act as lubricate to manipulate the ingested food to facilitate swallowing. Moreover, secretions of the mucous lingual glands may act as a protective cover to the mucous membrane of the upper digestive tract whose activity is similar to those suggested by Samara et al. (2002).

In addition, sialic acid residues of carbohydrates are known to coat the mucosal surface of the tongue to provide a hydrophilic environment condition which could preserve hydration. However, salivary sulfo and sialomucins actively participate in the modulation of the oral mucosal calcium channel activity. This function is of paramount importance to mucosal calcium homeostasis (Slomiany et al., 1996).

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