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Histological and Histochemical Study on the Stomach (Proventriculus and Gizzard) of Black-tailed Crake (*Porzana bicolor*)

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Abstract.- Black-tailed crake is a little known species of bird in the Rallidae family. Its natural habitat is subtropical or tropical moist montane marshes at heights around 2100m. Black-tailed crake looks for food on the grass-land, picking up small grasshoppers, land-shells and small worms. In this study, the structure of the proventriculus and gizzard of adult black-tailed crake were examined at light microscopic level by using hematoxylin-eosin (H&E), and special staining for mucins *i.e.*, periodic acid schiff (PAS), alcian blue (AB pH 2.5), AB-PAS and combined aldehyde fuchsin- alcian blue (pH 2.5) (AF-AB). The results revealed that the epithelium of the proventriculus invaginated to form the simple tubular glands, which showed a weak positive reaction with PAS stain but an intense positive reaction with AB (PH 2.5) stain. With the AB (pH 2.5)-PAS and AF-AB (pH 2.5) staining procedures, the simple tubular glands appeared blue and purple in color, respectively, owing to the presence of sulfated mucins. The compound tubular glands were made up of 8-10 conical or oval lobules and accounted for 77.8%-80.4% of the radius of the proventriculus. The cells lining the secretory ducts and central cavities of the compound tubular glands showed intense positive reactions with all the histochemical stains, indicating a mixture of neutral and acidic mucins occurred and the acidic mucins were carboxylated. However, the cells of corpus glandulae showed negative reactions for mucins. In the proventriculus-gizzard junction, there were nearly no mucins and the compound tubular glands were absent. The surface of gizzard was covered by a layer of rough PAS-positive cuticle. The cells lining the tips and the crypts of the glands of gizzard were covered by a layer of carboxylated mucins. The other parts of the gizzard showed negative reactions for mucins.

Keywords: Black-tailed crake, proventriculus, gizzard.

INTRODUCTION

Crake, mainly ground-dwelling birds, belongs to the family of Rallidae and the order of Gruiformes, and often looks for food in paddy fields, cannas, shrubs and swamps. They are omnivorous, as their diets consist of chits, seeds, and some invertebrates such as insects, larvae and snails. The histological architecture of stomach of birds shows great diversities according to their respective dietary habits (Ogunkoya and Cook, 2009; Kadhim *et al.*, 2011). Enough knowledge exists on the morphology of the stomach of many big-sized birds including species of the pheasant family (Aitken, 1958; Pastor *et al.*, 1988; Imai *et al.*, 1991; Selvan *et al.*, 2008; Kadhim *et al.*, 2011), and Moussa, 2012), ostrich (Eidaroos *et al.*, 2008), burrowing owl (Rocha and Lima, 1998) and falcon (Abumandour, 2013), but nothing has been published the stomach of black-tailed crake, which is more widely distributed in Bhutan, China, India, Laos, Mynamar, Nepal, Thailand and Vietnam. It is mainly an eastern Palaearctic taxon and a high-elevation crake. The adult black-tailed crake is 21-24 cm in length with a green bill, black tail and reddish legs. Its head and neck are ashy grey. Black-tailed crake looks for foods on the grass-land, picking up small grasshoppers, land-shells and small worms (Inskipp and Round, 1989).

The epithelium of stomach of birds is covered with a layer of mucins which protects the underlying epithelium from chemical, enzymatic and mechanical damage, and pathogenic micro-organisms (Ogunkoya and Cook, 2009). Gastric mucins of birds present variations that depend on the dietary habits of each species. Clinical rescue of the black-tailed crake in the wild is handicapped by the paucity of the basic data of the stomach structure. As a result, a detailed description of the histological

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architecture and stomach mucins of the black-tailed crake was carried out, which would also be useful for the surgical and nutritional management of these birds in captivity.

MATERIALS AND METHODS

Eight adult black-tailed crakes of either sex used in the study were obtained from paddy field of Qujing, near the Qujing Normal University. This study was approved by The Animal Experimentation Ethics Committee of Qujing Normal University (Qu-2013012). The birds were checked for their health status before being euthanized with ethyl ether. For the histological study, fragments of the stomach were immersed in neutral formalin for 24 h and then submitted to the dehydration process with alcohol and embedded in paraffin wax (Saleem *et al.*, 2015). Serial sections, 5-6 µm in thickness, were cut. After being deparaffinised and hydrated, the sections were stained with hematoxylin-eosin (H&E) and Masson's trichrome stain to determine the general structure of stomach regions, the periodic acid schiff (PAS) for determinating neutral mucins, glycogen, and other periodate reactive carbohydrates (sialidaselabile, sialomucins and weakly sulphated mucins), alcian blue (pH 2.5) for identifying acidic mucins, alcian blue (pH 2.5)-Periodic acid Schiff (PAS) for differentiating acidic and neutral mucins, combined aldehyde fuchsin-alcian blue (pH 2.5) (AF-AB) for differentiating of acidic mucins with sulphate and carboxyl groups. Tissue sections from different stomach portions were examined by conventional light microscopy (BX51; Olympus, Tokyo, Japan) and were evaluated for characteristics of stomach (proventriculus and gizzard) and its mucins.

RESULTS

Histological characteristics

The wall of the proventriculus and gizzard consisted of three tunics: a mucous membrane (tunica mucosa gastris), a muscular layer (tunica muscularis gastris) and the serosa (tunica serosa gastris). The mucous membrane of proventriculus was lined by a simple columnar epithelium, invaginating to form the simple tubular glands (Fig.1A), which was constituted by cells with basal nuclei and acidophilic cytoplasm. The cells in the sulci of the glands were low columnar, while the cells lining the folds were tall columnar and mostly desquamated (Fig. 1B). The lamina propria was constituted by diffuse muscle fibers, lymphocyte infiltrations and blood vessels.

The wall of the proventriculus was 1.48-1.62 mm thick, and the simple tubular glands were only 0.11-0.20 mm in depth, while the compound tubular glands were 1.19-1.26 mm in depth, accounting for 77.8%-80.4% of the radius of the proventriculus. The compound tubular glands were made up of 8-10 conical or oval lobules which were separated by a thin layer of connective tissue. A glandular lobule was constituted by many glandular tubules, radiating to the interlobular connective tissue. The glandular tubules, constituted by cuboidal cells, drained into a central cavity, which released the mucins and pepsinogen to the lumen of the proventriculus through an excretory duct (Fig. 1A). The cells lining the excretory ducts and the central cavities were high columnar cells, with hyaline cytoplasm and flat nuclei (Fig. 2B). The lamina muscularis mucosa was constituted by a layer of longitudinal muscle, which had a 0.04-0.13 mm in thickness. The muscular layer was constituted by a 0.14-0.23 mm thick layer of circular muscle (Fig. 2A). The proventriculus-gizzard junction was characterized by the simple tubular glands, which were separated by the connective tissue to 3-4 independent structures, called peacock tail-liked glands. The compound tubular glands were absent (Fig. 3A, B).

The wall of gizzard was lined by a layer of serrated cuticle called koilin (Fig. 4A). The mucous membrane was lined by high-columnar cells with basal nuclei and hyaline cytoplasm. The epithelium invaginated to form folds and sulci, and the bottoms of the sulci were the openings of the simple tubular gizzard glands, surrounded by loose connective tissue. The cells lining the folds mostly shed and fell into the koilin (Fig. 4B). The koilin and the lamina propria protruded into the lumen of the gizzard and made the wall uneven. The histological architectures of the folds and sulci were different and appeared alternately (Fig. 4A). In the folds, the koilin was 0.20-0.24 mm thick, and the simple straight tubular

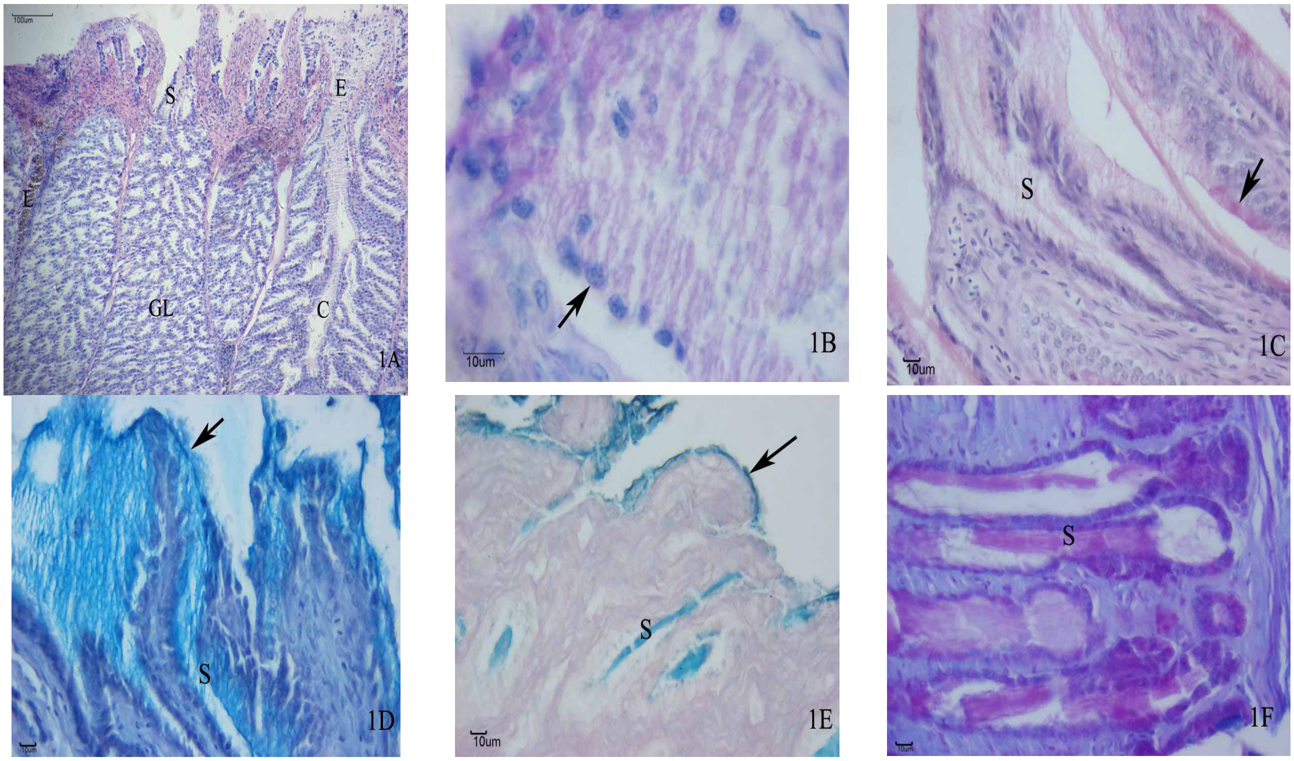

Fig. 1. Histological structure of the simple tubular glands in the proventriculus of the black-tailed crake. A, The mucous membrane of proventriculus was lined by a simple columnar epithelium, invaginating to form the simple tubular glands (S). The glandular lobules (GL) of the compound tubular glands were constituted by many glandular tubules, drained into a central cavity(C), which released the mucins and pepsinogen to the lumen of the proventriculus through an excretory duct (E). lymphatic nodules (L) were prominent within the connective tissue between the glandular lobules; B, The cells (arrow) lining the folds of the simple tubular glands were tall columnar and mostly desquamated; C, The epithelial cells (arrow) lining the sulci of the simple tubular glands (S) exhibited a weak PAS reactivity. D, The surface epithelium (arrow) and the simple tubular glands (S) showed an intense positive reaction with Alcian blue; E, The surface epithelium (arrow) and the simple tubular glands (S) appeared blue in color with AB (pH 2.5)-PAS stain. F, The simple tubular glands (S) giving a purple color with AF-AB (PH 2.5) stain.
Magnifications: A, 100x; B, F, 1000x; C, D, E, 400x.
Stain: A, B, H & E stain; C, PAS stain; D, Alcian blue (pH 2.5) stain; E, AB (pH 2.5)-PAS stain; F, AF-AB (PH 2.5) stain.

glands, occupying all the lamina propria, were 0.42-0.46 mm in length. The glandular cavities were empty. In the sulci, the koilin was only 0.16-0.18 mm thick, and the glands were only 0.26-0.28 mm in length and separated by the collagen fibers into two portions: the interior and exterior portions. The glands in the interior portion were straight and the contents were ejected, while the glands in the exterior portion were oval and packed. The lamina muscularis mucosa was absent. The muscular layer was thick and made up of an interior circular muscle of 0.26-0.42 mm thick and an exterior longitudinal muscle of 0.90-1.13 mm thick. The tunica serosa consisted of connective tissue with blood vessels.

Mucin histochemical profile

The cells lining the epithelium and the cavities of the simple tubular glands in the proventriculus showed a weak positive reaction with PAS stain (Fig. 1C), but an intense positive reaction with AB (pH 2.5) stain (Fig. 1D). With the sequent AB (PH 2.5)-PAS staining procedure (Fig. 1E), they appeared blue in color owing to the presence of acidic mucins. Furthermore, these structures giving

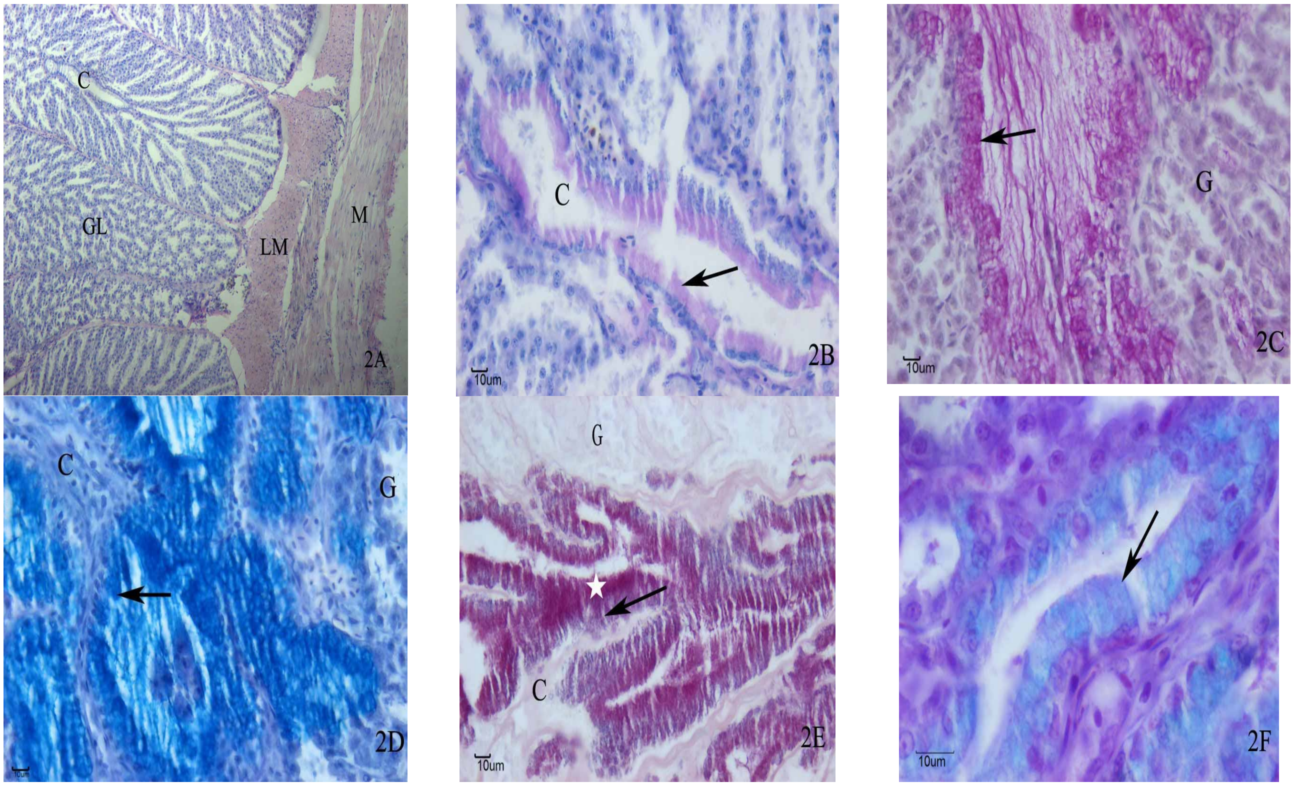

Fig. 2. Histological structure of the compound tubular glands in proventriculus of the black-tailed crake. A, The compound tubular glands of black-tailed crake occupied the thickest part of the wall in the proventriculus. The lamina muscularis mucosa (LM) was a layer of longitudinal muscle and the muscular layer (M) was constituted by a 0.14-0.23 mm thick layer of circular muscle. Glandular lobules (GL), Central cavity(C); B, The cells (arrow) lining the central cavity(C) were high columnar and with hyaline cytoplasm and flat nuclei; C, The cells (arrow) lining the central cavity exhibited an intense PAS reactivity, while the cells of corpus glandulae (G) were PAS-negative; D, The cells lining the central cavity and the secretory ducts (arrow) exhibited an intense AB reactivity, while the cells of corpus glandulae (G) and the connective tissue(C) were AB-negative; E, The tops (star) of the cells lining the central cavity (C) were stained reddish purple, whereas the bottoms (arrow) of these cells were stained bluish purple. The cells of corpus glandulae (G) were PAS-negative and AB-negative; F, The cells lining the central cavity (arrow) exhibited blue in color with AF-AB (pH 2.5) stain.
Magnifications: A, 100x; B, C, D, 400 x; E, F, 1000x.
Stain: A, B, H & E stain; C, PAS stain; D, Alcian blue (pH 2.5) stain; E, AB (pH 2.5)-PAS stain; F, AF-AB (PH 2.5) stain.

a purple color after the AF-AB (pH 2.5) staining procedure (Fig. 1F) showed that the acidic mucins were sulfated. The cells lining the secretory ducts and central cavities of the compound tubular glands showed intense positive reactions in both PAS (Fig. 2C) and AB (PH 2.5) (Fig. 2D) stains. The observation of the sections labeled with the AB (pH 2.5)-PAS staining method revealed that the tops of these cells were stained reddish purple, indicating the occurrence of mixed mucins that were more neutral than acidic, whereas the bottoms of these cells were stained bluish purple, implying the occurrence of mixed mucins that were more acidic than neutral (Fig. 2E). With the sequent AF-AB (pH 2.5) staining procedure, the cells exhibited blue staining (Fig. 2F), thus indicating that the acidic mucins were carboxylated. The cells of corpus glandulae showed negative reactions for mucins.

The cells lining the epithelium and the glandular cavities in the proventriculus-gizzard junction exhibited negative reactions in PAS (Fig.3C), AB (PH 2.5) (Fig. 3D), AB (PH 2.5)-PAS

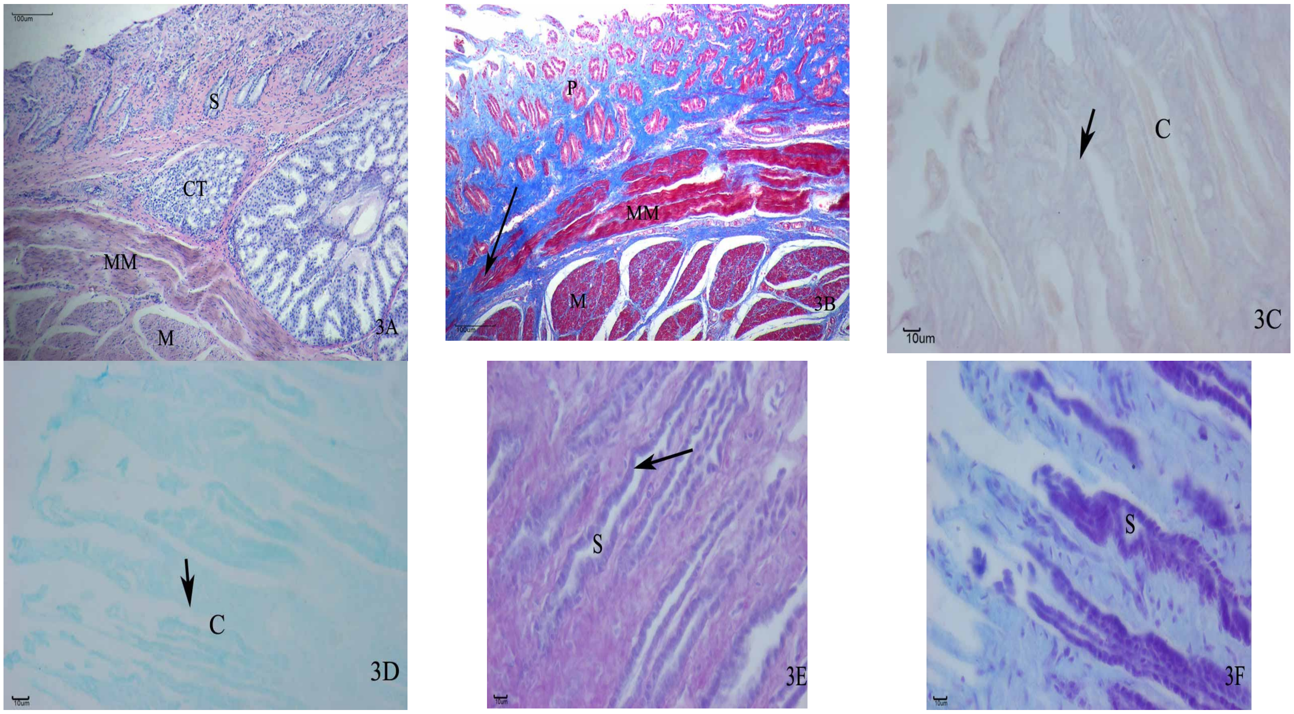

Fig. 3. Histological structure of the simple tubular glands in the proventriculus-gizzard junction of the black-tailed crake. A, The simple tubular glands(S) shortened, and the compound tubular glands (CT) were absent. M: muscular layer, lamina muscularis mucosa (LM); B, The simple tubular glands, which were separated by the connective tissue to 3-4 independent structures, called peacock tail-liked glands (P), and the lamina muscularis mucosae (MM) disappeared towards the gizzard (arrow). M: muscular layer; C, The simple tubular glands (arrow) and the contents(C) in the lumen showed a PAS-negative reactivity; D, The simple tubular glands (arrow) and the contents(C) in the lumen showed an AB-negative reactivity; E, The simple tubular glands(S) and the contents (arrow) in the lumen were PAS-negative and AB-negative. F, The simple tubular glands(S) showed negative reactions for mucins.
Magnifications: A, B, 100x; C, D, E, F, 400 x.
Stain: A, H & E stain; B, Masson's trichrome stain; C, PAS stain; D, Alcain blue; E, AB (pH 2.5)-PAS stain; F, AF-AB (pH 2.5) stain.

(Fig. 3E) and AF-AB (pH 2.5) (Fig. 3F) stains. In the gizzard, the koilin showed a positive reaction with PAS stain, but only its interior surface showed a positive reaction with AB (pH 2.5) stain. The surface epithelium and the cells lining the tips and crypts of the glands of gizzard showed moderate positive reactions for both PAS (Fig. 4C) and AB (pH 2.5) stains (Fig. 4D), whereas the secretory materials in the lumina only showed a moderate PAS reactivity. With the sequent AB (pH 2.5)-PAS (Fig. 4E) and AF-AB (pH 2.5) (Fig. 4F) staining procedures, these cells exhibited blue staining, thus indicating that the mucins were acidic and carboxylated respectively. The other parts of the gizzard showed negative reactions for mucins.

DISCUSSION

In birds, the parts of stomach accordingly have complementary roles. The proventriculus has a secretory function, whereas gizzard is essentially mechanical. The proventriculus receives food from the esophagus, and secretes mucus and HCl similar to what is seen in the mammalian stomach. The main function of mucus is to cover, lubricate, and protect the stomach surface from the corrosive actions of acidic gastric juices. In a number of birds, many studies have been carried out regarding the general morphology of proventriculus and gizzard and the histochemical nature of gastric mucus. The present study appears to be first description of the

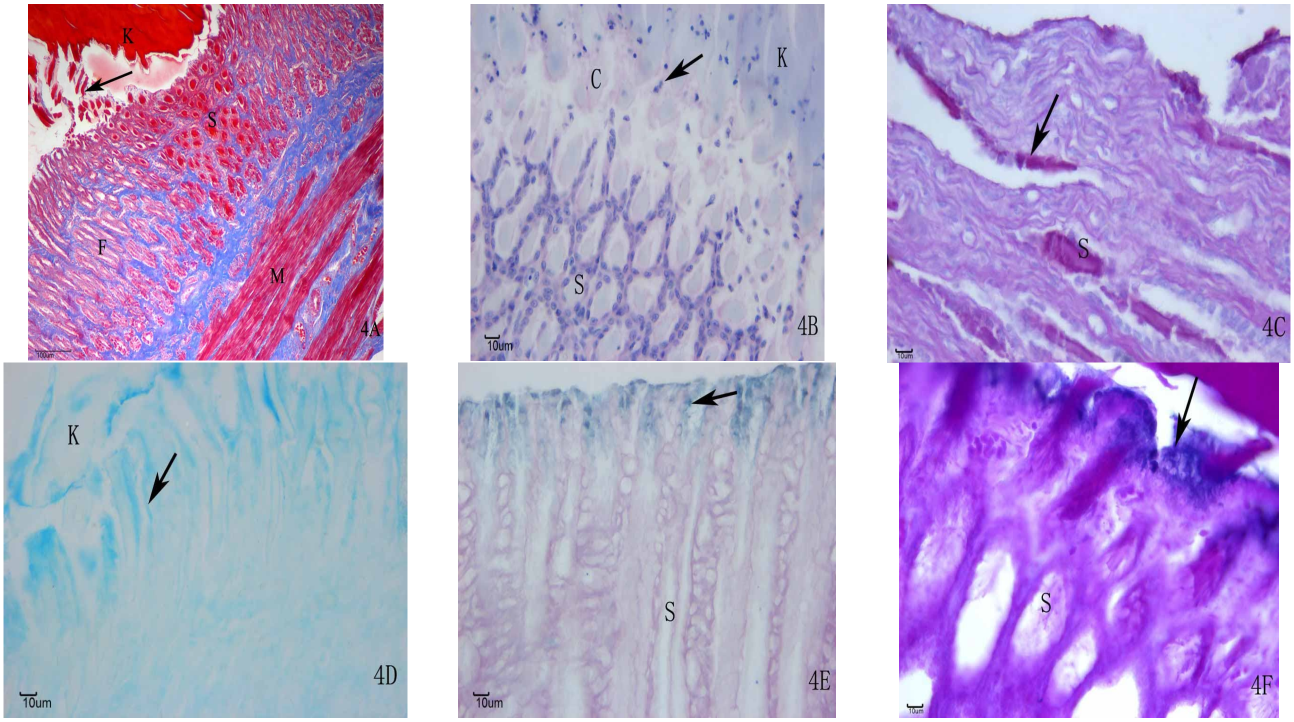

Fig. 4. Histological structure of the simple tubular glands in the gizzard of the black-tailed crake. A, The glands in the folds（F）were straight and the contents (arrow) were released to form koilin (K), while the glands in the sulci (S) were oval and packed. M: muscular layer; B, The cells (arrow) lining the folds of the glands(S) shed and the contents(C) fell into the koilin (K). Note the lamellae of koilin were perpendicular; C, The epithelial cells (arrow) and the glands (S) exhibited a moderate PAS reactivity; D, The cells lining the tips of the folds (arrow) and the interior side of the koilin (K) exhibited a moderate AB reactivity; the other parts of the gizzard were AB-negative; E, The cells (arrow) lining the tips and crypts of the glands(S) exhibited blue in color, indicating the occurrence of acidic mucins. The other parts of the gizzard were PAS-negative and AB-negative. F, The cells (arrow) lining the tips and crypts of the glands(S) exhibited blue in color, indicating the occurrence of carboxylated mucins.
Magnifications: A, B, C, D, E, 400 x; F, 1000x.
Stain: A, Masson's trichrome stain; B, H & E stain; C, PAS stain; D, Alcain blue; E, AB (pH 2.5)-PAS stain; F, AF-AB (pH 2.5) stain.

morphology and histochemistry of the proventriculus and gizzard of the black-tailed crake. The histological observation of the stomach of black-tailed crake demonstrated that the wall of proventriculus and gizzard consisted of three tunics, and this was in consistent with the findings of Zhu *et al*. (2013) in proventriculus of yellow-billed grossbeak, Rocha and Lima (1998) in gizzard of burrowing owl, and of Liman *et al*. (2010) in proventriculus and gizzard of Japanese quail, though more authors agreed that there were four layers in stomach of other birds (Hassan and Moussa, 2012; Ogunkoya and Cook, 2009; Abumandour, 2013).

Eidaroos *et al*. (2008) found that the mucosas of proventriculus of some birds were covered by a thin layer of cuticle or koilin, whereas in the proventriculus of black-tailed crake, this layer was not observed. In the proventriculus of black-tailed crake, there were two types of glands: simple tubular mucosal gland and compound tubular submucosal gland. Hodges (1974) stated that the simple tubular glands in proventriculus were artefacts of preparation, while Abumandour (2013) had not identified these glands existed in the proventriculus of falcon by using the same method. Ogunkoya and Cook (2009) stated that the surface epithelium invaginated into the lamina propria to form the simple tubular glands. Our findings here support the Ogunkoya and Cook's (2009) idea. Ogunkoya and Cook (2009) and Hassan and Moussa (2012) found that the simple columnar cells lining the folds were taller than those in the sulci. This was consistent with the results of our study.

It is known that feeding habits, diet quality and quantity of food affect the histology of the digestive system (Chikilian and Speroni, 1996). Like in other omnivorous birds, such as the red jungle fowl (Kadhim *et al.*, 2011), duck and pigeon (Hassan and Moussa, 2012) and *Coturnix coturnix* (Zaher *et al.*, 2012), the compound tubular glands of black-tailed crake occupied the thickest part of the wall in the proventriculus, while the muscular layer occupied the thickest part of the wall in the proventriculus of carnivorous falcon (Abumandour, 2013), which might because the big-sized foods need more power to be pushed into the gizzard. So, the size of the food might have an influence on the histological structure of the stomach too.

In mammals and birds, the mucosa of the stomach is covered by a layer of thick mucus, which is vital to protect the cell linings of that organ from the highly acidic environment within it. The major macromolecular constituents of normal mucus are the mucins. Mucins are complex carbohydrate secreted by different types of epithelial cells and glandular tissues of alimentary tract. In general, mucins are classified into neutral and acidic mucins (Cheah and Ramachandran, 1994). The latter are of 2 major types, sulphated (sulphomucins) and carboxylated (sialomucins). Mucins in mucus composition are used as lubricant for materials which must pass over membranes. Also, the mucins play an essential role in defensing against bacteria. Sulphomucins are acting as antiulcerogenic as they coat and protect mucosal surface while neutral mucins help the secretion of enzymes (Karambelkar *et al.* 2014). Until today, it is well known in many published articles in different birds that the carbohydrates of gastric mucus in the proventriculus and gizzard histochemical are different from each other (Aitken, 1958; Pastor *et al.*, 1988; Selvan *et al.*, 2008; Kadhim *et al.*, 2011; Prasad and Kakade, 1990; Hassan and Moussa, 2012). In our present study, histochemical reactions revealed the presence of sulphated mucins in the cells of luminal epithelium and the crypts of the simple tubular glands of the proventriculus. However, the neutral mucins were absent. This was in consistent with the findings of Chikilian and Speroni (1996) in tinamou, Suganuma *et al.* (1981) in four birds, Prasad and Kakade (1990) in domestic duck and Paster *et al.* (1988) in chicken. Contrary to our results Kadhim *et al.* (2011) found only neutral mucins in these cells of the red jungle fowl, whereas Inforzato and Sasso (1985) in *Columba livia*, Imai *et al.* (1991) in fowl and Selvan *et al.* (2008) in guinea fowl found that the cells of luminal epithelium had both acidic and neutral mucins. Our results showed the presence of sulphated mucins in the luminal epithelium and the crypts of the simple tubular glands which support the information in the literatures (Inforzato and Sasso, 1985; Pastor *et al.*, 1988; Selvan *et al.*, 2008; Ogunkoya and Cook, 2009). The mucins in the surface epithelial cells and glandular lumina might form a resistant mucosal barrier to protect the epithelium from the physical damage by the luminal contents and bacterial invasion.

In this study, it was determined that the cells lining ducts and central chambers of compound tubular glands contained a mixture of neutral and acidic mucins as they exhibited both PAS- and AB-positive reactions. This observation was similar to that reported in the guinea fowl (Selvan *et al.* 2008); though, it was not consistent with previous reports from Suganuma *et al.* (1981), Inforzato and Sasso (1985), Prasad and Kakade (1990), and Pastor *et al.* (1988), who had observed that neutral mucins were more than acidic mucins in the ductal epithelium. Furthermore, Imai *et al.* (1991) and Paster *et al.* (1988) both identified that the neutral and acidic mucins were of extremely small quantities in those of fowl. In present study, when the samples stained by using the AF-AB (pH 2.5) technique were studied, it was observed that the cells lining duct showed alcianphilia, thus indicating the occurrence of the carboxylated mucins (sialomucins), which was in consisted with the finding of Suganuma *et al.* (1981). Contrary to our findings were Imai *et al.* (1991) and Chikilian and Speroni (1996) identified only sulfated mucins present in the epithelium of the central chambers. The cells of corpus glandulae showed negative reactions for mucins. This coincided to previous observations by various workers (Inforzato and Sasso 1985; Selvan *et al.*, 2008; Pastor *et al.*, 1988), except Chikilian and Speroni (1996) who identified a moderate amount of glycoprotein in the secretory portion of the glands of tinamou. Sgambati *et al.* (1995) reported mucins in the glandular cells of the compound glands of the chick embryo, found and their histochemical properties were changed during incubation. These results indicated that the mucins in the proventricular mucosa might possess the functional differences. The proventriculus-gizzard junction showing negative reactions for mucins proved that it was a transition of the proventriculus and gizzard.

Compared with the birds studied, the diet of black-tailed crake mostly resembled that of the grey starling (Passeriformes). But the distribution and histochemical properties of the mucins in their proventriculi were significantly different (Suganuma *et al.*,1981). Furthermore, although the chicken, fowl, guinea fowl, Japanese quail and red jungle fowl were in the same family of Phasianidae and took similar foods, these differences still existed to varying degrees (Aitken, 1958; Pastor *et al.*, 1988; Imai *et al.* 1991; Selvan *et al.*, 2008; Kadhim *et al.*, 2011). On the other hand, the Japanese quail, domestic pigeon, grey starling and tree sparrow had different diets and were in different orders, but their mucins in the stomach had similar histological characteristics (Suganuma *et al.*, 1981). As a result, the differences in the distributions and histochemical properties of the mucins between the birds could not solely be attributed to various diets or species differences.

The contents in the cavities of the simple tubular glands were PAS-positive, which was in consistence with the findings of Selvan *et al.* (2008), Kadhim *et al.* (2011) and Zhu *et al.* (2013). The glandular cells were PAS-negative; Zhen (1995) stated that the matrix solidified in the cavities to form cuticula. These results revealed that the histological properties of the secreted materials might change with this procedure. The endothelial cells were both PAS-positive and AB-positive, but stained blue with PAS-AB stain, implying the absence of neutral mucins and the occurrence of acidic mucins. This was contrary to the findings of Inforzato and Sasso (1985) and Selvan *et al.*, (2008) who found that neutral mucins were predominant in the epithelium. According to the result of AF-AB stain, these cells stained blue implicating the presence of carboxylated mucins; however, Inforzato and Sasso (1985) only found the sulpho-mucins, and Pastor *et al.* (1988) identified the existence of both sialo- and sulpho-mucins. The other parts of the gizzard showed negative reactions for mucins. This finding was in accordance with the observations of many authors (Aitken, 1958; Suganuma *et al.*, 1981; Inforzato and Sasso, 1985; Pastor *et al.*, 1988 and Selvan *et al.*, 2008); except Chikilian and Speroni (1996) who reported that acidic mucins was present all the components of the gizzard of tinamou. It has been speculated that the function of the glands of gizzard was to form the cuticula, which replaced the defense function as the mucins in the proventriculus. The mucins between the endothelial cells and the koilin mostly served as lubricant. On the other hand, this was proved by the fact that carboxylated, sulfated and neutral mucins appeared simultaneously at the tubular glands of the chick embryo gizzard, whose cuticle had not formed (Sgambati *et al.*, 1996).

Akester (1986) and Kadhim *et al.* (2011) proposed that the koilin consisted of vertical rodlets produced by the gizzard glands and a horizontal matrix produced by the surface epithelial cells, and the lamellae of koilin were horizontal. On the contrary, the vertical matrix of black-tailed crake was constituted by the de-squamated cells lining the surface of the tubular glands and the materials secreted by the glands. As a result, the lamellae of koilin were perpendicular. Akester (1986) had proposed that the koilin in the glands might be pushed upwards by freshly secreted materials and carried upwards by movement of the glands themselves. According to the histological findings in this study, the release of the materials in glands of gizzard might also be due to the constriction of the muscular layer. The function of koilin in the gizzard was mostly compensation of the absence of teeth (Zhen, 1995), which may be because the koilin was loose, compared with that in the proventriculus-gizzard junction.

In conclusion, the present study demonstrated that the histological architecture of black-tailed crake's stomach and the distribution of its mucins resembled with those of other avian species, whereas some findings, as the arrangement of koilin and the histochemical properties of mucins in the proventriculus, were peculiar comparing with pre-existing reports.

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Statement about conflict of interest

The authors have no conflict of interest to declare.

REFERENCES

ABUMANDOUR, M.M., 2013. Morphological studies of the stomach of falcon. *Sic. J. Vet. Adv.*, **2**: 30-40.

AITKEN, R.N.C., 1958. A histological study of the stomach and intestine of the chicken. *J. Anat*., **92**: 453-466.

AKESTER, A.R., 1986. Structure of the glandular layer and koilin membrane in the gizzard of the adult domestic fowl (*Gallus gallus domesticus*). *J. Anat*., **147**: 1-25.

CHEAH, P.L. AND RAMACHANDRAN K., 1994. Alterations in mucin type: an indicator for suspicion of malignant gastric transformation. *Malaysian J Pathol*., **16**: 39-42.

CHIKILIAN, M. AND SPERONI, N.B.D., 1996. Comparative study of the digestive system of three species of Tinamou. I. *Crypturellus tataupa*, *Nothoprocta cinerascens*, and *Nothura rnaculosa* (Aves: *Tinamidae*). *J. Morphol*., **228**: 77-88.

EIDAROOS, H., YOSHIMURA, Y. AND HELMY, S.A., 2008. Distribution of the ghrelin hormone producing cells in the gastro-intestinal tract of some birds (immunohistochemical study). *J. Vet. Anat.*, **1**: 14-21.

HASSAN, S.A. AND MOUSSA, E.A., 2012. Gross and microscopic studies on the stomach of domestic duck (*Anas platyrhynchos*) and domestic pigeon (*Columba livia domestica*). *J. Vet. Anat*., **5**: 105-127.

HODGES, R.D., 1974. *The histology of the fowl*. London, Academic Press, pp. 35-88.

IMAI, M., SHIBATA, T., MORIGUCHI, K., YAMAMOTO, M. AND HAYAMA, H., 1991. Proventricular glands in fowl. *Okajimas Folia Anat. Jpn*., **68**: 155-60.

INFORZATO, D.L.M.A. AND SASSO, W.D.A.S., 1985. Histochemical detection of glycoproteins in the gastric epithelia of *Columba livia*. *Acta Histochem.*, **76**: 145-150.

INSKIPP, T.P. AND ROUND, P.D., 1989. A review of the black-tailed crake, *Porzana bicolor*. *Forktail*, **5**: 3-15.

KADHIM, K.K., ZUKI, A.B.Z., NOORDIN, M.M. AND BABJEE, S.M.A., 2011. Histomorphology of the stomach, proventriculus and ventriculus of the red jungle fowl. *Anat. Histol. Embryol*., **40**: 226-233.

KARAMBELKAR, R.R., SHEWALE, A.D., KARAMBELKAR, R.A. AND UMARJI, B.N., 2014. Histochemical study of mucosubstances of normal human parotid salivary gland. *J. Evol. Med. Dent. Sci*., **3**: 13079-13085.

LIMAN, N., ALAN, E. AND BAYRAM, G.K., 2010. The differences between the localizations of Muc1, Muc5ac, Muc6 and Osteopontin on quail proventriculus and gizzard may be a reflection of functional differences of stomach parts. *J. Anat*., **40**: 57-66.

OGUNKOYA, Y.O. AND COOK, R.D., 2009. Histomorphology of the proventriculus of three species of Australian passerines: *Lichmera indistincta*, *Zosterops lateralis* and *Poephila guttata*. *Anat. Histol. Embryol.*, **38**: 246-253.

PASTOR, L.M., BALLESTA, J., MADRID, J.F., TOMAS, R.P. AND HERNANDEZ, F., 1988. A histochemical study of the mucins in the digestive tract of the chicken. *Acta Histochem*., **83**: 91-97.

PRASAD, R.V. AND KAKADE, K., 1990. Histology and histochemistry of proventriculus of domestic duck (*Anas platyrhynchos Linnaeus*). *Mysore J. agric. Sci*., **25**: 506-511.

ROCHA, D.O.S. AND LIMA, M.A.I., 1998. Histological aspects of the stomach of burrowing owl. *Rev. Chil. Anat.*, **16**: 191-197.

SALEEM, S., NASEEM, N., HANNAN, A., NAGI, A.H., QAMAR, M.U. AND AKHTAR, N., 2015. Dose related histological changes in mouse kidney after administration of manuka honey. *Pakistan J. Zool.*, 47: 235-239.

SELVAN, P.S., USHAKUMARY, S. AND RAMESH, G., 2008. Studies on the histochemistry of the proventriculus and gizzard of post-hatch guinea fowl (*Numida meleagris*). *Int. J. Poult. Sci*., **7**: 1112-1116.

SGAMBATI, E., BRYK, S.G. AND GHERI, G., 1995. Histochemical characterization of the mucins of the epithelial cells in the chick embryo proventriculus. *Ital. J. Anat. Embryol*., **100**: 83-97.

SGAMBATI, E., BRYK, S.G. AND GHERI, G., 1996. Histochemical study of the epithelial mucins in the gizzard of the chick embryo. *Ital. J. Anat. Embryol*., **101**: 173-185.

SUGANUMA, T., KATSUYAMA, T., TSUKAHARA, M., TATEMATSU, M., SAKAKURA, Y. AND MURATA, F., 1981. Comparative histochemical study of alimentary tracts with special reference to the mucous neck cells of the stomach. *Am. J. Anat*., **161**: 219-238.

ZAHER, M., GHAREEB, A.W.E., HAMDI, H. AND

ABUAMOD, F., 2012. Anatomical, histological and histochemical adaptations of the avian alimentary canal to their food habits: I-*Coturnix coturnix*. *Life Sci. J*., **9**: 253-275.

ZHEN, G.M., 1995. *The birds*. Beijing Normal University Publishing, Beijing. pp.56-65.

ZHU, L., WANG, J.J., SHI, X.D., HU, J. AND CHEN, J.G., 2013. Histological observation of the stomach of the Yellow-billed Grosbeak. *Int. J. Morphol*., **31**: 512-515.

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