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Original Article

Gross, histological and histochemical investigation of the stomach of the Eurasian stone curlew (*Burhinus oedicnemus*) and pied king fisher (*Ceryle rudis*)

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ABSTRACT
The structure of the stomach is correlated to the type of the food of the organism. This study investigated the gross, microscopic, histochemical and ultrastructure of the stomach of the stone curlew and pied king fisher. Six adult stone curlew and six adult pied kingfisher of both sexes were used. The results revealed that, the proventriculus was truncated cone- shaped organ in curlew; while, it is very short tube-like in kingfisher. The mucosal surface of the proventriculus has proventricular papillae over its entire surface. The ventriculus was biconvex lens in shape in curlew; while, in kingfisher it is sac-like organ. The thick muscular wall in curlew consisted of the Crassus caudodorsalis, C. cranioventralis muscles, tenuis craniodorsalis and caudoventralis; while, the wall is thin in kingfisher so this organization is unclear. The proventriculus and ventriculus in both studied birds have folds of the tunica mucosa lined by columnar epithelium. Simple tubular glands occupied the lamina propria. The ventricular glands were lined by simple columnar cells. The proventricular glands were situated between the inner and outer layers of the lamina muscularis mucosae. The tunica submucosa was very thin in the proventricular wall; while, in the ventriculus, it was not separated from the lamina propria due to the absence of any lamina muscularis mucosae. Musclosa of ventriculus in kingfisher consisted of three layers: an internal longitudinal layer, a middle circular layer and an external layer of longitudinal smooth muscle fibers; while, in curlew the outer longitudinal layer was absent. In conclusion, the study detected many variations in the stomach structures of birds subjected to study and these differences could be due to the differences in food habit. Further studies should be carried out for more understanding the physiological process of digestion and nutrient absorption in these birds.

INTRODUCTION

Egypt is located at the Northeastern corner of Africa and occupies an area of about one million kilometers. It enjoys a unique strategic location, at the crossroads between Africa, the Middle East, and Europe. Egypt is divided into four geographical regions, namely, Nile Valley and Delta, Western Desert, Eastern Desert, and Sinai. The country is rich in wild bird species due to its wide range of habitats (Issa, 2019). The Eurasian stone-curlews, also known as dikkops or thick-knees, consist of 10 species within the family Burhinidae, and are found throughout the tropical and temperate parts of the world, with two or more species occurring in some areas of Africa, Asia, and Australia. Despite the group being classified as waders, most species have a preference for arid or semiarid habitats Bock (1994). It is a species of European conservation concern (BLI, 2012; BLI, 2015). The Eurasian stone-curlew is largely nocturnal, especially when singing its loud wailing songs. Its food consists of insects and other small invertebrates, and occasionally small reptiles, frogs and rodents (Dunning, 1992). Eurasian Stone Curlew Burhinus oedicnemus is a summer breeder in Turkey (Boyla 2016), a resident breeder in Cyprus (Bird Life International, 2015).

Pied kingfisher (*Ceryle rudis*) belongs to a family of ~90 species that range in size from the 9-g African Dwarf Kingfisher (Ceyx lecontei) to the ~500-g Laughing Kookaburra, *Dacelo novaeguineae* (Woodall 2001), widely distributed across Africa and Asia. It was one of the three most common types in the world. In ancient times believed this type descended from an ancestral American green kingfisher which crossed the Atlantic Ocean about 1 million years ago (Fry, 1980**;** Al-Mamoori, 2016). The Pied Kingfisher *(Ceryle rudis)* characterized by maculata plumage consisting of the black and the white color. It`s non-migratory birds, can be seen near lakes and rivers, and the feeds mainly on fish, crustaceans and large aquatic insects such as dragonfly larvae fish (Wanink, 1994; Al-Mamoori, 2016). Its characterized by ability to fly for a long time (fry et al, 1992; Dyce et al., 2010**;** Al-Mamoori, 2016).The digestive system in birds composed of buccal cavity, pharynx, esophagus, proventriculas, gizzard, small and large intestine and cloaca (Al Kinany, 2017). Clear variations are reported in avian digestive tract and type of food ingested (Al Kinany, 2017). The avian wall of digestive tract is composed mainly of four basis layers arranged from inner to outer, mucosa, submucosa, muscularis externa There is some variations in the microscopic structure and also thickness of these layers according to types of birds also types of ingested food (Rajabi and Nabipour, 2009).

Bird could be classified to three categories or group in relation to their stomachs function and food digestion process; first group; birds eat soft food, in this group, the gizzard acts as stock piling of food. Second group; birds eat hard diet and gizzard grinds the food and third group, birds eat intermediate diet and the gizzard acts for storage and physical digestion (Hassouna, 2001).

Due to the dearth of information on histological and anatomical structures of digestive system in wild birds, especially **Eurasian stone curlew and pied king fisher** birds. The aim of the present study was to illustrate the morphology, histology, histochemistry, and ultrastructure of the stomach of the Eurasian stone curlew and pied kingfisher. These observations will provide a basis for understanding the digestive physiology and help pathologists and nutritionists in future studies on diet and diseases affecting the species by facilitating the histopathological diagnosis of such diseases affecting bird digestive system.

Materials and Methods

Study area and Experimental birds

A total of twelve adult apparently healthy birds of both sexes from **Eurasian stone curlew and pied kingfisher birds,** were obtained from bird's hunters at Sharkeia governorate, Egypt between years of 2023-2024. Species identification and age determination were adopted according to keys given by Klos and Lang (1982). The current work was completed in accordance with animal welfare guidelines and the Faculty of Veterinary Medicine, Suez Canal University Ethics Committee, as well as Egyptian laws.

Gross morphological examination

Four birds from each species were selected, weighed, and anaesthetized with chloroform, slaughtered and allowed to exsanguinate. The ventral body wall of birds were longitudinally incised from the vent as far as the cranial end of the sternum, the flaps of the body wall were reflected on both sides sternum. The birds were laterally dissected, and the upper digestive tract were photographed in situ to provide a detailed description of its shape, position, and relationships with other organs, according the technique described by Basha et al. (2023).

Macro-morphometric measurements

The macro-morphometric measurements were performed as the following, the upper digestive tract birds were carefully resected. The stomachs were grossly examined in situ and carefully dissected, the isolated whole stomach, glandular stomach and muscular stomach were weighed, photographed. Gross morphometric measurements were conducted using Vernier's caliber following the procedures outlined by El Nahla et al. (2011) and El Mahdy et al. (2022)

Light microscopic examination

Small pieces of 1 cm^3 were taken immediately from same specimen of proventriculus and gizzard, that were used previously for the gross examination. specimens were fixed in 10% formaldehyde for one week, dehydrated at increasing ethanol concentrations, embedded in paraffin, sectioned at 5.0 µm with a rotary microtome and stained with hematoxylin–eosin (H&E), Masson´s trichrome stain, Verheofen van Gieson stain, Alcian blue (AB) and Periodic acid Schiff (PAS) stains according guidance and keys given by Bancroft et al (2008).

Scanning electron microscopy

The remaining birds from each species(two birds each) were utilized for scanning electron microscopic examination. The specimens were placed in 2.5% glutaraldehyde for 24 h at 4°C, then washed with phosphate-buffered saline (PBS, pH 7.4). Subsequently, the specimens were post-fixed in 1% aqueous osmium tetroxide for 4 h and rinsed in PBS (pH 7.4). after then the samples were dehydrated in ascending grades of ethanol and underwent critical point drying. The dried specimens were sputtered with a layer of gold at a thickness of 100 nm using a BIO-RAD sputter apparatus (Bio-Rad) and scanned using a scanning electron microscope (SEM, Model-JEOL ASID-10, Cambridge Ltd.) in the National Research Center, Cairo, Egypt. The scanning process was carried out According to instructions of manufacturer. The histological analysis and identification of stomach ultrastructures were performed by researchers and assistance from experts in the above mentioned center

RESULTS

Gross Morphological Structures

Glandular Stomach (proventriculus)

The result revealed that, the glandular stomach is an elongated, truncated cone- shaped organ in curlew; while, it is very short tube-like in kingfisher. It is directed craniocaudally somewhat ventrally and to the left, in the left ventral part of the body cavity. It extends between the levels of the 3rd and 7th in kingfisher, 3rd and 6th in curlew (Fig. 1C and D). Externally the junction of the glandular stomach and the esophagus is obvious in curlew; while, in kingfisher it is indistinct. Caudally, however, at the junction with the muscular stomach there is a distinct lighter colored constriction, the isthmus in kingfisher; while, it is unclear in curlew (Fig., 2A & B). The demarcation was by the appearance of the surface (the surface of oesophags was smooth with longitudinal folds; while, that of proventriculus showed densely packed elevations which represent the proventricular glands (Fig. 2 C, D, E & F). The color line of demarcation in oesophagus was whitish; while that of proventriculus was light brown (Fig., 2E & F) in addition, The wall of proventriculus was thicker than that of the oesophagus. Much of the left and ventral surfaces of the glandular stomach is close to the liver, and especially to the left lobe in which it produces an impression. The right side is caudo-dorsally close to the spleen; while, in kingfisher the spleen related to the left side of the junction of glandular and muscular stomach. The dorsal surface is related to the ventral surface of the lung (Figs., 1A & B). The caudal part of the dorsal surface is related to the left testicle in the male (and from the ovary and the cranial part of the oviduct in the female) (Figs., 1A & B).

Examination of the mucosal surface of the proventriculus revealed the presence of raised papillae, papillae proventricularis, over its entire surface (Fig. 1). The average number of the proventricular papillae in curlew was 167 ± 1.99 /$cm^{2,}$ whereas; in kingfisher was 229 ± 2.08 /cm^2. The mean length of the proventriculus in curlew was 1.885 ± 0.016 cm; whereas, in kingfisher was 1.071 ± 0.025 cm. The diameter of the proventriculus in curlew was 1.935 ± 0.027 cm; whereas, king fisher was 1.47± 0.038 cm. The other morphometrical measurements were illustrated in Table 1.

Muscular Stomach

The muscular stomach is a large organ shaped like a biconvex lens in curlew; while, in kingfisher it is sac-like organ. Its craniocaudal diameter is greater than its dorsoventral diameter. It lay in the left caudo-dorsal region of the thoraco-abdominal cavity (Figs. 1C & D) in all examined species. It is situated between approximately the levels of the 1st and 12th lumbosacral vertebrae in kingfisher and 1st and 10th lumbosacral vertebrae in the curlew. It lies essentially in the vertical plane. However, its craniocaudal axis is directed somewhat ventrally and to the right in the left ventral part of the body cavity, and its most ventral part often crosses the midline to the right side. Its right surface related to the right lobe of the liver and the descending lobe of duodenum in kingfisher the right lobe of liver covers the cranial third of the right surface of the ventriculus; while, in curlew it covers all the same surface (Figs. 1A & B). It joined the proventriculus by the cardiac sphincter and joined the hind gut by the pyloric sphincter. In the two examined species the cranial one third of the left surface is related to the left lobe of liver; while, the caudal two thirds were against the flank covered by the abdominal air sac (Figs. 1). The craniodorsal sac is ill-distinct in all examined species; while, the caudoventral blind sac is only visible in same extremity of the ventriculus of the curlew.

In the male and female, part of the jejunum also lies dorsal to the muscular stomach. The dorsal part of the right surface is separated from the intestine by the left abdominal air sac. The ventral part is close to the descending and ascending parts of the duodenum and the pancreas. Caudal to the muscular stomach are the loop of the duodenum, part of the jejunum and the distal parts of the ceca (Figs. 1). The dark-colored smooth muscle of the muscular stomach is moderately developed and can be separated into two lateral muscles (the dorsal and ventral muscles) of the body in kingfisher; while, curlew has additional intermediate muscle (the caudoventral muscles) of the blind sac. All muscles attach to extensive aponeuroses in the right and left walls (Fig. 2A & B). Of the two lateral muscles the dorsal muscle extends between the aponeuroses and over the dorsal surface of the muscular stomach. The ventral muscle is similarly distributed over the ventral surface. The lateral muscles are circularly orientated. The thickness, however, is asymmetrical since the dorsal muscle is thicker caudally and the ventral muscle thicker cranially. The intermediate muscle in curlew extend between the aponeuroses over the blind sacs. It is thinner than the lateral muscles. The caudoventral intermediate muscle in curlew is continuous with the ventral lateral muscle. These muscles responsible to crush the food content of the ventriculus. The pyloric region of the stomach, connecting the ventriculus and duodenum arose from the right face of the ventriculus. The inner aspect of the ventriculus in all examined species was lined by a hardened membrane, the cuticula gastrica, which is light orange in king fisher; while, in curlew it is grayish (Fig. 2A &B). The color of the mucosa of Isthmus gastric was pinkish in curlew; while, it is ill-distinct in kingfisher (Fig., 2C & D). No stones or grit were found in the three species examined (Fig. 2).

The mean length of the ventriculus in curlew was 4.28 ± 0.049 cm; while, in kingfisher it is about 2.937 ± 0.014 cm. The mean width of the ventriculus in curlew is 3.194 ± 0.018 cm; while, in kingfisher about 2.645 ± 0.075 cm . The mean wall thickness of the ventriculus in curlew was 0.879 ± 0.031 cm; while, in king fisher about 0.197 ± 0.001 cm. The other morphometrical measurements were illustrated in Table 1.

Histological findings

Proventriculus

The results of histological examination showed that the wall of the proventriculus and ventriculus consisted of four layers: a mucous membrane (tunica mucosa gastris), the submucosa (tela submucosa gastris), a muscular layer (tunica muscularisgastris) and the serosa (tunica serosa gastris). The mucous membrane (tunica mucosa) presented folds (plicae proventriculares) and sulci at its luminal surface (Fig. 3) in curlew. The folds varied in height. These folds are unclear in kingfisher. Some of the folds were confluent with each other. The folds were lined by a columnar epithelium, but the cells seemed to diminish in height, becoming cuboidal towards the base of the sulci. Sections stained by PAS showed a positive reaction in curlew for neutral mucin (PAS positive), especially in the upper part of the folds and the surface epithelia (Fig. 3); while, it gives negative reaction in kingfisher. From the base of these folds, in curlew short simple tubular glands extended through the lamina propria. The glandular cell lining was similar to that of the folds lining the epithelium. These glands in kingfisher are tubuloalveolar and too crowded in the lamina proprea. The lamina muscularis mucosae consisted of an inner layer, which appeared as longitudinal smooth muscle bundles lying along the inner surface of the lobules of the proventricular glands (Fig. 3), and an external layer of longitudinally disposed smooth muscle fibers that appeared thicker, especially opposite the areas between the lobules of the glands (Fig. 3). The lamina propria showed many mucous glands with lymphatic infiltration.

The proventricular glands occupied the main part of the proventricular wall. The lobules of the glands were conical or rounded in curlew and elongated oval in kingfisher and are demarcated from one another by connective tissue fibers rich in blood vessels (Figs. 3). Each glandular lobule consisted of tubulo-alveolar units. These secretory units were lined by cuboidal in curlew and kingfisher that were juxtaposed in their basal portions where they made contact only with adjacent cells, giving them a dentate appearance (Figs., 3). These cells mostly had a conical shape, with a nucleus located basally and the free surface of each cell extending into the lumen of the gland, but cells with wide straight apices and a centrally located nucleus were also seen. The collecting tubules and excretory ducts were lined by a tall, simple columnar epithelium; the glandular cells of the collecting ducts showed a dentate appearance similar to that of the glandular alveolar cells (Fig. 3). However, each glandular cell group drained into the main proventricular lumen through a single mucosal papilla. The nuclei of the cells of the proventricular glands were large, round and regular. The proventricular glandular cells showed a negative reaction with the blue–PAS technique for neutral mucins but the basal lamina showed positive reaction (Fig. 3); while, in curlew it is positive. The telasubmucosa was a narrow connective tissue layer sandwiched between the circular layer of the tunica muscularis and the main mass of the lamina muscularis mucosae (Fig. 3). The tunica muscularis consisted of an inner longitudinal, middle circular layer of smooth muscle fibres and an external longitudinal layer (Fig. 3) in the two species, the longitudinal layer is thicker. The tunica serosa consisted of connective tissue rich in blood vessels, a nervous plexus and adipose tissue, all covered by mesothelium (squamous cell layer).

Ventriculus

The ventriculus of the two species was characterized by an internal abrasion-resistant lining membrane (koilin) and a thick muscular tunic. The tunica mucosa was covered by an inner layer or 'horizontal koilin lining' which was a PAS-positive layer. The coilin is thick in curlew. The folds of the mucous membrane are lined by columnar epithelium in the two species (Fig. 4). In curlew, the cells at the tip of the epithelial folds appeared taller. The epithelial folds are longer in curlew. The cells at the tip of the epithelial folds appeared taller and seemed to have larger secretory vesicles in all species examined which stained green with Masson's trichrome stain, revealing their mucous secretions; they secret mucous which appeared mostly in the lumen and underneath the koilin lining (Fig. 4).

Simple tubular glands extended from the lamina propria to the surface epithelium. Each tubule consisted chiefly of cuboidal cells with large, round nuclei in a basophilic cytoplasm and a few, large, pale staining basal cells (Fig. 4). The glandular lumina were filled with PAS-positive vertical koilin material (basal and luminal secretions) that extended to the horizontal koilin lining (Fig. 4). The basal ends of the glands, which were situated deeply in the lamina propria, showed great coiling (Fig., 4). The lamina muscularis mucosae consists of thick layer of longitudinally arranged smooth muscle cells (Fig. 4). The ventricular glands obscured most of the lamina propria, terminating at a layer of dense connective tissue known as the tela submucosa (Fig. 4). The tunica muscularis in kingfisher consisted of three layers: an internal longitudinal layer, a middle circular layer and an external layer of longitudinal smooth muscle fibres; while, in curlew the outer longitudinal layer was absent. The cercular layer form most of the bulk of tunica muscularis in the two species. The muscle bundles were extensively surrounded by dense connective tissue fibres (Fig. 4). The tunica serosa consisted of connective tissue and ganglionic cells of the nervous plexus that were covered by mesothelium (Fig. 4).

Scanning microscopic findings

The epithelium and lamina muscularis mucosa of proventriculus are thicker in curlew than kingfisher (Fig. 5). The surface of the mucosa of gizzard was even in curlew; while, in kingfisher it was folded (Fig. 6). The opening of the tubular glands of gizzard were wide, circular with the same size and regularly distributed filled with vertical koilin in curlew; while, in kingfisher they were very small irregularly distributed with different sizes (Fig. 6). The tubular glands of gizzard are more numerous in kingfisher the curlew, while the epithelium and tunica musclosa is thicker in curlew than kingfisher (Fig. 6).

Table 1. Gross morphometrically measurements of the stomach in Stone curlew and pied kingfisher

Stomach`s part	Measurement	Stone curlew	Pied kingfisher
General	Whole weight of bird (gm)	387.5 ± 2.82	95 ± 1.8
	Whole GIT length (cm)	89.438 ± 0.049	91.50 ± 0.01
	Weight of whole stomach (gm)	11.68 ± 0.163	2.85 ± 0.07
	Weight ratio of whole stomach to body weight	3%	3%
	Length ratio/ GIT	12.90%	7.80%
	Wall thickness (cm)	0.26 ± 0.017	0.06 ± 0.002
Proventriculus	Width (cm)	1.935 ± 0.027	1.47 ± 0.03
	Length(cm)	1.885 ± 0.016	1.071 ± 0.02
	Length ratio /GIT	2.11%	1.17%
	Weight ratio to whole stomach	11.95%	16.10%
	Wall thickness (cm)	0.344 ± 0.026	0.102± 0.005
	Number of proventricular gland openings /cm^2	167 ± 1.99	229 ± 2.08
	Shape of the glandular opening	Rounded	Rounded
	diameter of the glandular opening (cm)	0.031 ± 0.003	0.04 ± 0.002
Ventriculus	Width (cm)	3.194 ± 0.018	2.645 ± 0.07
	Length (cm)	4.284 ± 0.049	2.937 ± 0.01
	Length ratio /GIT	4.79%	3.21%
	Weight (gm)	10.168 ± 0.014	2.348 ± 0.02
	Weight ratio to whole stomach	87.05%	82.18%
	Wall thickness (cm)	0.879 ± 0.031	0.197± 0.001

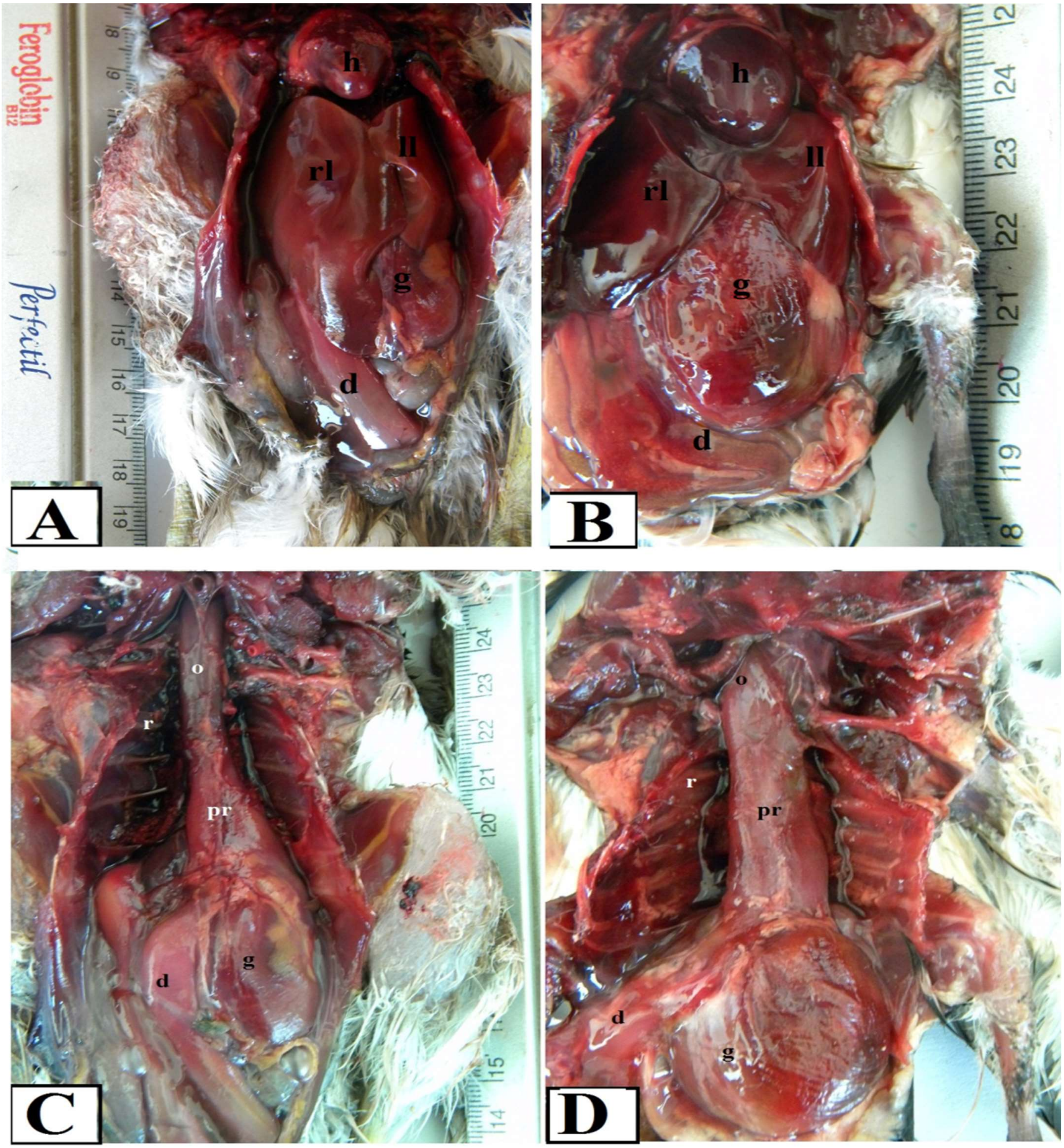

Fig.1.A photograph of ventral view of dissected thoraco-abdominof adult curlew (A) and kingfisher (B) and after removal of liver, lung and heart of adult curlew (C) and kingfisher (D) showing, esophagus (o), heart (h), Proventriculus (pr), Ventriculus (g), right lobe of the liver (rl), left lobe of the liver (ll), duodenum (d), ribs (r).

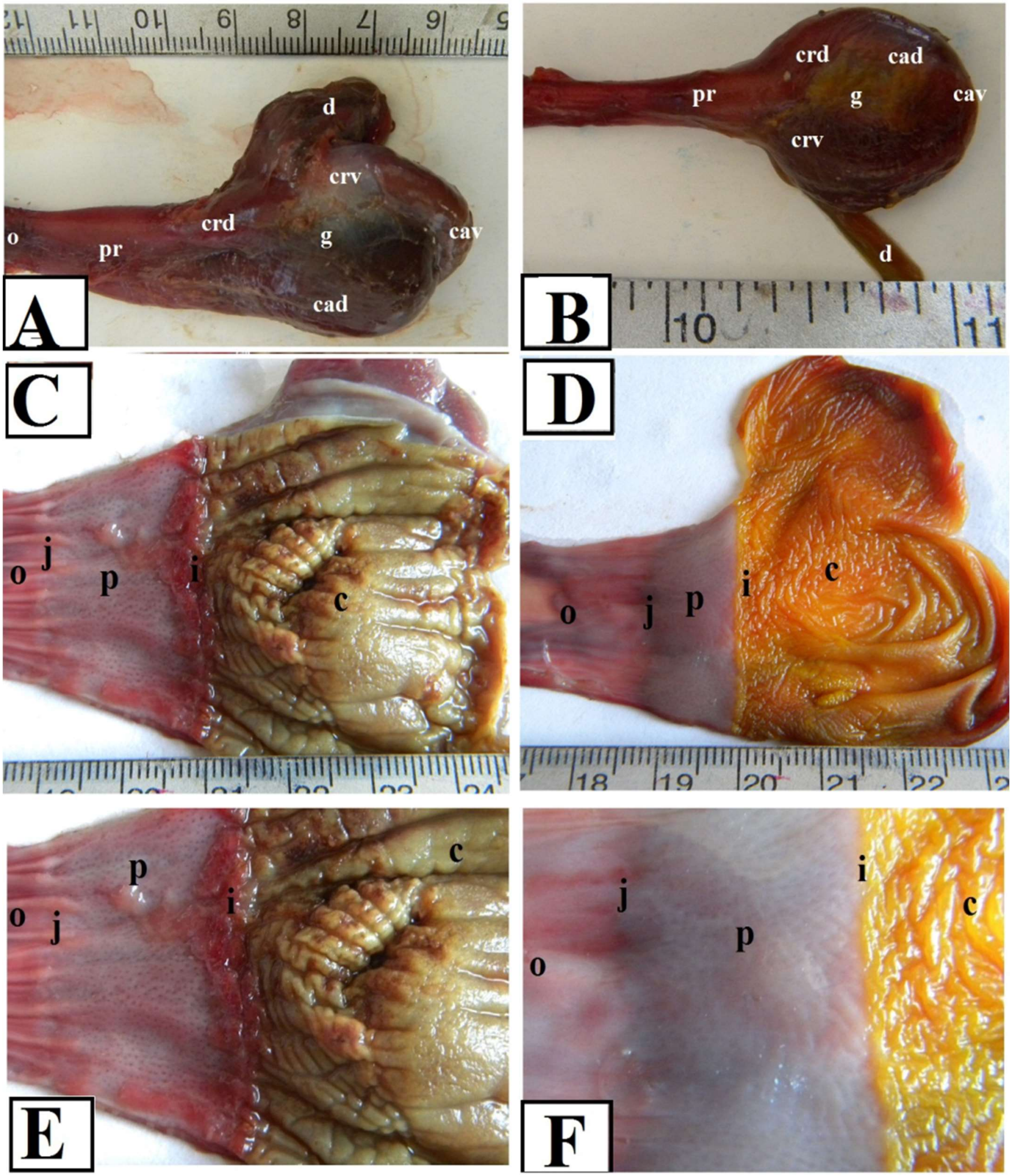

Fig.2. A photograph of intact stomach of adult curlew (A) and kingfisher (B). A photograph of the interior of the stomach of adult curlew (C&E) and kingfisher (D&F) showing, Esophagus (o), Proventriculus (pr), Ventriculus (g), M. crassus cranioventralis (crv), M. tenuis craniodorsalis (crd), M. crassus caudodorsalis (cad), M. tenuis caudoventralis (cav), isthmus gastris (i), Junctura esophago-proventricularis (j).

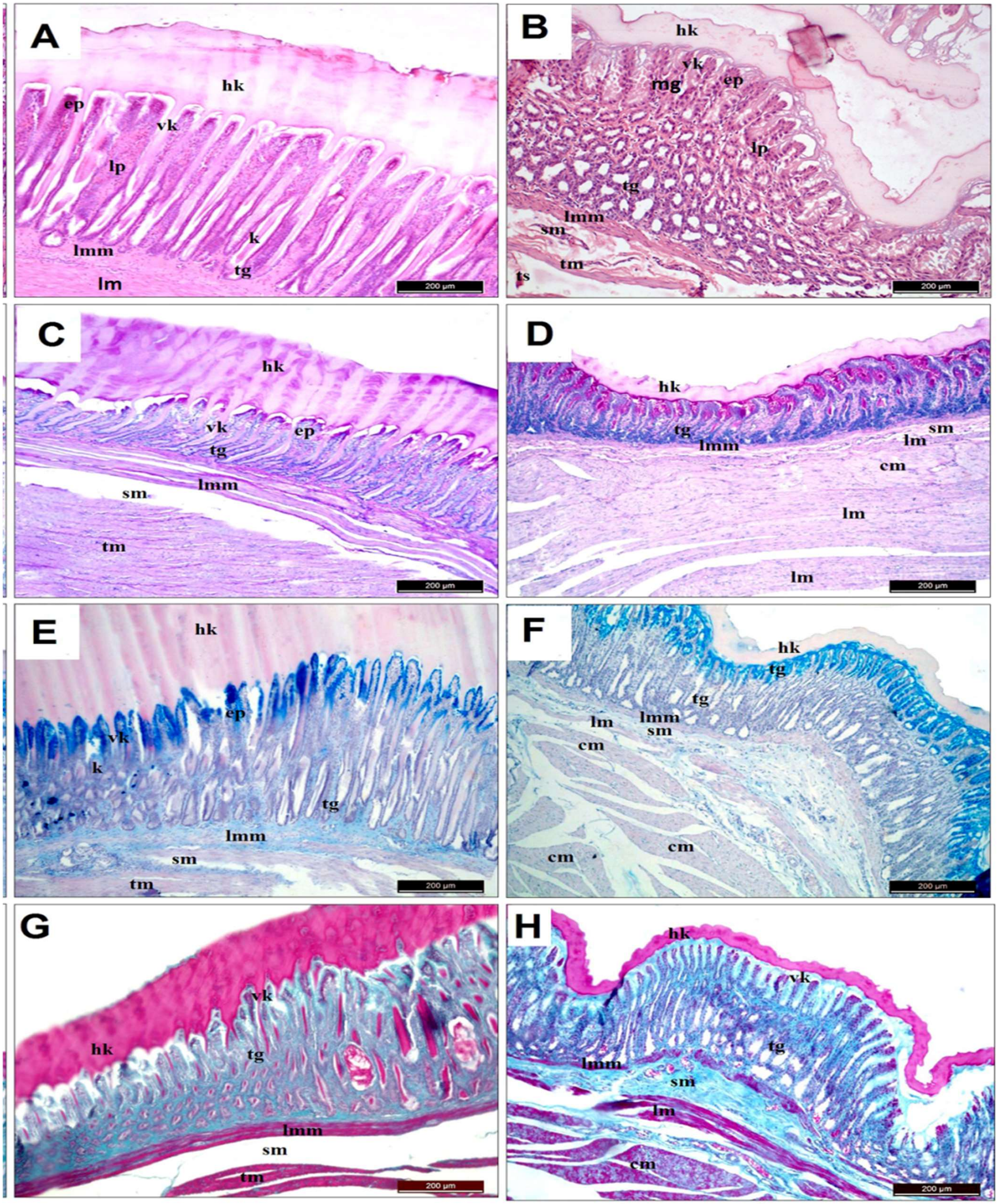

Fig. 3. A photomicrograph of a longitudinal section in the proventricular wall of adult curlew (A) and kingfisher (B) H and E, adult curlew (C) and kingfisher (D) PAS, adult curlew (E) and kingfisher (F) alcian blue and adult curlew (G) and kingfisher (H) massons trichrome showing, lamina epithelialis (ep), Lamina propria submucosa (lp), superficial proventricular simple tubular glands (tg), deep proventricular glands (pg), which had clear lumen (lg).

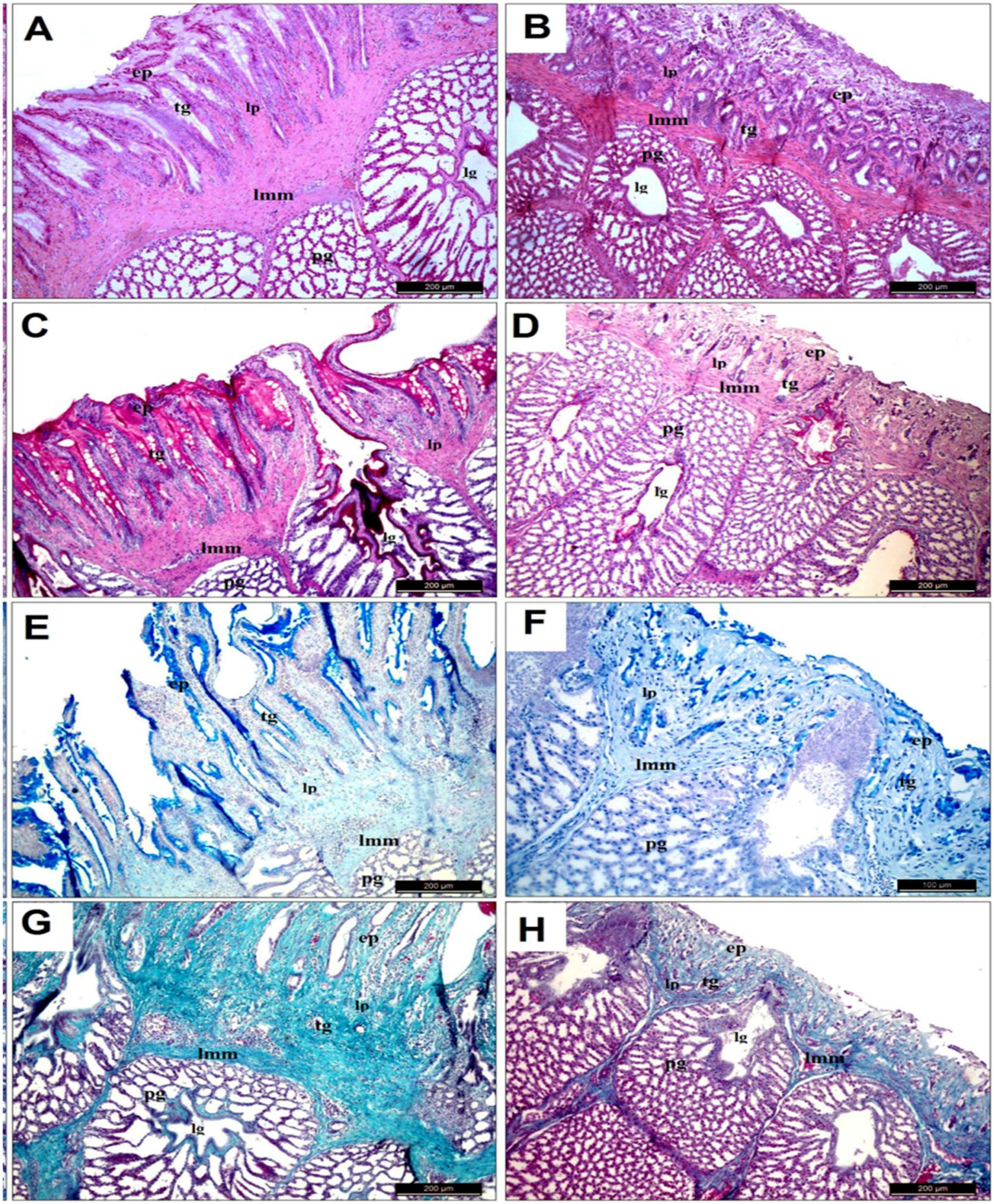

Fig. 4. A photomicrograph of a longitudinal section in the ventricular wall of adult curlew (A) and kingfisher (B) H and E, adult curlew (C) and kingfisher (D) PAS, adult curlew (E) and kingfisher (F) alcian blue and adult curlew (G) and kingfisher (H) massons trichrome showing, lamina epithelialis (ep), lamina muscularis mucosa (lmm), Lamina propria (lp), tubular glands (tg), muscular longitudinal layer (cm), smooth muscular circular layer (cm), submucosa (sm), horizontal koilin (hk), vertical koilin (vk), mucosal glands (mg).

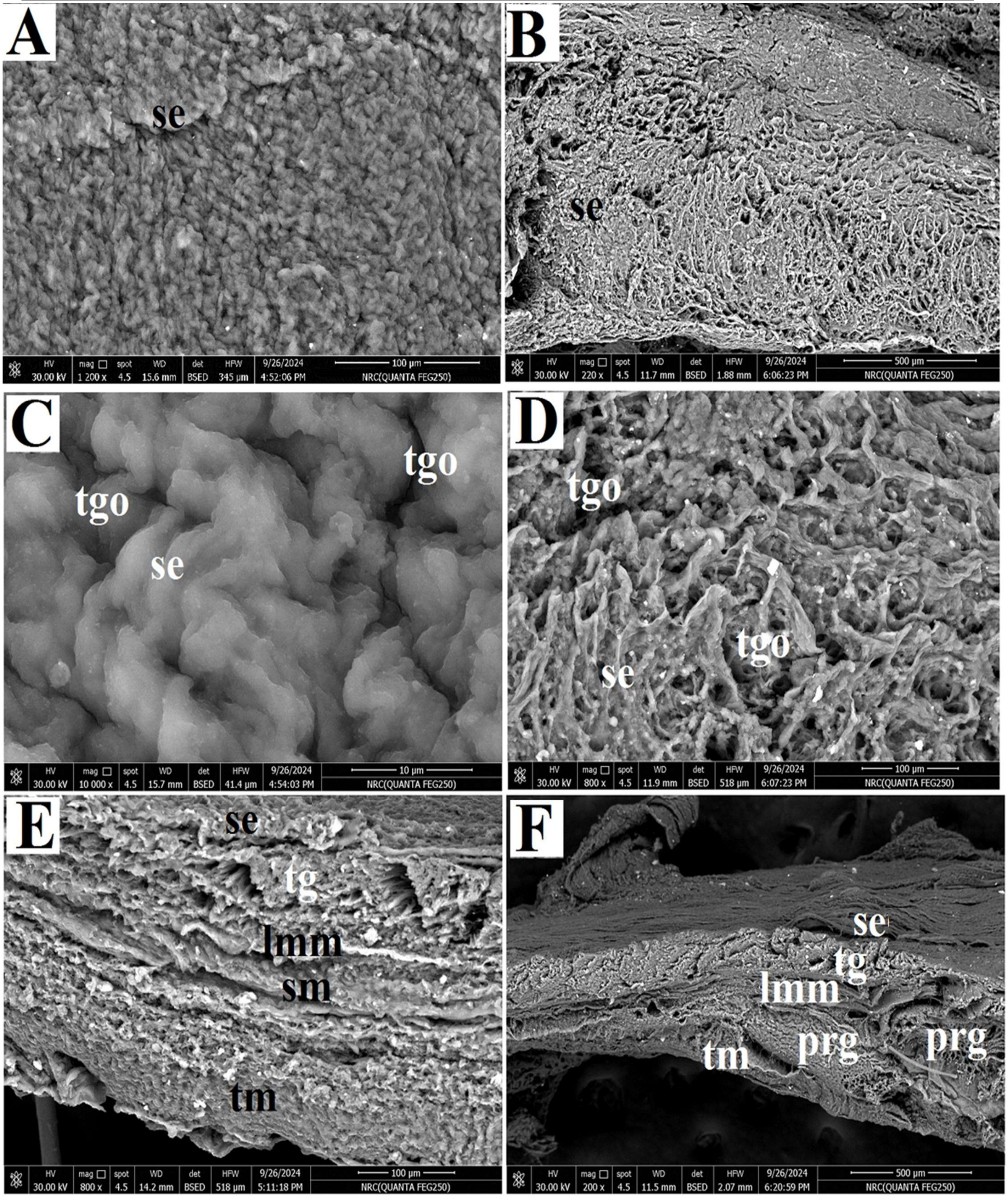

Fig.5. Scanning electron micrograph of the proventriculus of adult curlew (A) and kingfisher (B) showing, surface epithelium (se), openings of the tubular glands (tgo), tubular glands (tg), lamina muscularis mucosa (lmm), submucosa (sm), tunica muscularis (tm).

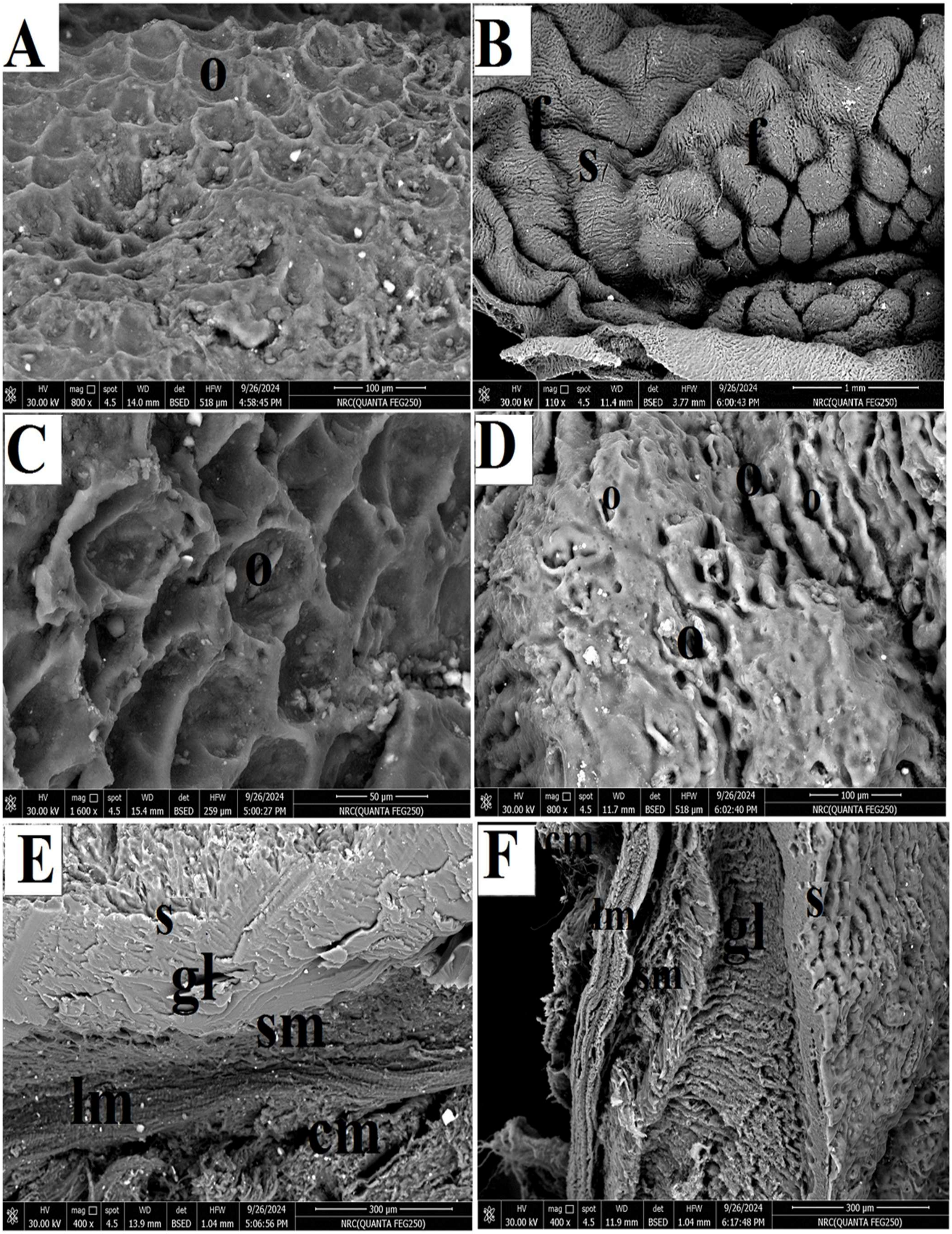

Fig.6. Scanning electron micrograph of the ventriculus of adult curlew (A) and kingfisher (B) showing, surface epithelium (s), fissure (f), openings of the tubular glands (o), tubular glands (gl), submucosa (sm), longitudinal muscular layer (lm), cercular muscular layer (cm).

DISCUSSION

The present work was carried out on 10 birds of two species, namely, Eurasian stone curlew and pied kingfisher in order to observe the morphological and histological as well as fine structure of stomach.

The stomach of the birds is divided into two chambers. The first stomach is proventriculus and the second chamber is called gizzard or ventriculus. Gizzard stomach is grinding function of grains by their muscles, so that it's facilities indigestive process (Rebecca, 2021). The two chambers of stomach are different in size and shape depending on the nature of birds' diets as carnivores, piscivorous and granivorous birds. The glandular stomach (proventriculus) is characterized thin layer based on the food force, grains (AL-Taai, 2022). The stomach in fowl is composed of glandular stomach (proventriculus) and muscular portion that is ventriculus or gizzard (Suganuma et al., 1981; Macari et al. (1994); Dyce et al., 1996; Baily et al., 1997; Bacha and Bacha, 2000), which are separated by an isthmus. The chicken stomach is located at the left of the median line and is situated dorsal to the liver. In carnivorous and piscivorous fowls that swallow big victuals very little distinction exists between the glandular and the muscular stomach (Sisson & Grossman, 1986; Baumel et al., 1993). The statements and findings of above workers are in line with results of this study. The results displayed that, the isthmus was ill-distinct in the studied birds of this study, this could be explained that the investigated birds are carnivorous in contrary with the grainvorous species the isthmus is clear as they eat small grains like chicken (Macari et al., 1994) and bustards (Baily et al., 1997).

Macari et al. (1994; AL-Taai, 2022), cited that, the avian proventriculus is a structure located between the lower esophagus and the ventriculus, lined by a glandular mucosa with secretory function, this statement is in accordance with results of current study. The long proventriculus in curlew and king fisher may due to the need of coarse food storage like what mentioned in ratites (Angel et al. 1996); while, the very short proventriculus in bee eater, this may due to that bees do not need much digestive enzyms to be digested (Basha et al., 2023). On the inner surface of the proventriculus of the bee eater (Basha et al., 2023), chicken (Sisson & Grossman, 1986, Banks, 1992, Melvin and Reece, 1996; Turk, 1982; Dyce et al., 1996) and bustards (Baily et al., 1997), there is papillae, low and wide, on the lumen; on the apex of each papilla opens one of the proventricular glands. However, in the curlew and king fisher proventriculus there are no grossly detected papillae, the ducts of the proventricular glands open in depressions on the mucosal surface. These findings are in agreement with results of the current study.

The mucosa is excessively folded, forming flat folds, Hassan and Moussa (2012) stated that the mucosal epithelium of the proventriculus of pigeon and duck was columnar and this is unlike the findings of Banks (1992) in fowl and Juliana et al. (2005) in partridge who stated that it is cuboidal. Banks (1992) in fowl and Hassan and Moussa (2012) in pigeon and duck and Basha et al. (2023) in bee eater observed that the lamina propria in the proventriculus is typical and it contains numerous lymphatic tissues, which are nodular or diffuse. An interrupted layer of guided fibers forms the muscularis mucosae longitudinally, and bunches are interdigitated between the mucous glands. Submucosa occupied by numerous submucosal glands, which are compound, ramified or tubular. Juliana et al. (2005) in partridge the gland lobules separated by connective tissue septa. These findings were on line with this study in curlew and king fisher.

In fowl, Banks (1992) and bee eater (Basha et al., 2023) recorded that tunica muscularis is formed of inner longitudinal, middle circular and outer longitudinal layers. Similar findings were recorded in this study; while, Juliana et al. (2005) in partridge and Hassan and Moussa (2012) in pigeon and duck stated that it is formed of inner longitudinal musculature and an outer circular layer. The tunica serosa in fowl (Banks, 1992), partridge (Juliana et al. 2005) and pigeon (Hassan and Moussa, 2012) is composed of connective tissue and a cuboidal cells layer; while, in the current study the cell layer was squamous as that recorded in duck (Hassan and Moussa, 2012). The differenced may be attributed to species and biological variation of the birds.

Ventriculus contains the acids and enzymes secreted in the gastric proventriculus (Turk, 1982; Macari et al., 1994). These findings in consistent with our results in curlew. considering the studied birds are carnivores, the reason behind that could be explained in view of Moawad et al., 2017, who stated that, the feeding activities of fishes and birds are classified according to the nature of food consumed by all fish and bird species into three categories; herbivores that eat plant material, omnivores which consume both plant and animal materials and carnivores one which consume animal material secreting acid and enzymes to digest their food.

Nickel et al. (1977) in avian, Juliana et al. 2005 in partridge as well as Baily et al. (1997) in bustards and Basha et al, in bee eater have described muscular stomach had format of a biconvex lens lying in the left dorsal and ventral regions of the thoracoabdominal cavity. Like that recorded by Chikilian and Speroni (1996) in *Nothura maculosa* and *Nothoprocta cinerascens,* the gizzard in kingfisher has round format; while, in *Crypturellus tataupa* it presents an oval format. The findings of above workers are partially in agreements with findings of current study. the contrast could be attributed to capacity factor and nature of food.

Baily et al. (1997) reported in chicken that, the ventriculus is constituted by four muscles, two thick and dark colored, the caudodorsalis and the cranioventralis; and two with fine thickness and clear colored, the craniodorsalis and the caudoventralis, that are responsible to crush the victuals ingested, These muscles are organized is ill-distinct in ours study as the species of the study are carnivors eating fish , whereas the fish are not so hard compared to grains which need high force for grinding.

Similar to our findings in curlew, Sisson and Grossman (1986) in chicken; Baily et al., (1997) in bustards and Hassan and Moussa (2012) in duck and pigeon indicated that the body of ventriculus separates the two tapering ends, the saccus cranialis and saccus caudalis; while, these two sacs are indistinct in kingfisher this may be due to that the muscular wall of the gizzard is ill-developed as they do not need much force to grind the fish.

In our study, the Ventriclus` weight recorded was 2.6% in curlew and 2.5% in king fisher body weight, these results are higher than findings reported in Houbara and Kori Bustards birds (1.3%), in chickens (1.9%), in duck (2.2%) of body weight (Hassan and Moussa, 2012), and lower than mean value reported by Basha et al. (2023) in bee eater (4.7%.) body weight. The discrepancies between our results and above findings could be attributed to the function of stomach in differs birds. Regarding the presence of stone in Ventriclus, Hassan and Moussa (2012) reported that presence of stones in ventriculi of duck and pigeon with different sizes. Moreover, they suggested that, the presence of stones probably ingested intentionally to assist with the grinding down of food; in contrast, the current study no stones were found in gizzards of all birds investigated, this could be explained that, investigated birds are carnivores and their food are fishes which needs only acid and enzymes to be digested.

Zhu, (2015) cited that, the ventriculus is internally lined by columnar epithelium has tubular glands open in crypts of the epithelium. Similarly, the inner layer of the muscular stomach of the birds subjected to investigation in this study, was lined by a thick cuticula gastrica, which is grayish in curlew and orange in kingfisher. Furthermore, the results revealed that, the cuticle is yellowish in most birds examined, this results are on parallel with findings recorded by Suganuma et al. (1981) in wild fowls and Juliana et al. 2005 in partridge, and Baily et al. (1997) in bustards.

Basha et al. (2023) stated that, in bee eater the lumenal surface of ventriculus is lined by a proteinaceous substance similar to keratin produced by mucous glands. These findings were similar to ours study findings in the two bird’s species studied. George et al. (1998) claimed that the glands produce the material of the hardened membrane of the gizzard are called koilin. Furthermore, Eglitis and Knouff (1962) demonstrated that the secretion of the tubular glands was a carbohydrate-protein complex; this established the non-keratin nature of the membrane, since a carbohydrate component is absent in pure keratin. George et al. (1998) in chicken recorded that there was a layer of elastic and collagen fibers is observed surrounding the ventricular mucosae, constituting the compact stratum, and externally is limited with the submucosa. The differences in nature of material produces by above glands could be attributed to needs of digestion process and protection the wall of stomach in various birds.

In accordance to the current study, Bennett & Cobb (1969) in their studies on birds demonstrated that the muscular stomach smooth muscle is mainly disposed in crossed bunches or layers, separated by connective tissue, in relation to the other visceral muscles. Banks (1992) in chicken, Basha et al. (2023) in bee eater researched to similar results on their studies, that the serosa outer to the tunica muscularis, composed of connective tissue lined by a squamous cell layer.

CONCLUSION

It could be concluded from this study, there are some variations between the stomach of stone-curlew and kingfisher. The stomach features undoubtedly correlated with its feeding habits. Further studies should be carried out for more understanding the behavior, digestion process and nutrient absorption of these birds.

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AUTHORS' CONTRIBUTION

Aref AM conducted the field survey under the supervision of Basha AAB, Aref AM and Basha AAB designed the study, prepared the primary draft; revised the manuscript; Ahmed, M.A. and Hassan, S.A. helped in processing of the specimen. All authors have seen and approved this version of the manuscript.

CONFLICTS OF INTEREST

No conflicts of interest have been declared.

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ETHICS APPROVAL

The study was approved by scientific research ethics committee, Faculty of Veterinary Medicine, Suez Canal University, Ismailia, Egypt.

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دراسة عيانية ونسيجية وكيميائية نسيجية لمعدة الكروان الحجري الأوراسي وصياد السمك

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الملخص
يرتبط تركيب المعدة دائما بنوع الطعام الذي يتناوله الكائن الحي. أجريت هذه الدراسة لمعرفة البنية التشريحية والمجهرية والنسيجية الكيميائية لمعدة الكروان الحجري وصائد السمك. تم استخدام ستة طيور بالغة من كروان حجري وستة طيور من صائد السمك من كلا الجنسين. كشفت النتائج ان المعدة الغدية عضوًا مخروطيًا مبتورًا في الكروان بينما كان يشبه الأنبوب قصيرًا جدًا في صائد السمك. يحتوي السطح المخاطي للمعدة الغدية على حليمات بطينية على كامل سطحه بكثافة متغيرة بين الأنواع المدروسة. كانت المعدة العضلية على شكل عدسة محدبة الشكل في الكروان بينما في طائر صائد السمك تشبه الكيس. يتم ربط المعدة العضلية بالمرىء عن طريق العضلة العاصرة الفؤادية وترتبط بالاثني عشر عن طريق العضلة العاصرة البوابية. يتكون الجدار العضلي السميك في الكروان من عضلات الخلفية العليا والامامية السفلى والامامية العليا والخلفية السفلى بينما يكون الجدار رقيقًا في صائد السمك لذا فإن هذا التقسيم العضلى غير واضح. كان السطح الداخلي للمعدة العضلية في كلا الطائرين مُبطنًا بغشاء متصلب يُسمى بشرة المعدة. اما في الدراسة لنسيجية، تم صبغ عينات من المعدة الغدية والمعدة العضلية باستخدام صبغة E&H وحمض شيف الدوري (PAS) وصبغة ماسون ثلاثية الألوان وصبغة فان جيزون. أظهرت النتائج ان المعدة الغدية والمعدة العضلية في كلا الطيور التي تمت دراستها تحتوي على طيات من الغلالة المخاطية المبطنة بظهارة عمودية. كانت الغدد البطينية مبطنة بخلايا عمودية بسيطة. تقع غدد المعدة العضلية بين الطبقات الداخلية والخارجية للصفيحة العضلية المخاطية. كانت الغلالة تحت المخاطية رقيقة جدًا في جدار المعدة الغدية بينما في المعدة العضلية لم يتم فصلها عن الصفيحة المخصوصة بسبب عدم وجود أي صفيحة عضلية مخاطية. تتكون الغلالة العضلية للمعدة الغدية من طبقة داخلية سميكة من ألياف عضلية ملساء دائرية وطبقة خارجية رقيقة من ألياف عضلية طولية. تتكون عضلات المعدة العضلية في صائد السمك من ثلاث طبقات: طبقة طولية داخلية وطبقة دائرية متوسطة وطبقة خارجية من ألياف عضلات ملساء الطولية بينما في الكروان تكون الطبقة الطولية الخارجية غائبة. خلصت الدراسة الى ان هناك العديد من الاختلافات في تركيب المعدة للطيور التي خاضعة للدراسة ويمكن أن تكون هذه الاختلافات بسبب الاختلافات في طريقة تناول الغذاء ونوعه. توصى الدراسة بإجراء المزيد من الدراسات لفهم العملية الفسيولوجية للهضم وامتصاص العناصر الغذائية في هذه الطيور.

الكلمات المفتاحية: **الكروان الحجري الأوراسي ، دراسة عيانية ونسيجية وكيميائية، صياد السمك**

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