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Original Research

Adaptive Interplay between Feeding Preference and Structure of the Upper Digestive Tract in African Green Bee-eater (*Merops viridissimus cleopatra*)

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Abstract

Wild bird research, particularly investigations of the interplay between feeding habits, diet, and alimentary tract anatomy, offers a captivating avenue for scientific exploration. While numerous studies have delved into the upper digestive tracts of various avian species, there remains a dearth of data on the upper digestive tract anatomy of the African green bee-eater (AG bee-eater, *Merops viridissimus cleopatra*). This study aimed to bridge this knowledge gap by elucidating the gross, microscopic, and histochemical features of the esophagus and stomach in AG bee-eaters, shedding light on their food preferences, and feeding habits. Ten adult, apparently healthy AG bee-eaters were examined, revealing structural organizations of the esophagus, proventriculus, and gizzard that parallel those observed in other avian species. Key findings encompass a protective mucous layer in the esophagus and proventriculus, coupled with a moderately thick cuticle, guarding against harm from stinging insects like bees and wasps. The upper digestive tract houses numerous mucous-secreting glands, secreting both protective acidic mucin and enzymatic-neutral mucins. The proventriculus, featuring a thin wall and abundant glandular activity, equips AG bee-eaters with vital gastric enzymes for digesting their high-protein diet. This adaptation aligns with the bird's compact upper digestive tract, well suited for processing relatively small food particles. Additionally, the ventriculus's muscular layer, moderately thick, aligns with the moderately coarser texture of the bee-eater's dietary preferences. Overall, this study unveils crucial anatomical adaptations enabling AG bee-eaters to thrive on a diet dominated by stinging insects.

KEYWORDS
African green bee-eater, Anatomy, Feeding habits, Upper digestive tract, Wild bird.

INTRODUCTION

Wild birds' investigations considered one of the most exciting research areas especially studying the relationship between their feeding habits, type of diet and the digestive tract anatomy. The avian digestive tract, unlike other animals, had undergone many morphological modifications to adapt to a diverse range of food varieties (Klasing, 1999).

Numerous previous investigations have been conducted on the upper digestive tracts, specifically the esophagus and stomach, in various bird species. However, there is no available data on the upper digestive tract anatomical features in the African green bee-eater (AG bee-eater).

Bee eaters are insectivorous birds eat flying insect, particularly bees and wasps that are hunted in the air by sallies from an open perch; it pursues an insect in a swift fly, capturing it, and then returning to the perch to kill it before eating it. Honeybees are their main food source, although they will pursue any flying insect (Wasnik, 2014). The bird consumes 200 to 400 bee-sized insects per day to meet both its own needs and those of its young (Cramp, 1999).

The present study focused on elucidation of the gross, microscopic, and histochemical features of esophagus and stomach in AG bee-eater in correlation with its food preference and feeding habits.

MATERIALS AND METHODS

Experimental animals

Ten adult, apparently healthy African Green Bee-eater of both sexes were captured using net traps in San-Elhagar, Sharkeia governorate of Egypt. The study adhered to animal welfare standards and was conducted with the endorsement from the Ethics Committee within the Faculty of Veterinary Medicine at Suez Canal University, Egypt (Approval No. scu 2023067).

Gross morphological study

All birds were weighed then five birds were anaesthetized with urethane, slaughtered and allowed to exsanguinate, then a ventral longitudinal incision was performed in the abdominal wall of three birds. The other two birds were laterally dissected, and the upper digestive tract were photographed in situ and to provide a detailed description of its shape, position, and relationships with other organs and the skeleton. The anatomical terminology employed in this investigation adhered to the standards outlined in the Nomina Anatomica Avium (McLelland, 1979).

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Light microscopic study

Small pieces of 1cm3 were taken immediately from the esophagus, proventriculus and gizzard. Subsequently, the specimens were fixed in a 10% neutral buffered formalin solution for a week and then processed using conventional histological methods to create paraffin blocks. Thin sections, approximately 5µm in thickness, were stained utilizing various techniques including hematoxylin and eosin stain (H and E), Masson's trichrome stain, Periodic acid Schiff (PAS), and Alcian blue (AB) stains, following the protocols described by Bancroft and Gamble (2008). For imaging, Leica DM 1000 Microscope (Leica Microsystems GmbH, Germany) were used.

Morphometric study

For the macro-morphometric measurements, the upper digestive tract of other five birds were carefully resected, the isolated esophagus, whole stomach, glandular stomach and muscular stomach were weighed, photographed. Gross morphometric measurements were conducted following the procedures outlined by El Nahla *et al.* (2011). Stereomicroscope (M6C-9) was used for counting the number of the proventricular opening per 1 cm^2. For the micro-morphometric measurements, three paraffin sections from each part then three microscopic field at magnification x10 were randomly selected, photographed and analyzed for the thickness of all layers using Fiji ImageJ software (Schindelin *et al.*, 2012).

Statistical analysis

All measurements were recorded as means ± SEM and statistically analyzed using SPSS® software (version 18, IBM).

RESULTS

Gross anatomical findings

Esophagus

The cervical part of the esophagus adjoined anteriorly with the oropharynx and extended along the S-shaped vertebral column in the neck (Figs.1 a and c). In the thoracic cavity, the thoracic part extended caudally dorsal to the trachea and the base of the heart to terminate in the glandular stomach (Fig. 1b). The esophagus represented about 68% of the upper digestive tract length. There was no crop detected in this specie (Fig. 1c). The esophageal internal surface carried numerous longitudinal folds (Fig. 1f).

Glandular Stomach (Proventriculus)

The glandular stomach of the AG bee-eater was a very short tube directed caudally extending between the levels of the 4^{th} and 6^{th} thoracic vertebrae in the left side of the thoraco-abdominal cavity (Figs. 1b and c). Externally, the connection between the esophagus and proventriculus was clearly obvious; however, caudally the junction with the muscular stomach was less distinct (Fig. 1d). The majority of the glandular stomach's left surface was close to the left hepatic lobe (Fig. 1a) while its right surface correlated to the spleen. Moreover, it was related to the ventral surface of the lung and to the left testicle in the male or the ovary and the cranial part of the oviduct in the female caudally (Fig. 1a). The proventricular diameter was larger than its length and the proventriculus as a whole represented about 4% of the upper digestive tract weight (Table 1). Observation of the inner surface of the proventriculus unveiled the existence of elevated numerus papillae on its interior (Figs. 1e and f).

Table 1. Gross morphometrical measurements of the upper digestive tract in the African green bee-eater.

I. General measurements	
a. Whole weight of bird	47.5±0.94
b. Whole GIT length	31.879±0.063
c. Upper GIT length	9.656±0.063
d. Upper GIT length/whole GIT ratio	30%
e. Weight of whole stomach	2.482±0.024
f. Weight ratio of whole stomach to body weight	5.22%
II. Esophagus measurements	
a. Whole length	6.767±0.154
b. Whole length/whole GIT ratio	20.50%
c. Whole length/upper GIT ratio	68.00%
d. Cervical part length	5.65±0.053
e. Thoracic part length	1.1±0.011
f. Diameter of cervical part	0.61±0.044
g. Diameter of thoracic part	0.34±0.02
h. Wall thickness	0.125±0.005
III. Proventriculus measurements	
a. Diameter	0.888±0.005
b. Length	0.377±0.012
c. Length/GIT ratio	1.18%
d. Length/upper GIT ratio	4%
e. Weight	0.148±0.025
f. Proventriculus weight to whole stomach	5.96%
g. Wall thickness	0.198±0.006
h. Number of proventricular gland openings	245 3.1
i. Shape of the glandular opening and diameter	Rounded (0.034±0.0032)
IV. Gizzard measurements	
a. Dorsoventral diameter	1.329±0.027
b. Craniocaudal diameter	2.345±0.017
c. Length /GIT ratio	7.36%
d. Length /upper GIT ratio	24%
e. Weight	2.272±0.037
f. Weight to whole stomach ratio	91.53%
g. Wall thickness	0.290 ±0.012

Note: All measurements are expressed in means (µm)±SEM except, number of proventricular Glands opening measured per (1 cm2)±SEM and weight is expressed in (gram)±SEM.

Muscular Stomach (Gizzard or Ventriculus)

The muscular stomach of the AG bee-eater looked like a biconvex lens and occupied the space between the level of 1st and 12th lumbosacral vertebrae (Figs. 1a, b, c and d). The ventriculus was covered by the right and left hepatic lobes, cranially and by the left abdominal air sac, caudally (Fig. 1a). The ventricular musculature was moderately developed and consisted of four smooth muscle: two thick cranio-ventral and caudo-dorsal dark-colored smooth muscles and two thin caudo-ventral and cranio-dorsal light-colored smooth muscles. All muscles attached to extensive aponeuroses in the right and left surfaces called centrum tendineum. The pyloric part of the stomach was not detected (Figs. 1b and d). Internally, the cuticle was thin, slightly hard and dark brown (Fig. 1f). Inspection of the ventriculus content disclosed the presence of bee, its preferred food, with no stones or grits (Fig. 1e).

The most important gross morphometrical measurements of the esophagus, proventriculus and gizzard were illustrated in Table 1.

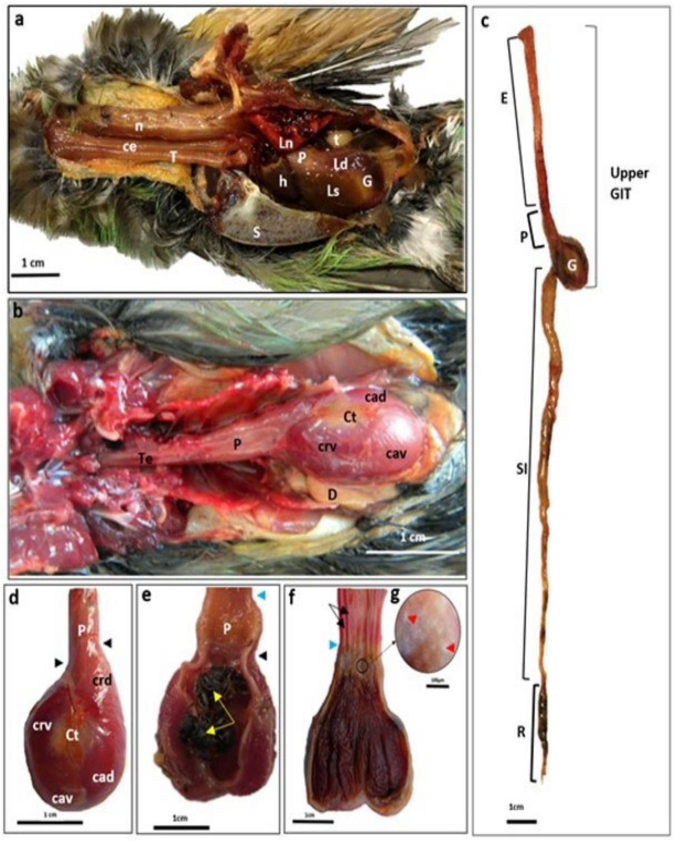

Fig. 1. A photograph of the cervical region and thoraco-abdominal viscera of a dissected adult African green bee-eater left lateral view (a) and (b) ventral view showing the position and relations of the upper GIT parts. (c) A photograph of eviscerated gastrointestinal tract (GIT) from the beginning of esophagus to the end of the rectum showing upper GIT and other parts. (d) Exterior of the stomach showing the two parts of the stomach, (e) A photograph showed the content of the stomach; bee (yellow arrow) (f) A photograph of the interior of the stomach showed mucosal lining of the upper GIT parts, (g) A stereomicroscopic photograph showed the papillae of the Proventricular glands (red head arrow). Trachea (T), Neck (n), Esophagus (E), Cervical part of the esophagus (ce), Thoracic part of the esophagus (Te), Heart (h), left lung (Ln), Proventriculus (P), Ventriculus (G), right lobe of the liver (Ld), left lobe of the liver (Ls), Left testis (T), Sternum (keel bone) (s), Duodenum (D), M.crassus cranioventralis (crv), M.tenuis craniodorsalis (crd), M. crassus caudodorsalis (cad), M. tenuis caudoventralis (cav), Centrum tendineum (Ct), small intestine (Si), Rectum (R), isthmus gastris (black head arrow), Junctura esophagoproventricularis (blue head arrow), esophageal longitudinal folds (black arrow).

Light microscopic findings

Esophagus

The esophageal wall consisted of four layers: Tunica mucosa, Tela submucosa, Tunica musculosa and Tunica adventitia (Fig. 2a). The innermost layer was a thick non-keratinized stratified squamous epithelium. A thin basophilic layer of mucous was detected covering the epithelium (Figs. 2a, b and c). Lamina propria formed of dense collagenous connective tissue layer (Fig. 2e) containing diffuse lymphocytic infiltration and numerous large mucous secreting tubulo-alveolar esophageal glands (Fig. 2a). The glandular epithelium were high columnar cells with basal oval nuclei and its cytoplasm filled with vacuoles, exhibited a robust positive reaction to Alcian blue and PAS staining (Figs. 2c and d) moreover, PAS positive secretion found to be emitted in the lumen of the esophagus (Fig. 2d). Lamina muscularis mucosa clearly identified as a longitudinally oriented smooth muscle layer. Moreover, Tunica submucosa was detected as thin layer of loose connective tissue (Fig. 2e). The muscular layer consisted of an inner longitudinal and thicker outer circular layer of smooth muscle (Fig. 2a) and represented about 45% of the esophageal wall thickness (Table 2). The outermost layer, Adventitia, was made up of a loose connective tissue that contained nerves and blood vessels. (Figs. 2a and e).

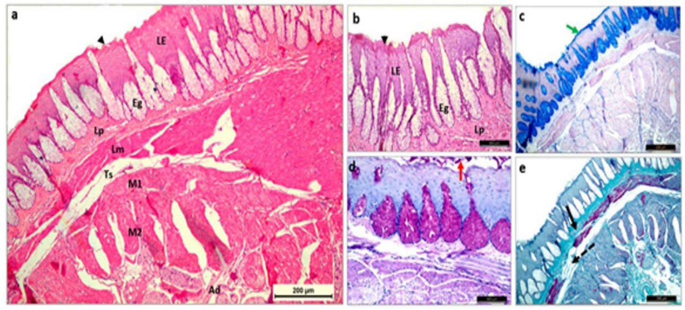

Fig. 2. A photomicrograph of a longitudinal section in the esophagus of the African green bee-eater (a) and (b) clarified its layers, H and E. The innermost layer, lamina epithelialis (LE), lined by a mucous layer (black head arrow) and enclosed the excretory ducts of esophageal glands (Eg). Lamina propria (Lp), lamina muscularis mucosae (Lm), Tunica submucosa (Ts), inner longitudinal muscular layer (M1), outer longitudinal muscular layer (M2) and outermost layer, Adventitia (Ad). A photomicrograph of the esophageal glands(c) Alcian blue and (d) PAS stains showed a strong positive reaction to both stains moreover, a thin strong basophilic layer of mucous (green arrow) and PAS positive secretion (red arrow) from the esophageal gland appeared directed toward the esophageal lumen. (e) A photomicrograph shows the dense collagenous connective tissue layer of lamina propria (black arrow) while sub mucosa was a thin layer of loose connective tissue (dashed black arrow), Masson's Trichrome stain.

Proventriculus

The proventricular wall consisted of three tunics: mucosa gastris, muscularis gastris and serosa gastris. The mucous membrane exhibited numerous folds and sulci (Fig. 3a). The folds varied in height, interwoven together and lined by a columnar epithelium (Fig. 3a), these cells strongly reacted to Alcian blue stain and appeared to be covered with a thick layer of basophilic mucous (Fig. 3d), this layer was negatively reacted to PAS (Fig. 3b). The upper parts of the epithelial folds reacted positively to PAS however, this reactivity diminished toward the sulci (Fig. 3 b). Short simple tubular superficial proventricular glands located in lamina propria submucosae, lined by simple cuboidal epithelium, and showed negative affinity to PAS stain (Fig. 3b), however; positively reacted to Alcian blue stain (Fig. 3d). The lamina propria submucosa comprised of tightly packed dense collagen fibers enclosing many compounds tubulo-alveolar secreting glands, named deep proventricular glands (Fig. 3a and e). These glands were lined by columnar cells that showed a week affinity to PAS and Alcian blue stains however, their excretory ducts were lined by a single layer of tall columnar cells that positively reacted to both stains (Figs. 3a and c). The tunica muscularis constituted approximately half of the proventricular wall's thickness (Table 2) and composed of an internal longitudinal and an outer thicker circular layers of smooth muscle fibers. The serosa was a layer of connective tissue abundant in blood vessels (Fig. 3a).

Ventriculus (Gizzard)

The ventricular wall of the African green bee-eater showed three tunics: Mucosa gastris, Musculosa gastris and Serosa, with absence of lamina muscularis mucosae. The cuticle was moderately thick, PAS-positive layer and its deeper layer was stuck within the ducts of the ventricular glands (Figs. 4a, b and d). The mucous membrane was thrown into folds covered with a layer of columnar epithelium that exhibited strong reactivity when subjected to both PAS and Alcian blue staining (Figs. 4d, e, f and g). Numerous tubular ventricular glands located deep within the lamina propria, that lined by cuboidal cells with prominent, rounded nuclei (Figs. 4a and c) that reacted positively to PAS (Fig. 4d) but negatively stained with Alcian blue (Figs. 4f and g). The tunica muscularis was moderately thick which represented about 60% of the ventricular wall thickness (Table 2) and exhibited two layers: an inner longitudinal layer and an outer thicker circular layer. The muscle bundles were surrounded by loose collagen fibers (Fig. 4i). The serosa was composed of connective tissue, neural cells, and blood vessels (Fig. 4a).

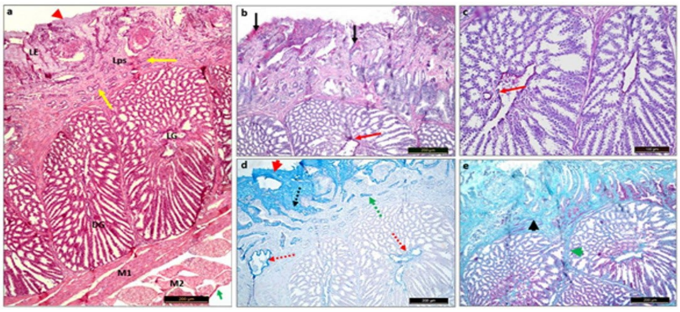

Fig. 3. A photomicrograph of a longitudinal section in the proventricular wall of the African green bee-eater (a) showed its layers, H and E. The innermost layer, lamina epithelialis (LE) presented folds and sulci at its luminal surface, covered by a mucous layer (red head arrow). Lamina propria submucosa (Lps) contained superficial proventricular simple tubular glands (yellow arrow) while the bulk of this layer was formed by the deep proventricular glands (DG), which had clear lumen (LG). The muscular layer was formed of an inner longitudinal muscular layer (M1) and an outer longitudinal muscular layer (M2). The outermost layer, Tunica serosa (green arrow). A photomicrograph of sections stained with PAS stain (b) and (c) showed a weak positive reaction in the upper part of the folds and the surface epithelia (black arrow) and the lumen of the deep proventricular (red arrow). (d) The epithelial lining cells strongly reacted to Alcian blue stain (dashed black arrow) and appeared to be covered with a thick layer of basophilic mucous (red head arrow) in addition, the superficial proventricular gland (dashed green arrow) and lumen of the deep proventricular (dashed red arrow) showed positive reactivity. (e) A photomicrograph shows the dense collagen fibers within the lamina propria submucosa (black head arrow) and the deep proventricular glands demarcated from one another by dense collagen connective tissue fibers (green head arrow), Masson's Trichrome stain.

All Histo-morphometric measurements of the esophageal, proventricular, and ventricular walls were recorded in Table 2.

Table 2. Histo-morphometrical measurements of the upper digestive tract in in the African green bee-eater.

I. Esophagus measurements	
a. Mucous layer	10.113±0.93
b. Lamina epithelialis	108.696±5.6
c. Esophageal gland surface area	6461.89±12.5
d. Lamina propria	60.7918±2.9
e. Lamina muscularis mucosae	36±6.4
f. Tunica submucosa	30.133±2
g. Tunica musculosa (inner longitudinal layer)	47.46±1.7
h. Tunica musculosa (outer circular layer)	153.6±1.77
II. Proventriculus measurements	
a. Mucous layer	143.84±0.92
b. Epithelial folds height	237.191±1.3
c. Lamina propria submucosa	155.443±2.7
d. Superficial proventricular gland surface area	655.44±5.05
e. Deep proventricular glands lobes surface area	237189.2±2.5
f. Tunica musculosa (inner longitudinal layer)	228.796±4.5
g. Tunica musculosa (outer circular layer)	324.99±3.72
III. Gizzard measurements	
a. Cuticle	46.3308±1.9
b. Epithelial folds height	333.298±2.9
c. Ventricular tubular glands surface area	1300.1±4.62
d. Lamina propria submucosa	774.1994±1.5
e. Tunica musculosa (inner longitudinal layer)	83.552±4.02
f. Tunica musculosa (outer circular layer)	1495.1±4.35

Note: All measurements are expressed in means (μm)±SEM except, surface area of gastric Glands was expressed in (1 μm2)±SEM

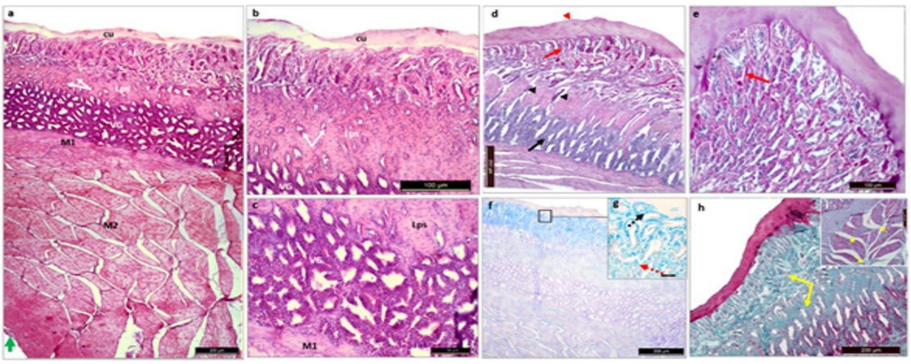

Fig. 4. A photomicrograph of a longitudinal section in the ventricular wall of the African green bee-eater (a, b and c) showed its three tunics, H and E. Lamina epithelialis (LE) was covered by a moderately thick cuticle, (cu). The mucous membrane arranged in epithelial folds (Ef) lined by columnar epithelium. Lamina propria submucosa (Lps) contained simple tubular ventricular glands (VG) that lined with cuboidal cells with large, rounded nucleus and its excretory ducts (white arrow). The muscular layer was formed of an inner longitudinal muscular layer (M1) and an outer longitudinal muscular layer (M2). The outermost layer, Tunica serosa (green arrow). A photomicrograph of sections stained with PAS stain (d) and (e) where, the cuticle appeared as PAS-positive layer (red head arrow) and its deeper layer was stuck within the glandular epithelium (black head arrow) in addition, the cells at the tip of the epithelial folds (red arrow) and the glandular epithelium reacted positively to PAS (black arrow). Views (f and g) clarified that the epithelial lining cells at the top of the folds (dashed black arrow) were strongly reacted to Alcian blue stain however, the reactivity diminished gradually toward the base of the epithelial folds (dashed black arrow). (h and i) A photomicrograph show the dense collagen fibers of lamina propria submucosa (yellow arrow) and the muscle bundles were surrounded by loose collagen fibers (dashed yellow arrow), Masson's Trichrome stain.

DISCUSSION

The upper digestive tract of the AG bee-eater, an insectivorous bird, was thoroughly examined and the findings were correlated with the mechanisms of adaptation to its feeding preference and feeding habits. The esophagus consisted of long cervical and short thoracic parts similar to partridge, duck (Das and Biswal, 1967) and moorhen (Hanafy, 2021), however, other studies recorded that the thoracic part was longer than the cervical part in chicken (Sisson and Grossman, 1986) and in garganey (Hanafy *et al*., 2020). In AG bee-eater, gulls and penguin (Nickel *et al*., 1977), house sparrow (Rajabi and Nabipour, 2009) and garganey (Hanafy *et al*., 2020), the crop was absent. On the other hand, the crop was well developed in granivores (Fisher and Dater, 1961), *Galliformis* and *Falconiformes* (Kobryń and Kobryńczuk, 2004) and pigeon (Batah, 2009). The crop had a feed storage function (Klasing, 1999) and a role in nestlings rearing through production of crop milk as in pigeons, parrots, finches (Lumeij, 1994) however, this function seems to be of less important in AG bee-eater. Examination of the interior of the esophagus revealed presence of numerous longitudinal folds, which may allow expansion of esophagus during swallowing especially because the food was swallowed without chewing in birds.

The histological examination of the AG bee-eater esophageal wall clarified the presence of four tunics, Mucosa, Submucosa, Musculosa and Adventitia. The innermost layer of the esophageal folds was thick non-keratinized stratified squamous epithelium, to protect the underlying tissues from injury during swallowing its food, which is consistent with the observation of Hanafy *et al*. (2020) in garganey and, Alsanosy *et al*. (2021) in domestic fowl and kestrel. However, Zaher *et al*. (2012); Hamdi *et al*. (2013) and Al-taee (2017) confirmed the presence of the true cornification in the esophagus in quail, black-winged kite, and brown falcon, respectively. The epithelial layer in AG bee-eater was covered by a thin basophilic layer of acidic mucopolysaccharides secretion from the esophageal glands however, this mucous layer was absent in garganey (Hanafy *et al*., 2020) and, in domestic fowl and kestrel (Alsanosy *et al*., 2021). Numerous tubulo-alveolar esophageal mucous glands were detected within lamina propria similar to the findings recorded by Eurell and Frappier (2013) and Hanafy *et al*., (2020) in garganey and Alsanosy *et al*. (2021) in domestic fowl and kestrel. These glands showed strong positive reactions with PAS and Alcian blue stains indicating their acidic and neutral mucopolysaccharides secretions. Similar reactivity was detected in house sparrow and rock dove (Rajabi and Nabipour, 2009) and kestrel and domestic fowl (Alsanosy *et al*., 2021). The acidic secretion seems to form the basophilic coating of the epithelium while the neutral mucins emitted to the esophageal lumen to be mixed with the food which, suggesting its role in lubrication during swallowing. The esophageal muscular tunic consisted of inner circular and outer longitudinal layers and covered externally by tunica adventitia, confirmed the findings of Eurell and Frappier (2013); Hanafy *et al*. (2020) and Alsanosy *et al*. (2021).

The stomach of most birds, including AG bee-eater, consisted of two parts; the glandular and the muscular stomach (Bacha and Bacha, 2000; Ince *et al*., 2010; Jassem *et al*., 2016) while Hodges (1974) and El Nahla *et al*. (2011) recorded a third part called the pyloric part in domestic fowl and cattle egret, respectively. Both proventriculus and gizzard located at the left side of the thoraco-abdominal cavity where, the proventriculus extended between levels of the 4th- 6th thoracic vertebrae and related to the spleen, lung, and gonads while the gizzard extended between the levels of 1st-12th lumbosacral vertebrae and related to the liver similar to that reported in domestic fowl (Sisson and Grossman, 1986).

The proventriculus of AG bee-eater and duck (Hassan and Mousa, 2012) was tubal in shape however, it was fusiform in chicken, pigeon and duck (Salem and Yousria, 2000), Eurasian Hobby (Abumandour, 2014), garganey (Hanafy *et al*., 2020) and moorhen (Hanafy, 2021) but, cone-shaped in pigeon (Hassan and Mousa, 2012). Moreover, the small size of proventriculus in the AG bee-eater strongly related to the small size of pray. Regarding to the interior of the proventriculus, numerous elevated papillae of the deep proventricular glands' openings were the gland secretion, Hcl and pepsin, emitted into the lumen (Dyce *et al*., 2002).

The proventricular wall of AG bee-eater formed of three layers, namely mucosa, muscularis and serosa; similar finding was reported in yellow-billed grosbeak (Zhu *et al*., 2013). However, in other avian species such as cattle egret (El Nahla *et al*., 2011), red jungle fowl (Kadhim *et al*., 2011), duck and domestic pigeon (Hassan and Moussa, 2012), common starling (Sayrafi and Aghagolzadeh, 2020), domestic fowl and kestrel (Alsanosy *et al*., 2021), the proventriculus had four tunics, due to the spreading of lamina muscularis mucosae in these species. The mucous membrane presented proventricular folds of variable heights with columnar epithelium lining; agreed with Jassem *et al*. (2016), Das *et al*. (2017); Hanafy *et al*. (2020) and Alsanosy *et al*. (2021) however; simple cuboidal lining detected by Selvan *et al*. (2008) and Hassan and Mousa (2012). Histochemical examination of these cells in AG bee-eater, domestic fowl and kestrel (Alsanosy *et al*., 2021) confirmed their positive reactivity to both PAS and Alcian blue stains suggesting that both acidic and neutral mucin were secreted from the surface epithelial lining. However, Mahdy (2009) in ostrich and El Nahla *et al*. (2011) in cattle egret detected only the acidic mucopolysaccharides in the surface epithelium. On the other hand, Spicer (1965) found only neutral muco-substance in fowl. All these differences reflected the effect of feeding preference on proventriculus microstructure.

Moreover, a PAS-positive Koilin layer was detected covering the proventricular folds of ostrich (Eidaroos *et al*., 2008) and cattle egret (El Nahla *et al*., 2011). In this study, instead, an Alcian blue-positive mucous layer was detected which completely different from the previously mentioned, Koilin, in its consistency and histochemical reaction, suggests the role of the acidic mucin layer in lubrication and protection of the underlining proventricular tissue (Zhu, 2015). On the other hand, this covering was absent in domestic fowl and kestrel (Alsanosy *et al*., 2021).

Lamina propria submucosa was occupied by two types of glands; simple tubular superficial proventricular glands and compound tubuloalvealar deep proventricular glands; in agreement with Ogunkoya and Cook (2009) in passerines and Sayrafi and Aghagolzadeh (2020) in starlings. The superficial proventricular glands secretion, in AG bee-eater, cattle egret (El Nahla *et al*., 2011) and domestic fowl (Alsanosy *et al*., 2021), showed a positive reactivity to Alcian blue stain confirming the acidic nature of its secretion, although; Sayrafi and Aghagolzadeh (2020) in starlings and Alsanosy *et al*. (2021) in kestrel stated the affinity of these glands to both PAS and Alcian blue stains. The deep proventricular glands were compound tubuloalvealar type similar to passerines (Ogunkoya and Cook, 2009), red jungle fowl (Kadhim *et al*., 2011), and starlings (Sayrafi and Aghagolzadeh, 2020), while in yellow-billed grosbeak (Udoumoh *et al*., 2016) and African pied crow (Zhu *et al*., 2013) these glands were compound tubular glands. The excretory duct lining showed a positive affinity to both PAS and Alcian blue stains similar to the results recorded in kestrel (Alsanosy *et al*., 2021). However, in cattle egret (El Nahla *et al*., 2011) and domestic fowl (Alsanosy *et al*., 2021), these cells exhibited weak affinity to both stains. Although, Selvan *et al*. (2008) in guinea fowl and Sayrafi and Aghagolzadeh (2020) in starlings detected only PAS-positive reactivity of these cells. In AG bee-eater, the tunica muscularis consisted of inner thin longitudinal and outer thick circular layers; supports the finding of other studies such as Sayrafi and Aghagolzadeh (2020); Hanafy *et al*. (2020) and Alsanosy *et al*. (2021). However, in fowl (Banks, 1992), three layers of smooth muscles were detected. On the other hand, only one longitudinal layer of tunica musculosa was detected in cattle egret (El Nahla *et al*., 2011). The muscular layer, in AG bee-eater, represented the major structural component of the proventricular wall, about 50% of it thickness followed by the deep proventricular gland layer, agreed with Abumandour (2014) in Eurasian Hobby. This finding confirms the role of the proventricular musculature to push the gastric content into the gizzard while, the size of the proventricular glands strongly affected the amount of gastric secretion that depends on the type of food and its chemical composition, in accordance with the findings of Zhu (2015).

The gizzard of AG bee-eater, partridge (Rossi *et al*., 2005), duck, goose and pigeon (El-Ghazali, 2008), Eurasian Hobby (Abumandour, 2014), and garganey (Hanafy *et al*., 2020) looked like a biconvex lens. However, it was oval in kestrel and owl (Hassouna, 2001), hoopoe (El-Ghazali, 2008), rounded in ibis (El-Ghazali, 2008) and truncated elliptical in cattle egret (El Nahla *et al*., 2011), these differences may depend on the thickness of the muscular layer. The present study declared that the musculature of ventriculus in AG bee-eater was moderately developed and could be differentiated into four muscles, two thick and two thin smooth muscles; same muscle grouping was noted by Bailey *et al*. (1997) in fowl and Hassan and Moussa (2012) duck and pigeon. In contrast, the muscular layer was well developed in granivores as domestic pigeon and duck gizzard (Hassan and Mousa, 2012) and ill developed in cattle egret an insectivore (El Nahla *et al*., 2011). Comparing the later studies with our finding suggests that the development of the gizzard musculature simulates the hardness of the food particle coinciding with McLelland (1979) and Klasing (1999). Regarding to the interior of the ventriculus, hard and dark brown cuticle was covering the mucosal surface of the gizzard to protect it from the effect of the gastric enzymes and to minimize the physical damage caused by food particles. Moreover, examination of the ventriculus content revealed absence of any stones or grits, which only detected in granivores gizzard to help crushing of hard grains (Hassan and Mousa, 2012). The ventricular wall consisted of three tunics; Mucosa, Musculosa and Serosa, agreed with the findings of Aughey and Frye (2001) in carnivorous birds; El Nahla *et al*. (2011) in cattle egret. However, in house sparrow (Klem *et al*., 1983) and domestic fowl and kestrel (Alsanosy *et al*., 2021), four tunics was detected. An eosinophilic cuticle was detected covering the epithelial layer; similar finding was detected in domestic fowl and kestrel (Alsanosy *et al*., 2021). However, El Nahla *et al*. (2011) mentioned that the cuticle of cattle egret was Alcian blue-positive, while Mahdy (2009) confirmed positive reactivity to both Alcian blue and PAS stains in Ostrich cuticle. The chemical composition of the cuticle may depend largely on the nature of the ventricular gland secretion. The folds of the mucous membrane were lined by columnar epithelium, which had a strong affinity to both PAS and Alcian blue stains similar finding recorded in kestrel (Alsanosy *et al*., 2021); however, in cattle egret (El Nahla *et al*., 2011), ostrich (Mahdy, 2009) showed only Alcian blue-positive reactivity. On the other hand, in domestic fowl (Alsanosy *et al*., 2021) positive reaction to PAS stain only was only noticed. Deeply in lamina propria, numerus tubular PAS-positive glands were detected, similar result reported by Zhu *et al*. (2013) in yellow-billed grosbeak and (Hanafy *et al*., 2020) in garganey, suggesting the role of the ventricular glands in the cuticle formation. The musculosa of AG bee-eater represented about 60% of the ventricular wall thickness and consisted of two layers; an inner longitudinal, and an outer thicker circular layer as reported by Batah and Selman (2012) in coot bird. In contrast, Imai *et al*. (1991) detected three layers of the muscular layer in striated finch. The thickness of the muscular tunica in AG bee-eater as an insectivorous bird appears to be moderately developed compared with granivores well-developed gizzard or carnivores ill-developed gizzard that depends on the coarseness of the food in each group of birds (Kadhim *et al*., 2011; Abumandour, 2014; Sayrafi and Aghagolzadeh, 2020).

CONCLUSION

The present study indicated that the anatomical and histological structures of upper digestive tract of AG bee-eater closely resembled those of other avian species. Noteworthy findings such as; the mucous layer covering the interior of the esophagus and the proventriculus, in addition to, the moderately thick cuticle, act as protection barriers against harmful effect caused by ingestion of its special food type (bees, wasps and other insects). Moreover, many types of mucous-secreting glands distributed throughout the upper digestive tract secreting both acidic mucin (acting as an anti-ulcerogenic to coat and protect mucosal surfaces) and neutral mucins (assisting in enzymatic secretion). The crop was absent and seems to be of less important in AG bee-eater. The proventriculus is relatively thin-walled and more glandular for providing adequate gastric enzymes for digestion of high protein diet ingested by this bird. The small size of the upper digestive tract components is adapted for processing relatively small food particles. Additionally, the moderate thickness of the ventriculus muscular layer suits the coarser texture of the food consumed by this interesting remarkable species.

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CONFLICT OF INTEREST

The authors declare that they have no competing interests.

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