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ORIGINAL ARTICLE

LIGHT MICROSCOPIC STUDIES OF THE UPPER DIGESTIVE TRACT OF ADULT SPUR-WINGED GEESE (*PLECTROPTERUS GAMBENSIS*)

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Ethical considerations: When reporting experiments on animals Observation of the ARRIVE guidelines 2.0: Updated guidelines for reporting animal research, published on July 14, 2020 (DOI: 10.1371/journal.pbio.3000410), is applied. The authors ensure that all procedures were performed in compliance with the guidelines for animal care of their institutions or with national/international guidelines.

ABSTRACT

The present study elucidated the histoarchitecture of the upper digestive tract (UDT) of six Spur-winged geese (SWG), a wildfowl species of the Sudano-Sahelian wetlands of northern Nigeria. Tissue samples collected from different parts of the UDT were processed by the routine paraffin technique of light microscopy. The beak was lined by stratified squamous keratinized epithelium. The underlying dermis consisted of dense areolar tissue, blood vessels, and encapsulated nerve endings of Herbst corpuscles. The apical part of the tongue was lined by stratified squamous epithelium, which was heavily cornified on the ventral surface. The lingual submucosa comprised a mass of compound tubular mucous glands that exhibited moderate reactivity to Alcian blue. The esophagus was lined by stratified squamous non-keratinized epithelium, while the subepithelial area showed a few lymphatic nodules and polymorphic alveolar mucous glands. The mucosal glands were modified at the esophageal-proventricular junction into crypt-like folds. The proventricular wall comprised mainly proventricular glands, consisting of straight alveolar tubules lined by oxyntico-peptic cells, especially towards the basal portion. The proventricular plicae at the isthmus were modified into organized gizzard glands. The gizzard mucosa was lined by a hard cuticle layer. The gizzard-duodenal junction presented mucosal folds of variable heights, lymphatic nodules, and duodenal villi.

Key words: histology; spur-winged geese; upper digestive tract

INTRODUCTION

The spur-winged geese (SWG) of the family *Anatidae* are closely related to geese and shelducks but are distinct in a number of anatomical features, and thus are classed under the subfamily *Plectropterinae* [1]. The International Union for Conservation of Nature (IUCN) has classified these birds as a "least concern" species. They are herbivorous waterfowl, which usually eat seeds and vegetative parts of aquatic and terrestrial plants [2, 3]. The digestive system of birds, when compared to mammals, exhibits less interspecific variation [4] but evolves through a multitude of evolutionary changes to become a unique anatomo-physiological entity [5], presenting a number of significant structural differences between species and breeds [6].

There is a paucity of information on the detailed anatomic description of the digestive apparatus of the SWG. Knowledge of the functional anatomy of the digestive tract is essential for an improved understanding of the adaptive feeding habits in this species and for avian comparative anatomy. Thus, the present study was aimed at providing a detailed description of the upper digestive tract and some associated glands of the SWG, using histology and histochemical techniques.

MATERIALS AND METHODS

Sample collection

A total of six apparently healthy adult SWG, comprising three males and three females each, were used for this study. The birds were purchased from local sellers at the Moromoro local market and the *Zabarmari* ward of the Jere Local Government Area (LGA), Borno State (11.8024° N, 13.1931° E.), Nigeria. The birds were euthanized by a combination of diazepam (4 mg/kg) and ketamine HCl (60 mg/kg) intramuscularly as described by Onuk et al. [7]. The procedure was conducted as per the ARRIVE guideline and conformed to the guidelines of the Animal Use and Ethics Committee (AUEC) of the Faculty of Veterinary Medicine, University of Maiduguri, Nigeria. (Ethical approval number: AUP-R004/2023).

Histoarchitectural studies

Tissue samples (approximately 5 mm^3) from the upper and lower beaks, tongue (apex, body, and root segments), esophagus (proximal and middle segments), crop, proventriculus (esophageal-proventricular junction, middle, and proventricular-gizzard junction segments) and ventriculus (middle and ventricular-duodenal junction segments) were collected and fixed in 10% neutral buffered formalin for approximately 48 hours. The hard tissue (beaks and bony palate) was decalcified using the formic acid-sodium citrate method [8].

The tissues were further processed by a routine paraffin technique, and serial sections of 5-6 µm were cut and stained with Harris's hematoxylin and eosin (H&E) for general tissue architecture, Mallory's trichome stain for collagen fibers, and Alcian Blue (AB) (pH 2.5) for mucosubstances. All stained sections were examined, and photomicrographs were captured at different magnifications (x40, x100, and x400) using the I-SCOPE DN1177D LED microscope.

RESULTS

Upper beak

The upper beak was covered by an epidermis and the underlying dermis (Fig. 1a). The epidermis comprised a stratified squamous epithelium consisting of stratum basale, stratum spinosum, and stratum corneum (Fig. 1b). The stratum basale comprised 2-3 layers of basal cells, having a basophilic nucleus. The stratum spinosum comprised a layer of fusiform-shaped cells with flattened nuclei. The stratum corneum presented cells having pyknotic nuclei and few areas of vacuolations. These strata were covered by a very hard, dense cornified layer, and at some places, the free surface of the cornified layer presented a discontinuous layer of flattened cells (Fig. 1a).

The dermis consists of connective tissue with blood vessels and encapsulated nerve endings, the Herbst corpuscles. The Herbst corpuscle was ovoid-shaped, surrounded by a distinct cellular capsule. It presented a central axial nerve fiber surrounded by concentric lamellae (Fig. 1b).

The lamina epithelialis of the palatine mucosa comprised the stratum basale, a distinct stratum lucidum, and the stratum corneum (Fig. 2a). The lamina propria consisted of loose connective tissue, and the subepithelial area of the propria presented papillae of loose connective tissue, while the deeper layer was dense connective tissue and presented the palatine gland (Fig. 2b).

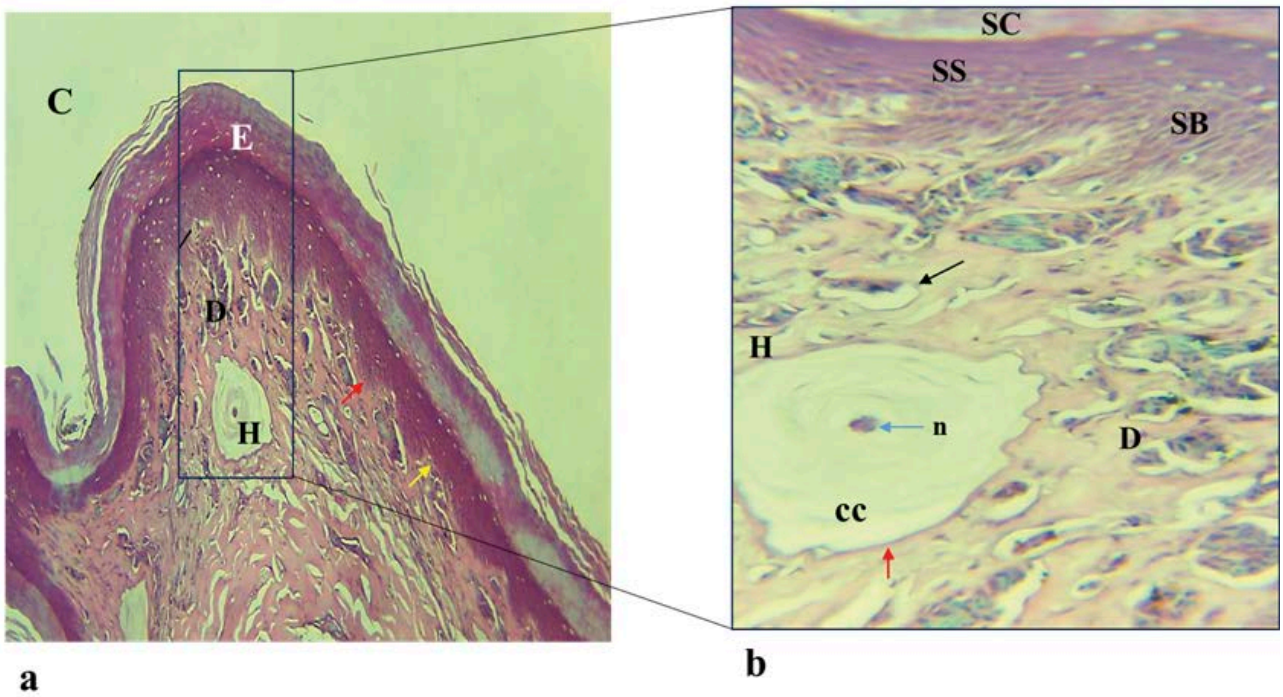

Fig. 1. Photomicrograph of upper beak showing: a. C. oral cavity, E. epidermis, D. dermis, epidermal peg (red arrow), dermal papilla (yellow arrow), H. Herbst corpuscle (H&E, ×100), b. higher magnification (H&E, ×400) of the epidermis showing: SB. stratum basale, SS. stratum spinosum, SC. stratum corneum, D. dermis with blood vessel (black arrow), cc. concentric core, and n. nerve of the Herbst corpuscle.

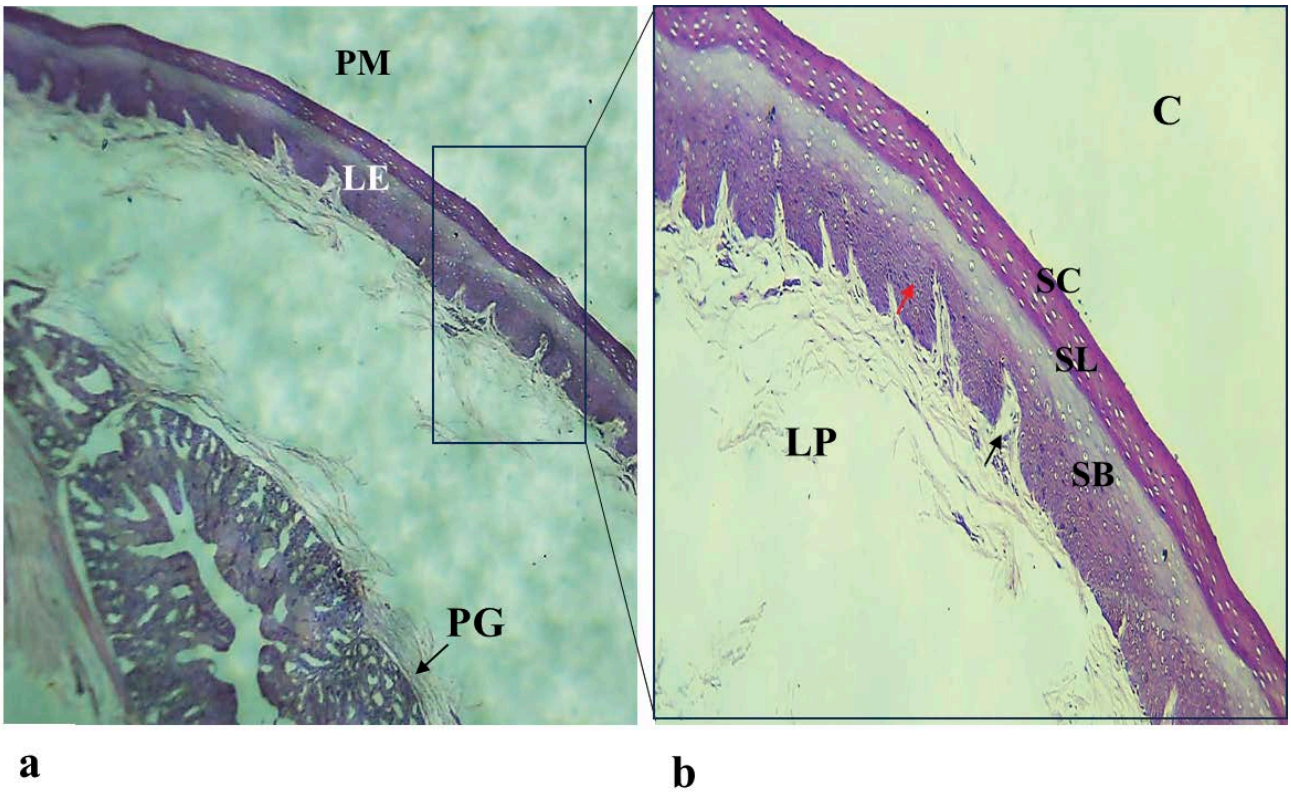

Fig. 2. Photomicrograph of cross section of the palate showing: a. PM. palatine mucosa, includes the LE. lamina epithelialis, PG. palatine gland deeper propria (H&E, ×40), b. higher magnification (H&E, ×400) of the lamina epithelialis showing: SB. stratum basale, SL. stratum lucidum, SC. stratum corneum, epidermal peg (red arrow), dermal papilla (black arrow).

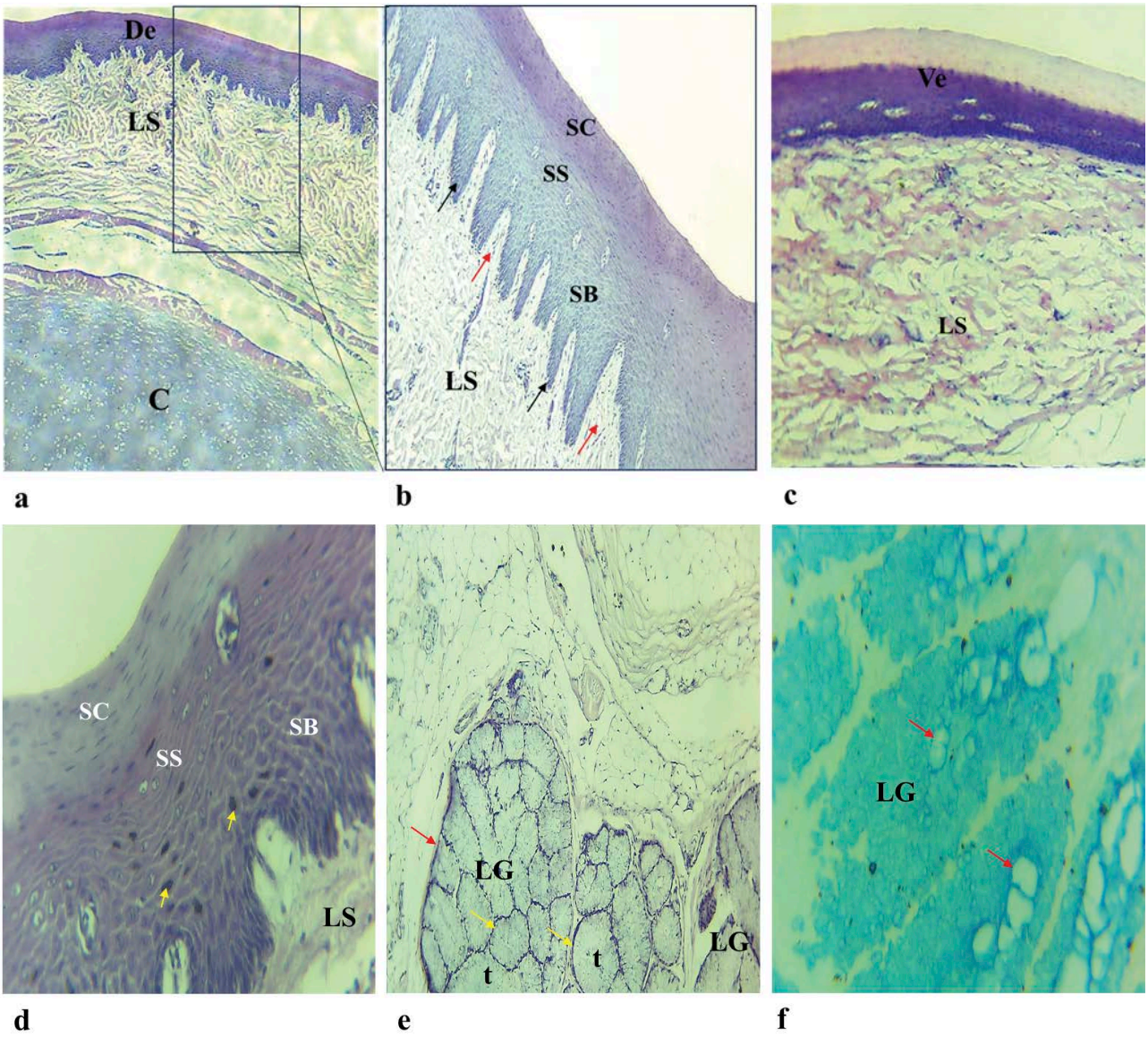

Fig 3. Photomicrograph of transverse section of the tongue (apex) showing: a. De. dorsal epithelium, LS. lingual submucosa, C. cartilage (H&E, ×40), b. higher magnification (H&E, ×100) of the dorsal epithelial surface showing: SB. stratum basale, SS. stratum spinosum, SC. stratum corneum, LS. lingual submucosa, epidermal pegs (black arrows), dermal papilla (red arrows), c. longitudinal section of the tongue (apex) showing: Ve. ventral epithelium, LS. lingual submucosa (H&E, ×100), d. transverse section of the tongue (body) showing: SB. stratum basale, SS. stratum spinosum, SC. stratum corneum, LS. lingual submucosa, melanocytes (yellow arrow) (H&E, ×400), e. transverse section of the tongue (root) showing; LG. lingual salivary glands, surrounded by connective tissue capsule (red arrow), t. glandular tubules, separated by connective tissue septa (yellow arrows) (H&E, ×100), f. transverse section of the tongue (root) showing: weakly sulfated lingual salivary glands (red arrows), LG. lingual gland. (AB ×100).

Tongue

The tongue was lined by a thick stratified squamous epithelium that was well developed dorsally (Fig. 3a). This epithelium is comprised of stratum basale, stratum spinosum, and stratum corneum. The free surface of the dorsal epithelium was straight, while the deeper layer presented a well-developed epidermal peg, alternating between papillae of the lingual submucosa (Fig. 3b). The entoglossum, forming the rostral end of the hyoid apparatus, was cartilaginous and surrounded by a perichondrium (Fig. 3a).

The ventral epithelium at the tongue apex was covered by a thick cornified layer of keratin (Fig. 3c). It was devoid of epidermal pegs but presented strata similar to the dorsal epithelium. The histoarchitecture of the body and root of the tongue was similar to that of the lingual apex. However, melanocytes were observed irregularly distributed within the cells of the stratum basale and stratum spinosum, especially at the tongue body (Fig. 3d). In addition, striated lingual muscle and lingual glands were observed towards the deeper surface of the lingual submucosa, adjacent to a

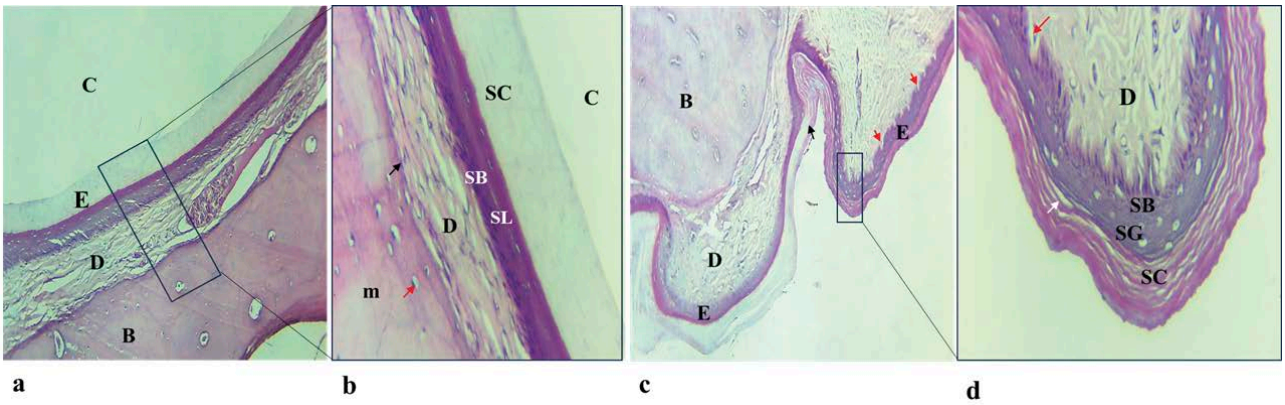

Fig. 4. Photomicrograph of lower beak showing; a. C. oral cavity, E. epidermis, D. dermis, B. dentary bone, (H&E, ×100), b. higher magnification (H&E, ×400) of the dorsal epithelial surface showing: SB. stratum basale, SL. stratum lucidum, SC. stratum corneum, D. dermis, periosteum (black arrow), m. bone matrix, osteocyte within lacunae (red arrow) (H&E, ×400), c. lateral lower beak showing: B. bone, E. epidermis, D. dermal papillae (red arrows), the hard cornified layer of the epidermis transitioning into softer form arranged in stacks (black arrow) (H&E, ×100), d. higher magnification (H&E, ×400) of lateral skin fold of lower beak showing: SB. stratum basale, SG. stratum granulosum, SC. stratum corneum, D. dermal papilla (red arrow).

cluster of adipose tissue (Fig. 3e). The lingual gland consisted of a compound tubular gland that exhibited low to moderate reaction to weakly sulfated muco-substance and sialo-mucin (Fig. 3f).

Lower beak

The free surface of the epidermis of the lower beak presented a very thick layer of relatively soft keratin that was translucent in appearance (Fig. 4a). The epidermis consists of the stratum basale, spinosum, and corneum (Fig. 4b). The bony core of the lower beak was composed of dentine bone; the bony matrix housed osteocytes with eccentric nuclei (Fig. 4b).

At the lateral commissure of the upper and lower beak, the hard cornified layer of the epidermis becomes soft and is arranged in irregular stacks (Fig. 4c). At the adjacent skin fold of the lower beak, the epidermis showed stratum basale and stratum corneum, whereas the dermis consisted of relatively loose areolar connective tissue and presented numerous dermal papillae (Fig. 4d).

Esophagus

The mucosal wall of the esophagus consists of 4 layers (Fig. 5a). The tunica mucosa is comprised of lamina epithelialis, lamina propria, and lamina muscularis mucosae (Fig. 5a-b). In certain regions, lymphoid follicles were observed in the sub-epithelial area, with infiltration of lymphoid cells toward the basal portion of the epithelium (Fig. 5a-b). The lamina propria was comprised of loose connective tissue and contained sub-epithelial glands that were compound alveolar glands. In some areas, a lymphoid nodule was seen adjacent to the mucosal gland (Fig. 5b-c).

The submucosa was poorly developed, comprising an indistinct layer of fine connective tissue. The lamina muscularis mucosae was situated immediately beneath the alveolar glands, and at some places, the fibers of the muscularis mucosae extended between the mucosal glands and sub-epithelial area of the mucosa. The tunica muscularis consisted of thick inner circular and thin outer longitudinal smooth muscles, separated by fine connective tissue fibers (Fig. 5c).

Pseudo-crop

The diverticulum of the pseudo-crop was lined by stratified squamous non-keratinized epithelium, and in some places lympho-epithelium was observed (Fig. 6a). A few mucosal glands were observed in the sub-epithelial area of the lamina propria (Fig. 6a). The body of the pseudo-crop presented a similar epithelial lining as the diverticulum, and the propria consisted of loose connective tissue, while mucosal glands were absent. The lamina muscularis mucosae was prominent, presenting a thick band of smooth muscle layer (Fig. 6b).

The esophageal-proventricular junction was a short transitory zone, where the esophageal mucous glands were modified into crypt-like folds of the proventriculus (Fig. 6c). A strand of muscle fibers passed inward over the anterior surface of the glandular lobule of the proventriculus and connects the main mass of the muscularis mucosa with the scant tissue of the submucosa (Fig. 6d). Heavy infiltration of lymphatic cells forming large foci in the lamina propria was observed.

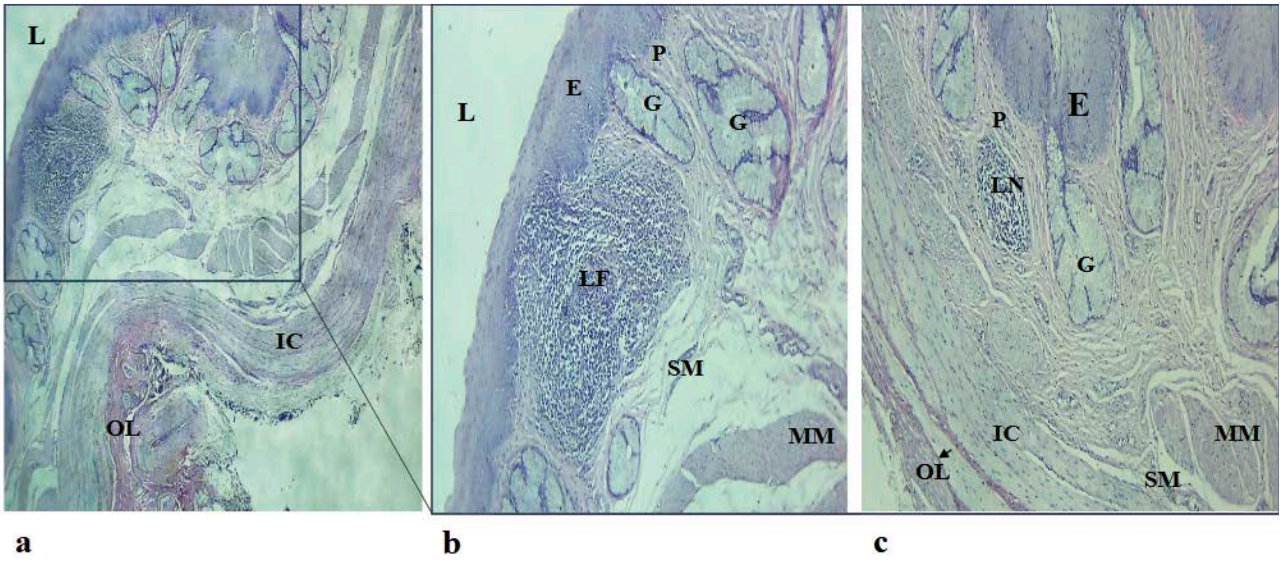

Figure 5. Photomicrograph of transverse section of esophagus showing: a. L. lumen, IC. inner circular and OL. outer longitudinal muscle layer of tunica muscularis, (H&E, ×40), b. higher magnification (H&E, ×100) of a transverse section of esophagus showing: L. lumen, E. stratified squamous epithelium, LF. lymphoid foci, LP. lamina propria, G. mucosal glands, MM. muscularis mucosae, SM. submucosa, c. transverse section of esophagus showing: E. stratified squamous epithelium, LP. lamina propria, LN. lymphoid nodule, G. mucosal gland, MM. muscularis mucosae, SM. submucosa, IC. inner circular and OL. outer longitudinal muscle layer of tunica muscularis (H&E, ×100).

Proventriculus

The mucosal lining of the proventriculus was thrown into folds of varying height. The greater part of the proventricular wall was composed of proventricular glands, presenting a number of rounds to polymorphic lobules. These lobules were surrounded by a connective tissue capsule, which separated the muscularis mucosa into a thin inner and a thick outer part (Fig. 7a-b).

The wall of each lobule was composed of numerous straight alveoli radiating from a central cavity of the lumen (Fig. 7b). The tunica muscularis consisted of a prominent inner circular and outer longitudinal muscle layer (Fig. 7b). The apical portion of the proventricular gland was lined by microscopic folds (plicae) of varying height. The plicae were lined with simple columnar epithelium (Fig. 7c). At the basal area of the proventricular gland, the inner and outer parts of the muscularis mucosae were prominent (Fig. 7d).

The straight alveoli joined to form a short tertiary duct and were lined by simple cuboidal epithelium (Fig. 7d). The central cavity of the lobule was lined by simple columnar ciliated epithelium with a few goblet cells. A sec-

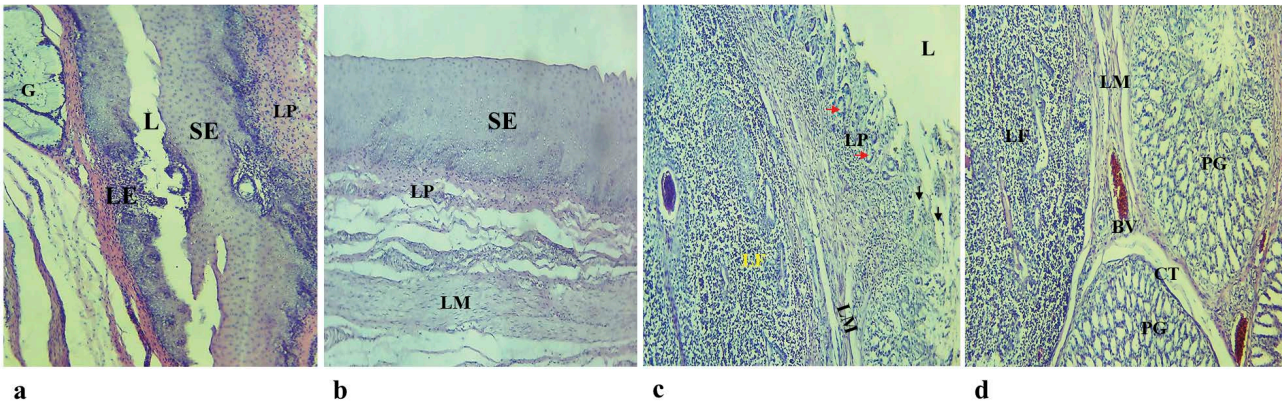

Fig. 6. Photomicrograph of transverse section of pseudo-crop (diverticulum) showing: a. SE. stratified squamous keratinized epithelium, L. lumen, LP. lamina propria, LE. Lymphoepithelium, G. gland (H&E, ×100), b. transverse section of pseudo-crop (body) showing: SE. stratified squamous keratinized epithelium, LP. lamina propria, LM. Lamina muscularis mucosa (H&E, × 100), c. transverse section of apical part of esophageal-proventricular junction showing: L. lumen, LP. lamina propria, LM. lamina muscularis mucosae, lf. lymphoid foci, esophageal mucosal glands (red arrows), crypt-like folds of proventriculus (black arrows), (H&E, ×100), d. transverse section mid-portion of esophageal-proventricular junction showing: lf. lymphoid foci, LM. lamina muscularis mucosae, BV. blood vessel, CT. connective tissue, Pg. proventricular gland (H&E, ×100).

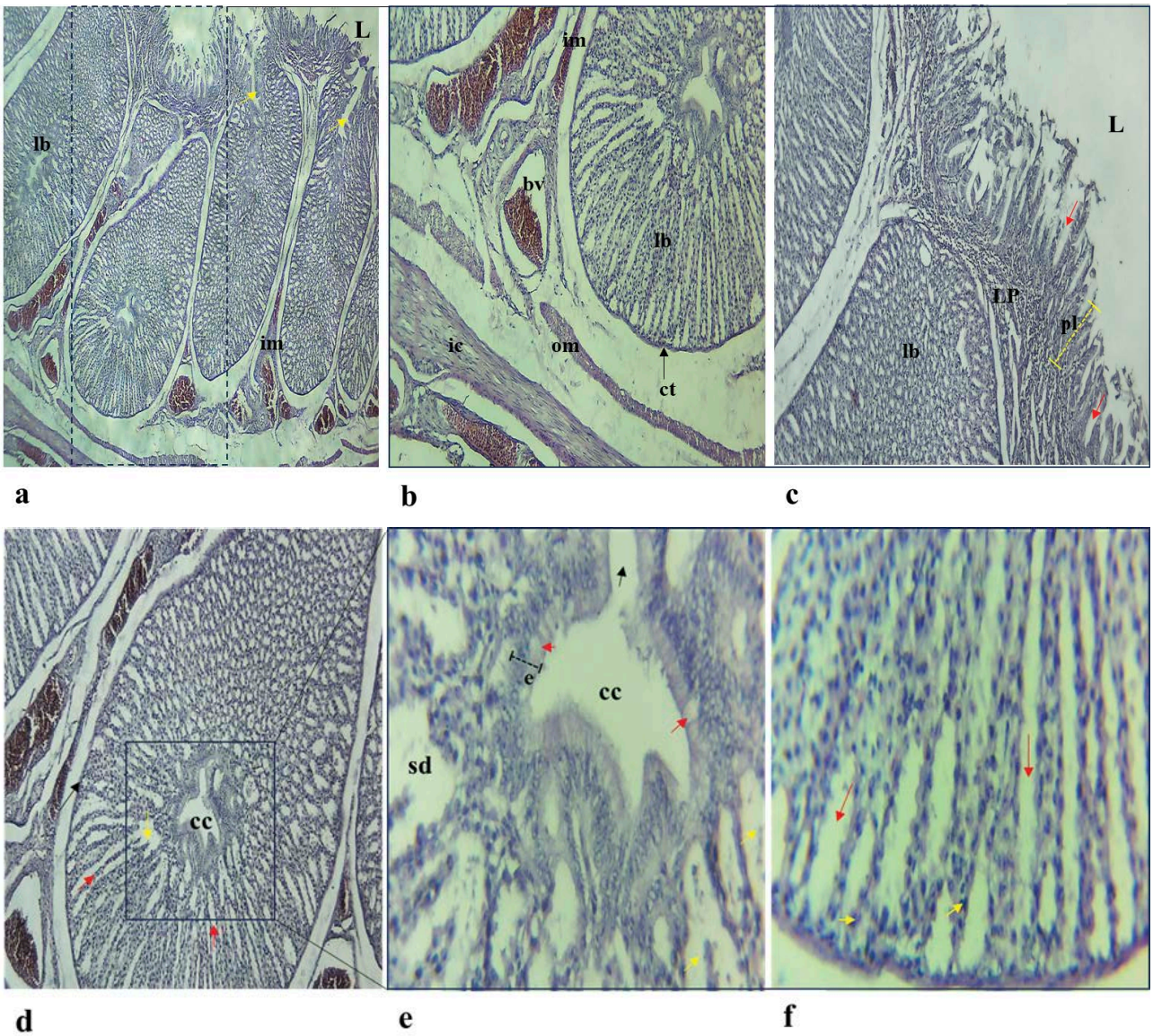

Fig. 7. Photomicrograph of transverse section of proventricular wall showing: a. L. lumen, lb. lobules of proventricular glands, primary ducts (yellow arrow), im. inner part of muscularis mucosae (H&E, ×100), b. higher magnification of transverse section of proventricular wall (basal part) showing: im. thin inner and om. thick outer part of muscularis mucosae, bv. blood vessel, lb. lobule of proventricular gland, ct. connective tissue capsule (black arrow), ic. inner circular muscle of muscularis externa (H&E, ×100), c. transverse section of proventricular wall (apical part) showing: L. lumen, pl. microscopic folds or plicae having sulci (red arrows), LP. lamina propria, lb. lobule of proventricular gland (H&E, ×100), d. proventricular gland lobule showing: cc. central cavity, glandular tubules (red arrows) joining to from a short tertiary duct (yellow arrow), each lobule is surrounded by connective tissue capsule (black arrow). (H&E, ×100), e. higher magnification (H&E, ×400) of the cc. central cavity, lined by columnar ciliated epithelium with Goblet cells (red arrows), sd. secondary duct, tubulus (yellow arrow), f. proventricular gland showing: straight tubules (red arrows) lined by low cuboidal epithelium, oxyntico-peptic cells (yellow arrows). (H&E, ×100).

ondary duct extended from the central cavity and was lined by the epithelium of the central cavity (Fig. 7e). The duct of the straight alveolar secretory units was lined by low cuboidal epithelium, and towards the basal portion of these alveoli, there were oxyntic peptic cells having a darkly basophilic oval nucleus (Fig. 7f).

The *isthmus* was a short transitory zone, in which the proventricular gland terminated abruptly. The proventricular plicae were gradually modified into the gizzard gland. The fine muscle fibers of the internal portion of the muscularis mucosa extended over the posterior edge of the last proventricular lobule to join the main mass of the muscularis mucosa (Fig. 8a). The outer portion of the muscularis mucosa merged with the circular layer of muscularis externa at the terminal portion of the proventriculus to form the main mass of the muscularis externa (Fig. 8b).

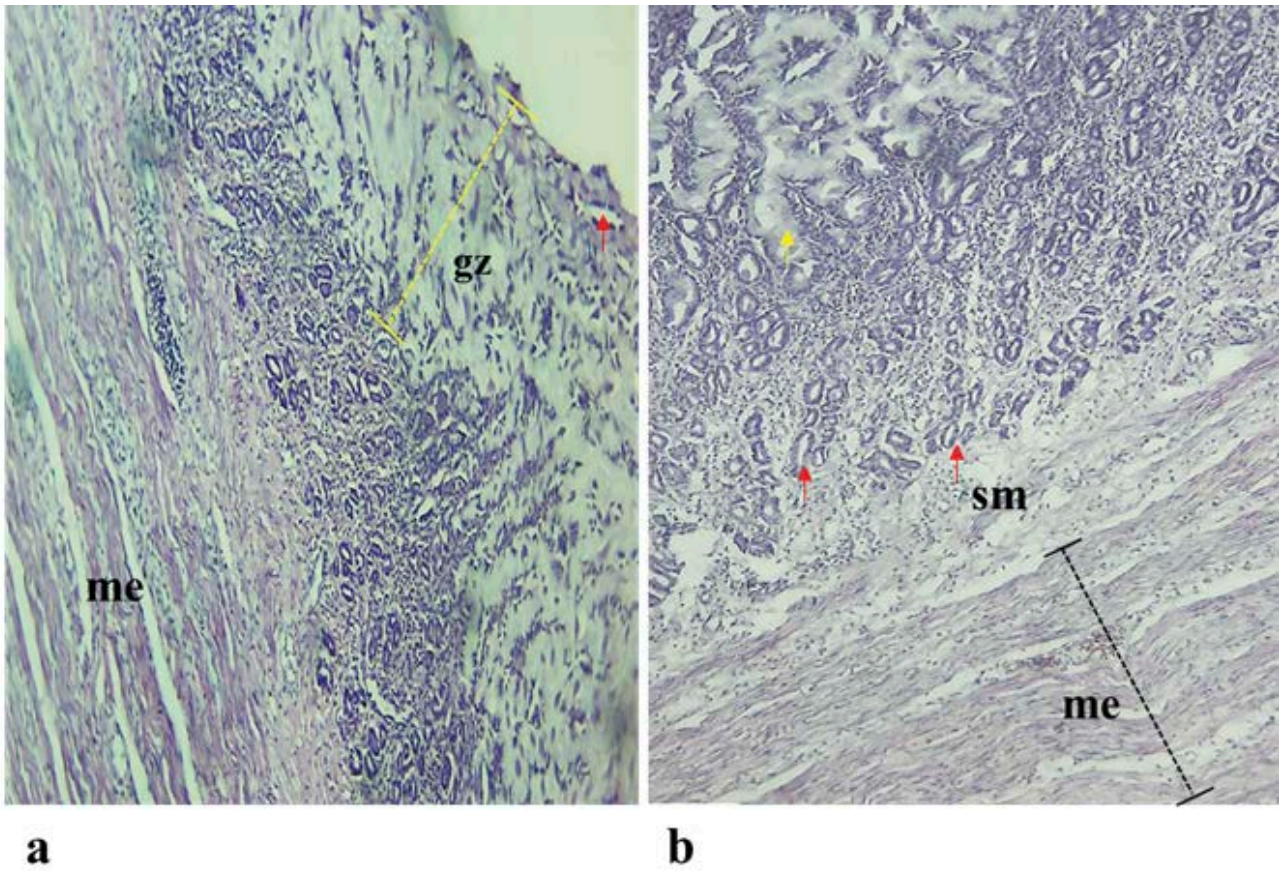

Fig. 8. Photomicrograph of proventricular-gizzard junction (isthmus) showing: a. gz. Gizzard lining covering the mucosa of the isthmus, proventricular plicae transitioning into the gizzard gland (red arrow), me. thick circular muscle layer, (H&E, ×100), b. mucoid secretory product (yellow arrow) lining the cuticle, gastric glands (red arrows), sm. submucosa, me. thick circular muscle layer (H&E, ×100).

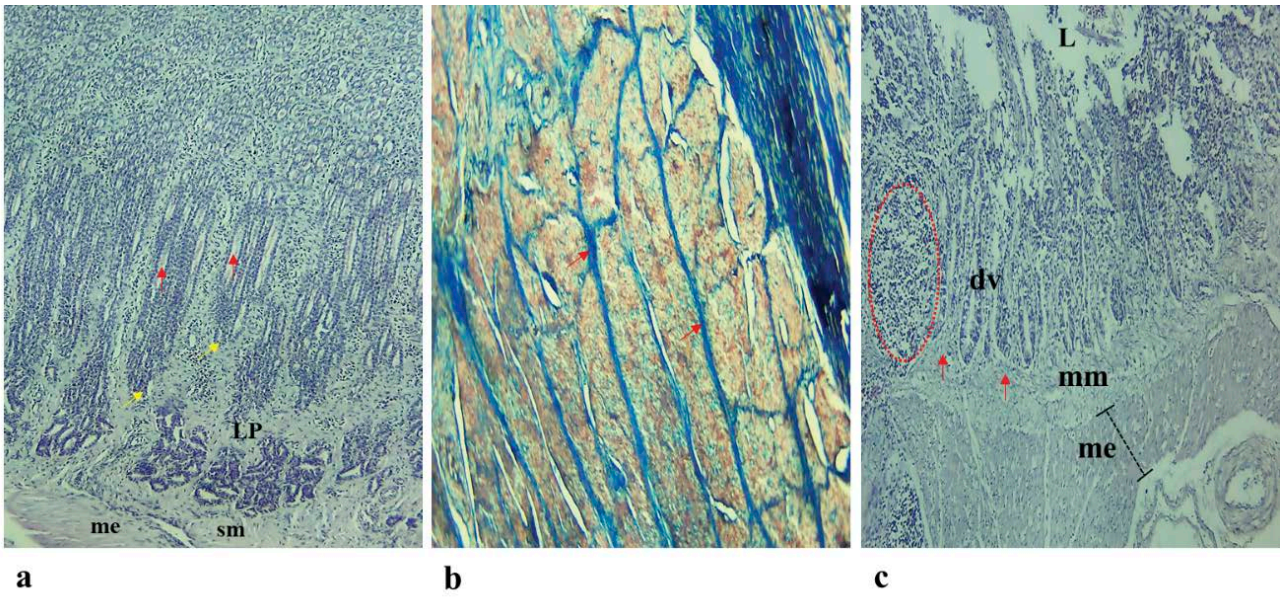

Fig. 9. Photomicrograph of gizzard showing: a. gastric pits (red arrows), gizzard glands (yellow arrows), LP. lamina propria, sm. submucosa, me. muscularis externa, (H&E, ×100), b. muscle fasciculi surrounded by connective tissue perimysium positive for collagen (red arrow), (MT ×100), c. gizzard-duodenal junction showing: L. lumen, dv. duodenal villi, lymphoid nodule (red dotted circle), mm. muscularis mucosae, having folds (red arrow), me. muscularis externa (H&E, ×100).

Ventriculus

The lining of the gizzard mucosa was formed by secretions of the gizzard gland oriented both parallel and perpendicular to the surface of the gizzard mucosa (Fig. 9a). The tunica propria was overlain by gizzard glandular tubules, while the submucosa was covered by a layer of dense connective tissue (Fig. 9a). The muscularis externa of the gizzard was well developed, comprising bundles of muscle fascicles surrounded by fine collagen connective tissue fibers (Fig. 9b).

The gizzard-duodenal junction was also a short transitory zone, where the mucosa was characterized by folds of variable sizes. There was also a lymphatic nodule that was both diffuse and nodular. The first few duodenal villi became discernible at this junction, and ventral to these villi, the duodenal mucosa formed a prominent fold of varying heights. The muscularis externa was prominent at this junction (Fig. 9c).

DISCUSSION

The true adult geese are essentially vegetarian [9]; thus, the anatomical adaptations of their digestive apparatus are a reflection of their feeding habit. For instance, the Herbst corpuscle observed in the upper beak of SWG was described as a sensory receptor used for selection and assessment of prehended foodstuffs, particularly in dabbling waterbirds [10]. Although SWG are surface feeders, they dabble on the surface of shallow waters for small invertebrates [9]. Furthermore, these Herbst corpuscles function as pressure-sensitive mechanoreceptors, detecting pressure and vibration from the surrounding environment [11].

The thick and keratinized epithelial lining of the SWG tongue corresponds to recent findings of Abdelhakeem et al. [12] in adult Egyptian geese. On the contrary, their studies reported the presence of lingual nail as thickened para-keratinized epithelium of the ventral surface of the tongue apex. In the SWG, the lingual nail existed as a gross cornified horny plate at the rostral tip of the premaxillary and the lower beak but was not a structure of the tongue. According to Jackowiak and Ludwig [13], a strongly keratinized epithelium was seen mainly in herbivorous and granivorous birds, while a lesser degree of keratinization was seen primarily in aquatic birds [14]. Furthermore, several lamellated sensory corpuscles and nerve endings were observed in the lingual nail area in the Egyptian geese [12]. In the SWG, sensory corpuscles were observed only in the dermis of the beak.

The compound tubular lingual salivary glands of SWG were similar to findings reported in Egyptian geese [12]. The lingual glands found in the Muscovy ducks and African pied crows are of the simple tubular type [15], while in the laughing dove and quail, they were tubulo-alveolar, having a strong affinity to AB stain [16, 17]. The lingual salivary glands function by secreting mucinous saliva, acting as a lubricant to aid swallowing of ingested food as a protective cover for the mucous membrane of the upper digestive tract [18].

The esophageal mucosal wall of SWG comprised four histological layers, as observed in many avian species [19], and the crop was indistinct, present in the form of a simple fusiform dilation, as reported in the common moorhen [20]. Generally, birds with a fully developed crop temporarily store ingesta, soften it, and predigest poorly digestible food particle [10].

The histo-structure of the crop mucosal wall showed species-specific characteristics. In the SWG, the mucosal lining was stratified squamous non-keratinized, as reported in pigeons, ducks, Japanese quail, and cattle egrets [21]. Similarly, compound alveolar mucosal glands were seen in the subepithelial area of the propria in SWG. In the cattle egret, the gland was of the simple branched alveolar type and compound tubulo-alveolar in domestic ducks [22]. Mucus produced by the mucosal glands of the esophagus and crop helps lubricate the lumen of the esophagus and crop. In addition, it provides a protective coat to the epithelium against friction [23].

The stomach of grain- and plant-eating birds, including the chicken, pigeon, geese, and ducks, is distinctly divided into the glandular proventriculus and the muscular ventriculus [10]. The gizzard in avians functions in grinding and macerating tough ingesta through powerful muscular development [24].

The wall of the proventriculus in SWG was mainly comprised of proventricular glands, as reported in domestic duck [25]. In the moorhen, these were divided into superficial and deep proventricular glands [20]. The gizzard tunica mucosa of the SWG was lined by the secretory cuticle of the gizzard gland, as reported in broilers [24], and had a yellowish layer of cuticle in domestic duck [21].

Dyce et al. [4] reported that cuticles are species-specific. Abumandour [26] reported that only a small area of the mucous membrane of the ventriculus of the eurasian hobby bird was covered by a cuticle layer, while the remaining luminal surface lacked this structure. The thickness of the cuticle was strongly correlated with the type of food: it was thin in frugivorous and nectarivorous birds, and thick in granivorous birds [27].

The gizzard tubular glands of SWG are mostly lined by chief cells, which stained basophilic. These cells secrete proteolytic enzymes called pepsin [28]. The muscularis layer of turkey gizzard was thick and consisted of bundles of parallel smooth muscle fibers, as observed in our findings.

CONCLUSION

The present study provided detailed microscopic features of the upper digestive tract of SWG, which revealed many similarities with the domestic duck. Noticeable features observed were compared with those of other avian species for a better understanding of the functional morphology of the digestive tract as it relates to the adaptive feeding habits in the SWG.

Data Availability Statement

The raw data that supports the findings of this study will be made available by the authors without undue reservation.

Ethical Statement

This study follows ethical guidelines and approval from the Animal Use and Ethics Committee (AUEC) of the Faculty Veterinary Medicine, University of Maiduguri (Approval no: AUP-R004/2023).

Conflict of Interest

The authors have no conflict of interest to declare.

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Generative AI Statement

The manuscript was prepared without the use of generative AI or AI-assisted technologies.

Authors' Contributions

IAG – Concept and results interpretation; SB and MMK – Sample collection and processing; YBM and MZ – Literature search/Resources; IAG and BGG – Manuscript writing and critical review.

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