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Histological, Histochemical and Amino Acid Profile Study of the Cervical Esophagus in the Iraqi Pin-Tailed Sandgrouse (*Pterocles alchata*) and Red-Wattled Lapwing (*Vanellus indicus*)

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ABSTRACT

The avian esophagus shows structural and functional differences related to feeding habits and habitat, with mucous secretions and amino acids playing key roles in protection and metabolism. The present study aimed to comparatively investigate the histological, histochemical, and amino acid profile characteristics of the cervical esophagus in the Iraqi pin-tailed sandgrouse (*Pterocles alchata*) and the red-wattled lapwing (*Vanellus indicus*). A total of eight adult individuals from each bird species (sandgrouse and lapwing) were collected from the Al-Samawah Desert, Al-Muthanna Province, and Al-Yusufiyah District, Baghdad Governorate, Iraq, respectively, between October 2025 and January 2026. Histological examination was performed using hematoxylin and eosin (H&E), Masson's trichrome, periodic acid–Schiff (PAS), and Alcian blue (AB, pH 2.5) stains. Amino acid profiles of cervical esophageal tissues were analyzed using high-performance liquid chromatography (HPLC). The histological results demonstrated that the cervical esophagus in both bird species consisted of three main layers: tunica mucosa, tunica muscularis, and tunica adventitia, while the tunica submucosa and muscularis mucosae were absent. The mucosa was lined by non-keratinized stratified squamous epithelium supported by a collagen-rich lamina propria. In the Iraqi pin-tailed sandgrouse, the esophageal glands were located mainly within the lamina propria, whereas in the red-wattled lapwing, they appeared as intraepithelial glands. Histochemical staining revealed strong PAS reactivity and moderate AB reactivity in the mucous secretions of both bird species, indicating the presence of neutral and acidic mucopolysaccharide. The HPLC analysis identified 18 amino acids in the cervical esophageal tissues of both species. Glutamine showed the highest concentration, whereas methionine exhibited the lowest concentration in both birds. Most amino acids did not differ significantly between the two species ($p > 0.05$). However, glutamic acid showed a significant increase in the Iraqi pin-tailed sandgrouse compared with the red-wattled lapwing ($p \leq 0.05$). The present findings suggest that the cervical esophagi of both bird species may exhibit structural and biochemical adaptations associated with feeding habits and ecological conditions.

1. INTRODUCTION

The avian digestive system exhibits remarkable structural and functional diversity that reflects adaptation to different feeding behaviors and ecological habitats. Among the organs of the digestive tract, the esophagus plays an essential role in food transport, temporary storage, lubrication, and facilitating swallowing [1]. In birds, the morphology and histological organization of the esophagus vary considerably according to dietary habits and the physical nature of ingested food [2]. Generally, the avian esophageal wall consists of tunica mucosa, tunica submucosa, tunica muscularis, and tunica adventitia [3].

The mucosal layer is commonly lined by non-keratinized stratified squamous epithelium and contains mucous glands that secrete lubricating substances to protect the esophageal surface from mechanical abrasion during food passage [4]. However, substantial interspecies variations have been reported in epithelial thickness, gland distribution, connective tissue organization, and muscular arrangement, particularly among birds with different feeding strategies [5, 6]. Histochemical investigations of the avian esophagus have demonstrated the importance of mucous secretions in protecting the mucosal lining and facilitating food movement. Periodic acid–Schiff (PAS) and Alcian blue (AB) staining techniques are commonly used to identify neutral and acidic mucopolysaccharides within esophageal glands and epithelial secretions [7, 8]. These secretory products contribute significantly to maintaining epithelial integrity and reducing friction during swallowing. In addition to histological organization, the biochemical composition of digestive tissues represents an important indicator of tissue function and

metabolic activity. Amino acids are essential structural and functional components of proteins and are involved in cellular metabolism, tissue repair, enzymatic activity, and physiological regulation [9].

Analysis of amino acid profiles in digestive tissues may therefore provide valuable information regarding tissue physiology, and adaptive metabolic responses may be associated with feeding behavior [10]. High-performance liquid chromatography (HPLC) is considered one of the most reliable analytical techniques for the qualitative and quantitative determination of amino acids in biological tissues due to its high sensitivity, accuracy, and reproducibility [11]. This technique has been widely applied in biochemical and physiological studies to evaluate metabolic activity, tissue composition, and nutritional adaptations in both vertebrate and avian species [12, 13].

HPLC-based amino acid profiling provides insight into tissue activity and physiological adaptations that may be associated with feeding behavior and environmental conditions [14]. The Iraqi pin-tailed sandgrouse (*Pterocles alchata*) is a granivorous bird inhabiting arid and semi-desert regions, which may be associated with their granivorous feeding habits [15]. In contrast, the red-wattled lapwing (*Vanellus indicus*) is an insectivorous and omnivorous bird commonly distributed in wetlands and agricultural areas, feeding primarily on insects, worms, and small invertebrates collected from moist soil surfaces [16]. These dietary differences may influence the histological and biochemical characteristics of the digestive tract, particularly the esophagus [17]. Although several studies have investigated the morphology and histology of the avian digestive tract, studies combining histological, histochemical, and amino acid profile analyses of esophageal tissues in wild birds remain limited, especially in bird species inhabiting the Iraqi environment [18, 19]. To the best of our knowledge, no previous study has comparatively investigated the cervical esophagus of the Iraqi pin-tailed sandgrouse and the red-wattled lapwing using both histological and biochemical approaches.

Despite the increasing number of anatomical and histological studies on the avian digestive tract, little information is available regarding the relationship between esophageal microstructure, mucous secretory activity, and tissue amino acid composition in wild birds. The cervical esophagus represents the first region exposed to the mechanical and chemical properties of ingested food and therefore plays an important role in food lubrication and mucosal protection. Furthermore, amino acid profiling may provide additional information regarding tissue metabolism and maintenance of esophageal integrity. Therefore, integrating histological, histochemical, and biochemical analyses may contribute to a better understanding of functional adaptations of the avian esophagus.

Therefore, the present study aimed to comparatively evaluate the histological organization, histochemical characteristics, and amino acid profile of the cervical esophagus in the Iraqi pin-tailed sandgrouse (*Pterocles alchata*) and the red-wattled lapwing (*Vanellus indicus*).

2. MATERIALS AND METHODS

2.1 Ethical approval

A total of 16 adult wild birds were obtained alive from local hunters between October 2025 and January 2026. Eight Iraqi pin-tailed sandgrouse (*Pterocles alchata*) were purchased from a hunter in Al-Samawah Desert, Iraq, whereas eight red-wattled lapwings (*Vanellus indicus*) were purchased from a hunter in Al-Yusufiyah district, Baghdad, Iraq. Species identification and specimen acquisition were conducted in cooperation with the Iraq Natural History Research Center and Museum, University of Baghdad, under Official Facilitation Letter No. 3064 dated 10 August 2025. The birds were obtained alive from local hunters between October 2025 and January 2026 and maintained under appropriate husbandry conditions until laboratory processing. All experimental procedures, including anesthesia, dissection, tissue sampling, histological processing, histochemical staining, and amino acid analyses, were conducted only after ethical approval had been granted by the Department of Biology, College of Education for Pure Science (Ibn Al-Haitham), University of Baghdad (Approval No. EC-93, dated 19 January 2026).

2.2 Bird collection and experimental design

The specimens described in Section 2.1 were used for gross anatomical, histological, histochemical, and amino acid analyses of the cervical esophagus. All birds were apparently healthy adults with no visible pathological abnormalities. The approximate age of the Iraqi pin-tailed sandgrouse ranged from 12–14 months, whereas that of the red-wattled lapwing ranged from 11–13 months. The mean body weight of the Iraqi pin-tailed sandgrouse (*Pterocles alchata*) was 211.25 ± 6.55 g, with body weights ranging between 195 and 225 g. The mean body weight of the red-wattled lapwing (*Vanellus indicus*) was 163.95 ± 6.69 g, with body weights ranging between 150 and 178 g. For histological and histochemical investigations, four birds from each species were examined, including two males and two females. Both sexes were included because previous avian histological studies reported no significant sex-related differences in the examined esophageal tissues and have successfully used mixed-sex sampling designs [20, 21]. Due to the limited availability of wild specimens during the collection period, sex-specific comparisons were not performed, which should be considered a limitation of the present study. The remaining four male birds of each species were used for amino acid analysis by HPLC. Male birds were selected exclusively for biochemical analysis to avoid potential physiological variations associated with female reproductive status and egg-production cycles, which may influence amino acid metabolism and tissue biochemical composition. The sample size was selected according to previous histological and anatomical studies on avian species using comparable sample numbers [22, 23].

2.3 Anesthesia and tissue sampling

Birds were anesthetized using ketamine hydrochloride (40 mg/kg body weight) combined with xylazine (5 mg/kg body weight) administered intramuscularly before dissection, according to standard avian anesthesia protocols [24]. Following complete anesthesia, birds were dissected under sterile laboratory conditions. The cervical portion of the esophagus was carefully excised, washed with normal saline solution to remove food residues, and immediately processed for histological and biochemical analyses.

2.4 Histological and histochemical procedures

Tissue samples designated for histological examination were fixed in 10% neutral buffered formalin for 48 h at room temperature (25 ℃). Following fixation, samples were dehydrated through ascending grades of ethanol (70%, 80%, 90%, 95%, and absolute ethanol), cleared in xylene, and embedded in paraffin wax according to standard histological procedures [7]. Paraffin blocks were sectioned at 5–6 µm thickness using a rotary microtome. Tissue sections were mounted on clean glass slides. For routine histological examination, sections were stained with hematoxylin and eosin (H&E), including hematoxylin staining followed by washing, differentiation, eosin counterstaining, dehydration, clearing, and mounting. Masson's trichrome staining was performed for collagen fiber identification according to standard protocols. Histochemical staining included PAS staining for the demonstration of neutral mucopolysaccharides and AB staining (pH 2.5) for acidic mucopolysaccharides according to previously established methods [7, 18]. Negative control sections were prepared by omitting the primary staining step where applicable. Histological sections were examined and photographed using a light microscope equipped with a digital imaging system.

2.5 Histomorphometric analysis

Histomorphometric measurements were performed using ImageJ software (National Institutes of Health, USA) after calibration with the scale bar. Mucosal thickness, mucosal fold height, gland diameter, gland density, and muscular layer thickness were measured from five randomly selected microscopic fields for each specimen. All measurements were expressed in micrometers (µm), except gland density, which was expressed as the number of glands per mm². Data were presented as mean ± standard error (Mean ± SE) and statistically analyzed to determine interspecific differences.

2.6 Histochemical scoring

The staining intensity of PAS and AB (pH 2.5) reactions was evaluated using a semi-quantitative scoring system based on the degree of staining observed in the epithelial lining and esophageal glands. The staining intensity was classified as follows: 0 = negative reaction, 1 = weak reaction, 2 = moderate reaction, and 3 = strong reaction. Five randomly selected microscopic fields from each specimen were examined, and the average score was used for comparative evaluation between the studied species.

2.7 Amino acid profiling by high-performance liquid chromatography

Free amino acid concentrations in cervical esophageal tissues were determined using HPLC after pre-column derivatization with phenyl isothiocyanate (PITC), according to the methods described by Zheng et al. [25] and Zhang et al. [26] with minor modifications.

Approximately 0.5 g of cervical esophageal tissue from each bird was homogenized in 5 mL of deionized water using a glass homogenizer under cold conditions. Protein precipitation was performed by adding 1 mL of sulfosalicylic acid solution (10%), followed by centrifugation at 10,000 rpm for 15 min at 4℃. The obtained supernatant was collected and adjusted to pH 2.0 using sodium acetate buffer.

Before chromatographic analysis, amino acids were derivatized using PITC. Briefly, 100 µL of sample supernatant was mixed with PITC reagent and incubated at room temperature for 20 min to allow complete derivatization. The derivatized samples were filtered through 0.22 µm membrane filters before injection into the HPLC system. Chromatographic separation was performed using a Shimadzu HPLC system equipped with a Shim-pack XR-ODS column (150 mm × 4.6 mm, 5 µm particle size). The column temperature was maintained at 30. Chromatographic separation was performed using a linear gradient elution program between solvent A (5% methanol in 0.1 N sodium acetate buffer, pH 7.0) and solvent B (methanol) over a total run time of 25 min at a flow rate of 1 mL/min. Detection was carried out at a wavelength of 254 nm, and the injection volume was 20 µL. Identification and quantification of amino acids (µg/g tissue) were achieved by comparing sample retention times and peak areas with those of standard amino acid mixtures analyzed under identical chromatographic conditions as previously described [27]. Calibration curves were prepared using serial concentrations of amino acid standards, and the linearity of calibration curves showed correlation coefficients (R^2) greater than 0.99 for all detected amino acids. Quantification of amino acids was performed based on peak area measurements and comparison with corresponding standard curves. To ensure analytical reliability, all samples were analyzed in duplicate to ensure analytical reproducibility. The external laboratory reported that LOD and LOQ assessments were based on signal-to-noise ratios of 3:1 and 10:1, respectively; however, the corresponding numerical values were not provided in the analytical report. Method performance characteristics, including recovery percentages ranging from 92% to 97%, were previously validated and reported by the study [13]. A mixed amino acid standard solution containing 18 amino acids at a concentration of 50 µg/mL for each amino acid was analyzed under identical chromatographic conditions (Table 1). Amino acid identification was achieved by comparing retention times, whereas quantification was based on comparison of sample peak areas with the corresponding standard peak areas.

Table 1. Retention times and peak areas of amino acid standards used for high-performance liquid chromatography (HPLC) identification

Amino Acid	Retention Time (min)	Standard Peak Area (a.u)
Asparagine	1.022	508104
Serine	1.875	515958
Glutamine	3.015	479841
Glutamic acid	3.870	523184
Threonine	4.930	455025
Histidine	6.007	432901
Citrulline	6.883	410423
Alanine	7.940	374910
Proline	8.775	436037
Glycine	9.853	393323
Arginine	11.012	377163
Tyrosine	12.112	362163
Valine	13.030	373215
Methionine	14.115	379762
Isoleucine	15.183	341591
Leucine	16.022	356542
Phenylalanine	16.853	427508
Lysine	17.918	338360

Note: Peak area values are expressed as arbitrary units (a.u.) generated by the HPLC integration software.

The HPLC analyses were performed by an external analytical laboratory using a validated PITC pre-column derivatization method. The chromatographic gradient program (linear gradient, 0–25 min) was provided by the laboratory and has been included in the revised manuscript. However, exact numerical values for the LOD and LOQ were not available in the analytical report.

2.8 Statistical analysis

Statistical analysis was performed using IBM SPSS Statistics version 26 (IBM Corp., Armonk, NY, USA). Data were expressed as Mean ± SE. Data normality was evaluated using the Shapiro–Wilk test before statistical comparisons. Comparisons between the Iraqi pin-tailed sandgrouse and the red-wattled lapwing were performed using the independent samples t-test. Statistical significance was considered at $p \leq 0.05$.

3. RESULTS

3.1 Histological and histochemical characteristics of the cervical esophagus

Histological examination of the cervical esophagus in the Iraqi pin-tailed sandgrouse (*Pterocles alchata*) (Figure 1) and the red-wattled lapwing (*Vanellus indicus*) (Figure 2) revealed that the esophageal wall consisted of three main layers: tunica mucosa, tunica muscularis, and tunica adventitia, whereas the tunica submucosa and muscularis mucosae were absent in both bird species (Figures 1(A) and 2(A)). The tunica mucosa was lined by non-keratinized stratified squamous epithelium supported by a lamina propria rich in connective tissue fibers and blood vessels (Figures 1(A, B) and 2(A, B)). Distinct longitudinal mucosal folds extending toward the esophageal lumen were observed in both species. In the Iraqi pin-tailed sandgrouse, these folds were more numerous and prominent in the anterior cervical region, whereas in the red-wattled lapwing, they appeared relatively uniform throughout the cervical esophagus. The epithelial surface of the red-wattled lapwing exhibited a greater number of glandular openings compared with the Iraqi pin-tailed sandgrouse, resulting in a more mucus-rich luminal surface (Figures 1(B) and 2(B)).

Lymphoid tissue aggregations were observed in the cervical esophagus of the Iraqi pin-tailed sandgrouse, particularly within the lamina propria and tunica adventitia (Figure 1(B)). In contrast, the red-wattled lapwing showed only scattered lymphoid cells without distinct lymphoid nodules (Figure 2(B)). The connective tissue of the lamina propria differed between the two bird species. In the Iraqi pin-tailed sandgrouse, the connective tissue was loose and highly vascularized, whereas the red-wattled lapwing exhibited dense connective tissue rich in collagen fibers, as demonstrated by Masson's trichrome staining (Figures 1(C) and 2(C)).

Mucous esophageal glands were observed in both bird species; however, their location and morphology differed markedly. In the Iraqi pin-tailed sandgrouse, the glands were mainly located within the lamina propria and appeared alveolar in shape with wide lumina (Figures 1(A)–(C)). In contrast, the glands of the red-wattled lapwing appeared predominantly as intraepithelial tubular glands arranged in one or two rows within the epithelial layer (Figures 2(A)–(C)). Histochemical staining using PAS and AB, pH 2.5, demonstrated strong positive reactions in the glandular secretions and epithelial mucous material of both bird species, indicating the presence of neutral and acidic mucopolysaccharides (Figures 1(D, E) and 2(D, E)).

The tunica muscularis in both species consisted of a thin inner longitudinal smooth muscle layer and a thick outer circular layer (Figures 1(A) and 2(A)). In the Iraqi pin-tailed sandgrouse, the circular muscle layer appeared as separated muscular bundles, whereas in the red-wattled lapwing, it formed a more compact and continuous muscular layer. The tunica adventitia consisted of loose connective tissue containing blood vessels and nerve fibers in both species. Diffuse lymphoid tissue was additionally observed within the adventitia of the Iraqi pin-tailed sandgrouse.

Histomorphometric analysis of the cervical esophagus revealed significant interspecific differences in several structural parameters (Table 2). The red-wattled lapwing exhibited significantly greater mucosal thickness (15.80 ± 1.19 µm) and mucosal fold height (47.82 ± 1.42 µm) compared with the pin-tailed sandgrouse (10.29 ± 0.67 µm and 30.96 ± 1.47 µm, respectively) ($p < 0.001$). In contrast, gland diameter was significantly larger in the pin-tailed sandgrouse (6.79 ± 0.49 µm) than in the red-wattled lapwing (3.32 ± 0.19 µm) ($p < 0.001$). Gland density was significantly higher in the red-wattled lapwing (4.03 ± 0.27 glands/mm²) than in the pin-tailed sandgrouse (2.45 ± 0.07 glands/mm²) ($p = 0.003$). However, no significant differences were observed in muscular layer thickness ($p = 0.30$) or adventitial thickness ($p = 0.80$).

Table 2. Histomorphometric measurements of the cervical esophagus in the studied bird species

Parameter (µm)	Pin-Tailed Sandgrouse (Mean ± SE)	Red-Wattled Lapwing (Mean ± SE)	p-Value
Mucosal thickness	10.29 ± 0.67^{b}	15.80 ± 1.19^{a}	<0.001***
Mucosal fold height	30.96 ± 1.47^{b}	47.82 ± 1.42^{a}	<0.001***
Muscular layer thickness	18.07 ± 1.76	19.97 ± 0.81	0.30 NS
Adventitial thickness	9.59 ± 0.51	9.79 ± 0.87	0.80 NS
Gland diameter	6.79 ± 0.49^{a}	3.32 ± 0.19^{b}	<0.001***
Gland density (No./mm²)	2.45 ± 0.07^{b}	4.03 ± 0.27^{a}	0.003**

Notes: Values are presented as mean ± standard error (Mean ± SE). Different superscript letters within the same row indicate significant differences at $p < 0.05$. NS = not significant ($p > 0.05$); ** $p < 0.01$; *** $p < 0.001$.

Table 3. Semi-quantitative evaluation of periodic acid–Schiff (PAS) and Alcian blue (AB) staining intensity in the cervical esophagus of the studied birds

Histochemical Stain	*P. alchata*	*V. indicus*
PAS reaction	3 (Strong)	3 (Strong)
AB reaction	2 (Moderate)	2 (Moderate)

Semi-quantitative assessment revealed strong PAS positivity in the epithelial lining and esophageal glands of both species, indicating abundant neutral mucopolysaccharides. AB staining showed moderate to strong reactivity, suggesting the presence of acidic mucopolysaccharides within the mucosal secretions (Table 3).

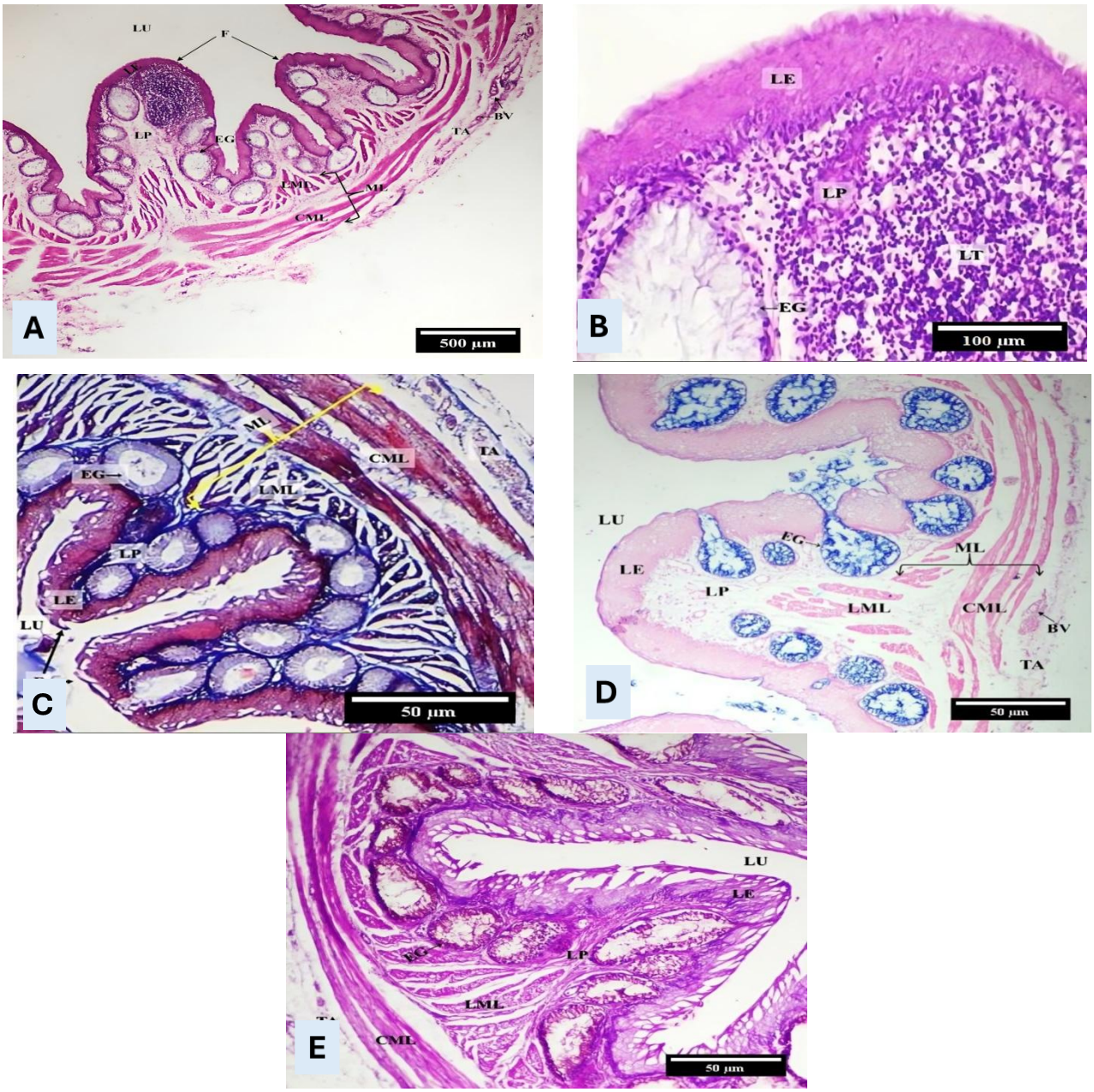
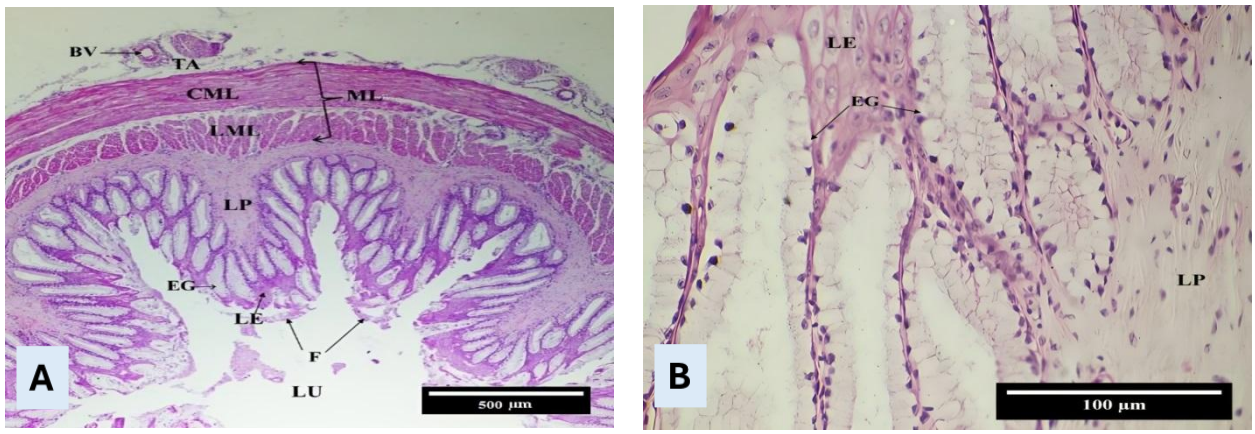


Figure 1. Histological and histochemical sections of the cervical esophagus in the Iraqi pin-tailed sandgrouse (*Pterocles alchata*). **(**A) General histological organization of the esophageal wall (H&E, 4×), (B) higher magnification showing the LE, LP, EG, and LT (H&E, 40×), (C) collagen fibers within the lamina propria stained with Masson's trichrome (MT, 10×), (D) Positive Alcian blue reaction in glandular secretions (AB, pH 2.5, 10×), (E) positive PAS reaction in mucous glands and luminal secretions (PAS, 10×)

Notes: Lining Epithelium (LE), lamina propria (LP), esophageal glands (EG), lymphoid tissue (LT), tunica mucosa (TM), tunica muscularis (ML), and tunica adventitia (TA), Lumen of Oesophagus (LU), Folds (F), Longitudinal Muscle Layer (LML), Circular Muscle Layer (CML), and Blood Vessels (BV).



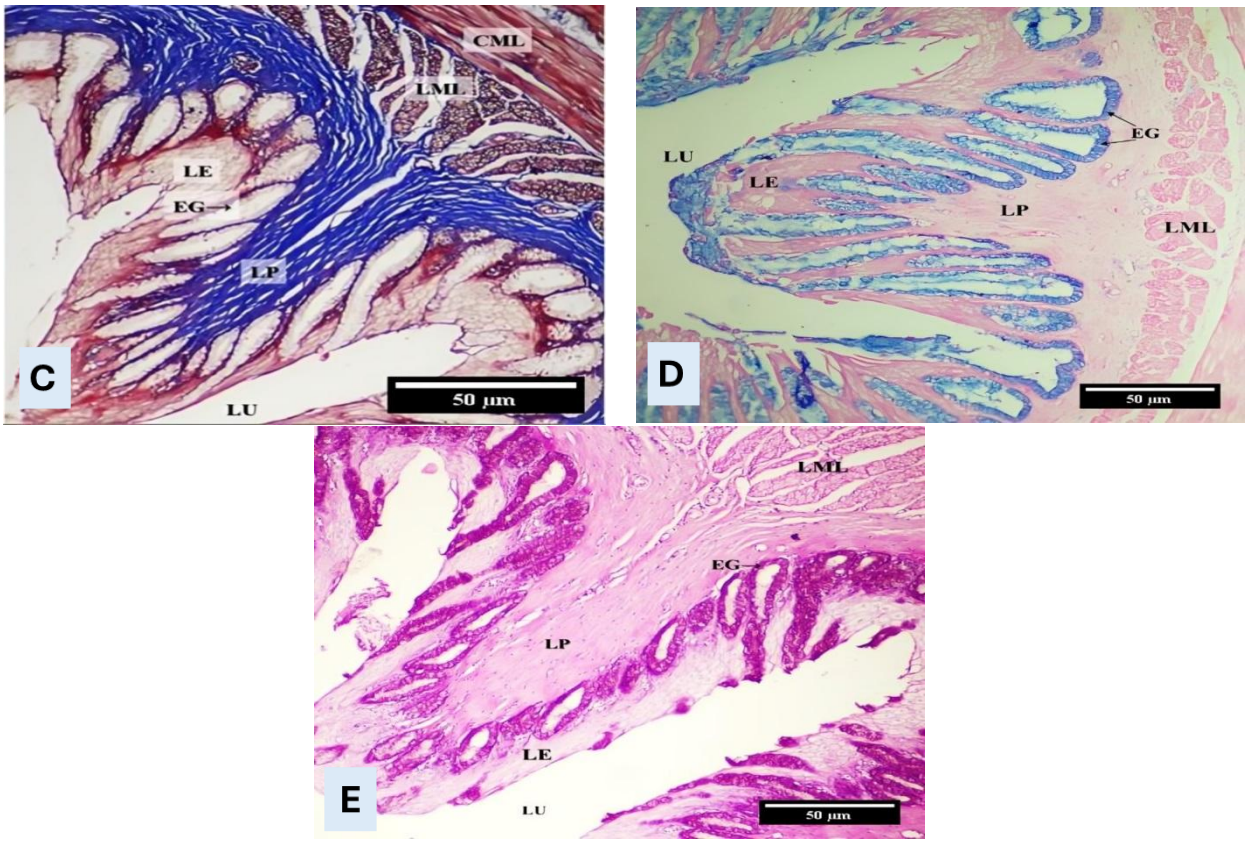


Figure 2. Histological and histochemical sections of the cervical esophagus in the Red-Wattled lapwing (*Vanellus indicus*). (A) General histological organization of the esophageal (H&E, 4×), (B) higher magnification showing LE, LP, and EG (H&E, 40×), (C) collagen fibers within the lamina propria stained with Masson's trichrome (MT, 10×), (D) positive Alcian blue reaction in glandular secretions (AB pH 2.5, 10×), (E) positive PAS reaction in mucous glands and luminal secretions (PAS, 10×)

Notes: Lining Epithelium (LE), lamina propria (LP), esophageal glands (EG), tunica mucosa (TM), tunica muscularis (ML), and tunica adventitia (TA), Lumen of Oesophagus (LU), Folds (F), Longitudinal Muscle Layer (LML), Circular Muscle Layer (CML), and Blood Vessels (BV).

3.2 Amino acid profile of the cervical esophagus

Eighteen amino acids were identified in the cervical esophagus of both bird species using HPLC. Although most amino acids showed numerically higher concentrations in *P. alchata* than in *V. indicus*, these differences were not statistically significant ($p > 0.05$). The only amino acid that exhibited a significant interspecific difference was glutamic acid, which was significantly higher in *P. alchata* (82.73 ± 12.47 µg/g tissue) than in *V. indicus* (48.23 ± 3.41 µg/g tissue) ($p = 0.037$) (Table 4, Figures 3, 4).

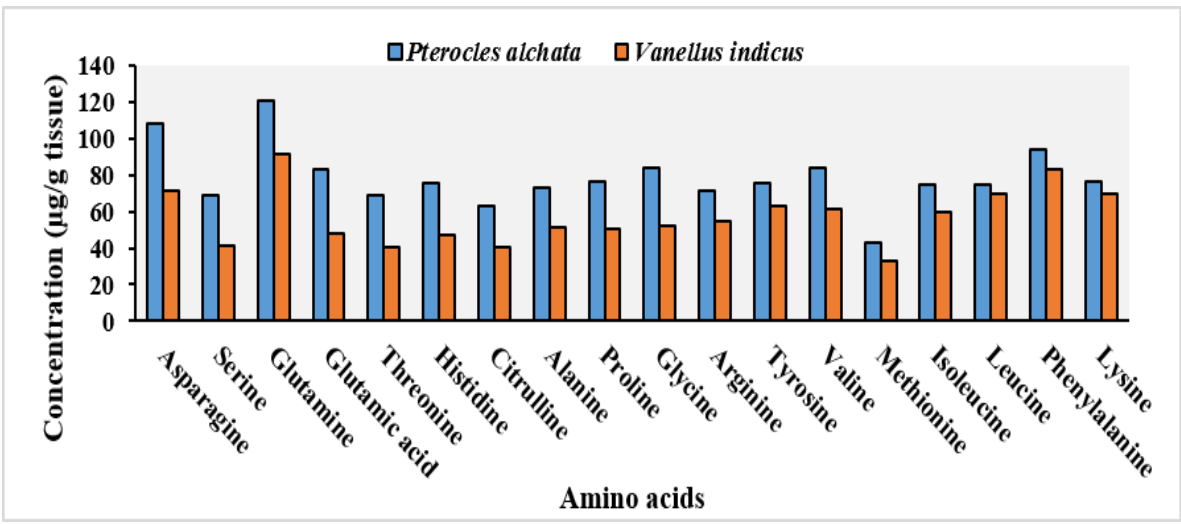


Figure 3. The average concentration of amino acids in the cervical esophageal tissue of the Iraqi pin-tailed sandgrouse and the red-wattled lapwing

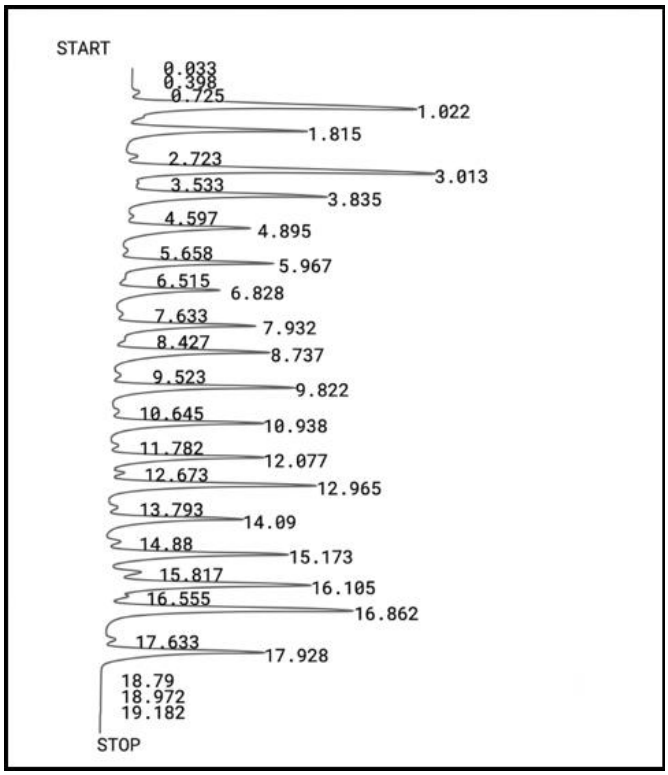


Figure 4. Representative high-performance liquid chromatography (HPLC) chromatogram of amino acids detected in the cervical esophagus tissue

Table 4. Amino acid concentrations (µg/g tissue) in the cervical esophagus of the Iraqi pin-tailed sandgrouse (*Pterocles alchata*) and the red-wattled lapwing (*Vanellus indicus*)

Amino Acids	Pin-Tailed Sandgrouse (Mean ± SE)	Red-Wattled Lapwing (Mean ± SE)	p-Value
Asparagine	108.30 ± 18.89	71.25 ± 6.80	NS
Serine	68.50 ± 16.36	41.41 ± 7.83	NS
Glutamine	120.55 ± 14.54	91.21 ± 9.70	NS
Glutamic acid	82.73 ± 12.47	48.23 ± 3.41	0.037*
Threonine	69.09 ± 13.35	40.78 ± 4.62	NS
Histidine	75.77 ± 11.68	47.15 ± 5.94	NS
Citrulline	63.02 ± 12.80	40.24 ± 6.61	NS
Alanine	72.90 ± 11.89	51.49 ± 5.45	NS
Proline	76.41 ± 13.11	50.27 ± 5.90	NS
Glycine	83.44 ± 13.77	52.44 ± 3.63	NS
Arginine	71.46 ± 10.27	54.71 ± 6.55	NS
Tyrosine	75.20 ± 8.04	62.85 ± 6.94	NS
Valine	83.58 ± 11.37	61.45 ± 2.44	NS
Methionine	43.05 ± 8.66	33.20 ± 0.49	NS
Isoleucine	75.01 ± 7.40	59.36 ± 4.24	NS
Leucine	74.74 ± 10.78	69.26 ± 3.60	NS
Phenylalanine	93.56 ± 6.71	83.09 ± 7.53	NS
Lysine	76.44 ± 10.11	69.61 ± 8.65	NS

Notes: Values are expressed as mean ± standard error (Mean ± SE). Different superscript letters within the same row indicate significant differences at $p < 0.05$. NS = not significant ($p > 0.05$); * = significant at $p < 0.05$.

4. DISCUSSION

The present study demonstrated clear histological and histochemical similarities and differences in the cervical esophagus between the Iraqi pin-tailed sandgrouse (*Pterocles alchata*) and the red-wattled lapwing (*Vanellus indicus*). In both bird species, the esophageal wall consisted mainly of tunica mucosa, tunica muscularis, and tunica adventitia, whereas the tunica submucosa and muscularis mucosae were absent. Similar observations have been reported previously in several avian species, including domestic fowl and wild birds, where the absence or poor development of these layers was considered a characteristic potential adaptation of the avian esophagus [1, 28].

The mucosal lining of the cervical esophagus in both species was composed of non-keratinized stratified squamous epithelium with prominent longitudinal folds. These folds increase the luminal expansion capacity and facilitate the passage of food materials during swallowing. Comparable findings were described by Indu et al. [29], who reported that the development of mucosal folds in birds may be associated with dietary habits and the physical characteristics of ingested food. The Iraqi pin-tailed sandgrouse has developed an adaptation through increased mucosal folding within its esophagus as a result of consuming dry, coarse food items (specifically seed and grain). This potential adaptation allows for increased distensibility (capacity to stretch) of the esophagus, which permits the smooth transition of particles through the esophagus from the throat to the stomach. Conversely, the relatively even folds of the red-wattled lapwing are indicative of an adaptation consistent with their primarily insectivorous diet, which consists mainly of softer, more hydrated (moist) food items.

Connective tissue organization differed markedly between the two species. In the Iraqi pin-tailed sandgrouse, the lamina propria consisted mainly of loose vascular connective tissue containing numerous mucous glands and lymphoid aggregations, whereas the red-wattled lapwing exhibited denser collagenous connective tissue. Similar structural variations among avian species have been associated with differences in feeding ecology and mechanical stress during food transport [2, 30].

The distribution patterns of the esophageal glands in these two avian species appear to be an indicator of functional adaptation to their different feeding habits. For example, the fact that the red-wattled lapwing has mostly intraepithelial glands may imply that it secretes mucus quickly and directly to the surface of its esophagus, which would aid in lubricating its moist invertebrate prey before swallowing. In contrast, the Iraqi pin-tailed sandgrouse has lamina propria glands that likely provide ongoing protection of its mucosa from physical injury due to the ingestion of dry, coarse grains. Similar epithelial glandular modifications have occasionally been reported in some aquatic and semi-aquatic bird species exposed to high mucosal secretory demands [31].

The present histomorphometric analysis demonstrated significant structural differences in the cervical esophagus between the two studied bird species. The significantly greater mucosal thickness and mucosal fold height observed in the red-wattled lapwing may increase luminal expansion and provide enhanced protection of the esophageal lining during food transport. Conversely, the larger gland diameter observed in the pin-tailed sandgrouse suggests greater secretory activity at the level of individual glands. The significantly higher gland density in the red-wattled lapwing indicates that mucus production may be achieved through a greater number of glands rather than increased gland size [32]. In contrast, the absence of significant differences in muscular and adventitial thickness suggests that the basic supportive and contractile

architecture of the cervical esophagus is relatively conserved between the two species. Collectively, these findings suggest species-specific morphological adaptations that may be associated with differences in feeding ecology and food-processing requirements; however, direct dietary investigations are required to confirm these interpretations [33].

Histochemical staining revealed strong positive PAS reactions and moderate positive AB reactions in the epithelial and glandular secretions of both species, indicating the presence of neutral and acidic mucopolysaccharides. Neutral mucopolysaccharides contribute mainly to lubrication and epithelial protection, whereas acidic mucopolysaccharides enhance water retention and mucosal viscosity. Similar reactions were documented in previous avian histochemical investigations by Alsanosy et al. [4], who emphasized the protective role of mucous secretions against mechanical injury and desiccation.

The muscular layer of the cervical esophagus differed slightly between the two bird species. In the Iraqi pin-tailed sandgrouse, the outer circular muscle layer appeared separated into muscular bundles, while in the red-wattled lapwing, it formed a compact, continuous layer. These variations may reflect differences in swallowing mechanics and food consistency. Granivorous birds generally require stronger esophageal motility to facilitate the transport of coarse and dry food particles compared with insectivorous or omnivorous birds [15].

Lymphoid tissue aggregations observed in the cervical esophagus of the Iraqi pin-tailed sandgrouse may indicate enhanced local immune defense against environmental pathogens and particulate materials associated with desert and semi-arid habitats. Similar lymphoid structures have been identified in the digestive tracts of wild birds exposed to dusty or contaminated environments [6].

Because of the exploratory nature of the amino acid analysis and the limited sample size, no multiple-comparison correction was applied. Therefore, the statistically significant difference observed for glutamic acid should be interpreted with caution and considered a preliminary finding requiring confirmation in larger studies.

The significant increase observed in glutamic acid may reflect species-specific differences in protein metabolism, tissue maintenance, or physiological adaptation. Glutamic acid is one of the major amino acids involved in cellular metabolism and protein synthesis, and its elevated concentration in *P. alchata* may be associated with the functional requirements of the esophageal tissue in this species [34, 35]. Granivorous diets rich in plant proteins may contribute to elevated glutamic acid concentrations compared with the more insect-based diet of the red-wattled lapwing [36].

The overall similarities in amino acid profiles between the two bird species may indicate comparable physiological requirements for maintaining esophageal structure and mucosal function despite differences in habitat and feeding ecology. Similar observations have been reported in biochemical studies of avian digestive tissues, where amino acid composition was found to be relatively conserved among bird species with moderate dietary variation [37].

The present findings provide important comparative information regarding the structural and biochemical adaptations of the cervical esophagus in wild Iraqi birds. Furthermore, the combination of histological, histochemical, and amino acid profiling approaches contributes to a broader understanding of functional adaptations of the avian digestive system in relation to feeding ecology and environmental conditions.

Overall, the current results indicated a high degree of similarity in the biochemical composition of esophageal tissue between the two studied bird species, with limited differences in certain amino acids. This may reflect a balance between the histological structure and the esophagus's function in transporting food and preparing it for passage to the subsequent segments of the gastrointestinal tract, as well as physiological adaptations may be associated with the birds' different feeding habits. The relatively small sample size represents a limitation of the present study because of the difficulty in obtaining wild bird specimens from natural habitats.

A limitation of the present study is that the exact numerical values of LOD and LOQ for the HPLC method were not available from the external analytical laboratory and, therefore, could not be reported.

5. CONCLUSIONS

In conclusion, both the Iraqi pin-tailed sandgrouse and the red-wattled lapwing exhibit species-specific structural and biochemical adaptations of the cervical esophagus that suggest possible structural and biochemical adaptations associated with feeding behaviour and habitat conditions. Structural differences in glandular organization, connective tissue composition, muscular arrangement, and amino acid profiles in the cervical esophagus of the studied species demonstrate a functional specialization to meet the dietary requirements of these birds. Through the use of histological, histochemical, and biochemical analyses, the current study provides novel comparative data that may be useful in future studies on avian digestive physiology and ecological adaptation.

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