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Comparative morphological histological & histochemical findings of the oropharyngeal cavity and tongue between male and female barn owls (*Tyto Alba*)

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Abstract

The study revealed clear morphological and morphometric as well as histological and histochemical differences in the oral cavity and tongue structure between male and female barn owls (*Tyto alba*), reflecting divergent anatomical adaptations consistent with differences in body lease size and feeding behavior between the sexes. The oral cavity in both sexes showed a longitudinal extension with a well-developed palatal cleft and gloss palatine process, suggesting mechanical adaptations that facilitate the ingestion of whole prey. Females displayed a significantly longer and wider oral cavity, as well as a longer and sharper beak, enhancing their ability to handle larger prey. The tongue in both sexes was characterized by a short, conical shape, of papillae, but it was longer, wider, and thicker in females. The study also described the histological and histochemical structure of the oropharyngeal cavity and tongue in male and female owls (*Tyto alba*), revealing clear differences between the sexes at the structural and secretory levels. Females were characterized by significantly greater thickness in the layers of the oropharyngeal wall (epithelium, lamina propria, and muscle), along with a higher density of mucous glands, particularly near the base of the tongue. On the tongue, females showed increased thickness in all studied layers, reflecting a more developed structure to support control during swallowing and handling of larger prey. Histochemical reactions using PAS and Alcian Blue stains revealed the presence of both neutral and acidic mucus, with stronger secretory activity in females.

Keyword: Barn owl, oral cavity, tongue

Introduction

The oral cavity and tongue are vital structures of physiological and adaptive importance in birds, playing a key role in food capture, ingestion, tasting, and assisting breathing in some species. Birds of various orders exhibit marked variation in the anatomical and histological structures of this part of the digestive system, reflecting the ecological and nutritional specialization of each species (Iwasaki, 2002; Meryom *et al.*, 2009). The owl (*Tyto alba*) belongs to the order Strigiformes and feeds on whole prey of small mammals, birds, and reptiles. Therefore, its oral cavity is characterized by anatomical features that facilitate direct swallowing without chewing, with high keratinization to protect the epithelium from tearing or abrasion. This necessitates morphological and histological adaptations that facilitate food selection, filtration, and moistening (Homberger, & Meyers, 1989; Wang *et al.*, 2019). One of the major differences between these two species is the presence of lingual papillae and mucous glands in geese, which are absent or rudimentary in owls. The degree of keratinization of the oral epithelium also varies between the two species, reflecting the nature of their diet and the mechanism of their consumption. Furthermore, histochemical studies using PAS and Alcian Blue stains help identify the types of mucous compounds within the glands and epithelium, highlighting the differences in the secretory and immunological functions of the oral cavity (Jackowiak & Godynicki, 2005; Jackowiak, & Ludwig, 2008) [17, 22, 14, 28, 18, 19].

This study aims to provide a detailed description of the morphological, microanatomical, and histochemical structures of the oral cavity and tongue in *Tyto alba*, to reveal the environmental and nutritional adaptations associated with the structure, and to expand knowledge about the evolution of the oral system in birds.

Materials and methods

Study Samples

The study included samples of twenty adult barn owls (*Tyto alba*) (10 male & 10 female). The samples were collected immediately after slaughter from local and veterinary markets and were confirmed free of any apparent diseases or injuries. The samples were transported in refrigerated containers to the Anatomy and Histology Laboratory at the Faculty of Veterinary Medicine for examination.

Morphological Study

A descriptive anatomical examination of the oral cavity and tongue was conducted using the following methods:

Manual dissection using fine dissection tools. Photographic documentation using a high-resolution digital camera. Lengths and dimensions were measured using a digital vernier caliper with an accuracy of ± 0.01 mm.

The following measurements were taken

Oral cavity length, mid-oral cavity width, tongue length, tongue base width. Measurements were performed according to the standards adopted in comparative anatomical studies (Iwasaki, 2002; Jackowiak & Godynicki, 2005; Alkhazraji, et al., 2023; Naser, et al., 2024).

Histological Study: Parts of the palate, and the tongue were removed from each bird and fixed in 10% neutral formalin (distal water 900 ml, Formalin (37% formaldehyde solution) 100 ml, Sodium phosphate, monobasic, monohydrate 4 g & Sodium phosphate, dibasic, anhydrous 6.5 g) (Sigma-Aldrich) for 48 hours. The samples were washed with running water, then passed through a graded alcohol series (70-100%) (Eupredia™ (Fisher Scientific)), then xylene (Sankyo Chemical Co., Ltd), followed by embedding in paraffin wax (Fushun Hengyi Petro & Chemical Co., Ltd) 5-micron-thick sections were cut using a microtome and placed on glass slides.

The sections were analysed for general tissue architecture using Hematoxylin and Eosin (H&E) and for collagen fibres with Masson's trichrome stain (Aniline Blue, Biebrich Scarlet/Acid Fuchsin, and Weigert's Iron Hematoxylin; Abcam). Histology evaluation was done using a light microscope with 40 $\times$ and 100 $\times$ objective lenses. The 100 $\times$ lens was used with immersion oil for better resolution and to see details of the cellular connective tissues. For the microscopic study, the epithelial type, glandular type, and muscle bundles were analysed using the method of Bancroft and Steven (2008) [17, 18, 3, 23].

Histochemical Study

Renewed samples from the same areas were dissected and fixed in the same manner. They were then stained with the following: combined Alcian Blue (pH 2.5) and PAS (Periodic Acid-Schiff) stain (Abcam): to detect neutral sugars and acidic mucopolysaccharide.

The slides were examined under a light microscope, and the intensity of the reaction (negative, weakly positive, moderately positive, or strongly positive) was recorded based on the intensity of the staining.

This analysis is based on the histochemical methodologies of Gargiulo *et al.* (1990) and Lillie & Fulmer (1976); Fischer *et al.*, 2008; Kzar, et al., 2023; Zghair, et al., 2020) [31, 32, 33, 34].

Statistical Analysis: Quantitative data (anatomical and histological measurements) were analyzed using SPSS (version 25). A t-test was performed to verify differences between the two types, with a P value of less than 0.05 considered statistically significant.

Results

Comparative morphological findings of the oral cavity and tongue between male and female Barn owls

The study revealed significant morphological, and morphometric differences between males and females in the structure of both the oral cavity and tongue, despite belonging to the same species. These differences are believed to reflect subtle variations in feeding strategies, differences in overall body size, and possibly sex-related competitive feeding behavior in some birds of prey.

The oral cavity

The oral cavity of both males and females is characterized by its longitudinal breadth and deep extension, with a curved palate and a relatively smooth roof of the mouth. The roof of the palate revealed a longitudinal palatal cleft extending posteriorly (Figure 1 & 2), separated by a distinct ridge from the nasal cavity, enhancing the efficiency of breathing during swallowing. An internal nasal canal opening (posterior palatine) was observed at the back of the palate, consistent with the respiratory function during ingestion. At the entrance to the pharynx, a relatively widened and developed palatine and lingual ridge was observed, indicating a mechanical adaptation to accommodate the passage of whole or large pieces of prey. The mucous membrane lining the oral cavity was also documented to be pale pink and shiny, appearing relatively dry due to keratinization, consistent with a nocturnal hunting and rapid feeding pattern. These morphological findings indicate that the Barn owl possesses distinctive oral and lingual adaptations that help it efficiently implement its specific feeding pattern, both in terms of capturing prey and directing and swallowing it. However, morphological measurements showed that females have a relatively longer and wider oral cavity than males, which is generally associated with the larger body size of females compared to males in most owl species (Figure 1, & 2). Characteristics of *Tyto alba* (table 1).

The upper beak is strongly curved in both sexes, covered with a hard horny layer, and appears longer and sharper in females (Figure 2).

There are specialized oral papillae or structures in both sexes, consistent with a feeding pattern based on devouring prey whole without chewing.

Tongue

- The tongue in both sexes is short, conical, devoid of papillae, and covered with keratinized epithelium on the dorsal surface (Figure 3,4).
- However, anatomical measurements have shown that the female tongue is relatively longer and wider, with a thicker base than that of males, which may be attributed to overall body size and a slight difference in oral control when handling large prey.
- Histological and histochemical findings of the oropharyngeal cavity and tongue in male and female barn owls:

First: The oropharyngeal cavity

General structure of the wall of the oropharyngeal cavity in both sexes consists of four main layers: the epithelium, the lamina propria, the muscularis, and the serosa or adventitia (Figure 5).

Epithelium: Lined with non-keratinized stratified squamous epithelium, with areas containing mucous-secreting cells observed, especially near the base of the tongue (Figure 5).
Mucous glands: Simple tubular glands were observed within the lamina propria in both males and females, and their density increased toward the posterior part of the pharynx. (Figure 7).

Voluntary striated muscles: Transverse muscles organized into bundles were observed to support swallowing functions (Figure 6, 7).

- Sex differences obvious histological differences were observed in the stratified structure or general organization of the oropharyngeal cavity and tongue between males and females higher in females than in males. A slight

increase in mucous gland density was observed in females compared to males, which may be related to physiological differences related to swallowing or saliva production during the incubation or feeding period (table 3&4).

- Females show higher thickness values in all layers, indicating that these differences are related to clear biological factors.
- Histochemical reactions confirm the presence of active mucous glands in the tongue, producing a mixed mucus of both neutral and acidic types, which enhances prey glide and protects the epithelium (Figure 8)
- The stronger reaction in females to both PAS and Alcian Blue indicates relatively higher secretory activity, which may support the hypothesis of dietary specialization or handling of larger prey (Figure 8).
- The Masson's trichrome staining results support previous morphological findings on the development of muscle and connective tissue at the base of the tongue in females (Figure 9, 10).

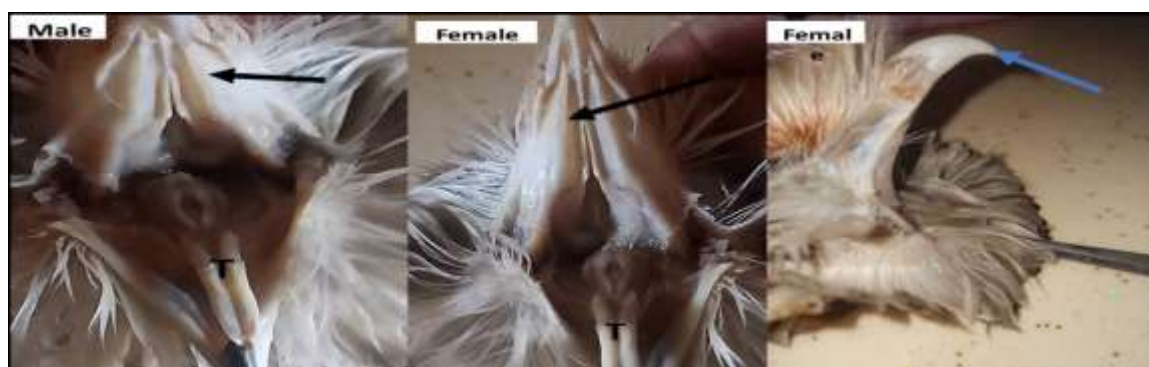


Fig 1: photo macrograph of the oral cavity in male and female owl

Left panel photo macrograph of the oral cavity in male owl showed roof of the oral cavity (black arrow), tongue (T)
 Middle panel photo macrograph of the oral cavity in female

owl showed roof of the oral cavity (black arrow), tongue (T)
 right panel photo macrograph of the oral cavity in female owl showed roof of the oral cavity (blue arrow),

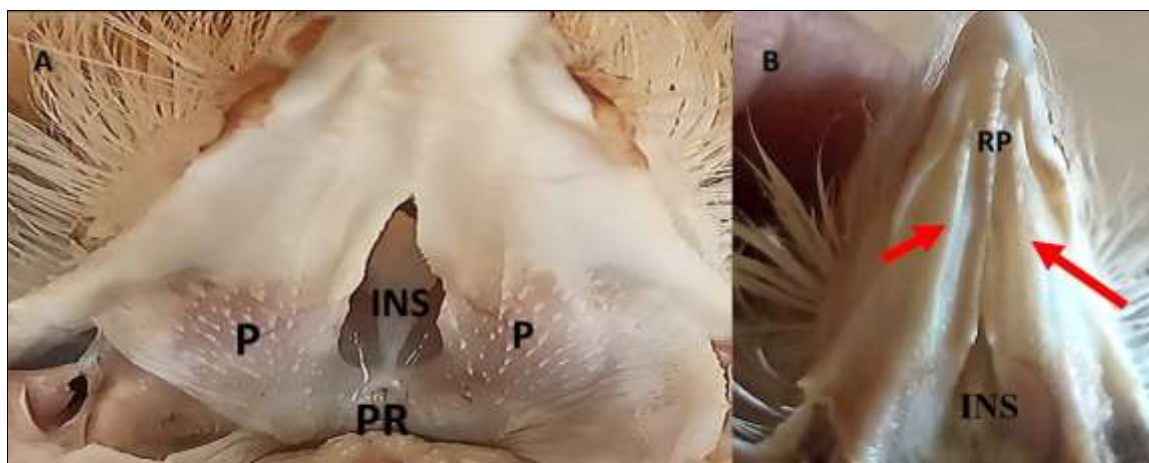


Fig 2: Photographs of the roof of oral cavity of the owl

- A: Photographs of the roof of oral cavity of the owl showed based papillae (P), choanal Clift (INS), and infundibular slit (RR),
- B: Photographs of the roof of oral cavity of the owl, Median palatine ridge (Rp), bilateral ridge (red arrows), choanal Clift (INS).

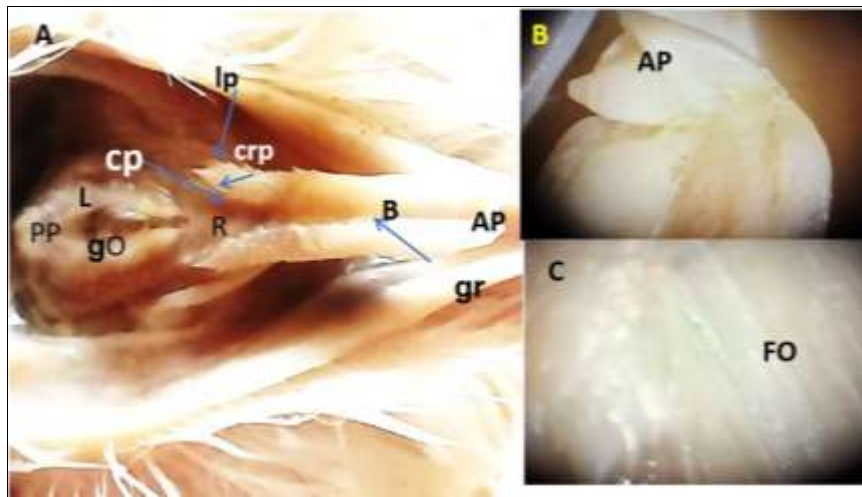


Fig 3: Photographs and dissecting image of the tongue of the female owl

A: Photograph of gross anatomical features of oropharyngeal cavity showed AP Lingual apex, B body, R root, gr median lingual groove, crp-shaped conical papillae crest with the mechanical caudally directed conical papillae (crp) and a rostral extension of the caudally directed papillae on the lateral lingual surface (lp), L laryngeal eminence with medial

opening referred to as the glottis (go) and with pharyngeal papillae (PP), a bifid lingual tip (AP). The U-shaped region of the lingual apex.

- B&C: dissecting micrographs of the parts of the tongue of the. AP Lingual apex, many folds (FO). X10

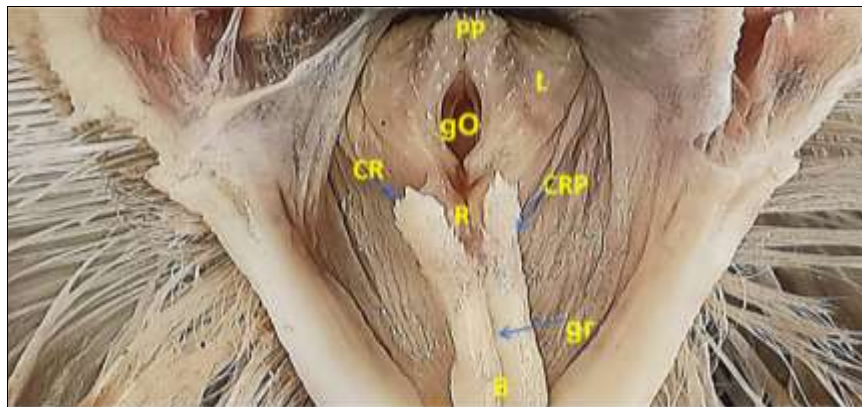


Fig 4: Photographs of the oropharyngeal and tongue of male owl

Photographs of the tongue of male owl showed: B body, R root, gr median lingual groove, CRP- conical papillae crest with the mechanical caudally directed conical papillae (CR) and a rostral extension of the caudally directed papillae on the

lateral lingual surface, (L)laryngeal eminence with medial opening referred to as the glottis (Go) and with pharyngeal papillae (PP).

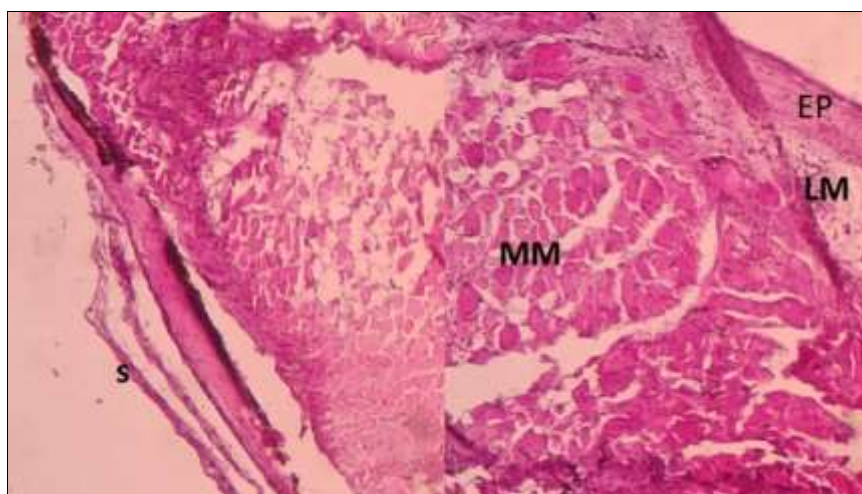


Fig 5: Microscopic image of longitudinal-section of the roof of oral cavity in male owl histology image showing longitudinal-section of the roof of oral cavity in male owl show: epithelium (EP), lamina propria (LM), muscularis layer (MM) and serosa (S) H&E stain; X200

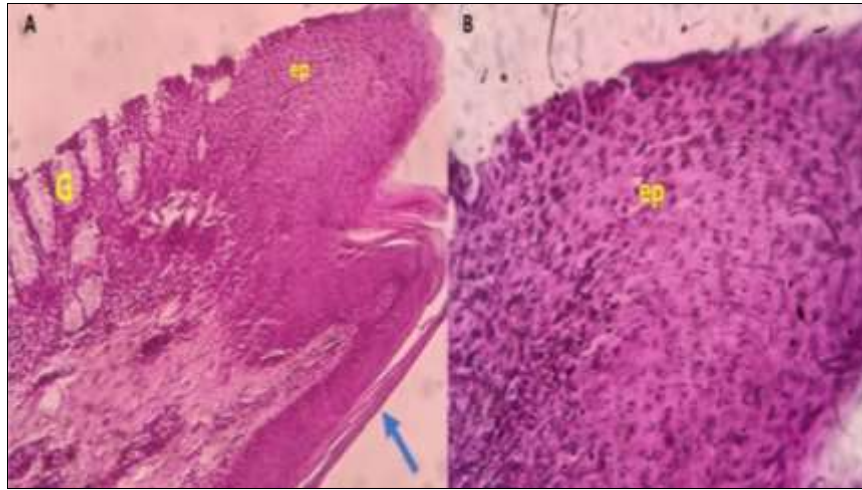


Fig 6: Microscopic image of longitudinal-section of the lateral tip & body of tongue in female owl

A: histology image of longitudinal-section of the lateral tip & body tongue in female owl showed simple branched tubular glands (G), tongue ventrum (Blue arrow) epithelium (ep), H&E stain A:X200

B: histology image of longitudinal-section of the lateral tip tongue in female owl showed stratified squamous epithelium (epi), H&E stain X400

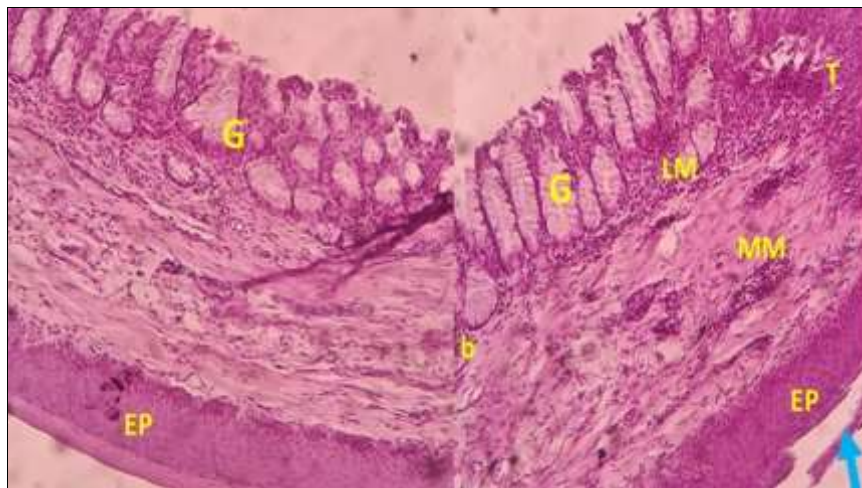


Fig 7: Microscopic image of longitudinal-section of the lateral tip& body tongue in male owl

- A: histology image of longitudinal-section of the lateral body tongue in male owl showed, simple branched tubular glands (G). epithelium (ep), H&E stain: X400
- B: histology image of longitudinal-section of the lateral tip & body tongue in male owl showed: tip(T) & body(b)

and glands demonstrating simple branched tubular glands (G), tongue ventrum (Blue arrow), epithelium (EP), lamina propria (LM), muscularis layer (MM) H&E stain; X200

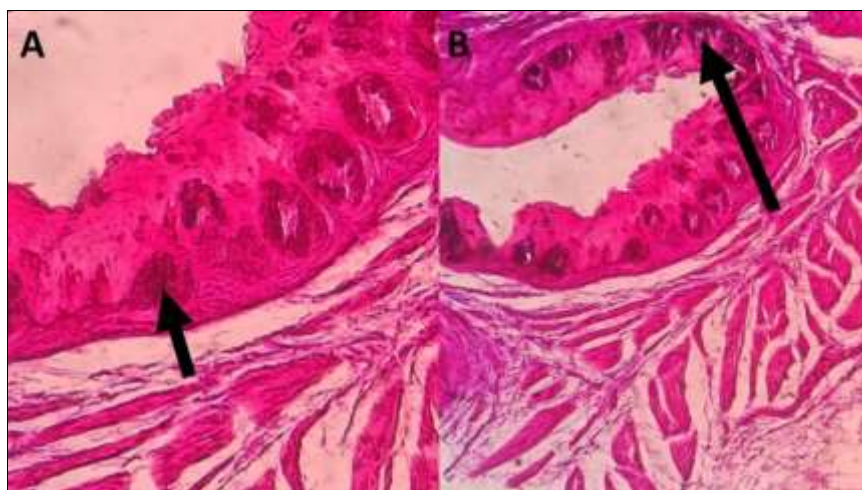


Fig 8: Microscopic image of longitudinal-section of the lateral tongue in Barn owl

A; histological image of longitudinal-section of the lateral tongue in Barn owl male showed: lingual glands (black arrow) positive reaction for combined AB-PAS stain; X200

B; histological image of longitudinal-section of the lateral tongue in Barn owl male showed: lingual glands (black arrow) positive reaction for combined AB-PAS stain; X200

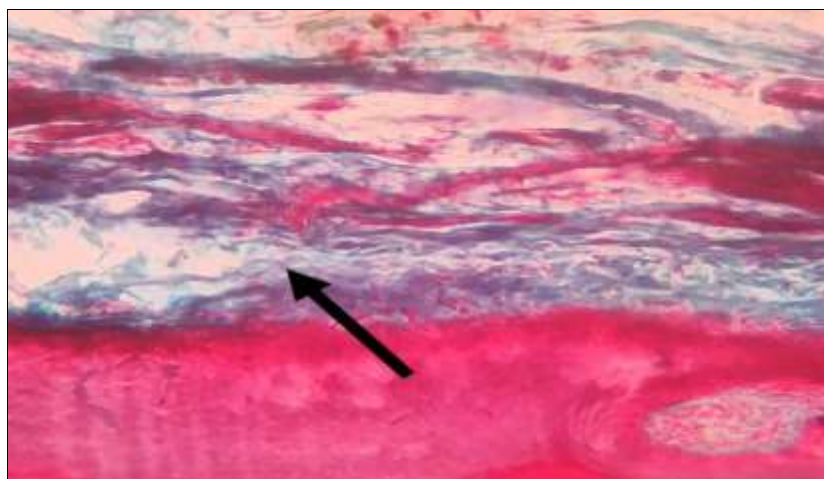


Fig 9: microscopic image of longitudinal-section of the oral cavity in male owl

Histological image of longitudinal-section of the oral cavity in male owl showed: collagen fiber in lamia propria positive reaction for masson trichrom stain X400

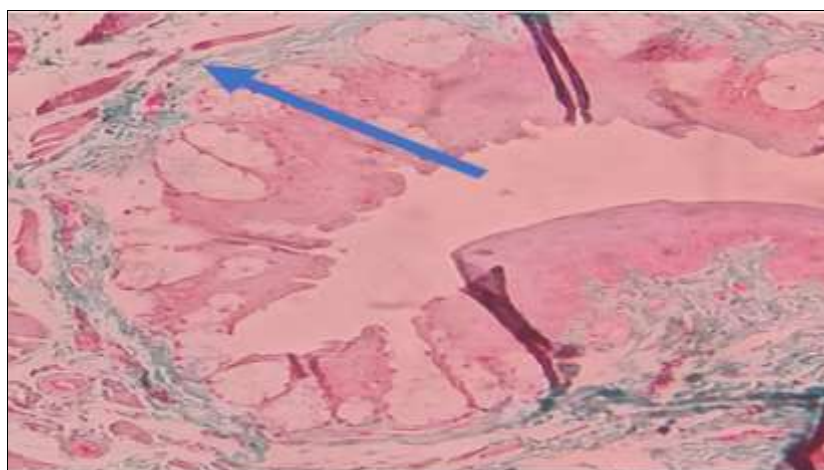


Fig 10: microscopic image of longitudinal-section of the tongue in female owl

Histological image of longitudinal-section of the tongue in female owl showed collagen fibre in lamia propria positive reaction for masson trichrom stain X200

Table 1: Anatomical measurement of oral cavity

	Male	female	T. test
Oral cavity length (mm)	27.2±1.6 B	30.8±1.5 A	4.36
Oral cavity width at midrib (mm)	9.8±0.5 B	12.5±0.9 A	2.87

Value represent Mean ±SE; LSD: least significant differences

Table 2: Measurement of tongue Morphometric measurements of male and female tongue of owl (mean± SE)

	males	females	T-test
Tongue length (mm)	9.8±0.4 B	11.3±0.7 A	3.53
Tongue base width (mm)	4.7±0.6 B	5.8±0.6 A	2.9
Tongue base thickness (mm)	3.5± 0.8 B	4.9± 1.5 A	2.4

Value represent Mean ±SE; LSD: least significant differences

Table 3: Histometric Measurements of owl Histometric Measurements of Oral Cavity Layer Males & Females (mean± SD) µm of owl

	Males (mean± SD) µm	Females (mean± SD) µm	t- test
Epithelium	37.4±2.5(b)	38.9±2.7(a)	2.24
Lamina propria	43.3±3.0(b)	48.6±2.9(a)	3.32
Muscular layer	56.1±4.6(b)	62.6±3.4(a)	2.36

Value represent Mean ±SE; LSD: least significant differences

Table 4: Histometric parameters of owl histometric Measurements of Tongue Layer Males & Females (mean± SD) µm of owl

Layer	Males (µm ±SD)	Females (µm± SD)	t- test
Epithelium	29.2±2.5	32.1±2.5	4.99
Lamina	36.8±2.1	38.1±3.5	3.46
Muscles	64.1±3.2	68.9±3.6	5.76

Value represent Mean ±SE; LSD: least significant differences

Discussion

The current study revealed that there is notable sexual dimorphism within the oral cavity and tongue of *Tyto alba*. While both sexes display similar general organizational structures across the oral cavity and tongue, structures and tissues are more developed within females. Such variations may pertain to functional adaptations correlating to body size, and possibly feeding mechanics, as previously noted in sexually dimorphic species of birds (Wikelski & Trillmich, 1997) [29].

The Oral Cavity

The oral cavity in both sexes presented a longitudinal palatal cleft and a posterior palatal cleft leading to the pharynx, which allows for dual respiration and deglutition during the act of ingestion. Similar adaptations were noted in predatory birds by İlğün *et al.* (2020), who underscored the role of the palatal region in providing a patent airway during the consumption of prey. Also, the pharyngeal palatine and lingual ridges were enlarged, which would be consistent with the ability to swallow prey in its entirety and is consistent with the feeding pattern noted in owls by Igwebuike and Anagor (2013).

The mucosa that is partly keratinized serves as an additional protective mechanism against the abrasion caused by the bones and fur of the prey, an attribute typical of nocturnal raptors (Ertaş, Parchami, & Dehkordi, 2011) [30]. The morphometric analysis showed that the oral cavity length and width were greater for females, which is consistent with the documented female-biased size dimorphism of owls (Tütüncü *et al.*, 2012). These structural variations are likely to facilitate functional resource partitioning between the sexes, thereby reducing intraspecific feeding competition (Parchami *et al.*, 2010) [29, 16, 15, 27, 25].

The Beak and Tongue

The Upper Beak

The upper beak was strongly curved in both sexes and in both sexes was covered with a keratinized sheath, indicative of its role in the grabbing and guiding of prey. This type of beak morphology is consistent with the modifications described for raptors by Al-Zahaby and Elsheikh (2014). The beak of the female is longer and sharper than that of the male, which, according to Skiersz-Szewczyk *et al.* (2012) likely relates to sexual size dimorphism and functional dietary differentiation [4, 26].

Tongue

The tongues of both sexes were short, conical, and covered on the dorsal surface by keratinized stratified squamous epithelium, which suggests a primarily mechanical function of directing the prey to the pharynx and not of mastication. Erdoğan *et al.* (2018) reported similar lingual adaptations in predatory birds. However, females exhibited noticeably greater lengths of the tongue and greater widths and thicknesses of the base. Such differences have been attributed by Bruce (1999) to the existence of complementary feeding roles between sexes, whereas Khaleel *et al.* (2023) stressed

that minute lingual structural differences are in alignment with prey size and feeding strategy.

Histology and Histochemistry of the Oropharyngeal Cavity and Tongue

Overall Structure of the Oropharyngeal Cavity

The oropharyngeal walls of both sexes were made up of epithelium, lamina propria, muscularis, and serosa, which is consistent with the layering seen and described in the walls of carnivorous birds (McLelland, 1979; Jackowiak & Godynicki, 2005). The stratified squamous epithelium and mucous glands indicate an adaptation for mechanical protection and lubrication of the walls of the oropharynx during the process of swallowing.

Mucous Glands and Secretory Activity

The simple tubular mucous glands that are located in the most posterior parts of the pharynx are most likely to have an important role in lubrication during the transport of prey (Duke, 1994). Females exhibited glandular densities slightly higher, coupled with stronger PAS and Alcian Blue reactions, indicative of more mixed mucous secretion. Magad and Zghair (2025) reported similar sex-related functional differences. The mixed neutral and acidic mucins in both sex active secretory functions, as reported by Bancroft (2008) [7, 8, 21, 8, 20, 6].

Histometric Differences between Sexes

Histometric analysis showed significantly more epithelium, lamina propria and muscular layers in females. Sex-related influence on tissue thickness has been reported for birds exhibiting specialized feeding Ali *et al.* (2003). More developed connective tissue and muscle fibers in females, especially more pronounced at the base of the tongue, were corroborated functionally by the more developed tissues of the swallowing apparatus, as also reported for raptors by Akalan *et al.* (2022) [2, 1].

Conclusion

There is considerable sexual dimorphism present in the mouth and tongue of *Tyto alba*. While males and females share the same structural organization, females show larger morphometric measurements, greater tissue layer thickness, increased density of mucous glands, and more intense histochemical reactions. These variations may indicate functional adaptations in response to size dimorphism and associated feeding requirements. These findings reinforce the significance of structural modifications that specialized predatory birds for feeding.

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Ethical Statement

All procedures were approved by the Animal Ethics Committee of the College of Veterinary Medicine, University

of Al-Qadisiyah, Al-Diwaniyah City, Iraq (Approval No.: 254). The barn owls were handled by trained personnel under veterinary supervision, and all efforts were made to minimize stress prior to the procedure. Euthanasia was performed humanely using a two-step veterinary protocol to ensure complete absence of pain and distress. Birds were first deeply anesthetized with inhaled isoflurane (5% in oxygen) administered via an induction chamber until loss of righting reflex and complete absence of pedal and corneal reflexes were confirmed. Following induction of deep anesthesia, euthanasia was completed by intravenous administration of an overdose of sodium pentobarbital (≥ 100 mg/kg body weight).

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Conflict of Interest

Not available

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Not available

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