A morphological and stereological investigation on the tongue of the merlin

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ABSTRACT

The study aimed to reveal the similarities and differences of the tongue of the merlin with other bird species. Merlin is the smallest bird of the *Falconidae* family and lives in America, the northern regions of Europe and Asia, the Middle East, and Central Asia. Since these species don't have teeth, lips, and cheeks, the tongue fulfills significant functions related to nutrition, and it differs morphologically as a result of differences in eating habits. In this study, the tongues obtained from five adult merlin (*falco columbarius*) were examined by morphological and stereological methods. It was determined that the tongue of the merlin was thin, long, and rectangular, the front part was oval, W-shaped *papilla linguales caudales* were found between the body and root of the tongue. The average length of the tongue was 26.32 ± 1.38 mm, the width was 7.26 ± 0.32 mm, and the thickness was 1.58 ± 0.14 mm. The histology of the tongue showed that the dorsal and ventral surfaces are covered with keratinized multilayered squamous epithelium; there are taste buds in the epithelial layer, the number of taste buds is higher especially on the root of the tongue; and the presence of *paraglossum*, which is in the hyaline cartilage structure. The volume of the tongue was on an average of 374.2 ± 14.08 mm^3.

Introduction

Merlin is the smallest bird of the family *Falconidae*. This raptor has 24-33 cm length and 53-69 cm wingspan. Facial features are weaker than hawks. They live in the northern regions of America, Europe, Asia, the Middle East, and Central Asia (19).

The tongue is located at the base of the mandible, containing various tissues such as cartilage and bone, glands, muscles, nerves, blood vessels, and connective tissue (3). The structure and function of the tongue are closely associated with the diet and adaptation of animals to nature (6). Teeth, lips, and cheeks are missing in avian species, so the tongue accomplishes significant functions such as capturing, separating, processing, and swallowing food (16). As a result of all these functions and different feeding habits in different birds, the tongue varies considerably in poultry morphologically (3, 27).

The tongue of the bird consists of three parts: the *apex lingua* (tip), the *corpus lingua* (body), and the *radix lingua* (root) (31). It has both mechanical and taste buds. The number and localization of these taste buds depend on the bird's diet or whether it is a flightless or water bird. These taste buds localization and number vary depending on the bird's diet and changes between water birds and flightless birds (33, 35). Morphology of tongues especially in bird species has been studied but most of these investigations are interested in the tongue of herbivores and omnivorous birds (26, 27). It appears that studies on the tongue of carnivorous birds are scarce (13, 25).

It was aimed to examine the morphological, histological, and stereological examination of the merlin tongue and reveal the similarities or differences with other bird species in this study.

Materials and Methods

Five adult Merlins (*Falco columbarius*) obtained from the Afyon Kocatepe University Veterinary Faculty Animal Hospital were used. This study was approved by the local ethical committee (Afyon Kocatepe University Animal Experiment Local Ethical Committee No: 49533702/41). Due to untreatable diseases apart from digestive tract diseases birds were euthanized by the department of surgery with a combination of ketamine (60 mg/kg) and xylazine (6 mg/kg). The cadavers were fixed in 10% formaldehyde solution.

For gross anatomical examination, five birds were decapitated and washed in running tap water. The tongues were examined in the oral cavity, and then they were cut and examined separately. Measurements were made with a digital caliper (Mitutoyo, Japan). Nomina Anatomica Avium was used for anatomic denomination (7).

The volume of the tongue was estimated by using The Cavalieri principle. Since the cadavers had already been fixed, the shrinkage ratio could not be calculated in the volume calculation. The slice thickness of the tongues was 2 mm, and a point-counting grid with 1 mm dot spacing was randomly left on the same face of each slice (Figure 1). The tongue volume was calculated by the following formula;

$$V = (t.\ a(p).\ \Sigma P)\ mm^3$$

V= volume; t: section thickness (2 mm); a(p): area associated with one test point (1 mm x 1 mm); and ΣP: Total number of points hitting the tongue section (20).

Slices corresponding to the *apex* (tip), *corpus* (body), and *radix* (root) parts of the tongue were subjected to histological tissue follow-up and embedded in paraffin blocks. The 5 µm thick histological sections were stained by the Hematoxylin-Eosin method. Histological examinations were carried out by a microscope camera (M-Shot brand, MDX4 model, Guangzhou, China) and M-Shot Digital Imaging System 9.3.3.1 software integrated into a light microscope (Olympus brand, MD2 model, Shinjuku-ku, Tokyo) with a motorized table (Lang brand, MS 316 model, Hüttenberg, Germany).

Results

It was found that the tongue was thin, long, and rectangular with an oval front (Figure 2). The tongue did not fill the floor of the oral cavity, there were gaps in the front and sides, and it was attached to the floor of the oral cavity via the *frenulum linguae* almost in the middle of the tongue.

Between the body (*corpus linguae*) (Figure 3a) and the root (Figure 3b) of the tongue, the *alae lingua* (Figure 3 black arrows) was observed being shaped by the *papilla linguales caudales* (Figure 3c) in a W-shaped direction towards the tongue body. Moreover, these *papillae linguae caudales* showed dark brownish-black pigmentation. The number of *papillae linguae caudales* was determined to be 14. These *papillae* were cone-shaped.

There were many salivary glands with draining ducts on the body and root of the tongue (Figure 2*). Larynx cranialis and glottis (Figure 2b, Figure 4*) were observed just behind the radix linguae. There were *papillae* (Figure 4 white arrows) located behind the *glottis* forming the *papillae pharyngis caudoventrales* and the number in each half was 17-18. Most of these *papillae* (except the medial one) possessed pigmentation close to black. Morphometric measurements of the tongues were shown in Table 1.

Figure 1. A point-counting grid with point spacing 1 mm used for the measurement of 2 mm tongue sections.

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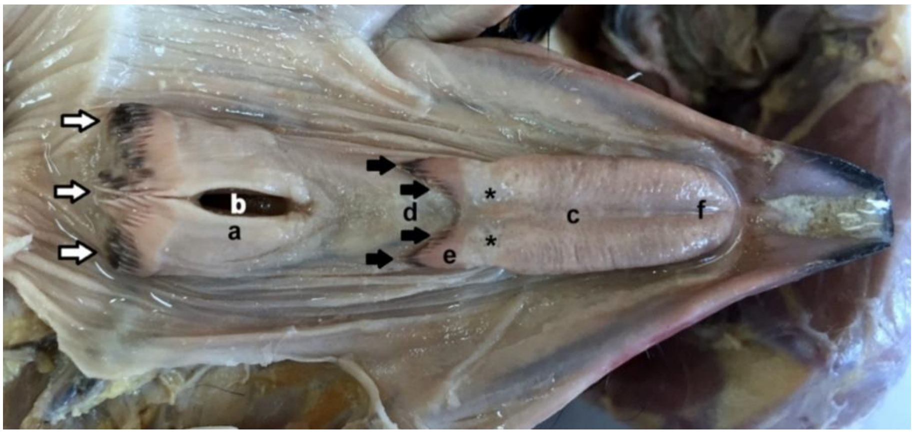

Figure 2. The merlin tongue view from inside the roof of the oral cavity (a: larynx cranialis, b: glottis, c: corpus lingua, d: radix lingua, e: alae lingua, f: apex lingua, *: orifices of gll. linguales, black arrows: papilla linguales caudales, white arrows: papilla pharyngis caudoventrales).

Figure 3. Dorsal view of alae linguae located between corpus and radix linguae (a: corpus lingua, b: radix lingua, c: alae lingua, black arrows: papilla linguales caudales).

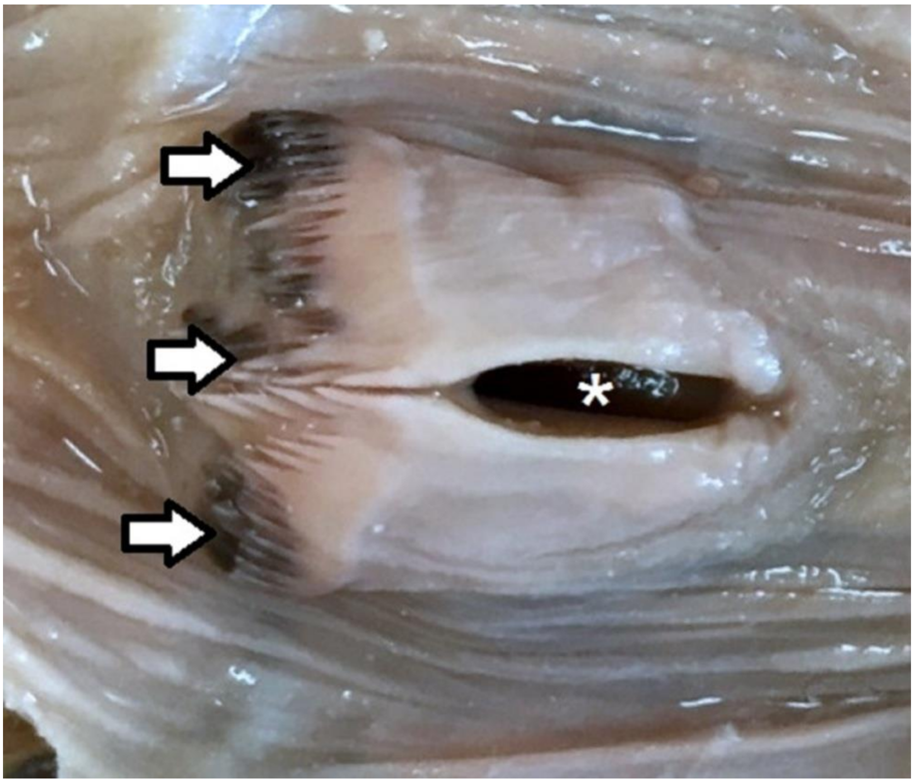

Figure 4. Dorsal view of papilla pharyngis caudoventrales (white arrows: papilla pharyngis caudoventrales, *: glottis).

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Table 1. Morphometric measurement values of animals.

Measurements (mm)	Min	Max	A.M.	S.E.	S.D.
Frenilum linguae length	13.9	15.7	14.77	0.3077	0.6881
Larynx cranialis length	11.4	12.6	12.02	0.1885	0.4214
Glottis length	6.8	7.23	7.06	0.0628	0.1405
Sulcus medianus linguae length	12.3	15.2	13.46	0.5162	1.1542
Tongue length	24.5	28.2	26.32	0.6167	1.3789
Tongue Width	6.9	7.6	7.26	0.1423	0.3183
Tongue Thickness	1.42	1.78	1.58	0.0604	0.1351

A.M.: Arithmetic Mean.
S.E.: Standart Error.
S.D.: Standart Deviation.

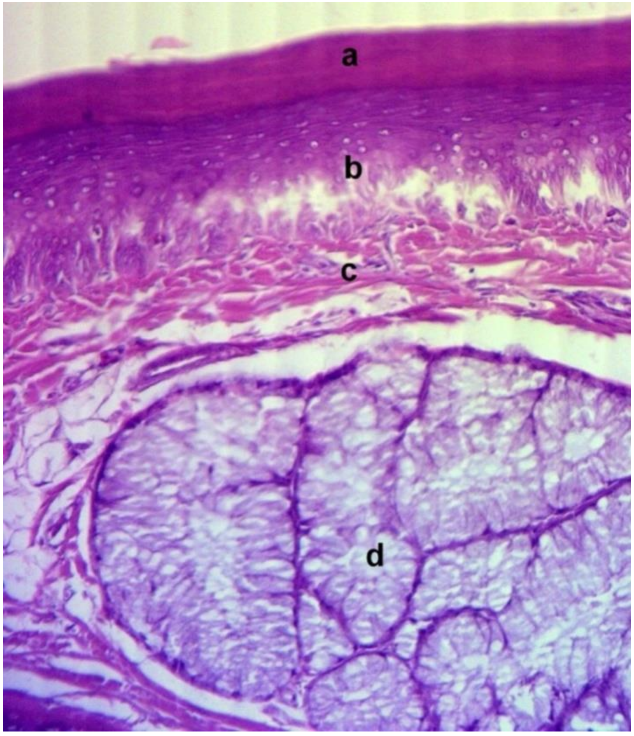

Figure 5. Dorsal surface of the merlin tongue (Hematoxylin-Eosin, 10X. a: keratinized layer, b: epithelial layer, c: connective tissue, d: gland).

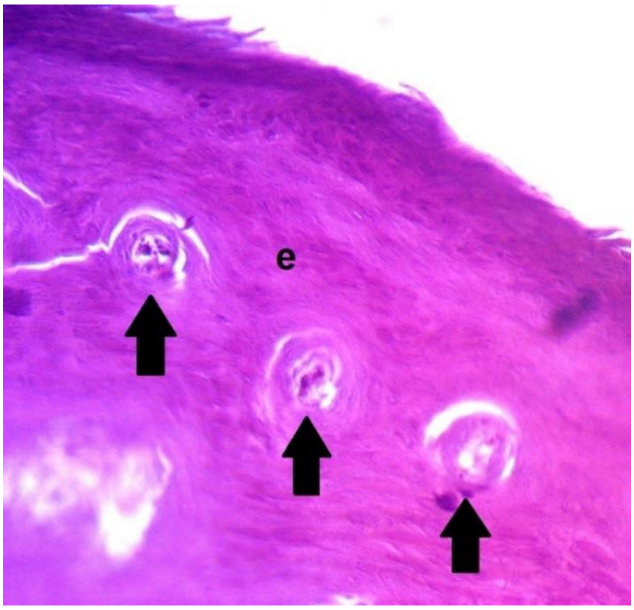

Figure 6. Taste buds (black arrows) within the epithelial layer (e) of the merlin tongue (Hematoxylin-Eosin, 100X).

Microscopic examinations revealed that the dorsal and ventral surfaces of the merlin tongue were covered with keratinized multilayered squamous epithelium (Figure 5a) and there were numerous taste buds in the epithelial layer. (Figure 6 black arrows) The number of taste buds was higher, especially on the root of the tongue. There was a richly vascularized connective tissue (Figure 7b) just below the multilayered squamous epithelial layer (Figure 7a). The *lamina propria* layer from the loose connective tissue (Figure 7b) made protrusions called *dermal papillae* (Figure 7 black arrows) towards the epithelial layer. There were many glands (Figure 5d) embedded in the submucosa layer and surrounded by connective tissue. These glands were found in the body (anterior salivary glands) and root of the tongue (posterior salivary glands). Skeletal muscle fibers seen in tongue tissue sections were seen in the transversal section. In other words, the orientation of the skeletal muscles were parallel to the long axis of the tongue. In addition to skeletal muscle and numerous vessels in the tongue tissue, the extension of the *paraglossum* (Figure 8c), in the hyaline cartilage structure was noted.

The average volume of the merlin tongue was calculated as 374.2 ± 14.08 mm^3 (Table 2).

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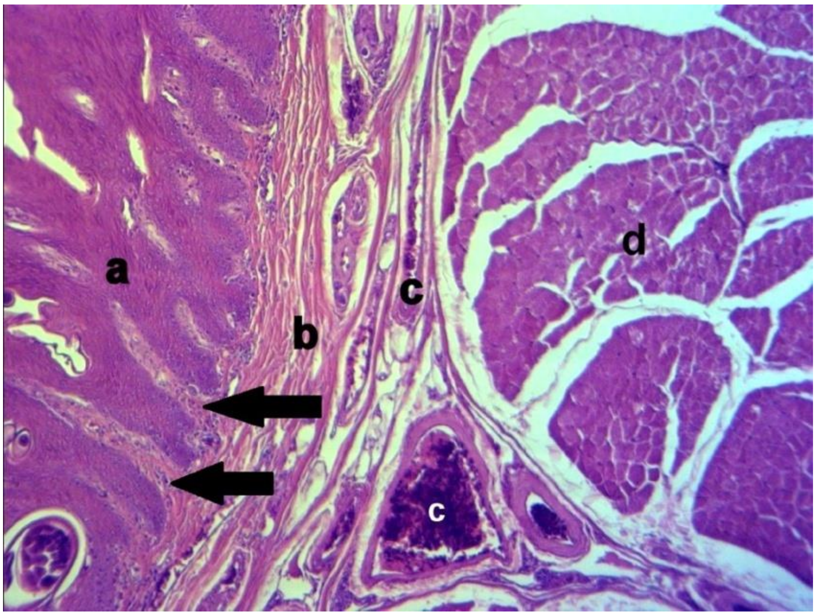

Figure 7. Cross-section of the merlin tongue (Hematoxylin-Eosin, 20X. a: epithelial layer, black arrows: dermal papilla, b: connective tissue, c: blood vessels, d: muscle layer).

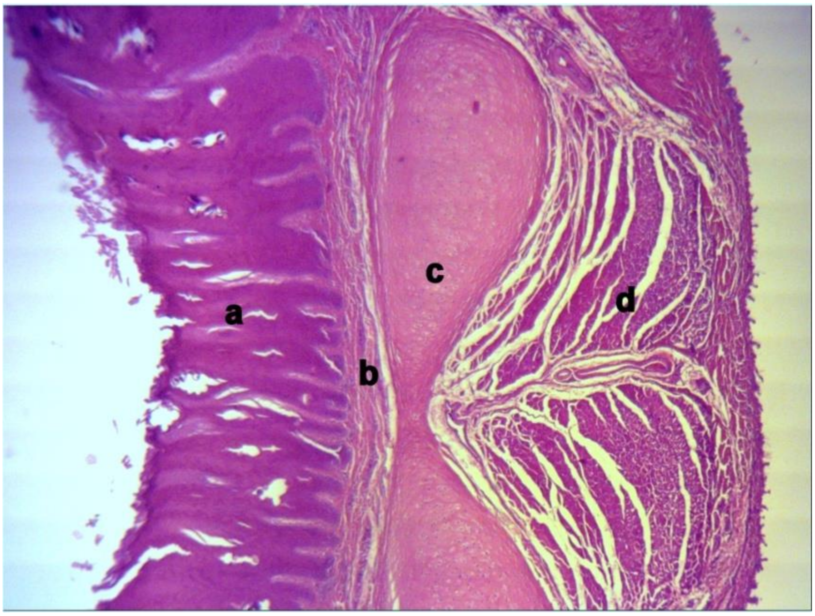

Figure 8. Cross-section of the merlin tongue (Hematoxylin-Eosin, 4X. a: epithelial layer, b: connective tissue, c: paraglossum, d: muscle layer).

Table 2. Tongue volume values of animals.

	Animals					
	I	**II**	**III**	**IV**	**V**	**A.M.**
Volume (mm3)	365	391	385	352	378	374.2
Standart Error						6.2953
Standart Deviation						14.0769

A.M.: Arithmetic Mean.

Discussion and Conclusion

Avian tongues are mainly responsible for taking food and swallowing. For this reason, the tongue differs depending on the nutrition, type of food consumed and the shape of the lower beak (3, 16, 31). In the morphological studies, it has been reported that the tongue is in the form of a triangle in seagull (24), white-eared bulbul (32), black francolin (28); toothpick like in Japanese pygmy woodpecker (13); protruding arrow like in the African pied crow (21); arrow like in the hooded crow (11); needle like in the heron (12); mushroom in hummingbird (34); spearhead like in the jungle nightjar (14); lip like in the scarlet macaw (15); and rasp like in penguins (29). The present study revealed that the merlin tongue was thin and elongated rectangular in shape and its tip was oval similar to the white-tailed eagle (25), golden eagle (33), and hawk (38) tongue. Some studies (3, 27) reported that the tongue fills the floor of the oral cavity, similar to our findings, the tongue did not fill the floor of the oral cavity in seagull (24), hawk (38), Euroasian coot (2). These differences in the shapes of tongues in poultry may be associated with the diet, lifestyle, and structure of the lower beak.

Studies on the tongue size of carnivorous birds are limited. The length of the hawk's tongue was 17 mm with 4 mm width, and 1.2 mm thickness on average (38). In this study, the average length, width, and thickness of the merlin tongue were 26.3 mm, 7.3 mm, and 1.58 mm, respectively. These differences between the tongue sizes of these birds, which have similar feeding habitats, could be related to the body size.

Erdogan and Iwasaki (16) stated that the presence or the number of papillae, or the shape of the papillar row were not associated with the feeding habit of birds. Moreover there was no *alae lingua* in the tongue of the omnivorous greater rhea (35), along with the carnivorous heron (12) and the stork (39). However, in herbivorous been goose (23) and long-legged buzzard (18), alae lingua with papilla linguales caudales exist in the form of the letter "V" and the same pattern can be seen in carnivorous white-tailed eagle (25). The alae lingua in the herbivorous zebra finch (9) and omnivorous Euroasian coot (2) show the letter "W" shape whereas the omnivorous hooded crow (30) and carnivorous cattle egret (4) possess the letter "U" shape pattern. It was determined that the *papilla linguales caudales* were in the form of the letter "U" in the Merlin tongue.

The number of *papilla linguales caudales* of the tongue was reported in long-legged buzzard (18), southern lapwing (17), hawk (38). In the present study, the number of *papillae linguales caudales* in the tongue of the merlin was found to be 14, similar to the Euroasian hobby (1). This similarity is not surprising since these birds are in the same genus (*Falco*).

In microscopic studies in various bird species, keratinization of the tongue epithelium was a common feature of birds (5, 27). Besides, tongue epithelial keratinization was lower in waterfowl (22). The tongues of common buzzard (10), cattle egret (5), and hawk (8) have keratinized epithelial tissue and that this keratinized tissue acts as a toothlike function in poultry, especially in helping to pluck plants. In our current study, it has been determined that multi-layered squamous epithelial tissue in the merlin tongue showed significant keratinization.

Kobayashi et al. (29) reported that there was no taste buds in the penguin tongue. On the other hand, the presence of taste buds in the epithelial tissue like the merlin tongue was mentioned in the African pied crow (22), common buzzard (10), and common kingfisher tongues (5). Loose connective tissue, skeletal muscle, blood vessels, and a large number of glands were found in the tongue tissue in various bird species (5, 8-10, 21). Microscopic findings in the merlin tongue were similar to these findings. The presence of *paraglossum* in the hyaline cartilage structure, which was located close to the ventral in the transversal sections of the merlin tongue, was reported in different bird species i.e. common kingfisher, long-legged buzzard, ostrich (5, 15, 26).

Volume calculations are generally determined by stereological methods on images obtained by imaging techniques such as Magnetic Resonance Imaging (MRI), Computed Tomography (CT), and Ultrasound (US) in the human tongue (37, 40). The tongue volume was determined as 2.1 mm^3 for the golden-winged sunbird, 1.1 mm^3 for the green-headed sunbird, and 0.6 mm^3 for the variable sunbird (36). In the current study, we determined the average volume of the merlin tongue as 374.2 ± 14.08 mm^3. These sunbirds consume the nectar from flowers and have a small size tube-like tongue. Also, it is thought that the body dimensions being smaller than Merlin may reveal this difference.

In conclusion, the morphological structure of the merlin tongue was revealed and its volume was calculated by the stereological method. We found that the multi-layered squamous epithelial tissue was keratinized, and there was a *paraglossum* in the hyaline cartilage structure in the body and root of the tongue. The data generated here could be useful for those who are interested in the morphology of the tongue of the carnivorous birds, particularly in the Merlin.

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Conflict of Interest

The authors declared that there is no conflict of interest.

Author Contributions

MAA, ACD, MAA, VÖ, İT and İD conceived and planned the experiments. MAA and ACD carried out the experiments. MAA, ACD and MSA contributed to the interpretation of the results. MAA took the lead in writing the manuscript. ID contributed to the editing and spelling of the article. All authors provided critical feedback and helped shape the research, analysis and manuscript.

Data Availability Statement

The data supporting this study's findings are available from the corresponding author upon reasonable request.

Ethical Statement

This study was approved by the Afyon Kocatepe University Animal Experiment Local Ethical Committee (Ethics committee number: 49533702/41).

Animal Welfare

The authors confirm that they have adhered to ARRIVE Guidelines to protect animals used for scientific purposes.

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