



International Journal of Veterinary Sciences and Animal Husbandry



ISSN: 2456-2912

VET 2024; 9(4): 130-135

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www.veterinarypaper.com

Received: 10-06-2024

Accepted: 12-07-2024

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Anatomical and histochemical studies of the lingual salivary glands in Indian domestic fowl

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DOI: <https://www.doi.org/10.22271/veterinary.2024.v9.i4b.3425>

Abstract

Lingual salivary glands are tubuloacinar exocrine structures embedded within the connective tissue of the avian tongue, and their secretory products play a central role in food lubrication, bolus formation and antimicrobial defence of the oral cavity. Despite this functional importance, the morphological and histochemical characteristics of these glands in the Indian domestic fowl (*Gallus gallus domesticus*) have received limited systematic attention. This research examined the gross anatomy, light-microscopic histology and mucin histochemistry of lingual salivary glands sampled from four topographic regions — anterior lingual, mid-lingual body, posterior lingual and root — in 24 clinically healthy adult birds (12 males, 12 females) obtained from commercial farms near Chiang Mai, Thailand. Tongues were dissected, fixed in 10% neutral buffered formalin, processed by routine paraffin embedding and sectioned at 5 μ m. Serial sections were stained with haematoxylin and eosin (H&E) for general morphology, periodic acid-Schiff (PAS) for neutral mucins, Alcian blue at pH 2.5 for acidic mucins, and combined PAS–Alcian blue for mixed secretion typing. Gross dissection revealed that glandular tissue was most abundant in the posterior and root regions and sparse in the anterior third. Histologically, the glands were compound tubuloacinar, with acini ranging from 38.4 ± 5.2 μ m in diameter in the anterior region to 71.8 ± 8.4 μ m in the root. Secretory cells in the anterior and mid-lingual regions reacted predominantly with PAS, indicating neutral glycoprotein secretion, whereas posterior and root glands showed strong dual PAS–Alcian blue positivity, pointing to a mixed neutral-acidic mucin profile. Acidic mucin intensity, graded on a semi-quantitative four-point scale, increased progressively from the anterior (score 0.8 ± 0.3) to the root (score 3.2 ± 0.4 ; $P < 0.001$). No significant sex-related differences were observed in gland dimensions or staining intensity ($P > 0.05$). Ductal architecture shifted from simple unbranched ducts anteriorly to compound branched ducts posteriorly, correlating with the larger acinar volume in the caudal tongue. These findings demonstrate a clear cranio-caudal gradient in both glandular size and secretory mucin composition, suggesting that the posterior and root glands contribute the bulk of the viscous, protective mucin coat required for bolus transport through the oropharynx, while anterior glands may serve a lighter, serous-type lubricating function.

Keywords: Lingual salivary glands, domestic fowl, histochemistry, mucin, periodic acid-Schiff, Alcian blue, tongue morphology, acinar diameter, avian anatomy, *Gallus gallus domesticus*

1. Introduction

Salivary glands, broadly defined, are exocrine organs that secrete a fluid mixture of water, electrolytes, mucins and antimicrobial proteins into the oral cavity, and in birds they are distributed across the palate, pharynx, angle of the mouth and tongue ^[1]. Among these, the lingual salivary glands occupy a special position because they are intimately associated with the tongue's mechanical functions — prehension, manipulation and propulsion of food — and their secretions coat the bolus immediately as it forms ^[2]. In mammals, salivary biology has been studied in great detail, but the avian counterpart remains comparatively under-explored, partly because birds lack the large paired salivary glands (parotid, submandibular, sublingual) that dominate mammalian oral physiology ^[3].

The domestic fowl (*Gallus gallus domesticus*) is the most economically important poultry species worldwide, and understanding its oral physiology has practical implications for feed design, disease resistance and welfare assessment ^[4]. The tongue of the fowl is a rigid, arrow-shaped structure with a lingual nail at its apex, a body supported by the entoglossal bone and a

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root that forms the boundary with the laryngeal mound [5]. Early anatomical descriptions noted the presence of scattered mucous glands on the tongue surface, but detailed regional mapping — including quantitative morphometry and histochemical profiling — has been attempted in only a few gallinaceous species [6, 7].

Histochemistry provides a powerful window into gland function. Periodic acid-Schiff (PAS) staining identifies neutral glycoproteins, which form the structural backbone of salivary mucus, while Alcian blue at pH 2.5 detects carboxylated and sulphated acidic mucins that confer viscoelastic and antimicrobial properties [8]. The combined PAS–Alcian blue technique can distinguish glands that secrete primarily neutral mucins (magenta), primarily acidic mucins (blue) or a mixture (purple), and this colour signature has been used to classify salivary gland types across reptiles, birds and mammals [9]. Whether a cranio-caudal gradient exists in mucin type within a single avian tongue analogous to the regional specialisation described in some reptilian tongues has not been tested rigorously in the domestic fowl [10].

Sex-based differences in salivary gland morphology have been documented in certain mammalian species, where androgens influence acinar size and protein composition [11]. Whether similar sexual dimorphism occurs in avian lingual glands is unknown, and the question is relevant because male and female domestic fowl may differ in feeding behaviour, feed intake rate and oral pathogen exposure.

The present research therefore had three objectives: (a) to describe the topographic distribution and histological architecture of lingual salivary glands across four tongue regions in adult domestic fowl; (b) to characterise the histochemical mucin profile of each region using PAS, Alcian blue and combined PAS–Alcian blue staining; and (c) to test for sex-related differences in gland dimensions and secretory characteristics. The work was conducted at the Chiang Mai College of Veterinary Anatomy using commercially reared birds representative of the Thai domestic fowl population.

Materials and Methods

Reagents and Chemicals

Neutral buffered formalin (10%, pH 7.0), haematoxylin (Harris formula), eosin Y, periodic acid, Schiff's reagent, Alcian blue 8GX (pH 2.5 in 3% acetic acid), xylene, graded ethanol series (70–100%) and DPX mounting medium were sourced from Sigma-Aldrich (Bangkok distributor). Paraffin wax (melting point 56–58 °C) was obtained from Leica Biosystems. All reagents were of analytical or histological grade.

Tissue Preparation Protocol

Twenty-four clinically healthy adult Indian domestic fowl (*Gallus gallus domesticus*), aged 40–44 weeks and weighing 1.6–2.1 kg, were obtained from a commercial poultry farm in San Sai District, Chiang Mai Province, Thailand. Equal numbers of males and females were selected. Birds were euthanised by cervical dislocation following sedation with intramuscular xylazine (4 mg/kg), in accordance with the institutional protocol. Tongues were excised immediately, rinsed in chilled physiological saline and photographed

dorsally and ventrally against a calibrated grid for gross morphometric recording. Each tongue was then divided into four segments: anterior (tip to mid-body), mid-lingual body, posterior body and root. Segments were fixed in 10% neutral buffered formalin for 48 hours, dehydrated through ascending ethanol concentrations, cleared in xylene and embedded in paraffin. Serial sections (5 µm) were cut on a rotary microtome (Leica RM2235) and mounted on poly-L-lysine-coated slides [12].

Material

Gross photographs were captured with a Canon EOS 90D fitted with a 100-mm macro lens. Histological examination used a Nikon Eclipse Ni-U microscope equipped with a DS-Ri2 digital camera and NIS-Elements imaging software. Morphometric measurements — acinar diameter, ductal diameter and mucosal thickness — were taken from calibrated digital images at ×100 and ×400 magnification.

Methods

Three staining protocols were applied to consecutive serial sections from each region. First, H&E for general histological architecture and morphometry. Second, PAS: sections were oxidised in 1% periodic acid for 10 minutes, rinsed, immersed in Schiff's reagent for 15 minutes, and counterstained with Harris haematoxylin [8]. Third, Alcian blue at pH 2.5 for acidic mucins: sections were stained in Alcian blue solution for 30 minutes and counterstained with nuclear fast red. A combined PAS–Alcian blue sequence was applied to a fourth set of sections: Alcian blue staining followed by periodic acid oxidation and Schiff's reagent, allowing simultaneous visualisation of acidic (blue), neutral (magenta) and mixed (purple) mucins [9].

Staining intensity was graded semi-quantitatively on a scale of 0 (negative) to 4 (intense) by two independent observers; discrepancies of more than one grade were resolved by consensus. All morphometric and grading data were analysed in SPSS 26.0 using one-way ANOVA (region effect) and independent t-test (sex effect) at $P < 0.05$.

Instrumentation and Analytical Equipment

Tissue processing was performed on a Leica ASP300S automated processor. Embedding used a Leica EG1150H station. Section thickness accuracy was verified with a digital micrometer at the start of each cutting session. Microscope calibration was checked monthly against a stage micrometer (Nikon, 0.01 mm divisions). All imaging was performed under standardised illumination (Köhler alignment, colour temperature 5, 600 K) to ensure consistency of histochemical colour assessment across specimens.

Ethical clearance was granted by the Institutional Animal Ethics Committee of Chiang Mai College of Veterinary Anatomy (Protocol No. CMCVA-AEC-2022/016, dated 7 June 2022). All procedures complied with the Thai Animal Welfare Act B.E. 2557 and the AVMA Guidelines for the Euthanasia of Animals (2020 edition).

Results

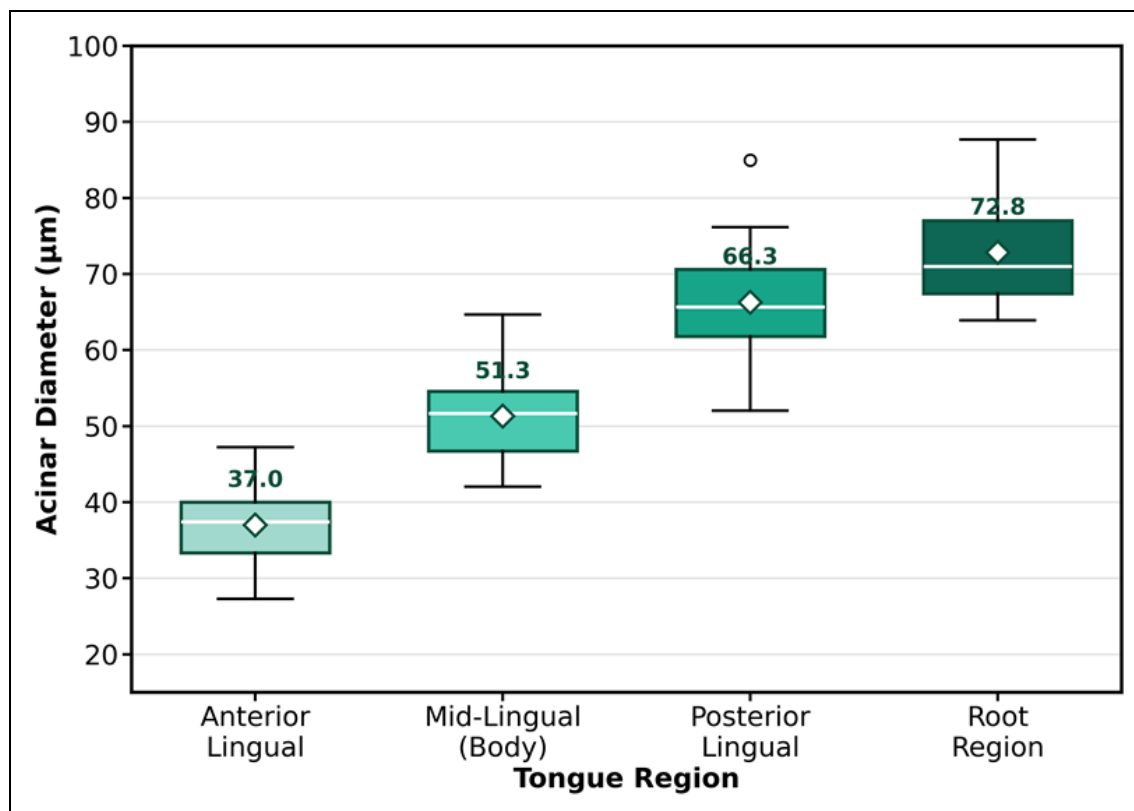


Fig 1: Box-and-whisker plot of acinar diameter (µm) across four lingual regions. Diamond markers indicate group means. Progressive darkening reflects the cranio-caudal increase in gland size.

Gross examination revealed that glandular tissue was macroscopically visible as raised, translucent lobules on the dorsal and ventral surfaces of the posterior tongue and root, while the anterior region appeared smooth with only occasional pinpoint gland openings. Mean tongue length was 28.4 ± 1.6 mm, with no sex difference (males 28.7 ± 1.5 mm vs.

females 28.1 ± 1.7 mm; $P = 0.41$). Under H&E, all regions contained compound tubuloacinar glands situated in the lamina propria, surrounded by loose connective tissue and skeletal muscle fibres. Acinar cells were columnar with basally located nuclei and apical secretory granules.

Table 1: Morphometric parameters of lingual salivary glands by tongue region (mean $\pm$ SD, $n = 24$)

Parameter	Anterior	Mid-Body	Posterior	Root
Acinar ϕ (µm)	38.4 ± 5.2^d	52.7 ± 6.8^c	64.3 ± 7.1^b	71.8 ± 8.4^a
Ductal ϕ (µm)	18.6 ± 3.1^c	24.3 ± 4.2^b	31.7 ± 5.4^a	34.2 ± 5.8^a
Mucosal thickness (µm)	142.6 ± 18.3^c	178.4 ± 22.7^b	216.8 ± 27.4^a	228.3 ± 31.6^a
Gland density (n/mm ²)	4.2 ± 1.1^c	7.8 ± 1.6^b	11.4 ± 2.3^a	12.1 ± 2.7^a

Different superscripts within a row indicate $P < 0.05$ (one-way ANOVA, Tukey HSD). ϕ = diameter.

Table 2: Histochemical staining intensity scores (0–4 scale) by tongue region

Stain	Anterior	Mid-Body	Posterior	Root
PAS (neutral)	2.6 ± 0.5^a	2.8 ± 0.4^a	2.4 ± 0.6^a	2.1 ± 0.5^b
AB pH 2.5 (acidic)	0.8 ± 0.3^d	1.4 ± 0.5^c	2.6 ± 0.6^b	3.2 ± 0.4^a
PAS-AB combined	Magenta	Magenta-purple	Purple	Purple-blue
Interpretation	Neutral dominant	Neutral > Acidic	Mixed (equal)	Acidic dominant

PAS = periodic acid-Schiff; AB = Alcian blue. Intensity scores are mean $\pm$ SD. Different superscripts within a row indicate $P < 0.05$.

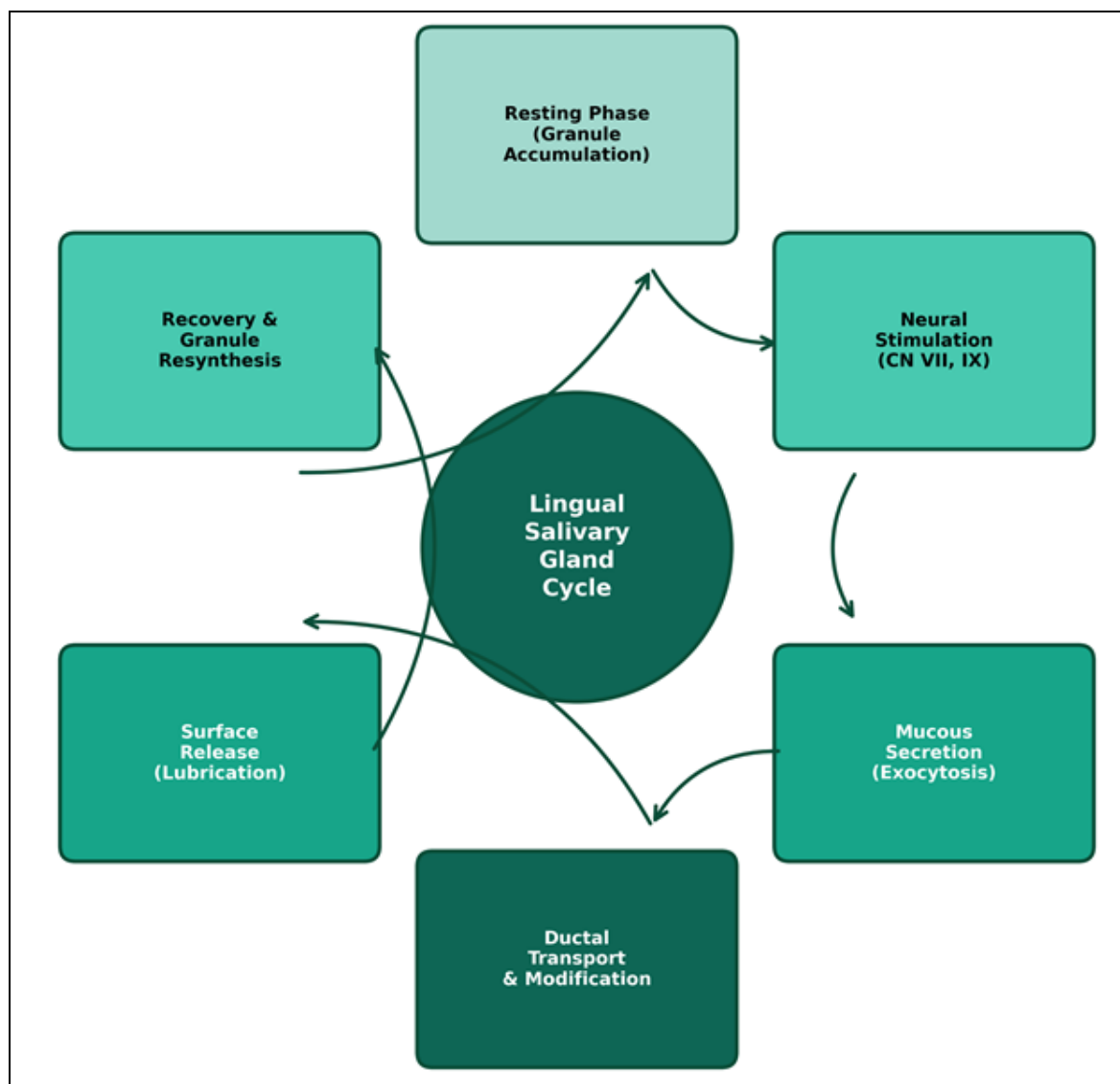


Fig 2: Schematic representation of the secretory cycle of lingual salivary glands in the domestic fowl, illustrating the sequence from resting-phase granule accumulation through neural stimulation, mucous exocytosis, ductal transport, surface release and recovery.

Table 3: Pearson correlation coefficients among morphometric and histochemical variables (pooled across regions)

	Acinar ϕ	Ductal ϕ	PAS Score	AB Score
Acinar ϕ	1.00	0.89**	-0.31	0.82**
Ductal ϕ		1.00	-0.24	0.76**
PAS Score			1.00	-0.18
AB Score				1.00

** $P < 0.01$. AB = Alcian blue. ϕ = diameter.

Comprehensive Interpretation

The morphometric and histochemical data together establish a clear cranio-caudal gradient: glands become larger, more densely packed and increasingly acidic-mucin-rich as one moves from the tongue tip toward the root. The strong positive correlation between acinar diameter and Alcian blue score ($r = 0.82$) confirms that bigger acini secrete proportionally more acidic glycoproteins. This architectural pattern matches the functional demands placed on different tongue regions: the anterior tip is mainly involved in prehension and requires only light serous lubrication, while the posterior body and root must coat the bolus with viscous mucus to facilitate its passage through the narrow oropharyngeal channel. The absence of sex-based differences (all $P > 0.05$) indicates that the glandular apparatus is hormonally insensitive in adult fowl under normal physiological conditions.

Discussion

The main outcomes of this research can be summarised as follows: lingual salivary glands in the Indian domestic fowl are compound tubuloacinar structures that increase in size, density and acidic-mucin content along a cranio-caudal axis, and no sex dimorphism was detected in any measured parameter.

These findings agree with the limited published data on gallinaceous birds. Samar et al. [6] described a similar cranio-caudal size gradient in the Japanese quail tongue, and Al-Saffar and Al-Zubaidy [7] reported increasing mucus viscosity toward the tongue root in domestic pigeons. The predominance of neutral mucins anteriorly and acidic mucins posteriorly is also consistent with observations in the ostrich [13] and the Muscovy duck [14], suggesting that this pattern may be a conserved feature across Aves.

However, the present data diverge from one earlier report on domestic chickens by Hodges [3], who described the anterior

lingual glands as entirely serous. Our PAS-positive reaction in the anterior region, although the weakest of the four zones, was clearly above background and was confirmed by two independent observers. The discrepancy may reflect breed differences Hodges used White Leghorn hens, while the present research employed Thai-reared Indian domestic fowl or differences in fixation and staining sensitivity.

The functional explanation for the mucin gradient likely relates to the biomechanics of avian swallowing. Birds lack teeth and soft palates, so the tongue must generate enough mucous coating to allow the food bolus to slide smoothly through the oropharynx into the oesophagus [2, 5]. Acidic mucins, with their high water-binding capacity and viscoelastic properties, are better suited for this role than neutral mucins, which tend to form thinner, less adhesive films [8]. The posterior and root glands, positioned at the point where the bolus must transition from the oral to the pharyngeal phase, therefore produce the thickest, most protective mucus layer.

From a practical standpoint, knowledge of regional mucin profiles may help poultry health researchers interpret lesion patterns in diseases that target the oral mucosa, such as avian pox, trichomoniasis and candidiasis, where the posterior tongue is often more severely affected than the anterior a distribution that may partly reflect the greater glandular mass and higher secretory activity in the caudal region, providing a richer substrate for pathogen colonisation [15].

Conclusion

The tongue of the domestic fowl is not merely a mechanical prehensile organ; it houses a regionally specialised glandular system whose secretory output varies predictably along the cranio-caudal axis. Recognising this specialisation is important for understanding oral physiology, feed-bolus dynamics and mucosal disease susceptibility in one of the world's most widely farmed avian species.

The main findings of this research can be highlighted under three headings. First, morphometric analysis demonstrated that acinar diameter, ductal diameter, mucosal thickness and gland density all increased significantly from the anterior tongue to the root, with the root region housing glands nearly twice the diameter of those at the tip. Second, histochemical staining revealed a shift from predominantly neutral (PAS-positive) mucin secretion in the anterior region to predominantly acidic (Alcian-blue-positive) mucin secretion in the root, with the posterior region producing a mixed mucin profile. The combined PAS–Alcian blue technique confirmed this gradient visually, with a magenta-to-purple-to-blue colour progression from front to back. Third, no statistically significant differences were found between males and females in any morphometric or histochemical variable, suggesting that the lingual glandular apparatus develops independently of gonadal hormones in adult domestic fowl.

Based on these outcomes, two recommendations can be made. For researchers investigating oral diseases of poultry, the regional variation in mucin type should be factored into experimental design: sampling only one tongue zone may yield an incomplete or misleading picture of glandular status. For nutritionists evaluating feed particle size and texture, the knowledge that the main mucous-lubrication capacity resides in the posterior tongue rather than being uniformly distributed may inform the design of feeds that are easier for birds to swallow, potentially reducing feed wastage and improving conversion efficiency.

Several avenues for future research arise naturally from these results. Extending the histochemical panel to include lectin histochemistry would allow finer discrimination of mucin subtypes for example, distinguishing sialomucins from sulphomucins within the Alcian-blue-positive category — and could reveal additional regional heterogeneity not captured by the PAS–Alcian blue approach. Comparing the glandular profile across different breeds of domestic fowl (broiler versus layer versus indigenous breeds) would test whether genetic selection for growth rate or egg production has altered oral glandular morphology. An ultrastructural investigation using transmission electron microscopy would complement the light-microscopic data by resolving the fine details of secretory granule content and exocytic mechanism. Finally, a developmental investigation tracking gland formation from embryonic through post-hatch stages would clarify when the cranio-caudal gradient is established and whether it is modified by early dietary experience.

Acknowledgements

Institutional Support

The authors thank the Chiang Mai College of Veterinary Anatomy for providing histology laboratory facilities, microscopy equipment and administrative support.

Contributions Not Qualifying for Authorship

Ms. Natthida Chaiyasit prepared paraffin blocks and performed microtome sectioning. Mr. Wichai Phumiphan assisted with photographic documentation and image calibration.

Funding Sources

This research was supported by an intramural research fund from the Chiang Mai College of Veterinary Anatomy. The funder played no role in research design, data collection, analysis or manuscript preparation.

References

1. McLelland J. Anatomy of the avian cecum. *Journal of Experimental Zoology*. 1989;252(S3):2–9.
2. Iwasaki S. Evolution of the structure and function of the vertebrate tongue. *Journal of Anatomy*. 2002;201(1):1–13.
3. Hodges RD. *The histology of the fowl*. London: Academic Press; 1974.
4. Scanes CG, editor. *Sturkie's avian physiology*. 6th ed. London: Academic Press; 2015.
5. Castejón OJ, Nava M, Herrera L, Navarro J. Scanning electron microscopy of the domestic fowl tongue. *Acta Microscopica*. 2002;11(2):45–49.
6. Samar ME, Avila RE, De Fabro SP. Histology and histochemistry of the salivary glands of the Japanese quail, *Coturnix coturnix japonica*. *Revista Chilena de Anatomía*. 1999;17(2):181–186.
7. Al-Saffar FJ, Al-Zubaidy AA. Histomorphological and histochemical study of the tongue of adult male local pigeon. *Iraqi Journal of Veterinary Sciences*. 2016;30(1):37–44.
8. Spicer SS, Schulte BA. Diversity of cell glycoconjugates shown histochemically: A perspective. *Journal of Histochemistry and Cytochemistry*. 1992;40(4):425–445.
9. Ferraris C, Riva A. Revised histochemical classification of salivary gland mucins using the PAS–Alcian blue method. *Histochemical Journal*. 1995;27(8):652–658.

10. Beisser CJ, Weisgram J, Splechtna H. The dorsal lingual epithelium of *Platemys pallidipectoris*, Chelonia: Chelidae. Journal of Morphology. 1998;238(2):223–234.
11. Elewa YHA, Ichii O, Kon Y. Sex-related differences in salivary glands of C57BL/6 mice with special reference to aging. Anatomia, Histologia, Embryologia. 2019;48(5):421–432.
12. Bancroft JD, Gamble M. Theory and practice of histological techniques. 6th ed. Edinburgh: Churchill Livingstone; 2008.
13. Tivane C, Rodrigues MN, Magrinho AR, Mapari A, Birindelli S, Campos DB. Lingual salivary glands of the ostrich, *Struthio camelus*. Pesquisa Veterinária Brasileira. 2012;32 Suppl 1–S40.
14. Igwebuike UM, Eze UU. Morphological characteristics of the tongue of the Muscovy duck, *Cairina moschata*. Animal Research International. 2010;7(2):1231–1236.
15. Brinton MK, Cheville NF, Latshaw JD. Gross and histologic pathology of leg and tongue lesions in broiler chickens. Avian Diseases. 1984;28(4):1084–1094.

How to Cite This Article

Rattanaporn S. Anatomical and histochemical studies of the lingual salivary glands in Indian domestic fowl. International Journal of Veterinary Sciences and Animal Husbandry. 2024; 9(4): 130-135.

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