

Morphological Study of Oropharyngeal Cavity and Histochemical Characteristic of Palatine Salivary Glands in Domesticated Goose (*Anser anser domesticus*)

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Abstract

This research is planned to investigate the morphological structures of the oropharynx cavity and the microscopic features of palatine salivary glands in domesticated geese. Ten heads of adult healthy geese were collected from the slaughterhouse. Five heads were prepared for macroscopic examination, and five were processed for histochemical investigation of the palatine salivary glands. Anatomically, the oropharynx cavity was divided into roof and floor. The oropharyngeal roof comprises the rostral region (lamellae part) and the caudal region (papillae part). The median palatine ridge divided the lamellar part into two equal sides. Each side had three longitudinal rows of palatine round tubercles. The oropharyngeal floor is subdivided into the rostral part occupied by the tongue and the caudal part, including the laryngeal mound. Horny bristle lamellae existed on the lateral margins of the beak. Three clefts were observed, including choanae, infundibular cleft, and glottis. The tongue was flat, elongated, and rostrally carried by the lingual nail. The microscopic findings showed that the palatine salivary glands were mucus-secreting and simple branched tubular glands. A mucus cell was tall and columnar, with its nucleus located basally. Also, the mucus cell secreted neutral and acid mucocutaneous, but proteins and glycogens were absent. The palatine glands contained numerous lymphatic nodules, which protected the oropharyngeal mucosa from invasion by microorganisms. The characteristic features of the oropharynx and secretions of the palatine glands showed their adaptation based on the type of food and feeding behavior in domesticated geese.

Keywords: Domestic goose, Histochemical stains, Oropharynx cavity, Palatine salivary glands.

Introduction

The environment and different feeding habits of birds influence the morphological structures of their oral cavity, tongue, and beak (1). Unlike mammals, birds have no teeth; the upper and lower jaws develop into a cornified beak (2). Because the glossopalatine folds and soft palate are absent, the oral cavity combines with the pharynx to form an oropharyngeal cavity (3). Also, the oral and pharyngeal mucosa is modified to pick up and partially break up food (4). In Anseriformes species, adaptation is also evident according to feeding habits (5). The distribution of lingual and pharyngeal papillae in different numbers and locations facilitates food holding into the oropharyngeal cavity and moving toward the esophagus (6). The salivary gland is an essential component in birds. It produces saliva necessary for digestion, lubrication, moistening of food, and protecting of the oropharynx mucosa from desiccation, toxic materials, and microorganism development (7). Some birds use their saliva, a sticky secretion, to build nests, attach them to walls, and trap insects (8). Some birds use their saliva, a sticky secretion, to build nests, attach them to walls, and trap insects Therefore, the present study is undertaken to describe the oropharynx anatomy and histochemical characteristics of the palatine salivary glands in domesticated geese.

Material and Methods

Ten heads of healthy domesticated geese (both sexes, ten months old, weight 5kg) were obtained from the local Sulaymani slaughterhouse. Immediately after slaughtering, five heads were prepared by washing with normal saline and dissecting through the edge of the beak angle to see the oropharynx structures and determine the

position of the salivary gland. A digital camera was used to take the photos were examined, and the other five heads prepared for the light microscopic examinations. The samples taken from the caudal region of the oropharynx roof were preserved in (10% formalin) for 24 hours and processed by the general method. Then, (5 μ m thickness) sections were made by using a rotatory microtome, and lastly, they were stained with (hematoxylin and eosin) and (Masson's trichrome) techniques to examine the general microscopic organizations. In addition, different histochemical stains are used to identify the histochemical components of the palatine glands, including periodic acid-Schiff (PAS) for neutral mucin (9), Alcian blue at pH 2.5 for acid mucin (10), Best's carmine for glycogen (11), and Bromophenol blue for total protein (12). Finally, the slides were examined under the light microscope (Motic - China), and a digital camera (Am scope - China) was used to capture the histological images.

Results

Macroscopic findings: Anatomically, a glossopalatine fold and soft palate were absent. As a result, the oral cavity was combined with the pharynx to form the oropharynx cavity. The oropharynx cavity is composed of an oropharynx roof and an oropharynx floor. The oropharyngeal roof is subdivided into the rostral and caudal regions. The rostral region is also called the lamellar or hard palate part covered with a hard mucous membrane. On top of the rostral region, a maxillary nail and maxillary cushion were observed. The palatine ridge divided the rostral region into two symmetrical parts. Each part had three longitudinal rows of rounded palatine tubercles (maxillary spines) (Figure 1). On the lateral margins of the upper

beak transverse horny bristle lamellae existed that separated from the palatine tubercles by a lateral deep palatine longitudinal groove. Also, maxillary salivary glands are named based on the location of glands that are presented in the mucous membranes covering the maxillary bone found in the rostral region of the oropharynx roof. These glands discharge their secretions into the oropharynx cavity through 2-3 openings located caudal to the maxillary cushion (Figure 2a).

The palatine salivary glands are composed of the medial and lateral salivary glands. The medial palatine salivary glands were numerous and appeared around the choanae. The lateral palatine salivary glands are located just next to the medial salivary glands. The last salivary gland in the oropharyngeal roof was sephenoptregoid salivary glands around the infundibular cleft. The choanae and infundibular clefts were observed on the oropharyngeal roof's caudal region. The oropharynx cavity was combined with nasal cavities through the choanae cleft. The choanae is composed of a slit, narrow rostral region and a wider caudal region. Two rows of conical papillae surround it. with the infundibular cleft is located caudal to the choanae, which communicates with the middle ear and the oropharyngeal cavity. Also, it was surrounded by a row of conical papillae, but they were shorter than the conical papillae around the choanae cleft.

Numerous pharyngeal conical papillae directed caudally observed on the oropharynx roof which they oropharynx roof's caudal region, which assists food swallowing toward the esophagus (Figure 2b). The oropharynx floor is subdivided into the rostral and caudal regions. The tongue fully occupied the rostral region, and the caudal region was composed of the laryngeal mound. A mandibular nail on

the tip of the lower beak was observed. Transverse horny lamellae covered by thick mucus membranes existed on the lateral margins of the lower beak, and they were shallower and smaller than the upper beak lamellae. The tongue was elongated, narrow, and flatted, and the rostral portion of the tongue was modified to a spoon-like structure known as a lingual nail. The tongue had ventral and dorsal surfaces with two lateral borders.

Anatomically, the tongue is composed of the apex (rostral part or lingual apex), body (middle part or lingual body), and root (caudal part or lingual root). A shallow median lingual sulcus divides the dorsal surface of the tongue into two equal parts. At the caudal end of the lingual body, lingual prominence protruded that carried conical papillae directed caudally as V-shaped. Nine spiny keratinized directed caudally conical papillae projected from the rostro lateral border of the tongue followed by six giant horny conical papillae. Small conical papillae are directed caudally and located at the caudolateral end of the lingual body. In addition, two mucosal folds carried 3-4 spiny conical papillae at the tongue's root (Figure 1).

The lingual salivary glands are detected as rosary beads on the tongue's latero-ventral surface. The mandibular salivary glands are divided into three parts named according to location. The first part was the rostral mandibular salivary glands that presented as a single serious in both halves of the oropharynx floor. The second part was intermediates mandibular salivary glands; they were few in number and appeared as a small group near the angle of the beak. The third part was the caudal mandibular salivary glands, which were indicated as small groups

of caudal to the intermediate mandibular glands.

The caudal region of the oropharynx floor is composed of the laryngeal mound and glottis cleft, which communicates the oropharyngeal cavity with the tracheal tube. Other V- shape conical papillae appeared on the rostral portion of the laryngeal mound, but the opposite arrangement of V- shape conical papillae presented on the top of the lingual prominence.

The laryngeal mound exposed one row of conical papillae around the glottis cleft. The caudal portion of the laryngeal mound also displayed transverse rows of thick and spiny, caudally directed pharyngeal papillae (Figure 3). They were more condensed and larger than the pharyngeal conical papillae observed on the roof of the oropharynx.

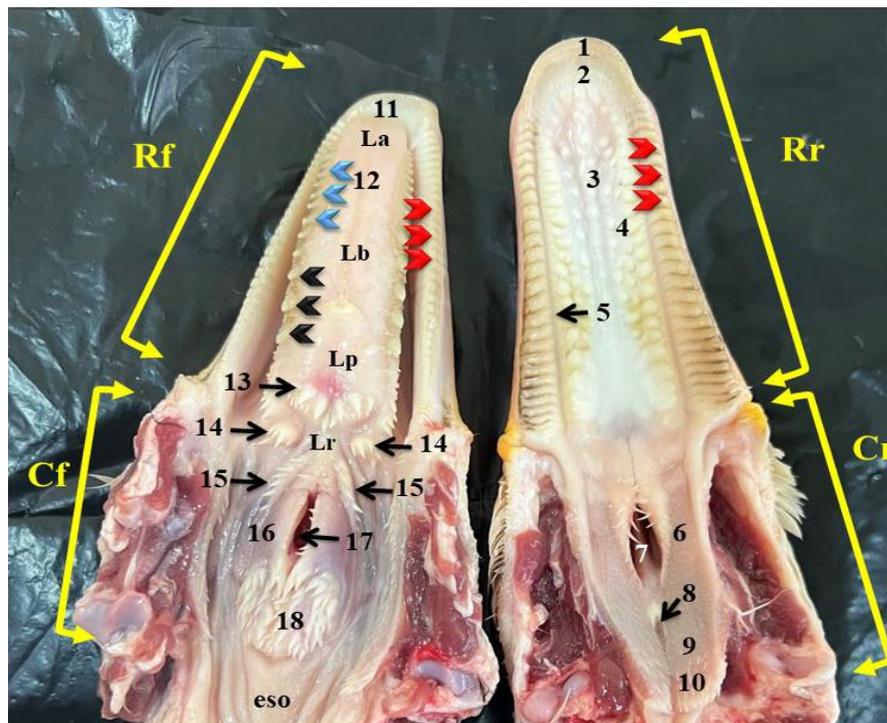


Figure 1 Morphological appearance of the oropharyngeal cavity. Rostral region of the oropharynx roof (Rr), Caudal region of the oropharynx roof (Cr), Rostral region of the oropharynx floor (Rf), Caudal region of the oropharynx floor (Cf), Horny lamellae (red arrow heads), Spiny keratinized papillae (blue arrow heads), Keratinized giant horny papillae (black arrow heads), Apex of the tongue (La), Body of the tongue (Lb), Root of the tongue (Lr), Lingual prominence (Lp), Initial part of the esophagus (eso), Maxillary nail (1), Maxillary cushion (2), Palatine ridge (3), Palatine tubercles (4), Lateral deep palatine longitudinal groove (5), Medial palatine salivary glands (6), Choanae (7), Infundibular cleft (8), Sphenopterygoid salivary glands (9), Caudal pharyngeal conical papillae (10), Mandibular nail (11), Median lingual sulcus (12), Conical papillae arrangement as V- shape that directed caudally on the lingual prominence (13), Two mucosal folds carried 3-4 conical papillae at the lingual root (14), Conical papillae around cranial end of laryngeal mound as an opposite V- shape (15), Laryngeal mound (16), Glottis (17), Caudally directed pharyngeal conical papillae on the laryngeal mound (18).

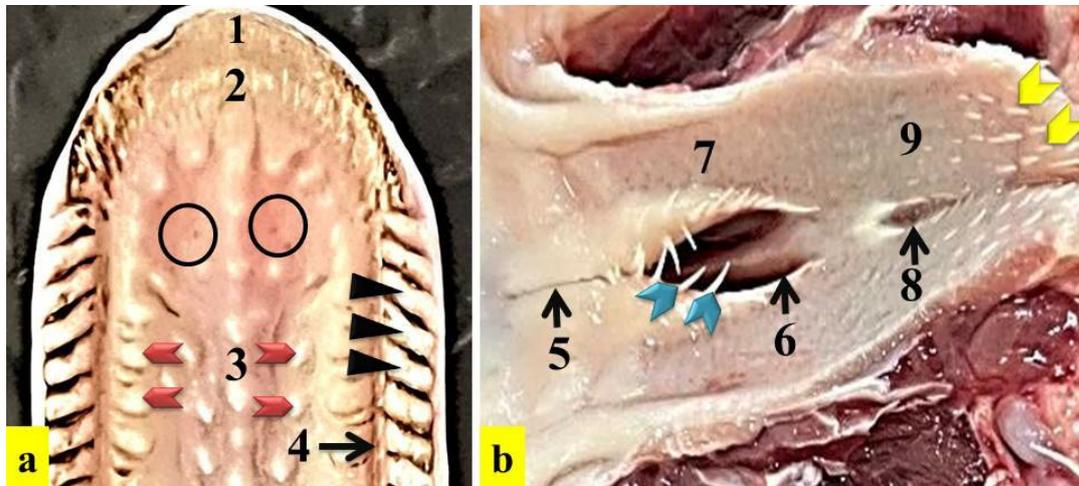


Figure 2 (a) Rostral region of the oropharynx roof, horny lamellae on the lateral margins of the upper beak (black arrow heads), Openings of the maxillary salivary glands (black circles), Round palatine tubercles (red arrow heads), Maxillary nail (1), Maxillary cushion (2), Palatine ridge (3), Lateral deep palatine longitudinal groove (4). (b) Caudal region of the oropharynx roof. Narrow rostral part of the choanae (5), Wide caudal part of the choanae (6), Medial palatine salivary glands (7), Infundibular cleft (8), Sphenopterygoid salivary glands (9), Single row of spiny shaped conical papillae around the choanae (blue arrow heads), Caudal pharyngeal conical papillae (yellow arrows heads).

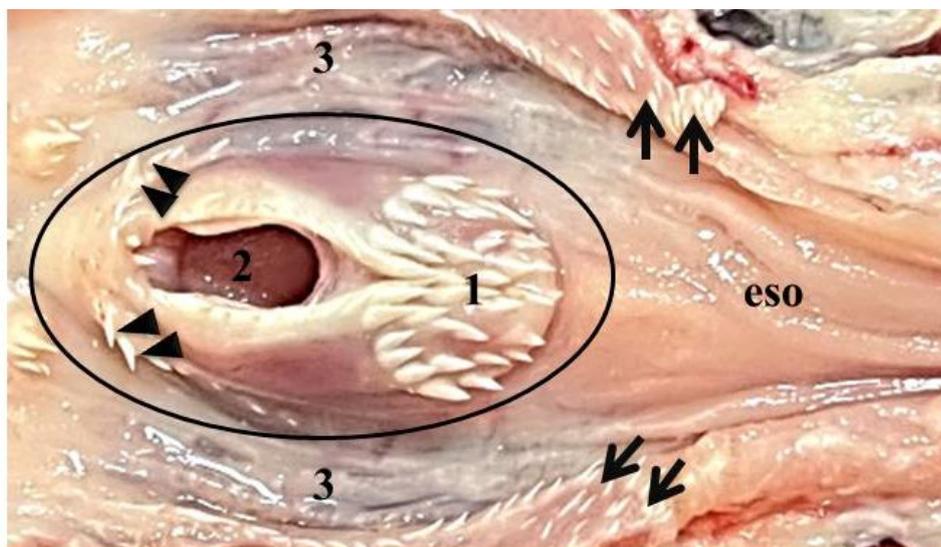


Figure 3 Caudal region of the oropharynx floor contained laryngeal mound (black circle), Caudally directed conical papillae on the caudal end of the laryngeal mound (1), Glottis (2), Floor of the pharynx (3), Opposite V- shape arrangement conical papillae at the rostral end of the laryngeal mound (black arrow heads), Pharyngeal conical papillae (black arrows), Esophagus (eso).

Microscopic findings

In the current study, routine stains and Masson's trichrome technique were applied to show the palatine salivary glands' general features. The histological results showed that the caudal region of the oropharynx roof covered by non-keratinized stratified squamous epithelium contained different sizes of conical papillae with underlying palatine salivary glands (Figure 4).

The palatine glands were composed of well-differentiated medial and lateral palatine salivary glands. The medial one is located along the midline of the palate around the choanae. The lateral palatine salivary glands were smaller than the medial palatine salivary glands and were found on both sides of the palate. However, they had identical microscopic structures. The palatine glands consisted of lobes of various sizes. The lobes are entirely enclosed by capsules that separate the lobes from each other. Each lobe is divided into (3-5) lobules via connective tissue septa that extend from the capsule. The connective tissue capsule consists of collagen fibers, blood vessels, and nerves. The accumulation of secretory epithelial cells constitutes each lobule. These cells in the lobules are arranged as a simple branched tubular mucous gland. The glandular cells were tall and columnar in shape, with foamy vacuolated cytoplasm and flattened basally located nuclei resting on a delicate basement membrane. Lymphoid nodules also appeared inside most the lobules. They were essential for protecting the oropharynx mucosa from invasion of microorganisms to the underlying

tissue structures. These lymphoid nodules reacted negatively with all special stains. Apart from lymphoid nodules, numerous individual lymphocytes were scattered throughout the lobules of the glands. The secretion from each lobule drained directly in the center of each lobule into a main excretory duct, passing through the epithelial layer, and opened separately into the end of the oropharyngeal cavity. The epithelial lining of the excretory duct consisted of simple columnar cells, as illustrated in (Figure 5).

In addition to routine staining, various histochemical staining has been used to identify microscopic components of the palatine glands. The results of histochemical examinations revealed that the lateral and medial palatine salivary glands were tubular glands that secreted mucus. These glands were particularly significant due to their locations and the volume of mucus they produced, which plays a crucial role in lubricating and protecting the oropharyngeal mucosal layer. Also, it facilitates the swallowing of food toward the esophagus as they adapt to produce mucus-rich secretions and are highly specialized for their herbivorous feeding habits. Periodic acid-Schiff (PAS) stain was positively intense in the lateral palate salivary gland in comparison to the medial gland, which was specific for neutral mucopolysaccharides. Mucous-secreting cells in tubular glands revealed a strong positive result with Alcian blue stain at pH 2.5, which was specific for acidic mucin (Figure 6).

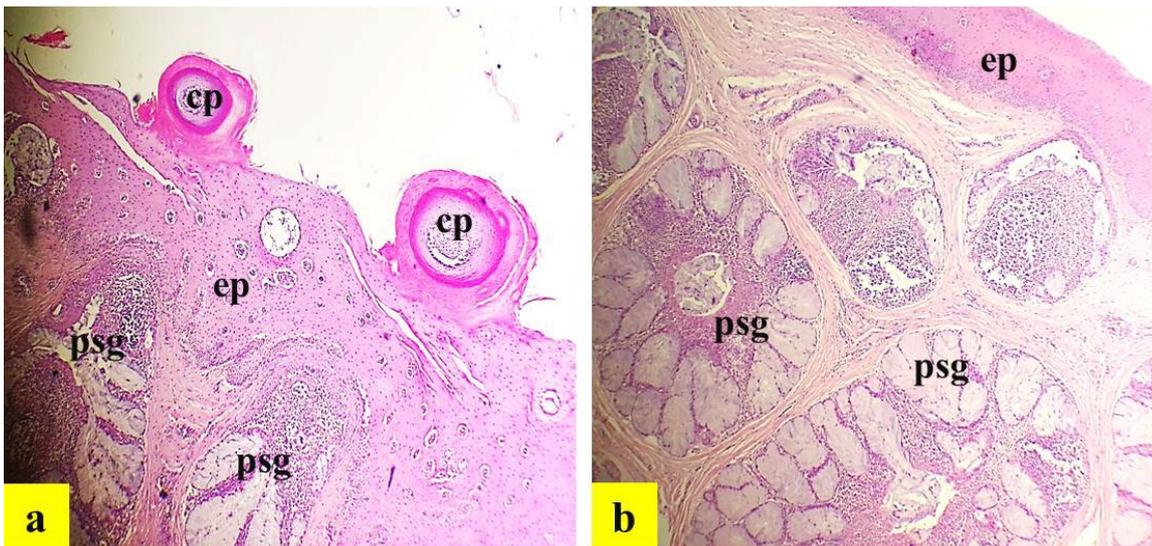


Figure 4 Photomicrographs of the sagittal section through the dorsal layer of palatine. (a) The surface of palatine covered by stratified squamous epithelium (ep) with keratinized conical papillae (cp). (b) Directly beneath the epithelial lining (ep) the palatine salivary glands (psg) located. H&E stains, (a, b) x40.

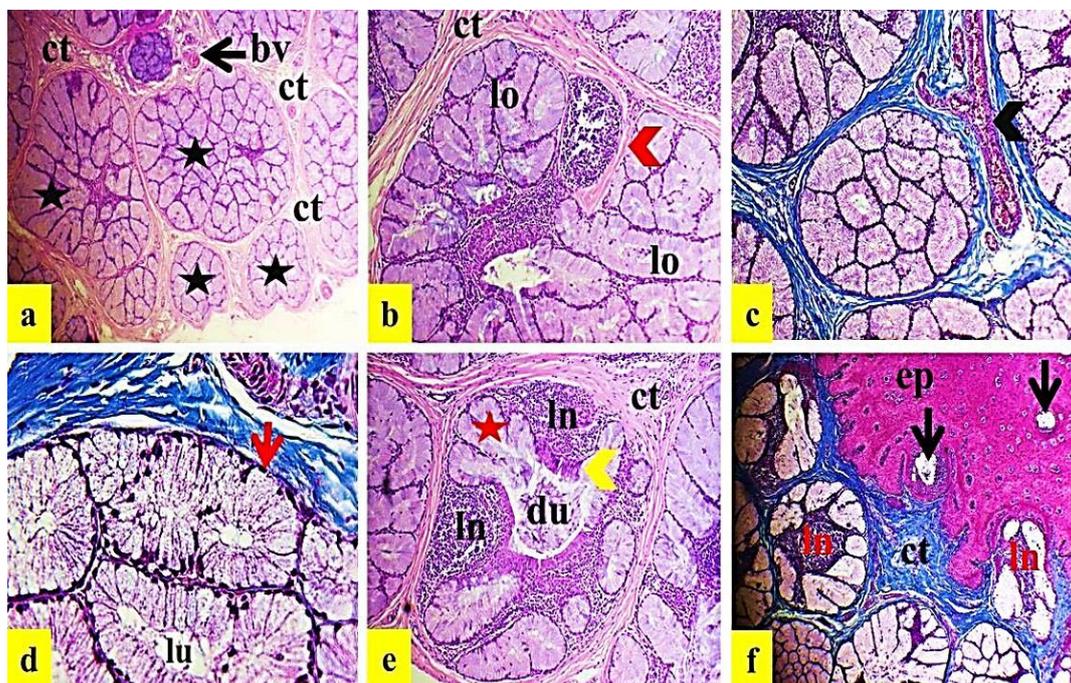


Figure 5 Photomicrographs of the sagittal section of the palatine salivary glands showed (a) Different size of lobes (black stars) enclosed by connective tissue capsule (ct) contained blood vessels (bv). (b) Each lobe divided into lobules (lo) by means of connective tissue septa (red arrow head) extended from connective tissue capsule (ct). (c) Connective tissue surrounding lobes consisted of collagen fibers in blue color and large blood vessel (black arrow head). (d) Large lumen (lu) of simple branched mucous gland constituted by tall columnar mucus cells with basally located flattened nucleus (red arrow), (e) Simple branched tubular gland (red star) excrete mucous secretion into excretory duct (du) which was lined by simple columnar epithelium (yellow arrow head) at the center of lobe, lymphoid nodules (ln) located between secretory tubules. (f) Excretory duct (black arrows) passing through epithelial lining (ep) of the palate and opened into the oropharyngeal cavity. (a, b, e) H&E stain, (c, d, f) Masson's trichrome stain, (a, f) x40, (b, c, e) x100, (d) x400.

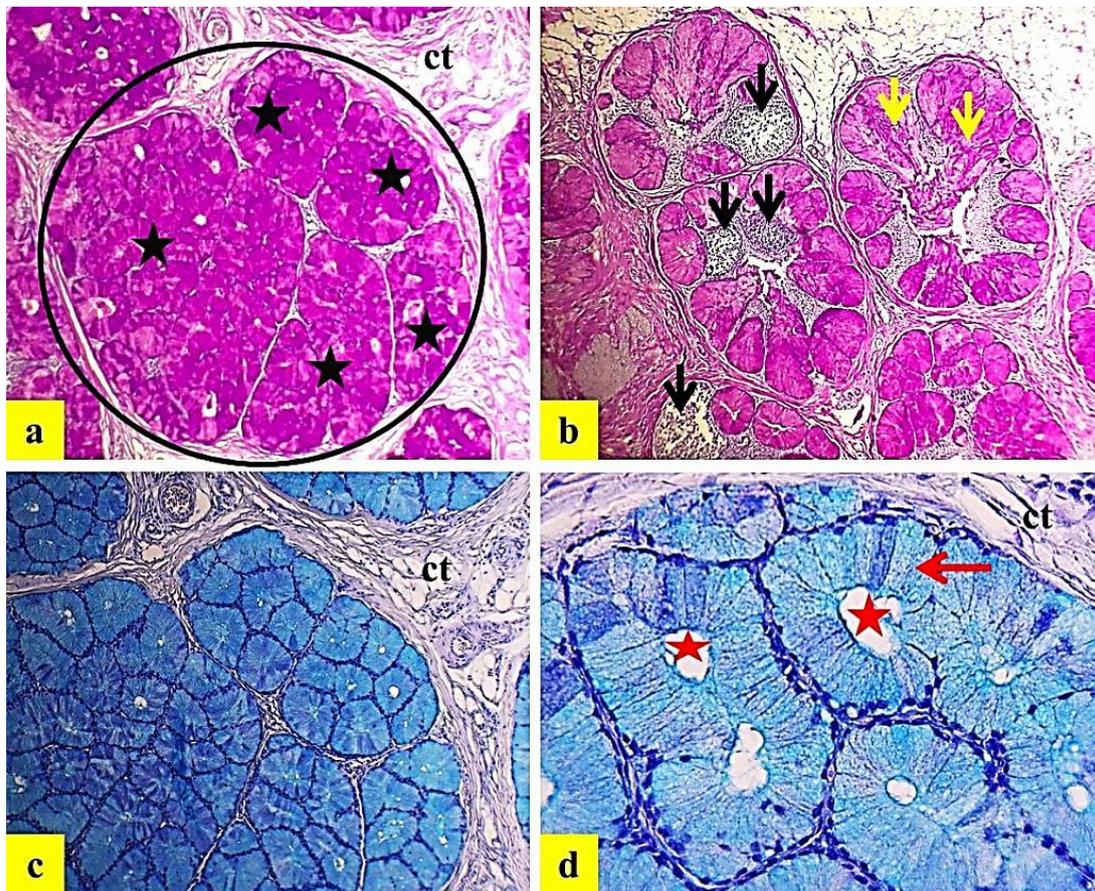


Figure 6 Photomicrographs of the palatine salivary glands stained with different histochemical stains showed (a) Strong positive PAS reaction of lobular gland (black stars) in one lobe (black circle) which was encapsulated by connective tissue (ct). (b) Simple tubular glands (yellow arrows) positively react to PAS stain excrete their mucous secretion into excretory duct in the center of the lobe, lymphoid nodules (black arrows) located between secretory glands negatively react to PAS stain. (c) Higher magnification from section (a) showed a positive reaction of mucous glands to Alcian blue stain at pH 2.5. (d) Higher magnification from section (c) showed positive stained columnar cells (red arrow) with Alcian blue, lumen (red stars) of tubular glands. (a, b) PAS stain (c, d) Alcian blue stain at pH 2.5, (a, b) x40, (c) x100, (d) x400.

Discussion

This study showcased the morphological structures of the oropharynx and conducted a histochemical examination of the palatine salivary glands in domesticated geese. The current findings revealed that the oral cavity and pharynx were not separated because the glossopalatine folds and soft palate were absent; instead, a common oropharynx cavity was formed, in agreement with the results of previous authors described in domestic geese by (3, 13). Previous studies correlated food adaptation mostly with the morpho-functional characteristic of the tongue (4, 14, 15). Four different types of food intake are

detected, including filter-feeding, pecking, grazing, and drinking according to the research on the mechanism of behavior feeding of Anseriformes (5). The current macroscopic results confirm the presence of transverse horny lamellae on the beak of domesticated geese, which they use to grasp and break off grass. In addition, on the lateral borders of the tongue, six small spiny and nine giant horny conical papillae existed, which related to the process of grazing, our observation is similar to the result of (4, 5, 15). The tongue occupies the entire rostral region of the oropharyngeal floor, and it modifies the rostral

portion of the tongue to form a ladle-like structure known as the lingual nail. According to previously researched oropharynx anatomy of parrots, chickens, and white-tailed eagles, the lingual nail in the Anatidae family relates to the transverse horny lamellae on the beak to pick up small food particles and grab the grains (16, 17, 18). The tongue of domesticated geese is a narrow, elongated, flat shape adapted to the length of the lower beak; similar results were described in domesticated geese and ducks (14, 19). In contrast to (20), it was shown that tongue of the Eurasian hoopoe bird is quite short which not occupy the lower beak length, and (21) revealed that woodpecker's tongue is longer than the length of the lower beak. According to our study, domesticated geese have well-developed palatine

salivary glands, which is consistent with the general morphology observed in the majority of bird species (22). The histological findings in the present study revealed that the rostral region of the palate was lined by stratified squamous keratinized epithelium, but the caudal region toward the choanae lined by non-keratinized epithelium contained conical papillae in accordance with the result of (23) in the chukar partridge. The palatine glands play a critical role in mucous secretion, moistening, lubrication, and swallowing food. Additionally, these glands protect the oropharyngeal cavity from microorganism invasion because they contain large amounts of lymphatic tissue, as stated in reference(15).

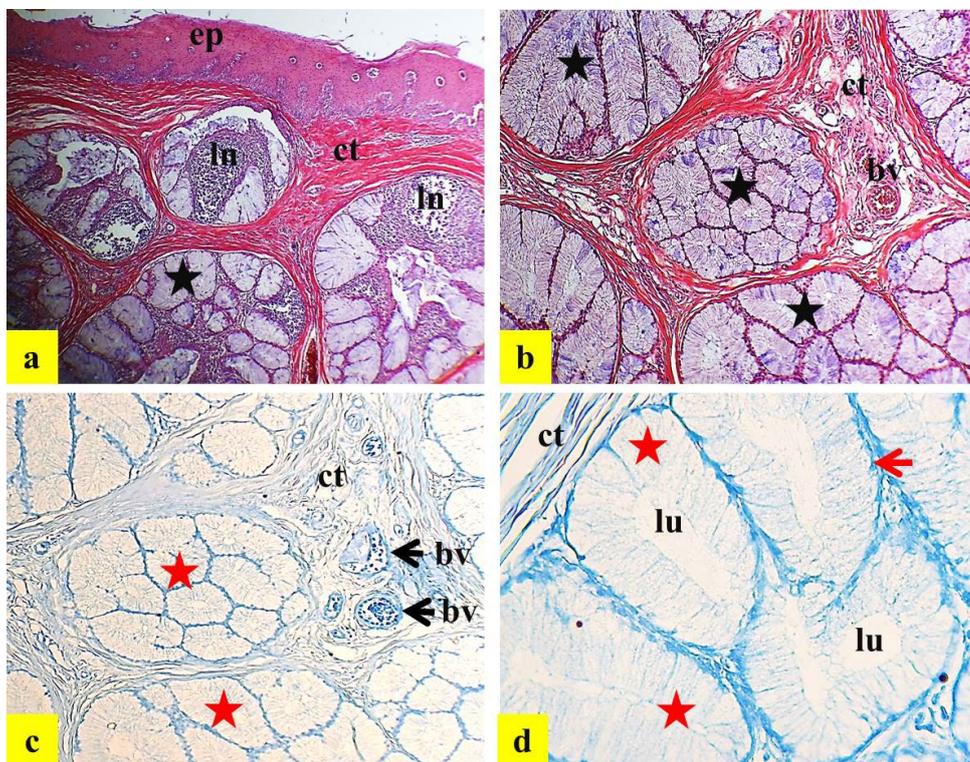


Figure 7 Photomicrographs of the palatine salivary glands stained with different histochemical stains showed (a, b) Negative reactivity in simple mucous glands (black stars) to Best's Carmine stain indicating the absence of glycogen in their secretion. (c, d) Negative reactivity in simple mucous glands (red stars) to Bromophenol blue stain indicating the absence of protein in their secretion. Stratified squamous epithelium (ep), connective tissue capsule (ct), lymphoid nodules (ln), blood vessels (bv), lumen (lu) of tubular glands, nucleus of columnar cell (red arrow). (a, b) Best's Carmine stain (c, d) Bromophenol blue stain, (a) x40, (b, c) x100, (d) x400.

In reference to our histochemical outcomes, the lateral and medial glands showed strong positive results for PAS stain, indicating the presence of the glycoconjugates containing vicinal diol groups in the secretory granules in the mucous cells in partial agreement with (24) stated that the cells and lumen of the lateral palatine salivary glands in the chicken produced strong positive results for PAS stain, while the apical portion of the secretory cells of the medial palatine salivary glands showed PAS reactions of varying intensity. In agreement with the results of another study (25) documented that the mucus cells of the medial and lateral palatine salivary glands in penguin showed PAS positive glycoconjugates. The results of the present study indicated that the palatine glands showed an intensive positive Alcian blue reaction at pH 2.5, indicating the presence of acid mucin. This finding supported the results of (24), which reported that the secretory cells and lumen of the lateral palatine glands produced a strong positive Alcian blue reaction at pH 2.5 in chickens. The best carmine stain was used to identify the presence of glycogen. The lateral and medial palatine glands yielded negative results for the Best Carmine stain, suggesting a deficiency in glycogen, which aligns with the findings in Chukar Partridge (23). The palatine glands showed a negative reaction to the bromophenol blue staining method, indicating the absence of protein, in contrast to results reported in the lingual salivary glands of some bird species (26).

Conclusions

In the current study, we concluded that the anatomical structures of the oropharynx are related to the feeding habit in domesticated geese. The morphology of the tongue and distribution of the lingual and pharyngeal

papillae facilitate food swallowing toward the esophagus. Mucous secretions of the palatine salivary glands lubricate food particles and protect the oropharynx from dehydration and microorganism invasion.

Conflicts of interest

The authors declare that there is no conflict of interest.

Ethical Clearance

This work is approved by The Research Ethical Committee.

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دراسة مورفولوجية للتجويف البلعومي الفموي والخصائص الكيميائية النسيجية للغدد اللعابية الحنكية

في الإوز المستأنس (*Anser anser domesticus*)

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2-قسم الأحياء المجهرية، كلية الطب البيطري، جامعة السليمانية، العراق.

الخلاصة

هدف هذا البحث الى دراسة البنى المورفولوجية للتجويف البلعومي الفموي والخصائص المجهرية للغدد اللعابية الحنكية في الإوز المستأنس. تم جمع عشرة رؤوس من الإوز البالغ السليم من المسلخ. تم تحضير خمسة رؤوس للفحص العياني وتم معالجة خمسة رؤوس للدراسة الكيميائية النسيجية للغدد اللعابية الحنكية. تشريحياً، ينقسم التجويف البلعومي الفموي إلى سقف وأرضية. يتكون سقف البلعوم الفموي من المنطقة الأمامية (الجزء الصفائحي) والمنطقة الخلفية (الجزء الحليمي). يقسم الحرف الحنكي المتوسط الجزء الصفائحي إلى جانبيين متساويين. يحتوي كل جانب على ثلاثة صفوف طولية من الخديبات الحنكية المستديرة. تنقسم أرضية البلعوم الفموي إلى جزء أمامي يشغله اللسان وجزء خلفي يتضمن التل الحنجري. توجد أشواك قرنية صفائحية على الحواف الجانبية للمنقار. لوحظت ثلاث فتحات تشمل المنخرين الداخليين، والشق القمعي، والمزمار. اللسان مسطح ومستطيل ويحمل في مقدمته الظفر اللساني. أظهرت النتائج المجهرية أن الغدد اللعابية الحنكية كانت مفرزة للمخاط وغدداً أنبوية متفرعة بسيطة. الخلية المخاطية عمودية طويلة مع نواة تقع في القاعدة. كما أفرزت الخلية المخاطية مواد مخاطية متعادلة وحمضية، لكن البروتينات والجليكوجين كانت غائبة. احتوت الغدد الحنكية على عُقيدات لمفاوية عديدة تحمي الغشاء المخاطي البلعومي الفموي من غزو الكائنات الدقيقة. أظهرت السمات المميزة للبلعوم الفموي وإفرازات الغدد الحنكية تكيفها بناءً على نوع الغذاء وسلوك التغذية في الإوز المستأنس.

الكلمات المفتاحية: الإوز المستأنس، الصبغات الكيميائية النسيجية، التجويف البلعومي الفموي، الغدد اللعابية الحنكية.