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EMİNE ÜMRAN ÖRSÇELİK

MURAT ERDEM GÜLTİKEN

DİNÇER YILDIZ

DURMUŞ BOLAT

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Ultrastructure of the tongue and histochemical features of the lingual salivary glands in buzzards

Emine Ümran BOZKURT¹, Murat Erdem GÜLTİKEN², Dinçer YILDIZ¹, Durmuş BOLAT^{1*}

¹Department of Anatomy, Faculty of Veterinary Medicine, Kırıkkale University, Kırıkkale, Turkey

²Department of Anatomy, Faculty of Veterinary Medicine, Ondokuz Mayıs University, Samsun, Turkey

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Abstract: The macroscopic characteristics of the tongues of 8 long-legged buzzards are described and scanning electron microscopy was used to examine the epithelial tissue of the tongue's surface. Crossman's modified triple staining method and hematoxylin and eosin dye were used to determine the locations and general histological features of the lingual salivary glands. The nature of the glandular secretions was examined by staining with PAS, AB pH 1.0, AB pH 2.5, and PAS-AB pH 1.0 and pH 2.5, and diastase enzyme digestion. PAS staining and weak, moderate, and strong methylation procedures as well as sialidase and hyaluronidase applications were performed. We report the finding of focal accumulations of the anterior lingual salivary glands at the tongue's base with numerous duct openings in this area. This finding, in conjunction with the absence of taste papillae in the tongue epithelium, suggests that the role of the tongue in buzzards is to mix food with saliva and to move the food bolus rapidly to the back of the oral cavity. The acidic secretions of the lingual salivary glands not only have antimicrobial effects but also facilitate the swallowing process and may influence the digestive process in the distal parts of the alimentary tract.

Key words: Tongue, salivary glands, morphology, scanning electron microscope

1. Introduction

The tongue has many functions including ingestion, partial digestion, saliva secretion, and taste. In birds, the morphological features of the tongue such as shape, epithelization, and degree of keratinization of the epithelium are related to the type of diet and environmental conditions (1,2). Epithelial characteristics are associated with diet, and keratinization is associated with habitat, diet, and postingestion food processing (3,4).

Extensions like the finger of the connective tissue attach the propria to the epithelium (2,5,6).

It has been reported that taste buds in poultry are not localized to specific papillae on the tongue, but may sometimes be found in the deep epithelial layer. These taste buds are attached to the surface of the tongue by long channels (7). Dehkordi et al. (8) reported that there are no taste buds in the epithelium covering the dorsal aspect of the tongue in zebra finches, but they have been reported in the epithelial layer of the body and base of the tongue in red-legged partridges (9).

The basic function of salivary glands is to produce saliva, which wets the ingested food, forming a smooth bolus. In addition, the saliva forms a barrier on the mucosal surface, protecting the tongue against pathogenic

bacteria (10). Although there are numerous salivary glands in grain-fed birds, wild poultry have poorly developed salivary glands and salivary glands may even be absent in fish-eating species (11). Nevertheless, it has been reported that salivary glands, which are closely associated with the taste buds in the lingual epithelium, may play a role in taste sensations (12).

Salivary glands are a good model for understanding the relationship between glandular structures and cellular secretions. It has been reported that histochemical differences between the salivary glands in different species are determined by the structure-function characteristics of the digestive system rather than diet (13).

Despite a significant body of literature on the salivary glands of poultry fed in different ways (12–19), the number of studies conducted in wild poultry is limited (12,18,20,21).

The present study aimed to determine the effects of morphological features of the tongue and lingual epithelium and the characteristics of oral cavity salivary glands via macroscopic, microscopic, and scanning electron microscopy (SEM) examination of the tongue, as well as histochemical examination of lingual salivary glands, in long-legged buzzards.

* Correspondence: bolatdurmus@yahoo.com

2. Materials and methods

The tongues of eight client-owned long-legged buzzards, euthanized at the faculty clinic following unsuccessful treatment, were collected. All procedures were performed according to the requirements of the Kırıkkale University Animal Experiments Local Ethical Committee (Permit Number: 11/2011). Tongues were removed just after death and fixed with 10% formaldehyde solution for examination under light microscopy.

Photographs (Nikon Coolpix P7100) were taken to determine the macroscopic characteristics of the tongues. The photographs were displayed in ImageJ and the pixel values of the photographs were converted into actual measurements (mm). Statistical analysis of the measurements was performed using descriptive statistics found in GraphPad Prism 6.0. The results are presented as mean $\pm$ standard deviation (SD) in the text.

The tongues were fixed with 0.5% glutaraldehyde solution in 1% sodium acetate buffer containing 3% HgCl_2 for SEM examination and were dried using a Polaron Critical Point Drier. Carbon and gold coating was achieved with Polaron Sputter Coater, and then the samples were examined under a JSM5600 30-kV scanning electron microscope and were photographed.

In order to determine microscopic characteristics and histochemical features of the tongue and lingual salivary glands, 100 serial longitudinal sections at 5–7 μm thickness were obtained from the tongues of eight buzzards, and groups of consecutive sections were selected by random sampling for staining.

Crossman's modified triple staining method and hematoxylin and eosin dyes were used to determine the localization and general histological features of the lingual salivary glands. PAS (395B, Sigma-Aldrich, St. Louis, MO, USA) was used to expose histochemical features; AB pH 1.0 (22) and AB pH 2.5 (23) were used to determine acidic mucins; PAS-AB pH 1.0 (23), diastase (from *Aspergillus oryzae*) (09962, Sigma-Aldrich), -PAS (24), hyaluronidase (from bovine testes) (H3506, Sigma-Aldrich) and -AB pH 2.5 (23) were used to discriminate acid from neutral mucins; weak, moderate, and strong methylations (25) were used to discriminate sulfomucin from sialomucin; and sialidase (from *Clostridium perfringens*) (N2876, Sigma-Aldrich) and -AB pH 2.5 (23) were used to determine whether sulfomucins were sensitive or resistant to sialidase.

The preparations were examined and photographed using an Olympus SC100 camera system connected to the CX21 Olympus binocular microscope and the cellSens Entry computer imaging system. Sections taken from rat colon and bronchi were used as the controls for AB and hyaluronidase procedures.

3. Results

The length of tongue from the tip of apex to the caudal margin of corpus was 18.75 ± 1.28 mm (mean $\pm$ SD, $n = 8$) (Figure 1). The coefficient of variation (CV) was 6.82%. All tongues were long and narrow, with an oval tip. They extended throughout the oral cavity with the width preserved along the length (Figure 1, A). Conical papillae were present in a single line and in the shape of a V with the wide side towards the caudal aspect (Figure 1, CP). There were at least 8 conical papillae on each side of the tongue, and the more lateral of these were longer (more than 1 mm in length) than those positioned more medially. There were frequent salivary gland channel openings on the upper surface of the radix and on the posterior half of the corpus (Figure 1, white arrows). A wide lingual sulcus was observed in the radix in the middle of the dorsal tongue surface (Figure 1, S). No laryngeal mons or laryngeal prominence was observed except for the lingual prominence (prominentia lingualis) in the region of the radix on the corpus.

SEM examination demonstrated a squamous epithelial layer with indistinct cell margins covering the upper surface of the tongue (Figures 2A and 2B). Parallel salivary gland secretory channels coursed beneath the upper surface of the tongue (Figures 2C and 2D, arrows) before opening onto the surface.

Where the ends of the channels opened onto the epithelium there were remarkably voluminous protuberances (Figure 2C, star). The largest openings, up to 190 μm , were located in the radix (Figures 2D and 2E, arrows), and the openings in the radix were narrower and usually had no protuberance, although they were more plentiful than in the corpus (Figure 2D).

Histological examination of the tongue revealed salivary glands to be simple tubuloalveolar glands, surrounded by a connective tissue capsule (Figure 3, CC) in the submucosa. They occurred in a small cluster in the corpus (Figure 3, ASG) but a large group was present close

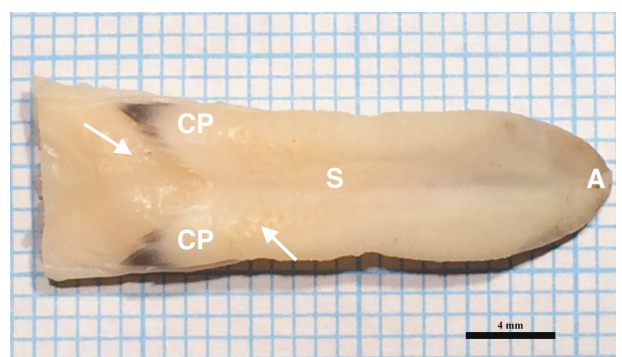


Figure 1. Macroscopic view of tongue. Apex (A), conical papillae (CP), openings of lingual salivary gland ducts (white arrows), groove (S).

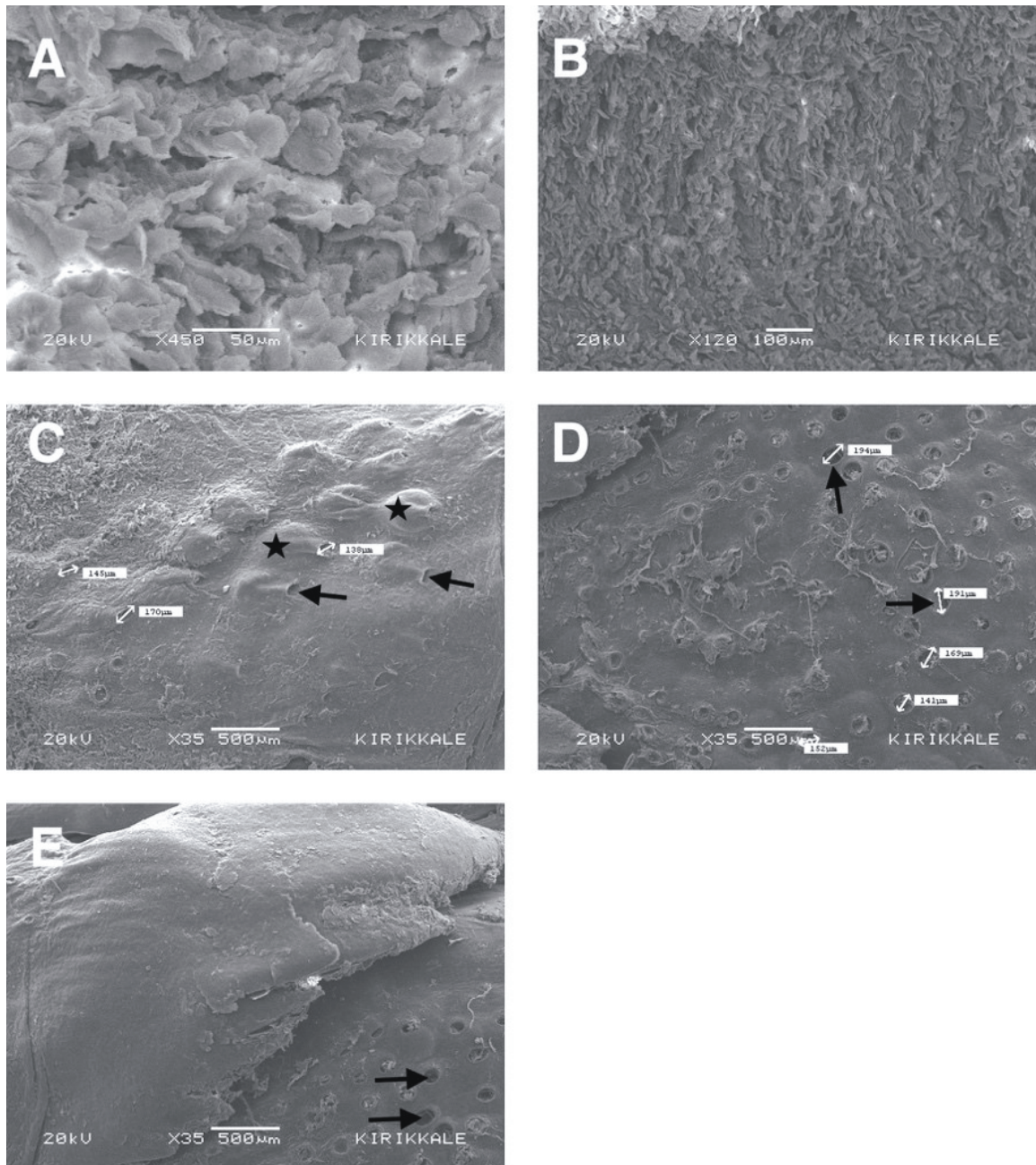


Figure 2. SEM appearance of buzzard tongue. SEM examination demonstrated a squamous epithelial layer with indistinct cell margins covering the upper surface of the tongue (A and B). Parallel salivary gland secretory channels coursed beneath the upper surface of the tongue (C and D, arrows) before opening onto the surface.

to the upper lingual surface in the radix linguae (Figure 3, PSG). The glands in the caudal group were underpinned by skeletal muscle fibers (Figure 3, M). The openings (Figure 3, O) of salivary gland ducts on the upper surface of the tongue (Figure 3, C), as well as duct enlargements (Figure 3, DE), were visualized.

Throughout the tongue the stratified keratinized epithelium on the ventral surface was thinner than that

on the upper surface. The epithelium covering the dorsal surface of the tongue (Figure 3, DEp) was thickest at the apex (400 μm) and the corpus (250 μm), thinning to about 100 μm in the posterior aspect of the corpus and in the radix. The thickness of epithelium on the ventral surface of the tongue was 55 μm (Figure 3, VEp). While the keratinization on the dorsal surface was 11 μm , it was 15 μm on the ventral surface of the tongue. The

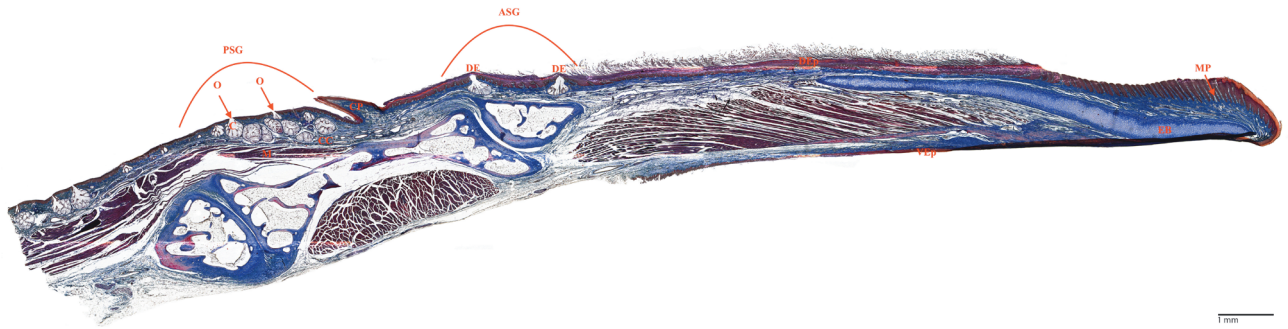


Figure 3. Reconstructed histological appearance of buzzard tongue using the virtual slice tool of Stereo Investigator under 2.5× objective (Crossman's modified triple staining, bar: 1 mm). Connective tissue capsule (CC), anterior lingual salivary glands (ASG), posterior lingual salivary glands (PSG), skeletal muscle fibers (M), openings of the salivary gland ducts (O), ducts of salivary glands (C), duct enlargements (DE), dorsal lingual epithelium (Dep), conical papilla (CP), microscopic papilla (MP), entoglossal bone (EB).

degree of keratinization on the ventral surface was 20% of epithelium thickness but it was highly variable on the dorsal surface of the tongue although always less than the ventral surface. The thickness of the epithelium covering transverse conical papillae (Figure 3, CP) was not different from those in the posterior aspect of the corpus and radix, and the degree of keratinization was also similar in these regions. It was observed that connective tissue extended into the epithelial tissue on the dorsal surface in the form of microscopic papillae (Figure 3, MP). These were small, short papillae (less than 100 μ m) in the corpus and radix, but they were longer in the apex due to increased epithelial thickness. The entoglossal bone (os entoglossum) (Figure 3, EB) extended as cartilage through the entire corpus and there were longitudinal skeletal muscles beneath the bone. No taste papillae or taste buds were found in the lingual epithelium.

Histochemical examination of lingual salivary glands revealed strong positive PAS staining (Figure 4A). Remarkable color loss was observed in the sections stained with PAS following diastase enzyme application, which converts glandular tissue glycogen into sugar such as maltose (Figure 4B).

The carbohydrate content of lingual salivary glands showed strong positive reactions with AB pH 1.0 and AB pH 2.5 and stained brilliant blue (Figures 4C and 4D). The brilliant blue color persisted with PAS staining after AB pH 1.0 and AB pH 2.5 treatment (Figures 4E and 4F), but completely disappeared in the sections stained with AB pH 2.5 when exposed to hyaluronidase enzyme. When salivary glands with AB pH 2.5 staining were treated with weak or moderate methylation procedures there was no color loss (Figures 4G and 4H), whereas color loss occurred in AB pH 2.5 sections exposed to strong methylation (Figure 4I). Absence of color loss with AB pH 2.5 after exposing the sections to sialidase enzyme digestion was observed (Figure 4J).

4. Discussion

The rounded oval tip of the tongue apex in long-legged buzzards does not reflect the shape of the lower beak, which tapers to the tip. A large lingual sulcus on the dorsal surface of the tongue and the transverse papillae present in a single layer were similar to those in white-tailed eagle (20) and goshawk (26). Although no lingual sulcus is found in falcons, kestrels (1), and owls (27,28), conical transverse papillae have been found in a quite wide area of the tongue. Therefore, it is thought that the line of conical papillae helps prevent food from returning to the anterior region.

The coefficient of variation of measurements (not including sex difference) was 6.82%. Therefore, no significant difference in terms of total tongue length is expected between females and males.

Iwasaki et al. (2) stated that extreme keratinization in the lingual epithelium has a tooth-like function in poultry to aid in plucking plants. The less extreme keratinization of the tongue surface in buzzards was attributed to the fact that, in this species, food does not stay long in the mouth and is swallowed without manipulation.

The localization and number of lingual salivary glands, forming two groups as anterior salivary glands (glandula salivarius anterior) in the corpus and posterior salivary glands (glandula salivarius posterior) at the tongue base, were consistent with literature reports (11,15,16). The openings of secretory channels of the lingual salivary glands in buzzards were found on the dorsal surface of the tongue's body where food contact is maximal, but these openings were not present on both sides of the radix as reported by Jackoviak and Godynicki (20) in white-tailed eagles. Protuberances/enlargements formed by the secretory channels of the salivary glands just beneath the surface epithelium have not previously been mentioned in the literature (Figure 2C, star) (1,20,21,27,28). These enlargements may represent small sacs trapping saliva

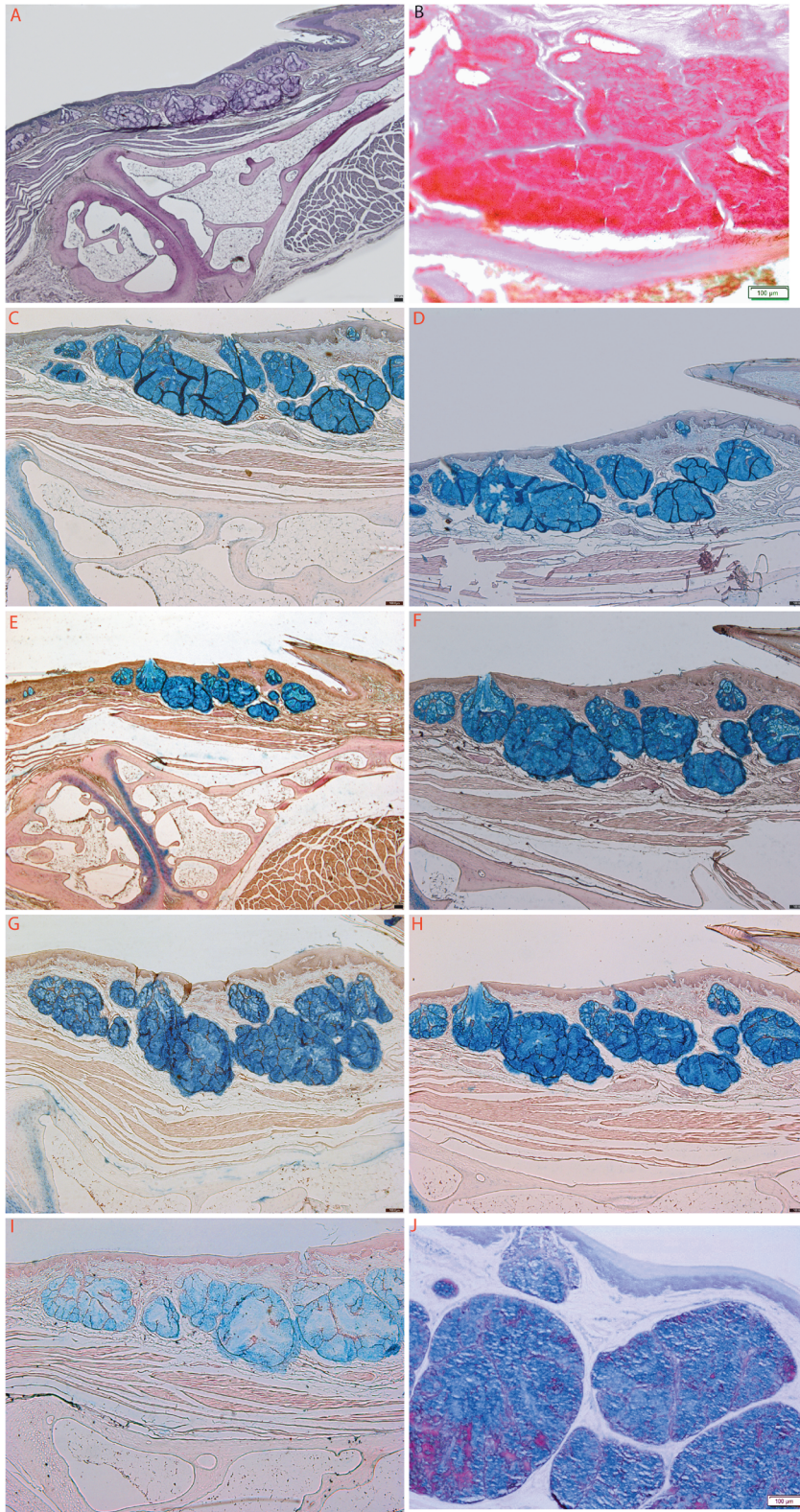


Figure 4. Histochemical reactions of lingual salivary glands in buzzard. PAS-positive (A), diastase-sensitive (B), AB pH 1.0 (C), AB pH 2.5 (D), AB pH 1.0-PAS (E), AB pH 2.5-PAS (F), weak methylation-AB pH 1.0 (G), middle methylation-AB pH 2.5 (H), strong methylation-AB pH 2.5 (I), sialidase enzyme digestion-AB pH 2.5 (J).

in this region to facilitate more rapid coating of the food bolus.

It has been reported that lingual salivary glands and secretory channels are heterogeneous in quails (19). The results of the present study with regard to the secretions of the anterior and posterior lingual salivary glands are in accordance with the general statements for canary, poultry, and exotic poultry, such that the salivary glands do not produce serous secretions but rather a secretion rich in carbohydrates and that the composition of glandular cells is homogeneous (14,15,29).

In the present study, macroscopic, histological, and SEM examinations in buzzards failed to show any structure that could represent taste papilla. Furthermore, no types of cell were detected in the submucosa of the tongue that could help determine the position of food on the tongue and subsequent direction of food passage as stated by Jackowiak et al. (3).

The strong positive reaction with PAS led us to conclude that lingual salivary glands have high glycogen content. Brilliant blue coloring in the sections stained with AB pH 1.0 and AB pH 2.5 indicated that salivary glands contain sialidase-sensitive acidic sialomucins and/or hyaluronidase-resistant sulfomucins. Persistence of this brilliant blue color after AB pH 1.0 staining followed by PAS shows that the carbohydrate content of salivary gland contains sialidase-sensitive sialomucin. The negative results obtained with AB pH 2.5 staining after hyaluronidase enzyme digestion indicate that the carbohydrate content of glands comprises substantial

amounts of strongly sulfated connective tissue mucins. The absence of color loss in weak and moderate methylation procedures, suggests that these were sulfate-containing carbohydrates rather than carboxyl group-containing carbohydrates and these sulfomucins were acid-resistant. The homogeneous staining in the anterior and posterior lingual salivary glands and in the cells of the same gland group suggests that the carbohydrate contents of these structures are the same.

The absence of salivary glands in the apex of the tongue and the intense clustering of lingual salivary glands with numerous channel openings at the tongue base, as well as the absence of taste papillae in the lingual epithelium and absence of a specific structure for touch and thermal sensations, suggest that the primary function of the tongue in long-legged buzzards is to allow contact of the food with saliva and to rapidly move the food bolus backwards.

The carbohydrate contents of the lingual salivary glands in buzzards are very similar to those in the canary (14), although these two species belong to different taxonomic orders and feed in different ways. Acidic secretions of lingual salivary glands facilitate the swallowing process and also probably have an antimicrobial effect. These secretions may also affect the digestive process in the distal parts of the alimentary tract.

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