Functional morphology of the tongue in the domestic turkey (*Meleagris gallopavo gallopavo var. domesticus*)

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ABSTRACT Nowadays, microstructural and ultrastructural analysis of organs of the avian beak cavity points to new aspects of adaptation to food intake through the various feeding groups. These data should undoubtedly be considered in the time of mass production of compound feed in poultry, when many studies analyze the optimal size of food particles and their doses. Galliformes possess complex mechanisms of food collection and transport in the beak cavity. They collect food by pecking and transport food by using catch-and-throw and slide-and-glue mechanisms. The aim of current research is to conduct functional analysis of the tongue in poultry such as domestic turkey in context of type of food, method of food intake, and transport to the esophagus. The study involves observations of macroscopic and microscopic structures of the tongue mucosa by light microscopy and scanning electron microscopy techniques with histochemical analysis of lingual glands. The obtained results showed that the tongue in domestic turkey fills two-thirds of the beak cavity. The lingual structure responsible for pecking is a rigid plate called lingual nail that works similar to a shovel to collect food. The median groove presented on surface of the tongue indicated path of food transport. The conical papillae on border between the lingual body and root are responsible for the last stage of food transport, while the papillae on the sides of root stabilize the path of food transport. For the first time, the presence of 2 types of cornified mucosal epithelia, orthokeratinized and parakeratinized epithelium, was presented. The analysis of occurrence of complex tubular lingual glands indicates production of mucous secretions composed of neutral mucopolysaccharides, with addition of sialomucins and sulfomucins. Mucous secretions moisturize surface of the tongue, thus facilitating the transport of dry food. The presence of sulfur mucopolysaccharides responds to protective function. To sum up, the tongue in domestic turkey is adapted to collect fine or coarse ground feed in form of mash or pellets through pecking and its transport to the esophagus using the slide-and-glue and throw-and-catch mechanisms.

Key words: turkey, tongue, lingual nail, orthokeratinized epithelium, parakeratinized epithelium

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INTRODUCTION

The macroscopic and microscopic structure of a bird tongue is the object of interest of many researchers owing to the wide morphologic diversity of the lingual mucosa structures resulting from the type of food intake and the complex mechanisms of food collection and transport in the beak cavity.

In the current literature, the most attention has been devoted to the structure of the tongue of wildlife and domesticated representatives of Galliformes (chicken – Iwasaki and Kobayashi, 1986; Homberger and Meyers, 1989; common quail – Parchami et al., 2010; red jungle fowl – Kadhim et al., 2011; Uppal et al., 2014; Chukar partridge – Erdogan et al., 2012; Japanese quail – Pourlis, 2014; Guinea fowl – Sundaram et al., 2015).

Macroscopic observations showed that the tongue of these birds has a triangular shape and is characterized by the presence of conical papillae at the border of the lingual body and root (Iwasaki and Kobayashi, 1986; Homberger and Meyers, 1989; Parchami et al., 2010; Kadhim et al., 2011; Erdogan et al., 2012; Pourlis, 2014; Uppal et al., 2014; Sundaram et al., 2015). Observations in some representatives of the Galliformes established the presence of median groove, lingual nail, an additional 2–3 conical papillae at the edges of the root of the tongue and filiform papillae on the dorsal surface of the tongue (Iwasaki and Kobayashi, 1986; Homberger

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and Meyers, 1989; Parchami et al., 2010; Erdogan et al., 2012; Pourlis, 2014; Uppal et al., 2014).

Microscopic examination of the lingual mucosa in Galliformes concerning 2 functionally important structures, that are the epithelium covering of the tongue and lingual glands located in the lamina propria, is less often undertaken.

As per some researchers, only the dorsal surface of the apex of the tongue is covered with the keratinized epithelium (Parchami et al., 2010; Kadhim et al., 2011; Uppal et al., 2014), and as per another, this epithelium occurs on both dorsal and ventral surface of the tongue (Iwasaki and Kobayashi, 1986; Erdogan et al., 2012).

Contrary to these studies, the new publications delivery the data about coexistence of 2 types of keratinized/cornified multilayered mucosal epithelia in birds, that are orthokeratinized and parakeratinized epithelium (Iwasaki et al., 1997; Jackowiak and Godynicki, 2005; Jackowiak et al., 2010, 2011; Skieresz-Szewczyk and Jackowiak, 2016; Skieresz-Szewczyk et al., 2014, 2017, 2019). These epithelia cover different areas of dorsal surface of the tongue and have a close relation with the mechanism of feeding and intraoral transport of collected food. The orthokeratinized and parakeratinized epithelia differ in height, presence/absence of cell nuclei in the cornified layer, and content of alfa and beta keratins (Jackowiak and Godynicki, 2005; Jackowiak et al., 2010, 2011; Skieresz-Szewczyk and Jackowiak, 2016; Skieresz-Szewczyk et al., 2017, 2019).

Research to date, regarding the lingual glands in Galliformes, indicates the presence of typical anterior and posterior lingual glands in the lamina propria of the lingual mucosa (Iwasaki and Kobayashi, 1986; Homberger and Meyers, 1989; Gargulio et al., 1991; Kadhim et al., 2011; Erdogan et al., 2012; Pourlis, 2014; Uppal et al., 2014). Whereby, 2 research teams, Gargulio et al. (1991) and Kadhim et al. (2011), describe that the anterior lingual glands in the domestic chicken and red jungle fowl are divided into 2 groups: lateral and medial glands.

Conducting research on the structures of the oral cavity in Galliformes, it should be mentioned that this group of birds is characterized with complex mechanisms for food collection and food transport, such as pecking, slide-and-glue mechanism and throw-and-catch mechanism (Zweers et al., 1994). In previous morphologic studies, no explanation has been made to clarify to what extent the type of food, the way of food collection, and its transport in the beak cavity structured the lingual mucosa in Galliformes. It should also be noted that some of the results were obtained only on the basis of macroscopic observations, and in the case of microscopic examinations, it was often limited to the tissue samples collected only from 1 area of the tongue, namely the lingual body.

During analysis of the morphologic structure of the tongue in birds, attention should also be paid to the type of food consumed. Poultry are fed fine or coarse ground feed in form of mash or pellets. Many researchers believe that feed particle size influences feed intake, feed conversion ratio, and BW gain (Lott et al., 1992; Kilburn and Edwards, 2001; Amerah et al., 2008). At the same time, recent research indicates that not only grain size but also additional supplementation with amylase and nonstarch polysaccharide improves feed conversion ratio and the nutritive value (Kaczmarek et al., 2014; Yin et al., 2018).

Current research aims for the first time to perform a comprehensive analysis of the microscopic and macroscopic structure of the lingual mucosa in poultry thoroughly in Galliformes on an example of the domestic turkey. Observations will include analysis of the macroscopic structure of the tongue, the distribution of each types of mucosal epithelium, and its structural features as well as the distribution and microstructure of the lingual glands along with histochemical analysis of gland secretions. The obtained results will be analyzed in the terms of adaptation of the lingual mucosa in the domestic turkey to the type of food consumed, the method of food intake by pecking, and transport of food to the esophagus.

MATERIALS AND METHODS

The research material consisted of 9 tongues of adult male domestic turkey, which were slaughtered from local breeder (average BW 5.5–6 kg). The study was conducted in accordance with the guidelines set out by the Ethics Commission at the Poznan University of Life Sciences and the national guidelines of Poland.

The tongues after dissection were washed in saline and fixed. Three tongues were collected for light microscopy analysis and fixed in Bouin solution. Six tongues were taken for scanning electron microscopy studies and placed in 4% buffered formalin. The tongues fixed in 4% buffered formalin were photographed using a digital camera Nicon DS 126311 (Sony) and a stereo microscope SteREO Discovery v.20 (Zeiss). Tissue samples were taken from each tongue from the same locations, such as lingual apex and body, conical papillae, and root of the tongue.

Tissue samples for light microscopy analysis were submitted for a standard procedure for the preparation of paraplast blocks, which were then cut to a thickness of 4.5–5 μm. Histologic sections were stained by Masson-Goldner trichrome (Romeis, 1989) and histochemical staining (Romeis, 1989), that is periodic acid-Schiff (PAS) reaction, AB (alcian blue) (pH = 2.5), alcian-blue-periodic acid-Schiff (AB-PAS) (pH = 2.5), and high iron diamine-alcian blue (HID-AB) (pH = 2.5). The microscopic observations of the histologic sections were made using an Axioscope 2 plus light microscope (Zeiss, Germany). Photomicrographs were used on 5 histologic sections. On each histologic section, 4 measurements were made to determine 20 measurements of the height of the epithelium, its keratinized layer, and diameter of the lingual glands using a MultiScan computer morphometric system (ver. 10.2; CSS, Warsaw, Poland). Histologic measurements were statistically analyzed using Statistica (ver. 12.5; StatSoft, Poland) software. For each morphologic feature, the following parameters were calculated: the mean value, the minimum value, and the maximum value.

Three of the 6 tongues fixed in 4% buffered formalin were proceeded to 7-day maceration in 10% NaOH at room temperature to remove the epithelium and to show the three-dimensional structure of the connective tissue in the lamina propria of the mucosa. After maceration, the tissue samples were washed in several changes of the distilled water. Macerated and nonmacerated tissue samples were dehydrated in a series of ethanol (70–96%) and acetone (96% – abs) and dried at critical point using liquid CO_2 (Critical Point Dryer EM CPD300; Leica, Germany). All specimens were mounted on aluminum stubs covered with carbon tabs, sputtered with gold (Sputter Coater S 150B; Edwards), and observed under the LEO 435 VP (Zeiss) at an accelerating voltage of 10–15 kV.

RESULTS

Macroscopic Observations

The tongue in the domestic turkey has a triangular shape and is attached to the bottom of the beak cavity with a lingual frenulum. The tongue fills the beak cavity except for the free space in the rostral part of the bill (Figure 1A). On the tongue, the apex, body, and root of the tongue are distinguished (Figure 1A).

The total length of the tongue is 4.7 cm. The length of individual parts of the tongue is apex 0.6 cm, body 2.9 cm, and root 1.2 cm, whereas the width of the tongue on the apex is 0.5 cm, on the body 1.4 cm, and on the root 1.1 cm.

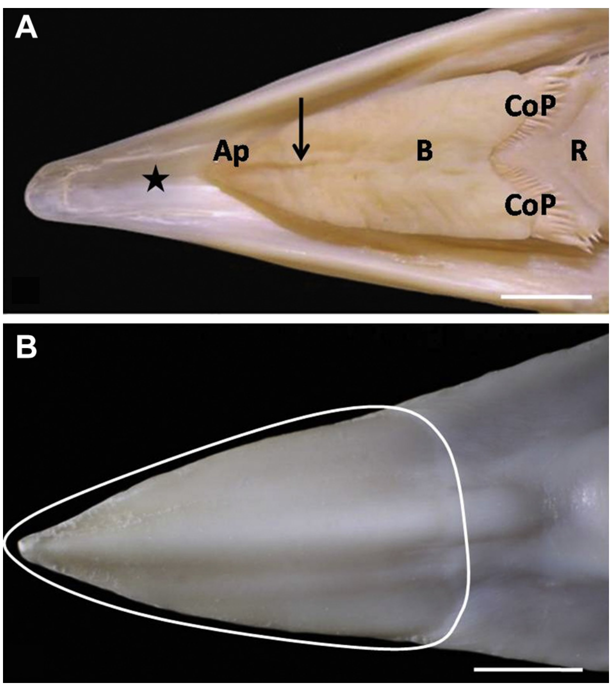

Figure 1. (A) Dorsal view of the tongue and bill in the domestic turkey. Arrow points the median groove. Asterisk shows the free part of the rostral part of the bill. Scale bar = 1 cm, (B) Ventral view of the lingual apex. Solid line marks the plate of the lingual nail. Scale car = 0.5 cm. Abbreviations: Ap, apex; B, body; CoP, conical papillae; R, root of the tongue.

The dorsal surface of the lingual mucosa on the apex and body is smooth and free of lingual papillae (Figure 1A). A median groove was observed in the medial part of the apex and body of the tongue, which divides the tongue into 2 equal-sized parts (Figure 1A). The posterior edge of the body take a shape of the letter V. Along this edge, symmetrically on the right and left sides from the median groove, 14–15 conical papillae are arranged individually (Figures 1A and 2C). The height of the conical papillae increases toward the lateral edge of the lingual body and ranges from 630.5 μm to 1,709.5 μm. The width of the base of the papillae is on average from 220.5 μm to 401.0 μm. On the posterolateral surface of the body of the tongue, there are 2 conical papillae directed toward the root of the tongue (Figure 2A). Observations of the both lateral surfaces of the lingual body showed the presence of about 11–12 openings of the lingual glands, which in the anterior part of the body are arranged linearly and in the posterior part are arranged in 2 rows (Figure 2A).

On the ventral surface of the lingual apex and the anterior part of the lingual body, there is a triangular plate of epithelium called the lingual nail, whose length is 2.1 cm and width is 1.4 cm (Figure 1B). This structure is white intravitally.

On the medial part of smooth surface of the root of the tongue, 8–11 openings of the lingual glands were observed Figure 2E). On the lateral edges of the root of the tongue, there were 3 conical papillae directed toward the posterior part of the tongue (Figure 2A). The height of these papillae ranges from 1,270 μm to 2,794 μm, and the width of the base of the papillae on average is from 482 μm to 1,121 μm.

Microscopic Observations

In the mucosa of the apex and body of the tongue is the presence of paraglossal cartilage of the hyoid apparatus, which is surrounded in the region of the lingual apex by yellow adipose tissue and in the body by the lingual glands (Figures 3A and 3B).

The lingual mucosa is covered with multilayered epithelium. Depending on the part of the tongue, 3 types of epithelium are distinguished, that is, parakeratinized epithelium, orthokeratinized epithelium, and nonkeratinized epithelium (Figures 2B, 3A, and 3B).

A multilayered parakeratinized epithelium is present on the dorsal surface of the apex and body of the tongue. This epithelium is composed of basal, intermediate, and cornified layers (Figure 4A and 4D). The cells in the basal layer are cylindrical in shape and have oval cell nuclei. The cells in the intermediate layer are polygonal, and their oval cell nuclei are arranged horizontally. The cells of the cornified layer are strongly flattened, elongated, and characterized by the presence of cell nuclei (Figure 5A). The cytoplasm of single cells of the cornified layer is colored red by Masson-Goldner. The cells of the

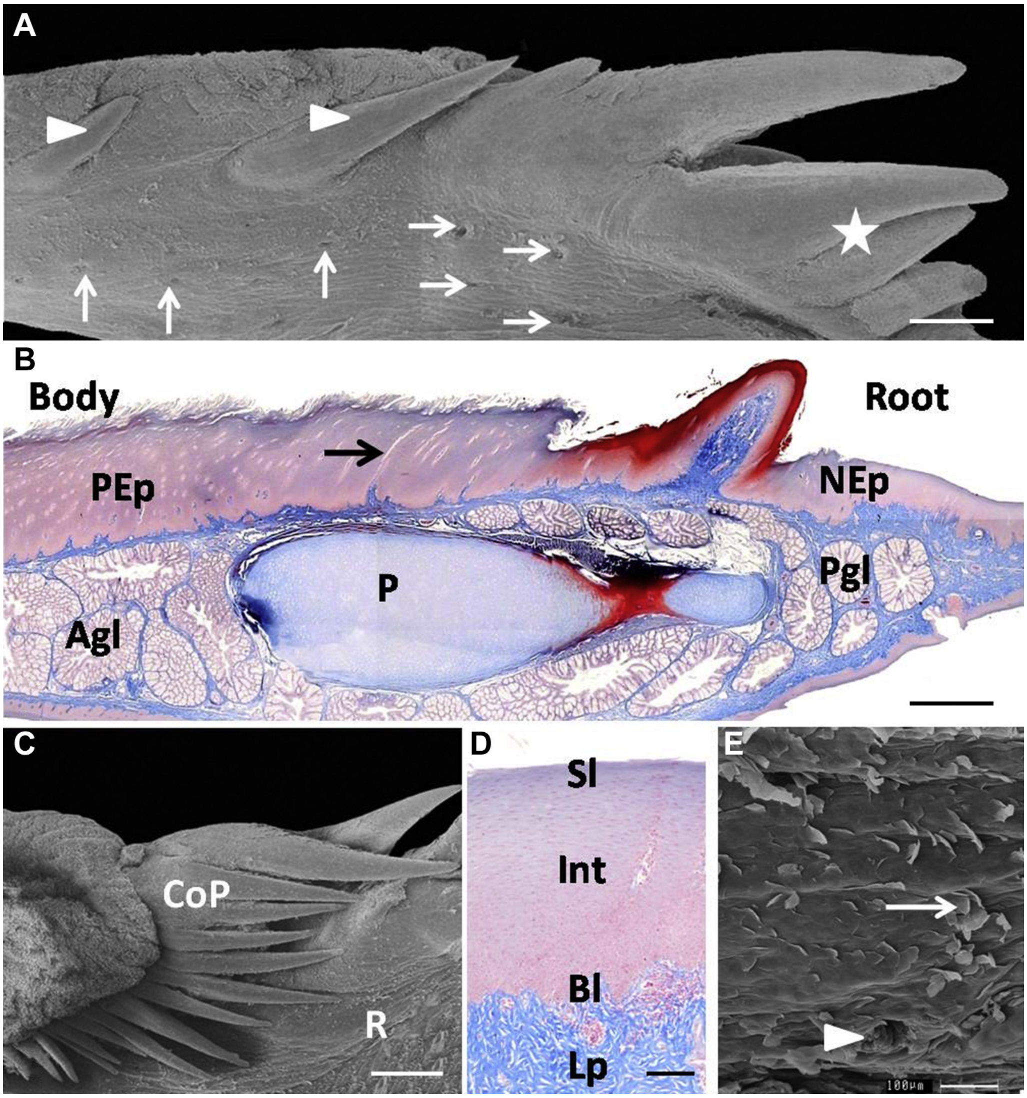

Figure 2. (A) Lateral view of the body and root of the tongue. Arrowheads show the papillae located on the posterolateral surface of the body of the tongue. Arrows point the openings of the anterior lingual glands. Asterisk marks the conical papillae located on the lateral sides of the root of the tongue. Scale bar = 1 mm. (B) Cross section of the lateral side of the lingual body and root of the tongue. Scale bar = 1 mm. (C) Dorsal view of the conical papillae and root of the tongue. Scale bar = 1 mm. (D) Cross section of the nonkeratinized epithelium of the root of the tongue. Scale bar = 100 μm. (E) Surface of the root of the tongue. Arrow marks single exfoliated superficial cells. Arrowhead points glandular opening of the posterior lingual glands. Abbreviations: Agl, anterior lingual glands; Bl, basal layer; CoP, conical papillae; Int, intermediate layer; Lp, lamina propria; Nep, nonkeratinized epithelium; P, paraglossal cartilage; Pep, parakeratinized epithelium; Pgl, posterior lingual glands; R, root of the tongue; Sl, superficial layer.

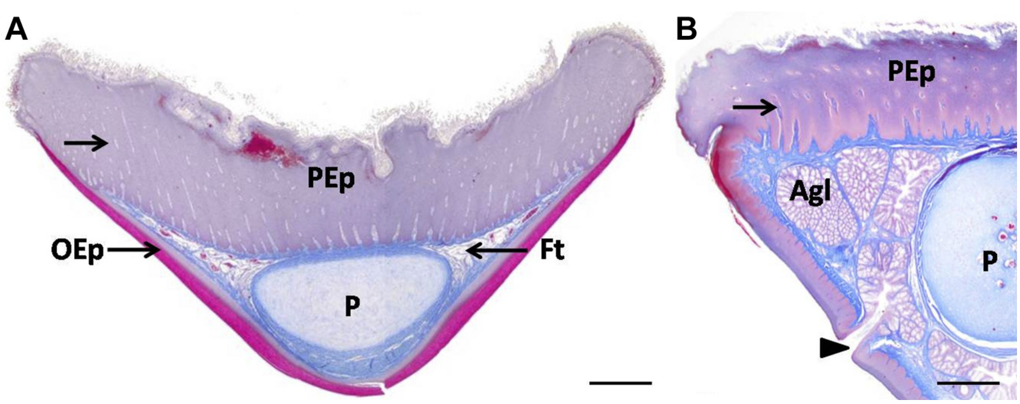

Figure 3. (A) Cross section of the lingual apex. Arrow points the connective tissue core. Scale bar = 1 mm. (B) Cross section of the lingual body. Arrow points the connective tissue core. Arrowhead shows the glandular openings of the anterior lingual glands. Scale bar = 1 mm. Abbreviations: Agl, anterior lingual glands; Ft, fat tissue; OEp, orthokeratinized epithelium; P, paraglossal cartilage; Pep, parakeratinized epithelium.

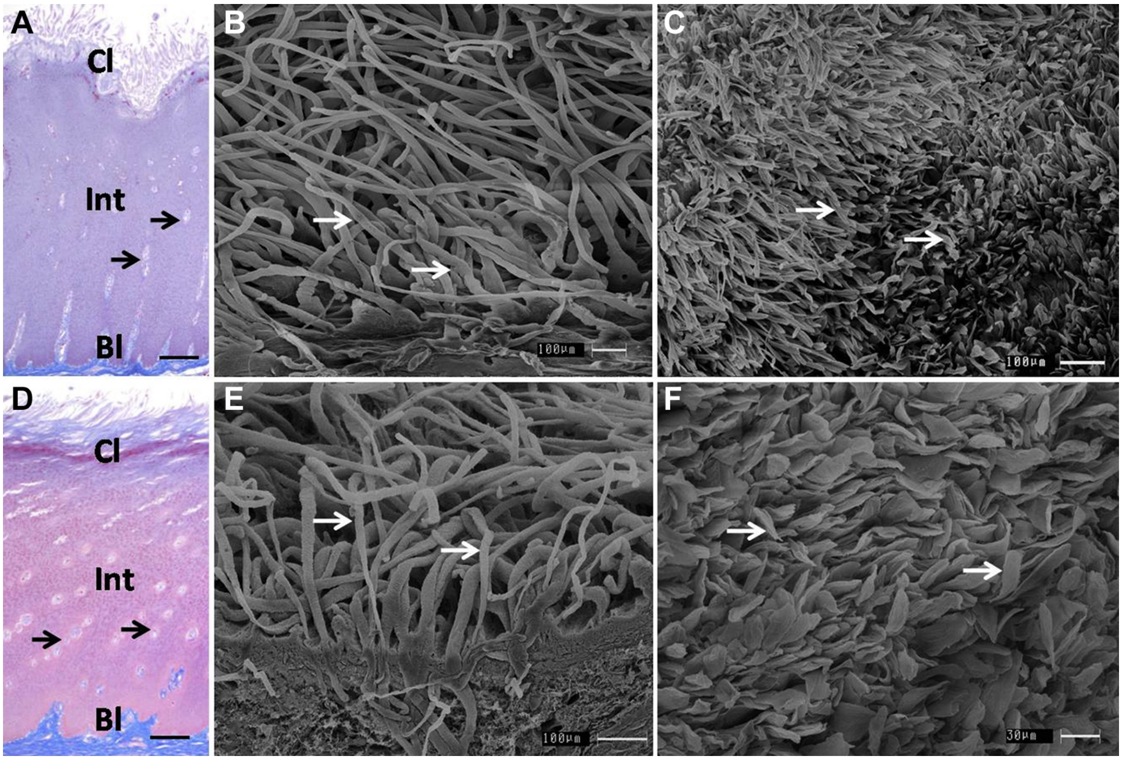

Figure 4. (A) Cross section of the parakeratinized epithelium of the dorsal surface of the apex of the tongue. Arrows show the connective tissue core. Scale bar = 200 µm. (B) Higher magnification of the connective tissue cores (arrows) of the parakeratinized epithelium of the dorsal surface of the lingual apex. (C) Surface of the parakeratinized epithelium of the dorsal surface of the apex of the tongue. Arrows point the elongated cornified cells which desquamate perpendicularly. (D) Cross section of the parakeratinized epithelium of the body of the tongue. Arrow shows the connective tissue core. Scale bar = 200 µm. (E) Higher magnification of the connective tissue cores (arrows) of the parakeratinized epithelium of the lingual body. (F) Surface of the parakeratinized epithelium of the body of the tongue. Arrows point the elongated cornified cells which desquamate perpendicularly. Abbreviations: Bl, basal layer; Cl, cornified layer; Int, intermediate layer.

cornified layer of the parakeratinized epithelium of the apex and the anterior part of the body of the tongue are massively exfoliated in the form of single, vertically arranged scales (Figures 4C, 4F and 5B). In the posterior part of the body of the tongue, elongated and flattened cells of the cornified layer of the parakeratinized epithelium exfoliate in the posterior direction (Figure 2B).

The average height of the epithelium on the dorsal surface of the apex of the tongue is 1,486.4 µm, and the height of its cornified layer is 237.2 µm (Table 1). The height of the epithelium on the anterior part of the body of the tongue is on average 1,241.5 µm and on the posterior part of the body of the tongue is 1,475.1 µm. The height of the cornified layer on the anterior and posterior part of the lingual body reaches 135.7 µm and 114.8 µm in height, respectively (Table 1).

The lamina propria of the lingual mucosa penetrates the parakeratinized epithelium of the apex and body of the tongue forming connective tissue cores (Figures 2B, 3A, 3B, 4A, and 4D). These connective tissue cores in the epithelium of the apex and anterior part of the body of the tongue are directed to the lateral edges of the tongue, while in the posterior part of the body are arranged to the root of the tongue (Figures 2B, 3A, 3B, 4A, and 4D). Scanning electron microscopy observations of macerated tissue samples have shown that connective

Table 1. The morphometry of the mucosal epithelium and its cornified layer in the particular part of the tongue in the domestic turkey.

Part of the tongue	Height of the mucosal epithelium (µm) X Min – Max	Height of the cornified layer (µm) X Min – Max
Apex of the tongue – dorsal surface –parakeratinized epithelium	1,486.4 1,158.8–2,038.4	237.2 220.6–257.4
Apex of the tongue – ventral surface –orthokeratinized epithelium	261.4 256.5–267.5	141.0 135.8–146.0
Body of the tongue – anterior part –parakeratinized epithelium	1,241.5 1,096.1–373.2	135.7 96.1–78.0
Body of the tongue – posterior part –parakeratinized epithelium	1,475.1 1,285.0–1,612.2	114.8 112.4–125.2
Root of the tongue – nonkeratinized epithelium	452.8 402.4–480.7	-

Abbreviations: Max, maximum value; Min, minimum value; X, mean value.

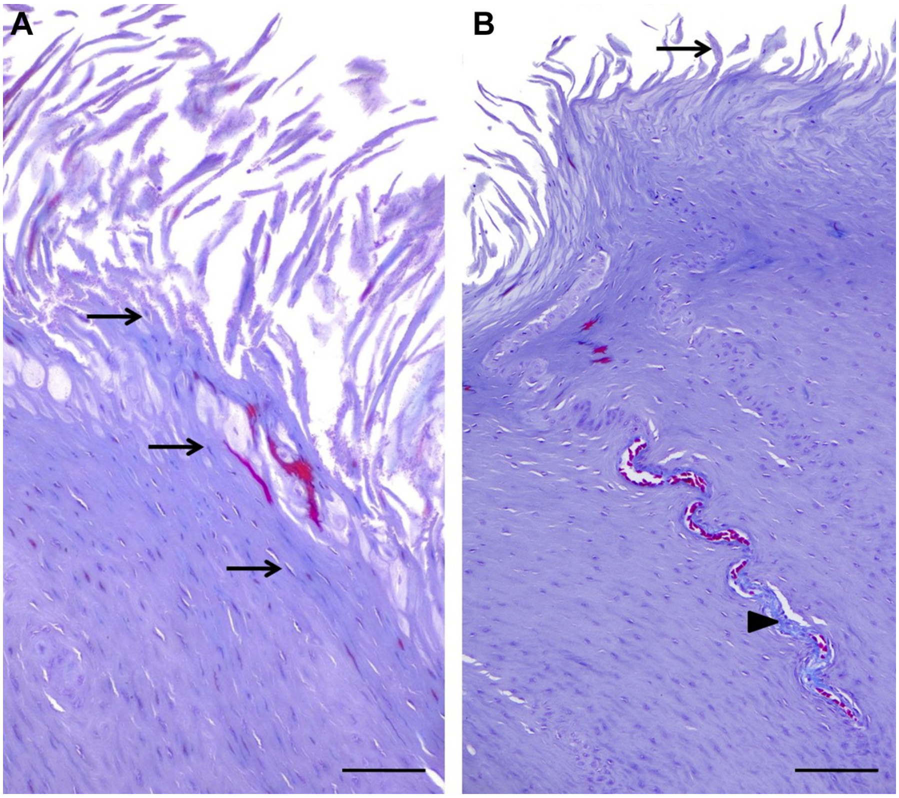

Figure 5. (A) Higher magnification of the cornified layer of the parakeratinized epithelium on the dorsal surface of the apex. Arrows point cell nuclei. Scale bar=100 μm. (B) Higher magnification of the cornified layer of the parakeratinized epithelium on the body of the tongue. Arrow points the elongated cornified cells which desquamate perpendicularly. Arrowhead shows connective tissue core with capillary blood vessel. Scale bar=100 μm.

tissue cores are in the form of single structures, which are twisted in the apical part (Figures 4B and 4E). The connective tissue cores contain convoluted capillaries (Figure 5B).

The height of the connective tissue cores in the parakeratinized epithelium of the apex, anterior, and posterior parts of the body of the tongue is 1,137.7 μm, 821.7 μm, and 1,274.5 μm, respectively. The width of connective tissue cores varies in the cross section and at the base of the papillae ranges from 28.9 μm to 42.6 μm, and at the apical part, it averages 16.2 μm.

The multilayered orthokeratinized epithelium occurs on the ventral surface of the apex of the tongue and covers the conical papillae (Figures 2B and 3A). This epithelium consists of basal, intermediate, and cornified layers (Figure 6A). The cells in the basal layer are cylindrical in shape and have oval cell nuclei. Polygonal cells of the intermediate layer have oval cell nuclei horizontally arranged. The cells of the cornified layer are strongly flattened and devoid of cell nuclei, and their cytoplasm, as a result of Masson-Goldner staining, is colored intensively red (Figure 6A). The cells of the cornified layer peel off as single scales (Figure 6B). The lamina propria of the tongue mucosa forms low connective tissue cores in the epithelium of the orthokeratinized epithelium, whose height is about 15.2 μm and width is constant over its entire length and averages 4.8 μm.

The average height of the epithelium on the ventral surface of the apex of the tongue is 261.4 μm and its cornified layer, which forms plate of the lingual nail, is 141.0 μm (Table 1). The orthokeratinized epithelium of the conical papillae reaches 280.5 μm in height and the height of the cornified layer on average is 117.8 μm.

The multilayered noncornified epithelium covers the lingual mucosa of the root (Figure 2B). This epithelium is composed of basal, intermediate and superficial layers (Figure 2D). The basal layer consists of cylindrical cells with oval cell nuclei. The cells in the intermediate layer are polygonal, and their oval cell nuclei are arranged horizontally. The flat cells with flat cell nuclei of the superficial layer peel off individually (Figures 2D and 2E). The average height of the noncornified epithelium is 452.8 μm (Table 1).

Microscopic observations of the cross section of the body and root of the tongue revealed the presence of the anterior and posterior lingual glands in the lamina propria of mucosa. The anterior lingual glands are located on the sides and under the paraglossal cartilage over the whole area of the lingual body (Figures 2B and 3B). The posterior lingual glands are present in the lamina propria of mucosa just below the conical papillae and on the entire dorsal surface of the root of the tongue (Figure 2B). The secretory units of the lingual glands are complex; tubular glands are arranged in packets, surrounded by thin bundles of loose connective tissue (Figures 2B, 3B, 7A and 7B). The secretory units of the lingual glands pass to the collective ducts and then to the short and wide excretory units, whose diameter in the anterior

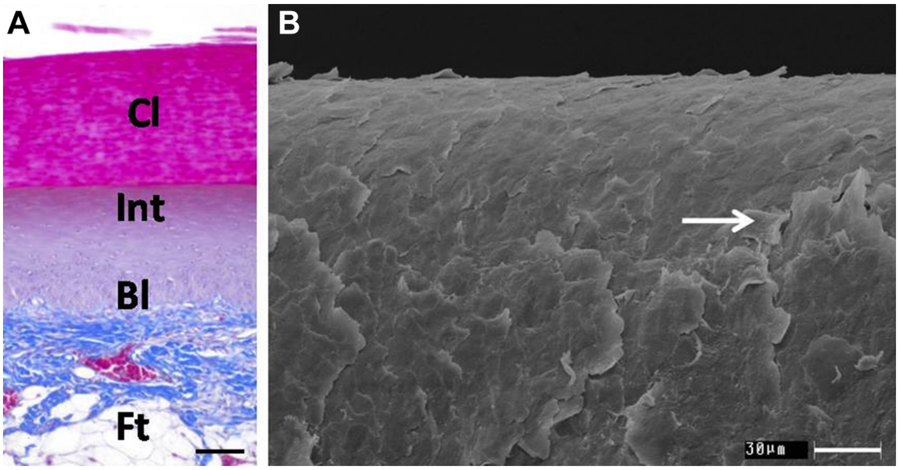

Figure 6. (A) Cross section of the orthokeratinized epithelium of the ventral surface of the apex of the tongue. Scale bar = 50 µm. (B) Surface of the orthokeratinized epithelium of the ventral surface of the apex of the tongue. Arrow points single desquamate cornified cell. Abbreviations: Bl, basal layer; Cl, cornified layer; Ft, yellow fat tissue; Int, intermediate layer

lingual glands is on average 126.5 µm and in the posterior lingual glands 72.0 µm. The mean value of the diameter of a single secretory units of the anterior lingual glands is 48.9 µm and of the posterior lingual glands is 66.5 µm. The packet of the anterior lingual glands has an average diameter of 766.0 µm and the posterior lingual glands has about 585.9 µm.

Histochemical PAS staining has shown a positive staining reaction in cells of all the secretory units of the anterior and posterior lingual glands (Figures 8A and 8B; Table 2). Whereby, in both types of glands, some cells of secretory units within the gland packet and the epithelium lining, the collective ducts are characterized by a stronger PAS staining reaction (Figures 8A and 8B).

The results of histochemical AB (pH=2.5) staining were different in the anterior and posterior lingual glands. In the area of the anterior lingual glands, there are single secretory units characterized by a blue staining reaction (Figure 8C; Table 2). The remaining secretory units did not stain blue, or only single epithelial cells of the secretory units showed a weak or strong staining reaction. In the posterior lingual glands, all secretory units in the gland packet showed a positive staining reaction (Figure 8D; Table 2). The epithelium lining of the collective ducts of the posterior lingual glands was not stained.

Histochemical staining of AB-PAS (pH=2.5) of the anterior lingual glands showed the presence of dark blue staining in single secretory units (Figure 8E; Table 2). In some secretory units, only single cells stained dark blue were present. The remaining cells in the secretory units and in the epithelium lining, the collective ducts were stained purple. In the posterior lingual glands, all secretory units were dark blue in color (Figure 8F; Table 2). At the same time, the cells of the epithelium lining of the collective ducts were purple colored.

As a result of HID-AB staining (pH=2.5) within the packet of the anterior lingual gland, single secretory units stained brown and secretory units in which only single cells stained brown were observed (Figure 8G; Table 2). In the posterior lingual glands, all secretory units are colored brown (Figure 8H; Table 2). At the same time, it was observed that within individual secretory units, there are secretory cells not colored brown.

DISCUSSION

The main factors influencing the wide morphologic diversity of the structure of the bird's tongue are the type of food and the way of food collection. Harrison (1964) distinguished 3 groups of tongues with special structural

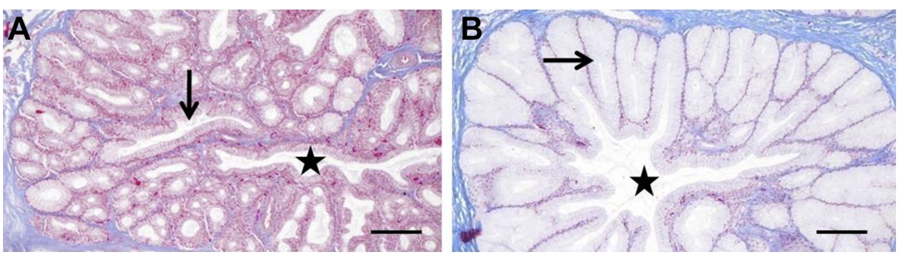

Figure 7. (A) Cross section of the anterior lingual glands. Arrow points the tubular secretory units. Asterisk shows the collecting duct. Scale bar = 100 µm. (B) Cross section of the posterior lingual glands. Arrow points the tubular secretory units. Asterisk shows the collecting duct. Scale bar = 100 µm.

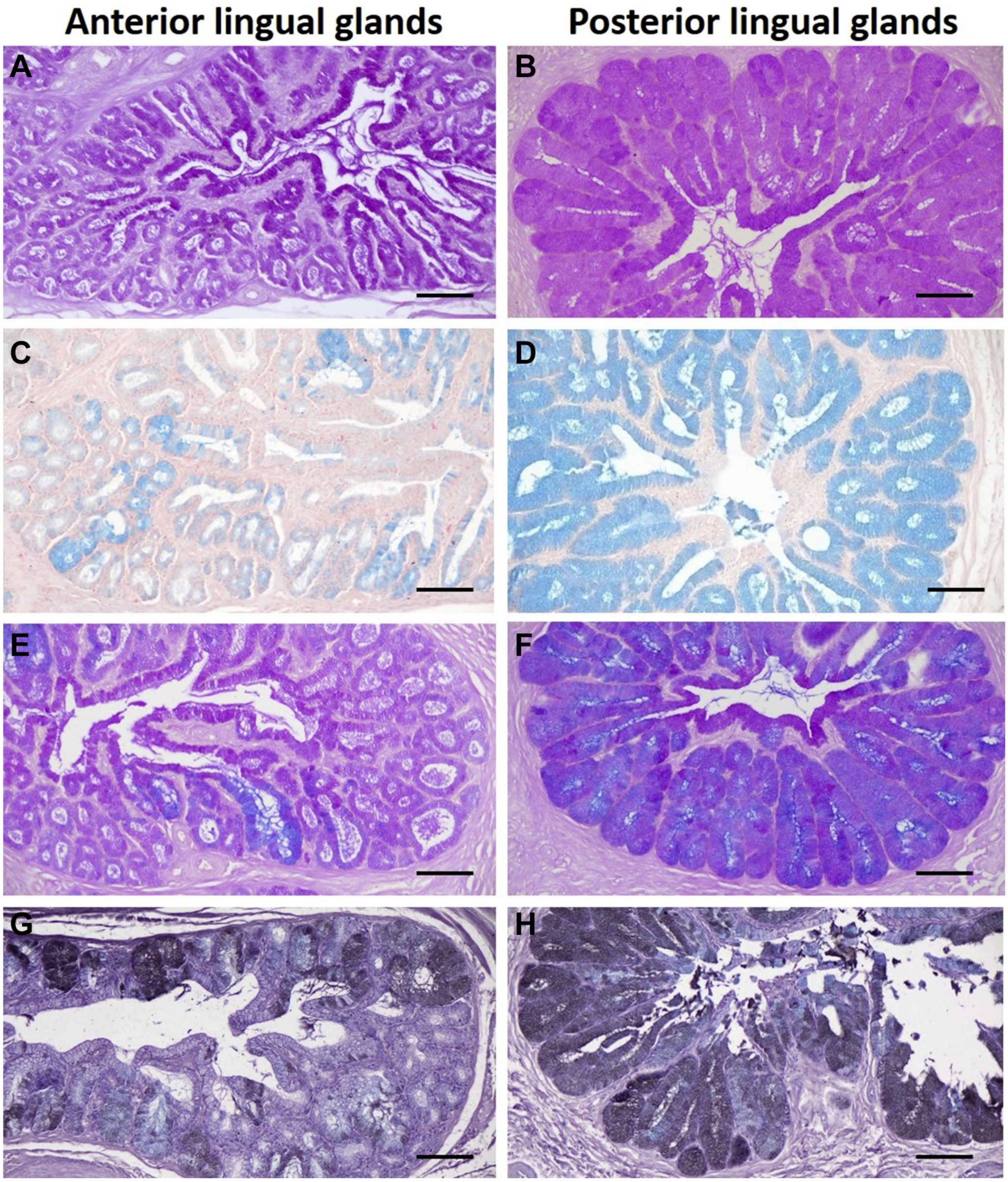

Figure 8. (A) PAS reaction. Scale bar = 100 μm. (B) PAS reaction. Scale bar = 100 μm. (C) AB (pH=2.5) reaction. Scale bar = 100 μm. (D) AB (pH=2.5) reaction. Scale bar = 100 μm. (E) AB-PAS (pH=2.5) reaction. Scale bar = 100 μm. (F) PAS-AB (pH=2.5) reaction. Scale bar = 100 μm. (G) HID-AB (pH=2.5) reaction. Scale bar = 100 μm. (H) HID-AB (pH=2.5) reaction. Scale bar = 100 μm.

adaptations in relation to their functions. The first group composes elongated and narrow tongues, which extend from oral cavity thanks to well-established hyoid apparatus. The second group consists of tongues with numerous lingual papillae, present on the sides of the tongue or covering the entire dorsal surface of the tongue, as well as tongue with well-established intrinsic lingual muscles. The third group is made up of tongues characterized by a deep set in the oral cavity and low mobility.

Table 2. Results of the histochemical staining PAS, AB, AB/PAS, and HID/AB of the lingual glands.

Type of lingual glands	PAS	AB	AB/PAS	HID/AB
Anterior lingual glands	+++	+	+	+
Posterior lingual glands	+++	+++	+++	++

Abbreviations: +++, strong staining reaction; ++, medium staining reaction; +, weak staining reaction.

The analysis of the structure of the domestic turkey tongue showed that the triangular tongue, deeply attached to the bottom of the beak with the lingual frenulum, fills the beak cavity apart from the free space in the anterior part of the beak. These features indicate low mobility within the beak cavity and allow qualifying the tongue in the domestic turkey to the third group of tongues according to Harrison (1964).

Poultry such as domestic turkey are fed fine or coarse ground feed in form of mash or pellets. Behavioral observations made by Zweers et al. (1994) indicate that Galliformes collect food by pecking. Upper and lower bills are used to catch food particles, which are placed in the free space between the anterior edge of the beak and the apex of the tongue. During pecking, the tongue is used to position and transport of food to the esophagus (Bels and Baussart, 2006). According to Zweers et al. (1994), food transport in Galliformes consists of 2slide-and-glue mechanism and catch-and-throw mechanism. During the slide-and-glue mechanism, the tongue is protracted and contacts with the food. Coordinated forward and backward movements of the tongue cause the food to be transported along the surface of the tongue to the esophagus. The catch-and-throw mechanism occurs when the head is moved upward and the beak is widely open. The tongue is retracted and the food moves posteriorly. To transport the food to the esophagus, the bird must perform several transport cycles using both the transport mechanisms or just the slide-and-glue mechanism (Zweers et al., 1994).

By analyzing the type of food intake and the way it is collected and transported in relation to the morphologic structures of tongue in the domestic turkey, we can conclude that the main tool used during pecking is the apex of the tongue with the lingual nail, which works similar to a shovel and is slipped under the different food particles during the slide-and-glue mechanism. It should be emphasized that the plate of the lingual nail in the domestic turkey covers not only the apex of the tongue but also the anterior part of the body of the tongue and forms a well-developed exoskeleton. The paraglossal cartilage present in the mucous of the lingual apex additionally stiffens this part of the tongue during pecking.

When transporting the food to the esophagus, the median groove in the area of the body of the tongue marks the transport path along the surface of the tongue up to the area of the conical papillae at the border between the body and the root of the tongue. The papillae, directed posteriorly, stabilize the food transport path and, during the last retraction cycle of the tongue, approach the laryngeal prominence and the food is transported, above the closed laryngeal cleft, to the esophagus. Attention should be paid to the papillae present on the lateral surface of the lingual root, whose aim is to prevent food from falling out of the beak cavity.

The fact that the domestic turkey collect the fine of coarse ground feed using the described mechanisms remains in correlation with the occurrence of 2 types of cornified epitheliums, that is parakeratinized and orthokeratinized epithelium. The parakeratinized epithelium is present in places where food is transported that is on the dorsal surface of the apex and body of the tongue. The orthokeratinized epithelium is present in places where food intake takes place, like the ventral surface of the apex. In other birds, such as Anseriformes, the orthokeratinized epithelium builds those parts of the tongue that are associated with cutting grass and filtration of food from water (Jackowiak et al., 2011; Skieresz-Szewczyk and Jackowiak, 2016).

Both types of epithelia have a protective function. Taking into account the microscopic structure of these epithelia, we can conclude that there are 2 protective strategies of the epithelia. The first one is the formation of a thick cornified layer, which was observed in the orthokeratinized epithelium. The second strategy consists in the formation of a high epithelium with a thin cornified layer, which subject to intensive peeling in the form of single cells, as exemplified by the parakeratinized epithelium. It is also important to note that the presence of high connective tissue cores in the parakeratinized epithelium is responsible for the strong anchorage of this epithelium in the lamina propria of mucosa by increasing the borderline between connective tissue and the epithelium. Presence of long, convoluted capillaries in the connective tissue cores causes the enlargement of capillary surface for exchange of nutrient. The blood flow in such capillaries slows down and enables the efficient nutrition of the epithelium, which undergoes intensive regeneration because of loss of superficial cells.

During transport of the food to the esophagus, the conical papillae at the border between the body and the root of the tongue approach the laryngeal prominence, thus covering the root of the tongue, so that this part of the tongue in the domestic turkey has little contact with the transported food and is covered with noncornified epithelium.

The slide-and-glue and catch-and-throw mechanisms for transporting food of different consistency, that is as a mesh or pellets in turkey, also require adequate hydration of the beak cavity. The secretion of salivary glands of the beak cavity and the lingual glands is responsible for this process. In the domestic turkey, the lamina propria of the tongue mucosa in the area of the body and root of the tongue is tightly filled with glands, which secretion is collected in wide collecting ducts and escapes through short excretory units to the glands openings present on the side of the body of the tongue and on the dorsal surface of the tongue root.

Comparing the structure of the tongue of the domestic turkey and the other Galliformes representatives, we can distinguish the features typical for all the examined bird species, the features characteristics only for few representatives, and species-specific features.

The features typical for all Galliformes include the triangular shape of the tongue and the presence of conical papillae at the border of the tongue body and root (Iwasaki and Kobayashi, 1986; Homberger and Meyers, 1989; Parchami et al., 2010; Kadhim et al., 2011; Erdogan et al., 2012; Pourlis, 2014; Uppal et al., 2014; Sundaram et al., 2015). It should be noted that conical papillae, in all Galliformes species so far described, have a common base of papillae forming a comb of conical papillae (Iwasaki and Kobayashi, 1986; Homberger and Meyers, 1989; Parchami et al., 2010; Kadhim et al., 2011; Erdogan et al., 2012; Pourlis, 2014; Uppal et al., 2014; Sundaram et al., 2015). In the domestic turkey, conical papillae occur as single structures of the lingual mucosa, which indicates that it is a species-specific feature.

The presence of 2–3 conical papillae located on the sides of the tongue root was qualified as a feature characteristic for few Galliformes representatives, including the studied domestic turkey. These papillae were also recorded in the common quail and Chukar partridge (Parchami et al., 2010; Erdogan et al., 2012; Uppal et al., 2014). The second feature is the median groove observed in the common quail and Japanese quail (Parchami et al., 2010; Pourlis, 2014; Uppal et al., 2014). The third feature is the lingual nail, which has so far been described in the chicken and Japanese quail (Homberger and Meyers, 1989; Pourlis, 2014).

The species-specific features of domestic turkey tongue include the already mentioned structure of the conical papillae at the border between the body and the root of the tongue, as well as the 2 conical in shape papillae present in the posterolateral part of the body of the tongue, which are directed toward the root of the tongue.

Studies by Iwasaki and Kobayashi (1986) in the chicken indicate the presence of another type of tongue papillae, namely filiform papillae, which cover the entire dorsal surface of the tongue. Studies in other Galliformes did not confirm these observations (Homberger and Meyers, 1989; Parchami et al., 2010; Kadhim et al., 2011; Erdogan et al., 2012; Pourlis, 2014; Uppal et al., 2014). Own study in the domestic turkey did not show the presence of filiform papillae either. Light microscopy and scanning electron microscopy observations indicate that the mucosa of the dorsal surface of the tongue is covered with unique parakeratinized epithelium among birds, which has elongated surficial cornified cells that peel off massively. Iwasaki and Kobayashi (1986) performed analyses only by scanning electron microscopy, and structures observed on the surface are considered as filiform papillae. However, the size of these structures corresponds to the size of a single, flattened epithelial cell, which indicates that the structures observed by Iwasaki and Kobayashi (1986) on the surface of the chicken tongue are not filiform papillae.

An interesting feature of the exfoliating superficial cells of the parakeratinized epithelium of domestic turkey is the direction of exfoliation. At the apex and anterior part of the body of the tongue, the cornified cells are vertically peeled off and at the posterior part of the body of the tongue they peel toward the root of the tongue.

In the lamina propria of lingual mucosa of the avian tongue, there are alveolar or tubular alveolar lingual glands, which are divided into 2 groups, that is anterior and posterior lingual glands (McLelland, 1990; Nickel et al., 1992; Vollmerhaus et al., 1992). Only in Anseriformes, 3 groups of lingual glands were distinguished, that is anterior, posterolateral, and posteromedial lingual glands (Jackowiak et al., 2011; Skieresz-Szewczyk and Jackowiak, 2016). Research on Galliformes conducted by Gargulio et al. (1991) in a chicken and by Kadhim et al. (2011) in a red jungle fowl distinguishes the presence of 2 groups of anterior lingual glands that are the lateral and median groups. Own research in domestic turkey showed the presence of anterior lingual glands, without division into groups and posterior lingual glands, which is a classical division of glands among the studied species of birds.

Comparing the composition of the produced mucus in Galliformes with the results of own research in domestic turkey, it can be concluded that it consists of neutral and acidic mucopolysaccharides (Gargulio et al., 1991; Erdogan et al., 2012; Uppal et al., 2014). In addition, it was found that acidic mucopolysaccharides contain sulfomucins and sialomucins (Gargulio et al., 1991; Erdogan et al., 2012). It should be emphasized that in Galliformes and domestic turkey, there are differences in the intensity of histochemical staining reactions. Own research in domestic turkey showed that the anterior and posterior lingual glands showed the same staining reaction to neutral mucopolysaccharides, and the posterior lingual glands have a stronger staining reaction to sialomucin and sulfomucin than the anterior lingual glands. Studies by Gargulio et al. (1991) and Kadhim et al. (2011) in chickens showed that the lateral group of the anterior lingual glands characterize with weaker staining reaction for neutral and acidic muchopolysaccharides in comparison with the median group and posterior lingual glands. Common quails also showed weaker staining reaction for neutral muchoplisacharides in the anterior lingual glands than in the posterior, but the staining reaction for acidic muchoplisacharides is strong in both the anterior and posterior lingual glands (Uppal et al., 2014). Erdogan et al. (2012) reveals stronger staining reaction for neutral and acidic muchoplisacharides in the anterior lingual glands than in the posterior lingual glands in Chukar partridge.

The intensity of staining reactions to neutral and acidic mucopolysaccharides is species-specific and can be related to both the type of food intake and environmental conditions, as the mucins not only moisten the surface of the tongue and the food but also cover the surface of the beak cavity to prevent drying out. The presence of sulfur-rich mucopolysaccharides in the mucus causes that the mucus secretion has a protective function against pathogens (Suprasert et al., 1986; Tabak, 2006). Before swallowing, the food is stopped at the border of the body and root of the tongue and is surrounded by mucus. Therefore, the posterior lingual glands of the domestic turkey have a higher secretory activity toward sulfomucin.

In conclusion, macroscopic and microscopic analysis of the tongue of poultry such as domestic turkey qualifies it to the third group of the tongue as per the Harrison classification (1964), that is, tongues that are deeply embedded in the beak cavity and, above all, act as a platform for adhesion and movement of food in the beak cavity before ingestion. The structure of the turkey tongue at the same time indicates a functional adaptation for taking fine or coarse ground feed in form of mash or pellets by pecking and transporting to the esophagus by means of slide-and-glue and throw-and-catch mechanisms. It should be emphasized that the performed analyses, for the first time, indicated the presence of 2 types of cornified epithelia in Galliformes. The study also showed the presence of species-specific features such as the structure of separated conical papillae at the border of the body and root of the tongue and the presence of lingual papillae in the posterolateral part of the lingual body. At the same time, the present studies in poultry such as domestic turkey indicate a very important methodological aspect of the examination of avian tongues, namely the necessity to collect tissue samples from diverse areas of the tongue and the application of various techniques of macroscopic and microscopic analyses, which guarantee a comprehensive morphofunctional analysis. The obtained results can be used in the development of the most optimal feed compound for granivorous and also in veterinary practice.

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DISCLOSURES

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