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Ultrastructure of the Tongue of the German Mast Goose (*Anser anser*) by Scanning Electron Microscopy Before and After Plastination

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ABSTRACT

The aim of this study was to compare the micro anatomy of the tongue in German mast geese with other bird species by scanning electron microscopy (SEM) analysis. In addition, by applying the silicone plastination technique to the tongue, it is aimed to compare the SEM images before and after plastination. German mast geese used as study material were obtained from goose breeders. After the micro-anatomical features of the German goose tongue were determined, SEM (FEI-Quanta; FEG 250, USA) analyzes were made and their general structures were photographed. Then, silicone plastination processes were applied at room temperature. The SEM images of the fresh material and the plastinated material were compared by taking the SEM images again. As a result, it was determined that the features of the tongue were preserved macroscopically after the plastination process. The silicone plastination procedure of the German mast goose tissues took a total of 31 days. On the SEM plastination images, scattered dispersion was observed on the epithelium of the tongue surface, corresponding to the findings on the fresh material. The papillae on the SEM images were observed to be preserved as in the fresh material images.

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Authors' Contribution
SBB and ZEO Planned the study. BCG and SBB performed the material collection phase. BCG, SBB and RI performed the electron microscopy and plastination step. SBB contributed to the article writing.

Key words
German mast geese, SEM, tongue, plastination, Ultrastructure

INTRODUCTION

Most birds can fly but some cannot fly and have therefore adapted to different environments in respect of nutritional sources such as the coastline, ponds, small rivers, fields, and mountains. In the most extreme conditions, penguins feed on fish below the sea. Birds have different feeding habits reflecting different lifestyles and correspondingly, there are differences in the beak and tongue structures (Iwasaki *et al*., 1997). The mast goose in Germany is bred to be used for meat, eggs, and animal feed, and is a breed which has been developed with the properties of good meat flavour, vitality, fertility, and reproductive rates (Diken, 2022).

Some researchers (Zweers, 1974, 1982; Zweers *et al*., 1977; Berkhoudt, 1985) have reported synchronized interactions between the jaw and tongue when eating and drinking in some bird species. Homberger and Meyers (1989) reported a more comprehensive examination of the biomechanical interactions of the structural elements of the lingual apparatus related to feeding in Gallus gallus. However, there is very little data related to the cytology of the lingual epithelium of birds.

Plastination was first introduced to the medical world by (Pashaei, 2010) as a preservation technique for body tissue with great diversity in processes and development. In these processes, water and lipids in biological tissues are exchanged for mostly curable polymers, which will then be hardened and ultimately result in dry, odour-free, durable samples of natural appearance (von Hagens, 1986).

In recent years, there has been an increasing tendency for plastinated products. This increasing tendency is reflected in articles emphasizing the primary role and importance of plastinated samples as important educational, research, and cultural tools in the medical world, but there is still debate amongst anatomists about the utility of these tools (Jones and Whitaker, 2009).

With the exceptions of studies by Hassan *et al*. (2009) and Khalaf and Ahmed (2020) of Egyptian geese, and by Jackowiak *et al*. (2011) of local geese, there are insufficient data defining the characteristic morphology of the oropharyngeal cavity of different goose species.

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There are also very few studies about the morphological characteristics of the tongue in these species. The aim of this study was to present a full morphological definition of the tongue of the German mast goose (*Anser anser*), based on macroscopic and scanning electron microscopy (SEM) examinations before and after silicone plastination.

This study can be considered to make a valuable contribution to the literature as there is no previous study of SEM examination of the German mast goose tongue applied with silicone plastination.

MATERIALS AND METHODS

As the study material, the tongues of 8 adult geese were used and obtained fresh from the German mast goose farm slaughterhouses in the Elazig province. The tongues were separated as 4 for SEM examinations and 4 for plastination procedures. Following the dissection of the research material, a morphological examination of the tongues was performed.

For the SEM examinations, the tongue was fixed in 10% buffered formol, then different sections were dissected as 60-70mm^2 trims. These were then washed in 0.1M buffered solution and after approximately 6 hours in 2.5% glutaraldehyde solution were washed again 5 times in 0.1M buffered solution (Emura, 2008; Erdoğan *et al*., 2012; Elsheikh and Alzahaby, 2014).

For the second fixation of the tongue tissue, the samples were left for one hour in 1% osmium tetroxide solution. In the next stage, the tissues were left for 10 mins in each of the 25%, 50%, 75%, and 100% ethanol solutions, were then dried with a drying device (Nüve EC160, Turkey) and finally covered with gold dust (Emura *et al*., 2008; Erdoğan *et al*., 2012; Elsheikh and Alzahaby, 2014). In the Science and Technology Research Centre of Aksaray University, the samples were examined under a scanning electron microscope (FEI-Quanta, FEG 250, USA) and the important structures were photographed.

The silicone plastination procedures were performed in the Plastination Laboratory of the Anatomy Department, Firat University Veterinary Faculty. The SEM images were performed in the Science and Technology Application and Research Centrel of Dicle University were examined and photographed.

For the silicone plastination procedures, the tongues were first dissected and then fixed in 10% formaldehyde. The samples were then placed in acetone baths and the dehydration stage was continued until the acetone level reached >95% at -25°C. The fat removal stage was performed by leaving the samples in acetone at room temperature for 2 days. Mandatory impregnation was performed in a vacuum tank within a BIODUR S10+S3 mixture. Finally, gas curing was applied with BIODUR S 6 chemical, and the plastination procedure was completed.

Following the silicone plastination procedures, SEM images were obtained and comparisons were made of the pre and post-plastination images. Nomina Anatomica Avium (Baumel *et al*., 1993) was used for the terminology.

RESULTS

In the examinations of the SEM images of the German mast goose tongues, the dorsal surface epithelium was smooth in appearance. The apex was separate from the corpus and radix sections. It was covered with keratinized epithelium. The cartilaginous section was below the keratinized epithelium. The tongue tip apex structure was pointed and triangular in form when folded (Figs. 1A, B). Filiform papillae were observed on the corpus images at a mean number of 20.

At the edges, 6 papilla linguales were observed with a sharp-pointed projection extending to the right and left. On the radix section, there were 10 papillae conica arranged side by side, extended as many papillae filiformis between V-shaped, flat, pointed tips. At the edges were papillae linguales caudales with sharp pointed tips and a smaller projection (Fig. 1C, E).

The silicone plastination procedure of the German mast goose tissues took a total of 31 days. Macroscopically, no difference from fresh material was observed. Only the dimensions and weight ratio differed from the fresh samples as the tissue fluid had been withdrawn. There was determined to be a 27% decrease in size and a 65% decrease in weight. Other than size and weight, the anatomic properties were preserved, and dry, odorless, durable samples had been obtained, rendering the silicone plastination procedure appealing to researchers.

On the SEM plastination images, scattered dispersion was observed on the epithelium of the tongue surface, corresponding to the findings on the fresh material (Fig. 2A, D). The papillae on the SEM images were observed to be preserved as in the fresh material images. Although partial dispersions were formed on the silicone plastination SEM images, the preservation of the characteristics as the image with general lines makes silicone plastination important.

DISCUSSION

Pourlis (2014) examined the tongues of Japanese quail on SEM images and reported that the tongue resembled a triangle in shape, the apex, corpus, and radix lingual sections were separate, and the epithelial surfaces were

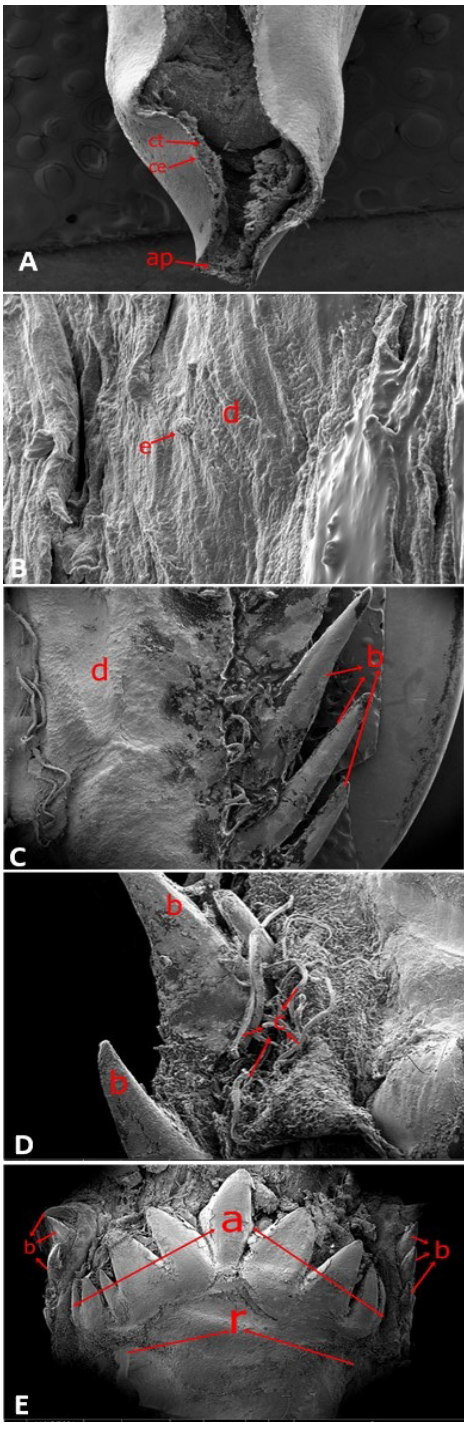

Fig. 1. Ultrastructure of German Mast excavation tongue. A, cross-sectional SEM view of the apex: ce, Keratimized cpithelium; ap, apex; ct, cartilago. B,C, corpus part: e, para creatimized ridge; d, surface epitholuim, b, papilla linguales; d, surface epitheluim. D, E, radix part: b, papilla linguales caudales; C, papilla filiformes, (a) Papilla conicae, (b) Papilla linguales caudales, (r) Radix.

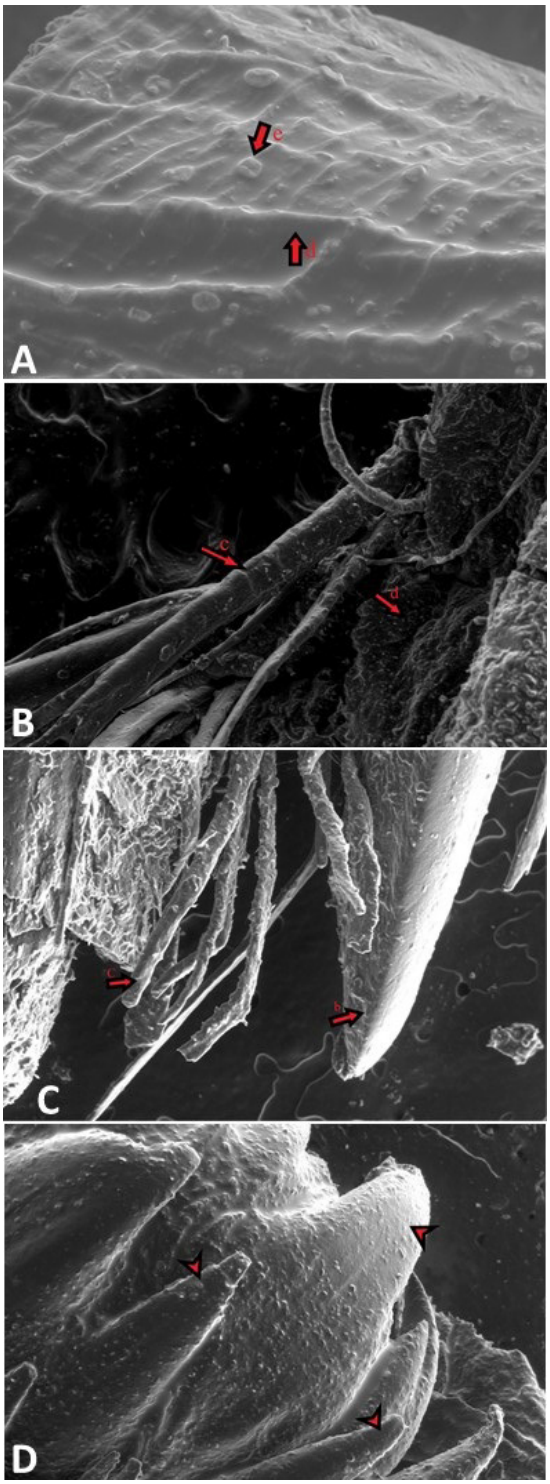

Fig. 2. Ultrastructure of corpus (A, B) and redix part (C, D) of German Mast excavated tongue (A) and excavation plastine tongue (A, C, D). b, papilla linguales caudales; c, papilla filiformes; d, surface epithelium. Papilla conicae (Arrowhead).

covered with multi-layered, smooth keratinized or non-keratinized smooth epithelium. In contrast, Ilgün *et al.* (2020) reported that in guinea fowl, the dorsal keratinised, multi-layered smooth epithelium was thicker than the ventral and the epithelial fibers were tight in appearance. The findings on the SEM images of the German mast goose in the current study were seen to be similar to those of the Japanese quail.

It has been stated in literature (Nickel *et al*., 1977; Erdoğan and Iwasaki, 2014; Onuk *et al*., 2013) that the condition of the dorsal epithelial surface of the tongue and the shape of the tongue have been shaped according to the form of feeding, the type of feed and the habitat. Ilgün *et al*. (2020) reported that the guinea fowl tongue on SEM images was in the shape of a long triangle with a smooth epithelial surface. The apex structure of the German mast goose tongue was pointed, folded and triangular in appearance on SEM images.

In studies of quail by Parchami *et al*. (2010), ostriches by Crole and Soley (2009a, b), Japanese quails by Pourlis (2014) and guinea fowl by Ilgün *et al*. (2020), it was determined from SEM images of the tongue epithelial surface that there were many epithelial micro ridges providing slipperiness of the tongue surface. On the SEM images of the German goose tongue, no epithelial micro ridges were determined.

In the literature on avian species (Crole and Soley, 2009a; Aytekin, 2010; Dursun, 2014; El-Bakary, 2011) the papillae in the dorsolateral of the tongue were fewer in number with smooth tips, and were named papillae linguales caudales in guinea fowl by Ilgün *et al*. (2020) and Tabasi and Mohammadpour (2019). Similar papillae were observed in the current study, but the end sections had a long, pointed, sharp appearance in the corpus region.

The conic-shaped papillae ends in the radix linguae arranged horizontally have been named in literature as papilla conica (Jackowiak and Godynicki, 2005; Igwebuike and Anagor, 2013). It has been reported in the literature (Crole and Soley, 2009a, b; Jackwiak *et al*., 2010; Erdoğan and Iwasaki, 2014), that in the majority of avian species, the papillae conica arranged in rows in the caudal of the tongue radix play a role in transferring food to the oesophagus and in regurgitation. There were stated to be no papillae conica in magpies by Igewebuike and Eze (2010) or in ravens by Erdoğan and Alan (2012). Ilgün *et al*. (2020) reported that there were 16-18 papillae conica in guinea fowl and Erdoğan and Alan (2012) stated this number to be 12-14 in partridge. In the current study material, papillae conica were determined in German mast geese, seen to be 10 in number arranged side by side with smooth pointed tips. In studies of partridge by Erdoğan and Alan (2012), of the white-tailed eagle by Jackowiak and Godynicki (2005), of quails by Parchami *et al*. (2010), of partridge by Regina *et al*. (2005), of geese by Hassan *et al*. (2009), of Nigerian guinea fowl by Igwebuike and Anagor (2013) and of guinea fowl by Ilgün *et al*. (2020), papillae conica have been reported to be in the root section of the tongue with a V-shaped arrangement. In the German mast goose tongue samples examined in the current study, the papillae conica were in the same section showing a similar arrangement.

Skieresz-Szewczyk and Jackowiak (2014) reported that filiform papillae only occurred in gaps between large and small conic papillae of the body in geese, whereas in ducks, filiform papillae in the rostral section of the body were fully consistent with small conic papillae and were formed of dense hairs which completely removed small food particles, and in the caudal section of the tongue, filiform papillae were located between large conic papillae, as in geese. The material in the current study was similar.

It has been reported that the epithelial layer of the dorsal surface of the tongue in birds is shaped according to the type of feeding of the avian species, the structure of the food consumed, and the habitat (Nickel *et al*., 1977; Onuk *et al*., 2010; Erdoğan and Iwasaki, 2014; İlgün *et al*., 2020).

Plastination has become an extremely helpful tool for the long-term preservation of anatomic samples without the need for preservative substances. When used for educational purposes, plastinates can be accessed at any time, and can be transported to distant learning areas outside the dissection room without the need to transport the samples in jars full of preservatives (Rahul *et al*., 2019).

In the same way, plastination allows organ and tissue examination by students, academicians, and researchers without any physical separation between themselves and the sample, as there is no need for gloves, masks, or any container (Latorre *et al*., 2016; Klaus *et al*., 2017; Rahul *et al*., 2019).

CONCLUSION

In the present study, plastinated samples do not prevent histological studies, even within the restrictions such as artefacts associated with shrinkage. In this study, the SEM images of samples preserved with plastination were compared with the SEM images of fresh material.

As there are insufficient morphological studies related to German mast geese and studies using SEM images of silicone plastination, this study can be considered of value due to the specific contribution to literature. Nevertheless, there remains a need for further studies on this subject.

Ethical statement

Permission for the study was obtained by the Fırat University Non-invasive Research Ethics Committee, session numbered 02/12/2020-426874

Statement of conflict of interest

The authors have declared no conflict of interest.

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