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Exploring the morphology of the glandula uropygialis in Denizli rooster: 3 tesla MRI and histological investigation

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ABSTRACT

The aim of this study was to examine the preen gland (uropygial gland) of Denizli rooster, an endemic species for Turkey, both macroscopic and by magnetic resonance imaging and to present its histological formation. Five adult male subjects were used for this study. Although some of the findings of the preen gland in Denizli rooster were quite similar to the previous studies on the various avian species, widely branching secondary sinuses of the both two lobes of the gland were formed different from those. It was observed that the sinuses of these two lobes were connected to each other by forming passageway at the isthmus region of the gland. MR images showed bright white hyper-echoic features due to high fat secretion of the preen gland. Therefore, it was determined that the preen gland could be easily isolated from the surrounding tissues. It was also observed in the MR images that the gland in the Denizli cock has a pair of symmetrical bilateral lobes and is located on both sides. This connection gave rise to thought that the problems occurred by the blockage of the one of the primary canals can be solved by the compensation of another canal connected through this passageway. Thus, the secretion of the gland could be maintained properly.

Denizli horozlarında glandula uropygialis morfolojisinin araştırılması: 3 Tesla MRG ve histolojik inceleme

ÖZET

Bu çalışma, Türkiye için endemik bir tür olan Denizli horozunun preen bezini (glandula uropygialis) hem makroskopik olarak hem de manyetik rezonans görüntüleme ile incelemek ve histolojik oluşumunu sunmak amacıyla yapıldı. Beş yetişkin Denizli horozunun kullanıldığı çalışmamızın sonuçları, şimdiye kadar bu konuda yapılan diğer çalışmalarla büyük oranda benzer olmasına karşılık, diğer türlerden farklı olarak, her iki loba ait her yöne dallanmış sekonder sinusların, bezin istmus bölgesinde birbirine açılarak iki lobu birbirine bağlayan geçiş kanalları oluşturduğu gözlendi. MR görüntülerinde preen bezinin yüksek miktarda yağ salgılamasına bağlı olarak parlak beyaz hiper-ekoik özellikler görüldü. Bu sebeple preen bezinin çevre dokulardan kolayca izole edilebildiği belirlendi. Denizli horozundaki bezin bir çift simetrik bilateral loba sahip olduğu ve her iki tarafta yer aldığı MR elde edilen görüntülerinde de gözlendi. Loblar arası geçiş sayesinde, primer kanallardan birinin herhangi bir nedenle tıkanması durumunda bezden dışarı salgı akışının devamlılığının sağlanabildiği ve böylece tıkanmaya bağlı olumsuzlukların engellenebildiği düşünülmektedir.

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1. Introduction

The Denizli rooster, a prominent domestic breed in Turkey, holds a distinctive position among other breeds due to its prolonged and continuous crowing, lasting approximately 20-25 seconds (1, 2). Renowned for its high-pitched, deep, and bass crowing, the Denizli rooster's voice is unique (3). In addition to its distinctive vocalizations, the Denizli rooster is admired for its aesthetically pleasing appearance, characterized by an almost black body color and various types of fleshy combs atop their heads (4, 5).

The uropygial gland is mostly known as the sebaceous gland, rump gland or preen gland, a skin gland unique to birds and not found in any other mammalian group. (6, 7, 8). It is always present in chickens and waterfowl. However, it may be absent in some parrots (Amazon, Anodorhynchus and Cyanopsitta), pigeons, ostriches and their relatives (ratites) and woodpeckers (9).

Morphological properties of the uropygial gland differ among the avian species and even individuals (10). In chickens and roosters, it is a gland topographically located on the upper side of the caudal root, approximately between the fourth caudal vertebra and the pygostylus, and can be seen with the naked eye (6, 9, 11, 12). The secretion of the gland consists of a combination of cells, ester waxes, fatty acids, fat, and sudanophilic secretory granules (13). It is reported that there are differences in gland mass relative to body mass due to different factors such as seasonal variation, habitat, body mass, individual variation and gender. The chemical composition of the gland secretion can vary depending on age, diet, sex and reproductive periods (14, 15).

Three main functions of this gland have been indicated by the researchers: Protects the keratin on the feathers which is essential for thermoregulation and flying, provides water tightness by covering the feathers, defends against pathogens and ectoparasites by protecting the microflora on the skin (6, 10, 12, 16). In addition, it has been reported in studies that it may also play a role in the storage of vitamin D and allow this vitamin to be taken up by the beak during grooming. The uropygial gland interferes in processes of sexual communication, via the production of pheromones. Another function is that saving against predator deterrence (17). This secretion works as an origin of chemo signals that evokes social and reproductive behaviors (11).

Preen gland generally consists of two lobes (10, 18). The appearance, size, and shape of the lobes vary between species (6, 10, 19). There is no statistically significant difference between hens and roosters or between right and left lobes in terms of morphometric parameters. Each lobe contains a special tissue that produces an oil-like secretion and a sophisticated canal system that transfers this secretion from the gland to the skin and the feather (6, 10, 20). The previous studies on the preen gland have indicated that it produces a holocrine secretion such in mammals and this helps to store the secretion in the canals and to release it through a small papilla when required (10, 16, 20, 21). This papilla is located right at the top of the tail (18, 20) and has a typical teat–like appearance (6). The papillar part distinctly differs from the lobar part of the preen gland by an intermediary area composed of a firm connective tissue, the isthmus region (22). Each lobe consists of a large number of follicles which are covered by secretory epithelium. When the preen gland is stimulated by the friction of the beak, the follicles start to excrete the secretion through the secondary sinuses and then the primary sinuses (6, 22). Each follicle can be observed in 4 different histological layers (6, 10, 11, 22). The germinative layer, the intermediate layer, the secretory layer and the degenerative layer (6, 8, 10, 11, 22, 23).

However there have been several studies about the uropygial gland since the middle of the thirteenth century (10), and the research which has been made by modern imaging techniques about this gland was very limited. In addition to the MR imaging technique, because of having any destructive effect, it has been frequently used as a non-invasive method (24). Especially tissues which have got high fat and water ratio can be readily visualized and quantified in virtual slices made with T1-weighted magnetic resonance imaging, and then summed across slices to calculate body composition (25). Besides it allows us to investigate the body part from a 3-dimensional capturing (26). It is a high incidence of intracranial tissue accumulations in domestic ducks with feather crests and is monitored for diagnosis

and management of liposarcomas (27, 28). It is used to image the normal anatomy of the brain and coelomic cavity of domestic pigeons (Columba livia domestica) on the other hand is also used for the eye and orbit of a euthanized screech owl (Otus asio) (29).

The aim of this study was to investigate the anatomical structures of the endemic Denizli rooster uropygial gland by both macroscopic and magnetic resonance imaging and to reveal its detailed histological structure.

2. Material and Methods

Pedigree embryonated eggs were obtained from the Denizli Food, Agriculture, and Livestock Directorate. The eggs were incubated in the hatchery of Ankara University, Faculty of Agriculture, Department of Animal Husbandry. Uropygial gland samples used in the study were obtained from 5 adult healthy roosters. Ethical approval (Decision No: 2012-24-139) was obtained from Ankara University Animal Experiments Local Ethics Committee.

Anesthesia was induced using a combination of xylazine, diazepam, and ketamine (30). The roosters were set in "prone" position and were scanned T1 and T2-weighted in 3 Tesla magnetic resonance imaging device (Siemens Magnetom, Germany). It was acquired in sagittal, transverse, and dorsal planes. In addition, 3-dimensional reconstructions were made from MR images in an electronic setting. T1 – weighted images were acquired using 0.8 slice thickness with a repetition time (TR) of 1100 ms and an effective echotime (TE) of 16 ms. T2 – weighted images were attained using 0.8 slice thickness with a repetition time (TR) of 8900 ms and an effective echotime (TE) of 85 ms.

For anatomical evaluation, topographic features of the glandula uropygialis were analyzed in situ. Macroscopic images of the glands were obtained from cadavers. The feather on the glandula uropygialis was removed and the dissection stage was started. The glands were carefully dissected and removed from the body without damaging the surrounding tissues. Images of the dissected glands were taken.

For the histological investigation, the uropygial gland was promptly excised and fixed in Bouin's fixative (composed of picric acid, saturated formaldehyde, and acetic acid in a ratio of 15:5:1, v/v) for 12 hours. Subsequently, a gradual dehydration process ensued, involving 1-hour incubations in 70%, 80%, 96%, and finally absolute alcohol. To facilitate material softening, the samples were immersed in methyl benzoate for 24 hours. Following this, the specimens underwent immersion in a benzene/paraplast (1:1) mixture and then in pure paraplast for 6 hours within a vacuum oven, ultimately resulting in embedding in paraplast. The prepared blocks were sectioned serially to a thickness of 5-6 μm using a rotary microtome (Leica RM 2025, Germany). Crossmon's Modified Triple stain (31) was applied to the sections for light microscopic (Leica DM 2500, Germany) examinations.

3. Results

An anatomical examination revealed that the glandula uropygialis were prominently situated at approximately the level of the fourth caudal vertebra in a dorso-median position (Figure 1). Dissecting and removing the overlying skin exposed the lobus glandula uropygialis, symmetrically organized into two right and left lobes, displaying a shiny appearance and light cream color (Figure 1). These lobes were discernibly separated by a interlobar septum. Each lobe featured an individual ductus glandula uropygialis and was positioned within the papilla uropygialis located caudally to the gland. These ducts, observed within the gland, were identified to open into the papilla uropygialis, a singular median protrusion adorned with small hairs. Notably, the ventral surfaces of the lobes appeared flatter than their dorsal counterparts. Importantly, the gland exhibited no structural connection with the pygostylus muscles.

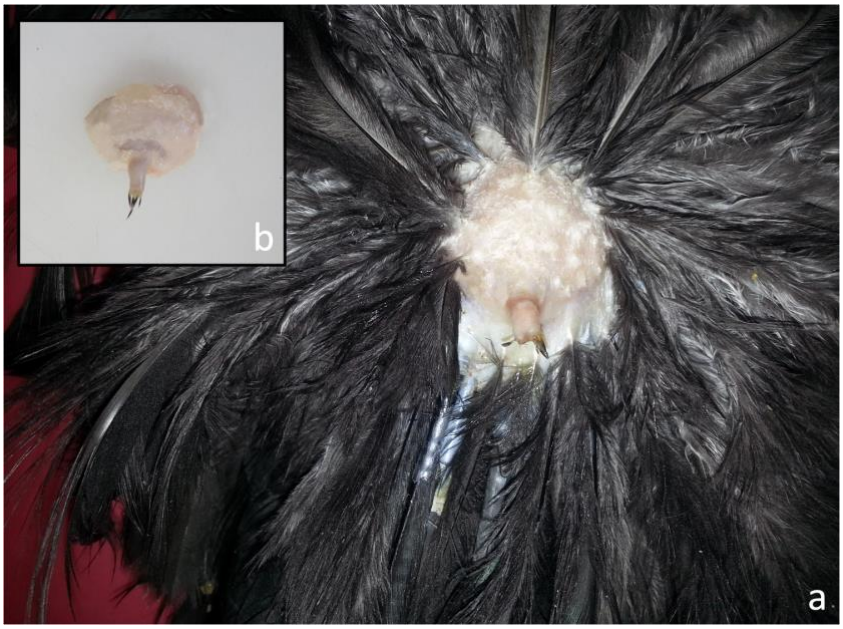

Figure 2: Before (a) and after (b) dissection view of glandula uropygialis in Denizli rooster
Şekil 2: *Denizli horozunda glandula uropygialis'in diseksiyon öncesi (a) ve sonrası (b) görünümü*

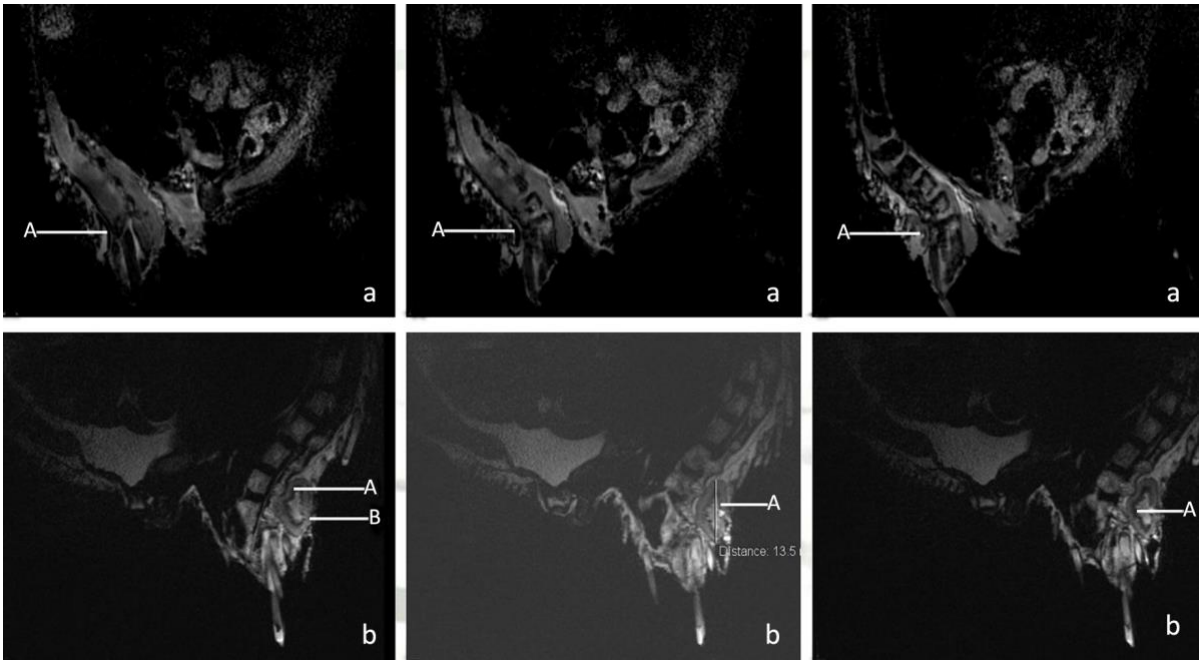

Figure 2: a-T1-weighted sagittal image in denizli rooster. b-T2-weighted sagittal image in denizli rooster. A-preen gland lobe. B-collecting duct.
Şekil 2: *a-Denizli horozunda T1 ağırlıklı sagittal görüntü: b-Denizli horozunda T2 ağırlıklı sagittal görüntü. A-lobus glandula uropygialis. B- ductus glandula uropygialis*

It was observed that anatomical details were visualized better in T1-weighted scans. The bib fabric was placed on the stern below the tail feathers. MRI images showed bright white hyper-echoic features due to the high amount of oil secretion of the preen gland. Therefore, it can be easily isolated from surrounding tissues. It was understood that the gland in the Denizli rooster had a pair of symmetrical bilateral lobes and was located on both sides. There is a channel for each lobe of the Denizli rooster. In transverse images, the width of the feather glands in the rooster at its widest level was determined to be 13.5 mm in the Denizli rooster (Figure 2).

In histopathological examinations, no pathological changes were observed. The histological appearance of the uropygial gland was identified. Accordingly, it was observed that the uropygial gland is surrounded by a wide capsule externally. The capsule also extended in between the two lobes and formed the interlobar septum in there. The thinner connective tissue prolongations arose from the outer capsule and the interlobar septum entered into the gland and formed the interfollicular septa. Complex secondary sinuses with a branching structure were observed in between the central cavity that stores the secretion, also termed as primary sinus, and the follicles. In the isthmus region where the lobes come close together, it was determined that transition channels connecting the two lobes by opening to each other in the isthmus region, shaped by branched secondary sinuses belonging to each lobe, were formed (Figure 3).

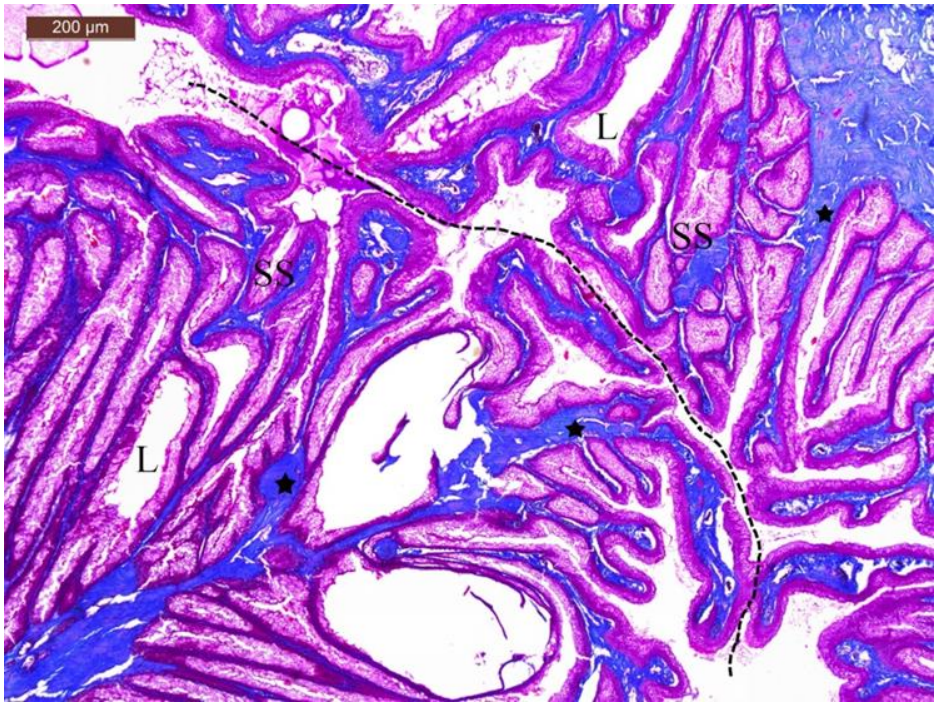

Figure 3: The histological image of the uropygial gland of the Denizli rooster. Interlobar septum separating into thinner connective tissue arms forming interfollicular septa (stars) entering into the gland, secondary sinuses (SS) consisting of branching structures, gland lumens (L), and a transition channel (dashed line) connecting the two lobes at the isthmus region of the secondary sinuses, stained using Crossmon's modified triple staining

Şekil 3: *Denizli horozuna ait üripygial bezin histolojik görüntüsü. İnterlober septumdan ayrılarak daha ince bağdoku kollarından oluşan interfoliküler septumlar (yıldızlar), Dallı budaklı yapıya sahip bezlerden oluşan sekonder sinuslar (SS) ve bez lümenleri (L) ile sekonder sinusların istmus bölgesinde birbirine açılarak iki lobu birbirine bağlayan geçiş kanalı (kesik çizgi), Crossmon'ın modifiye üçlü boyaması*

4. Discussion and Conclusion

The insights derived from morphological and radiological imaging studies of the uropygial gland hold significant importance in unraveling the gland's functions. The intricate structure of this avian gland necessitates heightened scrutiny of both its secretory processes and the mechanisms involved in its excretion. The increasing histological research on the preen gland not only enables us to observe the variations among the avian species but also is very important to explain the basic relationship between the secretion function and preening.

Jacob et al. (6) demonstrated some of the morphological variations of the preen gland in different bird species. However, the shape and the size of it vary up to the species, most of the researchers reported that the uropygial gland generally has two lobes (10, 18, 23, 32, 33). In accordance with that the uropygial gland of Denizli chicken was composed of two drop shaped lobes.

Many morphometric and macroanatomical studies have been performed on the glandula uropygialis (14, 34). However, there is no study on the evaluation of the glandula uropygialis using magnetic resonance imaging. Therefore, our study is the first study in this sense. Yılmaz et al. (19) in the morphometric study conducted in Assel breed roosters, the lengths of the right and left lobes of the glandula uropygialis were 13.95 and 12.93 mm, respectively. In our study, the width at the widest level of the feather glands in the rooster was determined as 13.5 mm in Denizli rooster in MR transverse images.

Previous studies stated that each lobe has its own primary sinus and a primary canal in connection with this sinus (19, 33, 34). However, researchers have emphasized that the uropygial gland has two sinuses and two openings basically, some exceptional species which have less or more openings were presented (6). It has been reported that the preen gland in hoopoe (Upupa epops) has 3 lobes and excretes its secretion to one main papilla with a wide opening (6, 12). On the other hand, the preen gland of European nightjar (Caprimulgus europaeus) has one lobe (6) and kiwi (Apteryx haastii) has two lobed gland with 4 primary sinuses and 4 primary canals for each lobe. Similarly to kiwi there are 4 primary sinuses in New Zealand bellbird (Anthornis melanura). However, hihi (Notiomystis cincta), tui (Prosthemadera novaeseelandiae) and saddleback (Philesturnus carunculatus) have 3 primary sinuses in each lobe (16). Our study demonstrated that preen gland in Denizli rooster had two lobes and a single primary sinus for each lobe. The primary sinuses were connected with the well-branched secondary sinuses and also opened out through the primary canals in the papilla.

The papillae of the uropygial gland differ from species to species in birds. Besides, Jacob et al. (6) identified different types of canals inside the papilla. In the compact type papillae, the connective tissue which is the continuation of the interlobar septum surrounds the each canal inside the papilla and wherefore makes the lumen diameter of the canals narrower. On the other hand the delicate type canals are void of this compact connective tissue mentioned above and therefore the diameter of the lumen can be much wider (6). In our study, a condensed and wide connective tissue had been observed around these two canals. And the canals were classified in compact type group.

Four main follicle layers have been emphasized in the previous researches. These are germinative layer at the basal part of the follicles and then intermediate, secretory and finally, at the inner part, the degenerative layer, respectively (6, 8, 10, 11, 16, 22, 23, 35). In our study, the follicle cell layers were similar to the other species analysed.

In some of the species, the skin at the upper part of the preen gland contains melanin granules and this induces a dappled appearance on the skin as result of pigmentation (6). However we didn’t observe such a pigmentation on the Denizli rooster.

As a result, the histological features of the preen gland in Denizli rooster is quite similar to those in other species. In addition it was observed that the well branched secondary sinuses of each lobe are connected to each other at the isthmus region and form passageway between the two lobes of preen gland. It has been thought that the

secretion of the gland could be maintained properly by this way and this probably helps to prevent the negative effects of the mechanic obstruction. In addition to these, in the study conducted in Denizli cock, the glandula uropygialis was visualised with magnetic resonance imaging for the first time and it was observed that the gland has a pair of symmetrical bilateral lobes, located on both sides and there is a duct for each lobe of Denizli rooster. It is thought that the presented data will be guiding for future studies of the glandula uropygialis with magnetic resonance imaging and can be analysed with three-dimensional reconstructions in more comprehensive studies.

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Conflict of Interest

The authors declared that there is no conflict of interest.

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Ethical Approval

The present study was approved by the Ankara UniversityAnimal Experiments Local Ethics Committee (Decision No: 2012-24-139, Ankara, Türkiye).

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