

MORPHOLOGICAL, MORPHOMETRIC AND HISTOLOGICAL STUDIES ON THE INTERDIGITAL GLAND (SINUS INTERDIGITALIS) IN AKKARAMAN SHEEP BREED

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Abstract. The aim of our study is to examine the sinus interdigitalis structure of Akkaraman sheep, a different breed of sheep bred in Turkey. For this purpose, without any gender factor, was examined sinus interdigitalis structure on the fore and hind feet of 10 Akkaraman sheep, weights between 52-68 kg where obtained from Aksaray Municipality Slaughterhouse after slaughtering small ruminants. In the study, sinus interdigitalis were dissected from the fore and hind feet. After morphometric measurements of sinus interdigitalis were taken, macroanatomical findings were examined and photographed. In all the feet, the gland extended between the phalanx proximalis and the phalanx media, was matt white, embedded in the fatty tissue, and the inner surface was covered with soft fine hairs. It was found that it resembles a thick fishing pin in shape with canal-shaped head with the tip pointing upwards, a neck that shows a sharp downward bend and bulging body part. According to the measurements, it was found that the measurements taken from the forefoot were higher than those of the hind foot. In the histological and histochemical tissue samples of the sinus interdigitalis, it was observed that the lumen of the gland was filled with secretions from the apocrine sweat glands and sebaceous glands. Important findings were revealed by different study methods of Sinus interdigitalis, which is an important anatomical structure in the fingers of Akkaraman sheep.

Keywords: Akkaraman sheep, Histological, Morphometrical, Sinus interdigitalis.

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1. Introduction

Akkaraman sheep which is a combined breed by efficiency that breeding in Turkey's central region is a domestic sheep breed that is generally white in color, large, long legs and drooping ears, with black spots on the head, nose, ears and feet, resistant to bad environmental conditions (Kaymakçı & Taşkın, 2008; <https://www.tarim.gov.tr/Haygem>). Skin glands have formed gl. tarsalis, gl. interdigitalis and gl. infraorbitalis specialized structures in various parts of the body in relation to reproduction in ruminants (Abbasi, 2009; Poulis, 2010; König & Liebich, 2013; Kara *et al.*, 2020). It has been reported that as embryological development, sinus interdigitalis occurs as a result of invagination of the ectoderm layer towards the mesoderm layer (Sivachelvan, 1992). It has been reported that the secretions produced by the sinus interdigitalis, as the reproductive pheromone in sheep, interact in reproductive behavior between the breeds in mating and enable sheep

to follow each other (Uğurlu, 1991; Misk & Misk, 2013; Kara *et al.*, 2020). Additionally, it has been reported that the secretion of the gland has fungicidal and bactericidal effects and has a protective feature against UV radiation (Abbasi *et al.*, 2009). In literature (Janicki *et al.*, 2003, Abbasi *et al.*, 2009), while sinus interdigitalis is found in all 4 feet that in sheep-like species, in some species only the hind feet. The sinus interdigitalis is located in the front of the foot, in the space between the toes, on both sides of the midline of the foot, and is also called the sinus biflex and there are channels that open out sweat and sebaceous glands secretions (Çalışlar, 1970; Kara *et al.*, 2020). It is resembled to a pipe with a long and narrow neck and a blunt pouch (Aslan *et al.*, 2010; Awaad *et al.*, 2015; Kara *et al.*, 2020). Histologically, the wall structure of this gland consists of epidermis, dermis and a capsule. The epidermis layer consists of squamous epithelium with a keratinized layer and the dermis layer contains muscle layer, hair follicles, sebaceous glands, sweat glands, and apocrine glands (Karahana *et al.*, 2007; Kara *et al.*, 2020). Although there are many studies in the literature about the morphological structure of sinus interdigitalis in various sheep and goat breeds (Parillo & Diverio, 2009; Avdic *et al.*, 2013; Gürbüz *et al.*, 2017; Kara *et al.*, 2020) a comprehensive study has not been found on our native breed Akkaraman sheep which has been raised as the most important source of income in the Central Anatolia region from past to present. In this study, it was aimed to determine the macroanatomical structure, morphometry and histology of the sinus interdigitalis in Akkaraman sheep. It is thought that the findings obtained from this study will shed light on further studies.

2. Materials and Methods

In the study, the fore and hind feet of 10 Akkaraman sheep, weighing between 52-68 kg, obtained after slaughtering sheep from Aksaray Municipality Slaughterhouse, regardless of gender, were used as material. Fore and hind feet were cleaned by waiting 30 minutes in 1% Sodyum hidroklorür added water. Firstly, sinus interdigitalis were dissected from the fore and hind feet for macroanatomical and morphological examinations. After the morphometric measurements of the sinus interdigitalis were taken with a digital caliper (A Brand, Germany), the macroanatomical findings were examined and photographed. *Nomina Anatomica Veterinaria* (Icvgan, 2017) was taken as the basis for the terms used for naming.

Histological and histochemical studies

In tissue sampling, interdigital gland tissue samples taken from sheep for histological and histochemical examinations were fixed for 24 hours in 10% buffered formol-saline solution (pH 7.4), then dehydrated, cleaned and embedded in paraffin blocks. Sections of 6 µm thickness taken from the blocks were stained with Crossman's triple staining methods (Hematoxylin eosin (H&E), Periodic Acid Schiff (PAS), Alcian Blue staining methods (Crossman, 1937; Culling *et al.*, 1985; Misk & Misk, 2013). All samples were examined under a light microscope (Leica DM2500, Leica Microsystems GmbH, Wetzlar, Germany) and photographs were taken with a 3.3 megapixel digital camera (Leica DFC 320).

Statistical analysis

SPSS version 22 was used for statistical analysis. T-Test pairs were applied in the analysis of the data obtained from the fore and hind, right and left feet. Mean and (\pm)

standard deviation values of all data were calculated. Statistical differences were admitted significant at $p < 0.05$.

3. Results

Fore and hind feet of Akkaraman sheep were dissected. There were sinus interdigitalis glands in both fore and hind feet. In all the feet, the gland extended between the phalanx proximalis and the phalanx media was matte white, embedded in the fat tissue, and the inner surface was covered with soft fine hairs. It was found that it resembles a thick fishing pin in shape with canal-shaped head with the tip pointing upwards, a neck that shows a sharp downward bend and bulging body part. The body part was in the middle of the phalanx media, and the hole of the canal was covered with fine hairs at the level of *Articulatio phalangis proximalis* (Figure 1, 2, 3). In all measurements on the head, neck and body parts of the sinus interdigitalis, it was found that the measurement values of the forefeet were higher than those of the hind feet.

Table 1. The statistics of measuring results of the sinus interdigitalis

	Forefeet (Mean \pm SEM)	Hind feet (Mean \pm SEM)	(p) value
Sinus interdigitalis weight	0.758 \pm 0.064*	0.534 \pm 0.050	0.013
Excretory duct lenght	25.495 \pm 1.023	22.861 \pm 1.057	0.090
Excretory duct width	3.439 \pm 0.187	3.118 \pm 0.178	0.230
Corpus lenght	15.518 \pm 0.739*	12.316 \pm 0.947	0.016
Corpus width	6.596 \pm 0.421	6.209 \pm 0.439	0.533
Flexura lenght	7.203 \pm 0.325	6.276 \pm 0.448	0.112

Significant ($P < 0.05$); not significant ($P > 0.05$).

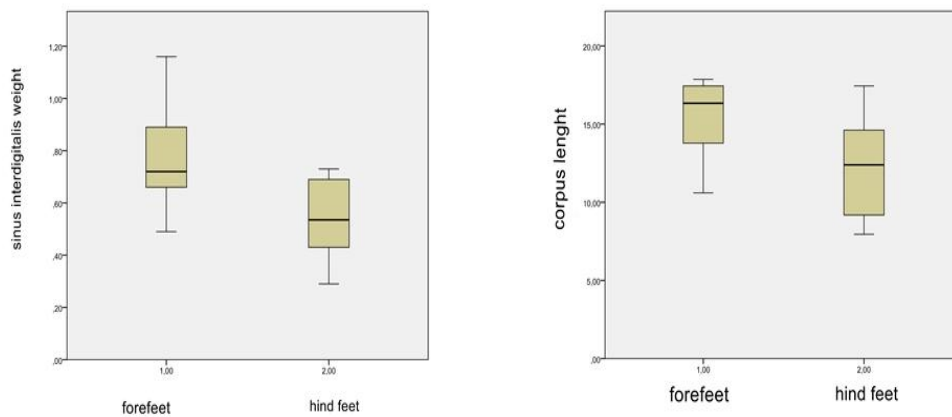


Figure 1. Statistically significant graphical views of the blot

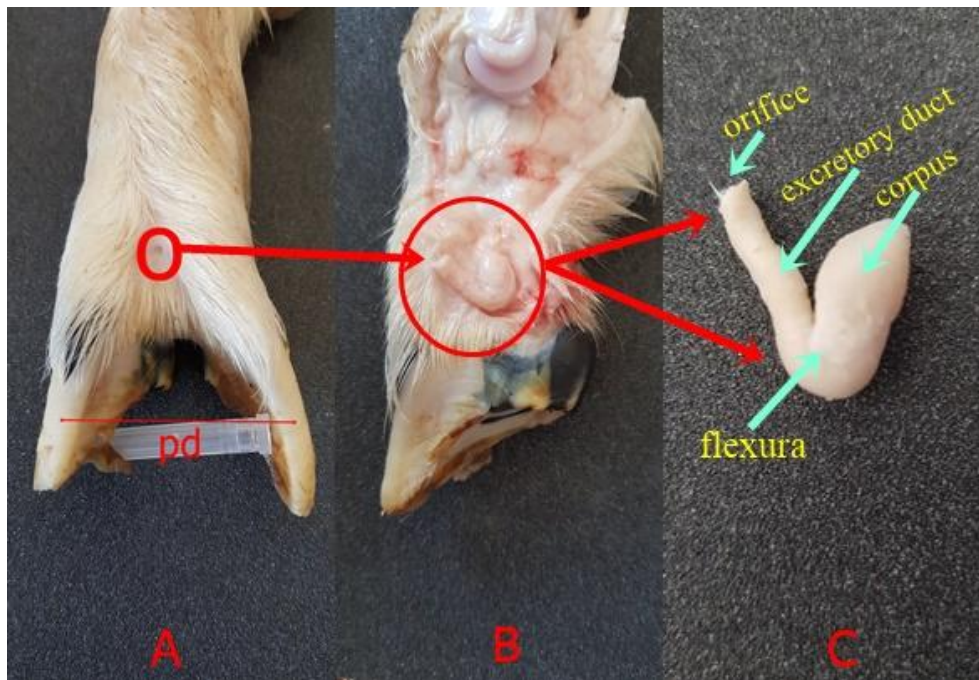


Figure 2. (A) Dorsal view of Akkaraman sheep's forefeet, (pd) digit, (B) Medial view of the interdigital region in Akkaraman sheep, (C) Appearance of the sinus interdigitalis

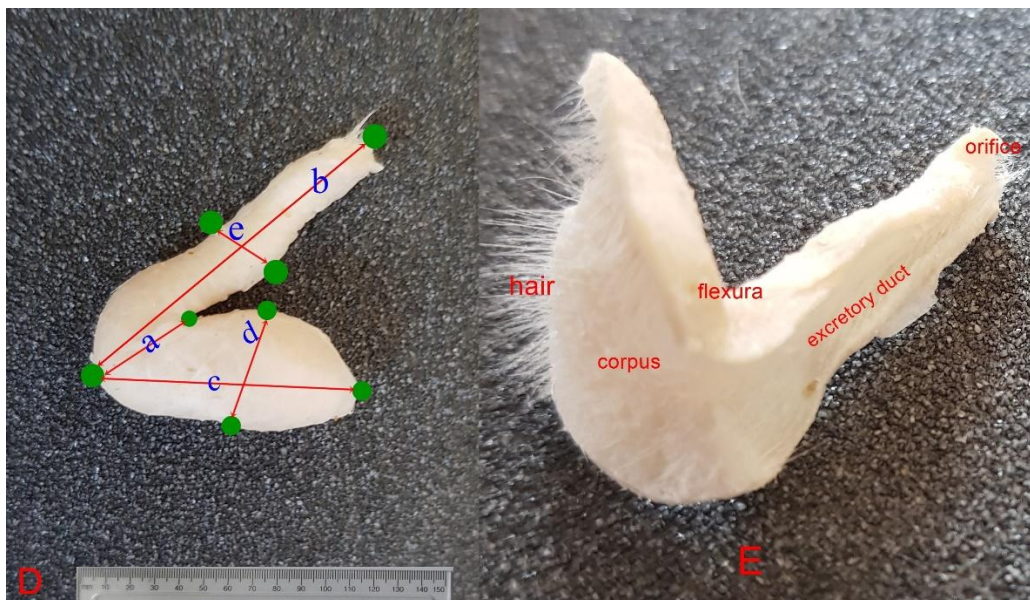


Figure 3. (D) Sinus interdigitalis and its parts (lateral of views). (a) flexura length, (b) excretory duct length, (c) corpus length, (d) corpus width, (e) excretory duct width. (E) Internal view of sinus interdigitalis

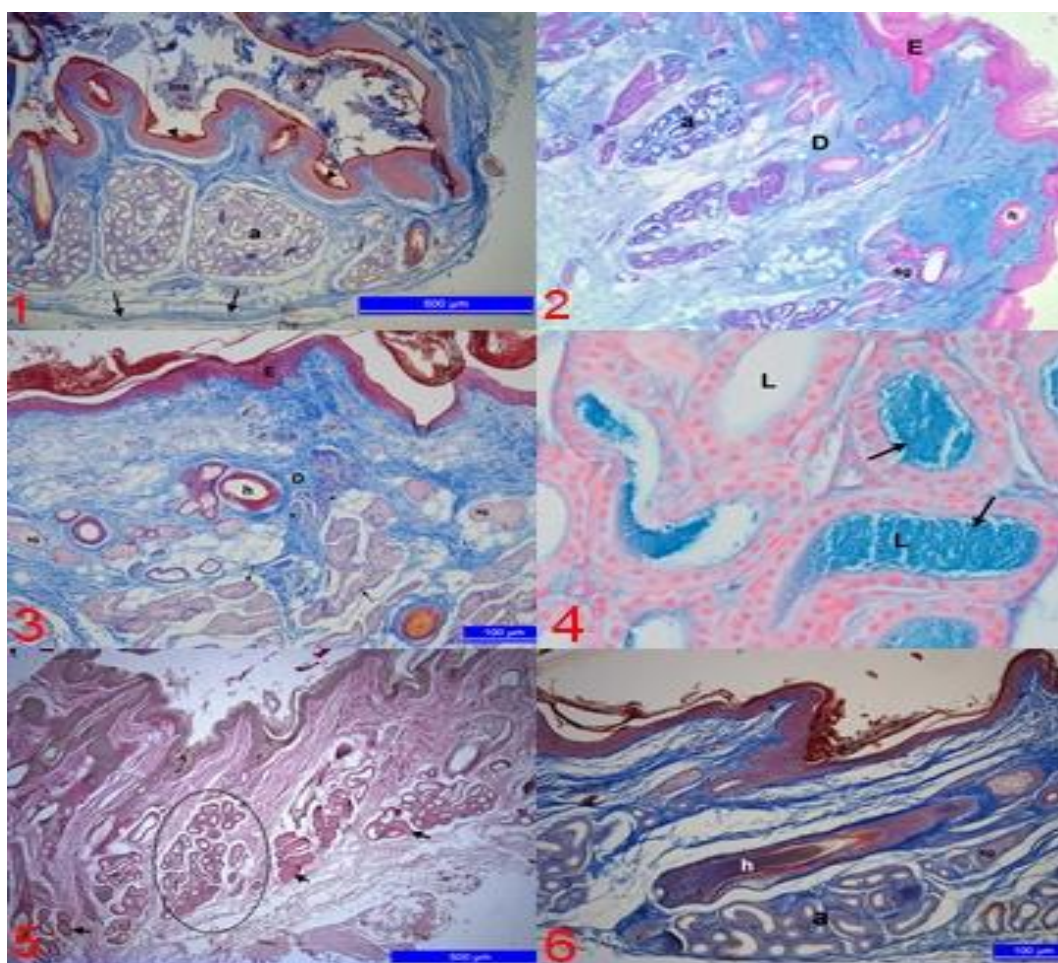


Figure 4. (1) Histologic structure of the sinus interdigitalis of sheep. Epidermis keratinized layer (arrowhead), apocrine sweat glands (a), interdigital lumen filled with mass secretion (ms), Collagen fibres (arrows) in the capsule. Crossman's triple staining. Bar: 500 μ m. (2). Epidermis (E), dermis (D), hair follicles (h), sebaceous glands (Sg), apocrine sweat glands of the interdigital sinus of the sheep (a), Alcian blue staining (Ph 2.5). Bar: 500 μ m. (3). Epidermis (E), dermis (D), hair follicles (h), sebaceous glands (Sg), apocrine sweat glands (arrows), arrector pili muscle (arrowheads), Crossman's triple staining. Bar: 200 μ m. (4). AB (+) secretion of apocrine glands in the sinus interdigitalis (arrows), the lumen of apocrine sweat gland (L), Alcian blue staining (Ph 2.5). Bar: 50 μ m. (5). PAS (+) secretion of apocrine glands in the sinus interdigitalis (arrows), apocrine sweat glands (circle shape), PAS staining. Bar: 500 μ m. (6). Hair follicles (h), sebaceous glands (Sg), apocrine sweat glands (a), Crossman's triple staining. Bar: 200 μ m.

In the statistical test measurements, it was observed that the weight and body length measurements of the forefoot sinus interdigitalis were statistically significant at $P < 0.05$ levels. It has been determined that the excretory duct length, excretory duct width, body width and flexura measurements between the fore and hind feet are insignificant (Table 1; $p < 0.05$).

On histological examinations, the lumen of the interdigital gland was filled with secretions from the apocrine sweat glands and sebaceous glands (Figure 3, 4). It was observed that the wall of the sinus interdigitalis consisted of three layers: epidermis, dermis and fibrous capsule. The epidermis layer consisted from squamous keratinized

epithelium. Hair follicles, sebaceous glands, muscle fibers, nerve plexus, arrector pili muscles and many large apocrine sweat glands were detected in the dermis. Each hair follicle of different sizes was surrounded by connective tissue, and arrector pili muscles and sebaceous glands were seen around the hair follicles (Figure 3, 4). The fibrous capsule surrounding the outside of the sinus interdigitalis was a tight connective tissue structure consisting of parallel collagen fiber bundles containing blood vessels, nerves and fat cells (Figure 4).

Apocrine sweat glands were located like in clusters in the lower part of the dermis (Figure 4). The epithelium of the apocrine gland separated by connective tissue regions consisted of single-layered cubic cells (Figure 4). Apocrine sweat gland secretion, PAS and AB reacted positively (Figure 4). This reaction shows respectively that the apocrine gland secretion consists of neutral mucopolysaccharides and sulfated acidic mucopolysaccharides.

4. Discussion and Conclusion

Janicki *et al.* (2003) reported that sinus interdigitalis was found only on the hind feet of the male roe and not on the forefeet. In other literature reviews, it has been reported that in sheep, the sinus interdigitalis is present in both the fore and hind feet, and it has an excretory duct flowing hole in its structure and resembles a pipe consisting of neck and body in shape (Awaad *et al.*, 2015; Behzad Mobini & Adermanabadi, 2017; Gürbüz *et al.*, 2017).

Çalışlar (1970) reported that there was no sinus interdigitalis in the feet of Ankara and hair goats, and some authors Karahan *et al.* (2007) and Bahadır & Yakısık (1988) reported that this gland was rudimentary in goats. In this study, it was determined that Akkaraman sheep were found on both fore and hind feet and resembled a thick fishing pin with its bulging body part. Some researchers have reported that in various sheep species such as Dubska Paramenka, Egyptian Native, Hemshin, Hasmer and Hasak sheep, the body part of the gland is in the middle of the phalanx media and the excretory duct hole is at the level of articulation phalangis proximalis. (Uğurlu 1991; Avdic *et al.*, 2013; Awaad *et al.*, 2015; Gürbüz *et al.*, 2017; Kara *et al.*, 2020).

The body part of the sinus interdigitalis and the place where the hole was located was also similar to the literature information in Akkaraman sheep.

The gland weight of sinus interdigitalis was reported respectively 1.05 g and 0.56 g in Hasak (Kara *et al.*, 2020), sheep for the fore and hind feet and 1 g and 0.6 g in Hasmer (Kara *et al.*, 2020) sheep and 1.88 g and 0.95 g in Hemshin (Gürbüz *et al.*, 2017) sheep and 1.25 g and 0.81 g in awassi sheep (Yılmaz *et al.*, 2017). In Akkaraman sheep, which are our research material, it was determined as 0.76 g in fore and 0.53 g in hind feet. According to this information, it was observed that Akkaraman sheep had lower fore and hind feet sinus interdigitalis weights than Hasak, Hasmer, Hemshin and Awassi sheep. It has been determined that the canal length (25,49 mm \pm 1,02) and diameter (3,43 mm \pm 0,1) of the sinus interdigitalis in the fore feet of Akkaraman sheep are longer and larger than the excretory duct length (22,86 mm \pm 1,05) and diameter (3,36 \pm 0,17) of the hind feet. According to the previous studies, fore and hind feet lengths and diameters were similar in some sheep breeds such as Hemshin, Iran Bakhtiari, Hasmer and Hasak sheep (Gürbüz *et al.*, 2017, Behzad Mobini & Adermanabadi, 2017, Kara *et al.*, 2020). However, it was determined that they did not differ on the fore and hind feet lengths of Kivircik sheep (Demiraslan *et al.*, 2014).

Body length and diameter of sinus interdigitalis in the fore and hind feet were reported (Kara *et al.*, 2020) to be 16.33 and 6.73; 12.89 and 5.24 in Hasak sheep (Kara *et al.*, 2020); 17.25 and 7.15; 13.67 and 5.59 in Hasmer sheep (Kara *et al.*, 2020), respectively. In our study, the body length and diameter of the sinus interdigitalis in the fore and hind feet of Akkaraman sheep were determined as $15,51\pm 0,73$; $6,59\pm 0,42$ and $12,21\pm 0,94$; $6,20\pm 0,43$, respectively. In appropriately with the literature data of our findings, it is concluded that the body length and diameter of the forefoot are larger than the hindfoot. Nevertheless, according to the literature information, Demiraslan *et al.* (2014) reports that the diameter of body of the sinus interdigitalis of the hind leg is bigger than that of the fore foot in Kivircik sheep, Uğurlu (1991) reports that there is no significant morphological difference between sinus interdigitalis in the fore and hind feet. In the literature, it has been reported that the wall structure of histological examinations of sinus interdigitalis: the gland consists of three layers: epidermis, dermis and fibrous capsule in Tuj (Aslan *et al.*, 2010), Akkaraman (Karahana *et al.*, 2007), Kivircik (Süzer *et al.*, 2016), the native sheep of Egyptian (Baladi sheep and goats) (Parillo & Diverio, 2009) and Iran native sheep (Abbasi *et al.*, 2009), Barbados black belly (Mohamed & Adogwa, 2016). Janicki *et al.* (2003), unlike the roe deer, reports that there is no connective tissue in the interdigital gland. In our study, the histological and histochemical findings of the layers of the gland were similar to those of Tuj (Aslan *et al.*, 2010), Akkaraman (Karahana *et al.*, 2007), Kivircik (Uğurlu, 1991), Barbados black belly (Mohamed & Adogwa, 2016) and native sheep of Egyptian (Baladi sheep and goats), and Iranian native sheep (Lori sheep) (Abbasi *et al.*, 2009).

In histological examinations, Süzer *et al.* (2016) is reported that sinus interdigitalis has hair follicles, sebaceous and sweat glands, and m. arrector pili in the dermis layer in Kivircik sheeps; Mohamed & Adogwa, (2016) is reported in Barbados black belly sheep. Aslan *et al.* (2010) reported that in Tuj sheep, there are lymph nodes in addition to these formations in the dermis layer. In our research material, similar structures were detected in the dermis in coherently with the literature (Süzer *et al.* 2016; Mohamed & Adogwa, 2016). But lymph nodes were not found. Histochemically, apocrine sweat gland secretion showed positive reaction in Hasmer (Kara *et al.*, 2020) and Hasak (Kara *et al.*, 2020) sheep, Awaad *et al.* (2015) Egypt native sheep (Baladi sheep and goats), Parillo & Diverio (2009) Red deer, whereas apocrine sweat gland secretion showed positive reaction to PAS, it showed a negative reaction with AB; Abbasi *et al.* (2009) has reported PAS negative and AB positive reaction in Iranian native sheep (Lori sheep). In our study, both PAS and AB were found to be positive.

The macroanatomical, morphometric, histological and histochemical features of the sinus interdigitalis of Akkaraman sheep showed significant similarities with other sheep breeds. However, it is thought that the difference in some findings may be due to the place of life, different diet and genetic characteristics. It is expected to be a resource for studies about Akkaraman sheep.

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