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Measurement of Aqueous Tear Production Rate with the Endodontic Absorbent Paper Point Tear Test: Reference Values for Akkaraman Sheep and Damascus Goats *

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To determine physiological reference values of aqueous tear production using the endodontic absorbent paper point tear test (EAPPTT) in healthy Akkaraman sheep and Damascus goats and to evaluate its clinical applicability in small ruminants. A total of 100 clinically healthy animals (50 Akkaraman sheep and 50 Damascus goats) were included. EAPPTT was performed bilaterally for 15 seconds using sterile, standardized size 30, 28-mm-long endodontic paper points without topical anesthesia or forceps. Measurements were obtained between 10:00 AM and 2:00 PM and performed by a single investigator to minimize variability. No significant differences were observed between right and left eyes (p=0.493 for sheep; p=0.077 for goats). Therefore, data were pooled (n= 100 eyes per breed). Mean ± SD EAPPTT values were 13.89 ± 2.35 mm/15 s in Akkaraman sheep and 13.21±2.24 mm/15 s in Damascus goats. The calculated 95% reference interval was 9.00–18.00 mm/15 s for both breeds. The test was well tolerated, with no ocular complications observed. The EAPPTT represents a rapid, atraumatic, and forceps-free method for assessing tear production in small ruminants. The reference values established in this study provide baseline data that may support clinical evaluation of the ocular surface and diagnosis of conditions such as keratoconjunctivitis sicca in both field and clinical settings.

Key Words: *Akkaraman sheep, damascus goat, endodontic paper point, reference values, tear production*

Endodontik Emici Kâğıt Uç Gözyaşı Testi ile Aköz Gözyaşı Üretim Miktarının Ölçülmesi: Akkaraman Koyunları ve Halep Keçilerinde Referans Değerler

Bu çalışmanın amacı, sağlıklı Akkaraman koyunları ve Halep keçilerinde endodontik emici kâğıt uç gözyaşı testi (EAPPTT) kullanılarak aköz gözyaşı üretiminin fizyolojik referans değerlerini belirlemek ve bu yöntemin küçük ruminantlarda klinik uygulanabilirliğini değerlendirmektir. Çalışmaya klinik olarak sağlıklı toplam 100 hayvan (50 Akkaraman koyunu ve 50 Halep keçisi) dahil edildi. EAPPTT, standart 30 numara ve 28 mm uzunluğunda steril endodontik emici kâğıt uç kullanılarak, topikal anestezi ve forseps uygulanmaksızın, her iki gözde 15 saniye süreyle uygulandı. Ölçümler, gün içi değişkenliği minimize etmek amacıyla 10:00–14:00 saatleri arasında ve gözlemci kaynaklı değişkenliği en aza indirmek için tek bir araştırmacı tarafından yapıldı. Sağ ve sol gözler arasında istatistiksel olarak anlamlı bir fark saptanmadı (koyunlar için p=0.493; keçiler için p=0.077). Bu nedenle veriler havuzlandı (her ırk için n = 100 göz). Ortalama ± standart sapma (SS) EAPPTT değerleri Akkaraman koyunlarında 13.89 ± 2.35 mm/15 sn, Halep keçilerinde ise 13.21 ± 2.24 mm/15 sn olarak belirlendi. Her iki ırk için hesaplanan %95 referans aralığı 9.00–18.00 mm/15 sn idi. Test, gözlemlenebilir oküler komplikasyon olmaksızın iyi tolere edildi. EAPPTT, küçük ruminantlarda gözyaşı üretiminin değerlendirilmesinde hızlı, atravmatik ve forseps gerektirmeyen bir yöntemdir. Bu çalışmada elde edilen referans değerler, oküler yüzeyin klinik değerlendirilmesi ve keratokonjunktivitis sikka gibi hastalıkların tanısında kullanılabilecek temel veriler sunmaktadır.

Anahtar Kelimeler: *Akkaraman koyunu, halep keçisi, endodontik emici kâğıt uç, referans değerler, gözyaşı üretimi*

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Introduction

The precorneal tear film plays a fundamental physiological role in maintaining ocular surface integrity, visual function, and overall welfare in farm animals (1-4). Disruption of tear homeostasis compromises ocular health, predisposing the eye to conditions such as keratoconjunctivitis sicca and epiphora, which may ultimately impair animal productivity (1, 3, 4).

Routine quantitative assessment of aqueous tear production is commonly performed using the 60-second Schirmer tear test (STT) and the 15-second phenol red thread test (PRTT) (3,4). However, the 5-mm-wide STT strips pose placement challenges in animals with small palpebral fissures (5) and induce mechanical discomfort, leading to reflex tearing that may mask basal secretion and reduce corneal sensitivity (6). Although the PRTT shortens application time, its highly flexible thread often requires forceps for proper placement in the conjunctival fornix, resulting in iatrogenic irritation, increased patient stress, and variable test outcomes (3, 7).

To overcome these methodological limitations, Lange et al. (8) introduced the endodontic absorbent paper point tear test (EAPPTT). This method utilizes standardized

size 30, 28-mm-long sterile paper points with a relatively rigid, conical structure, enabling rapid placement into the ventral conjunctival fornix without the need for forceps. As a result, the test can be performed by a single operator while minimizing iatrogenic irritation, particularly in manipulation-sensitive animals (3, 7-9). Furthermore, unlike the STT, EAPPTT preserves corneal sensitivity without altering the corneal touch threshold (6). By reducing stress-induced reflex tearing and improving ocular comfort, EAPPTT represents a practical, minimally invasive alternative for assessing tear production (6).

In veterinary ophthalmology, breed-specific reference values are essential, as tear production varies considerably across species and environmental conditions (10-12). Although normative data are available for several small ruminant breeds (4, 12, 16, 17), a clear gap exists for Akkaraman sheep and Damascus goats, which are indigenous breeds adapted to continental and arid climates, respectively (13-15). Accordingly, the primary objective of this study was to establish the first physiological EAPPTT reference values for these breeds and to evaluate the clinical applicability of the EAPPTT in small ruminant practice.

Materials and Methods

Research and Publication Ethics: Official permission for the study was obtained from the Provincial Directorate of Agriculture and Forestry, Şanlıurfa, Türkiye (Approval No: E59855012-325.04.02-19695294). The study protocol was reviewed by the Harran University Animal Experiments Local Ethics Committee, which determined that formal ethical approval was not required, as all procedures were classified as non-experimental clinical veterinary practices (Decision No: 2025/007/05). Written informed consent was obtained from all flock owners prior to the study.

Animals and Study Design: This study included 100 clinically healthy small ruminants, consisting of 50 Damascus goats and 50 Akkaraman sheep aged 1-8 years, sourced from private commercial flocks. The sex distribution was 37 females and 13 males in the Akkaraman sheep group, and 33 females and 17 males in the Damascus goat group. Systemic health was verified through routine clinical examination before inclusion. General attitude, body condition, mucous membrane appearance, capillary refill time, rectal temperature, heart rate, respiratory rate, and the absence of clinical signs of systemic disease were assessed. A complete bilateral ophthalmic examination was performed in all animals before EAPPTT measurements. The eyelids, conjunctiva, cornea, anterior chamber, and lens were evaluated using slit-lamp biomicroscopy (XL-1®, Shin-Nippon, Tokyo, Japan). Posterior segment evaluation was performed by direct ophthalmoscopy. Fluorescein staining was used to exclude corneal epithelial defects. Neuro-ophthalmic examination included menace response, dazzle reflex, and direct and consensual pupillary light reflexes. Animals showing epiphora, ocular discharge, blepharospasm, conjunctival abnormality, corneal opacity or ulceration, anterior segment abnormality, fundic abnormality, or clinical signs of systemic illness were excluded from the study.

All procedures were conducted in accordance with good clinical practices. Animals were gently restrained to minimize handling stress. All EAPPTT measurements were performed by a single trained investigator. The initial eye tested (right or left) was randomly assigned, and all measurements were conducted in the same environmental setting. Measurements were performed within the same daily time interval, between 10:00 AM and 2:00 PM, to reduce diurnal variation. Although measurements were carried out under comparable field conditions, objective environmental parameters, such as ambient temperature, relative humidity, and airflow, were not recorded.

Measurements were performed in awake animals in their natural head position without topical anesthesia. Standardized sterile endodontic paper points (Size 30; 28 mm length; Roeko Color, Langenau, Germany) (18) were inserted into the middle-to-lateral third of the ventral conjunctival fornix by gently everting the lower eyelid, without the use of forceps (Figure 1).

Although standard EAPPTT protocols use a 60-second retention time (8), high tear production in small ruminants rapidly saturates 28-mm paper points, resulting in a ceiling effect (2). To prevent saturation and maintain measurement sensitivity, the test duration was reduced to 15 seconds. The wetted length was immediately measured using a digital caliper (0.01 mm precision) and recorded as mm/15 s.

Figure 1. Measurement of aqueous tear production using the endodontic absorbent paper point tear test (EAPPTT) in an Akkaraman sheep

Table 1. EAPPTT values and breed-specific reference intervals in Akkaraman sheep and Damascus goats*

Breed	Measurement Unit	n	Mean EAPPTT ± SD (mm/15 s)	95% Reference Interval** (mm/15 s)	*p*-value***
Akkaraman Sheep	Right Eye (OD)	50	13.76 ± 2.65	N/A	0.493
	Left Eye (OS)	50	14.02 ± 2.00	N/A	
	Pooled Data (Both Eyes)	100	13.89 ± 2.35	9.00 – 18.00	N/A
Damascus Goat	Right Eye (OD)	50	13.62 ± 2.39	N/A	0.077
	Left Eye (OS)	50	12.80 ± 2.02	N/A	
	Pooled Data (Both Eyes)	100	13.21 ± 2.24	9.00 – 18.00	N/A

* SD: Standard Deviation, OD: Oculus Dexter, OS: Oculus Sinister, N/A: Not Applicable.

** Reference intervals were calculated from the pooled dataset (n=100 eyes per breed) using non-parametric methods (2.5^{th}–97.5^{th} percentiles).

*** Exact *p*-values for paired statistical comparisons between the right and left eyes of the same individuals.

Statistical Analysis: Statistical analyses were performed using GraphPad Prism version 9. Normality was assessed using the Shapiro–Wilk test. Bilateral comparisons were conducted using a paired Student's t-test or Wilcoxon signed-rank test, as appropriate. As no significant differences were observed between eyes, data were pooled (n=100 eyes per breed) to establish non-parametric 95% reference intervals (2.5th–97.5th percentiles) in accordance with CLSI EP28-A3c guidelines. No outliers were detected; therefore, all observations were retained for reference interval estimation. Data are presented as mean ± standard deviation (SD), range, and 95% reference intervals (RIs), with $p<0.05$ considered statistically significant.

Sample Size: For breed-specific reference interval estimation, 50 Akkaraman sheep and 50 Damascus goats were included. Because no significant difference was detected between right and left eyes, bilateral data were pooled, providing 100 ocular observations per breed for reference interval estimation. In addition, an a priori power analysis ($\alpha = 0.05$, $1-\beta = 0.80$) performed using G*Power 3.1, based on variance estimates from previous small ruminant tear studies (17, 19), indicated that 50 animals per breed were sufficient to detect clinically relevant bilateral differences. The study was not primarily designed or powered to evaluate sex- or age-related subgroup differences.

Results

EAPPTT measurements were completed in all animals, with no ocular complications observed. Detailed bilateral (OD and OS) tear production values are presented in Table 1. As no significant inter-eye differences were observed ($p=0.493$ for Akkaraman sheep; $p=0.077$ for Damascus goats), data were pooled (n=100 eyes per breed). Based on this pooled dataset, the mean EAPPTT values were 13.89±2.35 mm/15 s for Akkaraman sheep and 13.21±2.24 mm/15 s for Damascus goats. The calculated 95% reference interval was 9.00–18.00 mm/15 s for both breeds (Table 1).

Discussion

In this study, physiological EAPPTT reference intervals were established for clinically healthy Akkaraman sheep and Damascus goats. The absence of significant differences between right and left eyes allowed bilateral data to be pooled for breed-specific reference interval estimation. The EAPPTT was well tolerated in all animals, and no ocular complications were observed, supporting its practical applicability in small ruminant ophthalmology.

The EAPPTT offers several procedural advantages over conventional tear tests. Although the STT and PRTT are widely used in veterinary ophthalmology (3, 20, 21), STT strips may cause mechanical irritation and reflex tearing, whereas the flexible PRTT thread may require forceps for stable placement (3, 6, 22). In contrast, the relatively rigid and conical structure of the endodontic paper point enables rapid, atraumatic, and forceps-free placement by a single operator (2, 3, 8).

The maximum wetting value observed in this study was 18 mm, which remained below the 28 mm capacity of the paper points. This supports the use of the 15-second protocol, which was selected to prevent saturation and maintain measurement sensitivity in small ruminants with relatively high tear production.

Because previous EAPPTT data are not available for Akkaraman sheep or Damascus goats, the present findings were interpreted in relation to previously reported STT and PRTT values in small ruminants. Previous studies report 60-second STT means ranging from 10.6 to 18.8 mm/min (4, 17, 19), whereas 15-second PRTT values may exceed 20 mm in various breeds (4). The EAPPTT values obtained in the present study may reflect the high wicking capacity and narrow

surface area of the paper points, which can produce a greater wetting length for a given tear volume (23).

Beyond the physical properties of the EAPPTT material, the reference values observed in Akkaraman sheep may reflect breed-specific ecological adaptations. Variations in tear production reported in other ovine populations (12, 16) indicate that lacrimal physiology is influenced by both genetic and environmental factors (10, 12). Given the Akkaraman breed's adaptation to the harsh, drought-prone continental climate of Central Anatolia (13), the relatively high tear production observed in this study may represent a compensatory response to increased evaporation and the risk of ocular surface desiccation. This interpretation is consistent with lacrimal patterns described in other arid-adapted species, such as the Barbary sheep (24).

Similarly, the reference values observed in Damascus goats should be interpreted in the context of their adaptation to arid climates (15). Tear production values reported in other caprine species show considerable variation (17, 25). The values obtained in the present study are comparable to those reported for arid-adapted species such as the Nubian ibex (26). In combination with the high absorptive capacity of the EAPPTT material (2), this pattern may reflect an adaptive response of the lacrimal unit to increased thermal and evaporative stress, contributing to the maintenance of ocular surface homeostasis under harsh environmental conditions. However, these interpretations should be considered cautiously because the present study was not designed to directly test the effects of climate, season, age, or sex on tear production.

Although this study provides the first physiological EAPPTT reference values for Akkaraman sheep and Damascus goats, several limitations should be acknowledged. First, only clinically healthy animals were included; therefore, tear dynamics in ocular diseases such as infectious keratoconjunctivitis were not evaluated. Second, although measurements were performed within the same time interval and under comparable field conditions, objective environmental parameters, such as ambient temperature, relative humidity, and airflow, were not recorded. This may have introduced unexplained variability and reduced the reproducibility of measurements under different environmental conditions. Third, the study population included animals aged 1-8 years, but age-based subgroup analysis was not performed. In addition, although sex distribution was recorded, sex was not analyzed as an independent variable because the study was not primarily designed to evaluate sex-related differences in tear production. Moreover, the number of male and female animals was not balanced within each breed group, which limited the statistical reliability of sex-based subgroup comparisons. All measurements were performed by a single trained investigator to minimize procedural variability; however, inter-observer agreement was not assessed. Future studies should evaluate inter-observer reliability using approaches such as the intraclass correlation coefficient or Bland-Altman analysis and should include balanced sex distributions, age-stratified analyses, and objective environmental monitoring. Finally, the 15-second EAPPTT protocol requires further validation against the standard 60-second applications and conventional tear tests such as STT and PRTT.

In conclusion, the EAPPTT represents a rapid, atraumatic, and practical method for assessing tear production in small ruminant ophthalmology. The breed-specific reference values established for Akkaraman sheep and Damascus goats provide baseline data that may support ocular surface evaluation and the diagnosis of tear film disorders such as keratoconjunctivitis sicca in both field and clinical settings.

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