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Assessing chronic stress in sheep using wool cortisol: A comparative study of transhumant and intensive systems

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ABSTRACT

Chronic stress profoundly affects livestock welfare, productivity, and resilience, yet its dynamics across different production systems remain insufficiently understood. This study investigates chronic stress in Karayaka sheep within three distinct production systems—long-distance transhumance (LDT), short-distance transhumance (SDT), and intensive systems (IS)—using wool cortisol concentrations as a non-invasive biomarker. Wool samples were collected from 45 ewes and 30 lambs over an eight-month period (October, December, January, February, March, May, July, and September) to evaluate cortisol accumulation as a reflection of chronic stress. Cortisol levels were analyzed using ELISA immunoassay after steroid extraction. The results revealed significant impacts of production systems and sampling periods on cortisol concentrations ($P < 0.05$). The marginal mean of wool cortisol was higher ($P < 0.05$) in SDT production systems (5.49 ± 0.20 pg/mg) compared with IS (4.85 ± 0.20 pg/mg). Seasonal variations were prominent, with cortisol peaking during winter months (January–February) and declining in March ($P < 0.05$). Notably, cortisol concentrations decreased during high-altitude summer grazing but spiked post-migration (May). Lambs exhibited lower cortisol levels (5.11 ± 0.17 pg/mg) compared with adult sheep (5.34 ± 0.17 pg/mg). Statistical analysis underscored significant interactions between production systems, sampling periods emphasizing the interplay of environmental stressors and animal physiology. These findings highlight the linkage of stress to environmental transitions and the critical need for adaptive management strategies tailored to production systems to reduce chronic stress and improve animal productivity and to advance sustainable livestock management.

1. Introduction

Assessing the impact of production systems on chronic stress in sheep is a critical area of research with significant implications for animal welfare, productivity, and sustainable management. Chronic stress—distinct from acute stress, which is typically short-term and adaptive—results from the prolonged activation of the hypothalamic-pituitary-adrenal (HPA) axis, impairing immune function, reproductive efficiency, and overall health and performance (Ataallahi et al., 2022). While acute stress triggers short-term adaptive mechanisms (Narayan et al., 2021), chronic stress can have enduring negative effects. Cortisol, synthesized by the adrenal glands, has emerged as a reliable biomarker for evaluating stress in animals (Asencio et al., 2024). It is traditionally measured in blood, saliva, and feces to reflect acute physiological responses (Ghassemi Nejad et al., 2022). However, these biological matrices are inherently limited in capturing long-term stress exposure. The determination of cortisol levels through such samples may be invasive, often requiring the physical restraint and capture of animals, potentially inducing additional acute stress that could confound assessments of chronic stress.

In light of these challenges, numerous studies have validated the utility of non-invasive wool cortisol analysis for detecting chronic stress across various species (Koren et al., 2002; Davenport et al., 2006; Comin et al., 2013; Peric et al., 2013; Stubsjøen et al., 2015; Fürtbauer et al., 2019). Measuring cortisol in wool offers distinct advantages, particularly its capacity to reflect cumulative stress over extended periods,

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rather than transient fluctuations influenced by circadian rhythms or immediate stimuli (Salaberger et al., 2016; Burnard et al., 2017; Duran et al., 2017). Given that wool grows approximately 1 cm per month (Wennig, 2000), it serves as a retrospective archive, allowing researchers to estimate the timing of stress exposures without necessitating real-time sampling (Baumgartner et al., 1989). Burnard et al. (2017) further note that the continuous growth of wool fibers enables the construction of a longitudinal cortisol profile, enhancing the capacity to evaluate stress accumulation and physiological adaptation over time (Kotsampasi et al., 2024). Additionally, wool sampling is non-invasive and minimally disruptive, and the samples maintain their integrity over prolonged storage (Ashley et al., 2011; Carlitz et al., 2014; Duran et al., 2017; Peric et al., 2020).

Variations in wool cortisol accumulation across different production systems reflect the range of environmental and management stressors inherent to each system. While extensive systems allow for greater behavioral freedom, they expose animals to stressors such as extreme temperatures, inadequate shelter, resource scarcity, parasite load, and predation risk. These external factors may significantly modulate cortisol levels, highlighting the complex interaction between environmental and physiological stressors. In contrast, intensive systems may buffer environmental uncertainties but introduce stressors related to confinement, restricted movement, and limited behavioral expression (Ghassemi Nejad et al., 2022). These contrasting dynamics emphasize the importance of evaluating how different management regimes affect chronic stress physiology. Of particular interest are transhumance systems, traditional livestock management practices characterized by seasonal migration between lowland winter and highland summer pastures. These movements, typically conducted on foot, optimize forage availability and enable livestock to adapt to climatic variability.

To date, there is a paucity of research comparing wool cortisol concentrations across production systems while accounting for animal age as a factor in chronic stress assessment. This study aims to address this gap by examining wool cortisol concentrations in Karayaka sheep—a Turkish indigenous breed—managed under three distinct systems: long-distance transhumance (LDT), short-distance transhumance (SDT), and intensive system (IS). By capturing stress dynamics across an eight-month production cycle and incorporating physiological and environmental transitions, the present study offers a novel perspective on chronic stress in sheep. In addition to evaluating production systems, the study provides an in-depth interpretation of monthly climate variables across these systems, enabling a contextualized understanding of how environmental fluctuations interact with animal stress physiology. These findings are expected to inform the development of adaptive welfare strategies, promote sustainable livestock management, and support the resilience of Karayaka sheep within extensive production frameworks.

2. Materials and methods

2.1. Animal material and housing

This study was registered, and the protocol underwent review by Local Ethics Committee for Animal Experiments of Ondokuz Mayıs University, Turkey. Approval from an ethics committee was deemed unnecessary for this study (Reference No: 2019/52) since wool samples from both ewes and their lambs were collected non-invasively and without causing pain.

Three distinct production systems were utilized to assess chronic stress in pure Karayaka ewes and their lambs. The Karayaka sheep breed, indigenous to Turkey, is a resilient and economically significant dual-purpose breed well-adapted to the mountainous and semi-arid regions of Central Anatolia (Sen et al., 2011). Known for thriving on natural pastures with minimal supplementation, it contributes to sustainable agriculture and rural livelihoods.

A total of 75 Karayaka sheep (45 ewes, 30 lambs) were evaluated across three production systems: long-distance transhumance (LDT), short-distance transhumance (SDT), and intensive system (IS). Sample sizes were determined using power calculations to ensure statistical robustness. Group 1 (LDT) involved seasonal migration of ewes on foot (~240 km in 20 days) from Tokat (Erbaa province) to the Karagöl highlands (Giresun). Group 2 (SDT) involved a shorter migration (~80 km in 7 days) to the Niksar highlands (Fig. 1). Group 3 (IS) involved no migration; animals were kept year-round in permanent enclosures and grazed locally, with supplemental feeding during forage-limited periods. Table 1 presents the monthly maximum, minimum, and average temperatures (°C), relative humidity (%), and total precipitation (mm) recorded across the three sheep production systems—long-distance transhumance (LDT), short-distance transhumance (SDT), and intensive system (IS)—from September 2021 to September 2022. These environmental data were used to contextualize the physiological responses observed in animals, providing a basis for interpreting chronic stress patterns in relation to climatic variability across systems.

Transhumant flocks migrated on foot, accompanied by experienced herders and Kangal dogs for flock control and predator protection. Migration routes were planned around natural water sources; in dry areas, herders carried water in containers. Once at the summer pastures, flocks grazed freely under daily supervision, with grazing sessions timed to avoid heat stress. Ewes in each group ($n = 15$) were similar in live weight, body condition, and parity (≥ second parity). Lambs ($n = 10$ per group) were singletons, approximately 7–8 months old during the first sampling in October. The average ewe age was 2 years, with live weights of 57.1 ± 3.8 kg (SDT), 56.2 ± 4.1 kg (LDT), and 52.3 ± 3.5 kg (IS). Lamb weights averaged 43.0 ± 2.9 kg (SDT), 42.1 ± 3.2 kg (LDT), and 40.4 ± 2.7 kg (IS). Lambs from the same ewe were excluded to avoid genetic or maternal bias. Animals were not randomly assigned but selected from existing flocks within each production system. Selection was based on breed uniformity, age, parity, and body condition. Initially, 15 lambs were selected per group; however, two lambs died during the trial, and three were excluded due to illness and incomplete health records. All ewes in LDT and SDT systems were pregnant at the study's outset and lambed during winter housing (December–February) before migration. Feeding practices differed: transhumant sheep received alfalfa hay and ~1.7 kg of concentrate feed per day until April, then grazed exclusively on highland vegetation. Intensive-system sheep consumed ~2.5 kg of concentrate feed daily plus dry alfalfa hay. Lambs received ~1.2 kg of supplementary feed and followed similar pasture routines in the highlands. Key physiological, environmental, and management events across sampling periods are summarized in Table 3.

2.2. Wool sampling

Wool samples were collected on eight occasions throughout the 2021–2022 production cycle: October, December, January, February, March, May, July, and September. The first wool samples from lambs were obtained in October, when they were approximately 7–8 months old. Samples were obtained during routine farm activities and at critical physiological stages, rather than on a fixed monthly schedule, due to logistical constraints in transhumant systems. Extended migrations and temporary settlements limited accessibility and precluded frequent handling to avoid stress-induced alterations in physiological markers. Animals grazed on natural rangelands from early April to mid-October and were housed indoors during the remaining months. Farm and transport characteristics are detailed in Table 2.

2.3. Wool collection

Samples were obtained from the posterior vertex region of the neck, specifically between the cisterna magna and scapula bones, utilizing a cordless sheep clipper (Troy Clippers, China). Each sample measured approximately 2 cm in length and was collected from a designated area of 10×10 cm to ensure minimal damage to the skin. The wool samples

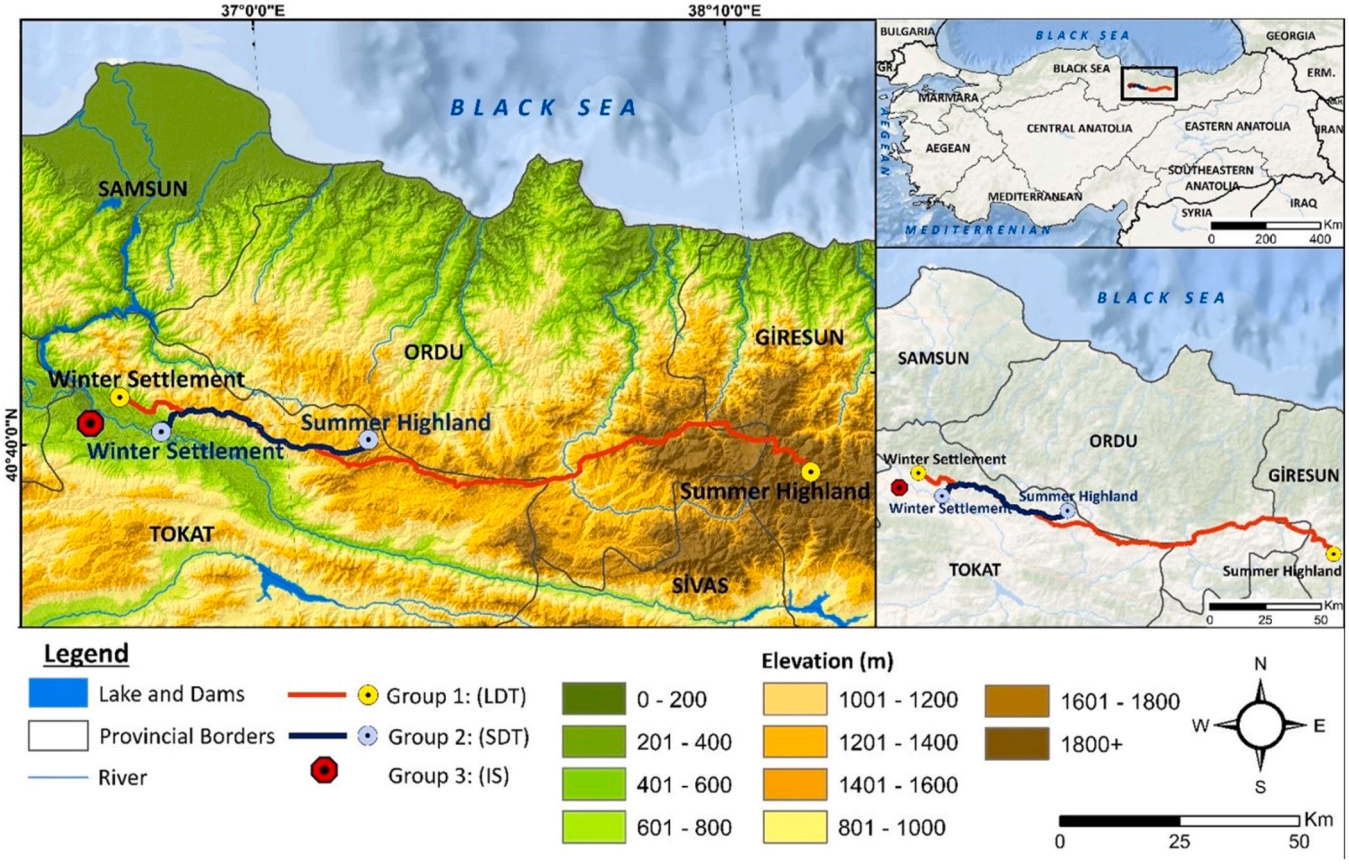

Fig. 1. Location map of production systems.

Table 1
Monthly climatic parameters (2021–2022) for the three production systems investigated in the study regions (MGM, 2025).

		2021				2022								
Production system	Climate Variable	Sep.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May	Jun.	July	Aug.	Sep
LDT	Max. temp.(°C)	28.6	27.1	26.1	23.4	20.0	21.7	23.1	29.4	30.2	28.5	29.0	31.1	40.3
	Min. temp (°C)	9.8	4.5	1.9	-4.8	-4.5	-0.5	-4.5	1.5	6.2	12.3	11.9	16.9	8.3
	Av. temp. (°C)	17.1	13.0	12.0	9.5	4.8	7.7	3.4	13.8	14.0	18.9	19.5	23.0	19.2
	Rel. hum (%)	80.1	80.6	69.6	58.5	70.2	66.1	78.8	62.5	72.5	82.5	80.0	81.7	75.5
	Total pre.(mm)	156.5	87.7	55.1	36.1	118.2	37.9	143.4	48.6	58.0	47.3	44.4	15.2	124.8
SDT	Max. temp.(°C)	25.8	23.4	18.9	13.0	8.3	10.0	13.3	24.5	26.8	29.9	26.0	32.3	33.7
	Min. temp (°C)	3.1	-0.4	-3.7	15.1	-13.8	-9.1	-14.1	-5.5	0.0	5.8	7.7	12.1	3.2
	Av. temp. (°C)	11.2	8.8	6.9	1.4	-3.6	-0.1	-3.2	9.5	9.0	14.7	14.0	19.0	15.3
	Rel. Hum. (%)	79.1	69.9	68.7	66.6	77.9	74.3	76.2	57.4	69.7	75.0	77.0	72.0	62.6
	Total pre. (mm)	55.9	30.1	62.1	39.1	97.6	40.1	52.1	35.0	62.3	64.3	18.9	20.6	48.6
IS	Max. temp.(°C)	32.9	29.2	26.0	22.7	19.5	21.8	25.2	33.3	36.5	34.4	35.2	38.7	39.8
	Min. temp (°C)	7.4	0.5	-1.9	-6.4	-13.9	-3.5	-4.3	2.2	4.0	13.8	11.4	17.1	7.0
	Av. temp. (°C)	18.9	13.8	9.3	7.7	3.1	6.5	5.0	14.9	16.2	22.0	22.3	26.5	21.1
	Rel. Hum. (%)	71.0	78.1	87.6	74.0	81.3	77.3	76.2	65.8	67.5	70.7	65.9	63.6	62.6
	Total pre. (mm)	32.7	16.6	25.2	19.5	121.4	38.9	52.5	33.3	44.6	64.1	2.2	0.7	23.8

LDT=Long-distance transhumace, SDT= Short- distance transhumance, IS= Intensive system, max. Temp.= Maximum Temperature, Min. Temp.= Minimum Temperature, Av. Temp. = Average temperature, Rel. Hum = Relative Humidity, Total pre= Total precipitation (mm), Sep= September, Oct=October, Nov=November, Dec=December, Jan=January, Feb=February, Mar=March, Apr=April, Jun=June, Jul=July, Aug=August.

were subsequently wrapped in foil, labeled with the animal ID and date of collection, and stored in a black plastic bag at room temperature until analysis.

2.4. Wool sample preparation and cortisol extraction

All of the wool samples were washed thrice in distilled water to remove external particles and left to air dry at room temperature for 24 h. Each wool sample (200 ± 30 mg) was washed with a rinse in 10 ml of 100 % isopropanol to eliminate any steroid residues from sweat or sebum, following methods proposed by Davenport et al. (2006) and then left to dry overnight in a desiccator. Dried samples were cut into 1–3 mm segments using sterile scissors, transferred to 10 ml Falcon tubes, and stored in dark, dry conditions at ambient temperature until processing. For cortisol extraction, 150 ± 10 mg of each wool sample was put in a labeled falcon tube with 5 ml of 100 % propanol and agitated for 3 min. The samples were then left to air dry on absorbent paper for two days. From these, 50 ± 3 mg dried samples were transferred to a Falcon tube

Table 2
Main characteristic of farms and the transport conditions from town to mountain rangelands.

	Group 1 (LDT)	Group 2 (SDT)	Group 3 (IS)
Winter housing	Concrete barn	Concrete	Semi open
Transport to mountain pasture	On foot	On foot	-
Duration, d	25	7	
Altitude, m	2233	1653	205
Mean slope, %	40	20	-
Supplements, kg/day	1.7/sheep 1.2/ lamb	1.7/sheep 1.2/ lamb	2.5/sheep

containing 5 ml of methanol, and the solution was incubated on a shaker at 37°C and 185 rpm for 18 h. This method, based on Koren et al. (2002), ensured effective cortisol extraction from the wool matrix, facilitating reliable quantification. After the extraction phase, each sample, along with its methanol component, was transferred into 15 ml Falcon tubes. Two Falcon tubes were designated per sample: one for centrifugation (containing the wool and methanol) and the other to facilitate methanol separation after centrifugation. The samples were then centrifuged at 2500 rpm for 15 min at 25 °C. Following this, the Falcon tubes with methanol were placed uncapped in a 37 °C incubator to ensure complete methanol evaporation, a process requiring approximately 5–6 days. Once fully dried, the samples were resuspended in 0.3 ml PBS using a vortex mixer and stored at −80 °C for subsequent ELISA analysis.

2.5. Wool cortisol enzyme-immunoassay

The quantification of cortisol levels in wool samples was conducted using enzyme-linked immunosorbent assay (ELISA) kits (Aviva Systems Biology, Cortisol ELISA Kit, catalog no: OKEH02541), adhering to the protocol specified by (Koren et al., 2002). Cortisol concentrations in samples were measured using an ELISA reader device (ThermoScientific, Renfrew, UK) adjusted to 450 nm wavelength. The cortisol concentrations were calculated and displayed in pg/mg (Meyer et al., 2014).

2.6. Statistical analyses

All statistical analyses were performed using SPSS 20.0 (licensed to Ondokuz Mayıs University). Data distribution was assessed for normality using the Kolmogorov-Smirnov test, while the homogeneity of variances was evaluated with Levene's test. The Kolmogorov-Smirnov test results showed that the wool cortisol concentrations shows the normal distribution ($p = 0.749$) and Levene test results showed that the variances were homogeneous ($p = 0.394$). Repeated measures analysis was used to evaluate the effects of production system (LDT=long distance transhumance, SDT=short distance transhumance, and IS=intensive system) and animal type (sheep and lamb) as a fixed factors where sampling periods were used as repeated time points. Mauchly's test confirmed sphericity (W=0.306; $p > 0.05$), validating the assumptions for repeated measures ANOVA. This approach ensured a robust evaluation of interaction effects and reliable interpretation of results. When significant effects were detected, Duncan's multiple range post-hoc test was applied to adjust for multiple comparisons and to identify specific group differences.

It should be noted that animals were not randomly assigned to production systems; instead, they were selected from naturally existing farm structures to reflect authentic management conditions. While this may introduce certain background variability, efforts were made to minimize confounding by selecting animals of the same breed, age, and parity. Consequently, the comparisons across systems should be interpreted as ecologically valid reflections of typical production settings rather than outcomes of a fully randomized design (Table 3).

Table 3
Sheep production timeline by sampling period.

Sampling period	Seasonal phase	Physiological status	Location	Management
October (OS)	Post migration, grazing on highland pasture	Non-lactating, pregnant	Highland pasture	Animals already migrated, adjusted to altitude
December (DS)	Winter housing, late pregnancy	late pregnancy	lowland winter housing	Barn housing, feed supplement
January (JaS)	Winter housing, peak lambing	lambing	lowland winter housing	Lambing, feed supplement, increased human interaction
February (FS)	Winter housing, lambing continues	lambing/early lactation	lowland winter housing	close monitoring, feed supplement
March (MS)	Winter housing, early lactation	lactating	lowland winter housing	In barns; feed supplement,pre-migration
May (MaS)	Post-migration, transition to highlands	lactating	highland pasture	Migration on foot, environmental stress
July (JS)	Highland grazing (mid-season)	dry	highland pasture	Free grazing, minimal human interaction
September (SS)	Highland grazing (late-season)	dry	highland pasture	Continued grazing; pre-migration

3. Results

Results indicated that the interactions of sampling period × production system × animal type ($p = 0.847$) and sampling period × animal type ($p = 0.387$) were statistically non-significant. Conversely, the interaction between sampling period and production system was found to be statistically significant ($p = 0.001$) (Table 4). Additionally, no significant interaction was identified between the production system and animal type (sheep/lamb). The interactions were subsequently evaluated using Duncan's multiple comparison test, where different letters in the same column denote statistically significant differences ($p < 0.05$).

Meteorological data collected for the study regions between September 2021 and September 2022 revealed notable climatic variation across the three production systems (Table 1). The LDT region exhibited moderate annual average temperatures, with summer maxima reaching up to 40.3°C and winter minima down to −4.8°C. The SDT region experienced the coldest winter conditions, with minimum temperatures dropping to −15.1°C in January and average temperatures frequently below freezing between December and March. In contrast, the IS region showed the highest summer temperatures, with maximum values exceeding 39°C, alongside winter minima as low as −13.9°C. Relative humidity varied moderately across systems, ranging from 57.4 % to 87.6 %, and total monthly precipitation ranged from 0.7 mm to 156.5 mm. These climatic differences characterize distinct thermal and hydric environments that correspond to the seasonal movements and housing practices in each production system.

The study's findings indicate a general reduction in wool cortisol trends across all sheep production systems in December, followed by an increase in January and February, and reached a secondary peak in March, particularly in LTD and SDT flocks, before declining sharply in June and rising again in September, with seasonal elevations during winter and early spring corresponding to physiological demands such as parturition. Lambs exhibited lower cortisol levels (5.11 ± 0.17 pg/mg) than ewes (5.34 ± 0.17 pg/mg) ($p > 0.05$), consistent with lower HPA

Table 4
Wool cortisol concentration according to sampling month and production system(pg/mg) pooled across ewes and lambs.

	Sampling month								
Production system	Oct	Dec	Jan	Feb	Mar	May	Jul	Sep	Sig.
LDT	5.91 ± 0.31[ab]	3.82 ± 0.51	5.73 ± 0.61	6.00 ± 0.43	4.82 ± 0.73[a]	7.00 ± 0.23[b]	3.33 ± 0.20	6.22 ± 0.23	0.001
SDT	6.50 ± 0.22[b]	5.10 ± 0.64	5.00 ± 0.32	4.90 ± 0.34	6.93 ± 0.55[b]	6.81 ± 0.73[b]	3.22 ± 0.20	5.65 ± 0.62	
IS	5.11 ± 0.52[a]	4.34 ± 0.52	4.44 ± 0.06	6.00 ± 0.71	4.70 ± 0.72[a]	4.74 ± 0.72[a]	3.41 ± 0.34	6.25 ± 0.45	
Sig.	0.037	0.199	0.173	0.224	0.030	0.015	0.749	0.575	

LDT=long distance transhumance, SDT=short distance transhumance, IS=intensive system
[a,b]The differences between the means shown with different letters in the same columns are significant ($p < 0.05$).

axis responsiveness in younger animals. Wool samples collected in May, after seasonal migration, showed elevated cortisol levels, indicating stress associated with the transition. In the transhumance system, cortisol levels decreased after moving to the highland pastures, with an increase observed again by late September.

Table 4 presents the differences in wool cortisol levels between the production system and the sampling period. The most notable finding is that the cortisol level was markedly elevated following the migration in April. This change was not observed in the intensive group. Accordingly, significant differences were identified in wool cortisol levels during the October, March, and May based on the production system. LDT sheep showed lower levels due to stable highland environments post-migration. The highest mean wool cortisol concentration was detected in the SDT group (5,49 ± 0,20; $P < 0.05$), while the lowest was recorded in the IS (4,85 ± 0,20; $P < 0.05$).

A limitation of the present study lies in the non-random allocation of animals to production systems, as group assignments were based on existing management practices of the farms involved. While this reflects real-world conditions and enhances the ecological validity of the findings, it may also introduce potential confounding factors such as differences in baseline flock management, forage availability, and farmer experience. Although all animals were of the same breed and similar in age and body condition at the start of the study, unmeasured farm-specific variables may have influenced stress levels across groups.

4. Discussion

The findings of this study provide compelling evidence on how production systems, seasonal dynamics, age and associated climatic conditions influence chronic stress in sheep, as assessed through wool cortisol concentrations. The most notable result was the significant effect of the interaction between production system and sampling period, underscoring that chronic stress is shaped by both management regimes and their alignment with seasonal transitions. In general, wool cortisol levels were highest in the short-distance transhumance (SDT) group, particularly during the winter months. This finding aligns with the meteorological data, which showed that SDT flocks experienced the coldest winter conditions, with temperatures dropping below −15°C. The combination of cold stress, indoor confinement, and heightened physiological demands during late pregnancy and lambing likely contributed to elevated HPA axis activity and consequent cortisol accumulation. These results support previous findings suggesting that cold exposure during sensitive physiological phases is a potent driver of chronic stress in small ruminants (Corrales-Hlinka et al., 2024; Ghassemi Nejad et al., 2022).

Contrastingly, the long-distance transhumance (LDT) group demonstrated more pronounced stress during migration and the immediate post-migration period in May, but subsequently exhibited reduced cortisol levels during summer highland grazing. While both LDT and SDT systems involve migration to highland pastures with favorable conditions—such as cooler temperatures, abundant forage, and enhanced behavioral opportunities—several factors may explain the lower summer cortisol levels observed in the LDT group. First, LDT animals grazed at higher altitudes (2233 m) compared with SDT (1653 m), potentially benefiting from reduced parasite loads, lower thermal stress, and richer alpine vegetation. Second, the extended migration period (20–25 days) may act as a physiological conditioning stimulus, moderating HPA axis reactivity through adaptive mechanisms. Third, the more structured migration logistics and experienced herders in LDT systems may minimize disruptions and enhance welfare. Finally, SDT flocks entered the highlands after enduring harsher winter exposure, which may have left residual physiological stress that dampened their recovery during summer. Together, these elements likely contribute to the more effective stress mitigation seen in the LDT system during the grazing season. In the intensive system (IS), animals exhibited overall lower cortisol levels compared with SDT and LDT, except during specific winter months when levels were comparably high. While confinement in IS may protect against environmental extremes, it likely introduces other forms of chronic stress, such as reduced movement, limited social interaction, and increased human handling. This duality underscores that intensive housing systems are not inherently less stressful; rather, the nature of stress differs based on system characteristics. These insights support the view that chronic stress arises not solely from external climatic exposure but also from restrictions in behavioral expression (Broom and Fraser, 2015).

Although animal type (ewe versus lamb) did not significantly affect cortisol levels, lambs showed consistently lower concentrations, which may reflect age-related differences in HPA axis sensitivity and adaptive capacity. This observation aligns with studies reporting attenuated stress responses in younger animals (Koolhaas et al., 2011). The sharp increase in cortisol levels following migration, especially in May, highlights the physiological burden of this transitional phase, which encompasses not only physical exertion but also environmental shifts and social restructuring within flocks. Interestingly, by July, cortisol concentrations declined sharply across all systems, pointing to a seasonal rhythm where summer grazing conditions—particularly in highland areas—offer a period of physiological recovery.

It is important to emphasize that animals in this study were not randomly assigned to production systems. Instead, they were selected from pre-existing flocks within each system to reflect real-world management practices. While this observational design introduces some background variability, several measures were taken to minimize potential confounding, including the selection of animals of the same breed (Karayaka), similar age, parity, and body condition. As such, the comparisons made across systems should be viewed not as outcomes of a controlled experimental design, but as ecologically valid insights into typical transhumant and intensive sheep production settings. Although most interactions between sampling period, production system, and animal type were not statistically significant, a significant interaction was observed between sampling period and production system. This finding suggests that temporal changes in wool cortisol are more strongly shaped by environmental conditions and management factors than by animal-related variables such as age. This observation aligns with previous research by Dulude-de Broin et al. (2019), which highlights the predominant role of external stressors in modulating cortisol levels in sheep. Prior studies have shown that wool cortisol levels can vary depending on age, environmental stressors, and specific husbandry practices. For example, stressors like high ambient temperatures and

water scarcity tend to elicit stronger cortisol responses in adult ewes than in lambs—likely due to the greater physiological demands of mature animals (Gupta et al., 2022).

5. Effect of production system on cortisol level

Our findings demonstrate that the production system has a significant impact on wool cortisol concentrations, with distinct patterns observed across different sampling periods. Cortisol, beyond serving as a biomarker for stress, plays a central role in regulating various physiological systems in response to environmental stimuli (Comin et al., 2011). Notably, sheep managed under short-distance transhumance (SDT) exhibited the highest overall cortisol levels, likely reflecting the stress induced by frequent environmental transitions and increased handling. In contrast, sheep in the intensive system (IS) displayed the lowest cortisol concentrations, suggesting reduced exposure to such external stressors. This pattern underscores the varying stress responses associated with management practices, as transhumant systems inherently involve seasonal movement and environmental change, likely contributing to increased physiological stress (Chávez-Espinoza et al., 2022). Cortisol levels varied significantly across production systems ($P < 0.05$), SDT sheep exhibited the highest cortisol levels, suggesting frequent environmental shifts and handling as primary stressors. Several factors may contribute to elevated wool cortisol levels compared with LDT. Frequent environmental transitions in SDT limit animals' acclimatization time, resulting in sustained stress. SDT systems may also involve denser pastures, increased handling, and rapid relocations with limited rest, each known to elevate stress markers in livestock. Additionally, inconsistent forage quality and heightened competition for resources in SDT settings can further increase physiological stress. By contrast, LDT animals benefit from a single extended migration followed by a stable highland environment, allowing them to adjust fully and experience reduced stress. These factors collectively suggest that SDT's structure may impose greater cumulative stress on sheep than the LDT system. Although higher cortisol levels were observed in sheep within SDT and LDT systems, this should not be unequivocally interpreted as evidence of poor welfare or distress related to environmental temperature. Cortisol secretion reflects activation of the HPA axis, which occurs in response to both negative (distress) and positive (eustress) stimuli. Importantly, sheep in transhumant systems often engage in more natural behaviors such as walking, foraging, and socializing within larger flocks—behaviors that are essential to their ethological well-being. As emphasized by Broom and Fraser (2015), the opportunity to perform natural behaviors can enhance welfare and may trigger physiological arousal that is not necessarily harmful. Thus, elevated cortisol in these systems may reflect the physiological demands of mobility and environmental engagement rather than chronic distress per se. This distinction underscores the need to interpret cortisol concentrations in conjunction with behavioral and contextual indicators when evaluating animal welfare.

In addition to environmental and management stressors, the role of shepherd dogs—particularly Kangal dogs used in both transhumant systems—merits consideration. While such dogs are critical for predator protection and flock guidance, their presence might theoretically be perceived as a stressor, especially by younger animals. However, in our study, lambs exhibited lower wool cortisol levels than adult sheep, suggesting that the dogs' presence did not provoke elevated stress in the younger cohort. This may be due to lambs' lower HPA axis responsiveness or habituation to the flock environment. Therefore, while guardian dogs are integral to transhumant practices, their influence on chronic stress appears minimal in this context, though this warrants targeted behavioral studies to isolate such effects. These findings are consistent with those of Comin et al. (2011). Poudel et al. (2022) who reported that migratory practices can induce stress responses as animals adapt to novel environments. Studies show that prolonged exposure to such harsh conditions raises cortisol levels, which can impair immune response, reproductive health, and growth performance (Dwyer and Bornett, 2004; Scocco et al., 2016). Additionally, extensive systems expose sheep to parasites and predators, leading to sustained physiological stress and greater disease susceptibility (Kilgour et al., 2008). Our findings are in alignment with those of the EFSA Panel (2014), offering valuable insights into the seasonal and management-driven variations in stress. For example, transhumant sheep display elevated cortisol levels during migration, which are attributed to the physical demands and environmental novelty of these movements (Tüfekci and Sejian, 2023). In intensive systems, environmental controls may reduce some external stressors, yet high stocking density, limited movement, and restricted social interaction can induce chronic stress. Confinement in such systems disrupts natural behaviors and, over time, can elevate cortisol levels, adversely affecting immune function and physiological balance (Caroprese et al., 2016). Notably, our findings also indicate significant changes in wool cortisol levels during OS, MaS, and SS, associated with the production system. Elevated cortisol levels in these periods might correspond to seasonal management changes, particularly as transhumant sheep move between pastures. Kilgour et al. (2008), EFSA Panel, (2014) and Pulido et al. (2019) report that such migratory transitions can induce stress due to unfamiliar environments and altered foraging conditions, supporting the observed peak in cortisol levels for transhumant animals in our study. Migratory transitions represent a critical period for livestock, as they adapt to unfamiliar environments and altered foraging conditions. These transitions are inherently stressful, with environmental changes disrupting established routines and imposing physiological challenges. Research indicates that transhumance induces significant stress responses, as animals encounter novel environments, variability in forage quality, and increased competition for resources. During such movements, livestock must adjust to unfamiliar terrain and forage availability, factors that not only affect nutritional intake but also elevate physiological stress markers such as cortisol (Kilgour et al., 2008). Elevated cortisol levels during migration reflect the activation of the hypothalamic-pituitary-adrenal (HPA) axis, a physiological response to sustained environmental stressors. Pulido et al. (2019) highlights that herd movement during migratory practices is associated with prolonged periods of stress, as livestock cope with physical exertion, climatic variability, and altered social dynamics. These stressors collectively affect not only immediate welfare outcomes but also long-term health and productivity. Moreover, the capacity of livestock to forage effectively in novel environments has a considerable impact on their stress levels. As animals adapt to new habitats, their foraging behaviour may initially be less effective, which can contribute to nutritional deficiencies and exacerbate the physiological stress associated with migration (Kilgour et al., 2008). This aligns with findings that stress-related cortisol peaks during migratory transitions are often attributable to the combined effects of environmental novelty, altered forage accessibility, and increased physical demands. Both transhumance systems arrived in the highlands by mid-October, rendering the cortisol data collected from wool samples at the end of October critically significant for evaluating the stress hormone burden associated with migration in these animals. Notably, the findings indicate that the migration process substantially elevates cortisol production. Conversely, the observed decline in cortisol levels in December is likely attributable to the cessation of migration and the subsequent secure housing of the animals in their enclosures since November. Furthermore, the increases in cortisol levels observed during January and February can be elucidated by the physiological changes associated with parturition and suckling, both of which contribute to elevated stress responses. The observed peaks in cortisol during migration emphasize the need for interventions, such as pre-migratory acclimatization and optimized forage/feed distribution, to reduce stress in transhumance systems. Intensive systems, while reducing environmental stressors, may require improved space management to mitigate confinement stress.

6. Seasonal variation in cortisol concentration

Wool cortisol concentrations followed a seasonal trend, with notable reductions across all systems in December and subsequent increases in January and February. This seasonal modulation is consistent with previous studies documenting a seasonal influence on cortisol, where winter reductions in cortisol may be attributed to decreased metabolic demands and reduced social stress (Comin et al., 2011). Similarly, of Kapustka et al. (2024) in their study on alpacas, demonstrated how seasonal stressors and environmental adaptation are reflected in hormone levels, reinforcing the use of cortisol as a biomarker for both chronic and acute stress across seasonal cycles. Moberg (2021) further supports this view, showing that cortisol secretion responds dynamically to seasonal physiological and environmental stressors. These findings are also consistent with those of Cobb et al. (2023), who found that wool cortisol concentrations in Australian Merino sheep increased during periods of climatic stress and declined during thermally milder seasons. The post-migration elevation in cortisol concentrations observed in May likely reflects the physiological cost of environmental transition following seasonal transhumance. This pattern is supported by Poudel et al. (2022), who reported increased cortisol levels in livestock shortly after relocation to new ecological zones, such as highland pastures. In our study, however, cortisol levels in transhumant groups declined in subsequent months, suggesting a successful acclimatization process to the highland environment. This reduction indicates physiological adaptation, wherein stress initially rises in response to novel environmental conditions but gradually diminishes as animals adjust. The long-term relevance of such seasonal and environmental stress dynamics is reinforced by Comin et al. (2011), who emphasized the role of wool cortisol in capturing slow-acting physiological changes and chronic adaptation responses in ruminants.

The climatic variability observed across the three production systems—LDT, SDT, and IS—provides essential context for interpreting the chronic stress patterns identified in wool cortisol profiles (Table 1). Each production region presents a unique combination of thermal, hydric, and seasonal dynamics that shapes animal welfare outcomes. Notably, the SDT system experienced the coldest winter conditions, with minimum temperatures reaching –15.1°C and average temperatures falling below zero from December through March. These data suggest a considerable cold stress burden, especially during late pregnancy and lambing, when energy demands are highest. Cold exposure is a well-documented stressor in sheep, triggering prolonged activation of the HPA axis, with negative consequences for immune competence, reproduction, and metabolic efficiency (Ghassemi Nejad et al., 2022; Corrales-Hlinka et al., 2024). While winter housing was provided in all systems, variations in insulation, airflow, and microclimate buffering may explain the relatively elevated cortisol levels observed in SDT system.

Conversely, the IS region experienced the highest summer heat load, with maximum temperatures exceeding 39°C in August and average temperatures consistently above 22°C from June through August. These thermal extremes, combined with relative humidity levels reaching 78 %, likely imposed significant heat stress, particularly under confined housing conditions that limit behavioral thermoregulation. As noted by Tüfekci and Sejian (2023), heat stress in small ruminants suppresses feed intake, increases water demands, and alters thermoregulatory capacity, with long-term welfare and productivity implications. Although average wool cortisol levels in IS sheep were lower than in transhumant groups, possible heat stress responses during peak summer months merit further investigation, especially through short-term biomarkers and behavioral indicators (Gupta et al., 2022).

The LDT system exhibited a more thermally moderate profile, with winter minima around –4.8°C and summer maxima peaking at 40.3°C in September, though average summer temperatures remained buffered by the high-altitude environment. Animals in LDT systems benefit from longer acclimatization periods post-migration, which likely contributes to more stable physiological transitions and lower mid-season cortisol levels. Relative humidity in LDT zones ranged between 58.5 % and 82.5 %, potentially mitigating thermal stress but also increasing risk for parasitic infections and respiratory irritation under prolonged humid conditions (Pulido et al., 2019). Moreover, LDT regions received the highest cumulative precipitation—up to 156.5 mm in some months—which may support pasture productivity but simultaneously compromise bedding quality and hygiene, particularly in shelters, thereby contributing to indirect stress (Caroprese et al., 2016). Taken together, the meteorological profiles of each system reveal a multi-dimensional stress landscape: SDT is characterized by severe cold exposure during winter, IS by high summer heat, and LDT by moderate but fluctuating highland conditions. These findings reinforce that environmental factors—particularly temperature amplitude, humidity, and precipitation cycles—interact with production system characteristics to shape chronic stress outcomes. Incorporating such climatic data into welfare assessment frameworks is essential for designing adaptive, region-specific management strategies that mitigate environmental stress and enhance animal resilience under changing climatic regimes.

7. Influence of age on cortisol level

Relatively few studies have investigated variations in wool cortisol levels across different age groups. While lambs exhibited lower cortisol concentrations than adult sheep, the difference was statistically non-significant ($p > 0.05$), suggesting that age did not play a dominant role in chronic stress variation under the studied conditions. This trend is consistent with observations in sheep reported by Fürtbauer et al. (2019) and has also been documented in other species, including alpacas (Kapustka et al., 2024), cattle, and pigs (Heimbürge et al., 2020). This outcome might be attributed to lambs' differing developmental physiology or lower sensitivity to environmental stressors compared to mature sheep. In line with this, Gregula-Kania et al. (2021) noted that younger animals often display distinct hormonal profiles, with lower baseline cortisol potentially due to undeveloped hypothalamic-pituitary-adrenal (HPA) axis responsiveness. While not statistically significant, these findings warrant further investigation to understand age-related variations in chronic stress markers across sheep populations. The lack of a statistically significant difference between lambs and sheep could indicate a shared resilience within this flock to the chronic environmental conditions they face, suggesting that the conditions and handling practices of this study were relatively consistent and low-stress across age groups. Such findings support the importance of stress-sensitive practices in transhumant and intensive systems to ensure welfare outcomes, especially given the physiological adaptability observed in different age groups. Additionally, wool cortisol levels are influenced by both immediate and cumulative stress exposures, and since lambs and sheep in transhumant systems often share similar grazing environments, their exposure to stressors may not differ enough to produce marked variations in cortisol levels. However, the findings of Zoratti et al. (2023) indicate that lambs aged between one to five months in various European breeds exhibit a notable decline in wool cortisol concentration and exhibit lower baseline cortisol levels than adult sheep due to developmental differences in HPA axis activity (Zoratti et al., 2023). Seasonal transitions such as lambing, suckling, and migration impose additional stress due to heightened metabolic and physiological demands (Ataallahi et al., 2022).

This observation suggests the existence of distinct metabolic strategies employed by these animals to cope with varying environmental stressors. In extensive or transhumant systems, where environmental stressors are often shared across age groups, differences in cortisol accumulation could also be attributed to the maturation of the HPA axis, which may alter the way stress is physiologically managed as animals age (Dulude-de Broin et al., 2019).

Given the observational design of this study, group assignment to production systems was not randomized but based on pre-existing flock

structures. Although this limits the causal interpretation of treatment effects, baseline animal characteristics were matched to enhance comparability. Accordingly, results reflect differences in temporal cortisol patterns across authentic production contexts, rather than experimental treatments. Statistical analysis focused on identifying interactions between sampling period and production system to capture these contextual differences.

8. Conclusion

This study provides compelling evidence that chronic stress in Karayaka sheep, as reflected in wool cortisol concentrations, is significantly influenced by the interaction of production systems and environmental conditions. Among the three systems examined, SDT was associated with the highest cortisol levels, likely due to more extreme winter temperatures and insufficient housing insulation. In contrast, LDT offered more stable highland grazing conditions that facilitated stress adaptation following seasonal migration. The IS, while buffered from environmental fluctuations, still presented seasonal thermal challenges, particularly during high summer temperatures. Notably, age did not significantly affect wool cortisol concentrations, indicating that both lambs and adult sheep responded similarly to chronic stressors within each system. These findings underscore the necessity of tailoring welfare strategies to regional climatic realities and production system constraints. Integrating climatic data into livestock stress assessments enables a more nuanced understanding of environmental burdens and informs the development of context-specific interventions—such as improved winter housing, pre-migratory acclimatization, and seasonal forage planning—to enhance animal welfare and resilience under varying ecological conditions.

CRediT authorship contribution statement

Sezen Ocak Yetişgin: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Hasan Önder:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Sen Ugur:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Büşra Akça:** Project administration, Methodology, Investigation, Formal analysis, Data curation. **Ceyhun Yücel:** Resources, Investigation, Funding acquisition, Conceptualization. **Ömer Faruk Yılmaz:** Software, Resources, Methodology, Investigation. **Hüseyin Mert Yüksel:** Project administration, Methodology, Funding acquisition, Formal analysis.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

Data will be made available on request.

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