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Research Article

Triaxial accelerometers and subcutaneous biologgers as tools to record diurnal and nocturnal changes in locomotor activity, body temperature, heart rate, and heart rate variability in melatonin-treated lambs *(Ovis aries)*

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ABSTRACT

The study of growth and development of lambs *(Ovis aries)* is essential in sheep farming, and melatonin plays an important role in the physiology of growing lambs. The effects of an exogenous melatonin treatment on several physiological characteristics in fattening lambs at weaning were studied. Eight lambs were assigned to one of two groups; those that did (melatonin group, $n = 4$) or did not (no-melatonin group, n = 4) receive two subcutaneous melatonin implants at weaning. At the end of the fattening period, to quantify locomotor activity (LA), each lamb was fitted with a triaxial accelerometer, for 7 days. Simultaneously, lambs received a surgically implanted subcutaneous temperature (T) and heart rate (HR) biologger, which was programmed to record data every 5 min. HR variability (HRV) variables (SDNN, the standard deviation of normal-to-normal R-R intervals, and RMSSD, the root mean square of consecutive deviations between normal heartbeats) were measured. All of the variables exhibited 24-h circadian rhythms. Time of day (daytime vs. nighttime) had a significant effect on LA, T, and HR, but considering both treatment with melatonin and time, differences between time for these variables were only observed in melatonin-treated lambs. Exogenous melatonin did not affect LA or T, but melatonin-treated lambs had lower HR, SDNN, and RMSSD than did non-treated lambs. In conclusion, the use of subcutaneous biologgers and triaxial accelerometers in growing lambs demonstrated circadian rhythms in LA, T, HR, and HRV, and melatonin treatment negatively affected HRV, but its effects on the other physiological variables differed between day and night.

1. Introduction

Sheep production plays a crucial role in global food systems by providing meat, milk, and dairy products that contribute to human nutrition and food security. The growth and development of lambs are essential in sheep farming, because they directly affect meat quality and the overall productivity of the farm (Fogarty et al., 2000). An integrative approach that combines veterinary expertise and advanced technologies for monitoring physiological variables provides a means of sheep management.

Melatonin influences circadian and seasonal rhythms (Pévet, 2003) and contributes to seasonal reproduction in sheep (Palacín et al., 2011). In addition, it is involved in various physiological processes that take advantage of its antioxidant and anti-inflammatory properties, and has a role in regulating energy metabolism (Ma et al., 2022). Research has shown that melatonin has multiple functions in the intra-uterine and neonatal stages of lamb development (Flinn et al., 2020). For instance, melatonin implants in pregnant ewes increase uterine blood flow and fetal oxygenation, which reduces neonatal mortality and increases birth weight (Flinn et al., 2020), and enhances the uterine microenvironment in early pregnancy in sheep (Viola et al., 2024). Furthermore, melatonin implants in ewes at lambing increase milk fat content throughout lactation, which increases growth rates and weights in lambs (Abecia et al., 2021), and ewes that had received a melatonin implant in the final trimester of pregnancy exhibited an increase in colostrum quality (Abecia et al., 2020). In addition, exogenous melatonin administered to

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fattening lambs at 30 days of age improves feed efficiency, particularly in females (Abecia and Canto, 2023), probably because of a reduction in metabolic activity, which is reflected in reductions in body temperature (T) and locomotor activity (LA) in melatonin-treated lambs.

The study of heart rate (HR) variability (HRV) in fattening lambs is important because it is a biomarker of stress and welfare (Kitajima et al., 2021), and it reflects the balance between the sympathetic and parasympathetic branches of the autonomic nervous system (ANS) (Turini et al., 2022). In assessing stress and the welfare of lambs, HRV reflects how lambs respond to stressors such as transport (Santurtun and Phillips, 2015), handling (Coulon et al., 2015) and weaning (Atkinson et al., 2022). Typically, reduced HRV indicates an increase in stress and a decrease in welfare, and an increase in HRV suggests resilience and adaptability (von Borell et al., 2007). Among the various indices used to quantify HRV, the standard deviation of normal-to-normal R-R intervals (SDNN) and the root mean square of successive differences (RMSSD) are considered the most informative and clinically relevant metrics. SDNN provides insights into overall autonomic balance and long-term health risks, and RMSSD offers a more focused view of parasympathetic activity and acute stress responses. Together, those indices provide a nuanced interpretation of HRV data, which improves clinical decision-making and patient management strategies (Soliman et al., 2013; Abolahrari-Shirazi et al., 2019).

Body temperature (T) and HR are reliable indicators of a lamb's response to a particular management procedure. Changes in body T in farm animals can indicate stress, which affects welfare and productivity (Hahn, 1989). The stress-induced hyperthermic response is a brief increase in body T that is triggered by stress (Bouwknecht et al., 2007). Furthermore, abnormalities in body T (e.g., fever, hypothermia) or HR can indicate illness, infection, or metabolic disturbances (Delano et al., 2007), and monitoring body T and HR helps in the evaluation of how well lambs respond to extreme temperatures or fluctuating weather conditions, which influences the choice of strategies used to improve their resilience (Sejian et al., 2022). In addition, the study of locomotor activity (LA) in lambs is important in understanding their behavior, physiology, health, and welfare (Abecia et al., 2024). Active lambs are most efficient in transforming food into muscle, which enhances growth and productivity (Price et al., 2022).

The development of sensors for monitoring physiological variables has greatly enhanced our ability to investigate how physiological conditions influence an animal's resilience to stressors. Subcutaneous biologgers are particularly valuable in veterinary and wildlife studies, where vegetation, landforms, or other animals can interfere with externally attached devices. Placed directly beneath the skin, biologgers minimize those external interferences and signal noise. Subcutaneous biologgers can measure T, HR, and HRV in natural environments with minimal impact on the animal's behavior, which provides a holistic perspective on health and improves our understanding of physiological processes. Furthermore, they have several advantages over alternative methods such as high accuracy, comfort for the animal, and high data quality. In addition, they are less susceptible to loss or damage than are externally attached devices, which increases the likelihood of consistent and uninterrupted data collection. As such, they are reliable and efficient tools for research in animal physiology and welfare. In addition, those devices allow for continuous, long-term monitoring, which is essential for capturing comprehensive data over prolonged periods (Twiss et al., 2021). We have used subcutaneous biologgers to assess the effects of grazing density on cattle (Palacios et al., 2021), to monitor changes in the physiology of sheep in intensive housing conditions (Abecia et al., 2022a), to record vaginal temperature by embedding the loggers in intravaginal sponges (Abecia et al., 2022b), to monitor changes in physiological variables after melatonin treatment in lambs (Viola et al., 2023), and to study body T and HRV, and their circadian rhythms in sheep (Abecia et al., 2025a).

Accelerometers have been used extensively to study human sleep, exercise, and daily activities (Emery et al., 2010) by collecting data on the quantity, frequency, and duration of movements. In addition, they can provide valuable insights into the intensity and patterns of activity throughout the day and night. The development of triaxial accelerometers has increased the benefits of this technology because it provides a non-invasive method for recording even the slightest body movements in real-world settings (Brown et al., 2013), including applications in veterinary medical research. Triaxial accelerometers have emerged as essential tools for monitoring animal behavior, providing insights into various aspects of animal welfare, health, and locomotor activity. Their capacity to capture movements in three spatial dimensions allows for more detailed assessments than does a uniaxial accelerometer, which is critical when measuring behaviors that require nuanced movement analyses such as lying, standing, and movement in animals. For example, triaxial accelerometers have proven to be effective in quantifying the lying behavior of domestic horses (Dubois et al., 2015), and in assessments of the health status of domestic cats based on their activity patterns (Smit et al., 2023). Elsewhere, we reported that accelerometers were effective in detecting changes in the circadian rhythms in LA in ewes and lambs (Abecia and Canto, 2023; Abecia et al., 2022a, 2024), and tri-axial accelerometers were effective in the identification and classification of the activities of captive tegus (Guadalupe-Silva et al., 2024).

By studying changes in T, HR, HRV, and LA in lambs in the fattening period, researchers and farmers can gain valuable insights into animal physiology, welfare, and management, which can contribute to healthier, more productive livestock systems. Earlier studies by our group demonstrated that exogenous melatonin can improve growth and conversion rates of lambs in the fattening period (Viola et al., 2023), lambs that received a melatonin implant at 45 days of age had higher growth and feed conversion rates in the fattening period than did lambs that received an implant at 15 or 30 days of age (Abecia et al., 2025b). We hypothesized that exogenous melatonin affects certain physiological processes in lambs during their growth and development. In particular, changes that reduce the animal's metabolism; e.g., a reduction in T, LA, or HR, might improve feed conversion rates and profitability. The aim of this study was to use subcutaneous biologgers and triaxial accelerometers to monitor changes in T, HR, HRV, and LA in lambs that were being finished for slaughter and either did or did not receive a melatonin treatment.

2. Material and methods

The Ethics Committee for Animal Experiments at the University of Zaragoza approved all of the procedures performed in the study. The care and use of animals were in accordance with the Spanish Policy for Animal Protection RD1201/05, which meets the European Union Directive 2010/63 on the protection of animals used for experimental and other scientific purposes.

2.1. Animals and experimental procedures

The experiment involved eight Rasa Aragonesa lambs (*Ovis aries*) (4 males, 4 females) born in December 2023 from eight ewes that had been synchronized in estrus by intravaginal sponges (30 mg fluogestone acetate, Sincropart, CEVA Salud Animal, Barcelona, Spain) and had been bred by one of six rams of the same breed on 7 July. Lambs were classified as "Ternasco de Aragón", the local lamb in northeastern Spain, under the European Protected Geographical Indication (PGI).

At weaning, at an age of 45 days (10–12 kg), lambs were assigned to one of two groups, which either did (melatonin group, $n = 4$) or did not (no-melatonin group, n = 4) receive two subcutaneous melatonin implants (18 × 2 mg, Melovine, CEVA Sante Animal, France). The groups were housed separately in 25-m^2 paddocks within the same building in a feed lot until they achieved slaughter weight (18–24 kg at 70–90 days of age). From weaning until the end of the fattening period (time necessary to achieve the highest growth rate and carcass yield in the shortest possible time - 45 days in our system - and to increase production per unit of resource), the lambs were fed a 14.8 % crude protein concentrate (Cadecor-2, Agroveco, Zaragoza, Spain) and barley straw ad libitum (Fig. 1).

At the end of the fattening period (75 days of age), for seven days, lambs wore triaxial accelerometers (46 × 33 × 15 mm, 19 g) that record high-resolution raw acceleration data (ActiGraph wGT3X-BT; ActiGraph, USA), which were attached to a neck collar. At a rate of 30 Hz, the sensors record accelerations (LA) based on the individual's amplitude (g) of movement along the three axes. The activity counts for the three axes were used to create minute-by-minute activity records (Vector Magnitude, VM), which were formed by the combination of the accelerations from the three axes. VM was calculated as follows:

$$VM = \sqrt{x^2 + y^2 + z^2}$$

In addition, lambs received a surgically implanted subcutaneous T and HR biologger (DST micro-HRT, Star Oddi, Iceland) (8.3 × 25.4 mm, 3.3 g), which had been programmed to record data every 5 min and was left in place for seven days. To program the biologgers, the Mercury software v5.83 (Star Oddi, Gardabaer, Iceland) was used via a communication box. HRV measurements were based on the raw ECG data that were collected every 5 min for 48 h. Biologgers were retrieved and the data were downloaded via the same communication box and software. The biologger measures HR through a leadless single-channel ECG, takes burst readings at predetermined intervals, and calculates the mean HR for each record. The sample frequency was 200 Hz, which is recommended for small ruminants.

As part of data validation, the Mercury software calculates a Quality Index (QI) for each burst. The algorithm that estimates the QI (Star Oddi, 2024) calculates each R-R interval (time elapsed between two successive R-waves of the QRS signal on the electrocardiogram), which is the cardiac contraction (systole) that begins with the Q wave, a negative deviation, and ends with the R wave, a positive (upward) deviation. The S wave refers to any negative deflection that occurs immediately following the R section. If there is $<$20 % variability within the R-R intervals, the grade is set to QI = 0 (good). If there is no R-R interval, QI = 3 (poor). If HR is $>$20 bpm and $<$ 600 bpm, QI = 3. Any data that did not meet those criteria were subjected to a second algorithm. In that step, each potential R wave is given a grade based on signal amplitude and "sharpness". A lower-level threshold (LLT) and a higher-level threshold (HLT) are identified based on the minimum and maximum grades. If those thresholds overlap (LLT $\geq$ HLT), all potential R waves are of a similar grade. In such cases, those R waves are used to calculate bpm, and Q1 = 1 (good), which is typical of high-quality recordings, particularly if arrhythmias or $>$ 20 % variation in R-R intervals occur within a single ECG recording. If the thresholds do not overlap, potential R waves that grade above the HLT, only, are included in the calculation of bpm. The QI remains at 1 (good), unless one or more potential R waves fall between the LLT and HLT, which introduces ambiguity and, therefore, the QI = 2 (fair). If a single R wave, only, exceeds the HLT, the QI = 3 (poor). HR measurements that have a QI = 2 or 3 are excluded from the analysis because they are deemed unreliable.

The Star-Oddi HRT Analyzer program (Star Oddi, Gardabaer, Iceland) computes HRV variables based on the raw ECG data. The system quantifies HR, QI, and HRV based on the SDNN (standard deviation of normal-to-normal R-R intervals) and the RMSSD (root mean square of consecutive deviations between normal heartbeats).

$$SDNN = \sqrt{\frac{1}{N-1}\sum_{i=1}^{N}\left(RR_i - \overline{RR}\right)^2} \quad \overline{RR} = \text{mean of RR intervals}$$

$$RMSSD = \sqrt{\frac{1}{N-1}\sum_{i=1}^{N-1}\left(RR_{i+1} - RR_i\right)^2}$$

2.2. Surgery

The biologgers were sterilized by immersion in a 0.55 % orthophthalaldehyde solution (CIDEX-OPA, Johnson & Johnson, New Jersey, USA) for 24 h. For the surgery, lambs were placed in a cradle in dorsal recumbency. A solution of povidone-iodine soap (Betadine Scrub 7.5 %, Alcon Laboratories, Inc., Fort Worth, TX) was used to prepare the skin. One ml of lidocaine hydrochloride (Anesvet, Ovejero, León, Spain) was injected subcutaneously as a local anesthetic. After an incision was made on the left thorax, just above the heart, a subcutaneous pocket was constructed to accommodate the biologger (Fig. 2). The electrodes of the

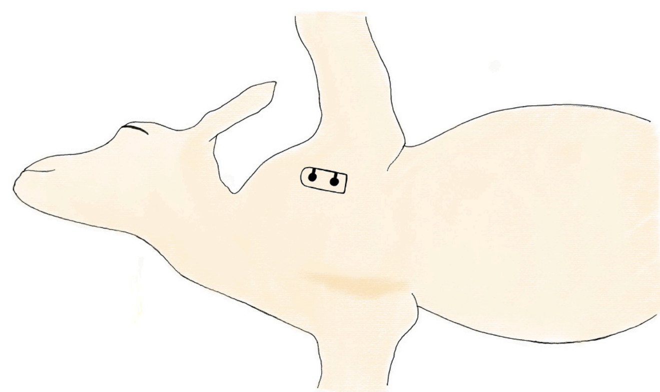

Fig. 2. Position of the subcutaneous biologger in the left thorax, just above the heart in Rasa Aragonesa fattening lambs. A pocket was constructed to accommodate the biologger after an incision was made on the left thorax. The electrodes of the biologger were placed in contact with the muscle layer nearest to the skin, and the sensor axis was aligned with the axis of the heart.

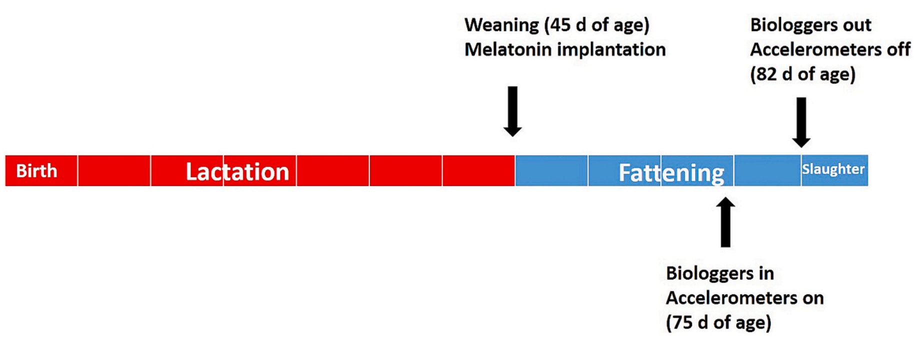

Fig. 1. Schematic representation of the experimental design. At weaning (45 days of age), Rasa Aragonesa lambs were assigned to one of two groups, which either did (group Melatonin, n = 4) or did not (group No-melatonin, n = 4) receive two subcutaneous melatonin implants. At the end of the fattening period (75 days of age), lambs wore triaxial accelerometers and received a surgically implanted subcutaneous biologger, for seven days. Lambs were slaughtered at 85 d of age.

biologger were placed in contact with the muscle layer nearest to the skin, and the sensor axis was aligned with the axis of the heart. The biologger was fastened in the pocket by a 2/0 absorbable suture (Novosyn, B-Braun, Melsungen, Germany) that was inserted through a tiny hole at the device's tip. After using two to three sutures to close the incision, aluminum spray (Aluspray, Vetoquinol, Madrid, Spain) was applied to the incision. A similar surgical approach was used to remove the biologger at the end of the experiment.

2.3. Statistical analysis

Before conducting the statistical analysis, the normality of the data was confirmed by a Kolmogorov-Smirnov Test. To evaluate the statistical significance of the effects of melatonin treatment and time of day (day vs. night) on the physiological variables, a multifactorial model was used that incorporated these variables as fixed effects and was based on the Least Squares Method via the GLM procedure in SPSS v.29. Significant differences within the fixed effects were confirmed by an ANOVA. The general structure of the model is y = xb + e, where 'y' is an Nx1 vector of observations, 'b' represents the fixed effects within the association matrix 'x', and 'e' is the vector of residual effects.

The mean (± S.E.) for each variable was calculated at hourly intervals. Circadian rhythms in VM were analyzed by fitting the time-series data from each lamb to a 24-h cosine curve based on the cosinor method provided by the Cosinor online platform (Molcan, 2019. Key rhythm variables including the Midline Estimating Statistic of Rhythm (MESOR, representing the average value around which the variable oscillates), amplitude (the difference between the peak and the average value of the wave), and acrophase (the timing of peak activity) were calculated for each individual and each variable. To assess rhythmicity, an F-test was performed to compare the reparametrized cosine model and a non-rhythmic model, with $P < 0.05$ indicating a significant 24-h rhythm. Subsequently, the data were aggregated, and the mean 24-h cosinor curve was computed for each variable. Significant differences in cosinor values among phases were confirmed by an ANOVA.

3. Results

3.1. Melatonin effects

The GLM indicated that the melatonin-treated lambs had significantly ($P < 0.001$) lower HR and HRV than did the untreated lambs (Table 2), but the two groups did not differ significantly in LA or T (Table 1).

3.2. Time of day effects (daytime vs. nighttime)

Lambs exhibited significantly higher LA (P < 0.001), T (P < 0.001) and HR ($P < 0.01$) at night than they did in the day, but the HRV indicators SDNN and RMSSD did not differ significantly between day and night (Table 2).

3.3. Interaction effects of treatment x time of the day

The interaction between melatonin treatment and the day-night cycle was significant ($P \leq 0.05$) for T, SDNN, and RMSSD, but not LA ($P = 0.08$). Within the melatonin-treated group, LA, T, and HR were significantly ($P < 0.001$) higher at night than they were in the day. In addition, the differences in LA and T between the two groups were statistically significant during the day, only (Table 3). In both groups, HR was significantly higher at night than it was during the day (P < 0.001). Both HRV variables (SDNN and RMSSD) were significantly higher in the untreated group than they were in the melatonin-treated group during the day and at night (Table 3).

Table 1
Statistical significance of the terms in a Generalized Linear Model (GLM) on the impact of melatonin implants on locomotor activity (LA), body temperature (T), heart rate (HR), and heart rate variability (SDNN and RMSSD) in Rasa Aragonesa lambs that either did or did not receive a melatonin implant at 45 days of age.

	LA	T	HR	SDNN	RMSSD
Treatment	$P = 0.60$	$P = 0.20$	P < 0.001	P < 0.001	P < 0.001
Day vs. night	P < 0.001	P < 0.001	$P = 0.005$	$P = 0.37$	$P = 0.21$
Interaction	P = 0.08	$P = 0.05$	0.721	P = 0.05	$P = 0.04$

Table 2
Mean (±S.E.) locomotor activity (LA), body temperature (T), heart rate (HR), and heart rate variability (SDNN and RMSSD) in Rasa Aragonesa lambs that either did (group Melatonin) or did not (group No-melatonin) receive a melatonin implant at 45 days of age (a,b indicate significant differences between groups or between day and night).

	LA (counts/min)	T (°C)	HR (bpm)	SDNN (ms)	RMSSD (ms)
No-melatonin	91.1 ± 7.5	39.0 ± 0.1	132.0 ± 1.1^{a}	18.5 ± 1.4^{a}	22.5 ± 2.0^{a}
Melatonin	87.5 ± 6.4	39.1 ± 0.1	124.5 ± 1.0^{b}	11.3 ± 0.5^{b}	12.5 ± 0.7^{b}
Day	70.1 ± 6.3^{a}	38.9 ± 0.1^{a}	124.6 ± 1.50^{a}	14.8 ± 0.8	17.7 ± 1.8
Night	105.1 ± 6.8^{b}	39.1 ± 0.1^{b}	132.4 ± 1.4^{b}	14.0 ± 1.2	15.9 ± 1.1

3.4. Circadian rhythms

Circadian rhythms in LA, T, and HR were similar in both groups, and they did not differ significantly in mean MESOR, amplitude, or acrophase (Table 4); however, the melatonin treatment significantly altered the circadian rhythm in SDNN and RMSSD such that the cosinor curves of the two groups had different shapes (Fig. 3), and the three variables differed significantly (P < 0.001) (Table 4).

3.5. Quality index

Among the 12,243 records that were downloaded from the eight biologgers, 94 % had Q0 or Q1 for the estimation of HR and HRV. The remaining 6 % ($n = 697$) were graded Q2 or Q3 and, therefore, were excluded from the analysis. For each of the eight lambs, the proportion of records that were Q0 + Q1 was 96 %, 91 %, 94 %, 96 %, 92 %, 95 %, 96 % and 94 %.

Table 3
Mean (±S.E.) locomotor activity (LA), body temperature (T), heart rate (HR), and heart rate variability (SDNN and RMSSD) during the day and at night in Rasa Aragonesa lambs that either did (group Melatonin) or did not (group No-melatonin) receive a melatonin implant at 45 days of age (a,b indicate significant differences between time of day within groups; x,y indicate significant differences between groups within time of day).

		No-melatonin	Melatonin
LA (counts/min)	Day	82.2 ± 11.4^{x}	61.0 ± 6.7^{ay}
	Night	98.6 ± 10.0	109.9 ± 9.3^{b}
T (°C)	Day	39.0 ± 0.1^{x}	38.9 ± 0.1^{ay}
	Night	39.0 ± 0.1	39.2 ± 0.1^{b}
HR (bpm)	Day	128.4 ± 2.5^{x}	121.8 ± 1.8^{ay}
	Night	135.0 ± 2.8^{x}	126.9 ± 1.1^{by}
SDNN (ms)	Day	20.5 ± 2.5^{x}	10.5 ± 0.5^{y}
	Night	16.8 ± 1.3^{x}	11.9 ± 0.8^{y}
RMSSD (ms)	Day	25.9 ± 3.6^{x}	19.6 ± 1.9^{x}
	Night	11.6 ± 0.6^{y}	13.2 ± 1.1^{y}

Table 4
Mean (±SEM) Midline Estimating Statistic of Rhythm (MESOR), amplitude (the difference between the peak and the mean value of a wave), and acrophase (the time of peak activity) of the cosine curve of a 24-h activity rhythm in locomotor activity (LA, counts/min), temperature (T, °C), heart rate (HR, bpm), Standard Deviation of Normal to Normal R-R Intervals (SDNN, ms), and the (Root Mean Square of Consecutive Deviations Between Normal Heartbeats (RMSSD) in Rasa Aragonesa lambs that either did (group Melatonin) or did not (group No-melatonin)) receive a melatonin implant at 45 days of age (a,b indicate significant differences between groups).

		No-melatonin	Melatonin
LA (counts/min)	MESOR	91.1 ± 6.3	87.5 ± 4.7
	Amplitude	118.9 ± 0.5	109.9 ± 1.5
	Acrophase (h)	23:10	23:14
T (°C)	MESOR	39.0 ± 0.1	39.1 ± 0.1
	Amplitude	0.1 ± 0.1	0.1 ± 0.1
	Acrophase (h)	22:50	00:12
HR (bpm)	MESOR	131.9 ± 2.8	127.7 ± 1.4
	Amplitude	5.82 ± 0.1	5.48 ± 0.1
	Acrophase (h)	02:47	03:03
SDNN (ms)	MESOR	18.5 ± 2.3^a	11.3 ± 1.0^b
	Amplitude	3.3 ± 0.1^a	1.6 ± 0.1^b
	Acrophase (h)	13:20	00:17
RMSSD (ms)	MESOR	22.5 ± 2.9^a	12.5 ± 1.4^b
	Amplitude	6.0 ± 0.4^a	1.8 ± 0.9^b
	Acrophase (h)	13:41^a	00:16^b

4. Discussion

4.1. Day vs. night effects and interaction with melatonin treatment

Treatment with exogenous melatonin at weaning reduced mean HR and HRV variables in Rasa Aragonesa lambs during the day and at night at the end of the fattening period; however, the reduction occurred in LA and T during the day, only. In another study (Viola et al., 2023), we monitored T and LA in lambs that had received an implant at 30 days of age and had retained until slaughter 50 days later. Those lambs exhibited a reduction in T and LA, but the effect of time of day was not investigated. The effects of melatonin implants on the T and LA of lambs during the day, only, has not been reported. Day is the natural time when endogenous melatonin levels are lowest (Arendt, 1998), which might amplify the effects of exogenous melatonin, and reinforce the biological signals associated with exogenous melatonin. Contrary to our expectations, however, treated lambs, only, exhibited significantly higher LA and T at night than they did during the day, which suggests that the exogenous melatonin might have acted in concert with the natural circadian rhythms to produce time-dependent effects. At night, when endogenous melatonin levels are high, the additional exogenous melatonin might influence temperature and activity, such that both are higher at night than they are during the day, which might reflect a complex interaction between exogenous melatonin and the body's natural rhythms.

Melatonin is important in seasonal thermoregulation because it is a transducer in mediating information about energy balance (Saarela and Reiter, 2005). Our experiment was carried out in December, when lambs were exposed to low ambient temperatures and, probably, were losing body heat to the environment, especially at night. Apparently, lambs that had received a melatonin implant had a higher capacity to thermoregulate in cold ambient temperatures at night than did those that were untreated. In fact, in a recent study (Viola et al., 2023), melatonin-treated lambs had a lower surface temperature than did untreated lambs, which suggests that the former had a greater level of vasoconstriction and, therefore, a higher capacity to retain body heat and maintain their core T than did the latter. In red deer, exogenous melatonin reduced HR in late spring and summer and increased it in autumn and winter (Domingue et al., 1992), which might reflect an energy-conserving mechanism that occurs when conditions are warm and, possibly, is associated with a reduction in metabolic demands, related to the reduced surface heat dissipation seen in lambs. Thus, the effects of melatonin on HR and vasoconstriction probably reflect an integrated seasonal adaptation to environmental challenges that balances energy conservation and thermoregulation.

In our study, melatonin-treated fattening lambs were less active at night than they were during the day, which might have been related to the reduced body T exhibited by the treated lambs at night. Probably, the elevated melatonin levels caused by endogenous nocturnal production plus the exogenous melatonin implants exacerbated the differences between day and night. In humans, sleepiness, reduced core temperature, increased heat loss, and other generally anabolic physiological functions have been described after treatment with melatonin (van den Heuvel et al., 2005). In a previous study, we showed that melatonin-implanted lambs consumed less concentrated compound feed than did untreated lambs (Viola et al., 2023), which suggests that the former had a reduced need to increase metabolic rate by consuming more food. That phenomenon might have being associated with the reduced LA exhibited by treated lambs at night in our experiment. A direct relationship between melatonin levels and a reduction in LA has been demonstrated in pinealectomized sparrows (Zimmerman and Menaker, 1975), rats that had been injected with pineal extracts (Ozaki et al., 1976), and rodents that received melatonin, which exhibited a tranquilized state (Golombek et al., 1996). The effect of exogenous melatonin on LA in Rasa Aragonesa lambs at night suggests that high levels of melatonin are needed to produce such an effect; i.e., endogenous and exogenous melatonin are necessary to exert a negative effect on LA in young lambs.

4.2. Effects on HRV

Probably, the most surprising finding of our experiment was the reduction in HRV after the treatment with melatonin. In general, melatonin is associated with beneficial effects on HRV because it can help regulate the autonomic nervous system, promote parasympathetic activity, which increases HRV (for review, see Arendt, 2019). Several factors might have contributed to why exogenous melatonin reduced HRV in fattening lambs. In our study, the lambs were still in a developmental phase when they received the melatonin implant and their autonomic nervous system was not fully mature, which might have caused them to respond to melatonin in a manner unlike that of adult animals. In fact, human infants do not exhibit a rhythmic excretion of 6-sulfatoxymelatonin in urine before 9–12 weeks of age (Kennaway et al., 1992). In lambs, a clear diurnal rhythm in melatonin concentrations in plasma does not emerge until 3–4 weeks of age (Nowak et al., 1990). Although lambs in our experiment received a melatonin implant at 6 weeks of age, individual variation in the maturation of the circadian rhythmicity in melatonin secretion might have influenced the results of our experiment. Wood et al. (1989) reported considerable individual variation in the rate of entrainment of melatonin secretion in lactating lambs when they were exposed to artificial changes in photoperiod.

4.3. Circadian rhythms

All of the physiological variables under study exhibited a 24-h circadian rhythm, as they did in lactating ewes (Abecia et al., 2022a), artificially reared lambs (Abecia and Canto, 2023), and adult ewes (Abecia et al., 2021; Abecia et al., 2025a) in previous experiments at the same location, based on data recorded by the same biologgers and accelerometers. Although melatonin implants had a significant effect on LA, T, and HR, they did not have a significant effect on the circadian rhythm of these variables, however, the acrophase of the SDNN and RMSSD were shifted ~12 h; i.e., a complete inversion of the circadian rhythms. In humans exposed to an altered photoperiod, repeated administration of melatonin phase-advanced the SDNN and RMSSD (Vandewalle et al., 2007). Melatonin rhythm is a valid phase marker of the circadian system in domestic species including sheep (Li et al., 2021) and, possibly, melatonin implants in young lambs might alter the

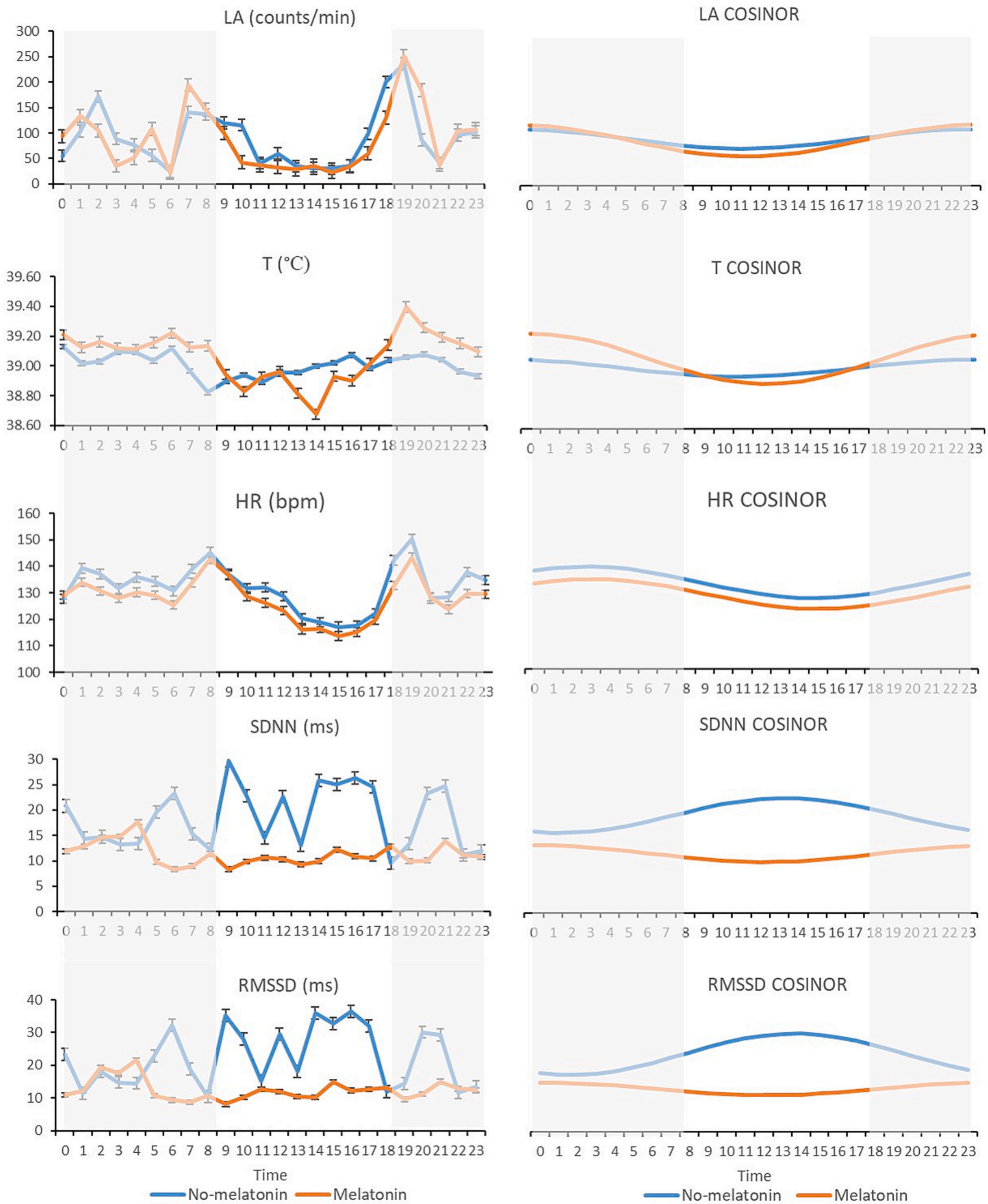

Fig. 3. Mean 24-h (left panel) locomotor activity (LA), body temperature (T), heart rate (HR), and heart rate variability (SDNN and RMSSD) in Rasa Aragonesa lambs at 75 days of age that either had (group Melatonin) or had not (group No-melatonin) received a melatonin implant at 45 days of age, and their corresponding cosinor curves (right panel) (grey areas indicated night).

synchronization of the biological rhythms of these animals by reducing HRV and shifting its circadian rhythm.

4.4. Quality index

In our study, 94 % of the HR records from the eight lambs graded as QI = 0 or 1 and, therefore, were included in the analysis. The extent of high-quality records was higher than it was in our other experiments (65 %, Abecia et al., 2025a), which suggests that the biologgers performed exceptionally well and the dataset was robust and reliable for analysis.

5. Conclusion

In conclusion, melatonin implants induced higher LA, T, and HR in treated lambs at night, and LA and T differed significantly from that of untreated lambs during the day, only. The use of subcutaneous bio-loggers and triaxial accelerometers detected circadian rhythms in LA, T, HR, and HRV in fattening lambs. The circadian rhythms in SDNN and RMSSD exhibited a phase-shift in the melatonin-treated animals. Future research should investigate the mechanisms responsible for those temporal differences, and confirm whether the differing effects of melatonin on the physiological variables under study are involved in the positive effects of this hormone on growth rate and food conversion in lambs.

CRediT authorship contribution statement

José A. Abecia: Writing – review & editing, Writing – original draft, Software, Resources, Project administration, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Isabella Manenti:** Methodology, Investigation. **Irene Viola:** Writing – review & editing, Methodology, Investigation, Formal analysis. **Paola Toschi:** Writing – review & editing, Investigation, Data curation, Conceptualization. **Carlos Palacios:** Writing – review & editing, Methodology, Investigation, Data curation. **Francisco Canto:** Writing – review & editing, Methodology, Investigation, Data curation, Conceptualization. **Silvia Miretti:** Writing – review & editing, Writing – original draft, Funding acquisition, Formal analysis, Data curation, Conceptualization.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that might 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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