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Effects of melatonin implants on locomotor activity, body temperature, and growth of lambs fed a concentrate-based diet

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ABSTRACT

Melatonin is involved in the regulation of circadian rhythms and is implicated in seasonal reproduction in sheep. In several physiological mechanisms it acts as an antioxidant and an anti-inflammatory molecule, regulating energy metabolism. This work investigated the effects of melatonin implants at 30 days of age on locomotor activity, body temperature, and growth of fattening female and male lambs. Sixty lambs were divided into two groups, one of which received melatonin (MEL, 15 males, 16 females) and a control group (CTR, 16 males, 14 females). In the melatonin group, 2 18 mg-melatonin implants were placed at 30 days of age. Lambs were fattened for 6 weeks from weaning (45 days of age) to slaughter (85 days). Feed conversion rate (FCR) was calculated based on live weight and the amount of concentrate consumed. Locomotor activity (LA) was measured weekly by actigraphy, and circadian rhythmicity (CR) was calculated. Rectal (Trec) and surface temperatures (Tsur) were recorded in the last week of fattening, and subcutaneous fat thickness (FT) over the *longissimus dorsi* muscle was measured by ultrasound scanning. Treatment did not affect FCR, although MEL female lambs consumed significantly ($P<0.001$) less concentrate than CTR lambs. Treatment and sex had a significant ($P<0.05$) interaction effect on FT; specifically, FT was significantly ($P<0.05$) higher in female MEL lambs (3.22±0.21 mm) than in female CTR lambs

(2.57±0.24 mm), but FT in males did not differ between MEL (2.77±0.21) and CTR (2.94±0.24 mm) lambs. Overall activity was significantly (P<0.001) lower in the MEL lambs (72.22±0.10 counts/min) than in the CTR lambs (78.89±0.12 counts/min). MEL lambs had a significantly (P<0.01) lower Trec (CTR: 39.00±0.07; MEL: 38.68±0.10) and Tsur for all body regions evaluated than the CTR lambs. In conclusion, treatment with exogenous melatonin at 30 days of age increased food efficiency in fattening female lambs, probably, because of the lower metabolism in treated lambs, which was reflected by the lower body temperature and locomotor activity exhibited by these animals. In addition, the study has demonstrated the effect of exogenous melatonin in the growth performances of post-weaning lambs, and that its effects depend on animal sex, which suggests that treatments that target females might be most appropriate in the fattening period.

Keywords:

sheep, fattening, actigraphy, thermography

Introduction

The development and growth of lambs play a crucial role in sheep husbandry because they influence the quality of the meat and the productive efficiency of the farms (Fogarty et al., 2000). For that reason, an integrative approach involving veterinary knowledge and the use of new technologies for recording physiological parameters provides an opportunity to improve sheep management.

Melatonin is involved in the regulation of circadian and seasonal rhythms (Pevet, 2003) and is implicated in seasonal reproduction in sheep (Palacin et al., 2010), and in several physiological mechanisms because it acts as an antioxidant and an anti-inflammatory molecule, and regulates energy metabolism (Ma et al., 2022). Studies have demonstrated that melatonin has several roles in the intra-uterine and neonatal life of lambs (Flinn et al., 2020), and melatonin implants in pregnant ewes improve uterine blood flow and fetal oxygen supply, which reduces neonatal mortality and increases birth weight (Flinn et al., 2020). Melatonin treatment of ewes at lambing increases the fat content of milk throughout lactation, which increases growth rate and weight of lambs (Abecia et al., 2021), and ewes implanted with melatonin in the last third of pregnancy show an improvement in colostrum quality (Abecia et al., 2020).

Cagnacci et al. (1997) reviewed the relationships between melatonin and core body temperature in humans and showed that the rhythms in both variables are caused by the circadian pacemaker located in the hypothalamic suprachiasmatic nuclei, and its effect on thermoregulatory centers, heat loss, and probably heat production are likely involved. Gianetto et al. (2016) reported that the daily rhythms of melatonin and rectal temperature in sheep and goats were synchronized, although they differed consistently from the rhythm in locomotor activity.

Exogenous melatonin has been shown to induce circadian rhythmicity in the locomotor activity of several species, such as rats (Redman et al., 1995; Martinet et al., 1996) and sparrows, which facilitates the synchronization of circadian rhythms to light (Hau and Gwinner, 1994). In addition, exogenous melatonin can reduce locomotor activity in humans (Zhdanova, 2005) and some birds (Mintz et al., 1998).

In the last 20 years, new technologies have been used to collect real-time data on the behavior of farm animals. For instance, accelerometer sensors are non-invasive devices for collecting locomotor activity, which can indicate a well-functioning system. A tri-axial accelerometer provides data on body motion and physical state continuously (Barwick et al., 2018) and have been used to measure posture in cattle for monitoring feeding and grazing activity and for detecting calving (Robert et al., 2009; Gonzàles et al., 2015). Those devices have been used to monitor activities in adult sheep (Giovanetti et al., 2017) and to detect lambing time in pasture-based sheep (Fogarty et al., 2020). In addition, actigraphy has confirmed that, in lambs, activity in the first week of life did not follow a circadian rhythm, and that twin-born lambs had stronger associations with their littermates than did singletons with other lambs (Abecia et al., 2022). Furthermore, actigraphy was useful in documenting the locomotion and feeding behavior of artificially reared lambs, which detected a reduction in circadian rhythmicity and the number of suckling sessions as the lambs aged (Abecia et al., 2023).

In small ruminants, the effect of gender on growth performances is mainly related to the quantity of fat deposited, deposition site, growth rate and carcass yield, so that females are more affected than males due to their higher precociousness

(Guerrero et al., 2013). In light of those findings, we hypothesized that melatonin implants affect growth and locomotor behavior in post-weaning lambs, and that accelerometers might be useful in confirming this hypothesis. The aim of this study was to compare the locomotor activity, body temperature, and growth of fattening lambs treated or not with melatonin implants from 30 to 85 days of age. We also determine if the effect of melatonin treatment varies according to the sex of the lambs.

Material and methods

Animals and experimental procedures

The study was conducted at the experimental farm of the University of Zaragoza, Spain (41°40' N 0°53' W). Sixty Rasa Aragonesa lambs (31 males, 29 females; 40 singles, 20 twins), born between Oct 13 and Nov 6 [mean live weight (LW) ±SD = 4.19 ±0.83 kg] from a synchronized mating in May, were assigned to one of two groups; one in which the animals received two subcutaneous melatonin implants (36 mg melatonin, Melovine, CEVA Animal Health, Barcelona, Spain) at 30±2 days of age (MEL) (n = 15 males, 15 females) and one in which they did not (CTR, non-implanted, control (n = 16 males, 14 females). From weaning (44±2 days of age) until the age of 86±3 d, the groups were housed separately within the same building, in 25-m^2 paddocks, and were fed a 14.8% crude protein concentrate (Cadecor-2, Agroveco, Zaragoza, Spain) and barley straw *ad libitum*. Lambs were managed in two batches, depending on the day of birth, so that implanting, weaning and slaughter dates were Nov 14, Nov 28 and Jan 12, and Dec 1, Dec 15 and Jan 29, respectively. Twin lambs were always separated into the two experimental groups. After weaning, lambs were fitted with commercially available sensors that record high-resolution raw acceleration data (ActiGraph wGT3X-BT; ActiGraph, FL, USA) (46 mm × 33 mm × 15 mm in size, mass = 19 g), which were attached to the dorsal side of a neck collar that remained in place until slaughter, five weeks later. At the end of each week, the recorded activity data were downloaded by the ActiLife software (ActiGraph, LLC, Pensacola, FL). Actigraph produces three columns of data; i.e., activity in the x-, y-, and z-axes. The activity counts for the three axes were used to create minute-by-minute activity (counts per 1-min intervals) data values (Vector Magnitude, VM), which is the magnitude of the vector that is formed from the combination of the sampled accelerations from the three axes on any device.

One week before slaughter, rectal temperature (Trec) was recorded by a conventional thermometer, and surface temperatures were recorded by a thermographic camera (Teso 883, Teso SE & Co. KGaA, Titisee-Neustadt, Germany). The areas delineated from the images are described in Fig. 1. The camera specifications are: temperature range -30 to +650 °C, resolution focus (manual) 320 x 240, image refresh rate 27 Hz, emissivity 0.01 to 1, with automatic recognition of emissivity and determination of reflected temperature. Images were taken at a distance of 1 m. Temperature data were analyzed by the IRSoft software. Ambient temperature and relative humidity when the images were recorded were 11°C and 52%, respectively. At the same time, a real-time ultrasound was used to measure the subcutaneous fat thickness over the *longissimus dorsi* muscle between the 12th and 13th ribs, and over the 3rd and 4th lumbar vertebrae. Scanning gel was applied to the scanning sites to improve conduction between the skin and the transducter. All measurements were taken on the left side by a portable ultrasound scanner (7.5-MHz transducer; EXAGO, France). Muscle depth and width were taken at the same points and the area was calculated. At the end of the finishing period, the concentrate residuals were weighed to calculate the total amount of concentrate consumed per group, and the feed conversion rate (FCR) was calculated as kg concentrate consumed/kg live weight gained.

Statistical analysis

For each of the five weeks of data collection, mean (±SE) VM for the seven days of each week that the lambs wore the sensors was calculated at hourly intervals. Circadian rhythms in VM were graphed by fitting the time-series measurements of each lamb to the cosine curve of a 24-h activity rhythm, which was obtained by the cosinor method at the Cosinor on-line platform (Molcan, 2019). Midline Estimating Statistic of Rhythm (MESOR, or the average around which the variable oscillates), amplitude (the difference between the peak and the mean value of a wave), and acrophase (the time of peak activity) were calculated for each individual. To test for rhythmicity, an F-test compared the (re-parameterized) cosine model and the nonrhythmic model. A $P<0.05$ indicated that the time series fit a 24-h rhythm. Thereafter, the data were pooled and the mean 24-h cosinor curve for each of the three parameters was calculated for each group, and the cosinor values of MEL vs. CTR groups were compared statistically by an ANOVA. To detect significant differences among the five weeks of fattening, a repeated-measures t-test was used.

The effects of week, lamb sex, type of parturition (single or twin), time of day (day or night) on activity-related variables were evaluated statistically based on a multifactorial model that included these parameters as fixed effects, and the Least Squares Method of the GLM procedure in SPSS v.26 (IBM Corp. Released 2019) was used. Within fixed effects, significant differences were identified by an ANOVA. A general representation of the model is as follows: $y = xb+e$, where y is Nx1 vector of records, b denotes the fixed effect in the model within the association matrix x, and e is the vector of residual effects. Before the statistical tests, a Kolmogorov–Smirnov Test was performed to confirm the normality of the data.

Results

LW, growth rate, and feed conversion rate (FCR)

The melatonin treatment did not have a significant effect on LW and growth rates throughout the finishing period (Tables 1 and 2); however, most of the LW and growth variables evaluated differed significantly between the sexes (Table 3), with no interaction between sex and treatment. Regarding type of parturition, single lambs had higher LWs than twins ($P<0.05$) (Table 3), with significant differences only for the growth rate between birth and weaning ($P<0.05$), with no interaction between type of parturition and treatment.

In the finishing period, melatonin-treated lambs consumed significantly ($P<0.001$) less concentrate per lamb per day (0.99±0.01 kg) than did non-treated lambs (1.03±0.01 kg), although the two groups had a similar FCR (3.24 vs. 3.34, resp.); however, there was a significant ($P<0.001$) effect of sex of the lamb, and a significant ($P<0.05$) interaction between treatment and sex on FCR. Specifically, males had a significantly ($P<0.001$) lower FCR (2.94) than did females (3.66), and there was a significant ($P<0.001$) effect of melatonin treatment on FCR in female (MEL: 3.47, CTR: 3.85), but not in male (MEL: 3.00, CTR: 2.89) lambs. No differences between single and twin lambs were observed either for the amount of concentrate consumed per day (single: 1.02±0.01;

twins: 1.00±0.01), or the FCR (single: 3.25; twins: 3.33). Considering the interaction between type of parturition and treatment, twin MEL lambs presented a significantly lower concentrate consumption (0.99±0.01 kg) than single MEL lambs (1.00±0.01 kg) ($P<0.05$), with no differences between single and twins for the CTR group.

Longissimus dorsi and back fat thickness scanning

Neither treatment nor sex of the lamb was correlated with the measurements of the *longissiums dorsi* (Table 4); however, a significant interaction between treatment and sex ($P<0.05$) was detected for backfat thickness; specifically, MEL (3.22±0.21 mm) and CTR (2.57±0.24 mm) females differed significantly ($P<0.05$), but male lambs did not (MEL: 2.77±0.21, CTR: 2.94±0.24 mm).

Locomotor activity

Week, sex of the lamb, time of day, and treatment with melatonin had a significant ($P<0.001$) effect on locomotor activity of lambs in the finishing period, and there were significant ($P<0.001$) interaction effects between treatment, time of day, and sex of the lamb. Overall activity was significantly ($P<0.001$) higher in the CTR (78.89±0.12 counts/min) than it was in the MEL group (72.22±0.10 counts/min), particularly, from week 1 through week 4 (week 1: 56.65±0.14; week 2: 66.51±0.16; week 3: 76.19±0.17; week 4: 81.21±0.18; week 5: 97.82±0.21). The differences between the MEL and CTR groups were more pronounced in the male lambs (CTR: 81.52±0.17; MEL: 69.20±0.14; $P<0.001$) than they were in the female lambs (CTR: 76.01±0.17; MEL: 75.26±0.14; $P<0.001$) (Fig. 2). Lambs in the MEL group were less active than were those in the CTR group (Fig. 3). Lambs increased their activity from week 1 to week 5 ($P<0.001$), and female lambs (75.58±0.11 counts/min) exhibited more movements than did male lambs (75.58±0.11 counts/min). Activity was significantly ($P<0.001$) higher in the day (90.10±0.13 counts/min) than it was at night (63.18±0.09 counts/min), and the differences between groups were greater in the day (CTR: 99.51±0.21; MEL: 82.57±0.17; $P<0.001$) than they were at night (CTR: 62.20±0.14; MEL: 63.95±0.12; $P<0.001$). No differences between single and twin lambs were observed, and no interaction between type of parturition and treatment was detected.

The 24-h evolution of activity each week is presented in Fig. 4. Both groups had peaks in activity at 0800 h and at 1800 h, which were significantly ($P<0.05$) higher in the control group than in the MEL group in week 4.

Circadian rhythms

All lambs exhibited a circadian rhythm in activity on every day of the fattening period, and melatonin treatment did not have a significant effect on mean MESOR or amplitude. Acrophase differed significantly ($P<0.05$) between MEL and CTR groups in week 3, only (Fig. 5). MESOR in the CTR group increased from week 1 to week 3 ($P<0.01$), and in the MEL group from week 1 to week 5. In the MEL group, only, amplitude increased significantly ($P<0.01$) from week 4 to week 5. In every week, CTR lambs experienced a significant ($P<0.001$) delay in acrophase, although significant changes in acrophase in the MEL group occurred from week 1 to week 3, only (Fig. 5).

Thermography

Trec was significantly ($P<0.01$) lower in the MEL lambs (38.68±0.10) than it was in the CTR lambs (39.00±0.07). In all the regions analyzed, surface temperatures were significantly ($P<0.01$) lower in the MEL group than they were in the CTR group (Table 5). Body temperatures did not differ significantly between the sexes.

Discussion

In this study, subcutaneous melatonin implants inserted at day 30 of age had a significant effect on the growth and activity in Rasa Aragonesa lambs in the fattening period. In addition, exogenous melatonin differentially affected the feeding conversion rate, locomotion, and body temperature of female and male lambs. Melatonin implants increased significantly the feeding conversion rate and fat thickness in female lambs, but not in males.

In post-pubertal beef heifers fed melatonin, the amount of fat on ribs, the growth of the longissimus muscle, and carcass fat increased significantly, which suggested that melatonin partitions nutrients toward fat accretion in later-maturing fat depots (i.e., im fat), an effect that is similar to that from exposure to short-day photoperiods (Zinn et al., 1988). An effect of melatonin treatment on adipose tissue metabolism and an increase in the cross-sectional area of longissimus muscle and back adipose tissue has been observed in lambs (Ma et al., 2022). Melatonin did not impact liver and kidney function, but did increase superoxide dismutase blood levels (Ma et al., 2022) as expressed by its antioxidant action (Rodriguez et al., 2004), which has a positive effect on muscle and adipose tissue development (Manchester et al., 2015). Furthermore, melatonin seemed to ameliorate the rumen microflora in lambs by enhancing nutrient absorption and increasing muscle and fat cell size (Ma et al., 2022). In a previous study, we observed an increase in the fat content of milk from melatonin-treated ewes, even though a direct effect on growth rate in implanted-newborn lambs was not observed (Abecia et al., 2021).

In a study designed to determine whether melatonin and light-dark schedule influence energy metabolism and performance in broiler chicken, Apeldoorn et al. (1999) found a reduction in energy expenditure for physical activity caused by melatonin supplements in the diet, which might have caused the increase in the feed conversion rate in melatonin-supplemented chickens. To our knowledge, our study is the first to demonstrate a direct effect of melatonin implants on post-weaning lambs and an interaction effect between melatonin treatment and animal sex, although it remains uncertain why it impacted adipose tissue development more in females than it did in males. Clearly, melatonin implants influenced energy balance in growing female lambs.

In our experiment, body temperature was lower in melatonin-treated lambs than it was in untreated lambs. In addition to its role in the timing of seasonal reproduction, melatonin affects seasonal thermoregulation; in particular, acting as a transducer in mediating information about energy balance (Saarela and Reiter, 1994). Considering that the temperature to which the animals were exposed at the time of the experiment, when the thermography was performed, was low (11ºC), lambs were losing body heat to the environment to compensate cold temperatures, so that it is likely that MEL lambs have a higher ability to thermoregulate their bodies under cold ambient temperatures. In the vasculature, melatonin predominantly acts through two membrane-bound receptors (MT1 and MT2), whose opposite effects have been demonstrated: the activation of MT1 elicits vasoconstriction and MT2, vasodilatation (Doolen et al., 1998). When ingested as a supplement in humans, melatonin blunts the cutaneous vasoconstrictor response during cooling (Aoki et al., 2008). As treated lambs presented a lower surface temperature, they likely had a greater level of vasoconstriction, therefore, a better capacity to retain body heat. Moreover, a greater level of vasoconstriction may be related to the fact that MEL lambs consumed less concentrate than CTR lambs, and thus, a lower need to increase metabolic rate by consuming more food, which in turn, can be related with the lower activity exhibited by MEL lambs.

In a study of the effects of exogenous melatonin on rectal and body surface temperatures in donkeys subjected to packing in the hot-dry season in Nigeria, Ake et al. (2023) demonstrated that melatonin had hypothermic effects on rectal and surface temperatures of the donkeys, and altered the circadian rhythms of these parameters through its biphasic effect; viz, its hypothermic effect in photophase and hyperthermic effect in scotophase. Ake et al. (2023) recommended that melatonin be administered to donkeys in the hot-dry season to improve the well-being and performance of working donkeys under heat stress conditions.

Environmental interference has been reported as a limitation of infrared thermography, so that inaccurate results caused by direct sunlight, humidity, or wind have been described (McManus et al., 2016). However, in the present study, rectal and body surface temperature measurements were well related -they were lower in treated lambs compared with the control animals-, so that it is likely that the ambient conditions where the images were taken allowed a good quality of the thermographic pictures.

In a study by Bouroutzika et al. (2020), pregnant ewes under heat stress treated with melatonin had a consistently lower body temperature than did untreated ewes. In addition, melatonin inhibits thyroid, adrenal, and corticosteroid hormones, which are linked indirectly to thermic modulation (Saarela and Reiter, 1994; Ma et al., 2022). In humans, the nocturnal peak in melatonin levels closely correlates with the nadir in core body temperature, and treatment with exogenous melatonin lowered it by 0.2-0.4°C (Cagnacci et al., 1992).

In our experiment, melatonin-treated lambs were less active than were non-treated lambs, which might have been related to the lower body temperatures exhibited by the implanted lambs because elevated melatonin levels from either endogenous nocturnal production or exogenous daytime administration are associated with effects such as increased sleepiness, reduced core temperature, increased heat loss, and other generally anabolic physiological changes in humans (van den Heuvel et al., 2005). The earliest evidence of a direct effect of the pineal hormone on the locomotor activities of vertebrates was found when the removal of the pineal gland from house sparrows maintained in constant darkness eliminated the free-running circadian rhythm in locomotor activity (Zimmerman and Menaker, 1975), and when a subcutaneous injection of protein-free pineal extract reduced running wheel activity in rats, and a pinealectomy increased activity (Ozaki et al., 1976). Furthermore, melatonin reduces pain and anxiety, which puts rodents into a tranquilization state (Golombek et al., 1996). Although cortisol levels were not measured in our study, it is plausible that a similar calming effect might have occurred in lambs. Given that locomotion is a large proportion of the energy budget in mammals (Bertram, 2016), and a small lowering of body temperature increases metabolic rate, probably, the reduction in temperature and locomotor activity caused by melatonin in our experiment was responsible for the reduction in concentrate ingested by treated lambs and, in females, an increase in food conversion rate. Melatonin treatments significantly reduced heat production in 2-week-old and 3-week-old broiler chickens, and improved feed conversion rate, especially in younger chickens, which suggests that melatonin reduces heat production by lowering body temperature and regulating heat dissipation (Zeman et al., 2001).

Conclusion

In conclusion, our study demonstrated that new-generation devices for monitoring daily activity can increase our understanding of lamb behavior in the fattening period and, in particular, detect the effects of exogenous melatonin on locomotor activity. Treatment with exogenous melatonin at 30 d of age in fattening lambs improved food efficiency rate, especially in females, probably, because of a reduction in the metabolism of treated lambs as indicated by the lower body temperature and locomotor activity exhibited by these animals. Furthermore, the reduction in the body temperature in lambs that received melatonin implants suggests the possibility of improving lamb performance in the summer fattening period, when lambs are exposed to heat stress. In addition, our study confirmed that melatonin plays an important role in post-weaning lamb metabolism, and that its effects depend on animal sex, which suggests that treatments that target females might be most appropriate in the fattening period.

Authorship statement

The idea for the paper was conceived by José Alfonso Abecia. The experiment was designed by José Alfonso Abecia, Irene Viola and Francisco Canto. The experiments were performed by Irene Viola and Francisco Canto. The data were analyzed by Irene Viola and José Alfonso Abecia. The paper was written by Irene Viola and José Alfonso Abecia. The final version to be submitted was approved by Irene Viola, José Alfonso Abecia and Francisco Canto.

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Ethical Considerations Statement

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.

Conflicts of interest

The authors declare no conflict of interest.

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Table 1. Mean (±SE) age (d) and live weight (LW, kg) of Rasa Aragonesa lambs that did (MEL, n=30) or did not (CTR, n=30) receive two 18 mg-melatonin implants at day 30 of age, and were fattened for five weeks from weaning (45 d of age) to slaughter (85 d of age).

	CTR		MEL	
	Age	LW	Age	LW
Birth	0.00±0.00	4.25±0.16	0.00±0.00	4.13±0.15
Implant	29.80±0.36	9.69±0.43	30.10±0.36	9.70±0.41
Weaning	44.57±0.34	14.47±0.57	44.90±0.36	14.39±0.56
60 days	60.03±0.39	19.41±0.75	60.30±0.37	19.56±0.74
75 days	74.27±0.44	23.56±1.10	74.50±0.40	23.95±0.83
Slaughter	85.73±0.55	27.61±0.86	85.90±0.49	27.36±0.0.86

Table 2. Mean (±SE) fattening parameters (kg/d) of Rasa Aragonesa lambs that did (MEL, n=30) or did not (CTR, n=30) receive two 18 mg-melatonin implants at day 30 of age, and were fattened for five weeks from weaning (45 d of age) to slaughter (85 d of age).

	CTR	MEL
Finishing period (d)	41.17±0.39	41.00±0.37
Growth birth-weaning	0.23±0.10	0.23±0.01
Growth weaning-60 d	0.32±0.02	0.34±0.02
Growth weaning-75 d	0.31±0.03	0.32±0.01
Growth weaning-slaughter	0.32±0.01	0.32±0.01
Growth birth-slaughter	0.27±0.01	0.27±0.01

Table 3. Mean (±SE) live weight (LW, kg) of Rasa Aragonesa male (n=31) and female (n=29) lambs that were fattened for five weeks from weaning (45 d of age) to slaughter (85 d of age) (a,b indicate significant differences at P<0.05).

	Males	Females	Single	Twins
LW	n=31	n=29	n=40	n=20
Birth	4.42±0.16^{a}	3.94±0.13^{b}	4.56±0.12^{a}	3.70±0.15^{b}
Weaning	14.97±0.61	13.84±0.49	15.50±0.51^{a}	13.02±0.52^{b}
60 days	20.50±0.76^{a}	18.41±0.66^{b}	20.58±0.72^{a}	18.06±0.66^{b}
75 days	25.69±0.83^{a}	21.69±0.97^{b}	24.70±1.03^{a}	22.52±0.76^{b}
Slaughter	29.33±0.87^{a}	25.51±0.67^{b}	28.63±0.84^{a}	25.98±0.78^{b}
Growth				
birth-weaning	0.24±0.01	0.22±0.01	0.24±0.01^{a}	0.21±0.01^{b}
weaning-60 d	0.36±0.02^{a}	0.29±0.02^{b}	0.33±0.19	0.32±0.01
weaning-75 d	0.36±0.01^{a}	0.26±0.02^{b}	0.31±0.02	0.32±0.01
weaning-slaughter	0.32±0.01^{a}	0.32±0.01^{b}	0.32±0.01	0.31±0.01
birth-slaughter	0.29±0.01^{a}	0.25±0.01^{b}	0.28±0.01	0.26±0.01

Table 4. Mean (±SE) dimensions (mm) of the *longisimus dorsi* muscle of Rasa Aragonesa lambs that did (MEL, n=30) or did not (CTR, n=30) receive two 18 mg-melatonin implants at day 30 of age, and were fattened for 5 weeks from weaning (45 d of age) to slaughter (85 d of age), as measured by real-time ultrasound between the 12th and 13th ribs and over the 3rd and 4th lumbar vertebrae.

	CTR	MEL
Depth	18.62±0.47	19.23±0.40
Width	42.55±2.99	47.52±2.50
Area	614.50±0.17	676.29±33.01

Fat thickness	2.77±0.17	3.00±0.16

Table 5. Mean (±SE) temperature (°C) of Rasa Aragonesa lambs that did (MEL, n=30) or did not (CTR, n=30) receive two 18 mg-melatonin implants at day 30 of age, and were fattened for five weeks from weaning (45 d of age) to slaughter (85 d of age) (a,b indicate significant differences at $P<0.05$).

	CTR	MEL
Hotspot (armpit)	38.51 ± 0.41^{a}	36.79 ± 0.53^{b}
Leg, ribs and shoulder	20.14 ± 0.26^{a}	18.32 ± 0.14^{b}
Rump and leg	19.16 ± 0.29^{a}	17.34 ± 0.13^{b}
Loin	18.30 ± 0.27^{a}	16.73 ± 0.16^{b}
Hind saddle	17.39 ± 0.30^{a}	16.51 ± 0.14^{b}
Back	18.51 ± 0.32^{a}	17.35 ± 0.17^{b}

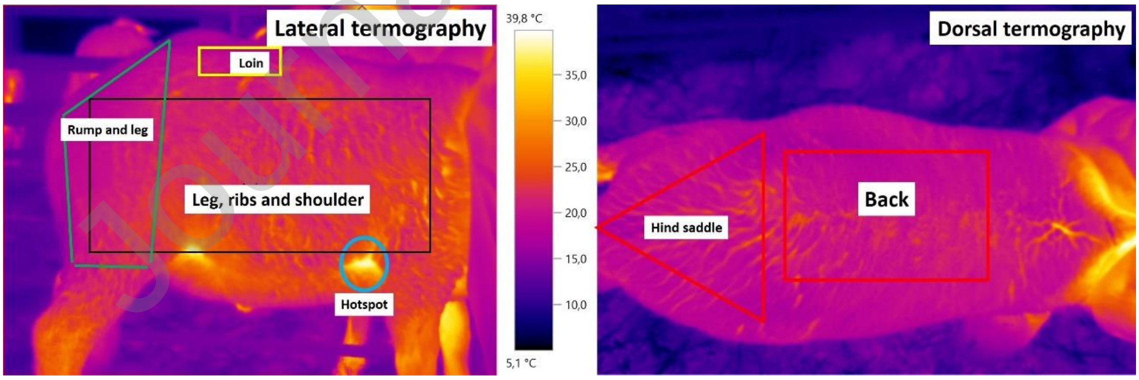

Figure 1. Body regions thermographed in Rasa Aragonesa lambs.

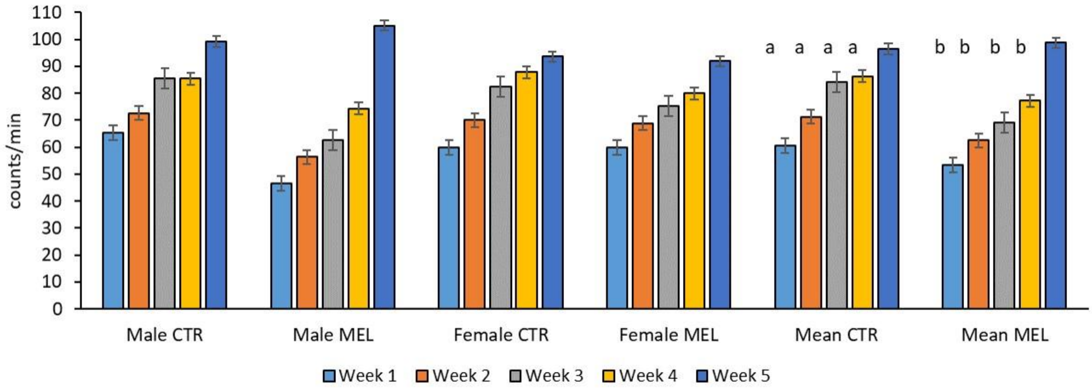

Fig. 2. Mean (±SE) Vector of Magnitude (counts/min) of Rasa Aragonesa lambs that did (MEL, n=30) or did not (CTR, n=30) receive two 18 mg-melatonin implants at day 30 of age, and were fattened for five weeks from weaning (45 d of age) to slaughter (85 d of age) (a,b indicate significant differences at P<0.05).

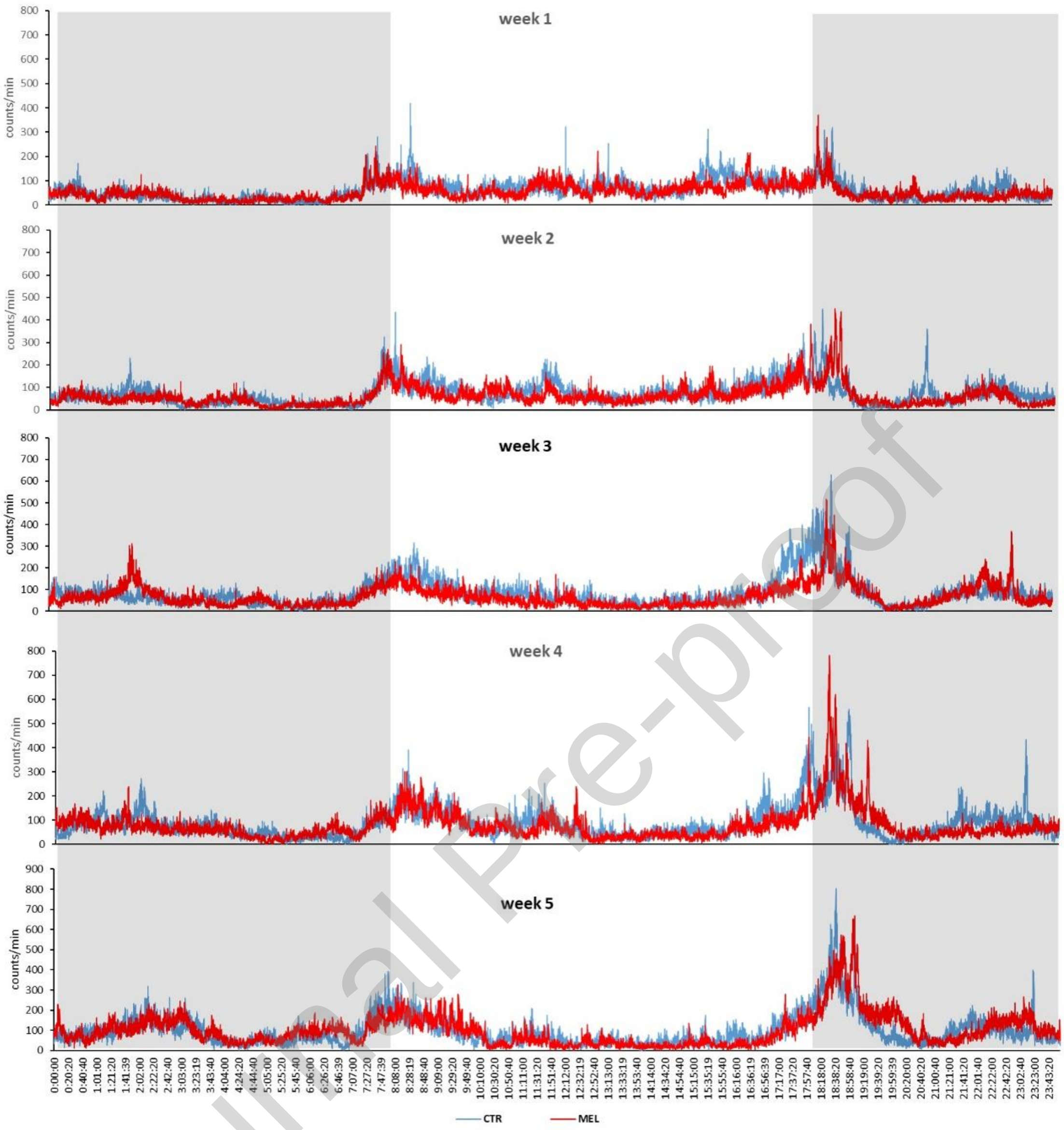

Figure 3. Mean 24-h locomotor activity (counts/min) of Rasa Aragonesa lambs that did (MEL, n=30) or did not (CTR, n=30) receive two 18 mg-melatonin implants at day 30 of age, and were fattened for five weeks from weaning (45 d of age) to slaughter (85 d of age) (gray areas represent night).

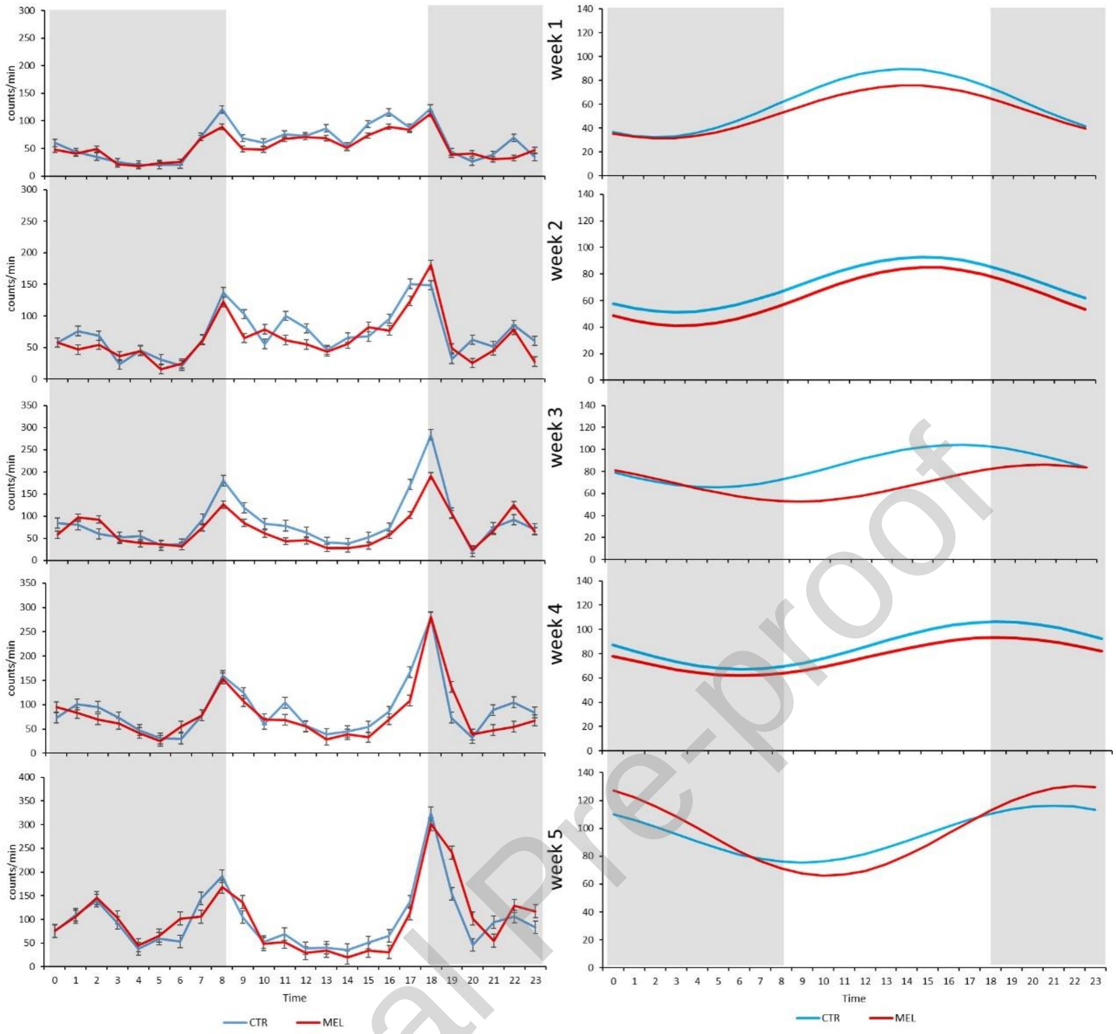

Figure 4. Mean (±S.E.) daily activity (counts/min/h) (left panel) of Rasa Aragonesa lambs that did (MEL, n=30) or did not (CTR, n=30) receive two 18 mg-melatonin implants at day 30 of age, in the 5-wk fattening period as measured by actigraphy, and the corresponding cosinor curves (right panel) of a 24-h activity rhythm (gray areas represent night).

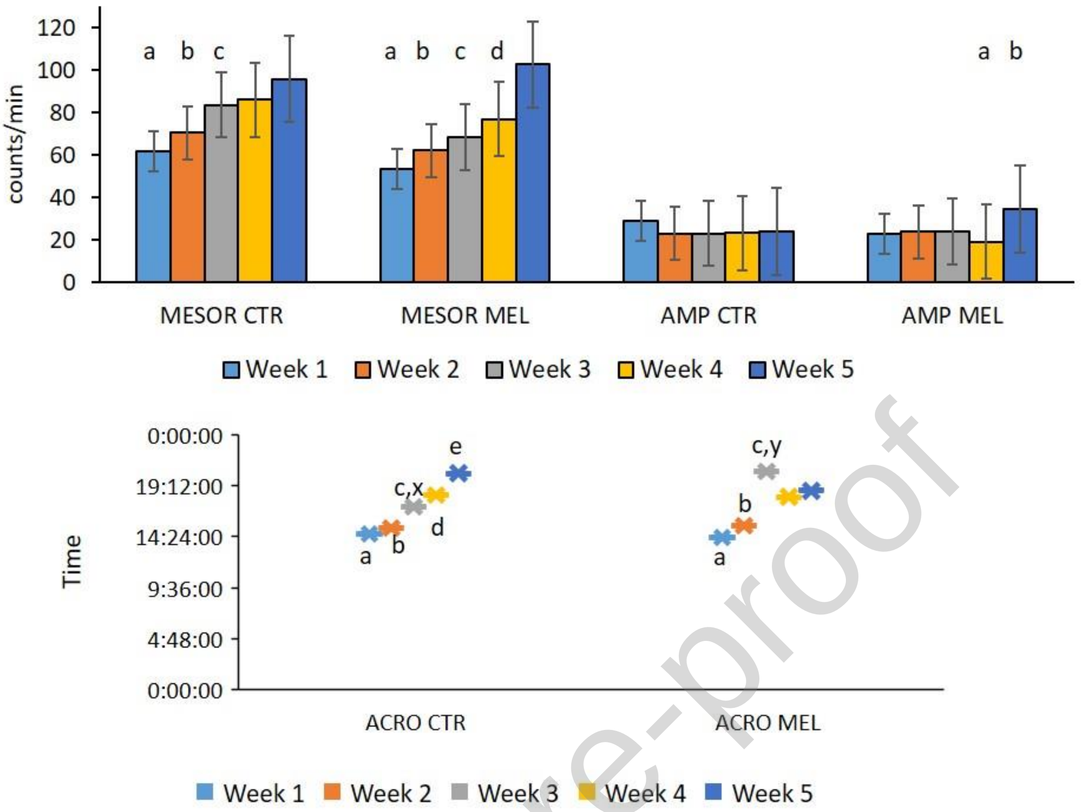

Fig. 5. Mean (±SE) Midline Estimating Statistic of Rhythm (MESOR), amplitude (the difference between the peak and the mean value of a wave), and acrophase (the time of peak activity) of the cosine curve of a 24-h locomotor activity rhythm of Rasa Aragonesa lambs that did (MEL, n=30) or did not (CTR, n=30) receive two 18 mg-melatonin implants at day 30 of age, and were fattened for five weeks from weaning (45 d of age) to slaughter (85 d of age) (a,b,c indicate significant differences at P<0.05 within groups; x, y indicate significant differences at P<0.05 between groups in the same week).

Ethical Considerations Statement

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.

Conflicts of interest

The authors declare no conflict of interest.

HIGHLIGHTS

- Melatonin implant diminished locomotor activity in fattening lambs
- Melatonin implant reduced rectal and surface temperatures in fattening lambs
- Female lambs treated with melatonin consumed less concentrate than not treated females
- Females treated with melatonin had a greater back fat thickness than not treated females