Locomotor activity measured by actigraphy as a means of estimating social mixing and animal density-induced stress in lambs

JOSÉ-ALFONSO ABECIA[1]*, JOSÉ-MARÍA BELLO[2], LAURA MARCO[2], IRENE VIOLA[3], FRANCISCO CANTO[1], GENARO C. MIRANDA DE LA LAMA[4]

[1] IUCA, Departamento de Producción Animal y Ciencia de los Alimentos, Universidad de Zaragoza, Miguel Servet, 177, 50013 Zaragoza, Spain
[2] NANTA S.A.U., Ronda de Poniente 9, 28460 Tres Cantos, Madrid, Spain
[3] Department of Veterinary Sciences, University of Turin, Largo Paolo Braccini 2, 10095 Grugliasco, Italy
[4] IA2, Departamento de Producción Animal y Ciencia de los Alimentos, Universidad de Zaragoza, Miguel Servet, 177, 50013 Zaragoza, Spain

SUMMARY

This study aimed to determine whether social mixing and density-induced stress affect locomotor activity in lambs. Eighteen 50-70 d lambs on a commercial farm were fitted with accelerometers for nine days. Lambs were housed with another 25 lambs in a 64- m^2 pen (density: 0.40 lambs/m^2; farm phase, FP). After nine days, lambs were transported to a feedlot and had the sensors affixed for another nine days. The 43 lambs were pooled with another 25 lambs that came from other farms, and were housed in a 10 x 10-m pen (density: 0.67; low-density phase, L-DP). After 5 d, another 49 lambs were added to the pen (density: 1.21; high-density phase, H-DP). The mean 24-h cosinor curve was calculated, and the cosinor values of each phase were compared statistically by Paired-sample t-tests. The effects of phase, lamb sex, time of day (day or night) on activity-related variables were evaluated statistically based on a multifactorial model; within fixed effects, significant differences were identified by an analysis of variance. Activity rates did not differ between FP and L-DP; however, lambs were less active in the H-DP than in the L-DP. Activity did not differ between males and females. The proportion of the lambs that exhibited a 24-hour circadian rhythm was higher in the FP than in the L-DP and the H-DP, which differed from each other. In conclusion, social stress, caused by social mixing and high densities can affect the locomotor activity and disrupt the circadian rhythms of lambs. This study demonstrated the usefulness and feasibility of using sentinel animals (fitted with sensors) in commercial feedlots to monitor changes in activity so that measures can be taken to improve animal welfare if necessary.

KEY WORDS

Feedlot, Lamb welfare, Social mixing, Stock density, Locomotor activity, Accelerometers.

INTRODUCTION

Accelerometers often have been used to monitor routine human behaviors including exercise and sleep [1]. In addition to information on daily activity and exercise intensity, the accelerometer provides data on the quantity, frequency, and duration of the activity. Triaxial accelerometers, which often have been used in veterinary medical research as a non-invasive means of recording all small-scale body movements of an animal, have undergone significant advancements, and many of the historical limitations of direct observations of animals in the field have been removed because of the advantages of accelerometry over other methods of quantify the activities of farm or companion animals [2].

The influence of different housing conditions and feeding schedules (grazing, housed in a box and fed ad libitum, or housed in a box and restrict feeding) on the daily rhythm of total locomotor activity in sheep has been studied using accelerometers [3]. Thus, an effect of time of day and the different experimental conditions on locomotor activity was observed, concluding that, even if locomotor activity is mainly entrained by photoperiod, the amount of activity may be influenced by housing conditions and food availability. Tri-axial accelerometers have also been used to study melatonin, rectal temperature and locomotor activity daily rhythms [4-5], or to investigate the influence of different reproductive stages and livestock management conditions on the pattern of the daily rhythm of total locomotor activity in dairy ewes [6]. We used actigraphy to quantify the circadian rhythmicity and locomotor activity in lambs in the lactation period. In addition, we quantified the relationships between lambs and their twins by using Bluetooth-enabled (BT) accelerometers that documented their proximity [7]. Recently, we used actigraphy to measure the locomotor and feeding activities of lambs raised artificially in their first three weeks of life [8].

In many countries, sheep production often is carried out in extensive or semi-extensive systems, mainly based on grazing [9]; however, typically, the finishing of lambs involves an intensive fattening process in industrial feedlots, with the aim of producing quickly animals of the size and weight demanded by the market [10]. The specialized fattening begins with the selec-

Corresponding Author:
José Alfonso Abecia (alf@unizar.es)

tion of lambs based on age, weight, size and or sex in the farms of origin, which are sent to the final fattening phase in private or cooperative feedlots, where the lambs are combined with animals from other sources for several weeks [11]. In those feedlots, the animals are expose to several sources of stress such as weaning or separation from their original flock-mates, artificial and novel environments, social mixing with unfamiliar animals, new feeding schedules and diets, and various density gradients [12]. Those sources of stress occur within a short period and, from a welfare perspective, create a drastic change for the animals from the natural farm environment to an artificial environment such as that experienced in a feedlot [13]. Under feedlot conditions, the social structure among the animals is severely disrupted, and lambs tend to establish a new dominance hierarchy through aggression [14], which can be exacerbated when animals are repeatedly mixed or access to resources (e.g., space, food, water, known flock-mates) is restricted or limited [15]. The size of the group, the age and sex of individuals, the degree of relatedness, the relationships among individuals, and the duration of association among group members influences the organization of a social group [16]. Although the effects of social mixing and high densities on the physiological and behavioral stress responses in lambs have been identified in several studies [12,14], understanding of the disruption of behavioral patterns is limited. New technologies such as accelerometer-based sensors provide a means of measuring locomotor activity and, because of the availability of continuous data, the circadian rhythms in behavior can be identified [17].

The effect of housing conditions on total locomotor activity in four domestic species (horse, sheep, goat and cattle) under natural 12/12 light/dark photoperiod, was measured by actigraphy, and a significant effect of species and housing conditions on all rhythmic parameters was reported, concluding that the influence of housing conditions on the characteristics of locomotor circadian rhythm should be taken into consideration in order to guarantee welfare in animal breeding [4]. In 30-day-old calves, housed in individual calf houses, the total locomotor behavior shows a diurnal daily rhythmicity, and the characteristics of rhythm were different from individual to individual and from day to day, so that the recorded intersubject variability must be taken in consideration during the monitoring of farm animals and justifies the application of the device to each animal [6].

This study used actigraphy to identify changes in locomotor activity as a means of assessing stress caused by social mixing and stocking density in commercial lambs.

MATERIAL AND METHODS

Animals

The study was performed at the experimental farm of the University of Zaragoza (Zaragoza, Spain; latitude 41° 41 N). In late Feb and early Mar 2023, eight-teen 50-70 d Rasa Aragonesa lambs (13 males, 6 females) (mean liveweight (±S.D.): 14.3±2.5 kg) on a commercial farm (Pinseque, Zaragoza, Spain; 41°43'N, 1°4'W) were fitted with commercially available sensors (46 mm × 33 mm × 15 mm in size, mass = 19 g) that record high-resolution raw acceleration data (ActiGraph wGT3X-BT; ActiGraph, FL, USA) that were attached to the dorsal side of a neck collar that remained in place for nine days. The sensors record accelerations (activity) based on the individual's amplitude (g) and frequency (Hz) of movement along three axes (x for front-to-back, y for side-to-side, and z for up-down). Sensors were programmed to collect data at a rate of 30 Hz (30 samplings/s). The 18 lambs were housed with another 25 lambs in a 64- m^2 pen, which created a density of 0.40 lambs/m^2 (farm phase, FP). After nine days, all lambs were transported to a feedlot (Franco y Navarro S.A.; La Joyosa, Zaragoza, Spain; 41°45'N,1°50'W), and had the sensors affixed for another nine days. The feedlot had three sheds (70 x 15 m) that housed 1750 lambs each. In the central shed, the lambs were classified based on age, body condition, and weight and housed until slaughter (25 kg). The 43 lambs were pooled among another 25 lambs that came from other farms and were kept in a 10 x 10-m pen, which created a density of 0.67 lambs/m^2 (low-density phase, L-DP). After five days, another 49 lambs were added to the pen, which created a density of 1.21 lambs/m^2 (high-density phase, H-DP). Sensors were removed four days later.

From the original farm to the feed-lot farm, the experimental lambs were transported in the middle of the bottom deck of the lorry with other nonexperimental animals, filling the vehicle's capacity of about 200 lambs. The entire trip took 30 minutes, including loading and unloading. The feed-lot company supplied the livestock lorry, which had been built specifically for transporting sheep. It had two axles, three floors, a robust, spring suspension, and forced ventilation. A hydraulic elevator was available on the vehicle for loading and unloading. Space was allotted at 0.32 m^2 per lamb. Feeding regime (Complecor, Nanta, 16% CP) and bedding conditions (straw) were similar during the three phases of the study.

Accelerometer data

The raw activity data were downloaded as activity counts per 1 min intervals to the ActiLife software (ActiGraph, LLC, Pensacola, FL), which generates three columns of data; i.e., activities on the x-, y-, and z-axes. The activity counts for the three axes were used to create minute-by-minute activity records; i.e, Vector Magnitude (VM), which is the magnitude of the vector that is formed by the combination of the accelerations from the three axes. VM was calculated as follows:

$$VM = \sqrt{(x\text{-axe})^2 + (y\text{-axe})^2 \ (z\text{-axe})^2}$$

Statistical analysis

Before performing the statistical tests, the Kolmogorov-Smirnov Test was used to confirm the normality of the data. For each of the three periods of data collection (on farm and at the feedlot), mean (±S.E.) VM 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 [18]. Midline Estimating Statistic of Rhythm (MESOR, 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 variable for each individual. To test for rhythmicity, an F-test compared the (re-parameterized) cosine model and the non-rhythmic 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, and the cosinor values of each phase were compared statistically by Paired-sample t-tests. The effects of phase, lamb sex, 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 [19] was used. Within fixed effects, significant differences were identified by an analysis of variance. 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.

RESULTS

Activity

Figure 1 shows the 24-h mean VM values in the three phases of the study. Lambs exhibited significantly higher mean locomotor activity in the day (101.08±0.17 counts/min) than they did at night (76.63±0.14 counts/min). Activity rates did not differ significantly between the FP (88.87±0.15 counts/min) and the L-DP at the feedlot (92.03±0.1 counts/min); however, at the feedlot, lambs were significantly (P<0.05) less active in the H-DP (78.12±0.23 counts/min) than they were in the L-DP. Activity rates did not differ significantly between males (87.33±0.13 counts/min) and females (88.58±0.19 counts/min).

Circadian rhythmicity

The F-test used to compare the cosine model and the non-rhythmic model, to determine whether the time series of VM fitted a 24-h rhythm, revealed that the proportion of the lambs that exhibited a 24-hour circadian rhythm was significantly (P<0.01) higher in the FP (71%) than it was in the low-density (29%) and high-density (0%) phases at the feedlot, and the proportions differed significantly (P<0.05) between the two densities at the feedlot. The hourly profile of the locomotor activities of the lambs were similar in the three phases (Figure 3); a peak near sunrise and another near dusk. The MESOR, amplitude, and acrophase of the COSINOR locomotor activity curves did not differ significantly between the FP and the L-DP at the feedlot (Table 1), but the MESOR and amplitude of their locomotor activity were reduced significantly (P<0.01) and their acrophase advanced in the H-DP (Figure 3).

DISCUSSION

Sheep are highly social and their social behavior influences many aspects of their lives such as grazing, spatial distribution, proximity to other group members and anti-predator strategies [20]. Our study showed that social stress (social mixing and high densities) can affect locomotor activity by disrupting the circadian rhythms of the lambs. Conducted under commercial conditions, our study demonstrated the importance of social behavior in sheep, not only in terms of social stability, but its impact on other motivations such as maintenance, which can affect animal welfare and health.

Changes in locomotor activity in farm animals are used com-

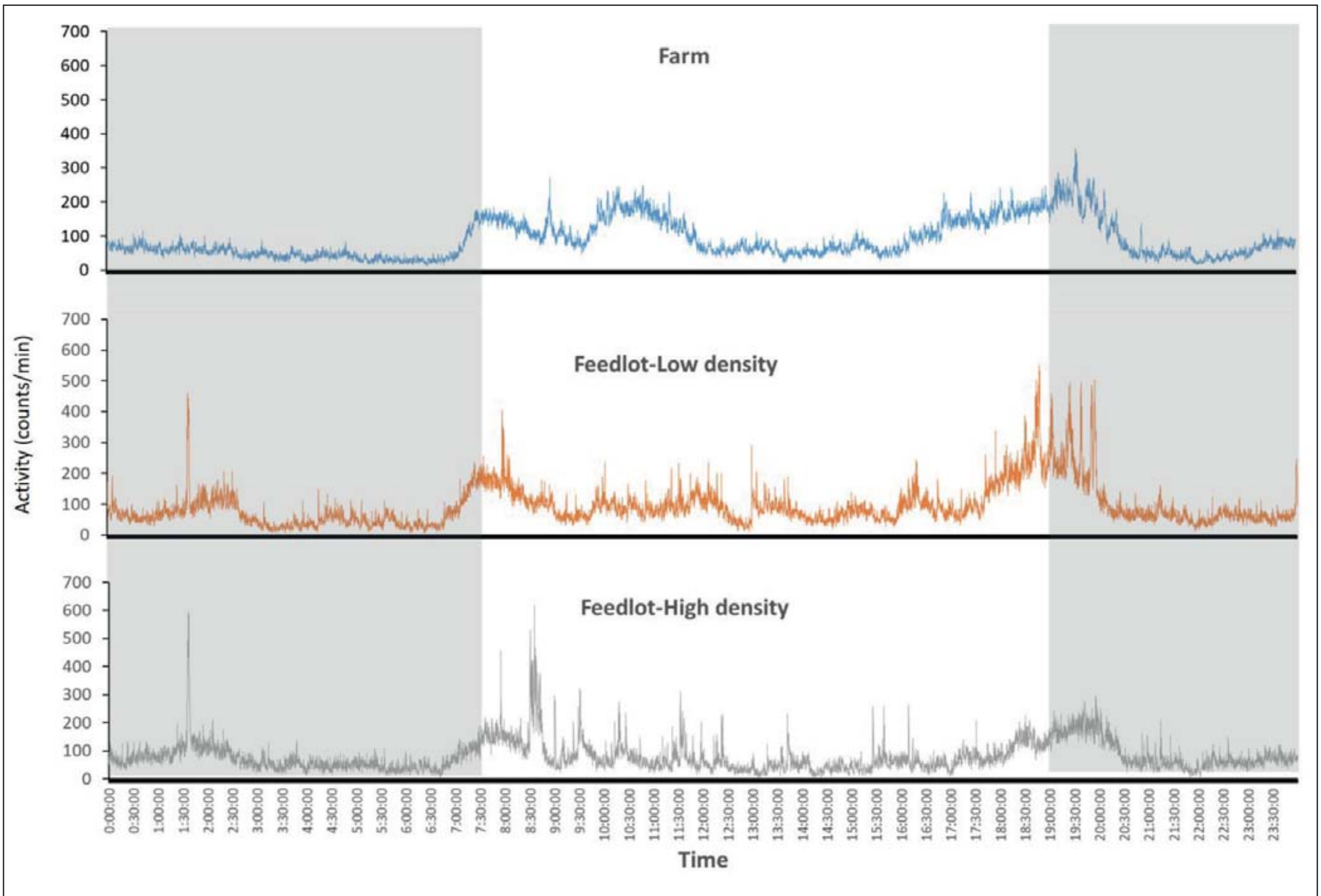

Figure 1 - Mean 24-h locomotor activity (counts/min) of Rasa Aragonesa lambs on their original farm (0.40 lambs/m²) and after they had been moved to a feedlot where they were housed at a low (0.67 lambs/m²) and a high (1.21 lambs/m²) density (grey areas represent night).

monly as an indicator of animal welfare [3,21]. Our study demonstrated significantly higher activity in lambs in the day than at night. The bimodal nature of locomotor activity in sheep was confirmed, which has been reported in other studies that have used accelerometry [7-8,22-23]. Although sex differences in locomotor activity have been reported in sheep, they were not evident in the lambs in our study. Females are known to have higher locomotor activity than do males in the estrus period and in risky environments [24]. Possibly, sexual differences in locomotor activity have not yet manifested in immature animals such as the lambs in our study. In another study that used the same technology to measure locomotor activity, we detected differences between male and female lambs [7]; specifically, females were more active than were male lambs when they were lactating with their mothers, but overall activity did not differ significantly between sexes when lambs were artificially reared [8].

Although no differences in activity were found between the farm and the feedlot at low densities, lambs were significantly less

Table 1 - 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 on their original farm (0.40 lambs/m^2) and after they had been moved to a feedlot where they were housed at a low (0.67 lambs/m^2) and a high density (1.21 lambs/m^2) (a,b indicates significant differences among housing conditions, P<0.001).

	Farm	Low-density	High-density
MESOR	89.13±4.53^a	91.44±6.09^a	78.81±4.9^b
Amplitude	33.11±3.74^a	30.79±3.58^a	13.12±1.87^b
Acrophase	14:30±0.62^a	16:20±0.71^b	8:07±2.00^b

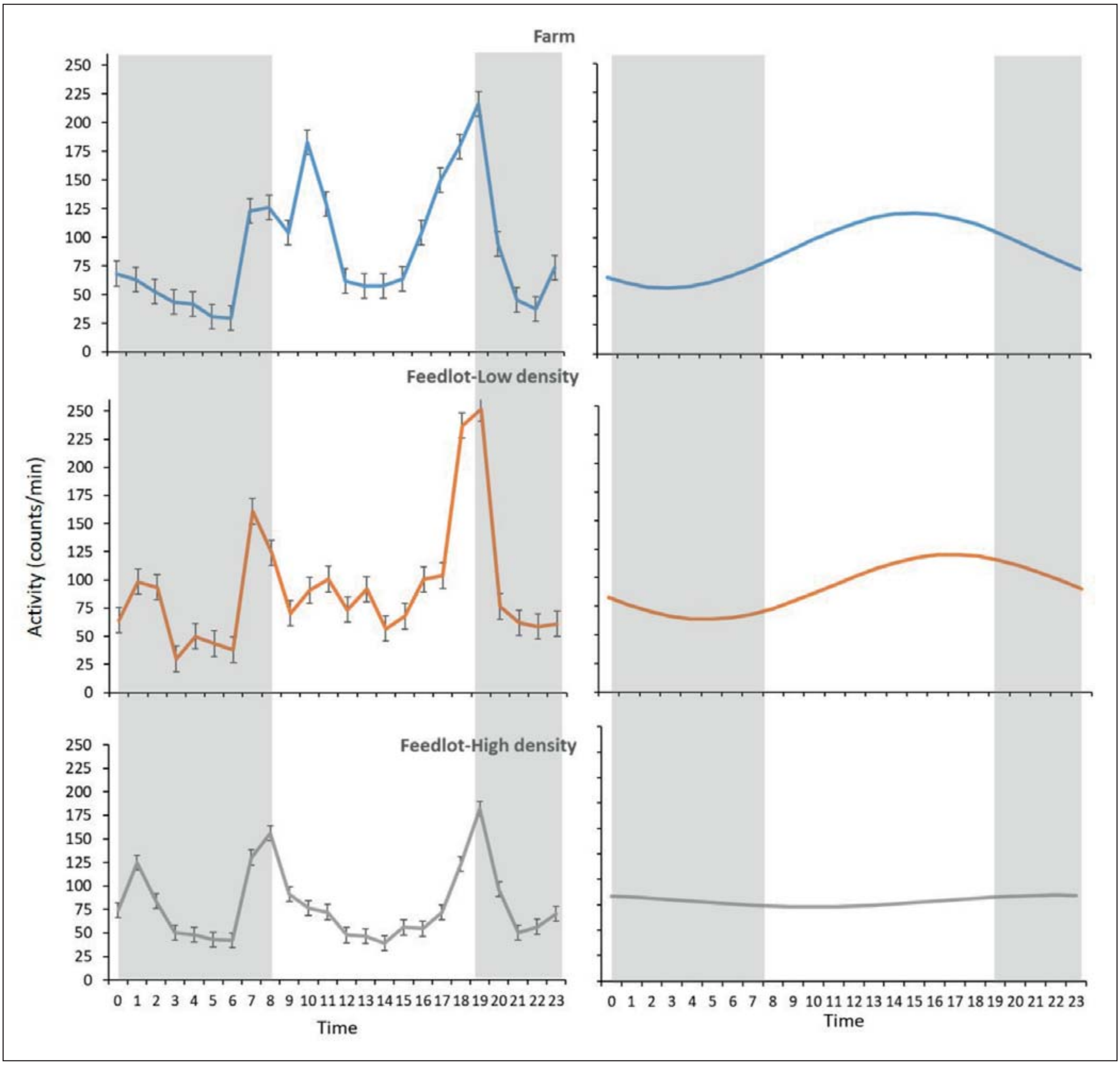

Figure 3 - Mean (±S.E.) daily activity (counts/min/h) (left panel) of Rasa Aragonesa lambs at their original farm (0.40 lambs/m^2) and after they had been moved to a feedlot and housed under a low (0.67 lambs/m^2) and a high (1.21 lambs/m^2) density, and the corresponding cosinor curves (right panel) of a 24-h activity rhythm (grey areas represent night).

active in the feedlot at high densities. In housed animals, the space available can influence activity [25] and, possibly, the reduction in time spent moving is caused by the proximity of conspecifics and the limitations on movement [26]. In addition, however, the high density (preceded by social mixing, transport, handling) might have caused an increased stress response and fatigue, which might have reduced the animals' motivation to move [27].

Circadian rhythms are nearly 24-hour oscillations that synchronize function and behavior (i.e., activity and locomotion) to adapt and optimize the physiology of organisms to environmental changes [28]. In our study, the proportion of lambs that exhibited a nictitating circadian rhythm was significantly higher in the FP than it was in the L-DP, and was virtually absent in the H-DP in the feedlot. The alteration or disruption of circadian rhythms can be a consequence of a pronounced stress response and has been associated with an increased risk of neurodevelopmental disorders in various animal models [29]. In intensive and extensive systems, sheep activity has been observed to have a bimodal frequency distribution (particularly, in summer) with peaks in activity around dawn and dusk [30], which was apparent in our study in each of the three phases. Unlike in extensive systems in which animals have relatively uniform access to resources, in feedlots space is significantly reduced, which forces animals to compete for space and leads to an increase in the incidence of lambs reducing their activity through social synchrony [31]. That might have caused the differences between the low- and high-density phases at the feedlot in our study. Possibly, when faced with an unpredictable environment over which they have little control, animals alter their circadian rhythm as a temporal habituation mechanism [12]. Future studies should investigate how high densities in the abattoir affect behavioral activity and weight gain in lambs throughout the fattening period.

CONCLUSION

Our study showed that social stress caused by social mixing and high densities, can affect the locomotor activity and disrupt the circadian rhythms of lambs. This study demonstrated the usefulness and feasibility of using sentinel animals (fitted with sensors) in commercial feedlots to monitor changes in activity so that measures can be taken to improve animal welfare if necessary. In addition, training programs incorporating scientific evidence (such as our study) need to be implemented to sensitize farmers and operators to the effects of social mixing and high densities on animal activity. Future studies should investigate the effects of disruptions in circadian rhythms on the immune response and weight gain of lambs under commercial conditions.

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Author contributions

JAA: conceptualization, funding acquisition, investigation, methodology, project administration, resources, writing-original draft; writing-review and editing; JMB, LM: investigation, methodology, writing-review; IV, FC: investigation, methodology, writing-review; GCM: conceptualization, writing-review and editing. All authors read and approved the final manuscript.

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Ethics approval

The study was conducted at the experimental farm of the University of Zaragoza, Spain (41° 63 N), following procedures approved by the Ethics Committee for Animal Experiments at the University of Zaragoza. The care and use of animals were by the Spanish Policy for Animal Protection (RD 53/2013), which meets the European Union Directive 2010/63 on the protection of animals used for experimental and other scientific purposes.

Competing interests

The authors report no declarations of interest.

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