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Research Report

Circadian rhythm of locomotor activity and body temperature in free-range laying hens as measured by triaxial accelerometers and subcutaneous biologgers

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SUMMARY

Biosensors are essential tools for monitoring temperature in poultry because they provide real-time data that can enhance animal welfare and productivity. The aim of this study was to evaluate the use of triaxial accelerometers and subcutaneous biologgers to monitor of locomotor activity (LA) and body temperature (BT) in free-range laying hens, to explore their potential for assessing circadian patterns relevant to welfare monitoring. Five hens were fitted with a harness that carried an accelerometer to record LA for 6 d. In addition, hens received a surgically implanted biologger to record BT every 15 min. Animals were housed indoors that had an artificial photoperiod (16L:8D) and an adjacent outdoor pen. Hen BT decreased between 1900 h and 2300 h, and was lowest 30 min before lights turned off at 2330 h. From that moment, BT increased throughout the night and until 0900 h in the morning; after which, it remained stable until the evening. Animals remained practically motionless during darkness, and began moving immediately after lights turned on. BT and LA exhibited a 24-h circadian rhythm. There was a significant correlation between BT and LA ($P < 0.01$), but BT was not correlated with ambient T. LA was correlated with indoor and outdoor ambient T ($P < 0.01$). In conclusion, free-range laying hens exhibited clear circadian rhythms in LA and BT, which were synchronized with the light-dark cycle. Ambient T influenced LA, which was correlated with BT. Those findings can contribute to the optimization of management practices that are intended to maximize welfare and productivity.

Description of problem

Global demand for eggs increased in response to population growth and increased awareness of the nutritional benefits of eggs (Henchion et al., 2021). In response, the poultry industry has shifted toward alternative housing systems such as free-range systems, which are perceived to offer living conditions for laying hens that are better for welfare than those offered by conventional cage systems. Free-range systems allow hens greater freedom of movement, access to outdoor areas, and opportunities to engage in natural behaviors such as foraging, dust bathing, and exploring (Lay et al., 2011). Often, eggs from those systems are marketed as being produced in more ethical and sustainable ways than those from conventional systems, which appeals to consumers who prioritize animal welfare; however, an understanding of how free-range conditions influence key welfare indicators such as locomotor activity (LA) and body temperature (BT) in laying hens is limited (Rodríguez-Aurrekoetxea et al., 2016). Although some studies have measured LA or BT, individually, few have assessed the relationship between these parameters in free-range hens, which has limited our ability to develop evidence-based guidelines for optimizing free-range systems. In addition to animal welfare, the importance of this research includes economic and sustainability considerations. Historically, free-range systems have had high production costs because of the need for more land and more complex management practices; however, these costs can be offset by improvements in hen health, productivity, and egg quality, which are closely linked to welfare. For example, hens that are least stressed and most active tend to have the strongest immune systems, lowest mortality rates, and highest egg production (Lay et al.,

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Fig. 1. Laying hens (n = 5) were fitted with commercially available tri-axial accelerometers (Axivity AX3 3-Axis, 23 × 32.5 × 7.6 mm; 11 g) that recorded raw acceleration data, which were housed in a plastic box (Hammond ABS, 50 × 35 × 20 mm, 18 g) that was attached to a dorsal harness.

2011).

LA, which includes walking, running, and stretching, provides information on the level of physical activity of hens, which can be indicative of welfare status when interpreted in the appropriate behavioral and environmental context. In free-range systems, hens have the most opportunity to move freely, which can promote physical health and reduce stress (Campbell et al., 2020). Understanding those locomotor patterns is essential for optimizing free-range systems so that hens fully benefit from the opportunities provided by those environments. BT regulation is another important indicator of hen welfare, particularly in free-range systems where hens are exposed to fluctuating environmental conditions (Campbell et al., 2021). In free-range systems, hens can experience greater temperature variability because they are exposed to outdoor conditions, which can affect their ability to maintain thermal balance. This can have significant effects on hen health, productivity, and overall welfare.

Advancements in remote monitoring technologies have made it possible to collect detailed data on hen behavior and BT in free-range environments (Casey-Trott and Widowski, 2018; Pullin et al., 2020). For instance, accelerometers have been useful in monitoring poultry in research settings, particularly, hens (Casey-Trott and Widowski, 2018; Pullin et al., 2020), although not yet in routine management. The sensors, which measure acceleration, are versatile instruments for tracking and recording vital data at research level, and have gained substantial attention because they can contribute to various aspects of poultry production; e.g., health monitoring. Pullin et al. (2020) used tri-axial accelerometers to record acceleration events in hens, which provided insights into the effects of pullet rearing on collisions and perch use in enriched colony cage layer housing. Casey-Trott and Widowski (2018) demonstrated that accelerometers can enhance animal welfare research by quantifying the effects of pain or sickness on activity, measuring daily activity patterns, and quantifying individual differences in general activity. Elsewhere, we quantified LA in three strains of laying hens based on data from triaxial accelerometers (Abecia et al., 2024).

Biologgers have become essential tools for monitoring temperature in poultry because they provide real-time data that can enhance animal welfare and productivity (Neethirajan, 2020). Those systems use various technologies, including wireless sensors and thermal imaging, to track temperature changes in poultry, which allow for timely interventions to prevent health issues (Hu and Tan, 2009).

The aim of this study was to demonstrate that LA and BT of free-range laying hens can be automatically monitored with biologgers and accelerometers under experimental-farm conditions. Our approach provides proof of concept that automated technologies yield reliable, continuous data on animal physiology and behavior, with minimal handling and without interfering with the animals' daily routine. Since the study was guided by the premise that ambient conditions (light, temperature, RH) influence LA and BT in free-range hens, the relationship between physiological and ambient parameters was also calculated.

Materials and methods

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. The care and use of animals were in keeping with 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.

Animals

Five hens (6 mo of age) from a commercial hybrid egg layer strain (Brown strain, Live weight (LW): 2.2 ± 0.3 kg; GRAPISA, Pinseque, Zaragoza, Spain) were used in the experiment. In mid-December, hens were fitted with a commercially available sensor that records high-resolution raw acceleration data (Axivity AX3, Axivity Ltd, Newcastle upon Tyne, UK) (23 × 32.5 × 7.6 mm; 11 g), which was housed in a plastic box (Hammond ABS, 50 × 35 × 20 mm, 18 g) that was attached to the back of the hen by a harness (Fig. 1). The activity monitors have been validated against direct behavioral observations, following the approach described by Murillo et al. (2020), ensuring that the accelerometer outputs reliably matched actual hen behaviors.

For six days, hens carried the sensor, which recorded (at 30 Hz) 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, hens received a surgically implanted subcutaneous T biologger (DST micro-HRT, Star Oddi, Iceland) (8.3 × 25.4 mm, 3.3 g), which had been programmed to record data every 15 min and was left in place for six days (Fig. 2). The biologgers were sterilized by immersion in a 0.55 % ortho-phthalaldehyde solution (CIDEX-OPA, Johnson & Johnson, New Jersey, USA) for 24 h. For the surgery, hens were placed in dorsal recumbency (Fig. 2). After removing some feathers to expose

Fig. 2. Biologgers (DST micro-HRT, Star Oddi, Iceland; 8.3 × 25.4 mm, 3.3 g) and some of the surgical procedures used to implant them subcutaneously in laying hens.

the skin, 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. A subcutaneous pocket was constructed with scissors to place the biologger on the superficial pectoral muscle, just beneath the skin (Fig. 2), which 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 or 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.

To program the biologgers, the Mercury software v5.83 (Star Oddi, Gardabaer, Iceland) was used via a communication box.

Hens were allowed 1 wk to acclimate to wearing the accelerometer before the start of the experiment. Animals were housed in an indoor hen house (3 m^2, density 0.6 m2/hen) that had an artificial photoperiod (16L:8D, light from 0730 h to 2330 h), feeders and automatic drinkers, perches, and a nest. The perches were made of wood, avoiding contact-induced cooling. The hens had access to an adjacent outdoor pen (4 m^2) that was equipped with dust baths. Access to the outdoors was through 35 cm x 24 cm doors. The walls between the indoor and the outdoor spaces had windows, which provided natural light in the facility. Water was available ad libitum. The hen coop was in a building that had air heating, which was turned on at 0900 h and turned off at 1700 h. At the time of the experiment, sunrise and sunset were at 0824 h and 1733 h, respectively. Ambient T (°C) and relative humidity (RH, %) were recorded by mini data-loggers (Testo 174H, Testo SE & Co. KGaA, Titisee-Neustadt, Germany), located both inside the indoor house and in the outdoor pen.

Statistical analysis

Shapiro-Wilk tests indicated that the distributions of the data of BT and LA did not follow a normal distribution. Mean (± S.E.) LA, BT, and ambient T were calculated at hourly intervals, and the significance in the differences between light and dark periods were assessed based on U Mann-Whitney tests. The Spearman coefficients of correlation among ambient T, BT, and LA were calculated.

Circadian rhythms were analyzed by fitting the time-series data from each hen to a 24-h cosine curve derived from the cosinor method provided by the Cosinor online platform (Molcan, 2019). The key rhythm

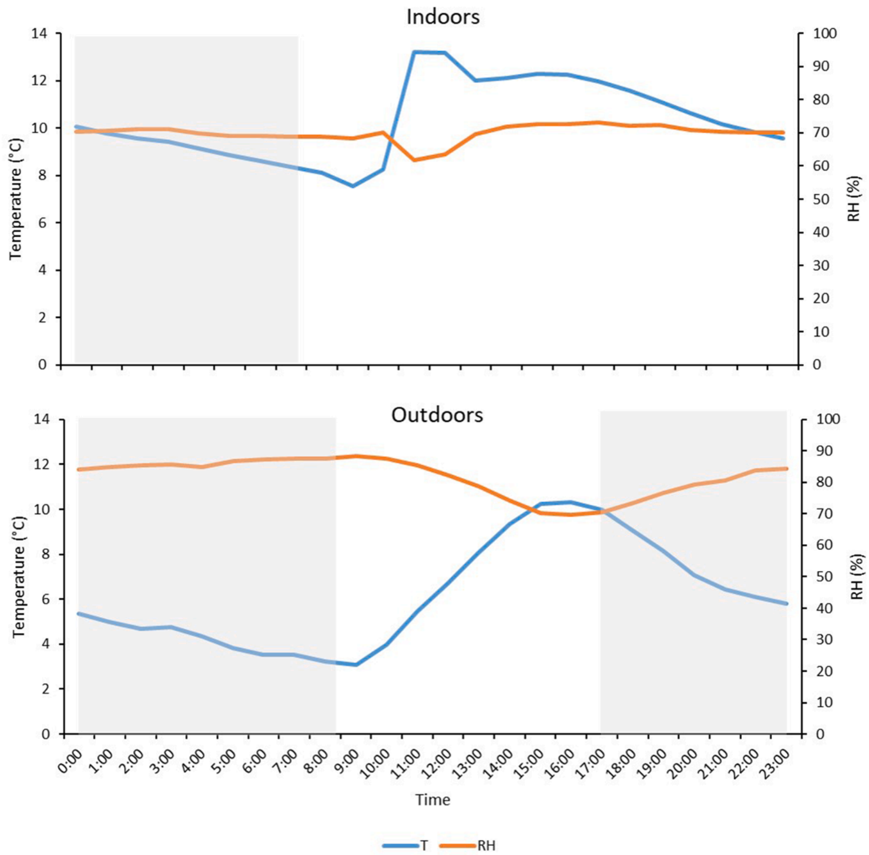

Fig. 3. Mean 6-day temporal changes in ambient temperature and relative humidity (RH) at the facility where laying hens were housed (the indoor hen house had an area of 3 m^2, with the feeders and automatic drinkers, perches, and a nest. The adjacent outdoor pen was 4 m^2 and was equipped with dust baths (grey areas represent dark period of the day).

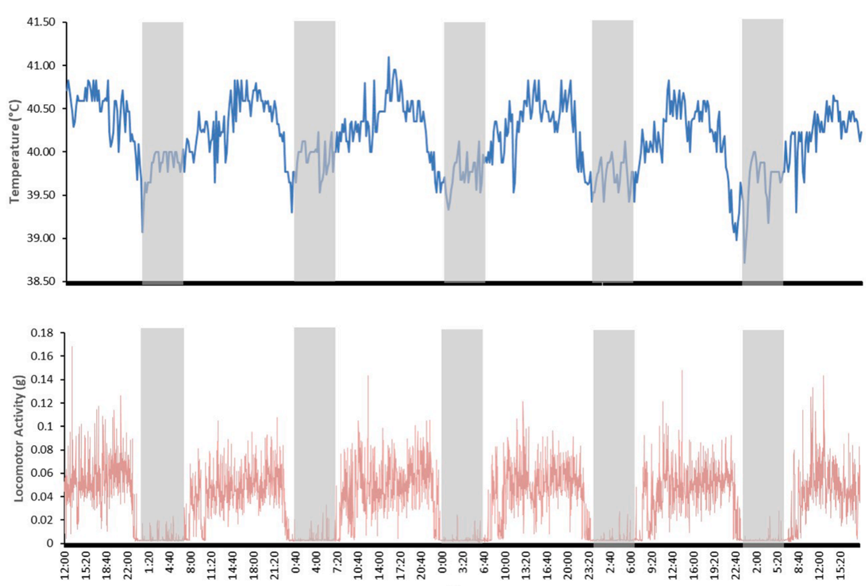

Fig. 4. Body temperature and locomotor activity of a representative laying hen throughout the 6-d study as recorded by subcutaneous biologgers and triaxial accelerometers, respectively (grey areas represent dark period of the day).

variables Midline Estimating Statistic of Rhythm (MESOR), (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

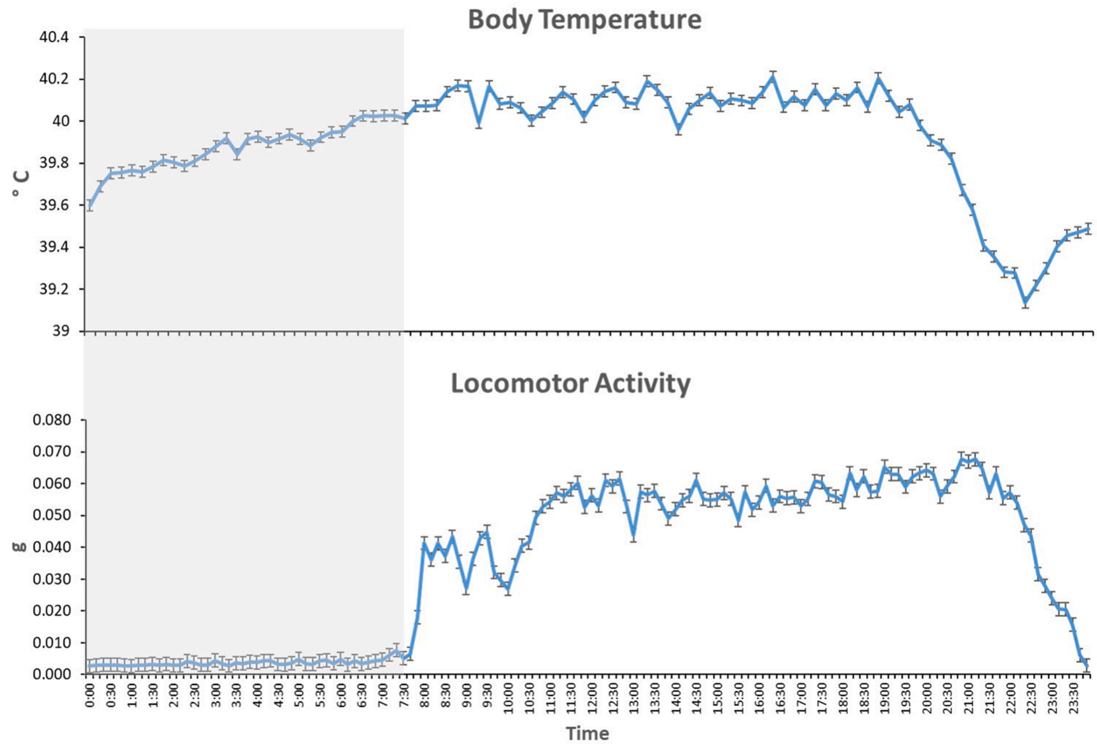

Fig. 5. Mean (± S.E.) 24-h body temperature and locomotor activity of laying hens as recorded by subcutaneous biologgers and triaxial accelerometers, respectively, for 6 d (grey areas represent dark period of the day).

Table 1
Mean (± S.E.) indoor and outdoor ambient temperatures (T) and relative humidity (RH), and BT and locomotor activity (LA) of laying hens as measured by subcutaneous biologgers and triaxial accelerometers throughout the 6-d study.

	Light	Darkness
Ambient T indoors (°C)	10.62 ± 0.31[a]	9.35 ± 0.26[b]
RH indoors (%)	69.79 ± 0.60	70.13 ± 0.68
Ambient T outdoors (°C)	5.52 ± 0.64[a]	7.15 ± 0.55[b]
RH outdoors (%)	82.99 ± 0.92	79.60 ± 0.75
BT (°C)	39.99 ± 0.01[a]	39.86 ± 0.01[b]
LA (g)	0.048 ± 0.001[a]	0.004 ± 0.001[b]

for each variable. Significant differences in cosinor values among phases were confirmed by an analysis of variance.

Results and discussion

Indoor ambient T increased and RH decreased after the heater turned on in the morning, and the opposite occurred after the heater turned off in the afternoon (Fig. 3). Outdoor ambient T increased from 0900 h to 1600 h, and decreased throughout the evening and night. Both indoor and outdoor ambient T were significantly ($P < 0.01$) higher in the day than they were at night, but RH did not differ significantly.

Fig. 4 presents an example of the patterns in BT and LA of a representative hen throughout the experiment. Hen BT decreased rapidly between 1900 h and 2300 h, and was lowest approx. 30 min before the lights turned off at 2330 h (Fig. 5). From that moment, BT increased throughout the night and until 0900 h in the morning; after which, it remained stable until the evening. BT and LA were significantly ($P < 0.001$) higher when lights were on than were lights were off (Table 1).

The animals remained practically motionless during the darkness, and began moving immediately after the lights turned on at 0730 h (Fig. 5). LA began to decrease at approximately 2100 h, in anticipation of the lights turning off. BT and LA exhibited a 24-h circadian rhythm (Fig. 6), with a MESOR, amplitude, and acrophase of 39.9°C, 0.27°C, and 1129 h for BT, and 0.035 g, 0.031 g, and 15:40 h for LA. BT and LA were significantly ($P < 0.01$) correlated; LA, but not BT, was significantly ($P < 0.01$) correlated with indoor and outdoor ambient T (Table 2).

This study demonstrated the clear circadian rhythms in LA and BT in free-range laying hens based on data recorded by triaxial accelerometers and subcutaneous biologgers. Thus, the novelty of this work lies in validating automated monitoring as a feasible and robust tool for welfare and management purposes, beyond the descriptive confirmation of diurnal patterns. LA and BT were higher in the day than they were at night and were synchronized with the artificial photoperiod (16L:8D). That pattern occurs in other diurnal birds and reflects the importance of light as the main zeitgeber of circadian rhythms (Aschoff, 1981; Gwinner, 1986; Daan and Aschoff, 2001).

In laying hens, LA increased abruptly after lights turned on (0730 h), acrophase occurred at 1540 h, and progressively decreased from 2100 h onwards, in anticipation of the dark period. That activity pattern is characteristic of hens in natural or enriched conditions, and reflects behaviors such as foraging, exploration, and socialization during6 the day (Blokhuis, 1983; Ross, 2018). The strongly expressed anticipation suggests a robust endogenous component, beyond a simple light response (Cassone, 2014). In fact, it has been reported that laying hens also anticipate darkness with a large increase in feeding behavior in the last few hours before lights out (Bessei, 1977; Tanaka and Hurnik, 1991).

Simultaneously, BT decreased markedly between 1900 h and 2300 h, before lights-out (2330 h), and decreased throughout the dark period before stabilizing at around 0900 h. Although it is likely that the superficial intramuscular placement of the sensor does not exactly reflect absolute core body temperature, this location has proven reliable for detecting relative changes and circadian rhythmicity. Minor fluctuations may be influenced by environmental contact (e.g., perches or litter during roosting), yet these interactions are consistent across individuals and periods, and therefore do not compromise the ability of the

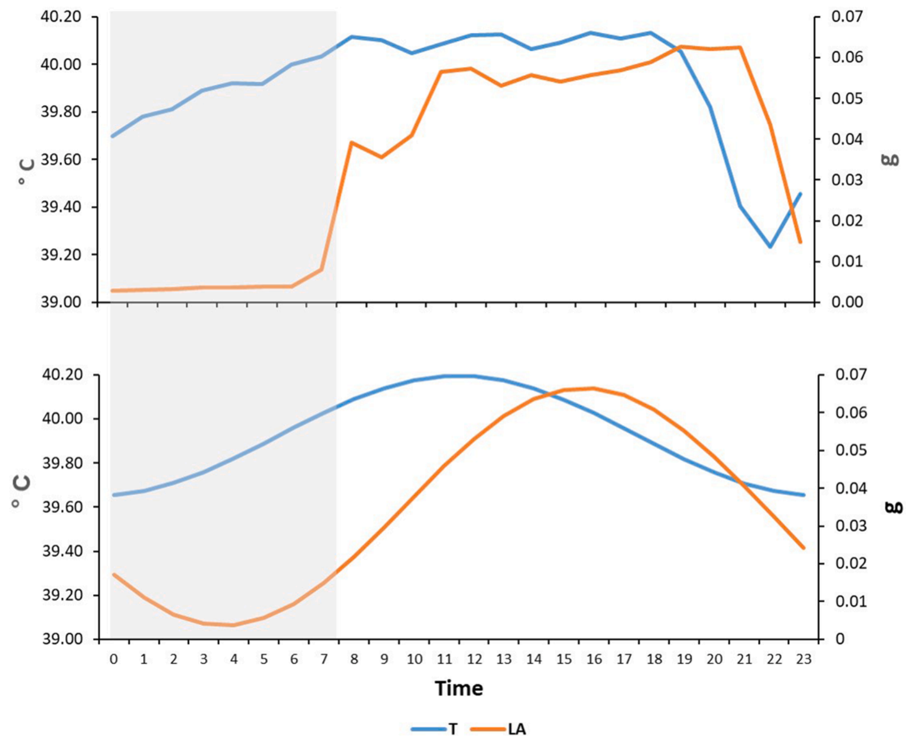

Fig. 6. Mean 24-h hourly body temperature (BT) and locomotor activity (LA) of laying hens, as recorded by subcutaneous biologgers and triaxial accelerometers, respectively, for 6 days, and the corresponding cosinor curves of the 24-h activity rhythms (grey areas represent dark period of the day).

Table 2
Spearman coefficients of correlation among indoor and outdoor ambient temperatures (T), and body temperature (BT) and locomotor activity (LA) of laying hens (** P<0.01).

	Ambient T indoors	Ambient T outdoors	BT	LA
Ambient T indoors		0.876**	0.345	0.746**
Ambient T outdoors	0.876**		0.302	0.838**
BT	0.345	0.302		0.595**
LA	0.746**	0.838**	0.595**	

biologgers to capture biologically relevant temporal patterns. The acrophase of BT (1129 h) preceded that of LA, which might have been caused by internal physiological factors such as basal metabolism, digestion, and hormonal rhythms that occur on the reproductive axis (Aschoff, 1981; Decuypere and Kühn, 1988), or even due to wearing the sensor. Ovulation, which is highly synchronized with the circadian rhythm, probably influences BT. Studies have demonstrated a relationship between pre-ovulatory hormonal peaks and thermal increases in birds (Etches, 1995; Rozenboim et al., 2004; Johnson, 2015), and others have shown that, in laying hens, ovulation and oviposition follow diurnal rhythms and, typically, occur in the early hours of the day, and that these physiological events are accompanied by transient increases in BT (Etches, 1995; Johnson, 2015). Such increases might have contributed to the temporal pattern in BT observed in our study, especially in the morning phase. Telemetry data from laying hens has demonstrated a cyclical pattern in BT that coincides with ovulation-related events. It has been reported that deep-BT follows a 28-hour cycle that had two peaks, one that coincides with egg laying and a another that, possibly, coincide with ovulation (Winget et al., 1965). Kadono et al. (1981) observed a marked increase in temperature at oviposition that was part of a cycle that was 25.2–25.3 h, which suggested that BT is temporally linked to reproductive events. Indeed, the superficial intramuscular placement of the loggers in the present experiment does not capture absolute deep core temperature, but it provides consistent relative measures that are suitable for detecting circadian fluctuations.

The significant correlation between LA and BT (r = 0.595, P < 0.01) suggests that increased physical activity generates metabolic heat (Brown-Brandl et al., 2003). In addition, in laying hens, LA was positively correlated with ambient T (both indoor and outdoor), which indicated that ambient temperature influences the motivation for birds to move. BT and ambient T were not significantly correlated, which suggests an efficient homeothermic capacity in hens, possibly maintained by physiological and behavioral mechanisms of thermoregulation (Yahav, 2019; Campbell et al., 2021).

Active indoor heating throughout the daylight period might have attenuated the influence of external thermal variations on BT, or the hens were able to thermoregulate to maintain a fairly constant BT. The significant effect of outdoor temperature on LA might have been because of the high variation in ambient conditions outside the coop, which should be studied in extreme environmental conditions such as heat waves or low winter temperatures.

Indeed, a limitation of this study is the relatively small sample size, yet the consistent patterns obtained indicate that the methods are robust; future studies with larger populations will allow further validation and strengthen the applicability of these automated monitoring tools.

Triaxial accelerometers and subcutaneous biosensors proved to be effective and reliable tools for continuous, non-invasive monitoring of hen behavior and physiological status, which validated their suitability for studies of animal welfare in alternative production systems (Casey-Trott and Widowski, 2018; Pullin et al., 2020; Casey-Trott et al., 2018; Neethirajan, 2020; Pullin et al., 2020. Those technologies allow for high-resolution data collection and are superior to traditional observational methods.

Conclusions and applications

1. The results confirm that biologgers and accelerometers can be used successfully to monitor circadian rhythms of body temperature and activity in animals under real housing conditions.
2. Free-range laying hens exhibited distinct circadian rhythms in LA and BT, with clear synchronization with the light-dark cycle.
3. Ambient T affected LA, which was strongly correlated with BT.
4. While these diurnal patterns themselves are well established in the literature, the contribution of this work is to show that these rhythms can be automatically detected with high precision, offering a pathway for precision livestock farming applications.
5. The implications are that farmers and researchers can adopt automated monitoring systems to obtain continuous, non-invasive data that facilitate the early detection of welfare problems, improve management decisions, and ultimately contribute to a more sustainable production system.
6. Future studies should measure reproductive hormones; e.g, progesterone, LH, to assess directly the influence of the ovulatory cycle on BT in laying hens, and the hourly distribution of indoor and outdoor activities recorded.

CRediT authorship contribution statement

José A. Abecia: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Writing – original draft, Writing – review & editing. **Francisco Canto:** Investigation, Methodology, Writing – review & editing. **Javier Plaza:** Methodology, Writing – review & editing. **Jaime Nieto:** Methodology, Writing – review & editing. **Carlos Palacios:** Conceptualization, Investigation, Methodology, Writing – original draft, Writing – review & editing.

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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