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Daily and estrous rhythmicity of body temperature in domestic cattle

Giuseppe Piccione[1], Giovanni Caola[1] and Roberto Refinetti*[2]

Address: [1]Dipartimento di Morfologia, Biochimica, Fisiologia e Produzioni Animali, Facoltà di Medicina Veterinaria, Università degli Studi di Messina, 98168 Messina, Italy and [2]Circadian Rhythm Laboratory, University of South Carolina, Walterboro, SC 29488, USA

Email: Giuseppe Piccione - giuseppepiccione1@virgilio.it; Giovanni Caola - giovanni.caola@unime.it; Roberto Refinetti* - refinetti@sc.edu

* Corresponding author

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Abstract

Background: Rhythmicity in core body temperature has been extensively studied in humans and laboratory animals but much less in farm animals. Extending the study of rhythmicity of body temperature to farm animals is important not only from a comparative perspective but also from an economic perspective, as greater knowledge of this process can lead to improvements in livestock production practices. In this study in cattle, we investigated the maturation of the daily rhythm of body temperature in newborn calves, characterized the parameters of the daily rhythm in young cows, and studied the oscillation in body temperature associated with the estrous cycle in adult cows.

Results: We found that the daily rhythm of body temperature is absent at birth but matures fully during the first two months of life. The mature rhythm had a mean level of 38.3°C, a range of excursion of 1.4°C, and was more robust than that of any mammalian species previously studied (90% of maximal robustness). Sexually mature cows also exhibited a robust estrous rhythm of body temperature. An elevation of about 1.3°C was observed every 21 days on the day of estrus. Small seasonal variations in this pattern were observed.

Conclusion: In conclusion, calves exhibit a very robust daily rhythm of body temperature, although this rhythm is absent at birth and develops during the first two months of life. Adult cows exhibit also 21-day rhythmicity in body temperature reflecting the duration of the estrous cycle.

Background

Biological rhythms have been extensively studied in animals. Two classes of rhythms that have received considerable attention are those related to the nycthemeron (daily rhythmicity) and those related to the reproductive cycle (estrous rhythmicity). Daily and estrous rhythmicities have been demonstrated in a variety of physiological and behavioral variables in many species [1,2]. Rhythmicity in core body temperature has been extensively studied in humans and laboratory animals [3,4] but much less in farm animals [5]. Rhythmicity in body temperature is an important physiological process both as a convenient and reliable marker of the operation of the biological clock [6,7] and as an indicator of the general health of an animal and of its energy metabolism [8,9].

Extending the study of rhythmicity of body temperature to farm animals is important not only from a comparative perspective (as most studies conducted so far concentrated on humans and laboratory animals) but also from an economic perspective (as greater knowledge of this process can lead to improvements in livestock production

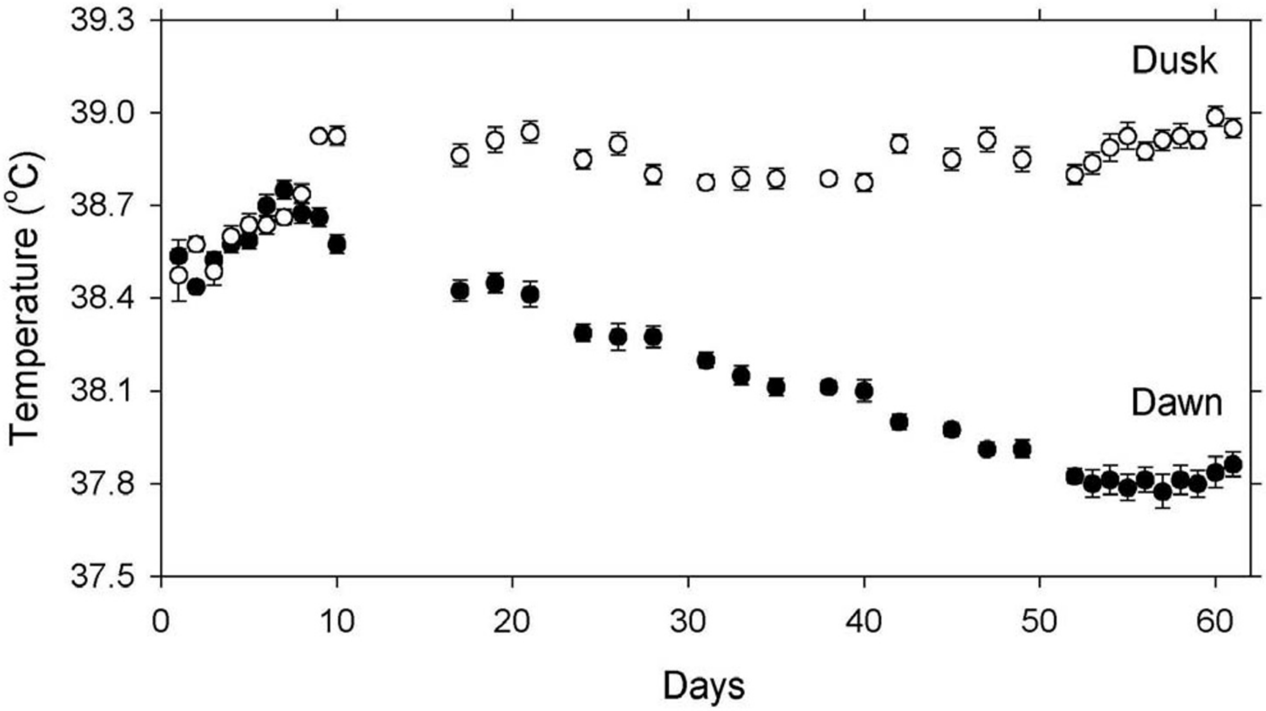

Figure 1
Mean body temperature of 8 calves measured daily from birth to 2 months of age. Values shown are means ± SEM. Measurements conducted at dusk are denoted by open circles. Measurements conducted at dawn are denoted by closed circles. As indicated by the missing data points, measurements were not conducted every day.

practices). In this study in cattle, we investigated the maturation of the daily rhythm of body temperature in newborn calves, characterized the parameters of the daily rhythm in young cows, and studied the oscillation in body temperature associated with the estrous cycle in adult cows.

Results

The mean body temperature of the 8 calves measured at dawn and dusk during the first two months of life is shown in Fig. 1. Measurements conducted at dawn and dusk were equivalent during the first 8 days of life. At both times of day, temperature rose about 0.3°C during this time. On the 9th day, the temperature measured at dusk rose another 0.2°C and remained relatively stable for the remaining of the recording period. In contrast, temperature measured at dawn fell about 0.9°C over several weeks, reaching a stable level on the 52nd day. Although ambient temperature was lower at dawn (22°C) than at dusk (28°C), the 6°C difference was rather small for a large homeotherm (75 kg by day 60) and unlikely to be responsible for the 1.2°C difference in body temperature between dawn and dusk. Furthermore, the 6°C difference in ambient temperature had no apparent effect on body temperature during the first 8 days of life, when thermal lability would be expected to be greater.

Greater temporal resolution of the oscillation in body temperature is provided in Fig. 2 for the first and last 10 days of the study. There was no daily rhythmicity during the first 8 days. A daily oscillation of about 0.5°C could be observed starting on the 9th day. By day 52, a regular pattern of daily oscillation had been established with a range of excursion greater than 1°C. Body temperature consistently rose during the day, reaching a peak at sunset, and fell throughout the night. The smooth rhythms give no indication that the body temperature rhythm could have been a collateral effect of the feeding schedule (food was provided 2.5, 7.5, and 12.5 h after sunrise daily). The daily rise in body temperature did not seem to anticipate (precede) the dark-to-light transition, although the 3-h resolution of our measurements would not allow the detection of anticipation by 1 or 2 h.

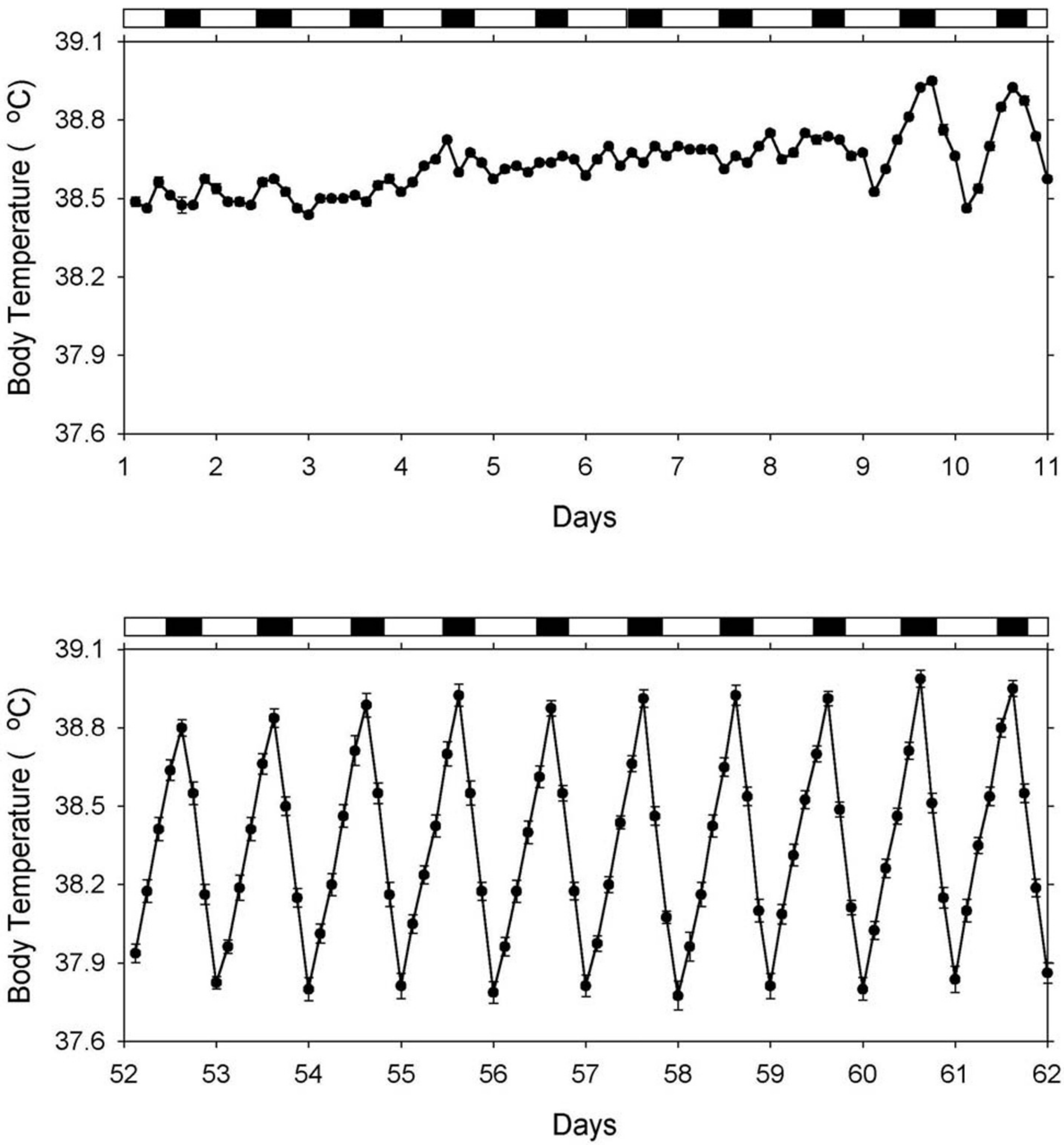

Figure 2
Mean body temperature of 8 calves measured every 3 hours. Values shown are means ± SEM of the body temperature of 8 calves measured every 3 h during the first 10 days of life (top) and during days 52 through 61 (bottom). The white and dark bars at the top of each panel indicate the duration of the light and dark phases of the light-dark cycle, respectively.

For consistency with the previous use of the chi square periodogram procedure in studies of circadian rhythmicity [14,24], the data were analyzed in 10-day blocks. Analysis of the rhythmicity of body temperature of the 8 calves revealed no significant daily rhythmicity during the first 10 days of life (mean $Q_P = 7$, $p > 0.05$), despite the incipient rhythmicity on days 9 and 10. Very significant 24-h rhythmicity was present during days 52 to 61 (mean $Q_P = 72$, $p < 0.001$). For this type of data (10 days with measurements at 3-h intervals), a perfectly rhythmic process yields a Q_P of 80. Thus, rhythm robustness was 9% of maximal robustness during days 1–10 and 90% of maximal robustness during days 52–61. The acrophase of the rhythm during days 52–61 was 18.6 ± 1 h (mean ± SEM), or 54 min before sunset. The mean level of body temperature was 38.64 ± 0.02°C for days 1–10 and 38.34 ± 0.02°C for days 52–61. This difference was statistically significant, $t(7) = 9.17$, $p < 0.001$. The range of excursion of body temperature was 0.74 ± 0.04°C for days 1–10 and 1.41 ± 0.04°C for days 52–61. This difference, which was reduced by the inclusion of days 9 and 10 in the first 10-day block, was also significant, $t(7) = 18.45$, $p < 0.001$. The range of excursion calculated for only the first 8 days was 0.28 ± 0.03°C.

Records of body temperature of four representative adult cows measured at dawn and dusk over three consecutive estrous cycles in the summer and winter are shown in Fig. 3. As expected, temperature measured at dusk was consistently higher than temperature measured at dawn. At both time points, a sharp rise in body temperature was observed on the day of estrus. The duration of the estrous cycle (computed by counting the days between two consecutive estrus episodes, as determined by observation of vaginal discharge, increased locomotor activity, and acquiescence to mounting by a bull) was exactly 21 days in all cycles of all animals in the summer. In the winter, there was some intra- and inter-individual variability. Cycle length ranged from 18 to 23 days with a mean of 21.3 days.

Mean values of three parameters of the estrous cycle of body temperature are shown in Fig. 4. Robustness was slightly but significantly higher in the summer than in the winter, $F(1, 21) = 22.92$, $p < 0.001$, but there was no significant effect of time of day, $F(1, 21) = 1.02$, $p > 0.05$, or of the interaction between season and time of day, $F(1, 21) = 3.07$, $p > 0.05$. For this type of data (3 estrous cycles with daily measurements), a perfectly rhythmic process yields a Q_P of 60. Thus, the robustness of the rhythm ranged from 43% of maximal robustness at dawn in the winter to 63% of maximal robustness at dawn in the summer. As expected, the mean level of the temperature cycle was significantly higher at dusk than at dawn, $F(1, 21) = 5806.50$, $p < 0.001$. It was also very slightly but significantly higher in the summer than in the winter, $F(1, 21) = 14.00$, $p < 0.002$, primarily at dawn (37.60 ± 0.02°C in the summer vs. 37.55 ± 0.02°C in the winter). The range of excursion was greater at dawn than at dusk, $F(1, 21) = 275.84$, $p < 0.001$, and greater in the winter than in the summer, $F(1, 21) = 17.92$, $p < 0.001$.

To evaluate the utility of body temperature measurements for the prediction of estrus, we analyzed the coincidence of temperature peaks with the direct measures of estrus. A peak in body temperature was consistently observed at dawn on the day of estrus (Fig. 3), although the practical utility of a same-day prediction is rather limited. We analyzed the changes in temperature on the days preceding estrus and found that an acceptable predictor of estrus could be computed as follows: 1) calculate the mean temperature at dawn for the past 5 days; 2) if today's temperature at dawn exceeds the mean of the preceding 5 days plus 3 standard deviations, then estrus will occur tomorrow. The use of this predictor led to the correct prediction of estrus in 32 of 41 episodes (i.e., a detection rate of 78%). The procedure also identified 5 episodes that did not happen (i.e., a false positive rate of 12%). Alterations of the procedure intended to reduce the false positive rate led to decreases in the detection rate. Similarly, alterations intended to increase the detection rate led to increases in the false positive rate.

Discussion

Although the daily rhythm of body temperature has been studied in detail in a variety of mammalian species [3,4], little attention has been given to farm animals [5]. Our results in 50–60 day old calves help characterize the daily rhythm of body temperature in domestic cattle. In animals maintained under environmental conditions of the mild Sicilian summer (temperature lows at 22°C and highs at 28°C; 15 h of sunlight per day), we found the mean level of the body temperature rhythm to average 38.3°C with a range of excursion of 1.4°C. This mean level is approximately 0.5°C lower than those obtained in four other studies conducted by other research teams [10–13]. Our animals were relatively young, but this cannot explain the difference, as our mature cows (1 year old) used in the study of the estrous cycle had a mean body temperature of 37.6°C at dawn and 38.5°C at dusk, which is consistent with a daily mean of 38.3°C. The higher mean level of the temperature rhythm in the other studies is likely due to hotter test environments and to the fact that several of them utilized lactating cows. In contrast to studies conducted on laboratory animals, studies on farm animals have often lacked standardized procedures [5]. Although we used a rectal probe instead of a telemetry device in this study, we followed a strict procedure with the probe inserted 9 cm (calves) or 15 cm (adults) into the rectum, a depth that is commonly used in veterinary practice. In large animals such as cattle, the minimal disturbance to the animals caused by the insertion of the probe does not produce alterations in body temperature as it does in small rodents.

We found the acrophase of the daily temperature rhythm to be about 1 h before sunset, which is consistent with the

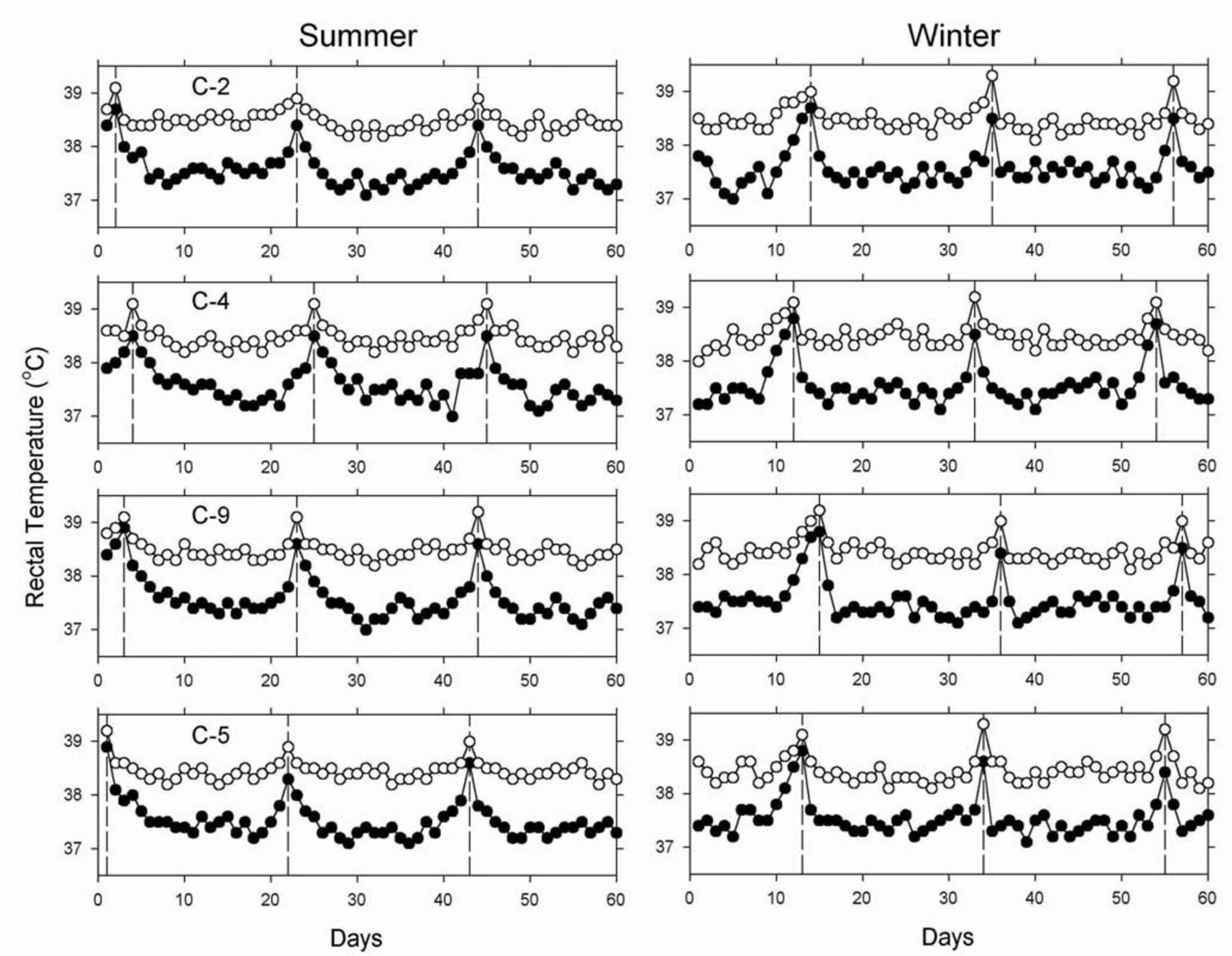

Figure 3
Daily measurements of body temperature over 3 estrous cycles. Shown are the records of body temperature of four representative cows measured at dusk (open circles) and dawn (closed circles) for 60 consecutive days during the summer (left) and winter (right). Vertical dashed lines indicate the days of estrus, as determined by observation of vaginal discharge, increased locomotor activity, and acquiescence to mounting by a bull.

results of the previous studies [10–12]. The robustness of the rhythm has not been previously investigated in cattle. We found it to be 90% of maximal robustness, which is possibly the highest robustness observed in a mammalian species. In six species of small rodents, the robustness of the body temperature rhythm was found to range from 48 to 77% of maximal robustness [14]. In sheep, goats, and horses, it ranged from 44 to 65% of maximal robustness [15,16].

Our results also provide important information about the maturation of the daily rhythm of body temperature. We observed no daily rhythmicity during the first week after birth. A dawn-dusk difference in body temperature was first noticeable on day 9 and increased gradually until a stable daily rhythm was attained approximately 2 months after birth. The greater daily range of excursion was achieved mostly by a lowering of temperature at dawn. Curiously, this pattern in calves is the opposite of the one we observed in lambs, where the difference in temperature between dawn and dusk was achieved almost exclusively by a gradual elevation of temperature at dusk, while the temperature at dawn remained unaltered [17]. The 2-month maturation process reported here in calves is

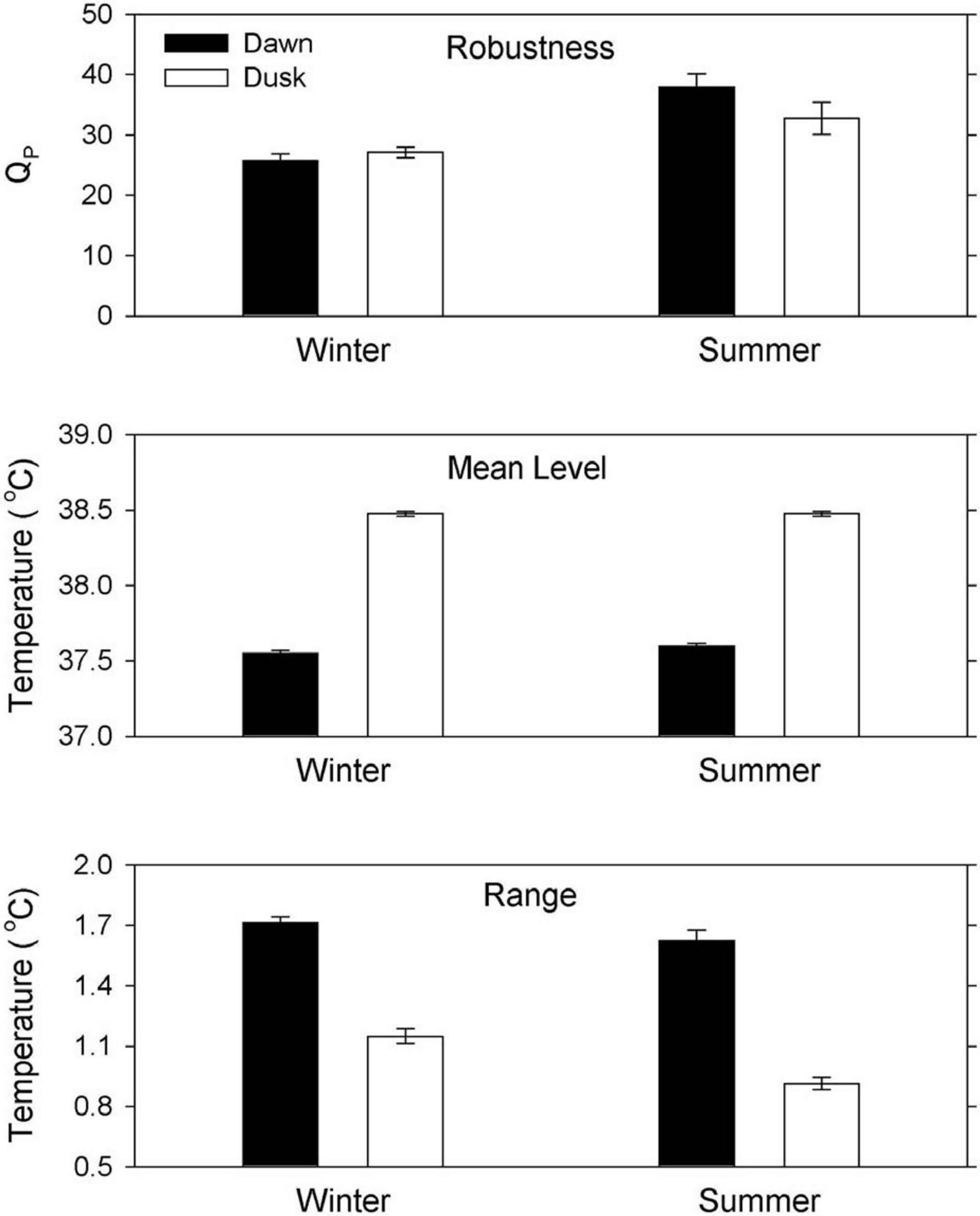

Figure 4

Parameters of the estrous rhythm of body temperature. Values shown are means ± SEM of three parameters of the estrous rhythm of body temperature of 8 cows (robustness, mean level, and range of excursion), as computed for measurements conducted at dawn (dark bars) and dusk (clear bars) during the winter and summer. For this type of data (3 estrous cycles with daily measurements), a Q_P value of 60 corresponds to a perfectly rhythmic process.

slightly slower than that observed in lambs and foals (1 month) [17]. The reasons for the differences in timing and mechanism between calves and lambs are not evident. These differences may be due to differences in the nursing behavior of the mothers of the two species (which we have not studied) or to differences in the thermoregulatory physiology of cattle and sheep. Different species of farm animals exhibit different parameters of body temperature rhythmicity in adults [5], and it would be reasonable to expect that differences also exist in the process of ontogentic development of rhythmicity.

In addition to the daily rhythm of body temperature, we investigated oscillations in body temperature associated with the estrous cycle. Body temperature measured at dawn was consistently lower than temperature measured at dusk and showed a greater range of excursion and similar rhythm robustness. The estrous rhythmicity of body temperature exhibited a smaller range of excursion but greater robustness in the summer than in the winter, possibly because of the difference in ambient temperature (mean lows of 22°C in the summer and 9°C in the winter). Greater rhythm robustness despite smaller range of excursion is not an unusual finding, as robustness and amplitude are independent (albeit interactive) parameters of a rhythm [4]. As a matter of fact, the greater robustness observed in the summer may have been a direct consequence of the smaller range of excursion if the latter resulted from fewer disturbances caused by environmental factors.

The duration of the estrous cycle (computed by counting the days between two consecutive estrus episodes, as determined by observation of vaginal discharge, increased locomotor activity, and acquiescence to mounting by a bull) was exactly 21 days in all cycles of all animals in the summer. In the winter, cycle length ranged from 18 to 23 days with a mean of 21.3 days. Our observation of a sharp rise in body temperature on the day of estrus is consistent with previous observations by other research groups [18–22], although we observed a larger range of excursion (1.3°C as compared to 0.2–0.6°C). Even though we did not collect quantitative data on locomotor activity, we observed a conspicuous elevation in activity on the day of estrus, in agreement with previous quantitative reports [20,21]. Because we did not manipulate this factor by immobilizing the animals, we cannot exclude the possibility that the estrus-related elevation in body temperature might be a consequence of the increased thermogenesis associated with increased activity. However, the fact that body temperature started to rise a few days before the day of estrus suggests the existence of an activity-independent process analogous to that observed in daily rhythms [23,24].

In every estrous cycle in our study, an elevation in body temperature was observed on the day of estrus. However, attempts to predict the day of estrus at least a day in advance had a detection rate of only 78% and a false positive rate of 12%. Although these figures are comparable to those attained in previous studies [18,19,21], they do not characterize the measurement of body temperature as a reliable method for the prediction of estrus.

Conclusions

In conclusion, calves exhibit a very robust daily rhythm of body temperature, although this rhythm is absent at birth and develops during the first two months of life. The mature rhythm has a mean level of 38.3°C, a range of excursion of 1.4°C, and is more robust than that of any mammalian species previously studied (90% of maximal robustness).

Adult cows exhibit also 21-day rhythmicity in body temperature reflecting the duration of the estrous cycle. The robustness of the rhythm is lower than that of the daily rhythm (43 to 63% of maximal robustness, depending on season) but its range of excursion is comparable (1.3°C).

Methods

The animals used in this study were cared for and experimented upon in accordance with current laws regulating research on agricultural animals in Italy and the United States. All animals were Frisian cattle (*Bos taurus*). They were housed in stables with windows at the veterinary facility of the University of Messina, in Sicily (38° 11' 30" North, 15° 33' 12" East, 120 m above sea level).

Eight female newborns (40 kg at birth) were studied during the summer under a natural photoperiod (sunrise at 0430 h and sunset at 1930 h) and natural ambient temperature (lows at 22°C and highs at 28°C). They were fed reconstituted milk three times a day at 0700, 1200, and 1700 h. Eight adult females (600 kg), not related to the 8 calves, were studied both in the summer and in the winter (sunrise at 0800 h and sunset at 1700 h, lows at 9°C and highs at 15°C). They were fed hay and concentrate (oats, corn, and barley) twice a day at 0700 and 1700 h.

Measurements of body temperature were conducted with a digital thermometer whose probe was inserted into the rectum to a depth of 9 cm (calves) or 15 cm (cows). Calves were studied during the first two months of life. Measurements were taken twice a day, at dawn (sunrise) and dusk (sunset), except that more frequent measurements (every 3 h) were conducted during days 1–11 and 52–62. Adult cows were studied for 60 consecutive days in the summer and 60 consecutive days in the winter. Rectal temperature measurements were taken twice a day, at dawn and dusk. Estrus was identified by observation of vaginal discharge, increased locomotor activity, and acquiescence to mounting by a bull.

For the analysis of daily rhythmic parameters, the body temperature data for each animal were evaluated for mean level, range of oscillation, acrophase (time of the daily peak), and rhythm robustness. The mean level of the body temperature rhythm was computed as the arithmetic mean of all values in the 10-day segment of data (80 data points) for each animal. The range of oscillation was computed as the difference between the highest and the lowest measurement. For computation of the acrophase (time of the daily peak of the rhythm), the 10-day segments of data were first averaged for each animal. Cosine waves were then fitted to the averaged 24-h rhythm, and the time corresponding to the peak of the best-fitting cosine wave was taken as the acrophase of the rhythm [25]. Finally, the robustness of the rhythm was calculated by the chi square periodogram procedure [26], where robustness is expressed as the Q_P statistic. The Q_P statistic reflects the strength or regularity of a rhythm independently of its amplitude. The greater the value of Q_P (up to that obtained for a perfectly rhythmic, artificial data set), the more robust is the rhythm. The Q_P value associated with P = 24 h (i.e., 24 h period) was used as the robustness index. This procedure was used also to compute the rhythm robustness of the estrous oscillation in the body temperature of the adult animals [27]. In this case, the Q_P associated with P = 20 days (i.e., 20 day period) was used as the robustness index.

The Q_P statistic of the chi square periodogram has a χ^2 distribution and, therefore, can be used to ascertain the existence of significant rhythmicity in the data set [26]. The significance of differences between means was tested by *t* tests for matched samples or by repeated-measures analyses of variance (ANOVA).

Authors' contributions

GP carried out the data collection procedures. GC supervised the data collection procedures and conducted bibliographic research. RR designed the study, conducted the data analysis procedures and drafted the manuscript. All authors read and approved the final manuscript.

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