Circadian rhythm of foot temperature assessed using infrared thermography in sheep

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ABSTRACT: The circadian rhythm of body core and surface temperature in 10 Comisana sheep kept under a natural photoperiod (06:30/19:00) was evaluated. Every 3 h for 24 consecutive hours rectal temperature (RT) and foot temperature were recorded. Particularly, foot temperature was recorded by infrared thermography, an ideal technique for evaluating the temperature not only at one point but also at the eight points as follows: in the right front of the interdigital area (FA1), in the left front of the interdigital area (FA2), in the right rear of the interdigital area (RA1), in the left rear of the interdigital area (RA2), in the right front of the interdigital line (FL1), in the left front of the interdigital line (FL2), in the right rear of the interdigital line (RL1) and in the left rear of the interdigital line (RL2). Two-way repeated measures ANOVA using SPSS, followed by Scheffé's test, showed a significant effect of the time of day and side of temperature collection ($P < 0.05$) on temperature values. The single cosinor procedure showed a daily rhythmicity of RT and foot temperature in all tested sides. The circadian oscillations of RT reflected the familiar circadian patterns of endogenous sources and the results of foot temperatures supported the idea that the daily rhythm was, at least in part, influenced by variation in the blood flow to the extremities. The infrared thermography providing more information on the development of disturbances in the peripheral circulation may be used with an advantage in occupational health examinations and in special clinical work.

Keywords: circadian rhythm; foot temperature; infrared thermography; rectal temperature; sheep

Circadian or daily rhythmicity is a ubiquitous and pervasive property of the mammalian physiology and behaviour (Turek and Van Reeth, 1996; Refinetti, 2006). Among many variables that exhibit circadian rhythmicity, body temperature has received a considerable attention (Refinetti and Menaker, 1992). In fact, it is well known that to maintain a constant body temperature, an animal has to satisfy the condition of "stationary equilibrium", in which the metabolic production of heat is equal to its loss (Piccione et al., 2008). In these conditions an increase of the metabolism allows the animal to adapt itself to different environmental conditions. So the regulation of temperature, one of the best studied functions affected by the circadian pacemaker, appears to be controlled by both homeostatic and circadian processes (Piccione et al., 2002b; Refinetti, 2010). A circadian pacemaker located in the suprachiasmatic nucleus (SCN) of the hypothalamus is responsible for an oscillatory process that is expressed in the body (Van Esseveldt et al., 2000; Moore and Leak, 2001). The examination of specific literature showed that a large number of studies were performed principally on humans (Reinberg and Smolensky, 1990; Brown et al., 2000) and on laboratory animals (Gordon, 1990; Refinetti, 1999; Jilge et al., 2001). The mechanisms involved in the temperature regulation and the synchronizers responsible for rhythmicity variations were also evaluated in horse, sheep and goat (Piccione et al., 2002a,b,c). All researches showed that the body temperature shows spontaneous and regular periodic oscillations over different periods of time. These oscillations are the result of complex mechanisms that witness the existence of endogenous and exogenous factors, within which the influence of age, season, feeding times and fasting, shearing and lactation was studied (Davidson and Fewell, 1993; Da Silva and Minomo, 1995; Mohor and Krzywanek, 1995; Jilge et al., 2001; Piccione and Caola, 2003; Piccione et al., 2003). The regulation of body temperature was also investigated in relation to physiological systems such as the respiratory, digestive, cardiovascular and motor system (Piccione and Refinetti, 2003). In addition to rectal temperature measurements, skin temperature was measured in different species of mammals. Skin temperature was measured in thigh, abdomen, ear and its daily patterns were observed in rabbit, goat, sheep, horse and cattle. All data showed statistical differences between body and skin temperatures (Piccione et al., 2005). Since changes in heat loss are primarily caused by variations in the skin blood flow, with consequent changes in skin temperature (Aschoff and Heiss, 1972), it is essential to evaluate if these skin temperature changes are affected by the circadian pacemaker, controlled by circadian processes. Moreover, the skin temperature and particularly foot temperature assume great importance as an indicator of diseases such as equine laminitis (Pollitt and Davies, 1998), foot rot and foot-and-mouth disease in cattle (Rainwater-Lovett et al., 2009). For this purpose the use of infrared thermography has a significant meaning because this technology is useful not only for assessing physiological responses to milking and feeding as observed in dairy cattle (Montanholi et al., 2008) but also for measuring the heat emitted from a surface (Dunbar et al., 2009). The technique of infrared thermography recognizes changes in the temperature of the entire body surface very quickly, in fact by the introduction of an image processing system, it is possible to obtain the average skin temperature of a specific region (Choi et al., 1997). In the present study we measured the foot temperature in sheep and the existence of the daily rhythm of foot temperature was evaluated. Particularly, the aim of this study was to observe the circadian rhythm of rectal temperature (RT) and foot temperature, measured not only at one point but also in the right and left front and rear of the interdigital area and interdigital line, using infrared thermography, in Comisana sheep.

MATERIAL AND METHODS

The study was conducted on 10 clinically healthy and not pregnant female sheep (Comisana breed, 2–6 years old, mean body weight 55 ± 2 kg). The health status of all animals, housed in a single box, was evaluated on the basis of behaviour, rectal temperature, heart rate, quality of respiration, cough, nasal discharge, eye discharge, appetite, faecal consistency, navel adspection and haematological profile.

As concerns feeding conditions, all animals were fed daily hay (2 kg), wheat straw (1 kg), wheat concentrate (0.5 kg) and water *ad libitum*.

During the trial period, all animals were kept under a natural photoperiod (sunrise 06:30, sunset 19:00), and air temperature and relative humidity (18–22°C; 70 Rh%) were recorded. Thermo-hygrometric recordings were conducted inside the pen throughout the entire study by means of a data logger (Gemini, Chichester, UK) placed in the middle of the boxes at 1.5 m height from the floor. All data were collected at 3 h intervals for 24 consecutive hours starting at 11:00 on day 1 and ending at 11:00 on day 2. On each subject rectal temperature was recorded with a calibrated electronic thermometer with the resolution of 0.1°C (Model HI-92740, Hanna Instruments, Bedfordshire, UK) with a probe inserted into the rectum at the depth of 9 cm while foot temperature was measured by infrared thermography. By means of Infrared Thermal Camera (ThermaCam P25 Model, Flir System, Wilsonville, USA) foot temperature was measured in the right front of the interdigital area (FA1), in the left front of the interdigital area (FA2), in the right rear of the interdigital area (RA1), in the left rear of the interdigital area (RA2), in the right front of the interdigital line (FL1), in the left front of the interdigital line (FL2), in the right rear of the interdigital line (RL1) and in the left rear of the interdigital line (RL2). The settings of the camera were as follows: emissivity of sheep's skin 0.98, distance between camera and skin surface 0.50 m.

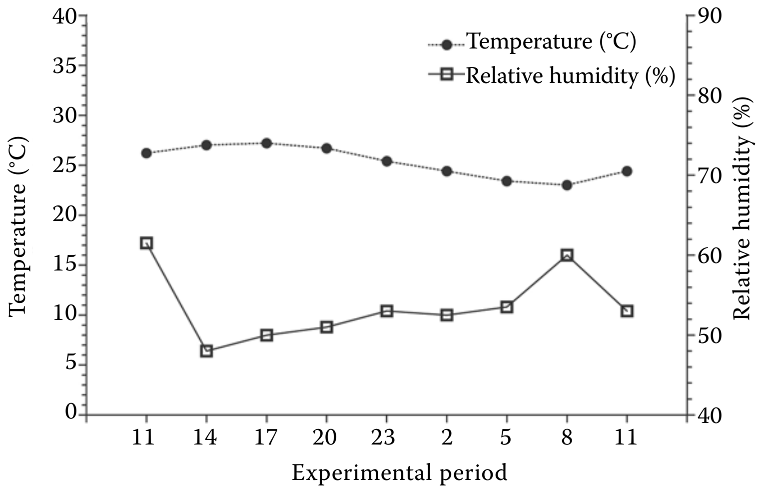

Figure 1. Air temperature (°C) and relative humidity (%) during the experimental period

The values of air temperature and relative humidity recorded continuously during the experimental period are shown in Figure 1. Temperatures were recovered by processing the thermographic images using the ThermaCam Researcher Basic Software (Flir System, Wilsonville, USA). Figure 2 shows the example of a thermographic image recorded in sheep exposed to natural photoperiodic and thermoperiodic conditions.

General animal care and welfare were carried out by professional staff not associated with the research team. All housing and care conformed to the standards recommended by the Guide for the Care and Use of Laboratory Animals and Directive 86/609 EEC.

All results were expressed as mean ± SD. Data was normally distributed ($P < 0.05$, Kolmogorov-Smirnov's test). Two-way repeated measures ANOVA was used to determine a statistically significant effect of the time of day and collection side on temperature. Scheffé's test was applied for post hoc comparison. P value < 0.05 was considered statistically significant. The data was analysed using the STATISTICA 8 software (StatSoft Inc., Tulsa, USA).

In addition, we applied a trigonometric statistical model to the average values of each time series so as to describe the periodic phenomenon analytically by characterizing the main rhythmic parameters according to the single cosinor procedure (Nelson et al., 1979). Four rhythmic parameters were determined as follows: mean level, amplitude (the difference between the peak and trough, and the mean value of the wave), acrophase (the time at which the peak of a rhythm occurs) and robustness (strength of rhythmicity). For each parameter, the mean level of each rhythm was computed as the arithmetic mean of all values in the data set (9 data points), the amplitude of a rhythm was calculated

Figure 2. A thermographic image recorded in sheep exposed to natural photoperiodic and thermoperiodic conditions

as half the range of oscillation, which in its turn was computed as the difference between the peak and trough. Rhythm robustness was computed as a percentage of the maximal score attained by the chi-square periodogram statistic for ideal data sets of comparable size and 24 h periodicity (Refinetti, 2004). Robustness higher than 40% was above the noise level and indicated statistically significant rhythmicity.

RESULTS

The application of two-way repeated measures ANOVA showed a significant effect of the time of day ($F_{(7.738)}$ = 317.06; P < 0.05) and collection side ($F_{(8.738)}$ = 17.99; P < 0.05). Mean values of RT, FA1, FA2, RA1, RA2, FL1, FL2, RL1 and RL2, obtained during the experimental period, are shown in Table 1. The application of Scheffé's test showed a significant difference of rectal temperature vs. foot temperature of the left front and left rear of the interdigital line, foot temperature of the left front and left rear of the interdigital area, foot temperature of the right front and left rear of the interdigital line and foot temperature of the right front and left rear of the interdigital area.

The application of the periodic model and the statistical analysis of cosinor enabled us to define the periodic parameters and their acrophases during the 24 h of monitoring (Table 2).

Circadian rhythms of FT, FA1, FA2, RA1, RA2, FL1, FL2, RL1 and RL2 are shown in Figures 3–5.

DISCUSSION

The results showed that rectal temperature and foot temperature changed with the time of day and in relation to the side of collection. In accordance with studies conducted on sheep's body temperature that reveal a daily rhythm with an ascent phase during the day and a descent phase during the night (Ayo et al., 1998; Piccione and Caola, 2003; Piccione and Refinetti, 2003), our results indicated the presence of a robust daily rhythm of rectal temperature. These circadian changes are probably due to a rhythmic input from the suprachiasmatic nucleus acting upon the hypothalamic thermoregulatory centres, modulating the set point (Aschoff, 1983; Refinetti and Menaker, 1992). According to an alternative view expressed by Refinetti (1997, 1998),

Table 1. The mean values (± SD) of rectal temperature (RT), foot temperature at the right front of the interdigital area (FA1), at the left front of the interdigital area (FA2), at the right rear of the interdigital area (RA1), at the left rear of the interdigital area (RA2), at the right front of the interdigital line (FL1), at the left front of the interdigital line (FL2), at the right rear of the interdigital line (RL1) and at the left rear of the interdigital line (RL2), obtained during 24 h of monitoring in healthy sheep

	Time (h)								
	11	14	17	20	23	2	5	8	11
RT	39.02 ± 0.23	39.08 ± 0.19	39.29 ± 0.20	39.50 ± 0.18	39.35 ± 0.18	39.20 ± 0.1	39.20 ± 0.18	39.10 ± 0.21	38.95 ± 0.28
FA1	30.87 ± 1.58	31.58 ± 1.26	32.06 ± 1.65	31.65 ± 1.12	30.98 ± 1.10	31.20 ± 1.17	30.74 ± 1.56	30.10 ± 1.26	30.51 ± 0.88
FA2	30.77 ± 1.40	31.66 ± 0.86	32.10 ± 1.15	31.55 ± 0.77	31.09 ± 1.45	31.25 ± 1.23	30.85 ± 1.33	30.22 ± 1.33	30.89 ± 0.69
RA1	30.92 ± 2.73	32.06 ± 1.13	32.28 ± 0.93	31.86 ± 1.58	30.82 ± 1.25	30.81 ± 1.36	30.67 ± 1.65	29.98 ± 1.35	30.03 ± 1.48
RA2	31.19 ± 2.23	31.72 ± 1.20	32.34 ± 1.15	31.62 ± 1.77	30.78 ± 1.26	30.53 ± 2.00	30.48 ± 1.66	30.00 ± 1.79	30.08 ± 1.32
FL1	31.32 ± 1.46	32.06 ± 1.11	32.83 ± 1.42	32.30 ± 1.20	31.94 ± 1.17	32.20 ± 1.16	31.43 ± 1.12	30.99 ± 1.30	31.64 ± 1.01
FL2	31.42 ± 1.34	32.08 ± 0.72	32.86 ± 1.06	32.28 ± 0.76	32.09 ± 1.07	32.03 ± 1.16	31.68 ± 1.14	30.80 ± 1.14	31.70 ± 0.83
RL1	31.58 ± 2.89	32.50 ± 1.07	32.98 ± 1.19	32.76 ± 1.52	32.80 ± 1.49	31.82 ± 1.40	31.80 ± 1.61	31.12 ± 1.33	31.27 ± 1.46
RL2	31.77 ± 2.14	32.25 ± 1.18	32.70 ± 1.41	32.00 ± 1.64	31.75 ± 1.25	31.53 ± 2.12	31.44 ± 1.53	30.90 ± 2.02	31.26 ± 1.39

Table 2. The mean values of four rhythmic parameters observed during 24 h of monitoring in healthy sheep

	Mesor (°C)	Amplitude (°C)	Acrophase (h)	Robustness (%)
RT	39.20	0.20	21:38	75.60
FA1	31.11*	0.73*	18:38*	63.10*
FA2	31.18*	0.66*	18:29*	60.80*
RA1	31.06*	0.99*	18:03*	60.50*
RA2	30.98*	0.97*	17:38*	62.20*
FL1	31.89*	0.67*	19:17*	63.40*
FL2	31.92*	0.68*	19:16*	61.40
RL1	32.00*	0.79*	18:39*	61.40*
RL2	31.74*	0.65*	17:56*	62.90*

*vs RT $P < 0.05$

the circadian body temperature rhythm is primarily under circadian control, bypassing the thermoregulatory set point, and is secondarily modulated by the thermoregulatory system. The robustness of this rhythm was 75.60% and the mean level was 39.2°C with a range of excursion of 0.1°C. The body temperature of sheep under a light-dark cycle was found to start its daily ascent at the time of sunrise, which was in agreement with the findings in different species of diurnal mammals (Refinetti, 1999). The acrophase of the rhythm occurred during the dark phase (21:38) of the light-dark cycle. Similarly like in rectal temperature, the obtained results clearly demonstrated the existence of a daily rhythm of foot temperature. The average mean level of the foot temperature rhythm ranged from 30.0°C to 32.9°C, showing an acrophase of the rhythm for all locations 2–3 h before sunset. As observed in humans, in sheep exists a daily rhythm distal skin temperature that was phase advanced by 140 to 180 min with respect to the circadian rhythm in rectal temperature (Krauchi and Wirz-Justice, 1994). In this respect it is of interest to remark that sheep, like goats, have long legs, which together with the large body, represent a mechanism for evaporative heat loss. In fact, distal regions of the body, the major sites for vasomotor heat loss, are rich in arteriovenous anastomoses, which adjust the blood flow and therefore play a key role in temperature regulation. In humans, there is a circadian rhythm of heat loss from the distal limbs, with rhythms of skin temperature and blood flow in these regions (Smolander et al., 1993; Krauchi and Wirz-Justice, 1994). Thus, the observed oscil-

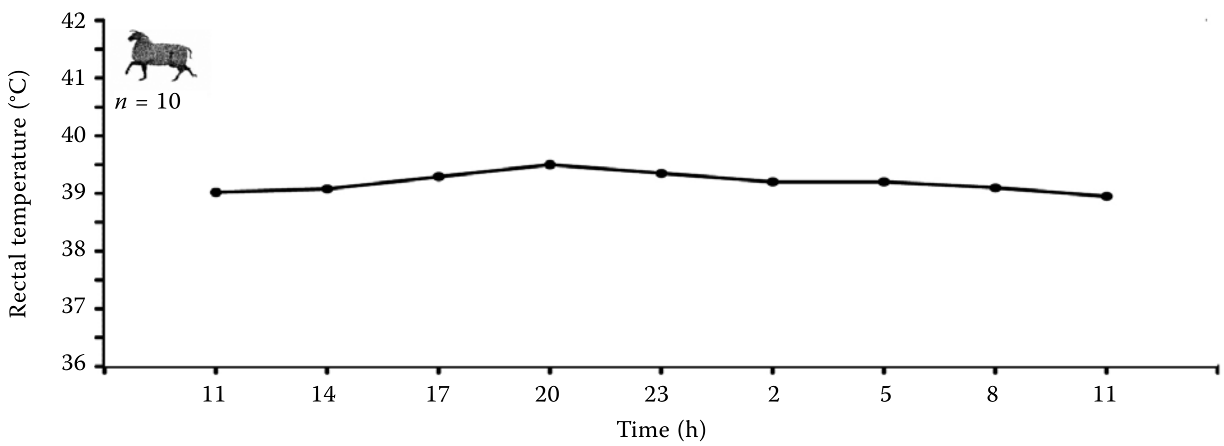

Figure 3. Circadian rhythm of rectal temperature (°C) in healthy sheep. Each point represents the obtained mean (n = 10) of rectal temperature

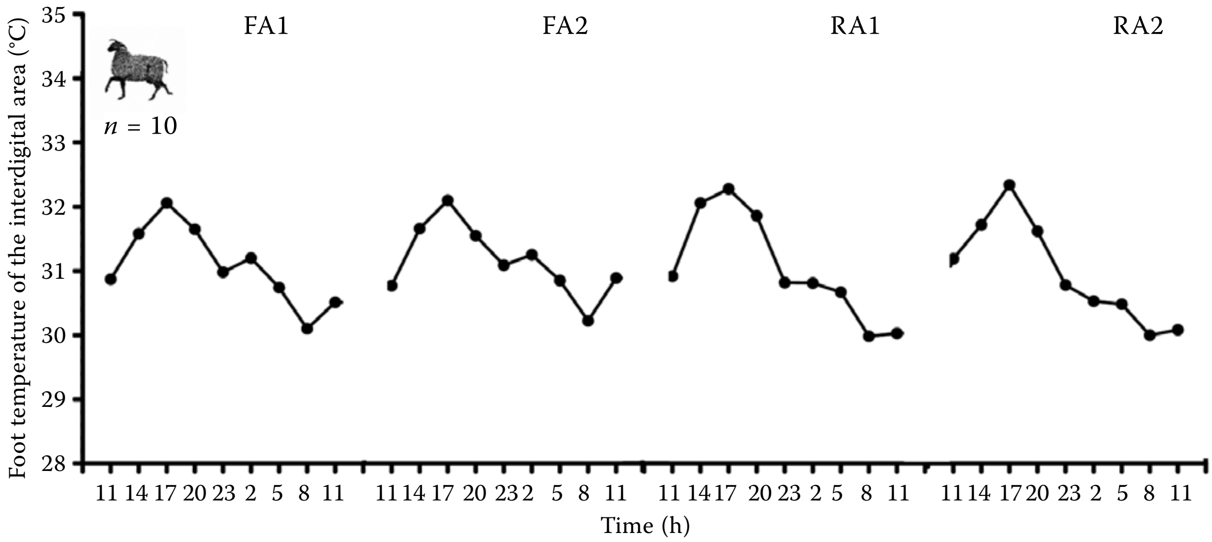

Figure 4. Circadian rhythm of foot temperature (°C) in healthy sheep. Each point represents the obtained mean (n = 10) of foot temperature at the right front of the interdigital area (FA1), at the left front of the interdigital area (FA2), at the right rear of the interdigital area (RA1) and at the left rear of the interdigital area (RA2)

lations of foot temperature are less robust than in rectal temperature, the robustness value is between 60.50% and 63.40%. Moreover, the amplitude of the foot temperature rhythm differs from the endogenous body temperature rhythm and it would be capable to establish the circadian gene expression de novo, though it could maintain previously induced rhythms (Weinert, 2010).

In conclusion, the obtained circadian oscillations of rectal temperature reflect the familiar circadian patterns of endogenous sources observed by Refinetti (2010), and the results of foot temperature support the idea that the daily rhythm is, at least in part, influenced by variation in the blood flow to the extremities. So the infrared thermography is a non-invasive tool to study animal welfare, which may provide more information on the development of disturbances in the peripheral circulation and may be used with an advantage in occupational health examinations and in special clinical work.

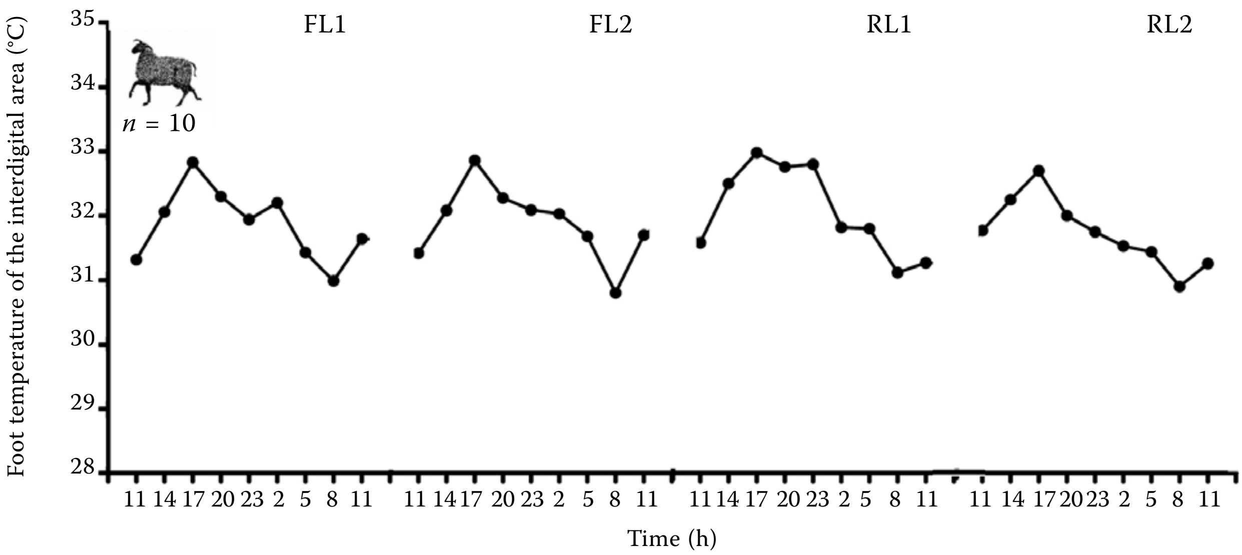

Figure 5. Circadian rhythm of foot temperature (°C) in healthy sheep. Each point represents the obtained mean (n = 10) of foot temperature at the right front of the interdigital line (FL1), at the left front of the interdigital line (FL2), at the right rear of the interdigital line (RL1) and at the left rear of the interdigital line (RL2)

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