Simultaneous recording of subcutaneous temperature and total locomotor activity in Bos-Taurus and Bos-Indicus raised in subtropical region of Argentina.

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Research Article

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Abstract

In this study, total locomotor activity and subcutaneous temperature was evaluated in 18 clinically healthy females' cows. All cows were divided in 3 groups: group A1 was constituted by 6 Holstein Bos-taurus, group A2 by 6 Herford females' cows Bos-Taurus and group B was constituted by 6 females Bradford Bos-indicus cows. Data recording of total locomotor activity (TLA) and subcutaneous temperature was performed by means of a subcutaneous thermometer (Thermocron) and an actimeter ACTIWATCH ® (Cambridge Neurotechnology Ltd) based on accelerometer technologies equipped on all subjects to record TLA. At the same time thermal and hygrometric records were carried out, considering the subtropical climate of Santa Fe. The application of GLM for statistical analysis showed a significant effect ($P<0.05$) on statistical model and time of the day on TLA and ST for all groups, no significative effect on animal parameter were found for ST and TLA except for Group B. Circadian parameters has been evaluated according to the single cosinor procedure of ST that showed a diurnal daily rhythmicity for all investigated groups and TLA which is focused almost during the photophase for group A1 and A2 and during scotophase for group B.

Considering different species and breeds, and different environmental conditions, this study suggested that some subjects may be much more able to adapt themselves to environmental stress than others.

During their evolution from Bos taurus, zebu cattle (Bos indicus) have acquired genes that confer thermotolerance. Subjects from Bos Indicus breeds are better able to regulate body temperature in response to heat stress than Bos-taurus subjects.

Introduction

Physiological mechanisms, in particular the adaptability to external environment conditions are important aspects of livestock performance during production and reproduction period together with food and water intake.

In cow breeding, the management factors and the variability of thermal environment has been showed to be the principal elements that can negatively influence cow's performance and welfare (Mazzullo et al. 2014; Rizzo et al. 2017). Animals housed outdoors are major exposed to environmental changes, such as ambient temperature, relative humidity, solar radiation and atmospheric pressure, which could affect the animal's biological system. Management conditions should guarantee animal comfort improving physiological mechanism, such as feed intake, milk production, reproduction activity and health, improving animal wellness and economic advantage (Rizzo et al. 2017).

The maintenance of many physiological processes is important contributes to the welfare of livestock animals. In all living organism, the circadian master clock, located in the suprachiasmatic nuclei ensures the metabolic homeostasis driving all physiological functions (Weinert and Waterhouse 2007; Mongrain et al. 2009).

Biological rhythms are the essential regulators of life (Foster and Kreitzman 2014; Smolensky et al. 2016), they are endogenously generated and influenced by environmental cues such as food availability, ambient temperature and in particular they are entrained by light-dark cycle (Van Esseveldt et al. 2000; Helfrich-Forster 2004; Del Sole et al. 2007). They have a free-running period close to 24h and for this reason they are so called “circadian rhythms” (Refinetti 2006).

The most studied physiological variables driven by the circadian master clock that are an index of health status and animal welfare are the body temperature and the total locomotor activity (Piccione et al. 2005). The rhythmicity of body temperature has been widely used to study the biological clock (Piccione and Refinetti 2003). Repeated measurements of body temperature over time allows the study of 24h rhythmicity in all animal species and its values are maintained relatively constant in mammals. The ability to thermoregulation is an evolutionary adaptation that allows homeotherms to predict environmental changes (Refinetti 2006).

Various ways to monitor body temperature have been applied at different locations of cows including rectum, reticulum, milk, and ear canal but the accuracy of body temperature measuring depend on test modality and the insulation of body temperature from the ambient influences (Lee et al. 2016).

Rectum is well protected by thick muscle and animal tail, and has a wide blood circulation.

Each methods have merits and limitations such as body location, fall off of tagged devices, system costs and patient invasive method. Another competitive location for monitoring body temperature has been under the skin (Brown et al. 1977, Hutu et al. 2009).

Skin has a 3 to 5 mm thickness, it’s well covered by hair, and it has a short of blood circulation, but easily affected by outside temperature fluctuations (Brown et al. 1977). Various subcutaneous temperature (ST) monitoring systems have been used in various experimental studies. The subcutaneous temperature measurement values depend on the implanting site as cattle hide is covered by a thick hair depends on communication distance (few cm) and on environmental influence (Brown et al. 1977; Lee et al. 2016; Scaglione et al. 2019). ST values showed daily circadian rhythm representing temperature difference between day and night (Lee et al. 2016).

In domestic animals changes in body temperature are usually attributed to environmental inputs, feeding and locomotor activity (Caola et al. 2010).

Like body temperature, total locomotor activity (TLA) including different behaviors, such as feeding, drinking, walking, grooming, and small movement during sleep, it has been investigated in many species and in various experimental conditions. It has been observed that it is influenced by different factors such as photoperiod (Bertolucci et al. 2008), different stabling conditions (Piccione et al. 2008), feeding schedules (Piccione et al. 2007) and environmental conditions (Rizzo et al. 2017) and it is controlled by endogenous circadian system (Berger 2008). Light suppresses locomotor activity in nocturnal animals and encourage activity in diurnal species. The direct effect of light on locomotor activity is promoted by a nonimage-forming visual pathway as suggested in previous studies (Piccione et al. 2011).

TLA ensures an optimal functioning of the biological system (Piccione et al 2010) monitoring behavioral changes in farm animals improving welfare and individual health (Muller and Schrader 2003).

Considering that environmental factors are major physiological stressor which affect the animal's biological system (Scaglione et al. 2018); the aim of this study was to evaluate the daily rhythm of locomotor activity and subcutaneous temperature in different species of cows (Bos Indicus and Taurus), and breeds (Holstein, Herford and Bradford) taken under subtropical environmental conditions.

Material And Methods

This study was carried out on 18 subjects belonging to the family of *Bovidae,* subfamily *Bovinae,* genus *Bos* were divided in 3 different groups on the basis of species and breed.

Group A was constituted by 12 Bos-Taurus, 6 Holstein females' cows (Group A1), with a mean body weight of 500±25 kg and 6 Herford females' cows (Group A2) with a mean body weight of 480±30 kg. Group B was constituted by 6 females Bradford Bos-Indicus with a mean body weight of 450±25 kg. In addition, all females will correspond in their productive and reproductive period with the timing of the measurement.

For each group, all animals delivered by about 45 days. Randomly chosen within their held. All animals were housed in their farm, kept in natural pasture with rotational of alfalfa meadow. They were milked two time daily (05:00 and 15:00).

The farms are free of zoonotic diseases such as brucellosis and tuberculosis as established by the national plan for the control and eradication of bovine brucellosis and tuberculosis.

The experiment was conducted in accordance with the regulations of the Guidelines for Guide for the Care and Use of Laboratory Animals (U.S. National Research Council, 2011).

Data recording of total locomotor activity (TLA) and subcutaneous temperature was performed at 5-minute intervals for 7 consecutive days. Total locomotor activity was defined as any movement the animal made, including different behaviors such a feeding, drinking, walking and grooming, indipendently of the animal's position, such as lying or standing (Sciabarrasi et al. 2014).

In order to record locomotor activity all animals were equipped with an ACTIWATCH ® (Cambridge Neurotechnology Ltd). This device is based on accelerometer technologies and has been validated for automatic 24h recording of activity in dairy cows (Muller and Schrader 2003). It recorded the amount, intensity and duration of movement in all directions by means of a piezo-electric accelerometer. This type of sensor integrates the degree and speed of motion and produce an electrical current that varies in magnitude. An increased degree of speed and motion produced an increase in voltage. Our relating results, according to corresponding voltage are converted, stored in the ACTIWATHC memory unit and transfered directly to the PC for further statistical analysis.

Subcutaneous temperature was recorded by means of a subcutaneous thermometer (Thermocron) set to monitor and store time and temperature records. All subcutaneous implants did not induce tissue reaction. Each subcutaneous device was placed in an incision of 2/3 cm into the skin in the area comprised among lateral neck, upper and lower scapula after local anaesthetic (10ml of Lidocaine 2%).

At the same time thermal and hygrometric records were carried out for the whole study by means of a data logger with a high reading accuracy and resolution (Model Tinytag Ultra 2 Gemini Data Logger, West Sussex, UK), considering the subtropical climate of Santa Fe with a minimum and maximum mean temperature between 20°C and 32°C on January for 7 days consequently of ambient temperature, solar radiation atmospheric pressure and relative humidity as showed in Table 2.

Statistical analysis

A multivariate analysis of variance (ANOVA) for repeated measures, General Linear Model (GLM), was applied on our recorded values during the whole study to establish the effect of animal, time of the day and day of monitoring on total locomotor activity parameter and subcutaneous temperature for all experimental groups. P values <0.05 were considered statistically significant.

Statistical analysis was performed using the SAS/STAT for Windows software package (SAS Institute, Inc. 1990). In addition, with "Temp" program of SAS software we describe the periodic phenomenon analytically, by characterizing the main rhythmic parameters according to the single cosinor procedure. Three rhythmic parameters were determinate: Mesor (mean level), amplitude (the difference between the peak, or trough, and the mean value of a wave) and acrophase (time of peak).

Results

The obtained data were expressed as mean ± standard deviation (SD).

Table 1 and 2 show the mean and SD of TLA, ST and environmental parameters recorded during the 7 days of monitoring in the three different groups.

The application of GLM, as showed in table 3-4 demonstrated a statistical effect on statistical model and time of the day on TLA and ST for all groups, no significative effect on animal parameter were found for ST and TLA except for Group B. No effect of day of monitoring was observed.

As represented in table 5, the application of single cosinor procedure showed a daily rhythm of TLA and ST in all experimental groups.

Figures 1-3 show the daily rhythmicity of subcutaneous temperature, total locomotor activity and all environmental parameters for Group A1 (Holstein), Group A2 (Herford) and Group B (Bredford), including the curve according to the application of cosenoidal fitting model during the hours of light and darkness. Subcutaneous temperature shows a diurnal daily rhythmicity for all investigated groups, Total locomotor activity is concentrated almost exclusively during the photophase for groups A1 and A2, confirming previous studies (Rizzo et al. 2017), while total locomotor activity shows an evident daily rhythmicity during scotophase for Group B.

Discussion

The results of the present study contribute to understanding the capacity of reaction and adaptation of animals to the environment.

Body temperature rhythmicity of farm animals is important from a comparative point of view (human beings and laboratory animals), but in particular from an economic aspect to guarantee the improvement in livestock production practices.

It is known that environmental temperature can affect skin surface temperature and influences the thermal exchange between the organism and the environment, especially in mild stress conditions (Martello et al. 2016).

The ideal ambient temperature (“thermoneutral” zone) for a cow is between 5°C and 25°C (Roenfeldt 1998). As ambient temperature increases, it becomes more difficult for a cow to cool herself adequately and she enters heat stress (Mazzullo et al. 2014). The climatic conditions in this region, show an extreme ambient temperature of more than 26.1°C. It’s a sub-tropical climate that can physiologically influence cows according to species and breeds.

Circadian patterns of some physiological parameters such as body temperature or locomotor activity have been identified in a variety of mammals, with the highest level monitored during the morning hours and the lowest level in the early evening for diurnal species. On the contrary for nocturnal animals the peak coincides with the onset of darkness. Our results confirmed previous studies showing a diurnal daily rhythm of locomotor activity (Giannetto et al. 2010; Bazzano et al. 2015; Rizzo et al. 2017) in cattle and Bos taurus species (Holstein and Herford). Cattle is known to have a marked diurnal behavior pattern; therefore, grazing is set to sunlight (Rizzo et al. 2017).

In diurnal species, locomotor activity increase during the morning and decreases during the evening and there is a preference for warmer ambient temperature in the morning and cooler ones in the evening (Piccione et al. 2011).

Our findings showed a circadian rhythm of total locomotor activity during scotofase in Bos indicus specie (Bradford). The Bradford breed was developed from crosses between Bos taurus and Bos indicus cattle (Utsunomiya et al. 2019).

Bos indicus was domesticated 2000 years later than was Bos taurus cattle. From the domestication site in the Indus Valley, Bos indicus spread across the globe, leaving a legacy of tropical adaptation to their descendants.

The adaptation of this specie to tropical and sub-tropical environments makes them more efficient in coping with heat, humidity, nutritionally poor food and disease challenges. Under warm conditions, these animals have better balance between heat production and heat loss than Bos Taurus cattles. This improved balance is achieved via intrinsic metabolic differences and efficient heat dissipation through conduction, radiation and evaporation by sweating and panting (Hansen 2004). Very little is known about the genetic and epigenetic control of heat tolerance in Bos indicus cattle but much promise comes from modern monitoring of body temperature, rumination, locomotor activity and other physiological parameters.

The absence of diurnal rhythm of total locomotor activity in group B of our study can be explained by the dependence of physiological pattern on the sleep-wake schedule. Generally, ruminants do not enter the deep state of sleep as do men or other domestic animals. Sleep periods in ruminants are linked directly to the digestive need of the animal since rumination requires both time and consciousness (Abebe and Scott 1990).

The presence of total locomotor activity nocturnal rhythm in Bos indicus is probably physiological related to the lack of deep sleep (Abebe and Scott 1992).

The destruction of the species-typical time organization of the individual animals forces them to shift the time of their main activity to the night (Mitloehner and Laube 2003).

A multi-step neuronal pathway from SNC to sleep/wake switch may allow the SNC output signal to be modulated and integrated with other physiological signals. Our results support this hypothesis by noticing that external stimuli may modulate a single oscillator (SNC) output switching phenotypes from diurnal to nocturnal (Phillips et al. 2013).

In this case the activity rhythms of our cows are primarily diurnal but group B of our study switches from diurnal to nocturnal behavior by changing how the SNC respond to light (Oster et al. 2002). It should be considered that the daily distribution of activity differs not only from species to species but also from individual to individual in the same species (Refinetti, 2006).

We can affirm that goats exhibit a daily rhythm of total locomotor activity, with the highest daily amount of activity during the photophase following the onset of light. The zeitgeber of the total locomotor activity is identified as the photic stimuli and the rhythm was diurnal (Giannetto et al. 2010). For other species of cows (Bos indicus) the total locomotor activity was expressed during the scotophase, probably because the zeitgeber is a non-photic stimulus (ambient temperature, environmental patterns or food availability), during food stress the animals were active at a time they previously associated with food availability (Giannetto et al. 2010).

Subcutaneous temperature showed a daily circadian rhythm for all groups reflecting temperature difference between day and night, it depends on skin and epidermis vascularization at the body surface.

Ambient temperatures and all environmental parameters affect thermogenesis by basal metabolic processes. Heat loss is enhanced by increasing peripheral blood flow, which would be expected to reduce surface temperature (Rey et al. 2015).

Physical activity leads to peripheral vasodilatation, typically to balance heat production, and affects both core and peripheral temperatures (Webb 1995) in a non-predictable manner. measuring subcutaneous temperature was previously discussed to estimates of the resting metabolic rates of endotherms (Rey et al. 2015).

Environmental conditions such as ambient temperature, solar radiation relative humidity and atmospheric pressure significantly affect locomotor activity and body temperature together with species and breeds.

The three cows' breeds behaved differently during the experimental period. Holstein and Herford showed a diurnal peak of the rhythm of TLA as opposite to the nocturnal in Bradford.

As an indigenous species, Bradford group showed a remarkable variability among the analyzed data and although the homogeneous group a statistical difference was observed among the subjects, unlike ST which showed its acrophase always during the light hours.

Herford group showed a significant variability in the amplitude of the rhythm, in particular for TLA, compared to the other species, while Holstein, being the breed that has been subjected to farm management for the longest time, is therefore more adapted to such conditions, in fact the circadian rhythm remained stable within the group and it did not show high oscillations of amplitude. It can be assumed that it had a better generational adaptation to the breeding conditions.

Some cows' species are better able to cope and adapt themselves to environmental stress. Considering the subtropical conditions suitable measures should be adopted in order to minimize environmental stress and to improve animal welfare.

Therefore, further studies are expected to establish how management conditions may affect the physiological parameters of animals living in subtropical regions.

Declarations

Data availability

The datasets used and/or analysed during the current study available from the corresponding author on reasonable request.

Ethics approval

This study was performed in line with the principles of the 1964 Declaration of Helsinki and its later amendments

Conflict of interest

The authors declare no conflict of interest

Author contribution

Hypothesis generation and experimental design, F. –F, F. –A, F. –A, A. –Z.; organizing and conducting the experiment, R. D. -C. and M.C. –S.; interpreting and analyzing the results R. D. -C. and M.C. –S; writing and revising the manuscript C. -G. and G. -P. All authors have read and agreed to the published version of the manuscript.

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Tables

Table 1. Mean values ± DS of subcutaneous temperature and total locomotor activity recorded in 3 groups (Group A1: Holstein, Group A2: Herford and Group B: Bradford) during the 7 days of monitoring.

		Total Locomotor Activity (Arbitrary unit)		*Subcutaneous Temperature (°C)*	
Holstein (A1)		Mean	SD	Mean	SD
Days	1	106.88	±223.38	37.60	±0.63
	2	115.56	±227.42	36.97	±0.92
	3	119.08	±229.20	37.04	±0.62
	4	110.41	±219.18	36.87	±0.77
	5	98.78	±202.43	37.06	±0.74
	6	84.67	±178.12	37.26	±0.62
	7	98.88	±208.57	36.85	±0.63
Bradford (B)		Mean	SD	Mean	SD
Days	1	213.39	±497.51	37.03	±0.67
	2	184.88	±397.67	36.87	±0.78
	3	160.03	±345.90	36.79	±1.01
	4	156.01	±301.70	36.76	±0.89
	5	156.41	±290.82	36.42	±0.95
	6	142.84	±260.82	36.31	±0.95
	7	193.21	±346.93	35.46	±1.26
Herford (A2)		Mean	SD	Mean	SD
Days	1	419.35	±691.24	37.52	±0.47
	2	534.74	±746.98	37.02	±1.02
	3	666.20	±821.51	37.23	±0.60
	4	727.72	±872.19	37.06	±0.59
	5	665.74	±851.73	36.95	±0.66
	6	561.78	±829.94	37.10	±0.72
	7	470.41	±724.38	37.40	±0.46

Table 2. Mean values ± SD of environmental conditions (ambient temperature, solar radiation, atmospheric pressure and relative humidity) in 3 groups of bovines (Group A1: Holstein, Group A2: Herford and Group B: Bradford) during the 7 days of monitoring.

		Ambient Temperature (°C)		*Solar radiation (W/m²)*		*Atmospheric pressure (mmHg)*		*Relative humidity (%)*	
Holstein (A1)		Mean	SD	Mean	SD	Mean	SD	Mean	SD
Days	1	27.21	±7.51	163.50	±169.94	995.63	±1.31	54.17	±22.52
	2	23.67	±2.97	130.42	±163.04	996.75	±2.25	64.46	±9.34
	3	23.46	±4.55	169.42	±171.38	1004.42	±1.72	67.58	±14.66
	4	25.08	±3.99	183.88	±188.16	1005.42	±1.69	54.63	±12.60
	5	26.50	±5.38	166.38	±171.92	998.92	±3.02	58.29	±11.28
	6	28.00	±3.56	128.54	±139.80	995.46	±1.25	64.04	±7.77
	7	24.46	±1.35	84.33	±116.80	998.00	±1.50	75.17	±5.22
Bradford (B)		Mean	SD	Mean	SD	Mean	SD	Mean	SD
Days	1	25.17	±6.06	168.58	±177.62	1008.25	±2.23	51.54	±21.48
	2	25.33	±4.52	146.79	±160.10	1003.67	±1.49	51.88	±13.43
	3	26.96	±5.03	165.92	±176.41	999.63	±1.88	47.17	±13.30
	4	28.21	±5.18	134.79	±149.16	997.13	±1.19	48.54	±9.83
	5	25.38	±3.08	121.17	±155.08	995.08	±2.48	67.38	±8.63
	6	24.67	±3.09	89.63	±121.98	990.54	±1.35	74.42	±10.58
	7	17.00	±3.62	164.13	±183.49	1000.79	±3.18	66.96	±14.98
Herford (A2)		Mean	SD	Mean	SD	Mean	SD	Mean	SD
Days	1	26.71	±3.90	131.71	±154.29	994.88	±1.94	70.17	±9.71
	2	23.08	±2.84	116.29	±154.38	996.75	±2.61	77.13	±6.91
	3	19.29	±2.71	89.46	±89.46	1004.92	±3.06	74.63	±4.42
	4	18.50	±0.72	46.88	±46.88	1006.08	±1.74	81.54	±3.89
	5	20.58	±2.10	97.21	±97.21	1002.00	±0.98	81.88	±5.02
	6	22.38	±3.24	144.33	±144.33	1002.25	±1.11	77.29	±7.62
	7	27.17	±4.52	141.88	±141.88	1001.21	±0.98	72.83	±11.26

Table 3. Results of GLM (General Linear Model) analysis expressing the effect of statistical model (free degree, f; and $P < 0.05$), animals and time of day on total locomotor activity in all groups.

	Statistical model		*Animal*		*Time of day*	
Total Locomotor Activity	f	P	f	P	f	P
Holstein (A1)	6.53	0.0001	3.26	0.0709	6.54	0.0001
Bradford (B)	5.76	0.0001	122.30	0.0001	5.35	0.0001
Herford (A2)	6.43	0.0001	3.50	0.0615	6.44	0.0001

Table 4. Results of GLM (General Linear Model) analysis expressing the effect of statistical model (free degree, f; and P< 0.05), animals and time of day on subcutaneous temperature in all groups.

	Statistical model		*Animal*		*Time of day*	
Subcutaneous temperature	f	P	f	P	f	P
Holstein (A1)	28.23	0.0001	0.87	0.3525	23.15	0.0001
Bradford (B)	23.32	0.0001	0.80	0.7621	20.48	0.0001
Herford (A2)	26.44	0.0001	0.90	0.1533	30.25	0.0001

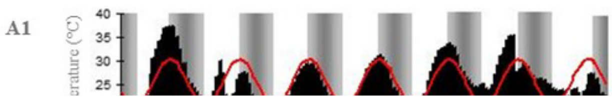
Table 5. Mean values ± DS of rhytmic parameters (mesor, amplitude and acrophase) expressed in their conventional unit, of total locomotor activity and subcutaneous temperature evaluated in 3 groups A1 (Holstein), A2 (Herford) and B (Bradford).

	Mesor (arbitrary unit)		*Amplitude (arbitrary unit)*		*Acrophase (hours)*	
Total Locomotor Activity						
Holstein (A1)	104.82	±1.94	21.68	±2.74	12.41	±0.29
Bradford (B)	172.52	±3.23	53.10	±4.57	2.23	±0.20
Herford (A2)	577.59	±7.13	226.56	±10.08	15.26	±0.10
	Mesor (°C)		*Amplitude (°C)*		*Acrophase (hours)*	
Subcutaneous temperature						
Holstein (A1)	37.10	±0.01	0.52	±0.02	18.10	±0.08
Bradford (B)	36.33	±0.02	0.69	±0.70	16.44	±4.41
Herford (A2)	36.89	±0.02	0.30	±0.03	17.49	±0.24

Figures

Figure 1

Representative 7-day recorded pattern of total locomotor activity (TLA) and subcutaneous temperature (ST) obtained in 3 groups: A1 (Holstein), A2 (Herford) and group B (Bradford). Grey and white bars indicate the scoto-e photo-phase of the experimental photoperiod.



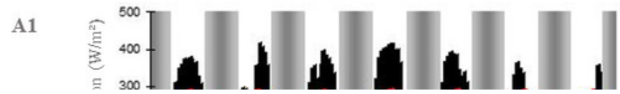


Figure 2

Representative 7-day recorded pattern of ambient temperature and solar radiation obtained in 3 groups: A1 (Holstein), A2 (Herford) and group B (Bradford). Grey and white bars indicate the scoto-e photo-phase of the experimental photoperiod.

Figure 3

Representative 7-day recorded pattern of atmospheric pressure and relative humidity obtained in 3 groups: A1 (Holstein), A2 (Herford) and group B (Bradford). Grey and white bars indicate the scoto-e photo-phase of the experimental photoperiod.