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Daily Rhythms of Some Physiological Variables in Alpaca (*Lama pacos*)

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Abstract: Circadian rhythmicity, an intrinsic characteristic of some physiological parameters in livestock, may be influenced by different exogenous synchronizers. The aim of the present study was to evaluate the influence of environmental temperature on the circadian pattern of some hormonal, haematochemical, urinary and physiological parameters in Alpaca (*Lama pacos)*. For this purpose, 6 clinically healthy adult female alpacas aged 7±1 years and bred under similar conditions on a farm 400 meters above sea level were used. Twice, at two different environmental temperatures (5-13.5°C *vs.* 11.5-33.5°C), blood samples were collected by means of a jugular puncture and urine samples were collected by means permanent catheters on each subject every 2 h during 24 h. A trigonometric statistic model has been applied to the records' mean values obtained during the experimental sessions to describe analytically the periodic phenomenon; furthermore, the singles Cosinor method was applied to the periodic parameters. The application of the periodic model enabled us to point out the circadian pattern of the following blood parameters: Melatonin, glucose, triglycerides, urea, phosphorus, magnesium and potassium. The same model was applied to the following urinary and physiological parameters: Creatinine, magnesium, sodium, fractional clearance of magnesium and potassiumand body temperature. Rhythm stability and periodicity keeping under natural environmental conditions (characterized by variable temperature, relative humidity and photoperiod), though with different acrophases, enable us to establish that periodical parameters pattern shows a strong rhythm. This one can be influenced by exogenous factors (daylength and temperature in this particular instance) able to modify its synchronicity but not its intrinsic periodicity.

Key words: Daily rhythm, physiological parameters, melatonin, *Lama pacos*, acrophases, periodicity

INTRODUCTION

Animals are characterized by average physiological functions with limited and regular fluctuations over the 24 h period. The 24 h endogenous rhythms, called circadian rhythms, are observable under constant environmental conditions. Within some limits, period, amplitude and phase of circadian rhythms can be influenced by cyclic variations of multiple environmental factors including daily light/dark cycle and environmental temperature. External stimuli have the capability to induce feedback mechanisms and furthermore, influence endogenous oscillator zeitgebers (time switches). Light, a fundamental zeitgeber, is actively involved in the regulation of circadian rhythms related to cyclic behaviour (e.g., sleep and wakefulness)and physiological functions (e.g., body temperature). Circadian and circannual temporal, structural and functional variations can be shown in all physiological systems.

Biological rhythms are regulated by the central nervous system and specifically, by mechanisms that are Suprachiasmatic Nuclei (SCN) activity dependent (Plautz *et al*., 1997; Delaunay *et al*., 2001; Lee *et al*., 2000; Whitmore *et al*., 2001). Biological rhythms regulate the activity of many hypothalamic nuclei and inform peripheral organs by endocrine (ACTH) and nervous (autonomic nervous system) messages. The SCN are important for the synchronization of endogenous rhythmic activities and environmental information (light, temperature) (Rensing and Ruoff, 2002; Piccion and Gaola, 2002). and for the definition of different-frequency rhythms (circadian, circatrigentan, circannual) (Nelson *et al*., 1979).

Daily oscillation in the levels of physiological variables has been described in a variety of species for a multitude of variables, including body temperature, heart rate, blood pressure, hormonal secretionand urinary excretion (Dunlap *et al*., 2004; Refinetti, 2005). Although

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the relative abundance of reviews on the biological rhythms of domestic animals (Komosa *et al*., 1990; Piccione and Caola, 2002; Piccione *et al*., 2003; Piccione and Refinetti, 2003) no literature is available on the circadian rhythms of haematological, urinary and physiological parameters of South American camelids, including the Alpaca (*Lama pacos*).

On the basis of this knowledge and as a continuation of our research, hormonal, haematochemical, urinary and physiological parameters in Alpaca were determined under normal schedules conditions in an attempt to gain an insight into the mechanisms of the circadian rhythms of body temperature and energetic metabolisms of this specie.

MATERIALS AND METHODS

Two clinical trials were performed on 6 female alpacas (aged 7±1 years, raised in Italy under similar conditions on a farm 400 meters above sea level), at two different environmental temperatures (5-13.5°C *vs.* 11.5-33.5°C) and with different photoperiod (light phase from 06.30 to 18.30 and from 05.00 to 21.00). On each subject, blood and urine samples were collected every 2 h for 24 h. All animals were given a clinical examination (rectal temperature, heart and respiratory rates) before collection of each sample. During the dark period, a red-filtered flashlight was used to facilitate venipuncture.

Blood samples were collected into heparinized vacutainer tubes by means of a jugular puncture. The heparinized samples were centrifuged at 3,000 rpm for 10 min to obtain plasma (stored at -20°C pending analyses). Plasma samples were analysed for glucose, triglycerides, urea, total bilirubin, total protein, albumine, creatinine, Phosphorus (P), Magnesium (Mg), potassium (K), sodium (Na), Alkaline Phosphatase (ALP) and Creatinine Kinase (CK) content using an automated chemistry analyser (Hitachi serum biochemical analyser, Boehringer Mannheim).

Urine samples were collected introducing permanent catheters, type GOLD 2 Way Sil 5cc 18Fr-Rusch Gold Silicone Coated Latex Foley Catheter-2 Way, Sterile, 2 opposed eyes, 5 cc balloon, Length 40 cm, Color coded, Single use-Rusch AG Germany. Urinary creatinine, calcium, phosphorus, magnesium, sodium and potassium concentrations were determined by UV spectrophotometry on the individual filtered and centrifuged urine samples. The fractional clearance of calcium, phosphorus, magnesium, sodium and potassium were calculated from the analysis carried out on individual serum and urine samples, according to the following formula:

$$Xu/Xs \times Crs/Cru \times 100$$

where Xu is the urinary concentration of the element, Xs is the serum concentration of the element and Cru and Crs are, respectively the urinary and serum concentration of creatinine.

Measurements of body temperature were conducted at the same hours of blood and urine collections with a digital thermometer whose probe was inserted 8 cm depth into the rectum.

All the results were expressed as mean±SD. Data were normally distributed ($p<0.05$, Kolmogorov-Smirnov test). A trigonometric statistical model was applied to the average values of each time series, so as to describe the periodic phenomenon analytically, by individuating the main rhythmic parameters according to the single cosinor procedure (Nelson *et al*., 1979). Mesor (Midline Estimating Statistic of Rhythm), expressed in the same conventional unit of the relative parameter, with the Confidence Interval (C.I.) at 95%, Amplitude (A), expressed in the same unit as the relative Mesor and Acrophase (Φ), expressed in hours with 95% confidence intervals. For each animal, the mean level of each rhythm was computed as the arithmetic mean of all values in the data set (12 data points). The amplitude of a rhythm was calculated as half the range of oscillation, which on its turn was computed as the difference between peak and trough. The acrophase of a rhythm was determined by an iterative curve-fitting procedure based on the single cosinor procedure. For each variable for each animal, a cosine wave was fitted to the data points according to the function $Y_t = M + A \bullet cos(\theta_t + \varphi)$, where Y_t denotes each data point in the time series, M is the mean level of the rhythm, A is the amplitude, θ_t is the trigonometric angle (in degrees) corresponding to time $t$and φ is the angle displacement for the acrophase. The value of φ was determined by iteration: The true value of φ was considered to be the one that produced the smallest sum of squares of the deviations between iterated cosine functions and the raw data.

RESULTS

One of the most important findings was the temporal variation of melatonin levels in plasma. Different levels were detected during the two trials, with an increase of diurnal circulating levels under conditions of high environmental temperature (Fig. 1). Some animals showed levels higher than other domestic ruminants in both periods (maximum value found at low temperature: 51,23 pg mL^{-1} and 198,38 pg mL^{-1}; maximum value at high temperature: 81,75 pg mL^{-1} and 150,42 pg mL^{-1} for diurnal and nocturnal sampling periods, respectively).

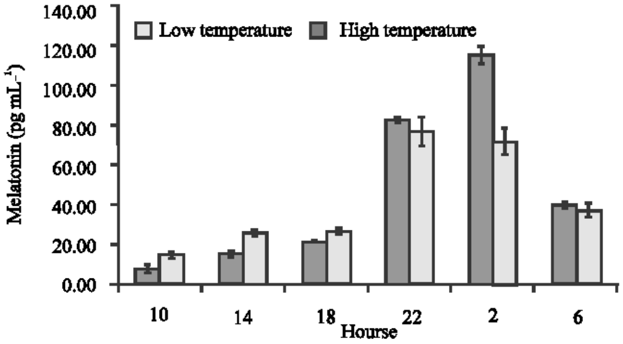

Fig. 1: Melatonin pattern under the influence of two different environmental temperatures in Alpaca raised in Italy

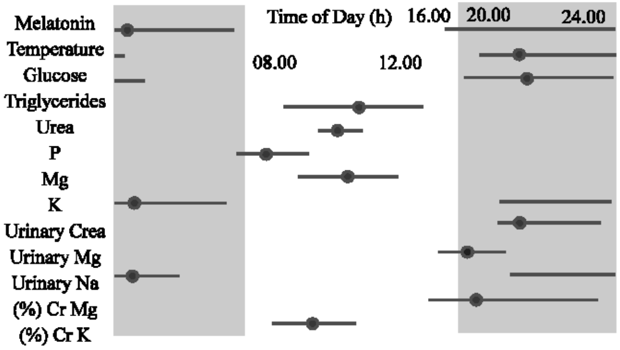

Fig. 2: Acrophases of the rhythms of 13 variables in Alpaca at low environmental temperature. Circles indicate the means. Horizontal lines indicate the 95% confidence intervals of the means. The grey columns indicate the duration of the dark phases of the light– dark cycle

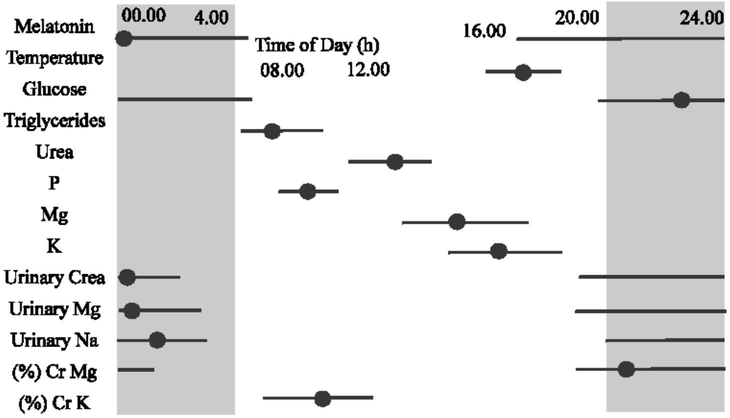

Fig. 3: Acrophases of the rhythms of 13 variables in Alpaca at high environmental temperature. Circles indicate the means. Horizontal lines indicate the 95% confidence intervals of the means. The grey columns indicate the duration of the dark phases of the light-dark cycle

The results of rhythmic parameters, are reported in Fig. 2 and 3. The application of the periodic model has permitted to point out, throughout the time series studied, the periodic progress of the studied parameters. The acrophases (with the confidence interval at 95%) were the follows: for urinary creatinine at 22.16 (21.00- 23.32) for the first experimental session and at 00.12 (20.56- 03.28) for the sec experimental session, for urinary magnesium at 19.32

(17.24- 21.40) for the first experimental session and at 00.28 (20.44-04.04) for the second experimental session, for urinary sodium at 00.48 (21.48-03.48) for the first experimental session and at 01.20 (22.20-04.12) for the second experimental session, for fractional clearance of magnesium at 19.52 (16.32- 23.12) for the first experimental session and at 23.08 (20.48-01.32) for the second experimental session, for fractional clearance of potassium at 10.00 (07.48-12.12) for the first experimental session and at 09.12 (06.36-11.48) for the sec experimental session, for glucose at 22.48 (19.12-02.16) for the first experimental session and at 02.00 (21.56-06.00) for the sec experimental session, for triglycerides at 12.28 (08.16-16.36) for the first experimental session and at 07.16 (05.20-09.12) for the sec experimental session, for urea at 11.32 (10.28-12.36) for the first experimental session and at 12.32 (10.44-14.16) for the sec experimental session, for phosphorus at 07.48 (05.48-09.52) for the first experimental session and at 08.48 (07.44-09.52) for the sec experimental session, for magnesium at 12.00 (08.44-15.08) for the first experimental session and at 15.56 (13.04-18.44) for the sec experimental session, for potassium at 01.16 (21.28- 05.20) for the first experimental session and at 17.52 (15.20-20.24) for the sec experimental session, for body temperature at 22.16 (20.08-00.20) for the first experimental session and at 18.36 (16.40-20.24) for the sec experimental session, for melatonin at 00.44 (17.44-05.44) for the first experimental session and at 00.04 (18.12-05.40) for the second experimental session.

DISCUSSION

Several studies pointed out the existence of a daily rhythmicity in different physiological variables such as liver function in sheep (Piccione *et al*., 2003), melatonin secretion in hamsters (Gunduz, 2002) and body temperature (Piccione *et al*., 2003; Piccione and Reffinetti, 2003). In some studies multiple variables were simultaneously monitored (Piccione *et al*., 2005; Fischetle *et al*., 1981; Honnebier *et al*., 1992; Jilge, 1985; Johnson *et al*., 1992; Krauchi and Wirz, 1994; Lefcourt *et al*., 1999; Meinrath and Amato, 1979; Moore *et al*., 1977; Robinson and Fuller, 1999; Scales *et al*., 1988), but only a few variables were monitored in each study. In order to better understand the multiple temporal relationships of physiological processes and the influence of external synchronizer, the simultaneous study of many variables in different environmental conditions is necessary. In the present study, the rhythmic pattern of 13 variables in alpacas kept at two different environmental temperatures and with different photoperiod has been investigated. With the exception of body temperature and potassium, all the studied parameters peaked in the same light/dark phase both at low and high environmental temperature. The results indicate that, different physiological variables exhibit different degrees of daily rhythmicity and reach their daily peaks at different times of the day; some of the studied variables (8 out of 13 variables at low environmental temperature and 6 out of 13 at high environmental temperature) peaked during the dark phase of the light-dark cycle. This pattern might be due to the different photoperiod of the 2 experimental session as described several authors (Marie *et al*., 2001; Lopez *et al*., 2006). Other external zeitgabers able to influence the daily rhythmicity of the studied parameters are the environmental temperature (Lowe *et al*., 2001) and the food intake (Marie *et al*., 2001; Lopez *et al*., 2006) It is known that timing of feeding and its energetic value affect in a significant way the shape and the amplitude of the daily rhythm of body temperature (Berman and Morag, 1971; Mohr and Krzywanek, 1990). Furthermore, the photoperiod as well as the food intake has an influence on melatonin secretion.

CONCLUSION

Our data point out the existence of a periodicity of the studied parameters during the two experimental sessions suggesting a rhythm stability, though with different acrophases, in animal kept under natural environmental conditions (characterized by variable temperature, variable relative humidity and variable photoperiod). Rhythmicity can be influenced by exogenous factors (like the environmental conditions) able to modify its synchronicity but not its intrinsic periodicity. Comparisons among different rhythms in different stabling conditions could led to further development of the circadian temporal organization in South American camelids.

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