Folia biologica (Kraków), vol. **56** (2008), No 3-4
doi:10.3409/fb.56_3-4.133-137

Circadian Activity Rhythm in Sheep and Goats Housed in Stable Conditions

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Accepted April 22, 2008

PICCIONE G., GIANNETTO C., CASELLA S., CAOLA G. 2008. Circadian activity rhythm in sheep and goats housed in stable conditions. Folia biol. (Kraków) **56**: 133-137.

The aim of this study was to establish the circadian rhythm of total activity in sheep and goats kept in housed conditions. For our study five Comisana breed sheep and five Maltese breed goats were kept in an individual box of 12 m^2 under artificial 12/12 light/dark cycle for 14 days. We equipped the animals with Actiwatch-Mini, by means of collars. A two-way ANOVA was used to determine significant differences between experimental treatments. A paired Student *t*-test was used to evaluate the differences between photophase and scotophase. The results show that activity in sheep and goats is mainly diurnal, the activity rhythm reaches its peak in the middle of the day. In conclusion small ruminants can be classified as diurnal animals, even though they show a variable amounts of activity.

Key words: Daily rhythm, total activity, stable conditions, *Ovis aries, Capra hircus.*

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The internal temporal coordination of physiological and behavioural processes of organisms involves the circadian timekeeping systems (JILGE 1993) which regulate several physiological systems acting on their rhythmic daily and seasonal activity (LOUDON *et al*. 2000). For long-term timekeeping, mammals use two types of mechanism: photoperiodism and circannual rhythm generation. Photoperiodism registers changes in day-length on an annual basis and translates it into a timed control of physiology and behaviour (TAMARKIN 1985) by means of photoinduction, a genetically programmed response to a change from short to long days, or *vice versa*. Photorefractoriness is an associated process that produces changes on a shorter basis (weeks or months) that may be a reversal of the initial response, producing a timed cycle. The circannual rhythm generation lasts several seasons and is a notable feature in many long-lived animals and shows some differences among animals. In fact in some animals the intrinsic annual cycle is predominant despite the photoperiod, while in others both circannual timing and photoperiodism are combined to regulate seasonality (LINCOLN *et al*. 2003). The sleep-wake cycles, many hormone rhythms and seasonal fatting, hibernation and reproductive cycles in wild animals are regulated in vertebrates by the circadian clock (LOUDON *et al*. 2000). An oscillator endogenously generates these circadian rhythms with a frequency close to 24 hours, but in the absence of temporal environmental cues for a long period of time, these rhythms will free-run. The usual environmental condition of a current, 24 hours light/dark cycle serves as an entraining stimulus or Zeitgeber, for setting endogenous period of the pacemaker at 24 hours (MOORE 1982).

The light stimuli act on the suprachiasmatic nucleus (SCN) entraining the circadian clockwork, acting on the waveform of clock genes and on the electrophysiological activity of SCN neurons (SUMOVA *et al*. 1995; NUESSLEIN-HILDESHEIM *et al*. 2000).

One of the most robust and best-studied classes of biological rhythms is associated with the alternation of day and night. The L/D cycle is the most potent cue for circadian entrainment in most organisms. Circadian rhythms probably evolved as adaptations that allowed organisms to prepare for relatively predictable environmental changes associated with the day-night cycle (PITTENDRIGH 1993); but also daily environmental and artificial temperature changes are characteristic Zeitgeber signals for the entrainment of the circadian clock in many organisms (RENSING & ROUFF 2002).

Daily oscillations in the level of physiological variables have been described for a multitude of variables, including locomotor activity, body temperature, blood pressure, haematochemical and haematological parameters, hormonal secretion, urinary output, sensitivity to pain, and short-term memory (MOORE-EDE *et al*. 1982; PICCIONE *et al*. 2002; 2004a, b, c; 2005; 2008; PICCIONE & REFINETTI 2003; REFINETTI 2006). The duration of daily locomotor activity varies with changing day-length. This rhythm has well characterised circadian outputs and is determined by the phenotype of the SCN (RALPH *et al*. 1990).

Daily rhythms of locomotor activity have been documented in a large number of species of mammals such as rodents, rabbits, cats, dogs and sheep (REFINETTI 2006; PICCIONE *et al*. 2007a, b; BARTOSZEWICZ & BARBACKA-SUROWIAK 2007), few investigations have been conducted in horses (GILL 1991; SCHEIBE *et al*. 1999; PICCIONE *et al*. 2008), and none in goats. The aim of this study was to establish if small ruminants (*Ovis aries* and *Capra hircus*) show the same total activity patterns while kept under intensively housed conditions, in which man controls most of the factors influencing ingestive behaviour such as the amount and type of food available, the time span during which it is available and the social context of its consumption.

Material and Methods

Five clinically healthy and not pregnant female sheep (Comisana breed, 2 years old, mean body weight 45±2Kg) and five clinically healthy and not pregnant female goats (Maltese breed, 2 years old, mean body weight 40±2 kg) were used. Animals were housed individually in a soundproof light-tight box of 12 m^2 equipped with an airflow system. The visual and acoustic isolation of each animal from others avoided the social entrainment of circadian behavioural rhythms (DAVIDSON & MENAKER 2003). The animals were put in the experimental box 30 days before starting the study to avoid changes in the behaviour and physiology of animals due to the state of fear induced by isolation (CARBONARO *et al*. 1992). Thermal and hygrometric records were carried out inside the box for the whole study by means of a data logger (Gemini, UK). Temperature during the experimental period was 15.5°C, minimum; 18.5°C, maximum; and mean humidity was 55-60%. Animals were kept under an artificial 12:12 L/D cycle (L:500 lx at the level of the head of animals; D: 0 lx) similar to the natural photoperiod in which the study was carried out (14th-27th of December). Full-spectrum cool fluorescent tubes (Osram, Germany) placed in the middle of the box at 2 m height from the floor were used as the light source. As standard farming practice, hay and water were available *ad libitum*.

In the present investigation, we recorded total activity of sheep and goats, which includes different behaviours, such as feeding, drinking, walking, grooming, ruminating as well as all conscious and unconscious movements. To record activity, we equipped the animals with Actiwatch-Mini® (Cambridge Neurotechnology Ltd, UK), actigraphy-based data loggers that record a digitally integrated measure of motor activity. This activity acquisition system is based on miniaturized accelerometer technologies, currently used for human activity monitoring but also tested for activity monitoring in small non-human mammals (MUNOZ-DELGRADO *et al*. 2004; MANN *et al*. 2005). Actiwatch-Mini® utilizes a piezo-electric accelerometer that is set up to record the integration of intensity, amount and duration of movement in all directions. The corresponding voltage produced is converted and stored

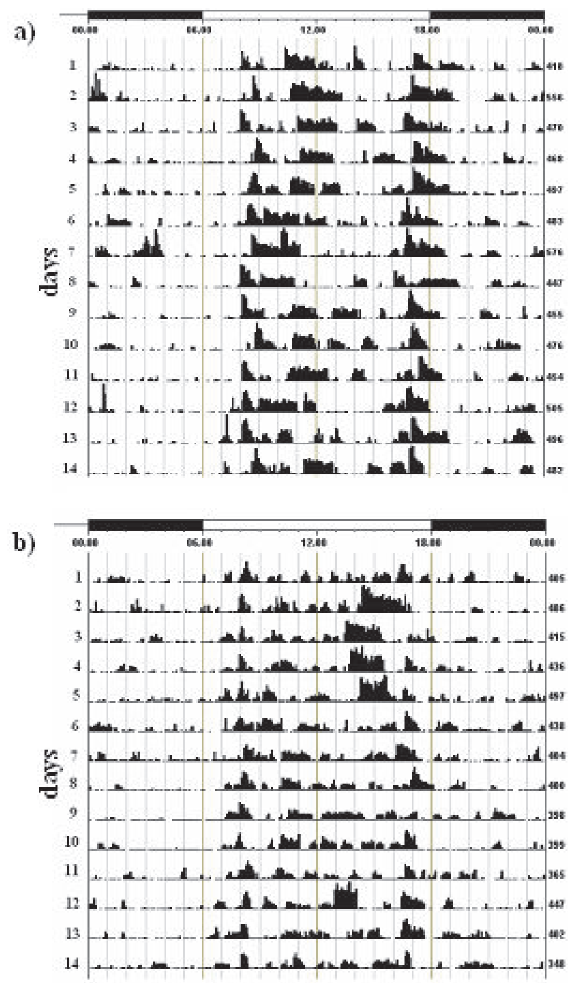

Fig. 1. Total activity record of a sheep (a) and of a goat (b) subjected to a 12:12 LD cycle. Each horizontal line is a record of 1 day's activity and consecutive days are plotted one after the other. White and black bars indicate light-dark periods of the 12:12 LD cycle.

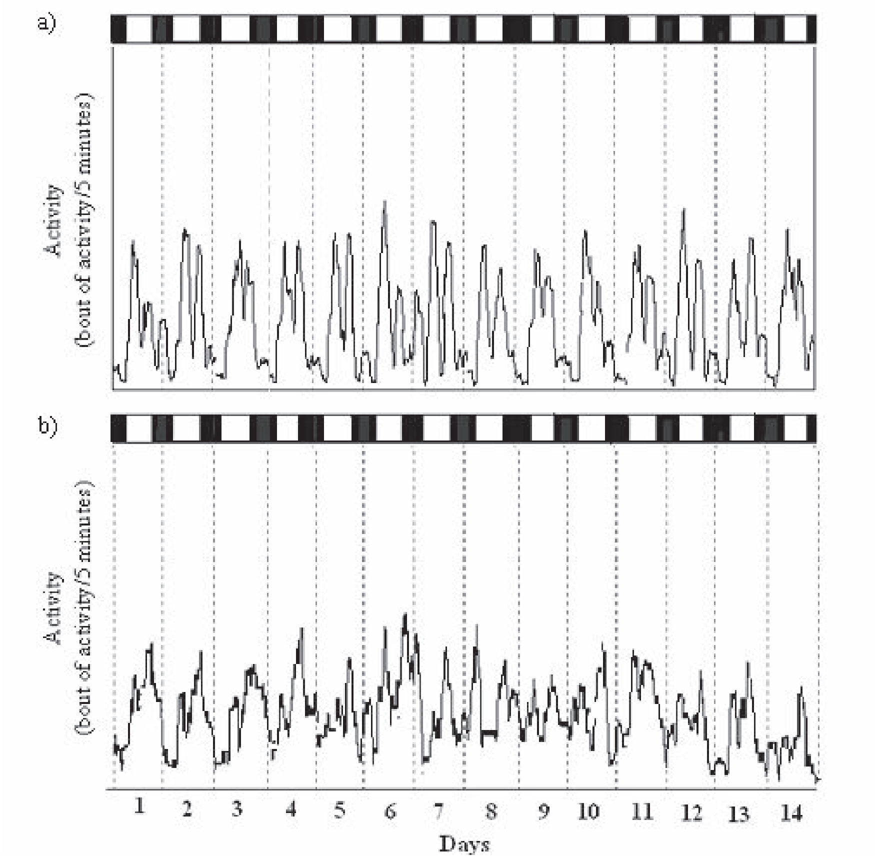

Fig. 2. Total activity rhythms showing acrphases, estimated time of day for peak activity in a sheep (a) and in a goat (b). White and black bars at the top of each graph indicate light and dark phases.

as an activity count in the memory unit of the Actiwatch-Mini®. The maximum sampling frequency is 32 Hz. It is important to stress that due to this improved way of recording activity data there is no need for sensitivity setting as the Actiwatch unit records all movement over 0.05 g. Actigraphs were placed by means of collars that were accepted without any apparent disturbance. Activity was monitored with a sampling interval of 5 minutes.

Actograms, a type of graph commonly used in circadian research to plot activity against time, were drawn using Actiwatch Activity Analysis 5.06 (Cambridge Neurotechnology Ltd, UK). The total daily amount of activity and the amount of activity during the photophase and the scotophase were calculated using Actiwatch Activity Analysis 5.06 (Cambridge Neurotechnology Ltd, UK). The Cosine peak of a rhythm (i.e., the time of the daily peak) was computed by cosinor rhythmometry (NELSON *et al*. 1979) as implemented in the Actiwatch Activity Analysis 5.06 program. A two-way analysis of variance (ANOVA) was used to determine significant differences between experimental treatments ($P<0.05$ was considered statistically significant). A paired Student *t*-test was used to evaluate the differences between photophase and scotophase.

All treatments reported above were carried out under the guidelines of the Italian Ministero della Salute for the care and use of animals.

Table 1

Average values and cosine peak of total activity ±SD during daytime, photophase and scotophase (12:12 LD cycle) recorded in five sheep and five goats during fourteen days

	Average	Light average	Dark average	Cosine park
Sheep	494.36±191.13	728.20±271.50	211.00±147.40	13.23±1.23
Goats	402.03±59.54	651.30±104.40	164.50±35.40	13.00±1.30

Results

Both sheep and goats were mainly diurnal, exhibiting greater activity during the photophase than during the scotophase (Sheep: photophase: 728.20±271.50, scotophase: 211.00±147.40, t_{69}=22.32, P<0.0001; Goats: photophase: 651.30±104.40, scotophase: 164.50±35.47, t_{69}=33.85, P<0.0001; paired Student *t*-test).

The total activity in sheep was significantly greater than in goats (Sheep: 494.36±191.13, Goats: 402.03±59.54; $F_{(1,112)}$=13.05, P≥0.0004, two-way ANOVA for repeated measures). This significant difference was present both during the photophase (Sheep: 778.16±271.50, Goats: 651.33±104.38; $F_{(1,112)}$=11.55, P≥0.0009, two-way ANOVA for repeated measures), and during the scotophase (Sheep: 210.98±147.37, Goats: 164.53±35.46; $F_{(1,112)}$=6.37, P≥0.012, two-way ANOVA for repeated measures) (Fig. 1). A two-way ANOVA for repeated measures showed that the cosine peak did not change between the two species ($F_{(1,112)}$=0.38, P≥0.53, two-way ANOVA for repeated measures). The cosine peak in sheep was between 12.00 and 14.45 and in goats was between 11.30 and 14.30. Figure 2 shows the value at each time point of the plot, representing the average for that time point over selected days of the study of a sheep and a goat.

Discussion

Animals have the ability to move in order to achieve activities necessary to sustain life such as search for food, avoid danger, etc. The ecological niche of each particular species demands a different behavioural strategy and consequently species differ between their use of habitat (CARSON & KINESIS 1985a). Perhaps the most fundamental, yet obscure, ecological issue in circadian physiology is an organism's adoption of a nocturnal niche or a diurnal niche (REFINETTI 2006). Sheep and goats, showing similar physiological correlations, live in the same zoogeographic zone due to a natural determination. But they show different social behaviour. For example, they have a different ingestive behaviour, sheep prefer grazing on level ground, whereas goats prefer fresh grass and are also able to reach inaccessible zones (PORTOLANO 1987). However, when sheep and goats are kept in captivity these different behavioural patterns persist.

The results obtained in this study outline a diurnal pattern regarding total activity both in sheep and goats. For both species, the cosine peak occurred in the middle of the photophase of the experimental L/D cycle, as previously observed in horse (PICCIONE *et al*. 2008) and sheep, at least when they were fed *ad libitum* (PICCIONE *et al*. 2007b). However, total activity in sheep was significantly greater than in goats (Fig. 1). Statistical differences were observed not only in average values of total activity, but also between photophase and scotophases. The Figure presented shows total activity recorded from a sheep and a goat during the recording period. In sheep total activity is prevalent during the photophase, from 07.00 to 18.30, while during the scotophase there are several activity peaks, mostly with lower intensity and shorter than during the photophase, with several cycles of sleep. In goats total activity is also prevalent during the photophase, from 06.30 to 18.00, but during the scotophase, several activity peaks alternated by several cycles of sleep were less frequent than in sheep.

The experimental stable conditions may inhibit many behavioural patterns in addition to exploration, e.g. social and sexual behaviour. Since many other behavioural patterns, e.g food-seeking, also change the animal's stimulus field, then the sensory deprivation of the animal must be severe if it is kept in the environment of a barren intensive farming system(CARSON 1985b).

In conclusion our results establish that in sheep and in goats housed in the same stable conditions, most activity is concentred in the photophase of the L/D cycle, although some activity is exhibited at any time of day. Thus the small ruminants can be classified as diurnal animals, the activity rhythm reaches its peak in the middle of the day, even if there are some differences in the amount of total activity between the two species.

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