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Daily rhythm of rumination in the goat (*Capra hircus*) is not driven by feeding

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Rumination shows a nocturnal rhythmic pattern in day-active ruminants. Although feeding has been shown to quantitatively modulate rumination, it is not clear yet if feeding rhythm plays a causal role in rumination rhythm. The present study was carried out to determine whether the daily rhythm of rumination in goats under natural environmental conditions is food-dependent or not, and whether it is modulated by the season. Rumination and locomotor activity (LA) were continuously monitored in four male Moroccan goats, fed once daily every morning, and then fasted for 4 days. The same experimental design was carried out in two different seasons (summer and winter). The results show that normally fed goats ruminate on average 281 ± 37 min in both seasons. Rumination shows a rhythmic 24-h profile with a late nocturnal peak. During the four-day food deprivation, rumination persisted while its total amount duration was significantly reduced in both seasons. Our results also illustrate the clear phase-opposition between the diurnal rhythm of LA and the nocturnal rhythm of rumination. This study characterized the daily rhythm rumination in goats according to seasons and fed/fasted states, and provided clear evidence that this rhythm is not generated by feeding behavior. Further investigations are required to determine whether rumination has an endogenous origin and is driven by the central circadian clock.

Keywords Rumination, Rhythm, Feeding, Food restriction, Locomotor activity

Rumination is the process by which fibrous digesta is regurgitated from the rumen to the oral cavity and re-masticated, ensalivated, formed into a bolus and then swallowed back into the rumen[1,2]. This peculiar physiological function is the main characteristic of a large group of mammals; the ruminants; whose biological, ecological and economic importance is of great value worldwide. Indeed, rumination enables these animals to attain high digestive efficiency, as well as the capacity to ingest large quantities of feed in a relatively short period of time, followed by rumination[3]. The time required for rumination to take place varies according to the nature of the ingested feed, thereby implying a variable retentiontime of the feed in the stomachs. The process requires, on average, 28.0 h for concentrated feed and 39.5 h for hay in cattle[4]. It can be observed that the rumination pattern is not a continuous process over time; rather, it is exhibited as repeated and interrupted episodes of rumination. A rumination episode is defined as a period of regular chewing were interspersed at regular intervals by inter-bout intervals lasting a few seconds of swallowing and regurgitation. The duration of a rumination episode can vary considerably, from a few seconds to 2 h in sheep and cows. The total duration of rumination episodes over a 24-h period represents the rumination time, which is the parameter generally used to measure and quantify rumination[5–8]. Several studies have demonstrated that the occurrence of rumination does not exhibit a uniform distribution throughout the day. Rather, it has been demonstrated that this behaviour shows a rhythmic profile, occurring mainly at night in diurnal ruminants[5,9–12].

At existing knowledge, the complete nervous determinism of rumination remains unknown. Earlier studies found that rumination is a reflex initiated by the presence of fibrous digesta in the rumen[13]. Subsequent research has demonstrated the existence of a latency period, or period of inhibition (lag period), after eating and before the onset of rumination[14]. This suggests that feeding activity is the primary factor influencing the initiation of rumination[14,15].

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It has been demonstrated in sheep that the physical form of unground roughage is the major stimulant of the rumination process and that the rumination rhythm is not dependent on the light–dark (LD) cycle[16,17]. Nevertheless, multiple studies have shown that rumination and its rhythm are not food dependent, but are mainly influenced by the photoperiod (LD cycles)[5,10,11], or at least partially by the photoperiod and by feeding[16]. In regard to the effect of light on rumination during the LD cycle, Oshiro and colleagues (1996) suggested that the decreased rumination effects observed during the photophase may be attributed to an increase in mastication (i.e., chewing associated with eating), which subsequently inhibits rumination until the scotophase[18]. Actually, a review of the literature reveals that the precise mechanisms underlying rumination and its rhythm remain elusive. In particular, the role of food as a necessary component in the display of this physiological process remains unclear.

Finally, the aim of the present study was to verify, experimentally, whether feeding is responsible for generating the rumination rhythm. To achieve this, outdoor goats were subjected to a period of food deprivation subsequent to normal feeding during a baseline period. Furthermore, to investigate the possible influence of seasons on rumination, daily rumination was studied in both fed and fasted states during the summer and the winter.

Results

Rumination

Goats display a clear robust and nocturnal rhythm of rumination that persisted even in the absence of feeding (Figs. 1 and 2; Table 2). Furthermore, actograms of rumination (Fig. 3) highlighted clearly the persistence of the nocturnal rumination rhythm throughout the fasting periods of stages 2 in both seasons.

A comparison of rumination duration between the two stages (fed and fasted) of each experiment was conducted. Post-hoc test has shown a significant decrease in the rumination duration between 01 to 09 am in winter. This decrease is only significant late at night and early morning in summer (Fig. 1).

During the fed state (stage 1), the 24-h total rumination time (Fig. 2) was observed to be constant between seasons, 280.8 ± 36.5 min and 280.5 ± 36.8 min respectively for summer and winter. Although goats were food deprived during stage 2, rumination episodes persisted throughout the four days of this stage in both seasons but with a significant ($F_{(3.\,9)} = 18.04$; $p = 0.006$) decrease in the time spent ruminating: 162.2 ± 38.9 min in summer and 128.7 ± 18.4 min in winter ($F_{(3.\,9)} = 18.04$; $p = 0.0016$) (Fig. 2). It is worth noting that comparing rumination time for a given stage between the two seasons has shown no significant differences (Fig. 2).

Moreover, two-way ANOVA between stages 1 and 2 of the two experiments showed that food deprivation did not alter the robustness of the rumination rhythm (Table 2). However, it significantly affected the amplitude and the mesor of the rhythm ($P < 0.05$) in both experiments (Table 2). Indeed, the amplitude decreased significantly from 23.76 ± 0.4 min to 16.26 ± 1.75 min in the summer ($F_{(3.\,9)} = 33.19$; $p = 0.0062$) and from 13.15 ± 1.07 min to 6.55 ± 1.12 min in the winter ($F_{(3.\,9)} = 33.19$; $p = 0.0089$). The mesor of rumination rhythm decreased significantly from 11.7 ± 0.7 min to 6.76 ± 0.64 min in the summer ($F_{(3.\,9)} = 18.03$; $p = 0.0059$) and from 11.67 ± 1.0 to 5.37 ± 0.9 min in the winter ($F_{(3.\,9)} = 18.03$; $p = 0.0016$) (Table 2).

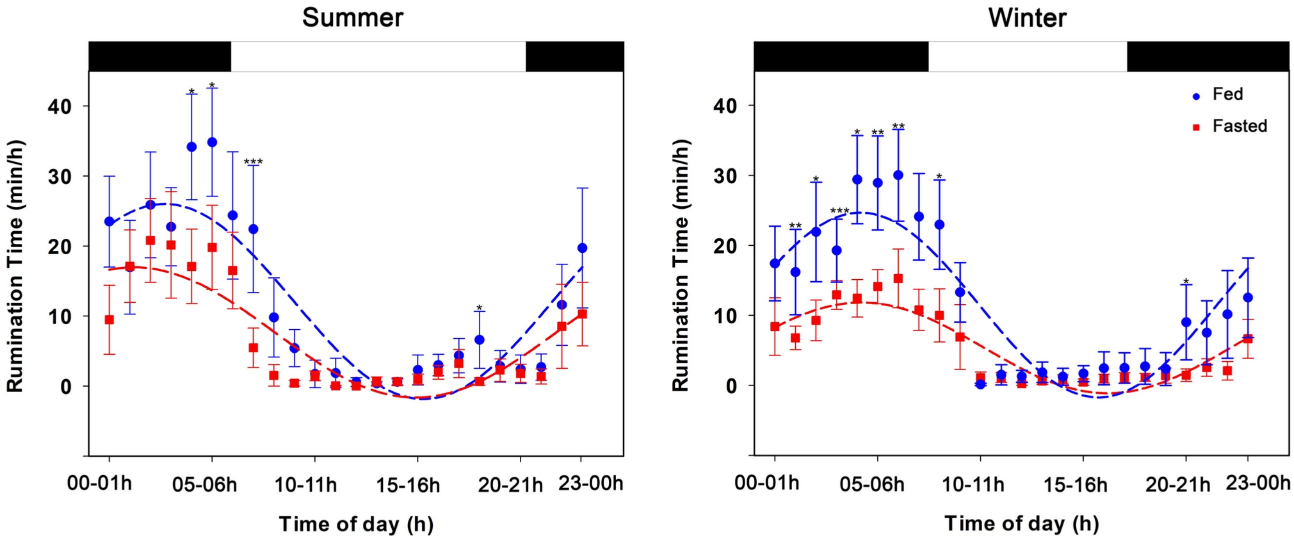


Fig. 1. Daily rhythm (Mean ± SEM) of rumination in fed and fasted goats in summer (left panel) and winter (right panel). Data (symbols) with fitted blue and red discontinued curves ($f = y0 + a^{*}\cos(2\pi^{*}(t\text{-}\varphi/\tau))$: f is the rumination at time t, y0 is the mesor, a is the amplitude, φ is acrophase and τ is the period). Each data point is calculated by averaging the rumination durations for each animal at 1h-time interval over the days of each stage and then averaging all animals for that interval. ***: $P \leq 0.001$ (Very highly significant differences), **: $P \leq 0.01$ (highly significant differences), *: $P \leq 0.05$ (Significant differences), ns: $P > 0.05$ (Non-significant differences).

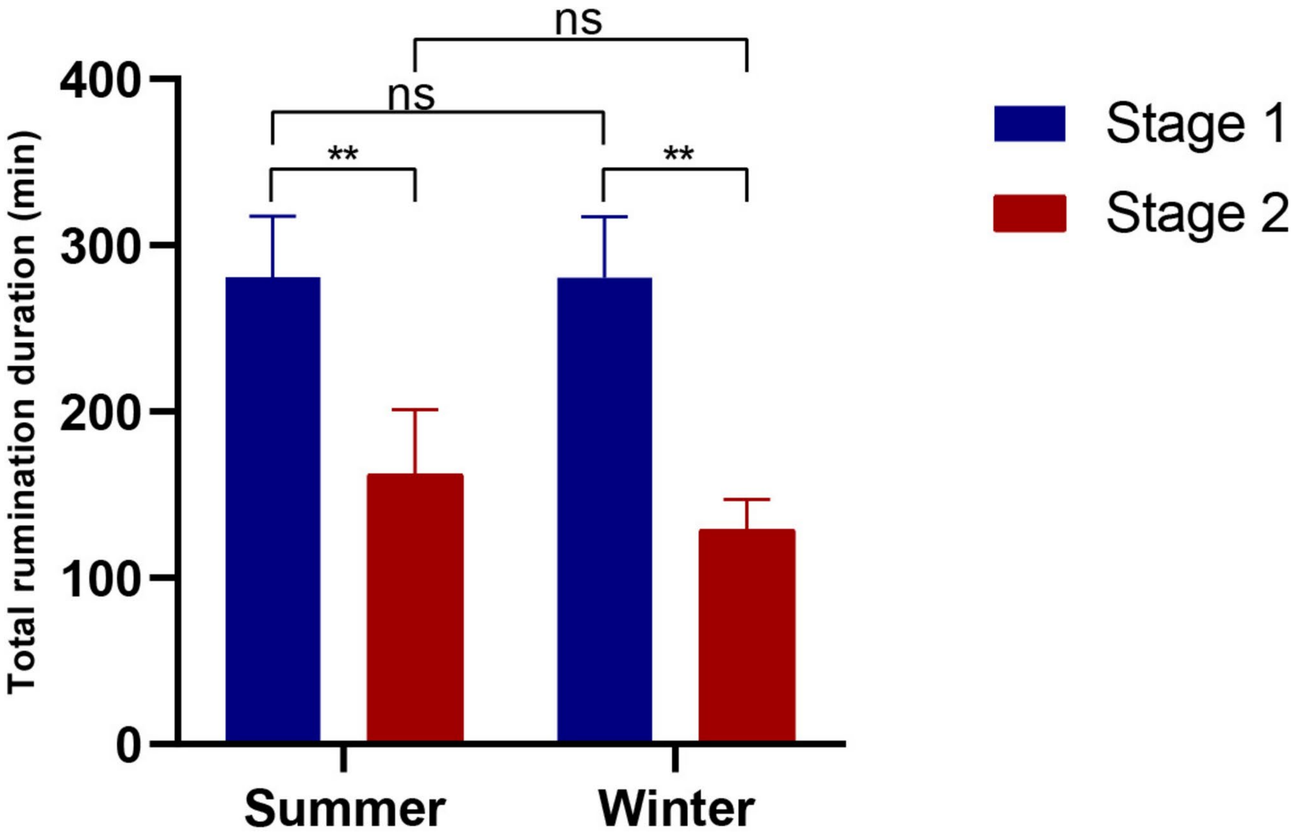


Fig. 2. Total rumination time (Mean ± SEM) per day in fed and fasted goats in summer and winter. For each animal, data of rumination duration were pooled for 24-h and then averaged over the days of each stage in each experiment and thereafter averaged for the 4 animals. Comparisons between stage 1 and 2 in each experiment and between the same stage of two different experiments were performed by using the Two-Way Repeated Measures ANOVA. **: $P \leq 0.01$ (highly significant differences), ns: $P > 0.05$ (Non- significant differences).

		Robustness	Amplitude	Mesor
Fed	Summer	42.55 ± 0.75% ^{Aa}	23.76 ± 0.4 min ^{Aa}	11.7 ± 0.7 min ^{Aa}
	Winter	44.43 ± 5.4% ^{Aa}	13.15 ± 1.07 min ^{Ba}	11.67 ± 1.0 min ^{Aa}
Fasted	Summer	39.3 ± 0.55% ^{Aa}	16.26 ± 1.75 min ^{Ab}	6.76 ± 0.64 min ^{Ab}
	Winter	43.10 ± 1.6% ^{Aa}	6.55 ± 1.12 min ^{Bb}	5.37 ± 0.9 min ^{Ab}

Table 2. Parameters of the rumination rhythm in fed and fasted states according to seasons. Two-way ANOVA analysis was used for the calculation of the statistical differences between the different rhythm parameters across different stages and experiments. Different superscript uppercase letters indicate significant differences between compared different seasons for the same stage (P ≤ 0.05) and similar superscript uppercase letters indicate no statistically significant differences (P > 0.05). Different superscript lowercase letters indicate significant differences between stages 1 and 2 in the same season (P ≤ 0.05) and similar superscript lowercase letters indicate no statistically significant differences (P > 0 .05).

The comparison of the rumination rhythm parameters between the two experiments showed that the season had few effects on the rumination rhythm (Table 2). In point of fact, during stage 1 (fed state), most parameters of the rumination rhythm did not change between the two seasons. Only the amplitude of the rhythm was significantly reduced ($F_{(3, 9)} = 33.19$; $p = 0.001$) in winter compared to summer (Table 2). This decrease occurred despite the fact that the total rumination time did not change between the two seasons (Fig. 2; Table 2). Regarding stage 2, under food deprivation, the rumination rhythm maintained the same robustness and mesor over both seasons. A significant decrease ($F_{(3, 9)} = 33.19$; $p = 0.0014$) in the amplitude of the rhythm was also observed in winter compared to summer despite the fact that the total rumination time did not change between the two seasons (Fig. 2).

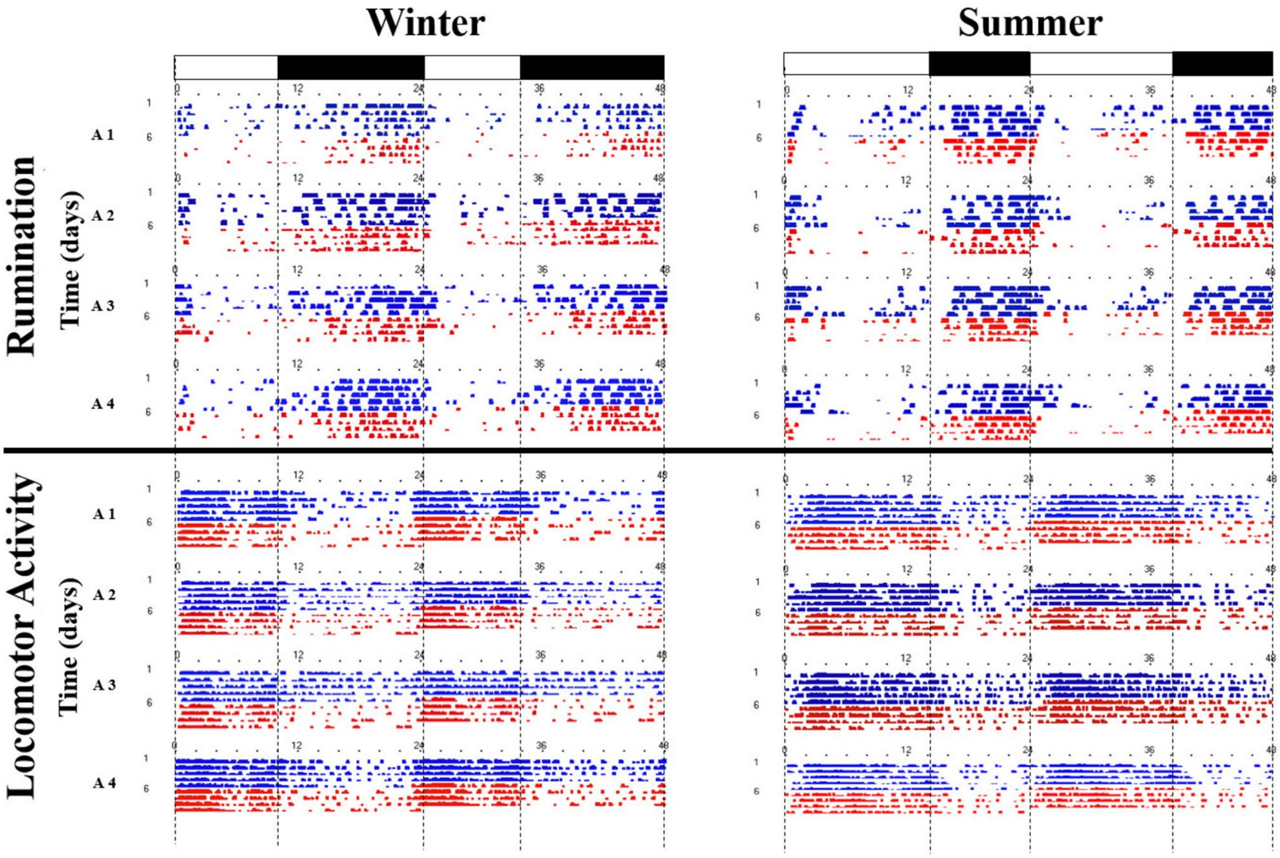


Fig. 3. Double-plotted actograms of locomotor activity and rumination rhythms in the four goats during 09 days of the two experiments. Each line corresponds to 24 h of locomotor activity or rumination starting at 06:25 h and ending at 06:25 h of the following day in summer and starting at 08:10 h and ending at 08:10 h of the following day in winter. The lines and blue or red points indicate the existence of a locomotor activity or rumination. Blue lines for stage 1 and red ones for stage 2. Scores of 1 to 5 for locomotor activity and of 1 to 5 for rumination (see methods section) induce an increase in the thickness of the line; while white or on-line vacuum denote of no activity and no rumination (Scores of 0). Top white and black bars represent the durations of the light and dark phases of LD cycle.

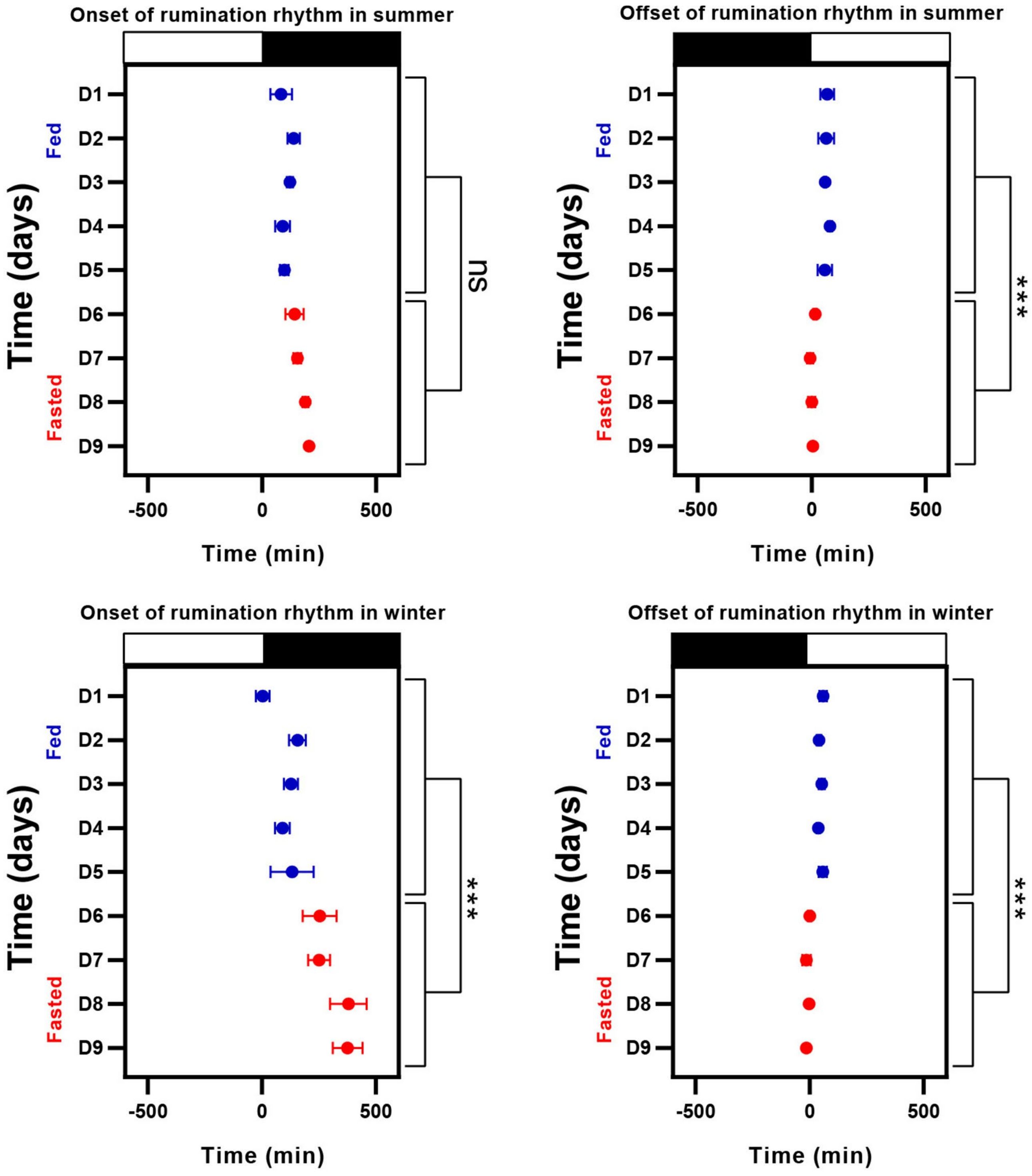
The onset of the rumination phase is always observed to occur after the day-night transition. In the winter, the onset occurs at 102 ± 26 min and 315 ± 36 min after the transition for the fed and fasted states, respectively. In the summer, the onset occurs 106 ± 10 min and 173 ± 14 min, respectively, after the transition for fed and fasted states. The two-way ANOVA showed that the onset of the rumination rhythm did not change throughout the stages in summer. However, in winter the onset was very highly significatively ($F_{(3.\ 48)} = 15.48$; $p < 0.0001$) increased (Delay) when goats were food deprived (Fig. 4). A statically significant change was also found in fasted state between the two seasons with increased onset during the winter compared to summer.

In fed state, the offset of the rumination phase has been demonstrated to occur 49 ± 4 min in winter and 65 ± 4 min in summer after the night-day transition. Food deprivation induced a statistically significant advance ($F_{(3.\ 48)} = 17.82$; $p < 0.0001$) in the offset of the rumination rhythm in both seasons (62.3 min in summer *vs* 57.5 min in winter). The Student test of conformity indicates that in the fasted state of both experiments, the offset of the rumination phase coincides exactly with the time of night-day transition. It is noteworthy that no statistically significant difference was observed in the offset of the same stage across different seasons. These results show that seasons do not affect the offset of the rumination rhythm.

In addition, food deprivation was shown to significantly reduce the rumination period (time between onset and offset) (Fig. 5). This duration decreased from 13.3 ± 0.8 h to 8.4 ± 0.6 h ($F_{(3.\ 9)} = 28.39$; $p = 0.0005$) in winter and from 9 ± 0.2 h to 7 ± 0.2 h ($F_{(3.\ 9)} = 28.39$; $p = 0.023$) in summer (Fig. 3 and 5). Season brings a very significant change ($F_{(3.\ 9)} = 28.39$; $p = 0.0008$) in the duration of the onset-offset rumination interval in normally fed animals (Stage 1) with a decrease from 13.3 ± 0.8 h in winter to 9 ± 0.2 h in summer. In addition, no change was observed in fasted goats (Stage 2) between the two seasons. Consequently, this result shows that the photoperiod has an effect on the duration of the rumination period when goat are fed normally.

Locomotor activity

Actograms showed that for both seasons, a robust diurnal LA rhythm persisted even in the absence of feeding (Stage 2) and was inversely correlated with the nocturnal rumination rhythm (Fig. 3).



Onset of rumination rhythm in summer

Offset of rumination rhythm in summer

Onset of rumination rhythm in winter

Offset of rumination rhythm in winter

Fig. 4. Means (± SEM) of the onset and the offset of the rumination rhythm (Blue for stage 1 and red stage 2) correlated to the dark–light and light–dark transitions during 09 consecutive days (D1, D2, D3, D4, D5: Stage 1 (normal feeding); D6, D7, D8, D9: Stage 2 (Food deprivation)) in each season (experiment). The onset of rumination was calculated as the phase-angle difference in minutes between the time of the beginning of rumination and the time of the dark–light transition (Sunset). The offset of rumination was calculated as the phase-angle difference in minutes between the time of the end of rumination and the time of the light–dark transition (Sunrise). The transitions of dark–light and light–dark are represented by the reference time 0 min. The black and white bars at the top of each figure correspond to a segment of the dark and light phases of the natural LD- cycle. Comparison of the means of the onset and the offset between stage 1 and stage 2 of each season was performed using the Two-Way Repeated Measures ANOVA. *** $P \leq 0.001$ highly significant difference, ns non-significant difference.

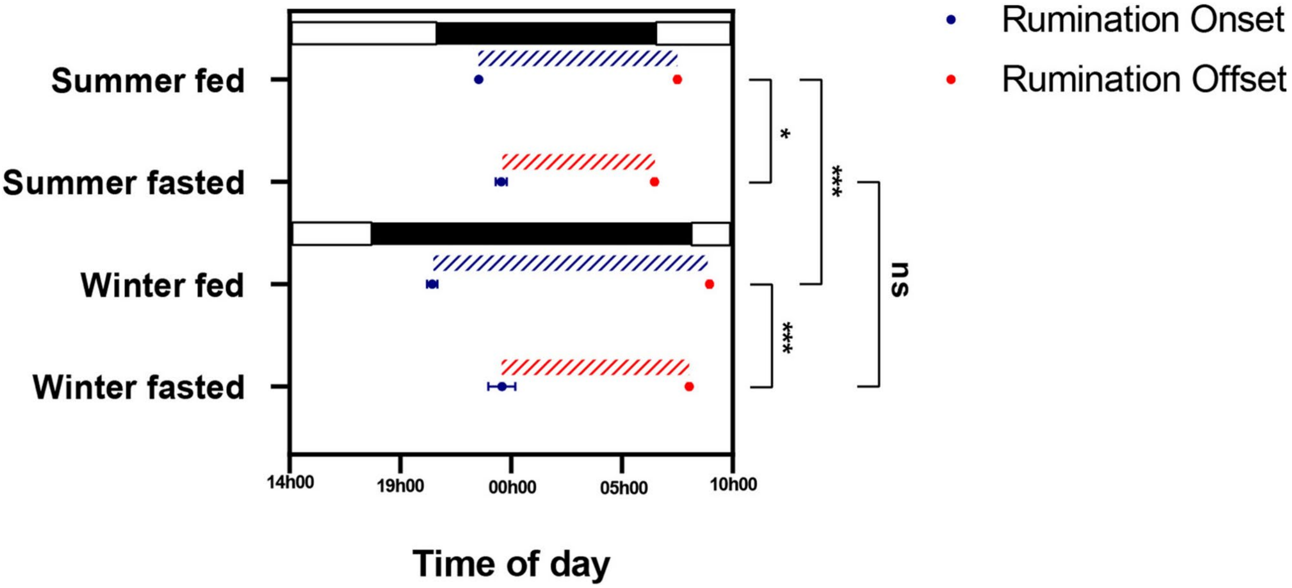


Fig. 5. Time of occurrence of the onset and the offset (means ± SEM) of the rumination rhythm of each stage in each season. The duration of the onset-offset rumination interval (Time period between onset and offset) was calculated and represented by blue and red–hatched bars for respectively stage 1 and 2. The means of the duration of the onset-offset rumination interval were compared between the different stages using Two Way Repeated Measures ANOVA. ***: $P \leq 0.001$ (very highly significant differences), *: $P \leq 0.05$ (Significant differences), ns: $P > 0.05$ (Non-significant differences).

		Robustness	Amplitude	Mesor
Fed	Summer	$24.1 \pm 0.62\%^{Aa}$	02 ± 0.5^{Aa}	1.17 ± 0.04^{Aa}
	Winter	$20.2 \pm 4.5\%^{Aa}$	2.05 ± 0.03^{Aa}	1.17 ± 0.03^{Aa}
Fasted	Summer	$10.5 \pm 1.6\%^{Ab}$	1.96 ± 0.07^{Aa}	0.81 ± 0.05^{Ab}
	Winter	$25.7 \pm 0.9\%^{Ba}$	2.19 ± 0.06^{Ba}	0.86 ± 0.02^{Ab}

Table 3. Parameters of LA rhythm in fed and fasted states according to seasons. Two-way ANOVA analysis was used for the calculation of the statistical differences between the different rhythm parameters across different stages and experiments Different superscript uppercase letters indicate significant differences between compared different seasons for the same stage ($P \leq .005$) and similar superscript uppercase letters indicate no statistically significant differences ($P > 0.05$). Different superscript lowercase letters indicate significant differences between stages 1 and 2 in the same season ($P \leq 0.05$) and similar superscript lowercase letters indicate no statistically significant differences ($P > 0.05$).

Two-way ANOVA comparison of LA rhythm parameters between fed and fasted states showed statistical changes in the mesor for both seasons and in the robustness only in summer (Table 3). Each season's robustness and amplitude were compared during stage 1 and stage 2 (Table 3) and the effect of food deprivation appears to be exacerbated by the seasonal effect.

In both seaosons, the amount of total activity mean was significantly higher (Summer fed state, $F_{(3. 48)} = 146.0$; $p < 0.0001$; summer fasted state, $F_{(3. 48)} = 146.0$; $p < 0.0001$; winter fed state, $F_{(3. 48)} = 204.0$; $p < 0.0001$; winter fasted state, $F_{(3. 48)} = 204.0$; $p < 0.0001$) during the day (Summer: 83.1 ± 4.5 in fed state, and 47.3 ± 5.4 in fasted state; Winter: 63.5 ± 2.7 in fed state, and 50.4 ± 1.1 in fasted state) than during the night (Summer:10.8 ± 0.7 in fed state, and 9.4 ± 0.9 in fasted state; Winter: 19.6 ± 3 in fed state, and 8.1 ± 1.2 in fasted state) denoting of a diurnal character of activity in the goat. The Two-way ANOVA showed that in both experiments, food deprivation led to a very significant (Summer $F_{(3. 48)} = 146.0$; $p < 0.0001$; winter $F_{(3. 48)} = 204.0$; $p < 0.0001$) decrease in the amount of total activity during the day (Fig. 6). During the night, a significant decrease of LA ($F_{(3. 48)} = 204.0$; $p < 0.0001$) was observed only in winter.

Rumenotomy

Laparotomy of food-deprived goats showed a filled rumen similar to that of normally fed goats (Deprived animals: 4.2 ± 0.4 L; normally fed animals: 4.8 ± 0.7 L) (Fig. 7). Rumenotomy revealed the presence of a very small amount of gas in the rumen of the fasted animal compared to the normally fed animal. The rumen content of fasted animals was entirely liquid, consisting of water mixed with very finely ground feed. However, in

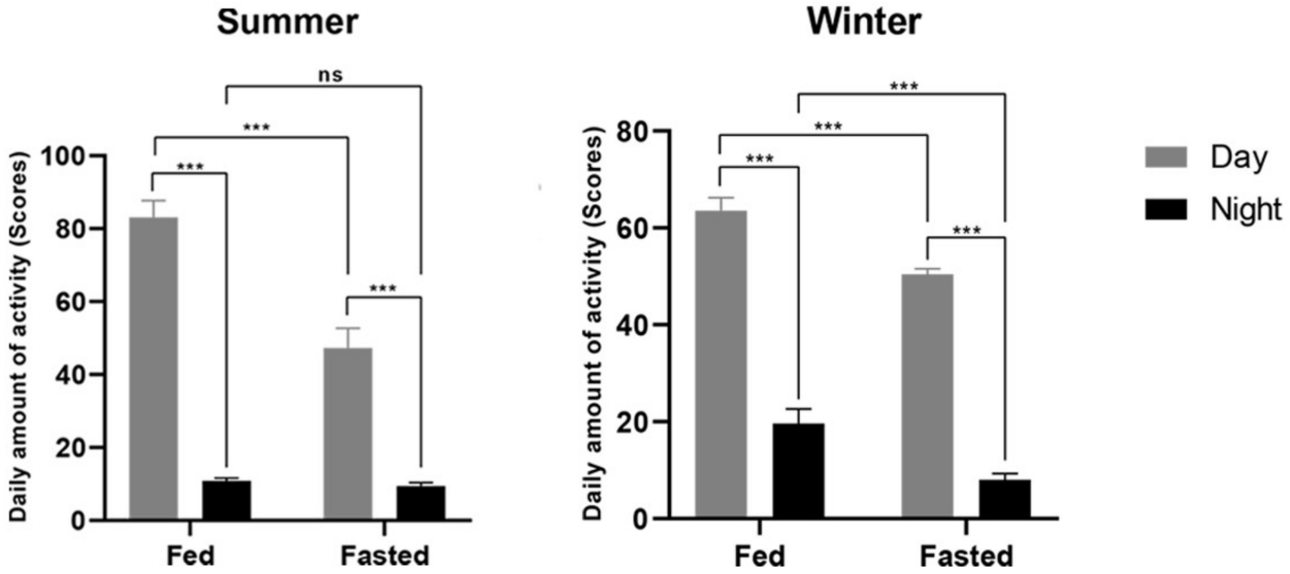


Fig. 6. Total amount of locomotor activity per day in fed and fasted goats during summer and winter. Total activity was calculated for each animal as the cumulative of the predominant scores 3, 4 and 5 observed for each 5 min, pooled for all nights then all days of each experiment and then averaged for the 4 animals. Comparison of the total activity mean between the day and night in each stage and between days and nights of different stages was performed using the Two-Way Repeated Measures ANOVA. *** $P \leq 0.001$ highly significant difference, ns non- significant difference.

Fig. 7. Left lateral view of the abdominal cavity of goat after cadaveric laparotomy. A: deprived of food; B: fed normally. a: dorsal sac of the rumen; b: ventral sac of the rumen; c: jejunum.

normally fed animals the liquid portion of the content was significantly reduced in volume and located in the middle portion of the rumen. The ventral part of the rumen contained a paste of ground feed (Fig. 8). This shows that food deprivation did not reduce rumen filling. However, it changed the physical nature of its contents. This resulted in the disappearance of the normal compartmentalisation of the ruminal contents, which consists of an upper layer of gases, an intermediate liquid layer and a lower solid layer.

Discussion

Previous studies have examined the occurrence of rumination in the absence of food[19,20], but all have focused on the quantitative characterization of this behaviour during short periods of food deprivation, rather than characterizing its rhythm. Indeed, to our knowledge, no study has provided data on the rhythm of rumination and how it is affected by food deprivation. This study is the first to use a chronobiological approach to characterize the daily rhythm of rumination under food deprivation and to investigate its relationship with the daily rhythm

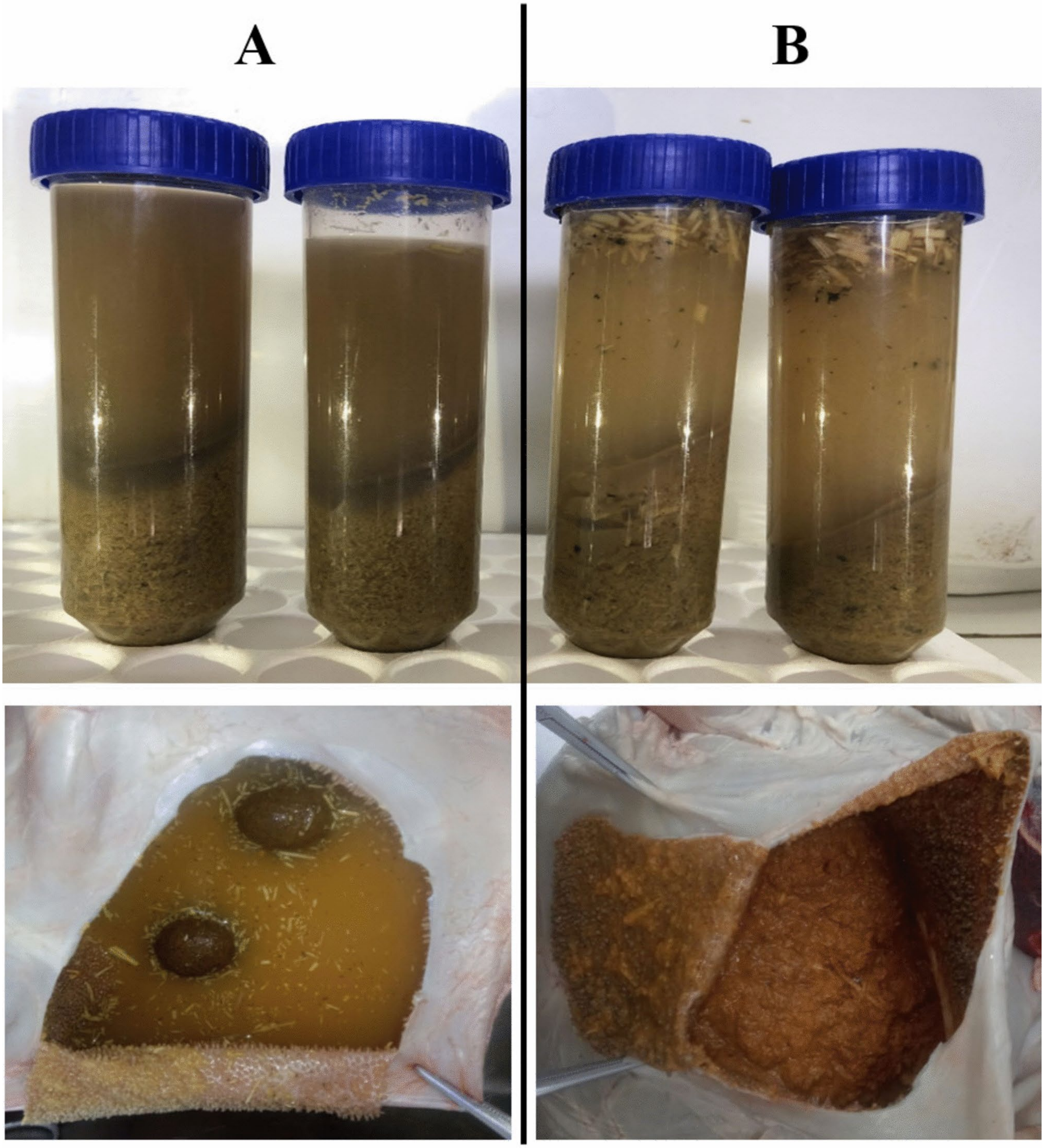


Fig. 8. Rumen content of goats. A: deprived of food; entirely liquid rumen content, consisting of water mixed with very finely ground feed. B: fed normally; a mixture of liquid and a solid portion of ground food.

of locomotor activity. The present work has experimentally demonstrated that the daily rhythm of rumination is not food dependent. However, feeding does quantitatively influence the amplitude of rumination rhythm. In addition, the seasons also modulate amplitude of rumination rhythm.

The results showed that the daily rumination duration in normally fed Moroccan black goats was comparable between summer (280.8 min/day) and winter (280.5 min/day). Compared to other goat breeds, the daily rumination observed in the present study appeared to be lower than that reported for the Saanen goat (329.8 min/day[20];), the English breed (464 min/day[11];) and the Indonesian breed Bligon (438.4 min/day[21];). These discrepancies exist despite the fact that all of the above studies, including the present one, used a single daily food supply and a comparable compound feed with fiber. The body weight may influence daily duration of rumination. In cattle indeed, rumination time is longer in high body weight breeds such as Holstein compared to low body weight breeds such as Jersey[22–24]. The Moroccan Black Goat has a relatively low average body weight compared to other goat breeds. Adult males weigh no more than 30 kg, as reported by Hossaini-Hilali and Benlamlih (1995)[25] and confirmed by the present sample. This may contribute to the observed discrepancy in total rumination time between our goats and those reported in other studies.

Despite the absence of food, rumination persists. However, its daily duration is decreased by 42% in summer and by 54% in winter. This is consistent with the reduction observed in Saanen goats during the second and third days of fasting[20]. However, in the later study, rumination showed a 137% increase on the first day of food deprivation, followed by a decrease during the second and third fasting days. In contrast, the goats in our study showed an immediate reduction in rumination time from the first day of fasting. This discrepancy may be due to differences in feeding management prior to the fasting period. Indeed, in the Saanen goat study, animals were fed ad libitum, whereas in our study, goats were fed once a day in the morning. This suggests that during the first day of starvation, the rumen of the Saanen goat was sufficiently filled with non-ruminated matter requiring therefore much time to digest and ruminate this content. In contrast, in our study, the goats were fed in the morning of the day before stage 2, thus allowing sufficient time for the animals to ruminate their ruminal contents during the night preceding the food deprivation. This subsequently induced a decrease in rumination time in the following day (the first day of starvation).

Welch and Smith (1968) found in sheep that food deprivation leads to a rapid reduction in rumination time. These authors have described rumination to be partially replaced by intermittent regurgitation patterns[19]. This is completely different from the behavior that we described in this study, which corresponds to clear and normal rumination, with bouts lasting up to 45 min. The ruminotomy we performed after 4 days of food deprivation showed a state of rumen filling similar to that of normally fed animals. This content consisted of water mixed with very finely ground feed. This suggests that in the absence of food, a minimum amount of fiber was retained in the rumen and has undergone several cycles of rumination.

Several previous studies have used changes in the timing of feed distribution or in the composition of the food ration to verify that rumination is not food-dependent[10,11,14,26,27]. Using a chronobiological analysis, the present work is the first to demonstrate that the daily rhythm of rumination is not food dependent because this rhythm persisted despite food deprivation for several days. The only changes observed were a decrease in the amplitude and mesor of the rhythm, which can be explained by the reduction in rumination time per day.

In normally fed animals (Stage 1), rumination in the summer ends (offset) later in the morning (i.e. 66 ± 4 min after sunrise) than in the winter (i.e. 50 ± 4 min after sunrise). However, in food-deprived animals (Stage 2), the offset of the daily period of rumination perfectly coincides with the sunrise in both seasons of the year. The offset of rumination seems to be modulated by the natural LD cycle when the food is removed.

Although the amount of time spent ruminating per day did not change over the seasons, the rumination phase seemed to depend on the length of the night. Indeed, phase duration (onset-offset duration) was significantly longer in winter (13.3 ± 0.8 h), when the night is long than in summer (09 ± 0.2 h). This was observed when the goats were fed normally (comparison winter stage 1 vs summer stage 1), but not in the case of food deprivation (comparison winter stage 2 vs summer stage 2). The fact that in normally fed animals, the rumination phase increased in winter compared to summer while the total rumination time did not undergo the same changes in winter can be explained by a winter decrease in the 1 h-amplitude of rumination (Fig. 1). Compared to winter, the phase duration in summer is shorter, but the goats ruminate more time per hour. From these data, it seems that there is a compensatory phenomenon in summer during which the goat condenses rumination to ruminate longer per hour to compensate the shorter total phase duration of this behavioural function.

It has long been assumed that rumination occurs during resting states of ruminants[28]. In 1973, Bell and Itabisashi used electroencephalography to try to establish a link between rumination and different states of vigilance (sleep, wakefulness, drowsiness) in goat. However, this study failed to find an exclusive link between rumination and any of the other vigilance states. In fact, rumination was shown to occur simultaneously with all states of vigilance except REM sleep and alert state[29]. In cattle, a weak positive relationship has been found between daily rumination time and lying time[30]. In camels, by using a non-invasive technique of polysomnography, we were not able to correlate rumination with sleep or other vigilance states[31]. This was due to the presence of myogenic artifacts, which precluded any interpretation of EEG tracings. Rumination was therefore considered as a separate vigilance state in camels and was observed to coincide with resting positions of this species. In the goat, the present study clearly illustrates that rumination is negatively correlated with locomotor activity. We show that rumination and LA rhythms have an opposite phase, which means that rumination does not occur during the active phase of LA rhythm.

To our knowledge, there is no previous study that has already investigated the effect of food deprivation on locomotor activity in ruminants. This study demonstrates that 4 consecutive days of food deprivation maintains the rhythm of LA, but with a significant reduction in the mean level in summer and winter. In both seasons, food deprivation led to a marked reduction (39.6% in summer *vs* 29.6% in winter) in the average amount of activity. This reduction in locomotor activity due to food deprivation has been reported in several laboratory animals including Mongolian Gerbils (*Meriones unguiculatus*) and Siberian hamsters (*Phodopus sungorus*)[32,33]. This result suggests that, as in the case of animals living in environments where food resources are scarce, the reduction of activity in Moroccan black goat is an adaptive phenomenon in order to reduce energy expenditure under conditions of food shortage.

The results also confirm the diurnality of the goat previously demonstrated in different studies[34–40]. This diurnality persisted during food deprivation, with activity starting with the beginning of the photophase and extending until beginning of the night.

The Arabian oryx (*Oryx leucoryx*), another desert mammal adapted to its harsh biotope, exhibits an adaptive strategy of temporal niche switching from diurnal activity in winter (cooler) to nocturnal/crepuscular activity in summer (hotter[41,42]. In another large desert mammal, the dromedary camel (*Camelus dromedarius*), by contrast, the diurnal pattern of LA does not change with seasons[43]. In the present study, during the hot summer and even under food deprivation, the diurnal pattern of LA persists in the Moroccan black goats. Both studies in camels and the present one in goats were conducted under a mesic environment which is completely different from the desert biotope. This suggests that the adaptive temporal niche switching of activity may indeed require extreme abiotic factors such as those found in the desert, including extreme heat and dehydration.

Conclusion

This study highlights the rhythmic nature of rumination. In addition, rhythmicity of rumination persisted under food deprivation, indicating that to occur, rumination does not require prior food intake. Furthermore, rumination in goats is a function temporally associated with nocturnal rest, and opposite in phase with diurnal period of activity and wake.

The daily rhythm of rumination is most likely of endogenous origin. Further studies in controlled environmental conditions are needed to determine the nature of this endogeneicity.

Methods

Animals

Four healthy adult male Moroccan black goats (*Capra hircus*) weighing 28.5 ± 1.3kg and aged around 3.5 years originated from the South of Morocco were used in this study. The animals were housed in-group in outdoor enclosures (4 × 3 m), moving freely under natural environmental conditions.

Experimental design

The study was designed as two experiments, the first one in July (corresponding to the summer season in Morocco) and the second in December (corresponding to the winter season in Morocco). The two experiments with the same design were conducted under natural environmental conditions at Hassan II Agronomy and Veterinary Institute of Rabat- Morocco (Latitude: 34°00′47″ N; Longitude: 6°49′ 57″ W). In order to reduce the number of animals and refine their use, the research was carried out using goats that were intended for dissection by veterinary students and for anatomy teaching in the veterinary school of the Hassan II Institute of Agronomy and Veterinary Medicine. All experimental procedures were reviewed and approved by the Hassan II Institute of Agronomy and Veterinary Medicine Local Ethics Committee (***Committee of Ethics for Animal Science and Veterinary Public Health: CESASPV***) under approval number: *CESASPV_2024_A05*, and were conducted in accordance with its guidelines and regulations. The study was also conducted and reported in accordance with ARRIVE guidelines.

In each experiment (Season), goats were maintained under natural environmental conditions of the LD cycle and ambient temperature, and subjected to two stages of feeding:

Stage 1 (fed): period of 5 days with a single food distribution per day in the early morning at 09 am GMT and water ad libitum. Food consisted of a complete compound food for goats (Maraa®, Alf-Sahel, Morocco) and wheat hay. The compound food was formulated with cereals (maize, barley), fibre (wheat bran), oilseed cake (soya, sunflower) and a mineral and vitamins premixe (Table 1).

Stage 2 (fasted): period of 4 days with no access to food but with water ad libitum. Vital functions of the animals were closely monitored by a veterinarian to ensure that goats are coping well with the food deprivation. A set of criteria for an experimental endpoint were defined, including hypothermia (Less than 38°C), abnormal cardiac and respiratory frequency, low vigilance score, decreased level of activity, and unusual findings of clinical and neurologic exam. These criteria were established to immediately halt the experiment before the animals displayed any signs of physiological distress. It is noteworthy that the local goat variety utilized in this experiment is well-adapted to the arid desert environment and is renowned for its ability to endure prolonged periods of food and water deprivation for extended periods. Vitamins and amino acids were supplemented intravenously before food deprivation.

Animals were monitored by video-recording using two high-resolution cameras equipped with infrared emitters (Dahua® HACHFW1200RM cameras made by Dahua Technology Co., Ltd in Zhejiang, China). The recorded videos were stored into a Digital Video Recorder (Dahua DVR (DHIHCVR5108H-S2, Dahua Technology Co., Ltd, Zhejiang, China) with the capacity to hold 4 Terabytes for subsequent sequences analysis of rumination and locomotor activity.

MINIMUM (%)	**Crude protein 14%**
	Crude fat 2%
	Phosphorous 0,3%
	Calcium 0,6%
MAXIMUM (%)	Minerals 10%
	Crude fibre 12%
	Water 13%
VITAMINS (International Units/1KG)	Vitamin A 1000 IU/KG
	Vitamin D 200 IU/KG
	Vitamin E 20IU/KG

Table 1. A summary of the chemical composition of the used food.

Rumination recording

The recorded video sequences were subjected to a visual analysis on a computer screen and a scoring of rumination for each of the four goats was conducted. The rumination was scored for a 30-s interval over the course of the nine-day experiment. A value of "1" is assigned as a score when rumination is observed, while a value of "0" is attributed when there is no evidence of rumination. This absence includes instances where the goats are chewing while feeding or when there is no evidence of mastication. In instances where a 30-s epoch encompassed two distinct scenarios, only the predominant one (exceeding 15 s) was considered. The duration of rumination was expressed in minutes per hour and minutes per day.

Locomotor activity (LA) measurements

The LA was recorded using the validated video-locomotion scoring technique[34], which is based on a visual attribution of scores to activity. The scoring system assigns a value of 0 to an animal in a sitting position and not moving, a value of 1 to an animal in a sitting position and moving its limbs beneath the body, and so on. In the absence of any observable movements while the animal in standing position is scored as 2. When the goat is observed moving its limbs but without locomotion, the score is 3. The activity is scored as 4 when the animal is observed walking and exploring the environment. Finally, it is scored as 5 when there is an intense movement or when it is agitated. For further details, refer to the following sources[34,44]:

Rumenotomy

Following the end of Stage 2 (unfed state), the goats have been returned to the aforementioned service of veterinary school. Animals were subjected to a scarification procedure for the purpose of anatomical teaching for the third class of veterinary students. Animals were sacrificed by exsanguination after they had been anaesthetized with intravenous Xylazine at a dose of 0.1 mg/kg and Ketamine 3 mg/kg). Subsequent to the sacrifice, a rumenotomy was performed posthumously to examine the rumen contents after 4 days of food deprivation. The ruminal content was compared then with that of normally fed animals. These animals were fed once a day in the morning and lived under the same conditions as the experimental animals.

Data analysis

Rumination and LA data were presented as mean ± standard errors of the mean (SEM) and plotted using the SigmaPlot® software (Systat.Sigmaplotv12.0, Systat) and GraphPad Prism software (GraphPad Prism 8.0.2).

Rumination and LA rhythm data were presented as individual actograms using the Actogram Plotter® software (Refinetti R, Circadian Rhythm Laboratory, University of South Carolina, http://www.circadian.org/softwar.html). A 5 min interval time-set was used to plot actograms. For Locomotor activity, the used scores of 0 to 5[34] denoting of different levels of activity have been attributed for constructing the LA individual actograms. While for rumination, scores of 0 to 5 representing a rumination lasting respectively from 0 to 5 min in each 5 min interval time set have been assigned to facilitate the design of actograms.

Calculation for rumination parameters included:

- **Rumination duration**, which represents the duration of rumination at 1 h-time interval. The rumination durations were averaged at 1 h-time interval for each animal over the days of each stage and then averaging all animals for that interval of 1 h, then represented as mean ± sem. Rumination durations at 1h-time interval over the days of each stage gives a rhythmic expression of rumination. It is this variation of time occupied by rumination in each 1 h that define the rhythm of rumination. Rumination rhythm analysis included:
- Mesor: An estimate of the central tendency (the average value) of the distribution of values of rumination.
- Amplitude: The difference between the peak and the mesor value of a 24 h-wave of rumination rhythm.
- Robustness: This refers to the consistency and stability (percentage of variance) with which the rhythm of rumination is expressed over time.

The short total duration of each stage (only of 4 to 5 days) in the present study doesn't allow, a comprehensive analysis for the period an acrophase.

The mesor, amplitude and the robustness of the rumination and LA rhythms were calculated by non-linear least squares regression using Acro® and Cosinor® softwares (Refinetti R, Circadian Rhythm Laboratory, University of South Carolina, http://www.circadian.org/softwar.html).

- **Total rumination time** (mean ± sem) was calculated by pooling data of rumination duration of each animal for each 24-h and then averaged over the days of each stage in each experiment and thereafter averaged for the 4 animals.
- **Onset and offset** of rumination were calculated in reference to the time of day–night and the night–day transitions, respectively. The onset corresponds to a phase-angle difference in minutes between the time of the beginning of rumination and the time of the day-night transition (i.e. sunset time) while the offset of rumination is the phase-angle difference in minutes between the time of the end of rumination and the time of the night–day transition (i.e. sunrise time). The onsets and offsets of rumination in each day of each stage and season were averaged for all goats and presented as mean ± SE.
- **Rumination period** was calculated as the lag between the onset and offset of rumination. This parameter was averaged for each animal and thereafter for the four animals in each stage and represented as a mean ± sem. Because rumination is exhibited as repeated and interrupted nocturnal episodes of variable lengths, rumination period reflects the temporal window of rumination from its beginning at night (i.e., Onset) to its end in the early morning (i.e., Offset) and hence is different from the total rumination time which represents the cumulative duration devoted to rumination bouts in a 24h- interval.

For locomotor activity, the **total activity** mean was calculated for each animal as the addition of the predominant scores 3, 4 and 5 observed for each 5 min interval, pooled for all nights then all days of each experiment and then averaged for the 4 animals.

Comparisons of total rumination time, of rumination duration and of the onset-offset rumination interval between the different stages and seasons was performed using Two-Way ANOVA with Repeated Measures followed by a Holm-Sidak post-Hoc test; in the same way, the comparison of the mean rumination duration between stages 1 and 2 of each experiment, was realized using the same test. The Student test of conformity was used to verify the statistical differences between the offsets of rumination rhythm in stage 2 of both seasons and the moment of the dark–light transition. The Two-Way Repeated Measures ANOVA followed by a Holm-Sidak post-Hoc test was as well used for comparison of the total activity mean between the day and night in each stage and between days and nights of different stages. For all these analysis, the statistical significance was set at $P \leq 0.05$.

Environmental conditions

The first experiment took place at the beginning of July. Sunrise was around 06h20 am (GMT + 1) and sunset was around 08h42 pm (GMT + 1). The average daytime temperature during the experiment was 28°C, and the night temperature was 18.5°C. Average relative humidity during the experiment was 64%. The second experiment took place at the end of december. Sunrise was around 08h28 am (GMT + 1) and sunset was around 06h21 pm (GMT + 1). The average daytime temperature during the experiment was 18°C, and the night temperature was 9°C. Average relative humidity during the experiment was 83%. During the two experimental periods there were no rainy days.

Data availability

The authors state that materials described in the manuscript, including all relevant raw data, will be freely available to any researcher wishing to use them for non-commercial purposes. Data will be available on request from the corresponding author.

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Author contributions

M.M.E Performed the experiments; Analyzed and interpreted the data and drafted the manuscript. B.Y Performed the experiments. M.R.A., F.H Analyzed and interpreted the data and drafted the manuscript. P.M Contributed reagents, materials, analysis tools or data and drafted the manuscript. P.P. and E.C. Conceived and designed the experiments; Analyzed and interpreted the data and drafted the manuscript. E.K. Conceived and designed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data and drafted the manuscript.

Declarations

Competing interests

The authors declare no competing interests.

Additional information

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