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Seasonal and circadian variation of the sexual behavior of Morada Nova rams in tropical environment

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ABSTRACT - The objective of this study was to evaluate the seasonal and daily variation in sexual behavior of Morada Nova breeders raised in the Brazilian semi-arid region. The data were collected from four rams and 114 ewes of the Morada Nova breed – 55 females in the dry season and another 59 in the rainy season. Observations were performed in the dry and rainy seasons, divided into four times of the day: morning (05:01 h to 11.00 h), afternoon (11.01 h to 17.00 h), evening (17.01 h to 23:00 h), and dawn (23:01 h to 05.00 h). Sexual behavior was assessed for a period of 24 h/day, in a breeding season. One ram was used each three days. The behavioral patterns of the rams in view of females were observed during the recognition (sniffing the female urine, bouts of anogenital sniffing, and flehmen reaction), preparatory (exposure of tongue and penis, leg-kicking, and low-pitched bleats), and copulatory (number of mounts, number of ejaculations, and refractory period) phases. The rams were more active during daytime. The animals in the rainy season showed more efficient sexual behaviors by the lower frequency of mounts per ejaculation and greater number of ejaculations in a shorter time. In contrast, courting behaviors such as exposure of tongue, number of mounts, low-pitched bleats, and leg-kicking were more frequent in the dry season. In the semi-arid region with latitudes close to 7°N, Morada Nova rams have a higher intensity of precopulatory behaviors and lower frequency of ejaculations in the dry season.

Key Words: dry season, food resources, libido, photoperiod

Introduction

The male sexual behavior is assessed through the libido by the recognition, preparatory and copulatory phases, when males are in front of one or more females in estrous and manifest signs of interest as they seek the female by courting with their paw, anogenital sniffing, signs of excitation like exposure of penis and tongue, attempted mounts, and copulation (Henry and Neves, 1998; Fonsêca et al., 2013).

The reproductive characteristics of sheep can be influenced by the photoperiod, wherein a period of high sexual activity can be observed as the hours of daylight are reduced, while libido in rams and ewes decreases as the photoperiod is increased (Mies Filho, 1989; Silva et al., 2005). In temperate regions, changes in photoperiod are one of the most important factors in seasonality, since they direct the secretion of gonadotropins, leading to periods of reproductive activity and inactivity (Arrebola et al., 2010).

However, in low-latitude regions, commonly found in tropical and subtropical environments, the effect of photoperiod upon reproduction is minimal and under these conditions sheep can be considered as non-seasonal. Therefore, seasonality in reproductive activity is primarily influenced by the quality and availability of food (Vieira et al., 1988; Mies Filho, 1989; Cheminau, 1994; Martin et al., 2002). Particularly, in semi-arid conditions, the summer is dry and little food resource is available, which can compromise the nutrition and reproductive performance of animals during this period.

Several studies of sexual behavior in male sheep have been conducted in artificial environments that differ from the natural context where the breed was selected. Moreover, they were conducted in controlled breeding systems, usually with a small number of females and an unrepresentative period of time (Machado et al., 1994; Silva et al., 2005; Alves et al., 2006; Pacheco et al., 2008). Thus, continuous observations conducted in open areas like that of the breeding season, provide a more reliable assessment of the reproductive capacity and libido of males, Because these

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situations allow for the observation of a larger repertoire of behavioral patterns, especially those related to the male's ability to identify females in estrous and other activities during courtship and copulation.

Therefore, the objective of this study was to evaluate the daily patterns of sexual behavior of Morada Nova rams raised in the Brazilian semi-arid region, in the dry and rainy seasons.

Material and Methods

The study was conducted in the municipality of São João do Cariri, Paraíba State, Brazil. According to Koppen's classification, the climate of the region is a semiarid BSh type, with an average annual precipitation of 450 mm. The climate of the region provides a typical vegetation classified as hyper-xerophytic caatinga distributed at low depths and on very stony soil.

Data were collected from four Morada Nova rams aged 4-6 years, in the breeding season in two periods of the year. In the rainy season (period from June 4 to 20, 2011), 55 Morada Nova females were used. In the dry season (period from January 3 to 21, 2012), another 59 females were used. For the data evaluation, the times of day were grouped into four shifts of observation: morning (05.01 h to 11.00 h), afternoon (11.01 h to 17.00 h), evening (17.01 h to 23.00 h), and dawn (23.01 h to 05.00 h). The accumulated precipitation was 509.6 mm (May, June, and July of 2011) and 1.6 mm (October, November, December and January of 2011-2012) for the rainy and dry seasons, respectively. During the two breeding seasons, 288 h of observations were performed for each season of the year, totaling 576 h of observation.

During the breeding seasons, males were housed in individual stalls, except for the ram used as breeder of the day. The male of the turn was released on the pasture with the females at 05.00 h and returned at 17.00 h to be supplemented and spend the night at the management center (20 m wide × 80 m long). Each ram remained three days with a group of females in the paddock (Figure 1).

Males that were housed received a diet containing sorghum silage and Tifton-85 hay and a concentrate diet based on corn, corn meal, soybean meal, cotton cake, wheat meal, and a mineral mixture (common salt, mineral mixture (macro and micro minerals), urea and dicalcium phosphate). Feed was supplied daily as a total diet, formulated according to the NRC (1985). The rams that were with the females had access to a natural pasture area with an arboreal stratum composed mainly of *Croton sonderianus* Muell. Arg., *Caesalpinia pyramidalis* Tull., Malva sp., and *Aspidosperma parvifolium* Mart and a herbaceous stratum with *Cyperus uncinulatus* schander, *Paspalum scutatum*, *Diodia teres* Walt, *Aristida adscensionis* L., *Chamaecrista desvauxii* (Collad) Killip, and *Evolvulus filipes* Mart. Upon returning in the afternoon, rams received the same concentrate supplementation as the female sheep.

A set of thermometers was placed in the interior of the management center at a height of 1.0 m from the floor to collect the environmental data, including ambient temperature and relative humidity. The climatic variables were measured every 2 h, 24 h/day (Figure 2). Behavioral assessments were performed from 05.00 h to 05.00 h the next day, totaling 24 h of assessment/day. These were quantified by the frequency and duration the behavioral states and events. The measurements were made by the direct method, continuous focal animal sampling (Martin and Bateson, 1986). Rams were marked with non-toxic paint on the left and right (A, B, C, and D) sides for easier viewing of the observers (Figure 1). The behavioral variables were divided into three phases, following a chronological pattern of sexual behavior of males.

Eight people conducted the observations and were divided into groups of two people who performed six-hour shifts. All observers were subjected to a confidence test for the records of behavioral activities. This test was performed with the use of videos in which each observer recorded the behaviors of interest. The records were then compared, with at least 95% correlations between the observers.

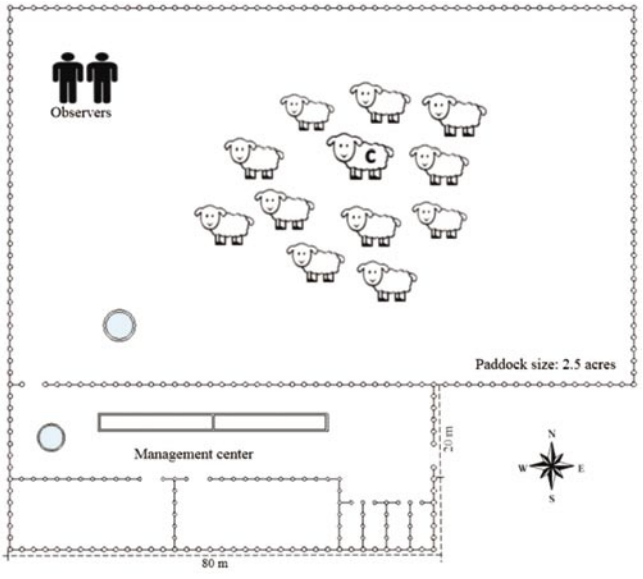


Figure 1 - Diagram of paddock and management center where the animals remained during observations.

The behaviors were divided according to the recognition, preparatory, and copulatory phases. For the recognition phase, the act of sniffing the urine of female (number of times that ram smelled the urine of a female on the ground), bouts of anogenital sniffing (number of times that ram inspected the urogenital region of a female), and flehmen reaction (number of times that the ram perfomed the characteristic movement with the upper lip) were evaluated. In the preparatory phase, the exposure of tongue (number of times that the ram exposed the tongue outside its mouth), exposure of penis (number of times that the male exposed its penis out of the prepuce cavity), leg-kicking (foreleg repeatedly directed toward the stimulus ewe in rapid succession), and low-pitched bleats (number of times that the ram vocalized with its mouth shut) were quantified. Finally, in the copulatory phase, the number of mounts (mounting without ejaculation or intromission), number of ejaculations (mounting with intromission and ejaculation), and the refractory period (time elapsed from a mating to another) were observed.

Exploratory analyses were conducted to characterize the data distribution. Normality distribution was checked by skewness and kurtosis coefficients. A generalized linear model (GLIMMIX procedure) was used to examine the main effects of the season of the year (rainy and dry season) and time of the day (morning, afternoon, evening, and dawn) on the ram sexual behaviors. Negative binomial (frequency data) and lognormal (data measured by the duration) distributions were used. Means were compared by the Tukey test. The statistical package used for all statistical analyses was SAS (Statistical Analysis System, version 9.3). The minimum level of confidence was $P<0.05$.

Results

There was interaction between factors (time of day × breeding season); thus, the results will be discussed together. During the recognition phase, there was a greater frequency in the act of the male sniffing the anogenital region and urine of female during the dry season (n = 3.64 vs. n = 6.28; n = 0.26 vs. 0.89; $P<0.05$; Figure 3). Regarding the daily variation for these activities, in the early dawn the animals expressed it in a lower frequency ($P<0.05$). There was no daily variation in the flehmen reaction in the rainy

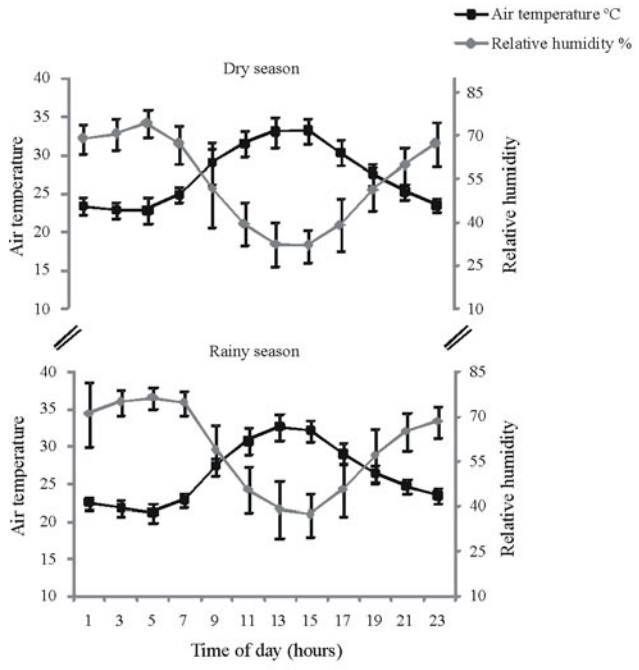
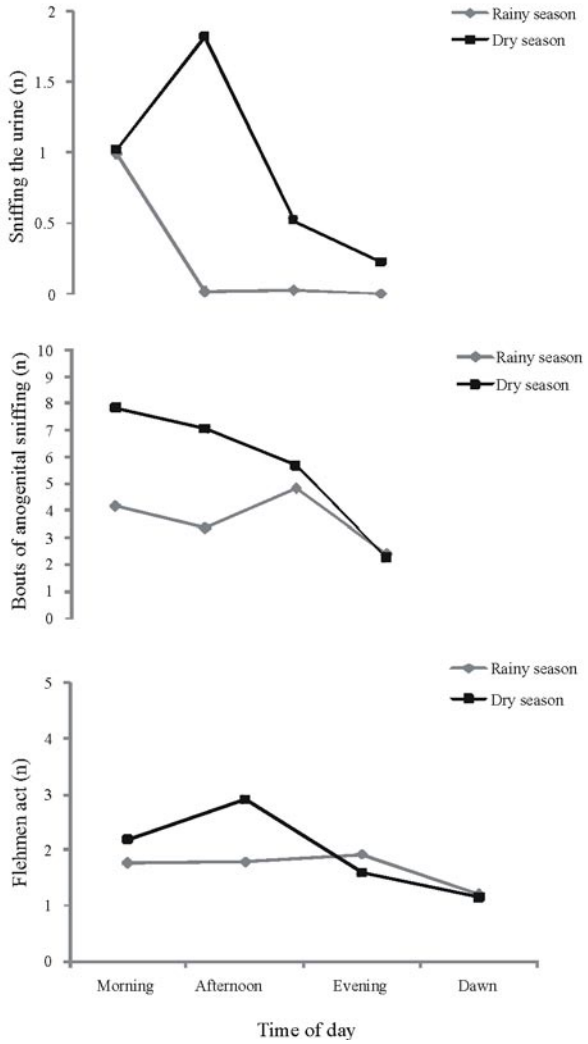


Figure 2 - Daily average air temperature and relative humidity in two-hour intervals during the experimental period.



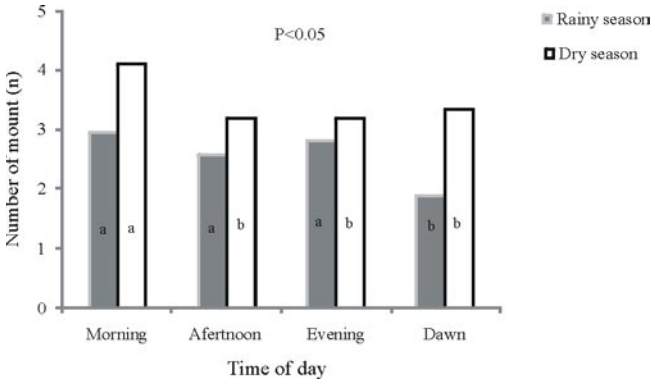
Variables are significantly affected by the interaction between season and time of day ($P<0.05$).

Figure 3 - Daily activities of the recognition phase of rams in the rainy and dry seasons.

season. However, this act was more (P<0.05) expressed in the morning (n = 2.19) and afternoon (n = 2.91) in the dry season of the year. Moreover, during the rainy season, males performed the flehmen reaction less often (n = 1.67 vs. n = 1.96; P<0.05; Figure 3).

For the activities of the preparatory phase, during the rainy season, vocalizations remained relatively constant, with a higher intensity in the afternoon (P<0.05; Table 1). This same behavior pattern was observed in the dry season. Comparing the frequency of vocalization between seasons, it was found also that during the dry season the expression for this activity was higher (n = 9.81 vs. n = 7.98; P<0.05; Table 1). The exposure of tongue and penis had similar patterns; in the rainy and dry season they were more frequent during the morning compared with the other times of the day (P<0.05; Table 1). Leg-kicking (P<0.05) was performed more often in the morning in the rainy season and in the afternoon in the dry season. During the dry season, leg-kicking was performed more often as compared with the rainy season (n = 6.09 vs n = 2.94; P<0.05; Table 1).

In the copulatory phase, in the dry and rainy seasons, the rams performed a greater number of mounts per ejaculation in the morning shift (P<0.05; Figure 4). However, in the dry season there was a greater number of mounts per ejaculation as compared with the rainy season (n = 4.46 vs. n = 2.56; P<0.05). The number of ejaculations was higher in the rainy season as compared with the dry season (n = 7.90 vs. n = 6.91; P<0.05; Figure 5). Regarding the distribution of the shifts, a greater frequency of ejaculations was observed in the diurnal period in both seasons (P<0.05). Consequentely, the refractory period was lower in the early hours of the day (morning) and the longest interval was observed during the night (P<0.05; Figure 5). Finally, there was no difference for the refractory period in the dry and rainy seasons, except for the dawn, when the time between two matings was longer in dry season (32.68 min vs. 28.13 min; P<0.05; Figure 5).



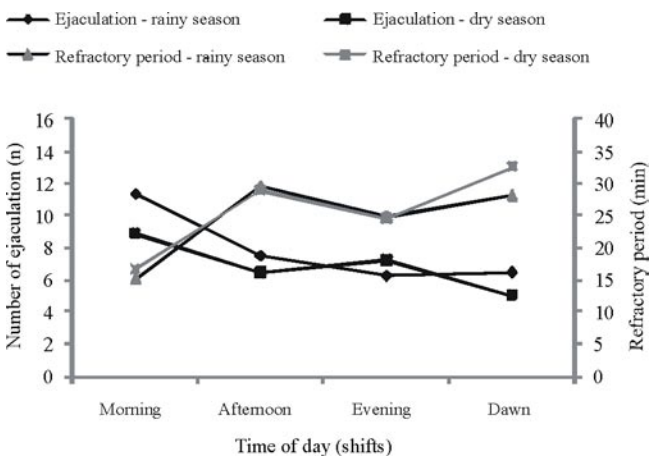
Lowercase letters represent differences between the times of day (P<0.05).

Figure 4 - Daily variation in the number of mountings in the rainy and dry seasons.

Table 1 - Behaviors of the rams during the preparatory phase

Variable/Time of day	Season		P-value		
	Rainy	Dry	Season	Time of day	Season × Hours
Low pitched bleats (n[1])			<0.01	<0.01	0.2340
Morning	7.80b	10.88b			
Afternoon	10.75a	13.51a			
Evening	7.13b	8.65c			
Dawn	6.24b	6.20d			
Leg-kicking (n)			<0.05	<0.01	<0.05
Morning	3.43a	6.19b			
Afternoon	2.95b	8.95a			
Evening	2.13b	5.32c			
Dawn	3.24a	3.88d			
Exposure of tongue (n)			<0.05	<0.05	0.0980
Morning	0.91a	3.89a			
Afternoon	0.37b	1.48b			
Evening	0.13b	0.23c			
Dawn	0.06c	0.00d			
Exposure of penis (n)			<0.05	<0.05	0.2667
Morning	2.15a	4.36a			
Afternoon	1.77b	3.11b			
Evening	1.47b	1.37c			
Dawn	1.52b	1.00c			

[1] Variable measured in frequency.
P-values are presented for the effect of season, time of day, and interaction between season and time of day.
Lowercase letters represent differences between the times of day.



Variables are significantly affected by the interaction between season and time of day (P<0.05).

Figure 5 - Number of ejaculations and refractory period during the copulatory period in the rainy and dry seasons.

Discussion

Libido can be quantified in male farm animals by measuring their motivation to seek out and remain in close proximity to potential mates and the frequency with which they engage in precopulatory and copulatory behaviors with mating partners. The ability of the male to detect females in estrus is an important characteristic to evaluate the reproductive capacity of rams (Galina et al., 2007; Katz, 2007). During the recognition phase, the stimuli are olfactory cues present in the urine of female, pheromones that attract males to investigate and test the responsiveness of the ewe. Females in estrus stimulate the males; they tend to shake and lift the tail and urinate more often, especially if the male is smelling it (Gill, 2008).

According to our results, it was observed that during breeding in the rainy season rams expressed investigative behaviors such as anogenital and urine sniffing of the female less often, except for the flehmen reaction, manifested in similar frequencies among times of day. According to Quirino et al. (2008), females that were not in estrus had a smaller amount of the pheromone releases, which causes greater intensity in identifying behaviors such as stalking, licking and sniffing. Such attitudes may promote less efficiency in mating, caused by physical wear of the male. Price et al. (1992), however, observed an increase in the frequency of the behavior of anogenital investigation when males were exposed to more receptive females. In the two breeding seasons, these investigative activities declined over 24 h and the males were more active during the morning as compared with the other times of the day. Despite being diurnal species, during the night (evening and dawn), breeders were active and expressed olfactory investigation behavior on the females. In contrast, the flehmen reaction was expressed at similar frequencies throughout the day.

The ethological basis to explain the flehmen behavior is related to stimuli of pheromones in the urine of the female, wherein by the act of smelling the perianal region or urine, the flehmen response will be triggered depending on the levels of these chemicals (Katz, 2007). Besides confirming the ewe's responsiveness, this behavior seems to enhance the male's libido to perform other activities in the courtship and copulation phases (Fonsêca et al., 2013).

Just as smell, hearing and all other senses of the animals during the breeding season are more sensitive (Gordon, 1999). According to Rivas-Muñoz et al. (2007), more sexually active males increase the frequency of vocalizations during courtship, consisting of an important hearing component. In the present study, more frequent bleats were observed during the dry season and in the morning and afternoon. Similarly, behaviors like leg-kicking and exposure of the penis and tongue were mostly in the dry season; also, throughout the day, there was a higher expression during daylight. This result can be attributed to the nocturnal rest of the animals, caused by an exhaustion state (Hafez and Hafez, 2004).

In both seasons, the number of mounts was higher at times in the morning and decreased throughout the day. Furthermore, in this study, the mounting behavior did increase significantly during the period of the year when breeding activity is lowest (dry season). According to Price et al. (1992), there is a negative correlation between the number of mounts and the number of ejaculations. The attempt to mount is a part of the sequence of events that occur during the preparatory and copulatory phases (Pacheco et al., 2008). Successive mounting attempts can cause excessive wear on the male. The reduction in the number of matings during the day is possibly due to the natural wear of the animal, since animals during the first two shifts (morning and afternoon) were loose in a paddock of 2.5 acres and in addition to reproductive efforts, activities of grazing, especially in the dry season, were more stressful due to the reduced availability of forage.

Ejaculation represents the success of all activities undertaken during the recognition and preparatory phases in the courting of female and seems to have an inverse relationship with the frequency of mounts, as observed in the present study. Typically, ejaculations are followed by periods of sexual inactivity (refractory period), and the duration of these periods increases with successive ejaculations.

The ejaculation is the conclusion of the sexual act and seems to be the best indicator to evaluate the libido and motivational state of a ram as compared with other measures of sexual motivation such as precopulatory behaviors, because in this study they did not have a positive relationship with the rate of ejaculation. The results do not support the hypothesis that the frequency of precopulatory behaviors in rams reflects their underlying sexual motivation. As noted, during the season with the lowest rates of ejaculation (dry season), there was greater expression of behaviors during the recognition and preparatory phases. Moreover, the daily average of females in estrus in the dry (4.35±1.2) and rainy (3.70±0.9) seasons was similar. Thus, the stimuli coming from the behavior of females were similar and the differences in male libido can be attributed to other external factors.

The results of this study support the hypothesis described by Galina et al. (2007). These authors observed, in the breeding season, that males spend a considerable time to identify females in estrus and that these features limit the time they spend in breeding activities such as copulation. Other courtship behaviors such as vocalizations, tongue exposure and leg-kicking also occupy a significant part time of males.

With respect to seasonal variations in male sheep libido, there are few studies in the literature in low-latitude regions, such as the locations of semi-arid climate. Research in different locations shows contradictory results regarding seasonal variations in testosterone levels and libido in goat and sheep breeding (Pepelko et al., 1965; Delgadilho et al., 1991; Sousa et al., 2002). Some studies conducted in mid-latitudes (>30°N<45°N) observed no significant changes, while others found higher testosterone levels at the time of the year with shorter days.

When studies were conducted in regions with latitudes below 20°S, the patterns of libido and hormonal levels were constant throughout the year in males and females, when supplemented at the time of low rainfall and food in the pasture (Silva et al., 2005; Souza et al., 2007; Dantas et al., 2011). In the present study, conducted in a region of semi-arid climate with low latitudes (7°S), changes in photoperiod are minimal throughout the year and do not seem to sensitize the biological rhythms of animals; however, it is a scientific fact that has to be better studied.

Other factors associated with season are capable of influencing the reproductive state of male sheep. Rosa and Bryant (2003) suggest that variations in temperature, relative humidity and rainfall distribution in tropical and subtropical regions affect the reproductive characteristics of sheep. In the Brazilian semi-arid region, the summer coincides with the dry season and the production of biomass in the caatinga during this period can be reduced to very low values, with losses that reach 60% of the production area (Mesquita et al., 1988).

Thus, it can be inferred that the availability and mainly the quality of forage, especially in semi-intensive farming systems, seem to be factors that most influence the reproductive activity in rams. In tropical regions of semi-arid climates, nutritional strategies during the dry periods of the year can improve the reproductive performance of male sheep in the breeding season.

Conclusions

The intensity of sexual behavior patterns of Morada Nova rams varies throughout the day and is more expressed in daylight.

In semi-arid regions with latitude close to 7°N, Morada Nova rams have a higher intensity of pre-copulatory behaviors and fewer ejaculations in the dry season.

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