



## Effect of concentrate supplementation and time scales of evaluation on behavioral and physiological responses of pregnant ewes on grazing system

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**ABSTRACT.** This study was realized to evaluate the effect of concentrate supplementation and time scales of evaluation on behavioral and physiological responses of pregnant ewes on grazing system. The experimental design was a randomized 2 x 2 x 3 factorial arrangement (two breeds: Morada and Santa Inês; two concentrate supplementation levels: 0.5 and 1.5% of body weight; and three time scales of evaluation: 10, 20 and 30 minutes) with six replicates per treatment. The physiological parameters were influenced by period of the day and breeds, with higher values in the afternoon to Santa Inês ewes. Also, influence of concentrate supplementation was found between the breeds with greater values in Santa Inês ewes. But, it did not alter the physiological parameters among animals of the same genetic group. The grazing activity was influenced ( $p < 0.05$ ) by the scales of observation intervals. Interval of 10 minutes was the most effective methodology to assess accurately the behavior of these animals due to higher number of the registries, offering higher data reliability. Morada Nova breed exhibited less time grazing ( $p < 0.05$ ) on the 10-minute scale compared to 20 and 30 minutes, which were similar ( $p > 0.05$ ) for such a feature. Thus, the pregnant ewes exhibited high adaptive capability with observations of 10 minutes for better description of grazing activity in Morada Nova.

**Keywords:** animal nutrition, behavior, native ewes, semi-arid conditions, thermoregulation.

### Efeito da suplementação concentrada e da escala de tempo de avaliação nas respostas fisiológicas e comportamentais de ovelhas prenhes em sistema de pastejo

**RESUMO.** Esse estudo foi realizado para avaliar o efeito da suplementação concentrada e das escalas de tempo de avaliação nas respostas fisiológicas e comportamentais de ovelhas prenhes em sistema de pastejo. Foi usando um arranjo fatorial 2 x 2 x 3 (duas raças: Morada e Santa Inês; dois níveis de suplementação concentrada: 0,5 e 1,5% do peso vivo; e três escalas de tempo de time avaliação: 10, 20 e 30 minutos) com seis repetições por tratamento. Os parâmetros fisiológicos foram influenciados tanto pelo período do dia como pela raça, com médias mais elevadas no período da tarde em ovelhas Santa Inês. Além disso, foi encontrada influência da suplementação concentrada entre as raças com superioridade em ovelhas da raça Santa Inês. A atividade de pastejo foi influenciada ( $p < 0,05$ ) pelas escalas de tempo de avaliação. Intervalo de 10 minutos foi a metodologia mais eficaz para avaliar com maior precisão o comportamento desses animais. Ovelhas Morada Nova apresentaram menos tempo de pastejo ( $p < 0,05$ ) na escala de 10 minutos em comparação com 20 e 30 minutos, que foram semelhantes ( $p > 0,05$ ) para essa atividade. Assim, as ovelhas prenhes apresentaram alta capacidade adaptativa com observações de 10 minutos para uma melhor descrição da atividade de pastejo em Morada Nova.

**Palavras-chave:** nutrição animal, comportamento, ovelhas nativas, condições semiáridas, termorregulação.

#### Introduction

Climate changes are intimately related to reproductive and productive performances in animals, mainly in the Semi-arid environment, which is characterized by high temperatures due to high incidence of solar radiation, low humidity and

high evaporation rates. The main feature of this region is the scarcity and high irregularity in the distribution of rainfall. Combination these factors can cause intense discomfort to animals (Costa, Takeda, & Lima, 2010; Silva Souza Júnior, Santos, Marques, & Torreão, 2013b, Rocha et al., 2014). In additional, climate changes can interfere in the

pasture availability, grazing time causing inefficiency of the production system.

Ewes exposed to heat stress promote physiological and metabolic adjustments to dissipate to heat load or to reduce thermogenesis (Marai, El-Darawany, Fadiel, & Abdel-Hafez, 2007). In response to the high ambient temperatures, ewes tend to increase physiological traits and modified your behavioral responses, with decrease dry matter intake (Marai et al., 2007; Silva et al., 2015b). Physiological and behavioral changes promote alterations not only in the level of blood metabolites (Silva, Marques, Torreão, Araújo, & Bezerra, 2015a; Silva et al., 2015c) but also in metabolic hormones (Marai, El-Darawany, Fadiel, & Abdel-Hafez, 2008; Sejean, Maurya, & Naqvi, 2010; Macías-Cruz et al., 2013). Given the climate change and gradual global warming scenarios, researches that evaluate the adaptive capability of ewes under unfavorable conditions are required, mainly in the arid and semi-arid environments. Despite being considered rustics animals, the use of native breeds in the system production is very important to the food supply in semi-arid regions, generating occupations, for subsistence of populations in agro-ecological zones, income and improved quality of life (Renaudeau et al., 2012; Shiotsuki, Oliveira, Lôbo, & Facó, 2014). It is also necessary to develop economic importance through little arable land by characterizing extensive production system. However, it results maximum production with minimum cost due to animals mostly depends on natural pastures and use of non-specialized genotypes (Shiotsuki et al., 2014).

Behavioural understanding is critical for the performance enhancement of sheep on grazing, mainly due to intense climate changes that can affect animal physiology. However, it is essential to enhance the utilization of pastures and defining strategies for nutritional management making the system highly productive.

Among these strategies, concentrate supplement aims to meet the nutritional deficit presented by these animals. Usually, available forage in the pasture does not contain all the essential nutrients in appropriate proportions to fully meet the requirements of grazing animals. Hence, supplementation with concentrates would support maintenance and fetal growth. The use of indigenous breeds under semi-arid regions, due to with high adaptability to different environments, including areas of high radiant heat load and development of several adaptations, since pigmentation of the mucosa crepuscular and grazing habits (Neiva, Teixeira, Turco, Oliveira, & Moura, 2004). Thus, these breeds may reach good

productive and reproductive rates, especially when subjected to energy and protein supplementation.

It is noteworthy to understand the animal behavior under supplementation in grazing system and to determine the optimal interval of observation of feeding behavior. The current study was conducted to evaluate the effect of concentrate supplementation and time scales of evaluation on the behavioral and physiological responses of native ewes during pregnancy under grazing system.

## Material and methods

### Animals, feeding and management

The experiment was conducted at the Professor Cinobelina Elvas Research Unit in Small Ruminants Campus (CPCE), Federal University of Piau , Teresina State, Brazil (FUPI), Bom Jesus - PI (9 04'28" south latitude and 44 21'31" west longitude) at an altitude of 277 m, with a semiarid climate (Bsh), with annual average rainfall of 900 mm, maximum and minimum temperatures at 36 and 18 C, and relative humidity at 55 and 75% respectively. The rainy season extends from November to May. The experiment was conducted with the approval of the ethical committee for animal experimentation of the Federal University of Piau , Teresina State, Brazil (ECAE/UFPI) under the number 91/2010 during the period of March to April 2012, with a total duration of 43 days and was preceded by a 15-day adaptation period to the experimental diet (0.5 and 1.5% based on body weight) and observers were performed.

In the present study twelve pregnant pluriparous ewes (third stage of pregnancy), six Morada Nova and Six Santa In s with an average initial body weight of  $52.6 \pm 6.54$  and  $31.3 \pm 3.7$  kg, respectively were used for this study. During the experimental period, the ewes remained in pasture paddock of *Andropogon* grasses (*Andropogon gayanus*), under continuous grazing, in an area with 1.8 hectares (ha) and approximately 0.44 animal unit (AU)ha<sup>-1</sup>, from 7:00 a.m to 17:00 p.m. They were collected in the late afternoon and allocated in individual pens measuring 1.75 m<sup>2</sup>. The sheep received concentrate supplementation (Table 1) that contained ground corn (70%), soybean meal (25%) and mineral mixture (5%).

Treatments were formed based on percentage of concentrate supplementation according to recommendations (NRC, 2007) for pregnant animals. A total requirement of protein and metabolizable energy was found to be 146 g day<sup>-1</sup> and 3.86 Mcal day<sup>-1</sup> respectively.

**Table 1.** Chemical composition of experimental ingredients, concentrate supplement and *A. gayanus*.

Ingredients	DM %	CP %	EE %	TDN %	NDF %	ADF %	Ca %	P %
Corn meal	87.19	9.98	5.19	67.50	24.55	5.87	0.05	0.49
Soybean meal	88.48	48.76	1.75	80.73	15.37	9.64	0.33	0.57
<sup>1</sup> Mineral mixture	97.91	-	-	-	-	-	18.00	13.00
Supplement	88.04	19.17	4.06	67.43	21.02	6.51	0.90	1.13
Andropogon	27.45	7.50	2.02	53.56	74.70	41.97	0.33	0.11

DM = Dry matter; CP = Crude protein; EE = Ether extract; TDN = Total digestible nutrients; NDF = Neutral detergent fiber; ADF = Acid detergent fiber; Ca = Calcium; P = Phosphorus. <sup>1</sup>Mineral mixture: 1.600 mg zinc, 600 mg copper, 1.500 mg manganese, 1.100 mg iron, 10 mg cobalt, 27 mg iodine and 22 mg selenium.

The animals were subjected to two nutritional plans: one plan in which the nutritional requirement of net energy was underestimated (ewes supplemented with 0.5% of concentrate supplementation based on body weight) and another plan in which the nutritional requirement of the net energy was overestimated (ewes supplemented with 1.5% of concentrate supplementation based on body weight) on natural matter basis.

#### Measurements of physiological parameters and environmental variables

Physiological parameters, respiratory rate (RR), heart rate (HR) and rectal temperature (RT) were measured in this sequence with the animals at rest in the morning (6:00-7:00 a.m.) and afternoon (13:00-14:00 p.m.) every seven days, totaling 4 samples during the same period of ingestive behavior evaluation. For animals on pasture, they were ushered into a smaller paddock to facilitate the collection of data, thus performing the least-jerky movements that could leave them stressed while recording these parameters.

Respiratory rate was measured in breaths per minute through direct observation of the movements of the left flank; HR was measured in beats per minute using a stethoscope placed between the third and fourth left intercostal space; the RT was registered using a clinical thermometer inserted directly into the rectum of the animals until the firing of the sounder.

Environmental variables, temperature (T) and relative humidity (RH) were measured with the aid of thermo-hygrometer and temperature of the globe-thermometer (thermometer Iconterm<sup>®</sup> 0-100°C inserted into a black globe with 150 mm diameter), at 55 cm deep into the soil close to the animals. Black globe temperature and humidity index (BGTHI) was used for the equation proposed by Buffington et al. (1981), in which  $BGTHI = 0.72 (WBT + BGT) + 40.6$  (where: WBT- Wet bulb temperature in °C; BGT- black globe temperature in °C).

#### Forage availability and chemical composition

To estimate the total forage availability, three collections of samples of the pasture were made in the respective day trial of ingestive behavior. For this

estimate, the forage was randomly divided into five areas within the pickets using a metallic square of 1.0 m<sup>2</sup> (1.0 × 1.0 m).

Samples from each square were weighed, divided into sub-samples, which were then used to form composite samples in a duplicate per period, packed in plastic bags, identified and frozen to assess the total availability of dry matter (DM). Out of each pair of forage samples, one was used for the separation of components into green leaf, stem green, dried leaf and stem dry, which were weighed, stored in plastic bags, frozen and subsequently analyzed to determine the chemical characteristics.

The mass of the leaf and stem material was identified through separation and quantification of the dry weight of each component. The samples were weighed fresh, placed in a forced-air oven and kept for 72h at 55°C for the quantification of the dry weight.

To evaluate the chemical composition of the forage consumed by the animals, samples were collected via the manual simulation of grazing. The processing of samples and chemical analyses were performed at the Laboratory of Animal Nutrition of the CPCE - FUPI. Analyses of the amount of dry matter (DM), crude protein (CP), ether extract (EE), neutral detergent fiber (NDF), acid detergent fiber (ADF), calcium (Ca) and phosphorus (P) in the concentrate and forage were performed. The determination of the potentially digestible dry matter (DMPD) was subsequently performed according to Paulino, Moares, and Zervoudakis (2005) as follows:  $DMPD (\% DM) = \{0.98 \times (100 - NDF) + (NDF - iNDF)\}$ , where NDF = neutral detergent fiber (% DM) and iNDF = indigestible neutral detergent fiber (% DM), where iNDF was determined according to the methodology described by Casali et al. (2009).

#### Measurements of ingestive behavior

The focal sampling method was used to record the amount of time spent on the adopted behavior in 10-minute intervals for 10 continuous hours with three replications (days), at weekly intervals, always starting 7:00 a.m and ending at 17:00 p.m, totaling

30 hours of evaluation and, consequently, 180 records per animal, according to the methodology described by Mezzalira et al. (2011). The ingestive behaviors have been considered mutually exclusive for each record; each animal was classified into only one activity (Carvalho et al., 2004). From these data, the average time spent Grazing (GRAZ), ruminating on foot (RUMF), ruminating lying down (RUML), standing idle (STAN), lying idle (LYIN), walking (WALK), urinating (URI), defecating (DEF) was analyzed. The data for each animal's behavioral activities were recorded by four trained observers using spreadsheets, who were positioned to interfere as little as possible with animal behavior.

Subsequently, we calculated these same variables simulating observations every 20 and 30 minutes from the original observations, every 10 minutes, considering the first observation of common day for all intervals, totaling 90 and 60 records per animal, respectively (Mezzalira et al., 2011).

### Statistical procedures

Ingestive behavior was analyzed by a randomized 2 x 2 x 3 factorial arrangement (two breeds, two supplementation levels and three times, 10, 20 and 30 minutes) with 6 replications per treatment, totaling 24 experimental units. The experimental data were analyzed using the computer program Statistical Analysis System (SAS, 2003). The data were evaluated by means of PROC MIXED procedure, and the means were compared with tukey's test at 5% probability, according to the statistical model:

$$Y_{ijk} = \mu + B_i + S_j + (BS)_{(ij)} + T_k + (BT)_{(ik)} + (ST)_{(jk)} + (BST)_{(ijk)} + e_{(ijk)},$$

where:

$Y_{ijk}$  = value observed for characteristic analyzed;

$\mu$  = overall average;

$B_i$  = breed effect  $I = 1, 2$ ;

$S_j$  = effect of concentrate supplementation level  $j = 1, 2$ ;

$T_k$  = temp effect  $k = 1, 2, 3$ ;

$BS_{(ij)}$  = effect of the interaction between breed  $i$  and concentrate supplementation level  $j$ ;

$BT_{(ik)}$  = effect of the interaction between breed  $i$  and temp  $k$ ;

$ST_{(jk)}$  = effect of the interaction between concentrate supplementation level  $j$  and temp  $k$ ;

$BST_{(ijk)}$  = effect of the interaction among breed  $i$ , concentrate supplementation level  $j$  and collection temp  $k$ ;

$e_{(ijk)}$  = aleatory error associated with the observation  $Y_{ijk}$ .

Physiological parameters and environmental variables were analyzed following a completely randomized split-split-plot design (two breeds, MN and SI, two levels of supplementation, 0.5 and 1.5% of body weight and two turns, morning and afternoon), with four measurements, repeated over time. The data were fitted to the statistical model:

$$Y_{ijk} = \mu + B_i + S_j + (BS)_{(ij)} + T_k + (BT)_{(ik)} + (ST)_{(jk)} + (BST)_{(ijk)} + e_{(ijk)},$$

where:

$Y_{ijk}$  = value observed for characteristic analyzed;

$\mu$  = overall average;

$B_i$  = breed effect  $I = 1, 2$ ;

$S_j$  = effect of concentrate supplementation level  $j = 1, 2$ ;

$T_k$  = turn effect  $k = 1, 2$ ;

$BS_{(ij)}$  = effect of the interaction between breed  $i$  and concentrate supplementation level  $j$ ;

$BT_{(ik)}$  = effect of the interaction between breed  $i$  and turn  $k$ ;

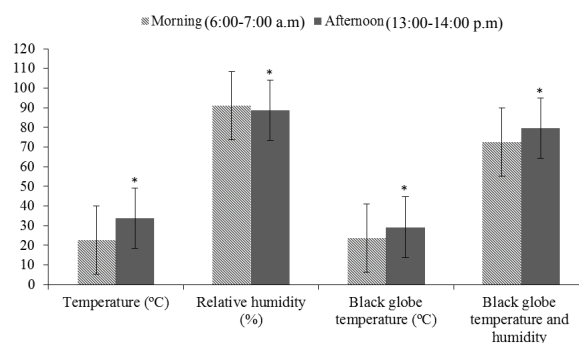
$ST_{(jk)}$  = effect of the interaction between concentrate supplementation level  $j$  and turn  $k$ ;

$BST_{(ijk)}$  = effect of the interaction among breed  $i$ , concentrate supplementation level  $j$  and turn  $k$ ;

$e_{(ijk)}$  = aleatory error associated with the observation  $Y_{ijk}$ .

### Results and Discussion

Average values of bioclimatic variables such as, air temperature (T), relative humidity (RH) and Black globe temperature and humidity (BGTH) assessed during the experimental period are shown in Figure 1.



**Figure 1.** Averages of environmental variables in different turns (morning and afternoon) during the experimental period. Asterisks indicates significant differences at  $p < 0.05$  when compared between turns.

There was a significant effect ( $p < 0.05$ ) between turns. It was found that T in the afternoon was beyond ( $3.6^{\circ}\text{C}$ ) the zone of thermal comfort for sheep (Marai et al., 2007; Pereira et al., 2014; Rocha

et al., 2014), mainly due to the large daily variation of environmental parameters in the Gurguéia Valley region. This climate change can result in a situation of thermal stress with negative effects in daily behavior of ewes impairing productive and reproductive efficiency of grazing animals. Silva et al. (2013a) reported that the climatic conditions of the Gurguéia Valley region produced significant changes in the physiological parameters of Morada Nova and Santa Inês ewes under concentrate supplementation with possible effects on behavior patterns of these sheep due to the heat increment. Relative humidity showed an opposite behavior compared to the T, with the morning shift above the critical limit, 50 to 70%, (Silva et al., 2013b). It was verified turn effect ( $p < 0.05$ ) on black globe temperature and humidity index with values above acceptable limits, characterizing an environment under extreme thermal stress (Sejian, Maurya, kumar, & Naqvi, 2013). According to Souza, Tinôco, Baêta, Ferreira, and Silva (2002), the values of BGTHI up to 74 define a comfortable situation, 74-78 as alert, 79-84 as danger and above 84 as emergency zone.

Physiological parameters showed ( $p < 0.05$ ) turn and breed effect, with higher averages in the afternoon and greater use of the mechanisms of heat loss by Santa Inês ewes (Table 2). Patterns of respiratory rate of the sheep were influenced ( $p < 0.05$ ) by day-shift and breed in the study. In the afternoon, higher values were shown for the species in the study. This result corroborate with Indu, Sejian, and Naqvi (2014) who reported influence of environment (simulated heat stress model *vs* natural environmental conditions) on physiological adaptability. Santa Inês ewes presented high use of respiratory thermolysis. Under conditions of high temperature and high relative humidity can occur little loss of body heat of latent and sensible form resulting in an increase in body temperature and thermal discomfort to the animals (Ortêncio, Barbosa, Shigueiro, Onorato, & Macedo, 2001), fact that was found in the present study (Figure 1 and Table 2), characterizing a situation of low-stress. Silanikove (2000) reported that the RR is an important parameter to quantify the severity of heat stress, being considered low stress when the frequency ( $\text{mov. min}^{-1}$ ) is between

40-60, mid-high between 60-80, high stress between 80-120 and severe for above 200 (Silanikove, 2000).

Similar values were found between the breeds but, the values differed between turns. During in the afternoon, was verified that climatic factors were more aggressive to the ewes due to high incidence of solar radiation, temperature outside the thermo-neutral zone and humidity outside the critical limit, (i.e) BGTH unfavorable for animal production with alterations on the physiological responses (Sejian et al., 2013).

HR is influenced by turn of the day and with a higher beat frequency in the afternoon, probably due to rise in thermal environmental parameters and with an increase in animal discomfort (Rocha et al., 2014). The increase in RR was result of a progressive accumulation of heat due to adverse environmental factors (Table 2). Once RT did not differ between the breeds ( $p < 0.05$ ), the mechanism of respiratory thermolysis was used to remove excess heat received by adverse environment, maintaining the TR within physiological patterns. Similar findings were reported by Sejian, Maurya, and Naqvi, (2010) and Indu et al. (2014). Among physiological parameters studied, the rectal temperature is one of the best indicators of thermal stress in an animal. An increase of  $1^{\circ}\text{C}$  in rectal temperature is enough to reduce performance in most species of domestic animals (McDowell, Hooven, & Camoens, 1976).

Physiological parameters were influenced ( $p < 0.05$ ) between the breeds (Table 3). However, the supplementation levels (0.5 and 1.5% of body weight) did not alter the physiological parameters between ewes of the same genetic group. Respiratory rate in Santa Inês ewes was above the physiological limit for sheep (Marai et al., 2007). Respiration is the most important variable to maintain homeostasis in sheep (Starling, Silva, Cerón-Muño, Barbosa, & Costa, 2002), being the physiological variable most affected in animals kept under heat stress conditions (Rocha et al., 2014). Environmental factors combined with heat increment of concentrate supplementation are factors that affect the physiological parameters and may cause loss of productivity of the studied breeds.

**Table 2.** Comparison among physiological parameters of sheep Morada Nova and Santa Ines in different turns (morning and afternoon) in the semiarid northeast.

Time of recording	Physiological variables					
	Respiratory rate (breaths $\text{minute}^{-1}$ )		Heart rate (beats $\text{minute}^{-1}$ )			Rectal temperature ( $^{\circ}\text{C}$ )
	MN	SI	MN	SI	MN	SI
Morning (6:00-7:00 a.m)	31.70 <sup>bb</sup>	41.83 <sup>ab</sup>	81.38 <sup>b</sup>	84.02 <sup>b</sup>	38.06 <sup>ab</sup>	38.15 <sup>ab</sup>
Afternoon (13:00-14:00 p.m)	36.80 <sup>ba</sup>	49.87 <sup>a</sup>	94.40 <sup>a</sup>	97.91 <sup>a</sup>	38.70 <sup>a</sup>	38.76 <sup>a</sup>
Standard error	2.25	2.14	1.66	1.59	0.07	0.06

Different uppercase and lowercase letters in columns and in rows indicate significant differences at  $p < 0.05$ . MN – Morada Nova; SI – Santa Inês.

**Table 3.** Comparison between physiological parameters of the Santa Inês ewes and Morada Nova subjected to concentrate supplementation in semiarid northeast.

Treatments	Physiological variables					
	Respiratory rate (Breaths minute <sup>-1</sup> )		Heart rate (Beats minute <sup>-1</sup> )		Rectal temperature (°C)	
	MN	SI	MN	SI	MN	SI
1 (0.5% BW)	32.25 <sup>hA</sup>	43.00 <sup>A</sup>	86.10 <sup>A</sup>	90.35 <sup>A</sup>	38.17 <sup>hA</sup>	38.44 <sup>A</sup>
2 (1.5% BW)	36.25 <sup>hA</sup>	48.70 <sup>A</sup>	89.68 <sup>A</sup>	91.58 <sup>A</sup>	38.19 <sup>hA</sup>	38.47 <sup>A</sup>
Standard error	2.35	2.14	1.74	1.59	0.07	0.06

Different uppercase and lowercase letters in columns and in rows indicate significant differences at  $p < 0.05$ . MN – Morada Nova; SI – Santa Inês; 0.5% BW – ewes subjected to 0.5% of concentrate supplementation based on body weight; 1.5% BW – ewes subjected to 1.5% of concentrate supplementation based on body weight.

Heart rate was not affected ( $p > 0.05$ ) by concentrate supplementation offered. In contrast to our results, Sejian, Bahadur, and Naqvi (2014) reported a decrease in HR according to increase of the feed restriction level and consequently decreased thyroid activity. However, its values are above the normality range (70 to 80 beats per minute). However, RT was influenced by breed, responding to the difference ( $p < 0.05$ ) found in RR values, as well as their patterns above physiological limits. Santa Inês ewes showed RR values above physiological limits due to the intense use of this mechanism to maintain body temperature, which is determined by balance between loss and heat gain. However, regardless of installation conditions and period of data collection, the breeds of this study change the behavioral and physiological mechanisms to maintain internal temperature, confirming the high adaptation physiology of native ewes (Sejian et al., 2014).

During the experimental period, there was high pasture availability (4260.2 kg dry matter (DM) ha<sup>-1</sup>) with greater composition in leaves (1345.3 kg DM /ha) and stems (2413.7 kg DM ha<sup>-1</sup>) green than leaves (779.9 kg DM ha<sup>-1</sup>) and stems (138.5 kg DM ha<sup>-1</sup>) dry, which offers to sheep a bulk of good digestibility with a greater use of this material at a level of rumen microorganisms. According to Menezes et al. (2010), animal performance can be optimized with the use of other grasses such as Aruana and Tanzânia grass, due to higher amount of leaf mass combined with best nutritional value of the forage. However, the

use of *Andropogon*, especially in the semiarid region, is justified by great resilience to the environment.

Assessing the feeding behavior of ewes in the last third of pregnancy in continuous grazing and receiving concentrate supplementation, grazing activity of sheep was influenced ( $p < 0.05$ ) by scales of observation intervals (Table 4). According to the activities performed by ewes during the observation period, the interval of 10 minutes is the most effective methodology to assess with accuracy the behavior of these grazing animals, mainly due to the greater number of the recordings offering higher data reliability. It is essential the evaluation these activities for improvement of animal performance and possible correction management, especially when using concentrate supplementation, significant factors to the efficiency of the production system. Our result differs from many studies (Carvalho et al., 2007; Mezzalira et al., 2011; Oliveira et al., 2011; Pinheiro et al., 2011; Marques et al., 2012), indicating the scale of 30 minutes for observation of the behavior of grazing, ruminating and idling. However, these study report that when it comes to evaluating related to the dynamics of dining activities, observations are needed every 5 minutes.

The time scales of grazing of 20 and 30 minutes did not differ ( $p > 0.05$ ) and were higher when compared with the grazing time rated on a scale of 10 minutes, thus promoting an overestimation of grazing time among these sheep.

**Table 4.** Times of behavioral variables of native ewes at grazing under supplementation.

Times scales	Behavioral variables (hours/observation period)							
	GRAZ	RUMF	RUML	STAN	LYIN	WALK	URI	DEF
10 minutes	5.98b	0.73a	0.59a	1.25a	0.53a	0.89a	0.02a	0.01a
20 minutes	6.44a	0.66a	0.49a	1.21a	0.40a	0.79a	0.01a	0.0a
30 minutes	6.43a	0.68a	0.50a	1.19a	0.39a	0.80a	0.01a	0.0a
Standard error	0.27	0.20	0.16	0.20	0.14	0.14	0.02	0.01

Different letters in columns indicate significant differences at  $p < 0.05$ . GRAZ-grazing; RUMF-ruminating on foot; RUML-ruminating lying down; STAN-standing idle; LYIN-lying idle; WALK-walking; URI-urinating; DEF-defecating.

In the diagnosis of other activities for the sheep throughout the 10 hours of evaluation, they were similar ( $p > 0.05$ ) for all rating scales, reinforcing that the scale of 10 minutes can infer more precisely the behavioral patterns exhibited by Morada Nova and Santa Ines sheep in an advanced state of pregnancy and receiving energy and protein supplementation

In general, the ewes showed a daytime behavior with greater intensity of activities performed for grazing, rest and rumination with averages of 6.28, 0.83 and 0.61 hours respectively. This result was expected given the fact that ruminants exhibit behavior over 24 hours that can be divided into similar grazing times, ruminating and idling (Roman et al., 2007). However, the realization of the feed consumption activity occurs during the day, always taking into account the hours with more favorable climate (Lin, Dickhoefer, Müller, & Susenbeth, 2011; Sejian, Maurya, & Naqvi, 2012).

The decline in pasture intake by sheep during periods of increased ambient temperature, is in parts, due to a decreased metabolic rate, resulting in signs of feedback, indicating lower energy expenditure requirements (Zanine, Vieira, Ferreira, Vieira, & Cecon, 2007; Sejian et al., 2012) or increased endogenous heat caused in parts by concentrate supplementation. This observation is important due to grazing system has a direct effect on intake rate and yield performance of the animal, allowing the creator to stream line management practices, aiming to reduce the cost and improve the quality of products (Medeiros, Pedroso, Jornada, Silva, & Saibro, 2007).

In addition to the above features, ruminants tend to divide their daily activities in similar periods in relation to time (Roman et al., 2007), the sheep of this study presented as activity of secondary priority the idleness standing, in which the animal does not perform the activity itself, an ethological condition

in which the animal may be evaluating the environment around it.

In analysis of rumination time, there was no effect ( $p > 0.05$ ) of evaluation methodology during experimental period. However, the sheep exhibited more time for rumination on foot, which was expected due to the ruminants have been characterized by use of night time to perform lying rumination activity (Figueiredo et al., 2013), to mitigate the effects of heat stress.

In Table 5 is showed the interaction of breed and scale of observation of behavioral variables assessed during the experimental period with effect ( $p < 0.05$ ) on grazing patterns.

Morada Nova ewes when evaluated on the scale of 10 minutes exhibited less time grazing ( $p < 0.05$ ) compared to times of 20 and 30 minutes, which were similar ( $p > 0.05$ ) for such a feature. The Results in our experimental conditions are supported by Marques et al. (2012) and Fish et al. (2000) who used an animal group kept with continuous observation to identify the best time interval for recording behavior.

Santa Inês ewes that received 1.5% of concentrate supplementation presented a decrease in roughage intake (Silva et al., 2010) in the three times of evaluation, being verified less time grazing ( $p < 0.05$ ), probably due to the substitutive effect caused by higher level of supplementation provided, promoting the supply of your daily requirement and consequently modifying the behavior. Silva et al. (2010) reported that concentrate supplementation up to 1% BW did not affect the grazing activity and rumination during the daytime. As Santa Inês ewes are larger and require greater dry matter intake, ruminants are able to regulate the daily voluntary intake based on their organic energy demand until the rumen reaches its physical ability of filling.

**Table 5.** Interaction between breed x time of evaluation x levels of supplementation about of behavioral variables of native ewes at grazing.

Behaviors (hours/observation period)	10 minutes				20 minutes				30 minutes				Standard error
	MN		SI		MN		SI		MN		SI		
	0.5%	1.5%	0.5%	1.5%	0.5%	1.5%	0.5%	1.5%	0.5%	1.5%	0.5%	1.5%	
GRAZ	5.47b	5.57b	5.63b	5.94b	6.36a	6.75a	6.87a	5.72b	6.44a	6.28a	6.78a	5.80b	0.39
RUMF	0.67	0.79	0.86	0.81	0.50	0.56	0.55	1.00	0.61	0.69	0.61	0.88	0.29
RUML	0.97	0.77	0.88	0.76	0.29	0.38	0.85	0.81	0.26	0.46	1.05	0.55	0.23
STAN	1.56a	1.37a	0.94b	0.92b	1.87a	1.37a	0.78b	0.81c	1.64a	1.35a	0.61b	0.89b	0.29
LYIN	0.58	0.80	0.73	0.68	0.39	0.29	0.40	0.51	0.33	0.33	0.34	0.55	0.19
WALK	0.65	0.70	0.94	0.87	0.59	0.59	0.52	1.15	0.72	0.89	0.61	1.33	0.20
URI	0.04	0.00	0.02	0.02	0.00	0.03	0.03	0.00	0.00	0.00	0.00	0.00	0.03
DEF	0.06	0.00	0.00	0.00	0.00	0.03	0.00	0.00	0.00	0.00	0.00	0.00	0.01

Different letters in rows indicate significant differences at  $p < 0.05$ . GRAZ-grazing; RUMF-ruminating on foot; RUML-ruminating lying down; STAN-standing idle; LYIN-lying idle; WALK-walking; URI-urinating; DEF-defecating. MN – Morada Nova; SI – Santa Inês; 0.5% – ewes subjected to 0.5% of concentrate supplementation based on body weight; 1.5% - ewes subjected to 1.5% of concentrate supplementation based on body weight.

In diets composed exclusively by bulky of lower quality, one of the most important factors in the control of voluntary intake is physical distension of the rumen. However, diet with high-concentrate, chemostatic and thermostatic mechanisms can act in regulation of the voluntary intake. Which will help to produce metabolite in the rumen or in the blood after a meal and stimulating chemically sensitive receptors that will act in stimulation of central nervous system responsible for satiety, causing the modification of feeding behavior.

The behavior of idleness of these sheep was also influenced ( $p < 0.05$ ) by breed in all times of evaluation (10, 20 and 30 minutes). Morada Nova ewes regardless of the level of concentrate supplementation offered, remained longer in standing idle, being verified again a more dynamic behavior of all day long.

There was not verified effect of concentrate supplementation and time scales of evaluation between breeds on RUM (on foot and lying down), LYIN, WALK, URI and DEF. These findings may explain a degree of similarity between breeds. Once, are native breeds of semi-arid region, characterized by high temperature, high incidence of solar radiation, low humidity and high evaporation rates. The interaction of these factors can cause intense behavioral and physiological alterations in animals raised on pasture (Sejean et al., 2010; Silva et al., 2015b). Further, inadequate and low quality feed is a major factor leading to under-production of animals in arid and semi-arid tropical regions.

These results show that Morada Nova ewes diversifies more their activities throughout the day, this breed is extremely adapted to nutritional management and environmental conditions, characteristics that make these animals of fundamental importance in the raising on small farms, constituting an indispensable source of protein in the diet of the rural population.

## Conclusion

Pregnant ewes exhibited high adaptive capability to the semi-arid environment with higher use of the thermolysis mechanisms by Santa Inês ewes.

For better description of ingestive behavior on Morada Nova ewes, in experimental conditions, observations of 10 minutes are recommended, mainly for grazing activity, indicating that this breed diversifies more their daytime activities. Furthermore, it's the most effective methodology to assess with accuracy the behavior of these grazing animals, mainly due to the greater number of the recordings offering higher data reliability. However,

for Santa Inês ewes, 30 minutes of interval can be utilized, not affecting the behavioral patterns of grazing, ruminating and idleness. This can improve native sheep production that has a direct influence on the income and quality of life for part of semi-arid population.

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