



Influence of availability of shade on testicular characteristics of Santa Ines rams

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ABSTRACT. The purpose of this study was to evaluate the effects of availability of artificial shading on testicular and seminal characteristics of Santa Ines crossbred rams. Twelve 8 month old rams at 38.1 ± 5.2 kg average weight, were used in a total random design with two treatments: areas with and without available artificial shading (black polyethylene mesh screen for a 50% reduction of solar radiation). The scrotal circumference, scrotal length, scrotal volume, rectal temperature and scrotal temperature were measured twice a week. Also a biweekly analysis of the semen was carried out, measuring the volume and spermatoc concentration as well as the percentage of normal spermatozoa. The results show that available shading provided for lower scrotal temperatures and higher sperm concentration, without influencing the scrotal volume and the percentage of normal spermatozoa. The testicular measures, such as the scrotal circumference and length were higher in the rams that were subjected to the no available shading treatment. The absence of differences, in some of the analyzed variables, between the two treatments, with and without available shading, could be due to high adaptability of the animals.

Keywords: semen, biometrics, sheep, shade, thermal stress.

Influência da disponibilidade de sombra sobre as características seminais de ovinos Santa Inês

RESUMO. Com a finalidade de avaliar o efeito do sombreamento artificial sobre as características testiculares e seminais de ovinos deslanados, foram utilizados 12 carneiros ($\frac{1}{2}$ sangue Santa Inês x SRD) com peso vivo médio de 38.06 ± 5.24 kg e faixa etária de cerca de oito meses. Os tratamentos foram disponibilidade ou não de sombreamento artificial, utilizando tela preta de polietileno com malha para bloquear 50% da radiação solar global. Duas vezes na semana entre 14h e 15h realizou-se a medição da circunferência escrotal (CE), comprimento escrotal (CC), volume escrotal (VE), temperatura retal (TR) e temperatura escrotal (TE). Quinzenalmente, foram realizadas análises quantitativas e qualitativas do sêmen. Os resultados obtidos permitem concluir que o sombreamento proporcionou menores temperaturas escrotales e maior concentração espermática, não influenciando o volume escrotal e a porcentagem de espermatozoides normais. Medidas testiculares como circunferência e comprimento escrotal foram mais elevadas ao sol. A ausência de diferenças em algumas variáveis analisadas nos grupos com e sem sombreamento, pode ser reflexo da rusticidade natural dos animais.

Palavras-chave: sêmen, biometria, carneiro, sombra, estresse térmico.

Introduction

Development of crossbred breeds, such as the Santa Ines in Brazil, can be an interesting alternative for increasing the efficiency of lamb meat production systems (CEZAR et al., 2004). However, the body size of the adult Santa Ines sheep, which is higher than that of many other native breeds, requires greater energy for maintenance, which, in tropical conditions, could compromise its reproductive efficiency, especially during the dry season where there is a food shortage (COSTA JR. et al., 2006).

High temperatures and solar radiation are stress factors that affect animal productivity. One of the most

relevant aspects to consider is the reduction in reproductive efficiency, which often occurs in animals subject to high radiant temperatures. In extensive systems with animals in grazing conditions, shading allows for reducing the radiant thermal load, and reduces the thermal stress caused by direct solar radiation.

The beneficial effects of providing shade shelter to sheep in improving their reproductive performance are also well established (STOTT et al., 1972; STEPHENSON et al., 1984).

The climate in northeast Brazil is characterized by high radiant temperatures during the whole year,

which tend to directly influence the testicular thermoregulation mechanisms, and consequently, the male reproductive functions (MOREIRA et al., 2001). The use of rams with greater testicular development, and consequently with high fecundation capacity, is important to ensure good reproductive efficiency of the flock (MAIA, 2002; EMSEN, 2005).

Rege et al. (2000) have observed that the yearly seasonal changes have a significant effect on the scrotal circumference, motility of spermatozoa and concentration and quantity of sperm defects. However, these authors have mainly associated these differences to nutritional aspects.

In sheep, seminal quality and male fertility tend to decrease during the hottest months (summer), possibly due to the seasonal effects on the hypothalamo-hypophyseal axis, or due to the direct effect of temperature on the testicles and epididymides (PINEDA, 1989).

Moreira et al. (2001) have verified, in a study of Santa Ines sheep, that changes in testicular length and scrotal circumference can be considered as viable indicators of the effect of thermal stress on the gonads.

The scrotal volume was measured to address the concern for increasing the accuracy of the selection of reproducers (MARTINS et al., 2003). In animals with long and thin testicles, the scrotal volume is a good indicator of the quantity of testicular parenchyma, as it is a more accurate measure, than the scrotal circumference, which in this case would undermine the reproductive potential of these animals (BAILEY et al., 1996). The testicular volume is a parameter that minimizes the errors of scrotal circumference when the testicles have different shapes (ALVES et al., 2006).

The scrotal volume of beef bulls has been estimated, quite accurately, using the formula of a cylinder, with the width of the testicles as the radius and their length as the height. However, that methodology seems to be not adequate for sheep due to testicular morphology (FIELDS et al., 1979; UNANIAN et al., 2000).

Testicular temperature is another important variable, when considering thermal stress and reproductive efficiency. An increase in testicular temperature would lead to seminal degeneration, which is related to reduction of male fertility (MOORE, 1924; PHILIPS; MCKENZIE, 1934; VOGLER et al., 1991 cited by MOREIRA et al., 2001). Despite the testicles own thermoregulation system, the testicular temperature depends on the body temperature, which can be measured through rectal temperature (SWENSON, 1988).

The purpose of this study was to evaluate the influence of artificial shading in testicular and seminal characteristics of Santa Ines crossbred sheep in semi-extensive production systems.

Material and methods

Study area and population

The study was carried out between July and October in the Goat and Sheep Production Sector of the State University of Southeast Bahia, Itapetinga Campus, Brazil. Located at latitude 15°18'00"S, longitude 40°15'32"W, and elevation 268 m.

The local climate is warm and humid with a dry winter season, with a mean annual temperature of 27.0°C and a relative humidity of about 76%.

During the experimental period, the accumulated monthly rainfall was 339 mm.

The study was carried out using twelve 8 mo-old cross Santa Inês - SI breed rams (½ SI x SRD - without well-defined type), with an average live weight of 38.1 ± 5.2 kg.

The animals were maintained in a semi-extensive production system, in about 960 m² areas with rotational grazing in the *Cynodon dactylon* cv. Tifton-85 pasture, with *ad libitum* water and mineral salt with urea. Concentrated feed, was distributed at a ratio of 1% of the weight and was provided twice daily, at 7:00 am and at 4:30 pm. The animals remained in the pasture during the day and in the evening were gathered into the pen.

Treatments

The twelve animals were divided into two groups for the treatments which were: 1) availability of artificial shading and 2) no available shading. For the treatment with available shading, a black polyethylene screen was installed for a 50% solar radiation reduction. The screen, with a height of 2.80 m, a width of 2.4 m and a length of 4.0 m, provided an approximate occupation of 1.6 animals. m⁻² of the shaded area. It was up to the animal itself to look for and remain in the shade (which represented 1% of the total available area). The search and the permanence for the shade (which represented 1% of the total area) was defined by the animal itself.

Data collection

The following parameters were measured twice a week, between 2:00 pm and 3:00 pm: scrotal circumference (SC), scrotal length (SL), scrotal volume (SV), rectal temperature (RT) and scrotal temperature (ST).

The SC was measured using a flexible measuring tape, in the median position of the scrotal, around the two gonads and the scrotal skin, at the point with the largest dimension. To measure the SL, the testicles were considered, excluding the tail of the epididymides towards dorsoventral axis. The SV, was calculated through water displacement,

according to Krause (1993), with immersion of the scrotum in a measuring recipient containing a predefined volume of water at approximately 27°C. The RT was measured using a digital clinical thermometer and the ST was measured using an infrared thermometer.

Quantitative and qualitative analyses of the semen were carried out every two weeks. In order to induce oestral behavior, estrogen was applied to a mount animal (mannequin) and the semen was collected through the artificial vagina. Immediately after collection, the ejaculate's volume concentration and percentage of normal spermatozoa (PNS) were evaluated.

After ejaculation, the semen samples were transferred immediately to the laboratory and assessed for volume was quantified using the graduated tube, which was adapted to the artificial vagina; mass motility: graded on 0-5 point scale and the concentration was determined using a spectrophotometer, previously calibrated with a hemocytometer ($\times 10^9$ sperms mL⁻¹); the PNS was measured using colored slides (eosin-nigrosine) (BARIL et al., 1993).

Data analysis

A completely randomized design was used and after verifying normality and homocedasticity an ANOVA unifactorial was calculated. In all the cases, a significance level of 5% probability was adopted.

The experimental data was processed using the NCSS (2004) statistical program and Microsoft Excel and Microcal Origin 6.0.

Results and discussion

During the experiment, the climatic variables presented the following mean values: air temperature of 20.2°C, relative humidity of 88%, black globe temperature of 19.9°C in the shade, black globe temperature of 20.2°C in the sun, global radiation of 9.776 MJ m⁻² day⁻¹ in the shade and global radiation of 17.536 MJ m⁻² day⁻¹ in the sun.

The obtained values of climatic variables during the experimental period (winter/spring) presuppose an environment within thermal comfort range, recommended for sheep (between 20 and 30°C) (BAÊTA; SOUZA, 1997; ALEXANDER, 1974).

At present there is not a chart on the black globe temperature-humidity index (BGT) for ovine (SOUZA et al., 2008).

This index probably represents one of the best indices to represent heat stress in open areas; nevertheless, it accounted for only 24% of the variance of heat stress-related milk yield depression in dairy cows (BUFFINGTON et al., 1983). This is in part

because of large variations between individuals and in part, because the animal is related to its environment in a much more complex manner than is represented by this index. Solar radiation has a major effect on the thermoregulation of grazing ruminants (GEBREMEDHIN, 1985).

The means and standard deviations for testicular characteristics and rectal temperatures, for each of the treatments, are presented in Table 1.

Table 1. Mean measurements gathered over an 11-wk period during the months of July and October 2009 for testicular and physiological characteristics of young Santa Ines rams.

Testicular and physiological measurements	Treatment			
	Shade		Sun	
	Mean (n = 6)	Standard Deviation	Mean (n = 6)	Standard Deviation
Scrotal Circumference (cm)	26.5*	0.9	27.4*	1.6
Scrotal length (cm)	16.5*	2.4	17.3*	2.8
Scrotal temperature (°C)	33.3*	1.5	33.8*	1.5
Scrotal volume (mL)	466.8	81.4	484.3	86.1
Rectal temperature (°C)	39.6	0.4	39.5	0.2

* Significant to 5% between columns.

It was observed that the treatment had a significant effect on scrotal circumference, length and temperature ($p < 0.05$). The animals in the without available shade treatment, presented higher values, with regard to these parameters, than those with artificial shading.

Even though air temperature was kept within the thermoneutral zone, availability of shade in the pasture was beneficial with regard to thermal comfort of the animals. The observed increase in scrotal temperature indicates that exposure to direct solar radiation had an influence on vascular activity in the scrotal area.

Godfrey et al. (1998), in a study that was carried out with wool-breed Caribbean rams, that were kept in pens with and without artificial shading, did not observe any changes in sexual characteristics. However, Moreira et al. (2001), in Santa Ines rams that were kept in the shade and subjected to localized heating of the testicles, observed a reduction in scrotal circumference, from 26.4 ± 1.1 cm to 21 ± 0.3 cm.

Analyzing body and testicular development of Santa Ines sheep, Pacheco et al. (2009) found a scrotal circumference of 23, 83, 27, 78, 28, 93 and 29, 74 cm, respectively, at 6, 8, 9 and 12 months of age; which are not higher than the values registered in this study, in a semi-extensive regime.

The shorter scrotal lengths in the sheep, in the available shade treatment, indicate a better thermal condition. In fact, a larger distance between the testicles and the abdominal cavity provides for maximizing heat loss in this area, which is an indication of greater thermoregulatory effort (JOHNSON, 1980).

Moreira et al. (2001) observed a mean scrotal length of 12 cm, in Santa Ines rams, before localized

heating of the testicles, which is less than the values observed in this study.

According to Bailey et al. (1998), long testicles, such as those of rams, provide for better heat dissipation, and facilitate thermoregulation, causing less harm to spermatogenesis, due to high temperatures.

The rams in the available shade treatment had lower scrotal temperatures ($p < 0.05$). However, the thermal gradient between the treatments was only 0.49°C .

Moreira et al. (2001), after localized heating of testicles, found scrotal temperatures of $37.2 \pm 0.17^{\circ}\text{C}$, which are above those observed in this study. Santos et al. (2005), studying different ram breeds in the semi-arid northeast Brazil, registered a scrotal temperature of 31.56°C , during the afternoon, which shows a high thermoregulatory capacity of the testicles in these rams.

The treatments did not have a significant influence ($p > 0.05$) on rectal temperature and scrotal volume.

The absence of an effect on rectal temperature, due to the treatment, reinforces the idea that the animals have adapted to the existing environmental conditions, and are close to thermoneutral conditions.

Figure 1 shows the variations in rectal (RT) and scrotal temperatures (ST), and their difference (RT - ST), throughout the experiment.

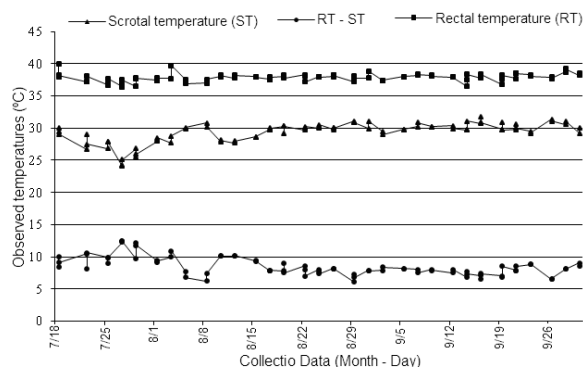


Figure 1. Comparison between rectal and scrotal temperatures of 12 Santa Ines rams during an 11-wk test period during July and October 2009.

The mean difference registered between the rectal and scrotal temperatures was $9.01 \pm 1.72^{\circ}\text{C}$ (Figure 1). These results are much higher than the values referred to in the literature for cattle and sheep, which are between 2 to 6°C (KASTELIC et al., 1995; WAITES, 1970). Maloney and Mitchell (1996), observing rams kept in ambient temperatures of 20 to 23°C , observed variations of $3.30 \pm 0.03^{\circ}\text{C}$ and concluded that regulation of scrotal temperature represents a high degree of independence from body temperature, which leads to scrotal temperatures that are always lower than body temperatures. The values of scrotal volume present great variability, with mean values of $474 \pm$

133 cm^3 . This reinforces the need for new studies which would make it possible to provide a better explanation for this variation.

Table 2 presents the mean and standard deviation values of the semen evaluation, as a function of the treatment.

Table 2. Mean measurement for gathered over an 11-wk period during the months of July and October 2009 for seminal characteristics of young Santa Ines rams.

Semen characteristics	Treatment			
	Shade		Sun	
	Mean (n = 6)	Standard deviation	Mean (n = 6)	Standard deviation
Sperm concentration (10^6 mL^{-1})	2.60*	1.22	2.29*	0.90
Ejaculate volume (mL)	1.14*	0.60	1.35*	0.55
Normal spermatozoa (%)	90.98	8.63	90.27	6.02

* Significant to 5% between columns.

A significant effect of the treatment on sperm concentration and ejaculate volume ($p < 0.05$) was observed.

Martins et al. (2003) have observed that the time the samples were collected and the individual characteristics of the animal had a significant influence on the obtained values for scrotal volume. Martins et al. (2003) studying the Santa Inês, noted a correlation of $r = 0.89$ between the scrotal circumference and volume.

The animals in the with available shade treatment had higher sperm concentrations ($p < 0.05$).

A significant effect of the treatment on sperm concentration and ejaculate volume was observed, which could indicate an influence of shading on the reproductive performance of the animals.

These values of sperm concentrations, together with the lower scrotal temperatures, make it possible to assume that the decrease in solar radiation, made possible by artificial shading, provided better conditions for spermatogenesis.

According to Lincoln (1998) the increase in sperm concentration occurs as a result of a higher quantity of seminiferous tubule, allowing the animal a greater spermatogenic activity.

The ejaculate volume of the animals in the no available shade treatment was higher ($p < 0.05$) than that of the animals in the available shade treatment. It was noted that the animals with the greater scrotal circumference were those that also had greater ejaculate volume.

According to Leal et al. (1998) the seminal volume of the crossbred sheep raised in the northeast of Brazil, increase in the rainy season and the spermatozoon concentration is inversely proportional to the spermatid volume. The authors suggest that the increase in fluid secretion in the epididymides and the accessory sex

glands leads to greater dilution of the semen. However, Moreira et al. (2001) did not find any differences in the spermatid volume of Santa Inês rams.

These values of percentage of the normal sperm could lead one to assume a good fertility rate, as they are higher than that recommended for sheep reproduction, which is 80% of normal sperm (CBRA, 1998; ROWE et al., 1993; BOUNDY, 1992).

There were no significant differences between the treatments with regard to the percentage of the normal sperm, with mean values of $90.6\% \pm 7.5$.

The absence of differences in some of the analyzed variables could be a reflection of the combined effects of the animals' high adaptability and the mild ambient conditions during the experimental period (winter/spring).

Conclusion

Spermatid activity of the scrotal region was affected by exposure of the animal to direct solar radiation.

The rams in the available shade treatment had lower scrotal temperatures, higher spermatid concentrations, smaller scrotal circumferences and shorter scrotal lengths.

However, the rectal temperatures, scrotal volumes and normal sperm percentages of the sheep were not affected by the absence of shading.

This study demonstrates that, even in mild conditions, availability of shade is an important factor in animal comfort, with potential benefits for reproductive efficiency.

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