Effect of shadow availability at pasture on reproductive traits of Nelore bulls (*Bos indicus*) raised in southeastern Brazil

*Efeitos da disponibilidade de sombra a campo sobre características reprodutivas de touros da raça Nelore (*Bos indicus*) criados na região Sudeste do Brasil*

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Abstract

Solar radiation is responsible for bull body temperature elevation. An alternative to minimize heat stress is to use artificial shade. Thus, this study aimed to evaluate the effect of thermal stress reduction, through shade availability, on reproductive characteristics of Nellore bulls (*Bos indicus*). For this, ten bulls were divided in: Available artificial shade (AS, n = 5) and Unavailable shade (US, n = 5). Each group was kept in two hectare paddocks, in which shade availability for group AS was artificially created. Animals were submitted to a clinical-reproductive evaluation and seminal analyses. No interaction was observed between treatments (AS and US) and time (8 collections) for all analyzed variables (P>0.05). No significant effect (P > 0.05) of treatment was observed for all parameters analyzed. So, it can be concluded that the absence of shaded areas during summer does not negatively affect reproductive characteristics such as: scrotal circumference, testicular consistency, progressive motility, percentage of rapidly moving cells (Computer Assisted Semen Analysis - CASA), morphology or sperm viability in Nellore bulls raised in southeastern Brazil, considering that results could be different in other regions of the country where average temperature is higher.

Keywords: Semen. Artificial shade. Nellore. Bull.

Resumo

A radiação solar é responsável pelo aumento da temperatura corpórea em touros. Uma alternativa para minimizar o estresse térmico é usar sombreamento artificial. Assim, este estudo teve como objetivo avaliar o efeito da redução do estresse térmico através da disponibilização de sombra artificial, sobre características reprodutivas de touros da raça Nelore (*Bos indicus*). Para isso, dez touros foram divididos em: Disponibilidade de sombra artificial (AS, n = 5) e Não-disponibilidade de sombra artificial (US, n=5). Cada grupo foi mantido em piquete de dois hectares cada, no qual a sombra para o grupo AS foi criada artificialmente. Os animais foram submetidos a avaliações clínicas-reprodutivas e análise seminal. Nenhuma interação foi observada entre os tratamentos (AS e US) e o tempo (8 coletas), para todas as variáveis analisadas (P > 0.05). Nenhum efeito significativo (P>0.05) de tratamento foi observado para os parâmetros analisados. Dessa forma, concluiu-se que a ausência de áreas sombreadas, durante o verão, não afetou negativamente as características reprodutivas como: circunferência escrotal, consistência testicular, motilidade progressiva, percentagem de células com movimentos rápidos (Computer Assisted Semen Analisys - CASA), morfologia e viabilidade espermática em touros da raça Nelore criados na região sudeste do Brasil, considerando que esse resultado pode ser diferente em outras regiões do país, onde as temperaturas ambientais são mais elevadas.

Palavras-chave: Sêmen. Sombreamento artificial. Nelore. Touro.

Introduction

Bovine reproduction can be affected by thermal stress because, under high temperatures and humidity, the thermoregulatory mechanisms are unable to increase heat loss leading to an elevation in body temperature above physiological limits (CHEMINEAU, 1994). Testicular temperature increase, caused by exposure

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Received: 28/05/2013
Approved: 18/12/2013

to high temperature environments or by scrotal thermal insulation interferes with spermatogenesis, resulting in decrease of sperm production and semen quality, consequences of which are testicular hypoxia and reactive oxygen species (SETCHELL, 1998).

In open environments, the main reason for body temperature elevation is the direct incidence of solar radiation. An alternative to decrease solar radiation and minimize heat stress is to use natural or artificial shade, which aids thermal comfort and favors homeothermy (TUCKER; ROGERS; SCHÜTZ, 2008).

In Brazil, beef cattle are maintained predominantly in native pastures and *Bos indicus* cattle are known to be more thermotolerant in response to heat stress than European breeds (HANSEN, 2004). This is one of the various reasons that *Bos indicus* (e. g. Nellore) is the elected cattle in this country.

However, studies to date with Zebu cattle were performed with the objective of comparing its adaptability to tropical climate with European breeds and to check its effects on the breeding soundness exam and semen freezability (BRITO; SILVA; BARBOZA, 2004; KOIVISTO; COSTA; PERRI, 2009). The effect of heat stress reduction was not yet experienced in reproductive characteristics in Zebu bulls. Thus, this study aimed to evaluate the effect of thermal stress reduction, through shade availability in paddocks on reproductive characteristics of Nellore bulls (*Bos indicus*).

Materials and Methods

The experiment was conducted from January to April 2008, at 21°57'0,6" south latitude and 47°27'01" west longitude, at 597 m above sea level.

The average environment temperatures were 24.6ºC, being 34.2ºC (maximum) and 13.2ºC (minimum), and average air humidity was 78.3%, (66.1% - 95.5%), during experiment period.

Ten Nellore bulls, aged 26 ± 2 months old, were randomly divided in two treatment groups, as follows: AS= with available artificial shade (n = 5) and US = unavailable shade (n = 5). Each group was kept in two hectare paddocks with predominantly *Brachiaria brizantha,* mineral supplementation (Fosbovi Protein Energetic – TORTUGA®) and *ad libitum* water, mimicking beef cattle husbandry conditions in Brazil.

For AS group, shade availability was artificially created using an area with polyethylene *Sombrite* (Polysack®), with 90% of solar light retention, a total of 10 m^2 of shade per animal.

The animals were submitted to eight semen collections and fortnightly examinations that included a clinical-reproductive evaluation (testicular consistency, scrotal circumference) and seminal analyses.

Sperm motility was assessed by CASA (Hamilton Thorne Biosciences, Ivos-Ultimate, Beverly, MA, USA). Its setup was pre-adjusted for bovine sperm analysis Semen sample was then diluted in Tyrode's albumin lactate pyruvate (TALP) sperm medium (BAVISTER; LEIBFRIED; LIEBERMAN, 1983), pre-warmed at 37ºC, to a final concentration of 25 x 10^6 spermatozoa/mL. The following variables were analyzed: progressive motility (%) and the percentage of rapidly moving cells (RAPID, %).

The integrity of plasma and acrosomal membranes, as well as mitochondrial function, were evaluated using a combination of propidium iodide (PI), fluorescein isothiocyanate conjugated *Pisum sativum* agglutinin (FITC-PSA), and 5,5',6,6'-tetrachloro-1,1',3,3'-tetraethylbenzimidazolcarbocyanine iodide (JC-1) fluorescent probes, respectively (CELEGHINI et al., 2008). A total of 200 sperm cells were examined per slide and classified according to the fluorescence emitted by each probe: IPIAH = intact plasma membrane, intact acrosomal membrane and high mitochondrial function (represents the "viable-cell" percentage in samples); IPIAL = intact plasma membrane, intact acrosomal membrane and low mitochondrial function; IPDAH = intact plasma membrane, damaged acrosomal membrane and high mitochondrial function; IPDAL = intact plasma membrane, damaged acrosomal membrane and low mitochondrial function; DPIAH = damaged plasma membrane, intact acrosomal membrane and high mitochondrial function; DPIAL = damaged plasma membrane, intact acrosomal membrane and low mitochondrial function; DPDAH = damaged plasma membrane, damaged acrosomal membrane and high mitochondrial function; DPDAL = damaged plasma membrane, damaged acrosomal membrane and low mitochondrial function.

To assess sperm morphology, semen was diluted and fixated in pre-warmed (37ºC) formaldehyde-PBS. Sperm cells ($n = 200$) were counted under differential interference-contrast microscopy (model 80i; Nikon, Tokyo, Japan) at a magnification of 1000x and were classified according to Blom (1973) and Barth and Oko (1989) into major defects and minor defects.

The results were analysed using the Statistical Analysis System (1989), the normality of the residues being verified by the tests of Shapiro-Wilk (PROC UNIVARIATE) and the variance compared with the Bartlett's test. The data that did not attend the statistical premises were submitted to the logarithm transformation [Log (X + 1)]. The original or transformed data, when this last proceeding was needed, were submitted to analysis of variance. Statistical analysis added to the factor of repeating measures in time, referring to several times of the sampling. The probability of interaction with the time was determined by the test – Greenhouse-Geisse, using the command REPEATED generated by proceeding – GLM (PROC GLM of the SAS). The experimental model used was as follows: $Y = \mu + Tr_i + T_f + (Tr_i T_f)_{if} + B_j + e_{ij}$, where Y is observation of treatments i and f in block j, μ the general mean, Tr_i the effect of treatment i, T_f the effect of time f, $(TrT)_{if}$ the effect of interaction between the treatment i and time f, B_j the effect of the block j formed in function of an index determined for each animal, and e_{ij} is the unexplained error. In all the statistical analyses, the level of significance considered was 5%.

Results

Testicular consistency and scrotal circumference

There was neither improvement in testicular consistency nor in scrotal circumference during the 8 weeks of collections after shade availability in the paddock. Mean testicular consistency during the experiment period was 3.9 ± 0.1 for the group with available shade (AS) and 3.7 ± 0.1 for the group without shade ($P = 0.60$). Scrotal circumference means was 30.5 ± 0.3cm for bulls with available shade and 31.7 ± 0.2 for bulls kept without shade ($P = 0.19$).

Sperm motility

Sperm motility data are shown in Table 1. No interactions were observed between treatments (AS and US) and time (referring to 8 times of the sampling) for variables progressive motility ($P = 0.35$) and percentage of rapidly moving cells ($P = 0.31$).

Simultaneous Determination of Mitochondrial Potential and Integrity of Plasma and Acrosomal Membranes

The percentages of spermatozoa with plasma and acrosomal membrane integrity and mitochondrial function (IPIAH) evaluated by a combination of fluorescent probes are demonstrated in Table 2. No interactions were observed between treatments (AS and US) and time (referring to 8 times of the sampling) for spermatozoa class IPIAH ($P = 0.64$), demonstrating that the unavailability of shade during 8 weeks did not lead to an increase in sperm membrane damage.

Sperm morphology

Data of morphological findings (abnormalities only) are included in Table 3. Shade in the paddock does not increase semen morphologic characteristics, as animals having shade access during a period longer than necessary for spermatogenesis (60 days) did not have improvement in semen major defects ($P = 0.32$) and minor defects ($P = 0.44$).

Table 1 - Mean ± S.E.M. of progressive motility (%) and rapid cells (%) in the semen of Nelore bulls, according to the treatment with available artificial shadow (AS) or unavailable shadow (US). Pirassununga – São Paulo – Brazil – January to April – 2008

Collection number	Progressive Motility[1]		Rapidly Moving Cells[2]	
	AS	US	AS	US
1	78.5 ± 2.8	81.1 ± 1.4	86.6 ± 3.3	81.1 ± 2.1
2	79.6 ± 1.6	72.0 ± 4.8	90.0 ± 1.6	72.0 ± 4.2
3	77.3 ± 5.3	81.6 ± 3.6	86.7 ± 3.2	81.6 ± 2.9
4	79.6 ± 3.3	83.3 ± 3.1	90.3 ± 2.6	83.3 ± 2.6
5	82.8 ± 2.0	82.6 ± 2.9	92.5 ± 1.5	82.6 ± 2.8
6	74.5 ± 3.7	79.1 ± 2.6	83.6 ± 4.3	79.1 ± 3.3
7	76.7 ± 3.2	77.7 ± 2.2	84.8 ± 3.5	77.7 ± 2.9
8	70.6 ± 5.4	77.2 ± 4.4	80.0 ± 6.4	85.5 ± 5.3
Mean	**77.7 ± 1.3**	**79.7 ± 1.2**	**86.6 ± 1.3**	**88.2 ± 1.2**

[1] P = 0.35; [2] P = 0.31

Table 2 - Mean ± S.E.M. of spermatozoa with integrity of plasma and acrosomal membrane with mithocondrial potential in semen of Nelore bulls, according to the treatment with available artificial shadow (AS) or unavailable shadow (US). Pirassununga – São Paulo – Brazil – January to April 2008

Collection number	IPIAH[1]	
	AS	US
1	58.1 ± 6.0	65.1 ± 5.1
2	67.4 ± 4.9	66.0 ± 5.6
3	67.0 ± 5.1	72.8 ± 2.6
4	66.1 ± 4.3	68.4 ± 1.4
5	75.1 ± 2.6	74.7 ± 3.9
6	71.0 ± 2.7	73.6 ± 4.3
7	65.6 ± 3.7	72.0 ± 1.0
8	63.8 ± 6.4	74.7 ± 3.23
Mean	**66.8 ± 1.7**	**70.9 ± 1.2**

[1] P = 0.64

Table 3 - Mean ± S.E.M. of major defects (%), minor (%), and total (%) of the spermatozoa of Nelore bulls, according to the treatment with available artificial shadow (AS) or unavailable shadow (US). Pirassununga – São Paulo – Brazil – January to April – 2008

Collection number	Major Defects[1]		Minor Defects[2]		Total Defects[3]	
	AS	US	AS	US	AS	US
1	12.1 ± 2.9	8.6 ± 2.4	10.6 ± 3.5	6.1 ± 2.0	22.7 ± 4.4	14.7 ± 4.0
2	10.9 ± 2.6	9.6 ± 2.4	16.3 ± 8.7	11.6 ± 1.0	27.2 ± 8.8	21.2 ± 1.8
3	10.5 ± 3.0	12.3 ± 3.3	5.5 ± 2.9	2.0 ± 0.6	16.0 ± 3.2	14.3 ± 3.5
4	12.1 ± 4.3	7.0 ± 0.6	21.5 ± 7.7	5.3 ± 1.5	33.6 ± 10.7	12.3 ± 1.6
5	16.0 ± 5.3	11.0 ± 1.5	10.8 ± 5.9	4.9 ± 1.5	26.8 ± 8.6	15.9 ± 2.8
6	14.0 ± 3.0	14.6 ± 2.1	6.0 ± 2.4	2.6 ± 0.4	20.0 ± 4.2	17.2 ± 2.8
7	14.9 ± 3.7	12.7 ± 2.3	7.5 ± 2.4	6.0 ± 1.6	22.4 ± 4.3	18.7 ± 3.4
8	11.6 ± 2.2	14.7 ± 3.6	9.6 ± 3.5	4.6 ± 2.5	21.2 ± 4.3	19.3 ± 5.8
Mean	**12.8 ± 1.2**	**11.3 ± 0.9**	**10.1 ± 1.9**	**5.39 ± 0.7**	**23.7 ± 2.3**	**16.7 ± 1.2**

[1] P = 0.32; [2] P = 0.44; [3] P = 0.28

Discussion

In the Brazilian beef cattle raising system, absence of natural shading in paddocks is a condition inherited from farms once used for agricultural activities, characterized by large areas without trees. It is important to say that no studies were performed with the objective of evaluating if the lack of shaded areas affects or does not affect reproductive characteristics of *Bos indicus* bulls, especially Nellore, which is the predominant breed in bovine breeding programs in Brazil.

In the present experiment, no effect of artificial shade availability was detected in the following reproductive parameters evaluated: testicular consistence, scrotal circumference, as well as sperm laboratory parameters. It was once believed that by decreasing direct sun exposure, a great part of body temperature elevation would decrease, thus minimizing heat stress and favoring homeothermy. No positive effect of shading on these characteristics was verified, so it can be speculated that Nellore bulls are really adapted to the tropical climate. Nellore bulls have a greater number of sweat glands in the scrotum, long, deep bodies presenting greater body surface area, thinner skin, and no scrotum hair, facilitating heat loss through convection in the environment. In addition, their greater testicular artery diameter increases blood cooling, and lower metabolic rate produces less heat (BRITO; SILVA; BARBOZA, 2004; HANSEN, 2004).

Mean temperature of 24.6ºC recorded during experimental period is within the limits of environmental temperature considered adequate for normal spermatogenesis in bovine, since critical environmental temperature for disrupting normal spermatogenesis is between 27 and 32ºC. However, continuous exposure to temperatures higher than 30ºC can determine a marked negative effect on sperm production (SKINNER; LOUW, 1966). This situation occurred during the experiment, as temperatures reached 34.2ºC. Bulls sought the shaded area when annoyed by direct sun radiation, especially during warmer periods of the day (close to 12:00).

It was hypothesized that animals without shade availability would have greater exposure to direct sun radiation, provoking greater heat stress, testicular degeneration, low sperm motility and moderate amount of cells with morphological defects.

Clinically in testicular degeneration, consistency and testicular area will be changed during palpation (VAN CAMP, 1997). Hence, one can affirm that the lack of shaded area in the paddock did not initiate a heat stress process which would culminate in testicular degeneration, since no alterations in scrotal circumference nor in testicular consistency were observed during the experiment in US group.

Bulls kept in paddocks without shaded area also did not show spermiogram alterations. There was no decrease in sperm progressive motility in CASA analysis and no increase in morphologic abnormalities was detected during the experiment; yet, no differences were found in plasma and acrosome sperm membrane integrity and mitochondrial function between the treatments US and AS. This confirms the absence of a testicular degenerative process due to heat stress during the experiment.

The integrity of sperm membrane is crucial for maintenance of its fertilizing capability, acrosome integrity as acrosome reaction is fundamental for sperm penetration into the zona pellucida and to oocyte plasma membrane fusion (OURA; TOSHIMORI, 1990; FLESCH; GADELLA, 2000). Finally, mitochondrial membrane potential, responsible for ATP production, is vital for flagellar beat and sperm motility (FLESCH; GADELLA, 2000). An alteration in spermatogenesis microenvironment can lead to greater reactive oxygen species production which alters sperm membrane stability (AITKEN; BAKER, 1995). If microenvironment changes occurred in seminiferous tubules in animals without shaded areas, certainly there would be an increase in sperm with damage in one of the cellular compartments, which would be detectable by the technique described by Celeghini et al. (2007).

Nichi et al. (2006) suggest that *Bos indicus* individuals have greater protection against lipid peroxidation generated by thermal stress compared with *Bos taurus.*

Greater resistance detected in *Bos indicus* to heat is not limited to adult animals. Lopes and Hansen (2003) observed that heat genetic adaptations are identified in cellular level in embryos from Brahman and Senepol breeds (*Bos indicus*). In this study, the exposure of embryos to 41°C for 6 hours reduced blastocyst development; however Brahman breed was the least affected.

However, it is worth noting that, the results obtained in the present experiment were from a region of the country that presented an average environmental temperature of 24.6ºC during the experimental period. It is possible that in other regions of Brazil, where average temperature may reach 10°C above that observed in this study, the effects of shadow unavailability on reproductive characteristics could be different.

Conclusion

With results obtained in this investigation, it can be concluded that the absence of shaded areas during summer does not negatively affect reproductive characteristics such as: scrotal circumference, testicular consistency, progressive motility, percentage of rapidly moving cells (CASA), morphology or sperm viability in Nellore bulls reared in extensive systems located in southeastern Brazil.

Acknowledges

The author thanks the PEC-PG Program and CNPq for the scholarship.

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