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Bioclimatic thermal stress indices and their relationships with andrological characteristics in hair rams

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Abstract

This study evaluated relationships among reproductive parameters and the bioclimatic indices: temperature and humidity index (THI), equivalent temperature index (ETI), black globe temperature and humidity index (BGTHI), and thermal comfort index (TCI), during the first 45 days of spermatogenesis (SP-45) and during the 15 days of sperm transit through the epididymis (STP-15) that preceded the reproductive assessments (ReA). Such information is useful in determining the optimal breeding season in Northeast Brazil. Santa Inês rams ($n = 25$) underwent two ReA in three periods of the year (D-P = dry; R-P = rainy and RD-P = rainy/dry transition), and the bioclimatic indices were calculated at the corresponding SP-45 and STP-15 timepoints prior to each ReA. Sperm kinetic parameters in D-P were depressed compared to R-P and RD-P ($P < 0.05$). The index values had an antagonistic relationship with most parameters and regression analysis demonstrated that the BGTHI and the TCI had a negative association with the progressive motility, curvilinear, straight line, and average path velocities, and a positive association with slow sperm in the ejaculate in SP-45 and STP-15 phases ($P < 0.01$). Semen quality kinetics is affected throughout the year by the environment and it is apparent that it is impaired in D-P and better in R-P and RD-P seasons. The BGTHI and TCI measured in the sperm production phase classified the environment most coherently and presented better association with the behavior of sperm kinetics.

Keywords Sperm kinetics · CASA · Semen · Sheep · Santa Inês

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Introduction

Heat is a major factor affecting animal reproduction and involves a combination of air temperature, humidity, and velocity of air and thermal radiation. Optimal conditions that are describe using these parameters are often referred to as the thermal comfort zone (Kingma et al. 2014; Cheng et al. 2022). Rams suffer heat stress when the environmental temperature deviates from the thermal comfort zone, negatively affecting their reproductive ability with a decrease in the amount of fertile sperm, and in extreme situations, damaging sperm in the epididymis and germinal epithelium (Marai et al. 2007; Van Wettere et al. 2021). Hair sheep breeds are more tolerant to heat stress due to their greater adaptability and efficiency in physiological thermoregulation compared to wool breeds (da Cruz Júnior et al. 2016; McManus et al. 2020). Despite greater tolerance of heat and heat stresses by hair rams, their semen quality is still affected by environments that are not in the thermal comfort zone (Kahwage et al. 2018).

More than ever, management and use of reproductive biotechnologies must be based on environmental conditions so that they show satisfactory results in favor of production, productivity, and sustainability of production systems. Thus, knowing the environmental stress and monitoring it throughout the year can be useful to improve reproductive performance of animals and define the best time to apply and adjust management and biotechnology techniques.

Such monitoring can be done by calculating the bioclimatic heat stress indices, which are estimated by meteorological measurement of the environment, and which have been studied and associated with thermal discomfort in animals of different species. The temperature and humidity index (THI), equivalent temperature index (ETI), black globe temperature and humidity index (BGTHI), and thermal comfort index (TCI) have been used to study the discomfort of animals, including sheep (Buffington et al. 1981; Barbosa and Silva 1994; Paim et al. 2012; Silva and Maia 2013). These indices are easier to calculate and implement in the workplace than other more complex indices which combine many environmental and physiological variables that require invasive measurements (Epstein and Moran 2006).

The advantages of THI and ETI are that they use easily accessible environmental variables, simple formulas, and are routinely used to monitor the environment in different regions of the world (Berman et al. 2016; Menegassi et al. 2016; Freitas et al. 2020). In contrast, BGTHI and TCI need more specific environmental variables for their calculation and are not always provided by meteorological stations. In addition, these indices include and consider thermal radiation in their formula, making it more suitable for evaluating extensive livestock production. (Neves et al. 2009; Paim et al. 2012).

Although correlations were found between the bioclimatic measurements and the quality of ram semen (Kahwage et al. 2018), no study has been conducted to understand the predictive nature of these indices with the reproductive potential of rams. Furthermore, the interaction of climate and spermatogenesis for a ram has not been evaluated from the perspective of these indices but should be considered because semen and sperm quality is a reflection of past events which come into being in the present moment. Therefore, we hypothesized that because spermatogenesis takes approximately 60 days in rams, monitoring heat stress via bioclimatic indices over multiple seasons would provide an understanding of ram reproductive capacity and quality.

Material and methods

Duration and site of experiments

This study was carried out over 12 months (2014–2015 years) in the Pedro Arle Experimental Field (CEPA) of Embrapa Tabuleiros Costeiros (Embrapa Coastal Tablelands), located in Frei Paulo city (Longitude: 37º 38′ 30.41″ West, Latitude: 10º 36′ 12.63″ South), Sergipe state, Brazil. The site has a photophase amplitude throughout the year, ranging from 12h46’on the summer solstice (11h14’ of darkness) to 11h29’ on the winter solstice (12h31’ of darkness), showing a difference of 1h17’ from the longest day in relation to the shortest day of the year. CEPA is located in the sub-region characterized as a transition zone between the Caatinga (stunted rather sparse forest exclusive to Brazilian territory that is leafless in the dry season and is widespread in areas of small rainfall in the northeast) according to AB’Sáber (1977) and the Atlantic Forest biomes which is in an area classified as a tropical savanna climate, according to Köppen (1936). According to the Instituto Nacional de Meteorologia (INMET 2023 – Brazillian National Institute of Meteorology), the historical records of annual average temperature and humidity of the 5 years that preceded this study (2010 to 2014) were as follows: 29.69 ºC and 72.28% (dry period = January to April); 26.12 ºC and 75.34% (rainy period = May to August) and 28.22 ºC and 73.25% (rainy/dry transition period = September to December).

Animals and handling

Twenty-five sexually mature Santa Inês rams were selected after being tested for libido by exposing them to ewes in heat. They had a live weight average of 65.87 ± 8.32 kg, a body condition score of 3.5 ± 0.5 on a scale of 0 to 5, and an age average of 48.65 ± 15.23 months. The rams were reared in a semi-intensive management system in which they were released at 0700 h and remained in the open field until 1600 h, daily. In the field they consumed native and cultivated fodder comprised of pangola grass (Digitaria decumbens L), star grass (Cynodon nlemfuensis), buffel grass (Cenchrus ciliares), and Panicum maximum of the aruana and green-panic cultivars. After this period in the field, the rams remained in covered stables to spend the night in an area of 2.0 m^2/ram from 1600 to 0700 h, where they received 300 g/animal/day of concentrate (18% crude protein) composed of soybean meal (21%), corn meal (77%) and limestone (2%). In the dry period of the year with little availability of forage in the field, in addition to the concentrate, the rams were supplemented with 2.0% of the live weight of corn silage (29.26% dry matter, 7.51% crude protein, 38.13% FDN, 4.47% FDA, 1.66 g/kg Ca and 2.03 g/kg P). During the entire experimental period, the animals had access to mineral salt and water ad libitum.

Reproductive assessments

Reproductive assessments (ReA) consisted of clinical-andrological and seminal analyses for each animal every 60 days, which is enough time for spermatogenesis to occur and to have a new sperm cohort (Brackett 2004). In total, six ReA were performed during the year, which were grouped in pairs, each pair representing one of three four-month periods as follows: D-P = dry period (January to April); R-P = rainy period (May to August) and RD-P = rainy/dry transition period (September to December). Thus, assessments of the 25 rams were performed every 2 months over a year, for a total of 150 assessments throughout the study.

Testicular biometrics

The rams were analyzed for testicular biometry by measuring the scrotal circumference (SC), circling both testicles at their greatest curvature with a tape measure. The testicular volume (TV) was obtained by measuring the testicular height (TH) and width (WD) with a caliper in the dorsoventral and lateromedial positions, respectively. Then, the TV of each gonad was obtained from the mathematical equation suggested by Bailey et al. (1998), considering the shape of the testicles: $TV = 2[4/3\ \pi\ (WD/2)^2\ (TH/2)]\ cm^3$, where: $\pi = 3.1416$.

Semen collection and assessment

Ram semen collection was performed using a heated artificial vagina (initial temperature of 45 ºC) and a ewe in natural estrus as a sexual stimulus. The ejaculates were immediately taken to the laboratory and kept in a water bath (37 ºC) until the seminal analysis was performed. The ejaculate volume (EV) was measured with the aid of a precision automatic pipette. Sperm concentration (SpC) was measured by adding an aliquot of semen to distilled water (1:400) and performing a sperm count with a Neubauer chamber and phase contrast microscopy at 400 × magnification.

The sperm morphology assessment was performed using a humid chamber. Semen samples (5 µL) were transferred to microtubes containing Dulbeco's solution (PBS) with 0.2% glutaraldehyde (500 µL at 37 ° C, Barth and Oko 1989). The sperm were analyzed using phase-contrast microscopy under oil immersion at 1000 × magnification. The morphology characteristics were observed for 200 cells, which were classified as either normal or having major (MaD) or minor (MiD) defects, according to the Brazilian College of Animal Reproduction (CBRA 2013).

Sperm kinetics were assessed using the Sperm Class Analyzer (SCA, Microptics, S.L. Version 5.1, Barcelona, Spain) set to default for rams but with a modification to the particle area from 3–70 μm^2 to 30–70 μm^2. An aliquot of semen was diluted in evaluation medium (Azevedo 2006) to a concentration of 20×10^6 sperm cells/mL and maintained at 37 ºC. Then, 2 µL of the diluted sample was pipetted into the Makler chamber (Sefi-Medical Instruments, Haifa, Israel) preheated at 37° C for image capture. Images were captured using a video camera (Basler Vision Technologies 602FC, Ahrensburg, Germany) connected to a phase-contrast microscope (Nikon 50i, Japan) at 100 × magnification. The following sperm kinetics parameters were analyzed: TM = total motility, PM = progressive motility; VCL = curvilinear velocity; VSL = straight line velocity; VAP = average path velocity; LIN = linearity; STR = straightness; WOB = wobble index; ALH = amplitude lateral head displacement; BCF = beat cross frequency; HYP = hyperactivity. The analysis of sperm subpopulations was performed according to the following parameters and criteria: RAP = rapid (VCL ≥ 200 µm/s); MED = medium (VCL ≥ 100 to 200 < µm/s); SLO = slow (VCL ≥ 20 to 100 < µm/s); STA = static (VCL < 20 µm/s).

Environmental assessment and calculation of bioclimatic indices

Air temperature (At), dew point temperature (Dpt), relative humidity of the air (Rh), wind speed (W) and thermal radiation (TR) were collected every hour throughout the year of the experiment at the CARIRA automatic weather station of Brazil's Instituto Nacional de Meteorologia (National Meteorology Institute—INMET 2014 – 2015), located in the same region as CEPA (Longitude: 37° 44′ 51.011″, Latitude: 10° 23′ 58.988″), and 21.4 km away from where the animals were kept (Table S1). From those variables, calculations were made to obtain the black globe temperature (Bgt), validated in the Northeast region of Brazil (da Silva et al. 2019), and the partial pressure of water vapor (Ppwv). The daily environmental variables were then used to compute the bioclimatic temperature and humidity index (THI), equivalent temperature index (ETI), black globe temperature and humidity index (BGTHI), and thermal comfort index (TCI) using the equations from Table S2. The bioclimatic indices were specifically determined using the values of the environmental variables collected every hour during the SP-45 and STP-15 phases. The SP-45, which represented the environmental data collected in the 45 days between the 60th and the 16th day that preceding the reproductive assessments, was indicative of the spermatogenesis phase (Cardoso and Queiroz 1988). The STP-15 assessments, which utilized environmental data collected during the 15 days between the 15th and 1st day that preceded the reproductive assessments, represented the sperm transit phase through the epididymis (Holt and Morrell 2013).

For the assessment of the environmental data, the values of the bioclimatic indices were stratified in their respective scales, and used to determine the occurrence or non-occurrence of different levels of stress by grade as follows: THI: <22.3, absence; ≥ 22.3 to $23.3 <$, moderate; ≥ 23.3 to ≤ 25.6, intense; > 25.6, extremely severe (Marai et al. 2007); ETI: ≥ 18 to ≤ 27, normal; > 27 to ≤ 32, caution; > 32 to ≤ 38, extreme caution; > 38 to ≤ 44, hazard; > 44, extreme hazard (Silva and Maia 2013); BGTHI: ≤ 74, comfortable environment; > 74 to ≤ 79, environment under alert; > 79 to ≤ 84, hazardous environment; > 84, emergency (Eigenberg et al. 2007); and TCI: ≤ 38, absence; > 38 to ≤ 45 uncomfortable environment; > 45, critical environment (Neves et al. 2009). From those interpretations, the frequency with which the values of the indices reached their respective scales was calculated.

Statistical analysis

Statistical analysis of the data was performed using SAS 9.4. Assumptions of normality and homoscedasticity of the data from the reproductive assessments that was used for parametric statistical analysis were verified by the Shapiro–Wilk ($P > 0.05$) and Hartley (Hc > H) tests and, when necessary, exponentiated. Data that met the assumptions of parametric statistics before or after the transformation were evaluated using a general linear mixed model from the PROC MIXED package. The statistical model associated with the analysis of the variables studied was represented by $Yijk = \mu + Ti + \beta j + T\beta ij + \gamma k + \varepsilon ijk$ where Yijk is the dependent variable, μ is the mean of all observations, the fixed effects are the evaluation date represented by Ti, the age of the ram represented by βj, the interaction between the evaluation date and the age of the ram represented by Tβij, the random effect is the animal (ram) represented by γk, and the experimental error represented by εijk. The means of the variables were compared using the Tukey test.

Variables that did not meet the assumptions of the parametric statistics even after the transformation were analyzed with nonparametric statistics using the Kruskal–Wallis test and, for multiple comparisons, Dunn's post-hoc test. Pearson's and Spearman correlation analyses ware used to correlate the bioclimatic indices with the reproductive parameters, and to determine their relationship. The regression adjusted by the PROC GLM package was used with the following model: $Y = \alpha + \beta + \alpha\beta + \gamma + \mu$ where Y is the evaluation parameter (dependent variable), α is the value of the bioclimatic index, β is the age of the ram, αβ is the interaction between the value of the bioclimatic index and the age of the ram (fixed effects), γ is the ram (random effect), and μ is the mean of the observations. For all analyses, a significance level of 5% ($P < 0.05$) was considered.

Results

In this study, the highest mean environmental values for At (ºC), W (m/s) and TR (w/m^2) and lowest Rh (%) were obtained in the D-P (Table S1). Table 1 presents the results of the bioclimatic indices from SP-45 and STP-15, which preceded the reproductive assessments. The average frequency with which the values of the bioclimatic indices reached levels of heat stress in the three periods of the year are shown in Figure S1.

The THI, ETI, BGTHI and TCI had higher means in D-P compared to R-P and RD-P ($P < 0.05$). On the other hand, taking into account only SP-45, the lowest THI and ETI were observed in RD-P ($P < 0.05$) and the lowest BGTHI and TCI were obtained in both R-P and RD-P ($P > 0.05$). The lowest THI, BGTHI and TCI in STP-15 were observed in R-P ($P < 0.05$). The ETI in turn presented lower values at STP-15 both in the R-P and in the RD-P in relation to the D-P ($P < 0.05$). In general, the maximum values of all bioclimatic indices observed in SP-45 and STP-15 were higher in D-P compared to R-P and RD-P. Except for ETI in STP-15, all other bioclimatic indices in SP-45 and STP-15 had the lowest minimum values in R-P and RD-P. The highest frequency with which the values of the bioclimatic indices reached the scales of stress considered extreme by the THI, precaution by the ETI, emergency by the BGTHI, and critical by the TCI were observed in D-P compared to R-P and RD-P.

The results of the reproductive assessments are presented in Table S3. The averages of the clinical-andrological parameters (SC, TV) and those related to the semen (EV and SpC) did not vary across the seasonal periods ($P > 0.05$). A few kinetic parameters (STR, WOB and MED) and sperm morphological parameters (MaD and MiD) did not differ ($P > 0.05$) across seasons. All other sperm kinetic parameters

Table 1 Bioclimatic indices throughout the three periods of the year measured in the phases that comprised spermatogenesis (SP-45) and sperm transit in the epididymis (STP-15) occurring before the reproductive assessments of Santa Inês rams

Bioclimatic index – sperm production phase	Periods of year						Mean ± SE	*P-valor*
	D-P		R-P		RD-P			
	Mean ± SE	Min–Max	Mean ± SE	Min–Max	Mean ± SE	Min–Max		
THI – SP-45	25.51 ± 0.05 a	19.72–32.18	23.03 ± 0.05 b	15.78–30.90	22.48 ± 0.06 c	14.99–31.19	23.67 ± 0.05	< 0.001
ETI – SP-45	26.81 ± 0.08 a	18.61–38.41	25.56 ± 0.08 b	14.52–31.04	23.93 ± 0.08 c	13.08–35.24	25.43 ± 0.08	< 0.001
BGTHI – SP-45	84.05 ± 0.14 a	69.55–96.91	78.79 ± 0.16 b	59.96–93.18	79.08 ± 0.17 b	61.09–93.99	80.64 ± 0.16	< 0.001
TCI – SP-45	42.23 ± 0.26 a	25.80–56.49	35.51 ± 0.22 b	18.68–52.98	35.70 ± 0.23 b	19.18–54.33	37.81 ± 0.24	< 0.001
THI – STP-15	26.30 ± 0.11 a	21.01–31.70	22.53 ± 0.11 c	16.90–27.28	23.54 ± 0.11 b	17.74–29.45	24.12 ± 0.11	< 0.001
ETI – STP-15	28.33 ± 0.16 a	14.34–36.16	25.21 ± 0.18 b	16.58–31.91	24.81 ± 0.13 b	18.12–31.24	26.12 ± 0.16	< 0.001
BGTHI – STP-15	83.67 ± 0.31 a	69.23–94.39	77.78 ± 0.28 c	62.48–85.95	81.57 ± 0.27 b	67.06–90.83	81.01 ± 0.29	< 0.001
TCI – STP-15	41.28 ± 0.46 a	26.81–53.54	33.46 ± 0.39 c	20.14–41.85	39.09 ± 0.42 b	24.22–49.24	37.95 ± 0.42	< 0.001

THI Temperature and humidity index; *ETI* Equivalent temperature index; *BGTHI* Black globe temperature and humidity index; *TCI* Thermal comfort index; *D-P* Dry period; *R-P* Rainy period; *RD-P* Rainy/dry transition period. *SE* Standard error; *Min* Minimum; *Max* Maximum. Values with different letters in the same row differ significantly ($P < 0.05$)

varied throughout the seasons of the year ($P < 0.05$). The MT, VCL, VSL, VAP and ALH parameters had lower averages in D-P when compared to R-P and RD-P ($P < 0.05$), which did not differ among themselves for those parameters ($P > 0.05$). PM had a lower average in D-P compared to RD-P ($P < 0.05$), which was not different from R-P ($P > 0.05$). LIN, BCF and HYP averages in D-P were lower than in RD-P ($P < 0.05$) and those in R-P did not differ from the other two periods of the year ($P > 0.05$). In D-P, lower RAP and higher SLO and STA averages were obtained in relation to R-P and RD-P ($P < 0.05$).

The results of the correlation between the bioclimatic indices and the reproductive parameters are shown in Table S4. No correlations were observed between the bioclimatic indices measured in SP-45 and STP-15 and the SC, TV, EV, SpC, MED and MaD parameters ($P > 0.05$). THI and ETI were negatively correlated ($P < 0.05$) with VCL, VSL, VAP ($r > 0.30$), WOB and BCF ($r \leq 0.30$), and positively correlated with STA ($r > 0.30$) in SP-45 and STP-15. In addition, THI was negatively correlated ($P < 0.05$) with, TM and PM in SP-45 ($r > 0.30$) and in STP-15 ($r \leq 0.30$), LIN, SRT in SP-45 ($r \leq 0.30$), ALH and RAP in SP-45 and STP-15 ($r > 0.30$), HYP in SP-45 ($r > 0.30$), HYP and MiD in STP-15 ($r \leq 0.30$), and positively correlated ($P < 0.05$) with SLO in SP-45 and STP-15 ($r \leq 0.30$). ETI was a negatively correlated ($P < 0.05$) with, TM, ALH and RAP in SP-45 ($r \leq 0.30$) and in STP-15 ($r > 0.30$), LIN in SP-45 and STP-15 ($r \leq 0.30$), SRT in SP-45 ($r \leq 0.30$), HYP in SP-45 and STP-15 ($r > 0.30$), and positively correlated ($P < 0.05$) with SLO in SP-45 ($r \leq 0.30$) and in STP-15 ($r > 0.30$). BGTHI and TCI were negatively correlated ($P < 0.05$) with TM, PM, VCL, VSL, VAP, ALH, HYP, RAP ($r > 0.30$), WOB, LIN and BCF ($r \leq 0.30$) in SP-45, and in STP-15 with MT, VCL, VSL, VAP, ALH and MiD ($r < 0.30$) and RAP ($r > 0.30$). BGTHI and TCI were positively correlated ($P < 0.05$) with SLO in SP-45 ($r > 0.30$) and in STP-15 ($r \leq 0.30$), and with STA in SP-45 and STP-15 ($r > 0.30$).

Use of regression analysis revealed the effect ($P < 0.05$) of the bioclimatic indices measured during SP-45 and STP-15 on reproductive parameters (Table S5). The parameters generated from the clinical-andrological (SC, TV), semen (EV, SpC) assessments, some of the sperm kinetics (STR, WOB, MED), and morphology (MaD, MiD) assessments that did not demonstrate variation in the evaluated periods ($P > 0.05$) were also not influenced by the bioclimatic indices assessed in SP-45 and STP-15 ($P > 0.05$).

Based on the regression analysis, we can see that the PM had negative and linear association ($P < 0.01$) with the THI in SP-45 ($R^2 = 0.17$) and with ETI in SP-45 ($R^2 = 0.20$) and STP-15 ($R^2 = 0.16$). The PM had a quadratic association with THI in STP-15 ($R^2 = 0.20$), and with BGTHI and TCI in SP-45 ($R^2 = 0.17$ and 0.20 respectively) and STP-15 ($R^2 = 0.20$). The VCL, VSL and VAP associated linearly and negatively ($P<0.01$) with THI in STP-15 ($R^2=0.16$, 0.10 and 0.14 respectively). They associated in the same way with BGTHI and TCI in SP-45 ($R^2=0.12$ to 0.17) and STP-15 ($R^2=0.03$ to 0.09). The HYP had negative and linear association ($P<0.01$) with THI in SP-45 ($R^2=0.08$) and with ETI in SP-45 and STP-15 ($R^2=0.11$). The SLO associated linearly and positively ($P<0.05$ to 0.01) with all bioclimatic indices in the different phases evaluated ($R^2=0.04$ to 0.09).

Discussion

The interaction between high air temperature, low humidity and high intensity of thermal radiation over a prolonged time, like D-P, is known to create a stressful environment for animals and this is the basis for all bioclimatic indices. Moreover, the vulnerability of the animal to heat stress is the product of the interaction between the magnitude and frequency of exposure to extreme environments, which can ultimately negatively affect reproductive physiology (Ebi et al. 2009). However, the applicability and interpretation of the indices is not universally understood or accepted. As an example, despite a comfort air temperature for hair sheep of up to 30 °C being reported (Kahwage et al. 2018), crossbred hair rams raised in an extensive livestock production in a semi-arid environment with air temperatures of 26 to 32 ºC showed a high frequency of degenerative changes in the testicle and epididymis that negatively affected their fertility potential (Costa et al. 2007). Likewise, in the current research, the high means and frequencies in which the stressor levels of the THI, BGTHI and TCI indices were obtained in the spermatogenesis (SP-45) and sperm transit (STP-15) phases, indicated that the environment of the D-P period is a stressor for rams. In addition, the high air temperature mean recorded in D-P (26.42 ºC) was pivotal in making the environment a stressor for the rams in this season. Contrary to this, the ETI classified the environments of these sperm phases during the same period as safe and precautionary, which exemplifies the point that identification of an appropriate model or further refinement of these bioclimatic models is necessary.

However, regardless of the bioclimatic model used, the impact of stressful environments on rams is well documented. Studies with hair rams raised in environments of intense stress like those presented in the SP-45 and STP-15 of the D-P classified by THI, showed an increase in sperm with minor morphologic defects in the Morada Nova breed and decrease in the number of sperm with mitochondrial potential in the Santa Inês breed (Kahwage et al. 2018). The environment represented by the THI with moderate to extreme stress had a negative influence, reflected in a low motility and viability of the spermatozoa in the semen of Kivircik rams, in relation to the control group kept in an environment without stress (Küçük and Aksoy 2020). Likewise, environments of heat stress classified on the same scale (emergency/BGTHI and uncomfortable/TCI) in our study, demonstrated a negative reproductive physiology influence on the animal. To combat heat stress, the animal directs the highest energy consumption to the respiratory system as a compensatory mechanism for heat loss. This kind of energy redirection/imbalance results in reduced glucose, cholesterol, and increases (cortisol) and decreases in hormone levels, compromising performance, decreasing productivity and negatively affecting the reproduction in Santa Inês and Morada nova hair sheep (Wagner et al. 2008; Neves et al. 2009; Costa et al. 2015; Tüfekci and Sejian 2023). Furthermore, it has been demonstrated that the energy and hormonal imbalance resulting from heat stress negatively affects the reproductive system of rams with a reduction in the kinetic quality of sperm (De et al. 2017). Thus, reproductive capacity is reduced by the fact that rams direct the use of energy to maintain homeotherm by activating thermoregulatory mechanisms such as body cooling, mainly by the respiratory and cutaneous systems of rams (Sejian et al. 2017; Morrison and Nakamura 2019) . Therefore, we hypothesize that this redirection of energy and consequential alteration of physiologic (metabolic and endocrine) balance manifested as the decrease in the quality of the sperm kinetic parameters TM, VCL, VSL, VAP, ALH, RAP, SLO and STA in D-P observed during SP-45 and STP-15.

Santa Inês rams are considered adapted to arid environments due to their resistance to heat stress when compared to sheep of wool breeds (McManus et al. 2020), but even these animals also need to make physiological changes when subjected to environments of high thermal stress for prolonged time that can impair their reproductive capacity. When subjected to periods of high heat stress, Santa Inês rams showed a reduction in semen quality with less progressive motility and less integrity of sperm plasma membranes (Kahwage et al. 2018).

Rams of the Santa Inês and Morada Nova hair breeds, when submitted to the emergency environment classified by the BGTHI, increased their respiratory rate from normal values (25 – 30 mov min^{-1}) to up to 65 mov min^{-1}, which is considered a reflex of moderate stress according to Silanikove (2000) (Titto et al. 2016). Morada Nova sheep submitted to a dry period in a stressful environment by the emergency classification of the BGTHI showed a decrease in the thyroid hormones triiodothyronine (T3) and thyroxine (T4) (Costa et al. 2015). The reduction of thyroid hormones decreases sperm motility (Vaghela et al. 2016), by reducing the cellular metabolic reaction and oxygen consumption in situations of stress (Agarwal 2011), which brings with it increased levels of reactive oxygen species (ROS) harmful to the membrane structure and mitochondrial sheath protein units that respectively ensure stability and energy for sperm motility (Nissen and Kreysel 2009). The increase in ROS due to bioclimatic stress has a negative effect on mitochondrial activity and the yield of ATP synthesis, which involves the dephosphorylation of glycogen synthase kinase-3α (GSK3α) and the interference of mitochondrial remodeling directly related to sperm motility (Gong et al. 2017). These physiological effects, such as an increase in ROS, may be increased in the semen of the rams collected during D-P, and consequently reducing sperm kinetics as observed in our results. We hypothesize that bioclimatic stress during D-P may also have negatively influenced sperm motility in the maturation phase in the epididymis because sperm located in the epididymis increase their levels of oxidative stress and decrease their antioxidant capacity in response to continuous and prolonged exposure to heat stress (Hamilton et al. 2016; Capela et al. 2022).

Our results substantiated the findings that link bioclimatic stress with reproductive performance and potential. In D-P there was a significant reduction in semen quality with a decrease in kinetic parameters relevant to reproduction such as TM, VCL, VSL and VAP observed in R-P and RD-P. Additionally, PM showed a significant reduction in D-P when compared to RD-P. Similarily, the semen of Santa Inês, Dorper, and crossbred rams demonstrated lower values of TM, VCL and VAP in the dry period in relation to those obtained in the rainy period (Frazão Sobrinho et al. 2014). In addition, Dorper rams raised in a semi-intensive system and INRA180 rams reared in a semi-arid environment similar to our study, showed a 25% reduction in progressive motility and low values of mass and individual sperm motility, respectively, compared to those submitted to environments without stress (Panyaboriban et al. 2016; Amiri et al. 2020).

The present study showed that the BGTHI and TCI indices had a better association with the behavior of sperm kinetics (PM, VCL, VSL, VAP and SLO), and classified the environment more congruently as a possible stressor in SP-45 and STP-15 compared with the THI and ETI indices. Despite the association between PM and SLO parameters with THI, and PM, HYP and SLO parameters with ETI, these indices were not congruent with the classification of the environment with the spermatogenesis and sperm transit phases. Antagonistic correlations ($r > -0.30$) between parameters PM, VCL, VSL and VAP and all bioclimatic indices obtained in SP-45, and correlation between VCL, VSL and VAP with THI and ETI ($r > -0.30$) and BGTHI and TCI ($r < -0.30$) obtained in STP-15 suggest that environmental stress during these periods negatively affects the quality of sperm kinetics. These kind of antagonist correlations were also observed for total motility ($r \leq -0.15$) and major defects ($r \leq -0.46$) of spermatozoa using THI and ETI indices, and likewise were observed during the 18 day spermatogenesis and 12 day epididymal transit phases of bull semen (Menegassi et al. 2016).

As can be seen in these results, the low linear association between THI values and sperm kinetics behavior may be due to the environment of the Agreste region of the Brazilian Northeast, the location in which the work was carried out, which presents an inverse association between humidity and temperature. According to Berman et al. (2016) the greater sensitivity of the THI in the classification of environmental stress is obtained in areas of high humidity and temperature. The lower association between ETI and sperm kinetic parameters in turn can be explained by the considerations made by Hahn et al. (2009) that this index can provide representative results for short-term heat challenges limited to 3 days, but that its average value, measured over long periods, as was the case with the SP-45 in our work, cannot truly reflect the state of environmental stress to which the animals were subjected.

Even though the stressor environment scales were reached during R-P and RD-P according to some of the indices, the quality of sperm kinetics was not significantly affected and was better in these periods in relation to D-P. Isolated environmental changes can be compensated for by animals to mitigate the effects of heat stress over short periods of time. The effects of bioclimatic stress on the testes can be minimized by altering its position relative to the abdomen and scrotum, increasing and decreasing scrotal surface area, and increasing sweating and respiratory rate to cool the spermatic cord, arteries, and venules of the pampiniform plexus (Cottle 2010). It is also possible that during short periods of heat stress, such as during R-P and RD-P, rams activate mechanisms to counteract the negative effects of heat such as the release of antioxidant enzymes (e. g. heat shock proteins, HSPs) present in seminal plasma in order to maintain sperm quality (Ji et al. 2012). According to Singh et al. (2017) HSP70 is differentially expressed depending on the time of year, and its concentration in plasma is higher in the most adapted breeds, such as the Santa Inês breed used in this work, which has a high degree of adaptability to tropical conditions (McManus et al. 2020) and to the place of its origin and selection in the Brazilian Northeast. Thus, it is likely that HSP70 was expressed at a sufficient level in R-P and RD-P to overcome the bioclimatic stress in our Santa Inês rams but may not have been sufficient during the D-P.

Based on the results obtained in the present study, and despite the fact that fertility trials with ewes were not performed, it is recommended that collection and use of semen in any form (cooled or frozen), from Santa Inês rams in the sub-region Agreste of the Brazilian Northeast is avoided during the dry period of the year or other periods with the same environmental characteristics. The bioclimatic characteristics of these periods impair sperm kinetics and consequently reduce the semen quality of these animals. Furthermore, we suggest that based on these results that all sheep producers, regardless of location, perform a similar analysis to determine their seasons for optimal and suboptimal ram production based on bioclimatic stress which will increase the productivity of their flocks.

Conclusions

The bioclimatic indices THI and ETI are less effective than BGTHI and TCI when classifying the impact of the environment on most ram sperm kinetic parameters from Santa Inês sheep. Furthermore, BGTHI and TCI are more effective at predicting the overall consequences of bioclimatic heat stress on the reproductive capacity of Santa Inês rams. In addition, the BGTHI and TCI indices were most effectively used to demonstrate the influence of bioclimatic stresses on the semen quality of Santa Inês rams during the dry period compared with the rainy and wet/dry transition periods of the year in the Agreste region of Northeast Brazil.

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Data availability The authors declare the availability of data to the manuscript "Bioclimatic thermal stress indices and their relationships with andrological characteristics in hair rams", when requested by the scientific journal International Journal of Biometeorology.

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Conflict of interest The authors declare that there is no conflict of interest in relation to the manuscript entitled "Bioclimatic thermal stress indices and their relationships with andrological characteristics in hair rams".

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