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REGULAR ARTICLES

Evaluation of thermal comfort, physiological, hematological, and seminal features of buffalo bulls in an artificial insemination station in a tropical environment

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Abstract This study aimed to assess the variation over time in thermal comfort indices and the behavior of physiological parameters related to thermolysis, blood parameters, and semen *in natura* of buffalo bulls reared in tropical climate. The study was carried out in an artificial insemination station under a humid tropical climate (Afi according to Köppen). Ten water buffalo bulls (*Bubalus bubalis*) were used during the 5 months (April to August) of study. The environmental Temperature Humidity Index (THId) and the pen microclimate Temperature Humidity Index (THIp) were calculated. Every 25 days, respiratory rate (RR), heart rate (HR), rectal temperature (RT), and Benezra's thermal comfort index (BTCI) were assessed in the morning and in the afternoon. A blood assay was performed every month, while semen was collected weekly. THIp did not vary over the months ($P>0.05$) and was higher in the afternoon than in the morning (77.7±2.6 versus 81.8±2.1, $P<0.05$). RR, HR, and BTCI significantly increased over the months and were different between the periods of the day ($P>0.05$) but within the physiological limits. RT varied between the periods of the day and decreased over the months, being the lowest in August (37.8±0.7 °C), time-impacted hematocrit, mean corpuscular volume, hemoglobin levels, and spermatic gross motility and vigor ($P<0.05$). Thus, buffalo bulls reared under a humid tropical climate may have variations in thermal comfort during the hotter periods but are able to efficiently activate thermoregulatory mechanisms and maintain homeothermy, hence preserving their physiological and seminal parameters at normal levels.

Keywords Animal welfare · *Bubalus bubalis* · Environmental effect · Hematological parameters · Semen

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Introduction

Raising water buffalo (*Bubalus bubalis*) significantly contributes to food production and traction force, mainly in developing countries (Singh et al. 2013). Currently, the worldwide domestic buffalo herd is of approximately 195.2 million animals, growing by 16 % between 2002 and 2011 (FAO 2011). Around 85 % of buffaloes are reared in intertropical areas, which are characterized by high-mean temperatures during most of the year and often high relative air humidity (Garcia 2013).

It is known that the climate is one of the main factors affecting animal productivity (Marai and Haeeb 2010) and that heat stress has the greatest negative impact since it challenges the animals' ability to maintain their energy, hormone, and mineral balances (Singh et al. 2012). Given the climate change and gradual global warming scenarios, research on the resilience and possible adaptation of animals to these new conditions are required, particularly in tropical and subtropical countries, in which the mean air temperature is expected to rise the most over the next decades (IPCC 2013). Despite being considered rustic animals, water buffaloes show signs of heat stress when subjected to high air temperature and relative air humidity and intense direct solar radiation (Koga et al. 2004; Gudev et al. 2007a).

In buffaloes, as well as in bovines, testicular temperature must be kept 2 to 6 °C below body temperature since higher testicular temperatures mean lower semen quality (Kastelic 2014) and poorer spermatogenesis (Koonjaenak et al. 2007). In buffaloes, lower body heat dissipation, even over short periods, may lead to testicular degeneration and lower semen quality (Garcia et al. 2010). Thus, the effects of the environment on the thermal comfort and physiological indices of buffalo bulls must be investigated, especially on animals kept in artificial insemination stations.

Therefore, this study aimed to assess the variation over time (April to August) in thermal comfort indices and the behavior of physiological parameters related to thermolysis, blood parameters, and semen *in natura* of buffalo bulls reared in tropical climate. These results will provide useful physiological information for decision-making in managing breeding buffaloes, especially regarding their thermal comfort.

Materials and methods

Experimental site and period

The trial followed the bioethics norms in animal experimentation, and the protocols adopted were previously approved by the Internal Technical Committee of Embrapa Eastern Amazon, Brazil. The study was carried out at the Center of Animal Reproduction Biotechnology (CEBRAN/UFPA), in Castanhal, PA, Brazil (1° 18′ 18″ S and 47° 56′ 36″ W). The site has megathermal humid tropical climate (Afi according to Köppen) with annual rainfall between 2300 and 2800 mm and monthly rainfall from 67 to 399 mm (Alvares et al. 2013; Valente et al. 2001). The experimental period comprised the months of April to August 2013 for a total of 22 weeks.

Animals and handling

Ten clinically healthy Murrah buffalo bulls (*Bubalus bubalis*) aged 55.0±8.9 months and weighing 701.4±82.8 kg were used (mean ± standard deviation). The animals were kept in collective pens with concrete floors and wire rope sides (18 m^2/animal). The pens' perimeter fences had forest species (*Ficus benjamina*) planted in lines spaced 8 m apart. The trees were approximately 14 m tall and had dense foliage, projecting 7 m^2 of shade/animal over the pens throughout the day. The animals adapted to the intensive handling and to the feed for 30 days prior to the beginning of the experiment. Feed was provided twice a day (8:00 and 16:00) and was made up of ground forage (*Pennisetum purpureum*) and concentrate based on bean residues (30 %), wheat meal (68.5 %), and mineral mix (1.5 %). Roughage was offered ad libitum, and the daily concentrate was equivalent to 1 % of the animals' live weight. The animals had free access to the feeding and drinking troughs.

Environmental data collection and Temperature Humidity Index calculation

Temperature, relative air humidity, rainfall, and solar radiation data were obtained by an automated meteorological station (1° 18′ 03″ S and 47° 56′ 51″ W) placed about 700 m away from the pens for climate characterization. Three data loggers (HOBO® U12-012, Onset Computer Corporation, Bourne, MA, USA) suspended 2.0 m above the soil in meteorological shelters protected from direct sunlight and water were used to characterize the temperature (°C) and relative air humidity (%) inside the pens (Mader et al. 2007; Malama et al. 2013). The data were recorded and stored by the data loggers every 15 min and were transferred by proprietary software (HOBOware® Lite 3.1.0, Onset Computer Corporation, Bourne, MA, USA). The Temperature Humidity Index was calculated according to the following formula: THI=(0.8Ta)+(RH/100) x (Ta-14.4)+46.4, where Ta is air temperature in a dry-bulb thermometer (°C) and RH is the air relative humidity (%) (Thom 1959). The daily THI (THId) was calculated throughout the experimental period using the temperature and relative air humidity values from the meteorological station. To calculate the pen microclimate THI (THIp) and later the correlation with the animals' physiological data, the means of the values recorded by the data loggers were used regarding the periods of the day when the physiological parameters were measured (morning: 6:00 to 9:00 a.m.; afternoon: 12:00 to 15:00 p.m.).

Physiological parameter collection and Benezra's thermal comfort index calculation

In order to measure the physiological parameters, the animals were led individually and gently to the squeeze chute located 50 m away from the pens. Respiratory rate (RR, mov/min), heart rate (HR, beats/min), and rectal temperature (RT, °C) were measured, in this order, in the morning (6:00 to 9:00

a.m.) and afternoon (12:00 to 15:00 p.m.). RR was determined by observing the thoracoabdominal region and counting the respiratory movements (mov/min). HR was determined by auscultating and counting the heart beats (beats/min). RT was measured using a veterinary clinical thermometer (Rosenberger et al. 1993). The physiological parameters were measured in seven campaigns on average 25 days apart for two consecutive days in the morning and afternoon for a total of 28 collections per animal (10 animals × 7 campaigns × 2 days × 2 periods = 280 collections). Benezra's thermal comfort index (BTCI) was determined using the following formula: BTCI=RT/38.33+RR/23, where RT is rectal temperature (°C) and RR is respiratory rate (mov/min). Values ≤2.0 represented a high degree of adaptability of the animal to the environment (Benezra 1954).

Blood collection and hematological analysis

Blood was collected once a month for each animal for a total of five samples per animal over the experiment (10 animals × 5 months = 50 samples). Blood was collected through jugular venipuncture into vacuum tubes containing anticoagulant (EDTA at 5 %). The samples were cooled and immediately transported and processed. The blood assays were performed using a BC-2800Vet® (Shenzhen Mindray Bio-Medical Eletronics, Nanshan, Shenzhen, China) automated counter, which counts red blood cells (RBC, $\times 10^6/\mu L$), leukocytes (Lc, $\times 10^9/L$), and platelets (Pt, $\times 10^3/\mu L$) through electric impedance, determines hemoglobin (Hb, g/dL) through the cyanmethemoglobin method, and calculates hematocrit (Ht, %) and the hematimetric indices of mean corpuscular volume (MCV, fl), mean corpuscular hemoglobin (MCH, pg), and mean corpuscular hemoglobin concentration (MCHC, %). The differential of leukocytes in lymphocytes (Lf, %), neutrophils (Nt, %), eosinophils (Eos, %), and monocytes (Mon, %) were performed in blood smears stained using rapid panoptic staining, and the results were expressed as percentages (França et al. 2011).

Semen collection and assessment

Ejaculates were collected weekly from the ten bulls during the 22 weeks of the trial, for a total of 220 ejaculates (10 animals × 22 weeks = 220 samples). Semen was collected using an artificial vagina and the ejaculates underwent physical and morphological evaluation. Volume (Vol, mL), spermatic concentration (Conc, $\times 10^9$ sptz/mL), gross motility (GM, 0–5), progressive motility (Mot, %), and vigor (Vig, 0–5) were assessed according to Vale (2002). The supravital staining technique with eosin-nigrosin, according to Khan and Ijaz (2008), was used to assess the plasma membrane integrity (PMI, %). Two hundred cells were analyzed per sample and the result was expressed as the percentage of cells with intact plasma membrane. The analysis technique and classification described by Blom (1973) were used to evaluate sperm morphology by counting 200 spermatozoa per sample. The defects observed were classified as minor defects (DMi, %) or major defects (DMa, %), whose sum corresponded to the total defects (DT, %).

Statistical analysis

The statistical treatment for the physiological, seminal, and blood parameter data from the ten bulls were descriptive statistics (mean ± standard deviation, minimum, and maximum) of all variables studied, normality test, and for the variables with non-normal distribution or with high coefficients of variation, transformations to the logarithmic scale were performed. For the physiological parameters (THIp RR, HR, RT, and BTCI), analysis of variance was adopted using procedure GLM of the software SAS version 9.3 (SAS 2011), considering in the model the effects of period of the day (morning and afternoon) and of the month (April to August). For the seminal parameters (Vol, Conc, GM, Vig, Mot, PMI, DMi, DMa, and DT) and blood parameters (RBC, Ht, Hb, MCV, MCH, MCHC, Pt, Lc, Lf, Nt, Eos, and Mon), the data underwent analysis of variance considering only the effect of the months. *F* test was used to compare the means between periods of the day, while Tukey's test was employed in multiple comparisons of the monthly averages. Pearson's correlation coefficient test was used to analyze the correlation between parameters (Garcia 2004) and only statistically significant correlations with bioclimatological indices were discussed. All statistical analyzes adopted a level of significance of $P<0.05$.

Results

The mean maximum air temperature was 31.5±0.8 °C, being ≥32.0 °C on 54 days (35.52 % of the period) (Fig. 1a). The relative air humidity ranged from 72.2 to 90.8 %, with an average of 81.3±3.8 % (Fig. 1b), while the mean THId ranged from 74.3 to 78.4 (Fig. 1c). The accumulated rainfall was the highest in April (284 mm) and the lowest in June (118 mm). Solar radiation had the lowest levels in April and May (620.5 and 651.1 MJ/m^2) and the highest in July (716.2 MJ/m^2) (Fig. 1d).

THIp was significantly different between the morning and afternoon ($P<0.05$), but no significant difference was found among the monthly averages (Table 1). THIp increased by up to three points (April and May) and from four to six points (June, July, and August).

The lowest values for RR were found in April and May (Table 2), with a gradual increase starting in June and reaching the highest values in July and August. The values increased between the periods of the day regardless of the month

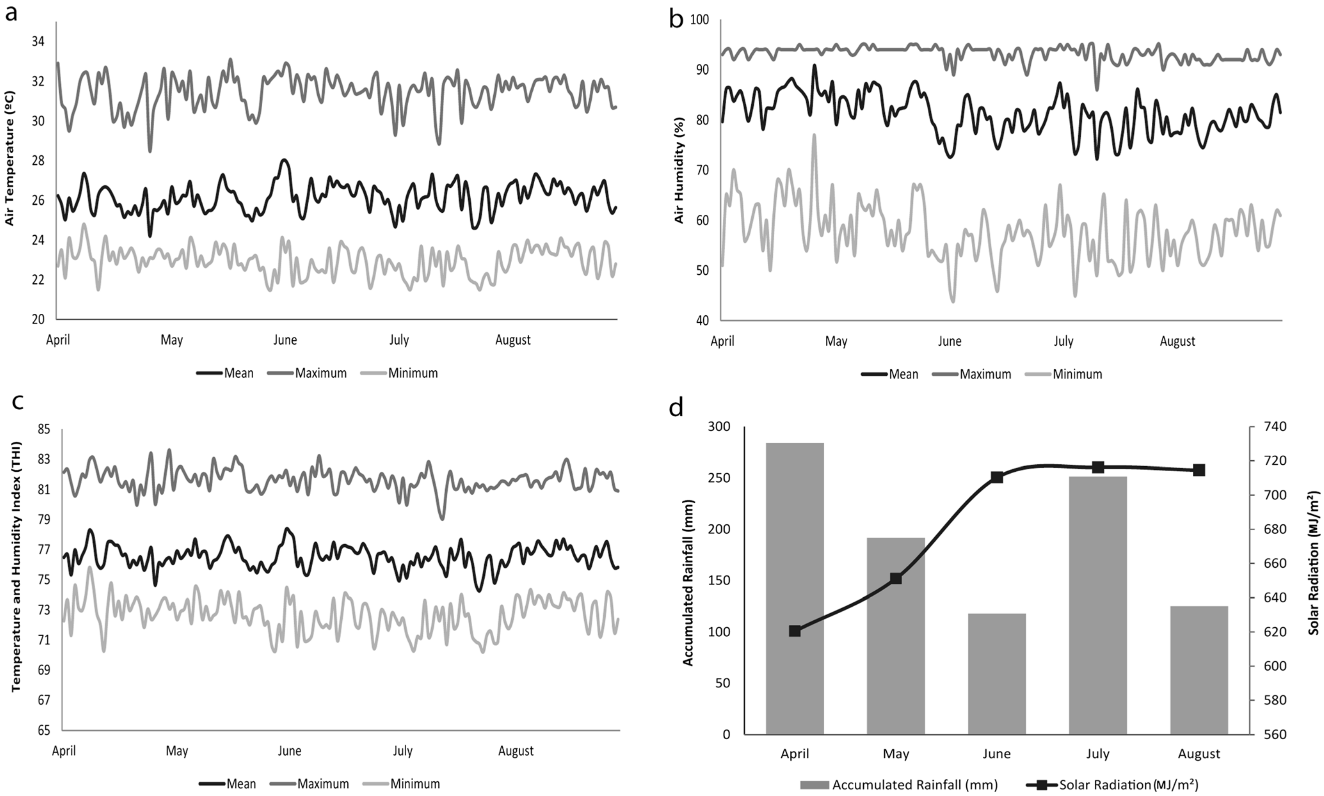

Fig. 1 Registered values for **a** air temperature (°C), **b** relative humidity (%), **c** Temperature Humidity Index, and **d** accumulated rainfall (mm) and mean solar radiation (MJ/m^2) recorded in an automated meteorological station in Castanhal, PA, Brazil (1° 18′ 03″ S and 47° 56′ 51″ W)

(P<0.05). The same pattern was observed for RH (P<0.05). In July and August, RT decreased (P<0.05) but increased on average 0.4 °C between the periods of the day (P<0.05).

Values for BTCI remained initially stable (1.96) but, starting in June, they gradually increased (P<0.05) and reached the highest values in July and August. Moreover, a statistical difference was observed between periods of the day (P<0.05). Regarding the thermal comfort indices studied, THIp was positively correlated with RT (0.63; P<0.001), while BTCI was highly correlated with RR (0.97; P<0.0001) and with HR (0.89; P<0.0001). HR and RR were also highly correlated between each other (0.87; P<0.0001). The other correlations between THIp, BTCI, RT, HR, and RR were not significant (P>0.05).

The monthly analysis of hematological parameters showed that the mean value of red blood cells (Table 3) varied slightly and was not significantly different over the months (P>0.05), which was also true for the hemoglobin levels. Hematocrit and MCV varied over the months and were the highest in June (P<0.05). MCH and MCHC varied monthly (P<0.05), with MCH peaking in May and MCHC decreasing in June.

The leukogram components and platelet values (Table 4) did not significantly vary over the months (P>0.05). The erythrogram, platelet, and leukogram values were not significantly correlated to THI. Among the erythrogram parameters, only MCH, MCHC, and Hb had a significant (P<0.05) and positive, albeit small, correlation with THI (0.48; 0.36, and 0.28, respectively).

Table 1 Mean values and standard deviations of the Temperature Humidity Index (THIp) inside the pens in the morning (6:00 to 9:00 a.m.) and afternoon (12:00 to 15:00 p.m.)

Period	April	May	June	July	August	General average
Morning	79.2±2.8	78.1±2.2	77.4±2.7	75.9±2.9	77.9±2.0	77.7±2.6^{d}
Afternoon	82.1±2.9	80.5±1.5	83.4±0.8	80.4±2.3	82.1±1.3	81.8±2.1^{c}
General average	80.6±3.1^{a}	79.3±2.2^{a}	80.4±3.61^{a}	78.2±3.4^{a}	80.0±2.7^{a}	79.7±3.1

^{ab} Means in the rows with different superscripts are significantly different (P<0.05)

^{cd} Means in the columns with different superscripts are significantly different (P<0.05)

Table 2 Mean values and standard deviations of the physiological parameters and BTCI of buffalo bulls (*Bubalus bubalis*) measured in the morning (6:00 to 9:00 a.m.) and in the afternoon (12:00 to 15:00 p.m.)

	Period	April	May	June	July	August	General average
RR	M	21.6±1.8	21.3±1.6	23.0±1.6	26.2±1.1	25.2±1.5	23.2±2.3^{e}
	A	22.6±1.7	23.3±1.2	24.8±1.4	31.6±2.3	30.1±3.1	25.9±3.8^{d}
	Av	22.1±1.8^{c}	22.3±1.7^{c}	23.9±1.8^{b}	28.9±3.3^{a}	27.6±3.4^{a}	24.5±3.4
HR	M	63.6±2.4	61.7±2.0	63.2±2.2	65.8±1.7	65.1±1.8	63.4±2.5^{e}
	A	63.6±1.8	64.1±1.6	65.6±2.1	68.1±2.9	66.7±2.7	65.5±2.6^{d}
	Av	63.6±2.1^{bc}	62.9±2.1^{c}	64.4±2.4^{b}	66.9±2.6^{a}	65.9±2.4^{a}	64.5±2.7
RT	M	38.0±0.7	38.0±0.3	38.2±0.3	37.8±0.4	37.6±0.7	38.0±0.5^{e}
	A	38.9±0.4	38.4±0.3	38.6±0.3	38.5±0.3	38.0±0.6	38.4±0.4^{d}
	Av	38.4±0.7^{a}	38.2±0.4^{ab}	38.4±0.4^{a}	38.1±0.5^{bc}	37.8±0.7^{c}	38.2±0.5
BTCI	M	1.93±0.09	1.92±0.07	2.00±0.07	2.12±0.04	2.07±0.07	2.00±0.10^{e}
	A	1.99±0.07	2.01±0.05	2.08±0.06	2.38±0.10	2.30±0.14	2.13±0.16^{d}
	Av	1.96±0.08^{c}	1.96±0.08^{c}	2.04±0.08^{b}	2.25±0.15^{a}	2.19±0.15^{a}	2.06±0.15

RR respiratory rate (mov/min), *HR* heart rate (beats/min), *RT* rectal temperature (°C), *BTCI* Benezra's thermal comfort index, *M* morning, *A* afternoon, *Av* average

[a,b,c] Means in the rows with different superscripts are significantly different ($P<0.05$)

[d,e] Means in the columns with different superscripts are significantly different ($P<0.05$)

The results of the monthly seminal parameters (Table 5) showed that volume and concentration did not vary ($P>0.05$). Gross motility slightly decreased in August, while vigor had its highest value in June ($P<0.05$). Progressive motility (68.7±10.2) and plasma membrane integrity (67.8±9.7) had very similar behaviors over time, but with no significant differences over time ($P>0.05$).

The results of minor, major, and total defects showed small, non-significant ($P>0.05$) variations. The lowest numeric values of minor, major, and total defects were found in June. The mean THIp had a significant negative correlation only with plasma membrane integrity (−0.17; $P<0.05$), and for all others seminal parameters, the correlations with THIp were not significant ($P>0.05$).

Discussion

The concomitance of high temperatures and humidity, besides intense solar radiation observed during the experiment, represents a challenging setting for the animals reared in tropical conditions (Garcia 2013). In effect, by considering values ≤74 as the most appropriate THI levels for buffalo rearing (Somparn et al. 2004), it can be seen that the daily average THId was above the recommended level on 96 % of the days of the experimental period.

Over the months, THIp was never below 75, even during mornings, which could indicate an alert or danger condition regarding the animals' thermal comfort according to the classic THI interpretation (Starr 1981). In theory, at the THI range found in the present study, the animals would show signs of

Table 3 Values of the erythrogram components of buffalo bulls (*Bubalus bubalis*) maintained in an artificial insemination station

Parameter	April	May	June	July	August	General average
RBC	6.3±1.8	6.4±1.5	6.8±1.6	6.9±1.6	6.8±1.3	6.6±1.5
Ht	36.3±8.9^{ab}	38.9±8.6^{ab}	49.0±11.0^{a}	36.6±7.0^{ab}	35.1±5.8^{b}	39.2±9.6
Hb	10.7±2.6	13.3±2.7	12.2±3.0	12.7±2.5	12.5±2.1	12.3±2.6
MCV	58.8±6.6^{bc}	60.4±4.0^{b}	72.6±5.8^{a}	53.7±5.4^{bc}	52.4±4.9^{c}	59.6±8.9
MCH	17.2±2.1^{b}	21.1±1.6^{a}	17.8±1.0^{b}	18.5±1.6^{b}	18.5±1.4^{b}	18.6±2.0
MCHC	29.4±0.5^{b}	34.4±2.7^{a}	24.7±1.2^{c}	34.6±0.7^{a}	35.4±0.7^{a}	31.7±4.4

RBC red blood cell (10^6/μL), *Ht* hematocrit (%), *Hb* hemoglobin (g/dL), *MCV* mean corpuscular volume (fl), *MCH* mean corpuscular hemoglobin (pg), *MCHC* mean corpuscular hemoglobin concentration (%)

[a,b,c] Means in the rows with different superscripts are significantly different ($P<0.05$)

Table 4 Values of platelets and the leukogram components of buffalo bulls (*Bubalus bubalis*) maintained in an artificial insemination station

Parameter	April	May	June	July	August	General Average
Pt	150.3±90.9	190.0±80.0	223.7±92.5	186.1±55.5	203.5±68.6	190.7±79.3
Lc	12.6±4.9	17.2±10.0	15.0±5.6	14.4±5.9	13.5±5.7	14.5±6.6
Lf	65.4±12.5	59.3±3.7	55.5±7.2	61.1±6.1	62.8±11.8	60.8±9.2
Nt	29.5±11.5	36.3±4.7	39.7±6.9	34.2±6.5	32.0±11.7	34.3±9.1
Eos	3.1±1.1	2.4±1.0	2.7±0.9	2.0±0.9	2.6±1.0	2.6±1.0
Mon	3.0±1.6	2.6±0.7	2.1±0.6	2.3±0.7	2.6±1.3	2.5±1.1

Pt platelets (10^3/μL), *Lc* leukocytes (10^9/L), *Lf* lymphocytes (%), *Nt* neutrophils (%), *Eos* eosinophils (%), *Mon* monocytes (%)

heat stress and a reduction in productive performance (Somparn et al. 2004). However, no evident signs of thermal discomfort were seen, which suggests that the animals had adapted to the experimental conditions or that a new interpretation of the THI scale must be done for buffaloes reared under tropical climate.

The results of RR, HR, and RT observed are within the normal physiological range (Garcia et al. 2011; Silva et al. 2011a) and were lower than those observed in animals challenged in an environmental chamber (Haque et al. 2012). The BTCI results obtained are similar to those found among adult bulls (1.7 to 2.4) maintained in silvopastoral systems with free access to natural shade (Garcia et al. 2011). RR, HR, and BTCI had a gradual increase over time, probably caused by the transition between the rainier and less rainy periods of the year (Silva et al. 2011a) and coincided with the period of higher solar radiation of more frequent spikes in air temperature, i.e., between June and August. Since water buffaloes are animals with intense epidermal pigmentation, their skins have low reflexivity and greatly absorb solar radiation (Marai and Haeeb 2010; Garcia 2013), which at times lead to undesirable caloric gains. Therefore, the increase in RR over time indicated a greater activation of thermolysis mechanisms (Moraes Júnior et al. 2010), following the increase in environmental heat.

RT had a downward trend over the months, with the lowest values found in July and August, always within the normal range for buffaloes (Silva et al. 2011a), which indicates a homeothermy condition. When associated, the RR, HR, BTCI, and RT data revealed the animals' ability to maintain the inner body temperature through their thermoregulatory mechanisms (Gudev et al. 2007b).

When the animals experience heat discomfort, expressed by the increase in BTCI at levels above 2.0 (Benezra 1954), they readily activate their thermolysis mechanisms. In buffaloes, the increase in RR is a common response in thermal discomfort situations as a way to dissipate heat (Marai and Haeeb 2010), followed by cardiac debt and peripheral vasodilation increase, that facilitates heat loss (Aggarwal and Singh 2008). These arguments were confirmed by the strong positive correlations found between BTCI and RR, between BTCI and HR, and between RR and HR. Thus, the lower RT value found in August, followed by the higher RR and HR means, show that the animals were highly efficient in activating physiological mechanisms that take part in thermolysis, which enabled them to maintain homeothermy. This rules out any sign of heat

Table 5 Monthly average of parameters of semen *in natura* of buffalo bulls (*Bubalus bubalis*) maintained in an artificial insemination station

Parameter	April	May	June	July	August	General average
Vol	2.1±1.5	2.3±1.6	2.2±1.2	2.4±1.5	2.1±1.3	2.2±1.4
Conc	1.3±0.5	1.3±0.6	1.3±0.5	1.4±0.5	1.3±0.5	1.3±0.5
GM	$2.7±1.0^{a}$	$2.7±1.0^{a}$	$2.6±1.2^{ab}$	$2.6±1.0^{ab}$	$2.0±1.0^{b}$	2.5±1.0
Vig	$3.1±0.7^{ab}$	$2.8±0.4^{b}$	$3.3±0.7^{a}$	$3.0±0.6^{ab}$	$3.0±0.4^{ab}$	3.0±0.5
Mot	67.9±12.0	67.0±9.5	71.9±9.5	70.9±8.8	65.6±11.0	68.7±10.2
PMI	66.9±14.2	66.8±7.6	70.5±7.2	69.0±6.4	66.0±13.2	67.8±9.7
DMi	5.9±6.3	6.6±3.6	4.5±2.9	5.7±4.1	6.1±4.5	5.8±4.3
DMa	13.2±8.0	14.6±6.3	13.1±4.8	14.4±6.9	14.4±9.4	13.9±7.1
DT	19.1±12.2	21.2±8.2	17.6±6.2	20.1±6.7	20.4±10.1	19.7±8.7

Vol volume (mL), *Conc* spermatic concentration (×10^9 sptz/mL), *Turb* gross motility (0–5), *Vig* spermatic vigor (0–5), *Mot* progressive motility (%), *PMI* plasma membrane integrity (%), *DMi* minor defects (%), *DMa* major defects (%), *DT* total defects (%)

[a,b] Means in the rows with different superscripts are significantly different ($P<0.05$)

stress during the experimental period. As for the periods of the day, the higher RR, HR, RT, and BTCI values observed in the afternoon were due to the increase in air temperature along the day, besides the fact that solar radiation increases in the morning and peaks at noon (Silva Júnior et al. 2006). This showed the animals' short-term response ability against heat, i.e., within hours.

The significant correlation between mean THIp and RT revealed an increase in inner body temperature as a function of the increase of air temperature and relative humidity. In bioclimatologic terms, the individual able to maintain body temperature regardless of the increase in environmental temperature can be considered more efficient (Scharf et al. 2010). In practice, individuals that had no or negative correlation between THI and RT should be the choice in animal breeding programs for food production in tropical areas, particularly bulls, which are responsible for a great part of a herd's genetic improvement (Marcondes et al. 2010).

Regarding hematological parameters, the red blood cells and hemoglobin values did not vary, matching the findings by Silva et al. (2011b), Gomes et al. (2010) and Vilela (2013). The increase in hematocrit in June may be explained by the higher mean air temperature, which led to a greater activation of thermolysis mechanisms and the consequent loss of body water through evaporative mechanisms, thus causing hemoconcentration (Silva et al. 2011b; Bernardini et al. 2012). The mean hematocrit values found were higher than those previously reported for adult animals reared in tropical environments in Brazil (Gomes et al. 2010; Silva et al. 2011b; Vilela 2013), in Colombia (Londoño et al. 2012), in Pakistan (Akhtar et al. 2007), and in the Philippines (Koga et al. 2004).

The mean MCV and MCHC observed varied but were similar to the mean values reported for adult bulls (Ciaramella et al. 2005; Gomes et al. 2010). The variation in MCH over the months may also be considered physiological since the values remained within the reference range for buffaloes, i.e., between 13.5 and 20.5 pg (Monteiro et al. 2013). The platelet values found are within the normal range for large ruminants (100 to 800 $\times 10^3/\mu L$) (Morris 1993) but are below the ones reported for adult female buffaloes ($300 \times 10^3/\mu L$) (Ciaramella et al. 2005; Gomes et al. 2010). Values for the leukogram components for buffaloes reared in tropical environments in previous studies match the current findings (Akhtar et al. 2007; Londoño et al. 2012; Vilela 2013).

The seminal parameters had no significant negative interferences over the months. The values of volume, gross motility, and vigor were a little lower of 3.6±1.9, 2.9±1.9, and 3.6 ±1.0 mL, respectively, than those previously described for buffalo bulls reared in humid tropical climate (Santos et al. 2014). However, progressive sperm motility and plasma membrane integrity were higher than those reported by the same authors of 59.3±20.5 % and 68.0±19.5 %, respectively. The results obtained match those reported by Koonjaenak et al. (2007). They are also in accordance with the data by Silva et al. (2014b) regarding plasma membrane integrity (68.18±10.59 %) and total defects (20.43±9.63 %), very important characteristics when it comes to bulls providing semen for cryopreservation. According to the andrology standards for buffaloes, the values described in the present study are within those recommended for raw semen collected with artificial vagina (CBRA 2013).

PMI values below 65 % were not observed, which characterizes appropriate semen conditions for use in animal reproduction biotechniques. However, the negative correlation found between PMI and mean THI shows that air temperature and humidity may negatively impact semen quality, mainly regarding sperm membrane structure, which is a requirement for successful fertilization, more specifically at the stages of spermatic capacitation, binding of the spermatozoid to the pellucid zone, and acrosome reaction (Santos et al. 2014). It is speculated that such correlation derives, at least partially, from individual differences in scrotal thermoregulation ability (Garcia et al. 2010), which may also impact ejaculate quality. Nevertheless, since scrotal temperature was not investigated in the present study, the inferences regarding the effective participation of testicular thermoregulation on semen quality become limited.

Conclusion

Buffalo bulls reared in humid tropical climate show variations in thermal comfort during the hottest periods of the day and also during the transition period between the rainier and less rainy periods of the year. However, the animals have an efficient thermoregulatory system, which is activated during the most critical periods. This enables them to quickly compensate caloric gains and preserve their homeothermy, thus maintaining physiological, hematological, and seminal parameters at normal levels. Since semen quality is, up to a point, correlated with oscillations in air temperature and humidity, a differentiated management can be recommended for bulls maintained in artificial insemination stations, such as offering naturally shaded areas for rest and the future selection of buffalo males with greater heat tolerance.

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