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Elevated temperature-humidity index induces physiological, blood and milk alterations in Holstein cows in a more pronounced manner than in ½ and ¾ Holstein × Gir

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Abstract *Bos taurus taurus* and *Bos taurus indicus* cattle subspecies present different capabilities in coping with situations of elevated temperatures, the latter being more tolerant to heat stress. Thus, some breeding programs crossed these subspecies to produce a high producing yet heat-tolerant breed (Girolando). Nineteen Holstein (H100) and 19 Girolando cows [(½ Holstein × Gir (H50) and ¾ Holstein × Gir (H75)] with similar milk production were used in a six-day experiment to evaluate the consequences of heat stress due to shade deprivation on their physiological, blood and milk traits. Cows were exposed to a non-shaded environment between morning (06:00h; GMT -3:00) and evening milking (14:30h; GMT -3:00) with access to water *ad libitum*. Procedures were conducted before morning and evening milkings. Physiological parameters related to mechanisms of heat dissipation were measured, as well as the milk composition. Blood traits were evaluated. The temperature-humidity index (THI) was calculated. Statistical procedures included analysis of variance, correlation, and principal factors. THI was elevated during the trial and negatively impacted physiological, milk, and blood parameters in H100, H75, and H50. Alterations in physiology, milk stability, milk composition, and blood traits were more pronounced in H100. Holstein cows presented changes in physiological parameters in a more pronounced manner and in some milk and blood traits related to the reduced capability of this breed in dealing with elevated THI. The similarity in milk production levels excludes this parameter as a justification for differences in heat tolerance, with genetic composition being the main reason for these results.

Keywords blood alterations, dairy cattle, heat tolerance, milk yield, physiology

1. Introduction

The deleterious effect of warm climates on overall animal performance became more important due to the introduction of temperate climate breeds in tropical environments during the 1920s and 1930s (Berman 2012). Cattle subspecies such as *Bos taurus taurus* (*B. taurus*) and *Bos taurus indicus* (*B. indicus*) present differences in their ability to cope with elevated temperatures and maintain their thermal stability, a characteristic defined as heat tolerance (McManus et al 2014). The former subspecies has its origin in Europe, while the latter derived from subspecies that evolved in warm zones of Asia. This distinction in breed evolution is responsible for the better adaptation of *B. indicus* to heat (Berman 2012). According to Hansen (2004), *B. indicus* cattle produce less heat, reduce cell damage when exposed to increased temperatures (compared to *B. taurus*), and increase dissipating heat to the environment. This same author attributed differences in heat tolerance to higher density and size of sweat glands in *B. indicus*, reduced tissue resistance to heat flow from the body core to the skin, along with hair coat characteristics. *Bos taurus* present thicker and denser coats, reducing heat flow via conduction and convection (McManus et al 2009). Also, *B. taurus*, especially Holstein, present increased milk production levels, representing an elevation in metabolic heat production and reduction in heat tolerance.

Crossbreeding has been used to increase milk production in warmer zones, obtaining satisfactory production indices (Daltro et al 2020), and is the basis for creating the Girolando breed. Its concept combines high

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production levels of *B. taurus* with increased heat tolerance of *B. indicus*, developing a high producing and heat-tolerant breed. In Brazil, the Girolando breed is becoming increasingly popular (Costa et al 2020), with nationwide distribution.

Physiological changes such as the increase in respiratory rate (Eigenberg et al 2005) and panting (Gebremedhin 2012), as well as in heart rate (Cerutti et al 2013), are key homeorhetic mechanisms mediated by the hypothalamus-pituitary-adrenal (HPA) axis (Collier et al 2006) that animals develop to cope with elevations in environmental temperature (McManus et al 2020). The efficacy of heat dissipation can be attested by the magnitude of changes in rectal temperature, which is the most reliable indicator of heat stress when compared with respiration rate (Hillman et al 2005). Alterations in blood parameters (packed cell volume, leukocytes, red cell distribution size, mean corpuscular volume, platelets, mean corpuscular hemoglobin concentration, among others) due to heat stress were also studied and were related to systemic stress (McManus et al 2009) and changes in energy metabolism and enzyme activities (Abeni et al 2007).

Along with physiological and blood traits changes, increased environmental temperatures may alter milk physical-chemical composition and overall production levels (West 2003). Bernabucci et al (2002) observed lower milk yield, lower contents of crude proteins and casein in Holstein milk during the summer. This reduction was reported during heat stress, although results differed among studies; a decrease in fat content was also reported (Giustini et al 2007). Cows with reduced feed intake and in negative energetic balance (NEBAL) due to heat stress, compared with cows in NEBAL due to other aspects (i.e. after parturition), did not show a reduction in plasma insulin, reduction in lipolysis, and consequent increase in plasma non-esterified fatty acids (NEFA) levels (Wheelock et al 2010). Thus, heat-stressed cows become metabolically inflexible and dependent on glucose for satiating energetic needs (Baumgard and Rhoads 2007). Such a scenario reduces glucose flow to the mammary gland and, in consequence, milk production levels are prejudiced.

Heat stress has a significant impact on cows' metabolism, production and milk characteristics, thus impairing the dairy production system. Since cows present different capabilities in coping with situations of elevated temperatures, this study aimed to evaluate the impact of elevated Temperature-Humidity Index (THI) on physiological, blood and milk traits and to compare changes in these traits in breeds with decreasing percentages of Holstein in their genetic composition (100% (purebred Holstein), 75% (¾ Holstein × Gir) and 50% (½ Holstein × Gir)) with similar milk production levels.

2. Materials and Methods

2.1. Local description, animals and management

Animal care procedures throughout the study followed protocols approved by the Ethics Committee for Animal Use (CEUA) at the Federal University of Rio Grande do Sul, number 22773/2012.

The experiment was carried out for six days during the summer (month of March) at Embrapa Gado de Leite (21°35'16"S and 43°15'56"W), in Coronel Pacheco, Minas Gerais State, Southeast region of Brazil. Animals used belong to Embrapa, thus, housing and feeding techniques were not altered nor established by the authors, except for heat stress induction.

Coronel Pacheco presents two seasons throughout the year: rainy, between October and March; dry: from April to September. Annual rainfall is approximately 1,440 mm. The annual temperature ranges from 15.6 to 25.6 °C, with an average of 20.6 °C.

Thirty-eight cows were used: 19 purebred Holstein (H100) and 19 Girolando with eleven ¾ Holstein × Gir (H75) and eight ½ Holstein × Gir (H50). Average days in milk (DIM) and milk production was 249.15 ± 68.19 DIM and 14.80 ± 2.59 l/day for H100; 169.3 ± 95.85 DIM and 14.62 ± 2.95 l/day for H75 and 95 ± 72.33 DIM and 12.40 ± 3.42 l/day for H50, respectively.

All experimental procedures were the same for H100, H75, and H50. The study induced heat stress by exposing cows to a non-shaded environment, with water and fresh feed *ad libitum,* between morning and evening milking. The temperature ranged from 22 to 35°C, and relative humidity ranged from 52 to 95%. H100 cows were housed in a free-stall barn at night, receiving a total mixed ratio of corn silage and concentrate (59% ground corn, 35% soybean meal, 3.5% protein-mineral-vitamin supplement, 0.5% mineral salt, 1% urea, and 1% bicarbonate) according to milk production (approximately 3 kg of concentrate per liter of milk produced). Between milkings, cows were kept on *Brachiaria brizantha* pasture. H75 and H50 were kept on *Pennisetum purpureum* pasture and fed concentrate before each milking (70% ground corn, 25% soybean meal, 3.5% protein-mineral-vitamin supplement, 0.5% mineral salt, and 1% urea) also according to milk production. All cows were kept in sunlight between milkings (7 to 8 hours exposed to the sun).

2.2. Milk collection and analysis

Cows were milked twice a day, at 06:00h (GMT -3:00) and 14:30h (GMT -3:00). Milk yield was recorded individually during each milking, and individual milk samples were taken to evaluate titratable acidity by titration with 0.1 N NaOH solution; ethanol stability of milk by mixing 2 mL of milk with 2 mL of an alcoholic solution in ascending concentrations (ranging from 50 to 98°GL) in a Petri dish - results were expressed as the minimal ethanol concentration that induced coagulation of milk proteins (clots formation). Milk samples were also collected in tubes containing Bronopol to evaluate concentrations of fat, protein, lactose, and nonfat dry stratum by an infrared analyzer (Bentley 2000® Equipment, Chaska, Minnesota, USA) and somatic cell count (SCC - transformed by $\log_{10}$) by flow cytometry with Somacount 300® (Bentley Instruments, Chaska, Minnesota, USA).

2.3. Physiological parameters assessment

Before morning and evening milking, held cows in pen, and physiological parameters were assessed: respiratory (RR) and heart rates (HR) through auscultation during 30 seconds (then multiplied by two); panting score (PS), by visual observation and in a 0 to 4 scale (Mader et al. 2006); rectal temperature (RT) with the use of a thermometer inserted 30 cm against the rectum wall during three minutes.

Respiratory rate and rectal temperature were used to calculate the Heat Stress Index (HT), according to Benezra (1954), in which higher values are worst:

$$HT = \left(\frac{RT}{38.33}\right) + \left(\frac{RR}{23}\right)$$

2.4. Blood collection and plasma analysis

Before each milking, but after physiological parameters assessment, blood samples were collected via caudal venipuncture in vacutainers containing EDTA. After sampling, blood was sent to the laboratory to evaluate the percentage of leukocytes in an automatic cell counter (CC550, Cellm$^{\text{TM}}$). Mean corpuscular volume (MCV; fL), mean corpuscular hemoglobin concentration (MCHC; g/dL), red cell distribution width (RDW; %), and mean platelet volume (MPV; fL) were determined by calculation. Platelets (thou/mm^3), percentage of segmented neutrophils, eosinophils, and monocytes were assessed by manually counting 100 cells in Wright-stained blood smears under an optical microscope.

2.5. Climatic indices

During experimental procedures, climatic parameters were collected hourly with the use of a black globe thermometer (Extech Instruments, Model HT30), including air temperature (AT, °C) and relative humidity (RH, %). Values were used to calculate a temperature-humidity index (THI) through the equation (NRC, 1971):

$$THI = (1.8 \times AT + 32) - [(0.55 - 0.0055 \times RH) \times (1.8 \times AT - 26.8)]$$

2.6. Statistical analysis

Individual cows were considered as an experimental unit, as a repeated measure. All physiological, blood and milk traits were compared, and the analysis of variance included THI as a covariable and genetic composition of the cow (%HOL) as a fixed effect (H100, H75, and H50). Data were analyzed using SAS 9.3 (SAS Institute, Cary, North Carolina, USA) by the procedures analysis of variance (PROC MIXED), principal factor analysis (PROC FACTOR), and correlation (PROC CORR). Response variables were divided into three groups: milk (MS, MP, TA, MF, MPr, NDS, SCC), blood (MCV, MCHC, RDW, MPV, PLAT, SN, EOS, LEUK), and physiological (RT, PS, RR, HR, HT)). Two-factor analyses were carried out: the first with THI, %HOL, milk, and physiological traits and the second with THI, %HOL, blood, and physiological traits. The rotation type was VARIMAX, and only variables with Kaiser-Meyer-Olkin (KMO) sampling adequacy greater than 0.5 were maintained in the analysis.

3. Results

3.1. Climatic, physiological and milk parameters

THI did not differ in the periods of analysis of the different breeds (Table 1). Nevertheless, changes could be observed in some physiological and milk traits according to breed conformation, H50 showed lower RR and HR, HT, and statistically equal to H75, presented lower RT and PS compared with H100.

Table 1 Climatic, physiological, and milk traits of cows with different percentages of Holstein breed in their genetic configuration.

Trait	H100	H75	H50	RMSE[b]	*P*-value
THI	79.52^{A}	79.20^{A}	80.32^{A}	6.2767	0.6356
Respiratory rate (breaths/min)	81.01^{A}	76.13^{A}	56.25^{B}	32.3133	<0.0001
Heat tolerance	4.56^{A}	4.33^{A}	3.43^{B}	1.4371	<0.0001
Heart rate (beats/min)	78.03^{A}	75.86^{A}	64.16^{B}	22.8805	0.0018
Rectal temperature (°C)	39.84^{A}	39.34^{AB}	39.14^{B}	4.7879	0.0020
Panting score	1.54^{A}	1.06^{AB}	0.52^{B}	1.4661	0.0003
Milk production (L/day)	13.74^{A}	13.60^{A}	12.60^{A}	3.0482	0.5383
Somatic cell count (cell/mL x 1000)	4.19^{A}	4.59^{A}	4.42^{A}	0.6179	0.9160
Milk fat (%)	4.19^{A}	4.59^{A}	4.42^{A}	1.1776	0.1034
Milk lactose (%)	4.40^{A}	4.44^{A}	4.36^{A}	0.2570	0.2770
Milk protein (%)	3.39^{A}	3.15^{B}	3.11^{B}	0.3571	<0.0001
Milk nonfat dry stratum (%)	8.68^{A}	8.48^{AB}	8.34^{B}	0.4893	0.0002
Titratable acidity (°D)	13.98^{C}	14.83^{B}	15.70^{A}	1.7708	<0.0001
Milk stability (°GL)[a]	72.55^{C}	82.45^{B}	85.78^{A}	7.8484	<0.0001

H100: purebred Holstein; H75: ¾ Holstein × Gir; H50: ½ Holstein × Gir. [a]Concentration of ethanol capable of inducing clots formation in milk. [b]Root mean squared error (RMSE). Means followed by different capital subscript letters are significant different by the Tukey test (*P* < 0.01).

Besides milk production, SCC, milk fat, and lactose were not different among the three groups. Milk protein was higher ($P < 0.05$) in H100, when compared with H75 and H50. Milk nonfat dry stratum concentration was not different ($P > 0.05$) between H75 and H100. Milk stability differed between groups, with H50 presenting highest and H100 lowest values. The same consideration can be made for titratable acidity.

Percentage of Holstein (100%, 75%, or 50%) presented low positive correlations with milk protein and nonfat dry stratum. In contrast, it showed a negative correlation with titratable acidity (low) and milk stability to the ethanol test (moderate) (Table 2). Elevation in THI and all physiological parameters were positively correlated with somatic cell count and milk fat. Still, all correlations were low and were negatively correlated with titratable acidity (mild to moderate), milk production (moderate), milk lactose (weak to moderate), and nonfat dry stratum (low to moderate correlations). Milk production was the most affected variable. Milk protein was weakly and negatively correlated with THI, HR, and RR. Milk stability, for its part, was not correlated with THI but presented a low and negative correlation with physiological parameters.

Table 2 Correlations between the percentage of Holstein, THI, heart rate, heat tolerance index, panting score, rectal temperature, and respiratory rate with milk parameters.

	% Holstein	THI	HR	HT	PS	RT	RR
SCC	-0.018^{ns}	0.204**	0.211**	0.201**	0.206**	0.207**	0.208**
TA	-0.366**	-0.302**	-0.422**	-0.443**	-0.417**	-0.448**	-0.454**
MP	0.068^{ns}	-0.619**	-0.483**	-0.565**	-0.560**	-0.568**	-0.569**
MF	-0.101^{ns}	0.372**	0.249**	0.338**	0.279**	0.344**	0.332**
MPr	0.318**	-0.173**	-0.159*	-0.129^{ns}	-0.088^{ns}	-0.092^{ns}	-0.134*
ML	0.039^{ns}	-0.242**	-0.183**	-0.175**	-0.137*	-0.211**	-0.179**
NDS	0.272**	-0.267**	-0.232**	-0.200**	-0.150*	-0.189**	-0.206**
MS	-0.581**	-0.021^{ns}	-0.165*	-0.182**	-0.228**	-0.167*	-0.189**

*$P < 0.05$; **$P < 0.01$; ns: non-significant; % Holstein: percentage of Holstein (100, 75 and 50%); HR: heart rate; HT: heat tolerance index; PS: panting score; RT: rectal temperature; RR: respiratory rate; SCC: somatic cell count (cell/ml); TA: titratable acidity (°D); MP: milk production (L/milking); MF: milk fat (%); MPr: milk protein (%); ML: milk lactose (%); NDS: nonfat dry stratum (%); MS: milk stability to the ethanol test (°GL).

Three principal factors were identified in milk component multivariate analysis (Eigenvalues ≥ 1.0). The first two explained 46.06% and 19.06% of the total variance observed, respectively. Milk lactose MSA (measure of sampling adequacy) value was below 0.5 and was removed from this analysis. A group was formed (THI group) comprehending THI and physiological parameters (PS, HR and RR, HT and RT), which indicates that these variables are highly correlated (Figure 1). Angles of 180° and 0° show elevated negative and positive correlation between variables, respectively. An angle of 90° indicates a low or null correlation. Thus, elevation in THI group values tends to reduce milk production efficiency. Titratable acidity was also negatively correlated with the THI group, but to a lower extent. Null or low influence of the group above on ethanol stability, milk protein, and nonfat dry stratum was observed. Due to the angle between THI group and milk fat, somatic cell count, and percentage of Holstein, a positive relationship between these variables can exist. It is expected that an increase in the percentage of Holstein would induce a reduction in milk stability to the ethanol test (angle of approximately 180°).

3.2. Climatic, physiological and blood parameters

Mild and positive correlations were observed between the percentage of Holstein and leukocytes, MCHC, and platelets concentration in blood (Table 3). Negative correlations were also found between the percentage of Holstein and MCV (low), segmented neutrophils (mild), and eosinophils (mild). None of the physiological parameters or THI were correlated with concentrations of segmented neutrophils, MCHC, RDW, platelets, and eosinophils. This same tendency was found for MCV, except for the PS, which presented a low and negative correlation with this blood parameter. Physiological traits were positively correlated with leukocyte content (low to mild correlations), except THI. THI presented a low and positive correlation with monocytes and MPV concentrations; this same consideration can be made for the correlation between HT and RR with MPV and monocytes. Some other positive correlations were estimated: PS with monocytes (low) and RT with MPV (mild correlation) (Table 3).

For blood traits, four principal factors were found (Eigenvalues ≥ 1.0). The first (32.90%) and second (16.23%) principal factors were responsible for 49.13% of the total variance observed. The THI group was formed and presented an almost null correlation with MCV, MCHC, RDW, eosinophils, and segmented neutrophils (Figure 2). Percentage of Holstein, segmented neutrophils, eosinophils, leukocytes, monocytes, and MPV formed another group. Percentage of Holstein, leukocytes, monocytes, and MPV were low and positively correlated with the THI group due to the angle formed between them and since variables are located at the right side of the Y-axis.

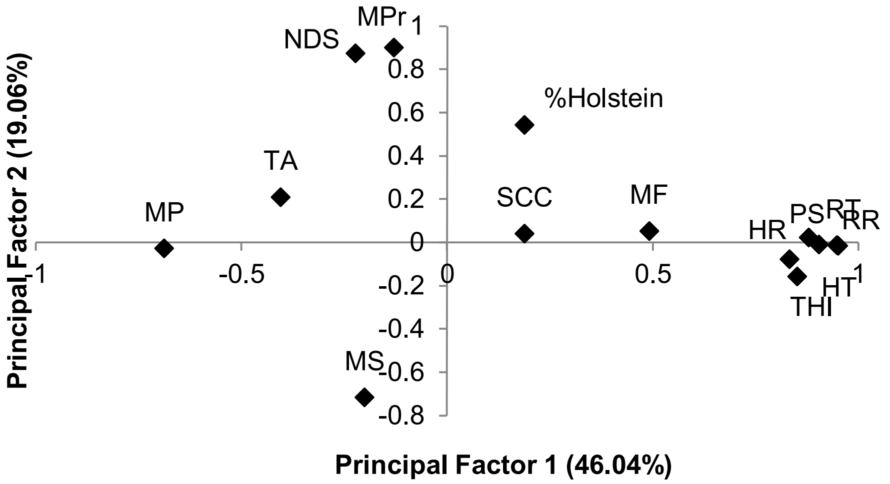

Figure 1 Percentage of Holstein, temperature-humidity index, physiological and milk traits projected in principal factors 1 and 2. MS: milk stability; MP: milk production; TA: titratable acidity; MF: milk fat; MPr: milk protein; NDS: nonfat dry stratum; % Holstein: percentage of Holstein in genetic configuration; SCC: somatic cell count; RT: rectal temperature; PS: panting score; RR: respiratory rate; HR: heart rate; HT: heat tolerance index; THI: temperature-humidity index.

Table 3 Correlations between the percentage of Holstein, THI, heart rate, heat tolerance index, panting score, rectal temperature, and respiratory rate with blood parameters.

	% Holstein	THI	HR	HT	PS	RT	RR
MCV	-0.158*	0.022^{ns}	-0.088^{ns}	-0.103^{ns}	-0.140*	-0.037^{ns}	-0.105^{ns}
MCHC	0.255**	-0.040^{ns}	0.031^{ns}	0.066^{ns}	0.016^{ns}	0.002^{ns}	0.063^{ns}
LEUK	0.295**	0.072^{ns}	0.211**	0.232**	0.196**	0.170*	0.229**
RDW	0.133^{ns}	-0.081^{ns}	0.020^{ns}	0.000^{ns}	0.023^{ns}	-0.048^{ns}	0.003^{ns}
MPV	0.070^{ns}	0.168*	0.103^{ns}	0.155*	0.096^{ns}	0.208**	0.155*
PLAT	0.231**	-0.069^{ns}	-0.017^{ns}	-0.041^{ns}	0.019^{ns}	-0.004^{ns}	-0.042^{ns}
EOS	-0.360**	0.127^{ns}	0.001^{ns}	-0.073^{ns}	-0.109^{ns}	-0.046^{ns}	-0.058^{ns}
SN	-0.322**	0.130^{ns}	0.058^{ns}	-0.042^{ns}	-0.039^{ns}	-0.029^{ns}	-0.041^{ns}
MONO	-0.117^{ns}	0.185**	0.118^{ns}	0.144*	0.157*	0.112^{ns}	0.152*

* $P < 0.05$; ** $P < 0.01$; ns: non significant; % Holstein: percentage of Holstein (100, 75 and 50%); HR: heart rate; HT: heat tolerance index; PS: panting score; RT: rectal temperature; RR: respiratory rate; MCV: Mean corpuscular volume (fL); MCHC: Mean corpuscular hemoglobin concentration (g/dL); LEUK: leukocytes (%); RDW: Red cell distribution width (%); MPV: Mean platelet volume (fL); PLAT: platelets (thou/mm^3); EOS: eosinophils (%); SN: segmented neutrophils (%); MONO: monocytes (%).

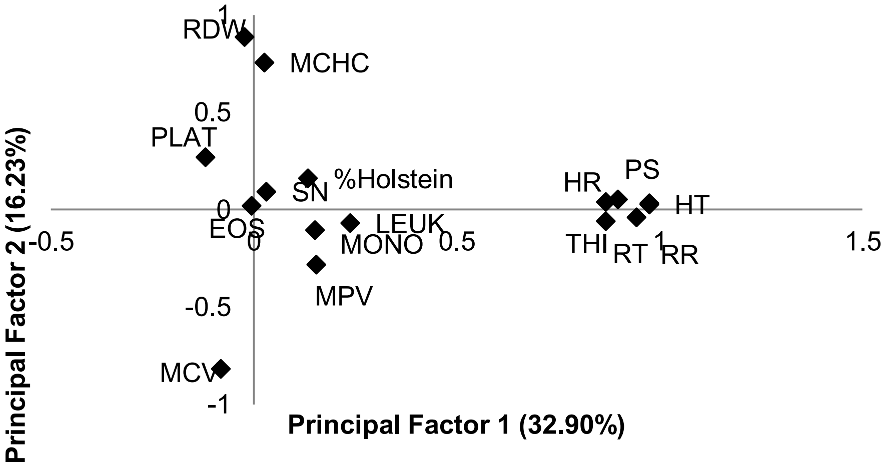

Figure 2 Percentage of Holstein, temperature-humidity index, physiological and blood traits projected in principal factors 1 and 2. MCV: mean corpuscular volume; MCHC: mean corpuscular hemoglobin concentration; RDW: red cell distribution width; MPV: mean platelet volume; PLAT: platelets; SN: segmented neutrophils; EOS: eosinophils; % Holstein: percentage of Holstein in genetic configuration; LEUK: leukocytes; RT: rectal temperature; PS: panting score; RR: respiratory rate; HR: heart rate; HT: heat tolerance index; THI: temperature-humidity index.

4. Discussion

Despite the similarity in THI, H100 and H75 cows had increased HR and RR, the latter being associated with evaporative cooling by the respiratory tract. Even though the RT was similar between H75 and H50, this parameter was combined with RR, generating the heat tolerance index, which was lower in H50. The panting score from the latter group was 50% lower in H50 than H75, as expected. Azevedo et al (2005) determined the upper critical THI for ½ HZ (Holstein - *B. taurus* × Zebu - *B. indicus*) as 80 and for ¾ HZ as 77. Based on these results, THI was slightly above the critical level for H50 (80.32). At the same time, it was 2 points higher than the critical level for H75 (79.2) and, consequently, even higher for pure Holstein cattle. For instance, Silva et al (2002) found harmful effects of THI 72 on milk production of Holstein cows.

Along with differences in critical THI for each group (H100, H75, and H50), Alfonzo et al (2015) showed that pure Holstein cows were larger than H75 and H50, and presented thicker coats and longer hair, such characteristics also contributed to the elevation in physiological parameters from H100.

Principal factor analysis showed that an elevation in THI represents a higher manifestation of mechanisms of heat dissipation. Increases in RR and PS were observed, as water loss through respiration can account for 15% of total heat loss (Maia et al 2005). Elevated HR in H100 and H75 represents another way to lose heat and prevent increased body temperature (McManus et al 2009). Thus, reduced HR in H50 demonstrated this group's higher adaptability to hot climates (Cheung and Mclellan 1998).

Rectal temperature from H100 was above (39.84ºC) and H75 slightly above (39.34ºC) the upper value, while H50 maintained its rectal temperature (39.14ºC) inside the normal range (38°C to 39.3ºC) as reported by Pires and Campos (2004). Also, principal factors indicated that elevation in the percentage of Holstein results in higher RT, which means that heat dissipation mechanisms were not sufficient to maintain homeothermy (Mota 1997) and that Girolando (H75 and H50) cows were more efficient in coping with elevated temperatures.

More productive animals present higher metabolic heat production (Purwanto et al 1990) and reduced capability to dissipate it to the environment. In our case, since cows were at the same production level (Table 1), physiological differences between breed composition may not be attributable to milk yield. Principal factor and correlation analysis show the capacity of elevated THI in reducing milk yield. Since ingestive parameters were not evaluated, it is impossible to infer how much of this decline in production is due to reductions in feed intake, but, according to Wheelock et al (2010), it may be between 35 and 50%.

Concentrations of fat from H100 (4.19%) did not differ from Girolando breeds, and protein content (3.39%) was higher than in milk from H50 and H75. The angle formed between the percentage of Holstein and milk protein in principal factors analysis (approximately 30º) and the positive correlation between these variables confirms the analysis of variance findings. Despite this, Holstein values stayed above the average values for the breed (fat: 3.5%; protein: 3.1%, Órdonez 2005). Holstein cows produced 13.74 l/day, below productions expected for this breed, but these cows were in the last third of lactation. It is possible that a concentration effect, in which, under heat stress, milk production reduces to a greater extent than fat (Lacy-Hulbert et al 1999) and protein (Mackle et al 1999) synthesis, turns these components more concentrated in the samples. In H100 cows, lactose levels stayed below the normal values (4.9%, Órdonez 2005). However, Silva et al (2018) showed that with an increase in somatic cell count, there is a decrease in milk production and lactose percentage and an increase in milk protein and fat percentages. Wheelock et al (2010) indicated that heat-stressed cows, which was the case of H100, secrete from 200 to 400g less milk lactose than thermal-neutral cows. Elevated glucose utilization by extramammary tissues may be responsible for such findings and be related to our results. H50 and H75 presented similar chemical compositions of milk. Protein and lactose concentrations stayed close to the expected for Girolando cows of 3.2% and 4.45%, respectively.

Milk stability to the ethanol test from H100 was below the values of Girolando (Table 1). Negative correlations between the percentage of Holstein and milk stability were also found in principal factors and correlation procedures. Milk stability from H100 was reduced when comparing values before and after the beginning of the experiment (75.36ºGL x 72.55ºGL). Reduced feed intake (Stumpf et al 2013) and deprivation of shade (Abreu et al 2020) reduce milk stability. Thus, a lower feed intake level due to higher THI conditions, although not measured, might be involved in our results. Shade deprivation did not affect milk stability from H50 and H75 since it stayed practically constant before (86.07 and 83.26ºGL, respectively) and during the experiment (85.78 and 82.45ºGL, respectively). Titratable acidity differed among groups but stayed inside the preconized range (14 to 18ºD) and in the same ascending tendency observed before starting the experiment: H100<H75<H50. In Abreu et al (2020), cows under stress and without shade showed a loss of stability associated with higher titratable acidity. Holstein cattle (H100) are more heat susceptible. They are subject to more hyperventilation, increasing exhaled CO_2 and more HCO_3- secretion from the kidney and saliva (Conte et al 2018), reducing titrable acidity. H100 was also at a later stage of lactation (although with the same milk producton as the other groups), leading to higher excretion of CO_2 in milk (Donnelly and Horne 1986), which may also explain these results.

There was a low but positive correlation between all physiological parameters and leukocyte content, also found in principal factor analysis. According to Lassen and Swardson (1995), this increase in leukocytes (leukocytosis) is found in animals under stress due to the liberation of epinephrine and corticosteroids, hormonal changes mediated by the HPA axis.

McManus et al (2009) compared different breeds' adaptation to heat and found that Holstein cattle, which presented slight leukocytosis, was the least adapted.

Neutrophilia often occurs in heat-stressed cows due to the mobilization of neutrophils from the bone marrow into the bloodstream (Jain 1993). Still, in our case, the correlation between segmented neutrophils and eosinophil numbers with THI and physiological parameters was null. Not all types of leukocytes were evaluated, but monocytes might be responsible for leukocytosis since they showed a moderate correlation with THI and some physiological parameters.

In agreement with the present experiment, McManus et al (2009) found no relation between RT, MCV, and MCHC. Our investigation suggested that elevated THI did not influence MCHC levels. El-Nouty et al (1990) found no alteration in MCHC from Holstein cows due to increases in THI ranging from 66.6 in winter to 81.9 in summer. Principal factors and correlation analysis showed that pure Holstein cows present a reduction in MCV compared with Girolando. Such reduction might be associated with depression in cellular oxygen requirements by Holstein cows to reduce cell metabolism and metabolic heat load (Lee et al 1976), thereby helping to deal with elevated temperatures. Red blood cell width (RDW) was unaffected by increases in THI and physiological traits, suggesting that heat did not change this cell configuration.

MPV increased following THI and physiological parameters (except for heart rate and panting score). According to Keating et al (1986), these results might be associated with heat stress, the release of platelets from the spleen, and the formation of new platelets, both larger than the normal circulating population (Corash et al 1978).

5. Conclusions

Alterations in physiological, milk and blood parameters following increases in the temperature-humidity index (THI) were observed and varied according to breed composition. Those changes indicated that cows with a higher percentage of Holstein genes could not deal with elevated THI even in similar milk production levels.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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