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Physiological adaptation indicators of three Colombian Creole cattle breeds

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Indicadores fisiológicos de adaptación en tres razas de bovinos criollos colombianos

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ABSTRACT

Keywords:
Climate change
Colombian creole cattle
Heat Load Index
Stress
Temperature-Humidity Index

The present study aimed to determine the physiological response and its relationship with the caloric impact (stress) index of three breeds of Colombian Creole cattle (Hartón del Valle, Blanco Orejinegro y Sanmartinero). These breeds have been considered adapted to low-tropical conditions and important to the sustainable meat and milk production in Colombia. To determine two heat tolerance indexes and two hormones and their relationship to the physiological response to heat stress in three Colombian Creole cattle breeds, Pearson's correlation methodology and mean comparison study were used. The adaptability indexes such as temperature-humidity index (ITH) and heat load index (HLI) and cortisol and T3 hormones were estimated and subsequently, the indexes were correlated with the hormones using the Pearson methodology, also the mean comparison study was used. Physiological variables were analyzed such as heart rate, respiratory rate, rumen patterns, and rectal temperature; during the study, serum concentrations of cortisol and triiodothyronine hormones were detected in seven different instances during the rainy season in the Piedemonte Llanero. ITH and HLI were used as indicators of thermal compensation. The HLI appeared to be a better indicator when the environmental conditions included wind speed and solar radiation. No variations were found between the breeds ($P \geq 0.05$), while the measurement periods did show variations ($P \leq 0.05$). It may conclude that the HLI provides better information to study physiological parameters and it may confirm that the animals are considered adapted to the conditions of the local environment.

RESUMEN

Palabras clave:
Cambio climático
Bovinos criollos colombianos
Índice de carga calórica
Estrés
Índice de temperatura y humedad

Se determinó la respuesta fisiológica y su relación con el índice de impacto (estrés) calórico en tres razas de bovinos criollos colombianos (Hartón del Valle, Blanco Orejinegro y Sanmartinero). Dichas razas son consideradas adaptadas a las condiciones de trópico bajo e importantes para la producción de carne y leche en Colombia. El objetivo del estudio fue determinar dos índices de tolerancia al calor y dos hormonas y su relación con la respuesta fisiológica al estrés calórico en tres razas criollas colombianas usando análisis de correlación y comparación de medias. Los índices de adaptabilidad ITH y HLI, las hormonas cortisol y T3 se estimaron y estos se correlacionaron con las hormonas y se compararon las medias. Se analizaron las variables fisiológicas: frecuencia cardíaca, respiratoria y ruminal y, temperatura rectal; igualmente se determinaron las concentraciones séricas de las hormonas cortisol y triyodotironina, en siete momentos en la época de lluvias en el Piedemonte Llanero. El índice de temperatura y humedad (ITH) y el índice de carga calórica (HLI) fueron empleados como indicadores de compensación térmica. El HLI mostró mejor relación con las condiciones ambientales al incluir el índice la velocidad del viento y la radiación solar. No se encontraron variaciones entre los grupos raciales ($P \geq 0,05$), mientras que los periodos de medición sí presentaron variaciones ($P \leq 0,05$). Se concluye que el HLI proporciona mejor información para el estudio de los parámetros fisiológicos y éstos reflejan la adaptación de los animales a las condiciones del medio.

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Euroamerican genetic resources are an important foundation of biodiversity and their differentiation patterns must be studied and conserved (Sponenberg, 2018). In Colombia, eight racial groups derived from animals that arrived on the second and third voyages of Columbus in the 16th century have been recognized, these constitute a valuable genetic bank of the *Bos taurus*, adapted to the Colombian tropical lowlands (Martínez *et al.,* 2009). Physiological adaptation of these bovines is reflected in their greater longevity and better reproductive rates achieved in difficult ecological environments, with high temperature and humidity, the presence of external and internal parasites, and the low nutritional quality of grazing forages. On the other hand, climate change opens a special window to analyze anthropogenic livestock farming processes (Saiz, 2010). However, in our environment, the adaptation processes of the Colombian Creole breeds and their potential against global warming have not been studied, since the adaptive process responds to homeostatic mechanisms, developed during more than five centuries of interaction with the tropical environment (Núñez-Domínguez *et al.,* 2016).

In cattle, the thermal stabilization mechanisms against a warm environment include the control of excess heat and respiratory rate, regulation of blood flow to the skin, and regulation of the consumption of dry matter and water (Sejian *et al*., 2018); variables that have been quantified according to their genetic component, through the determination of hormones, metabolites, or specific physiological parameters (Franzoni *et al*., 2018). Cortisol and thyroid hormones are indicators associated with chronic stress or thermal regulation (Kamal *et al*., 2018; Campos *et al.,* 2004). Since the temperature of an animal depends on the calories produced per unit mass and the rate of heat exchange, which in turn depends on the production of metabolic heat, the heat gains from external sources, and the rate of heat loss to the environment (Kamal *et al*., 2018), in the case of warm environments, body temperature tends to increase above normal values because of the effect ambient temperatures and solar radiation, as well as the heat from the ground. Air temperature and solar charge determine the speed with which an organism exchanges heat with the environment (Da Silva *et al*., 2015).

When the ambient temperature rises, bovines increase their respiratory rate (RF), pulmonary ventilation, and respiratory vaporization, but when temperatures reach 26 °C for *Bos taurus* and 35 °C for *Bos indicus*, especially when the relative humidity is greater than 75%, these mechanisms are not capable of dissipating all excess heat, given that the higher the relative humidity, the lower the ability to lose heat by convection (Sejian *et al*., 2018); for this reason, determining the caloric load indexes allows the understanding of the relationships derived from the physiological responses (ruminal, cardiac and respiratory frequencies, rectal temperature, bodily heat exchange) to climatic challenges such as wind speed, sunlight, humidity and air temperature (Polsky and von Keyserlingk, 2017), to elucidate the physiological mechanisms of adaptation of local native cattle. Consequently, this study aimed to determine two heat tolerance indexes and two hormones and their relationship to the physiological response to heat stress in three Colombian Creole cattle breeds using Pearson's correlation methodology and mean comparison study.

MATERIALS AND METHODS

The experiment was carried out at the La Libertad research center of AGROSAVIA in Villavicencio located at 4°03´ north latitude and 73°46´ west longitude, at 330 masl, the annual average temperature is 26 °C, relative humidity of 80%, the annual precipitation in this geographic area in the last 28 years exceeds 2900 mm, with a rainy season between April and November, followed by a dry period between December and March (IDEAM, 2017).

Blood samples were collected for hormones analysis, and physiological parameters were determined in four Sanmartinero breed cows, six Blanco Orejinegro breed cows, and five Hartón del Valle breed cows (Figure 1 A-C); with an age range of 3-12 years, and between one and five calvings, grazing in *Brachiaria decumbens* pastures with access to mineralized salt and water *ad libitum*.

Table 1 presents the weight, body condition score, production, and composition of the milk of the experimental cows. These animals were subjected to health prevention and control programs following the regular schedules and plans provided by the center, which included vaccination against foot-and-mouth disease and brucellosis in females and control of external and internal parasites twice a year for all

Figure 1. Colombian creole cattle breeds. A. Hartón del Valle. B. Sanmartinero. C. Blanco orejinegro. (Photographaps by G. Onofre R.)

animals. All experimental procedures were reviewed and approved by the animal ethics committee at the Faculty of Agricultural Sciences of La Salle University.

The collection of samples was carried out between April and September (rainy season) with seven evaluation periods, with an interval of 15 to 30 days. After a 60 min break, females entered the holding area, located under an artificial shade where cows could recognize the environment and relax for 15 min before starting the measurement of the physiological parameters and collecting blood samples carried out between 11:30 and 13:00 hours. Each cow's heart rate was measured by auscultation for 1 min, respiratory rate by observation of the lateral flank for 1 min also, ruminal motility by observing the left flank fossa for 2 min, and rectal temperature with a mercury thermometer for 3 min. Blood samples were taken by puncture of the coccygeal vein using 22-gauge needles, 25 mm long, and vacuum plastic tubes (Vacutainer®). Blood samples were centrifuged within 1 h after sampling to separate serum and were stored at -80 °C until processing in the laboratory. Cortisol and T3 hormones were analyzed by solid-phase radioimmunoassay (RIA), with a commercial kit (Siemens) labeled with ^{125}I radioactive isotope and cortisol by RIA through DPC kits (Los Angeles, CA); Reading was performed with a Nucleonics single-well equipment (Berkeley, CA) and the values of each hormone were obtained using the RIACALC software, from the University of Guelph (Canada).

Table 1. Mean values of body weight, body condition, dairy milk production, and lactation and milk composition of experimental cows

Variable	Hartón del Valle	Blanco Orejinegro	Sanmartinero
Body weight (kg)	475	520	450
Body condition score (1 to 5 scale)	3.0	3.0	3.0
Dairy milk production (kg d^{-1})	3.9	3.1	2.4
Total production per lactation (kg)	1002	737	633
Milk fat (g 100 g^{-1})	3.6	4.2	3.1
Milk protein (g 100 g^{-1})	3.5	3.6	3.7
Total solids (g 100 g^{-1})	12.6	13.5	12.8

The climatological data of the observation period were obtained from the meteorological station N°70 "La Libertad" of IDEAM located in the Research Center at latitude 4°04'N and longitude 73°28W. The information evaluated included the records of environmental temperature in the dry bulb, black bulb, relative humidity, wind speed, and solar radiation. With the data obtained, three ranges of caloric load (HLI) were established by Lees *et al.* (2019): 1 (low), 60-65; 2 (medium), 65.1-70, and 3 (high), 71-75.

The temperature-humidity index (ITH) was determined according to the equation: ITH = (1.8Ta + 32) – (0.55 – 0.0055RH) x (1.8Ta–26.8) HLIBG>25 = 8.62 + (0.38RH) + (1.55BG) – (0.5WS) + [$e^{2.4}$–WS]; where Ta is the ambient temperature (°C) and RH is the relative humidity expressed as a percentage (Dikmen and Hansen, 2009). To include the variables black bulb temperature and wind speed, the index Heat Load Index (HLI) was used. The HLI has two measurement points, for this experiment the (HLIBG) corresponding to the ambient temperature measured in a dry-bulb greater than 25 °C was used, which is calculated with the formula: HLIBG>25 = 8.62 + (0.38RH) + (1.55BG) – (0.5WS) + [$e^{2.4}$–WS]; where RH is the relative humidity, BG is black bulb thermometer temperature, "e" is the base of the natural logarithm (e=2.71828) (Das *et al.,* 2016) and WS is wind speed.

The experimental design and statistical analysis included a generalized linear model (GLM) and analysis of variance with three treatments (breeds) and seven periods, analyzing the caloric load factor. For all variables, measures of central tendency, variance, and standard deviation were calculated as measures of dispersion. The existence of possible associations between the variables studied was analyzed using Pearson's moment correlation coefficient. The statistical program SPSS version 23.0 (SPSS, 2015) was used for the analysis. Due to the homogeneity of the environment and having a different number of experimental animals for each treatment, the data was organized in a completely random arrangement (Petrie and Watson, 2013). The test was assumed to be significant if the probability associated with it was less than or equal to 5%. Duncan's test was used as a means comparison test.

In order to know the behavior of the physiological variables regarding the indexes of climatic adaptation (HLI and ITH), in the first phase, Spearman correlations were estimated to evaluate the relationships between the variables (P<0.05). Subsequently, multiple linear regression was performed, using the mixed model methodology. The regressors were all physiological variables (cortisol, T3, breed, rectal temperature, ruminal, heart, and respiratory frequency), the adaptation index (HLI or ITH) was the response variable, while the cow was considered as a random effect. In the first modeling attempts, it was observed in the partial residuals that the variable "ruminal frequency" included a quadratic behavior, so it was estimated and included as a regressor. To identify the physiological variables that most influenced the indexes, the "Backward" variable elimination method was used at P<0.05.

RESULTS AND DISCUSSION

Table 2 describes the climatic conditions that occurred during the experimental period, on the sampling days in which the physiological parameters were determined in the experimental animals. The ITH was higher ($P\leq 0.05$) in period 7 and lower in period 6 (Table 2), coinciding with the highest records of maximum temperature and relative humidity of 75%, records compatible with severed stress; however, the physiological parameters were found within the ranges expected for creole cows in conditions of the tropical lowlands (Table 3), but with

Table 2. Climatic variables, ITH and HLI associated with seven instances between April and September in the Experimental Center La Libertad (Villavicencio- Colombia).

*	1	2	3	4	5	6	7
Maximum Temperature (° C)	32.0 c	31.0 bc	31.6 bc	32.4 c	29.6 b	26.0 d	33.4 a
Minimum Temperature (° C)	22.2 b	22.0 b	21.6 c	23.4 a	22.2 b	22.0 b	23.2 a
Relative Humidity (%)	82 b	86 b	87.3 a	69.5 d	67.5 d	89 a	75 c
Win speed (m seg^{-1})	6.12 c	6.75 bc	7.28 bc	7.86 bc	11.07 a	2.57 e	4.68 d
Solar brightness (h)	6.6 b	9.4 a	4.1 c	6.5 b	3.7 c	1.1 e	2.6 d
HLI[1]	57.92 c	66.34 a	61.26 b	62.09 b	60.41 b	58.11 c	59.48 b
ITH[2]	78.5 b	78.9 b	78.3 b	78.2 b	75.9 c	74.2 c	81.2 a

*Sampling dates: 1=08/04; 2=22/04; 3=06/05; 4=20/05; 5=08/07; 6=12/08; 7=30/09
Different letters on each line indicate a significant difference ($P\leq 0.05$), through the Duncan test.
[1] HLI= Heat Load Index (Caloric Load Index). [2] ITH= Humidity Temperature Index

changes ($P \leq 0.05$) throughout the evaluation periods. The mean cortisol concentration found was 151.7 nmol L^{-1} and the greatest concentration (233.8 nmol L^{-1}) was found in period 3 and was greater ($P \leq 0.05$) than in periods 1, 6, and 7. The average value of the T3 concentration was 1.46 nmol L^{-1} but there were no differences between periods except for periods 3 with the greatest concentration (1.92 nmol L^{-1}) and 5 with the lowest (0.94 nmol L^{-1}) ($P \geq 0.05$). The HLI value was greater ($P \leq 0.05$) in period 2 and lower in period one, but there was evidence that the climatic variables recorded in the sampling periods corresponded to an HLI, from group 1 or lower in the range proposed (between 60 to 65).

Pearson's correlation coefficients between HLI and ITH were significant ($P<0.001$) with a value of 0.55. Since it is a medium and positive correlation, the HLI index was adopted because it included a greater number of climatic variables, which would theoretically make it more accurate.

The results for multiple linear regression showed that 3 variables T3 ($P<0.0502$), heart rate ($P<0.0102$), and quadratic ruminal frequency ($P<0.0167$) were equally significant for both indexes, although with a low R^2 (0.21). The only difference between the estimated models was found in the intercept 95.38 and 78.38 for ITH and HLI, respectively).

$$\text{HLI} = 78.38 \times -2.53 \times \text{T3} - 0.13 \text{ x heart rate} - 0.54 \times \text{ruminal frequency}^2$$

$$\text{ITH} = 95.38 \times -2.53 \text{ x T3} - 0.13 \times \text{heart rate} - 0.54 \times \text{ruminal frequency}^2$$

Table 3. Serum concentrations of cortisol, T3, physiological constants, and HLI, on seven different occasions between April and September (rainy season) in Colombian Creole cattle in the Piedemonte Llanero.

	1	2	3	4	5	6	7
Cortisol (nmol L^{-1})	126.6 b	159.5 ab	233.8 a	208.5 ab	162.0 ab	119.9 c	126.4 bc
T3 (nmol L^{-1})	1.46 ab	1.44 ab	1.92 a	1.45 ab	0.94 b	1.51 a	1.51 a
Heart rate (b min^{-1})	72.67 b	74.00 b	72.09 b	56.11 d	87.25 a	43.12 e	63.73 c
Respiratory rate (Mov min^{-1})	65.7 a	61.1 ab	51.0 de	42.2 de	29.9 f	33.1 e	52.4 cd
Ruminal movements (Mov min^{-1})	2.7 cd	2.8 cd	2.7 cd	2.7 cd	1.9 e	2.4 de	3.0 a
Rectal Temperature (°C)	39.75 a	39.81 a	39.54 a	39.50 a	39.35 a	39.41 a	39.66 a
HLI[1]	57.92 c	66.34 a	61.26 b	62.09 b	60.41 b	58.11 c	59.48 b

Different letters on each line indicate a significant difference ($P \leq 0.05$) through Duncan's Test.
[1] HLI= Heat Load Index (Caloric Load Index)

The variables analyzed between hormones, climatic information, and the HLI did not correlate ($P \geq 0.05$) between physiological parameters and the caloric load index (HLI).

Blood cortisol concentration was greater than those reported by Campos *et al.* (2009), in a study of metabolic behavior in the peripartum of Hartón del Valle cows, under tropical conditions, and exceed those found in the same breed, but with lactating cows, whose average values fluctuated between 94 - 94.1 nmol L^{-1} (Campos *et al.,* 2012). It showed a different racial behavior, similar to the case of the Sanmartinero racial group. Other values can be found in cattle; however, these are not completely comparable due to the racial type and the climatic conditions in which they are determined (Carvalho and Dupuy, 2017).

The blood concentration of T3 in the cows of this study was consistent with those found in the Colombian Creole lactating cows described by Campos *et al.* (2004). The role of thyroid hormones in the face of stress has been discussed (Kamal *et al.,* 2018), finding that lactating cows subjected to acute heat stress report values of 0.95 nmol L^{-1}. The thyroid and endocrine metabolic responses to climate change are not immediate (Gaughan *et al.,* 2019) and therefore, despite the existence of significant differences between sampling periods in the present study, there is no evidence indicating a direct relationship between caloric load and thyroid response.

First of all, there was no relationship between physiological parameters (respiratory, heart, and ruminal rates, and temperature) and blood cortisol concentrations within normal concentrations; the absence of a relationship may be because the determinations of the physiological constants were punctual (seven specific moments), when a process of adaptation should generally respond to a wider measurement window, which is not always possible because of the costs and permanent handling of animals that affect cortisol concentrations.

Secondly, the time of the year in which sampling was carried out (April to September -Table 2), corresponds to the rainy or wet season, which in the ecozone of study, is of monomodal type, where the rainy and dry seasons are markedly contrasting (Amézquita *et al.,* 2013), which could generate lower physiological efforts in the animals by having less radiation and better compensation for heat loss by refrigeration.

Thirdly, the number of experimental animals is an important limitation to extract information that can be extrapolated as a general parameter for Colombian Creole cattle, since only three of the eight breeds recognized as Colombian were studied in the experiment (Martínez *et al*., 2009), and among these groups, there was a limited and heterogeneous number of individuals. These cows came from the germplasm banks of the Research Center which limited access to a greater number of animals per breed, which have a reduced inventory; however, the responses for breeds were consistent throughout the evaluation period, which supports the results obtained.

The HLI was not different ($P\geq 0.05$) between dates in which the physiological parameters were determined. This index was considered low (level 1), with no environmental impact, and corresponds to the non-extreme climatic conditions registered, which were verified with the traditional ITH, that shows that the work area (Piedemonte Llanero) at the time of the measurements presented an ITH corresponding to the limit between the zone of moderate stress and severe stress zone, which is not considered significant for animals adapted to these environmental conditions. Only the seventh sample presented a qualification of the ITH as severe stress because in this period the environmental temperature was the greatest observed (33.4 °C) and the relative humidity was 75%, which generates a high-stress value according to the value of ITH for *Bos taurus* cattle under these conditions (Polsky and von Keyserlingk, 2017); however, the physiological responses of the cows were not altered, which raises questions about the value of ITH as an indicator of physiological response in bovines in the tropics and subtropics, as has been proposed by Dikmen and Hansen (2009).

According to Lees *et al.* (2018), the development of a heat stress index for livestock must be based on biological factors, which generates the need for a high number of data to develop and test an index. The biological parameter used should also be easy to measure and be a good indicator of the heat load. The physiological and behavioral changes were reliable indicators of the state of the heat load, demonstrated in the present work that the experimental animals were in conditions of adaptation since they did not present behavioral alterations or changes in their physiological parameters.

Heart frequency was between 40 and 94 beats per min. There was a positive correlation between heart frequency and heat load. Other authors found the same tendency (Peña *et al.,* 2011), in Patagonian Creole cattle. On the other hand, various alterations have been found in the physiological patterns that originated in adaptive changes in cattle under conditions of humidity and temperature similar to those of the experiment (Parra-Herrera *et al.,* 2017).

The rectal temperature remained within normal limits, which is to be expected if the thermoregulatory mechanisms are functioning. There were no differences in rectal temperature between treatments, although it increased at the maximum value with a greater caloric load. In the same sense, efficiency in thermal regulation is known when critical stress situations are not faced (Sejian *et al.,* 2018).

Regarding ruminal motility, the comparison presented differences ($P\leq 0.05$) in the number of ruminal movements under different caloric loads and, the linear regression analysis described a slight tendency to increase with greater caloric load, which would indicate that the consumption of dry matter did not affect, since the ingestion of grass was not compromised, which for native breeds of *Bos taurus* origin is positive because indirectly it can be associated with adaptation.

When the mechanisms of thermoregulatory response act in the bovine, ruminal movements are not altered, and this can clarify the significant positive correlation between respiratory frequency and skin heat loss processes. Pulmonary ventilation showed a trend line indicating that the higher the caloric index, the higher the respiratory frequency (significant correlation of 0.556). The reason why the high respiratory rate presented at high temperatures is that the cattle use pulmonary ventilation as a heat dissipation mechanism along with transpiration and behavior changes seeking a lower exposure to solar radiation (Das *et al*., 2016).

The physiological parameters (Table 3) presented values within the range known as "normal" for animals under the conditions of the experiment and did not present extreme outliers, except for the fifth sampling focal point, where there is no expected relationship between respiratory and heart frequency. The values for all animals did not present differences ($P \geq 0.05$) between the racial groups studied. This could indicate that under the experimental conditions the animals of the three racial groups exhibited similar physiological patterns and therefore high similarity of adaptive behavior associated with the interaction with the environment where they were kept. The main limitations for a conclusive connection between the relationship for adaptation with physiological variables lie in the number of animals included in the study and the homogeneity of animals per group.

Similar results were observed in Brazil by Lima *et al*. (2020) when evaluated the effect of temperature on Caracú (creole) and Nelore males in three different periods: morning (8:00 to 10:00), afternoon in the sun, and afternoon in a shaded environment (11:00 to 13:00). The animals of the two breeds exhibited no significant changes in heart rate, respiration rate, and cortisol level for the maintenance of thermal homeostasis. Researchers concluded that Caracú and Nellore are considered tolerant to sun exposure, indicating adaptation of these breeds to the high-temperature environments of the tropics. In northern Ethiopia was evaluated the effect of the season (dry and rainy season) on the physiological response to heat stress in the creole Fogera cattle (Abera *et al*., 2021). During the dry season was observed an increase in respiratory and heart rate, but not in rectal temperature.

Some authors suggest that is necessary a period of adaptation to evaluate response to heat stress. In India Yadav *et al*. (2021) developed a study to assess the acclimatization dynamics in dry crossbred creole cows (Haryana × Brown Swiss, Holstein, and Jersey). Cows were exposed daily to 25 °C and 40°C with a relative humidity of 40–50% in a climatic chamber from 10:00 to 15:00 h, for 21 days. Rectal temperature, respiratory and heart rate increased at 40 °C compared to 25 °C, but there was no effect on the T3 hormone. Acclimatization response in respiratory rate was first observed on day 11 and for T4 and cortisol hormones levels on day 11 and 16, respectively. The authors concluded that in extreme heat stress in creole crossbred cows is necessary a period of adaptation of 6 to 21 days for acclimatization to heat stress.

However, some physiological mechanisms of response to heat stress in tropical conditions are different between *Bos taurus* and *Bos indicus* or crossbred cattle (*Bos indicus* × *Bos taurus*). Moura *et al*. (2021) compared the physiological responses of an F1, Nellore x Angus to Nellore cattle kept under moderate thermal conditions (air temperature 16 to 30 °C). Slight differences in thermal storage and daily fluctuation of daytime rectal temperature (07:30 and 16:30), were observed in Nellore (39 to 39.30 °C) compared to Nellore x Angus (39.12 to 39.62 °C). In addition, a study in Brazil on Nellore heifers found that heat stress (34 °C) increased heart and respiratory rates (Moreno *et al*., 2021).

The climatic variables (Table 2) reflect the corresponding rainy season; thus, the relative humidity presented high values, and in at least one day a low wind speed was associated with greater rainfall, these values have been described by various authors for the geographical area called "Piedemonte Llanero" comprised approximately between 3°65' and 4°15' on the eastern slope of the eastern mountain range of Colombia (Amézquita *et al.,* 2013).

The relationship of climatic variables with physiological behavior was analyzed through the correlation between ITH and HLI, to integrate the animal response to the climatic conditions in which they live. The implications of unfavorable environmental conditions for livestock are presented in studies that describe how environmental stress negatively affects animal health and productivity (Herbut *et al.,* 2018; Polsky and von Keyserlingk 2017).

The first reference to the behavior of Creole cattle in various climatic conditions was based only on temperature and humidity changes and mechanisms morphological, behaviors, neuroendocrine, and cellular responses (Angel *et al*., 2018), which, although not invalidated, leaves reasonable doubt as to its current predictive value on physiological behavior. This paper uses the HLI that also contemplates solar radiation and wind speed, as a dynamic way to look for the best indicators that allow analyzing the physiological processes of adaptation (Das *et al.,* 2016).

The concept of the caloric load index generates a better understanding of the adaptation processes since the inclusion of the two climatic variables (black bulb temperature, which measures radiant energy or solar radiation, and wind speed), allows us to analyze the rates of heat loss or gain by the animal in its environment since this impact modifies the indexes in which the animal response is measured (Herbut *et al.,* 2018). Likewise, the low HLI values allow us to recognize that there are dynamic processes of physiological compensation in a moderately adverse environment (low HLI); on the contrary, the recording through ITH between 74 and 81.2 (Table 2) places the animals in front of dangerous classifications of alert, because they are close to the known limits for severe stress, which does not agree with the determined physiological constants.

When the simple interactions between biological variables were studied, through Pearson's correlation (Table 4), there is a wide variation between the values and statistical significance found, which does not always present a close correlation with the expected homeostatic behaviors or with the factor's compensatory physiological measurements between one measurement and another. Even though the present study found a better relationship between the HLI and physiological parameters compared to ITH, the applicability of the ITH had been demonstrated when the index was related to production traits. A study in Tanzania evaluated the effect of heat stress on milk production using the ITH on test-day milk records of smallholder dairy cattle herds (Ekine-Dzivenu *et al*., 2020), cows experienced heat stress between ITH values of 67 and 76. Similar results were observed in dairy cows in the tropical conditions of Costa Rica with a decrease in milk production when values of ITH exceed 72 (Ruiz-Jaramillo *et al*., 2019). In Italy Maggiolino *et al*. (2020) determined if a threshold of the ITH exists for milk production traits in Italian Brown Swiss dairy cows, researchers found that the Brown Swiss breed had higher thermal tolerance compared to Holstein cows. As ITH rose, Brown Swiss cows tended to produce the same volume of milk, but with a decreasing quality concerning components. This particular situation could be

Table 4. Pearson's correlations between physiological parameters, hormonal concentrations, and HLI in Colombian Creole bovines in Piedemonte (lowlands).

	HLI[1]	Cortisol	T[3]	HR[2]	ReR[3]	RuM[4]	RT[5]
HLI	1						
Cortisol	0.088	1					
T3	0.034	0.066	1				
Heart rate	0.361*	0.241*	-0.064	1			
Respiratory rate	0.556*	0.095	0.16	0.099	1		
Ruminal rate	0.064*	0.121	0.206	0.244*	-0.181	1	
Rectal temperature	0.161*	0.088	-0.054	0.057	0.209	0.27	1

* Values with an asterisk presented statistical significance $P \leq 0.05$)
[1] Heat Load Index; [2] Heart rate; [3] Respiratory rate; [4] Ruminal movements; [5] Rectal temperature

similar in creole cows adapted to tropical conditions for Fogera cattle of Ethiopia. Abera *et al.* (2021) found that the ITH value of 66 was considered optimum for high weight gain and normal physiological response to heat stress.

The literature reports several thermal indexes in addition to the HLI that can be used to predict the degree of heat stress on cattle by relating environmental variables to physiological parameters. Researchers in Brazil developed a model based on an artificial neural network, for individual assessment of the level of thermal stress in feedlot finishing Nellore cattle which includes both weather (dry and wet bulb temperature) and animal variables (rectal temperature, respiratory rate, and infrared body surface temperature). The thermal stress predicted by the neural model was correlated with rectal temperature, and the prediction was higher than the ITH method (Vieira de Sousa *et al.*, 2018). In addition, the Equivalent Temperature Index for Cattle (ETIC), which includes air temperature, relative humidity, air velocity, and solar radiation and their interactions was developed in 2018 for dairy cattle in Europe. The ETIC predicted better skin temperatures, core-body temperatures, and respiration rates compared to the ITH, the heat load index, and the comprehensive climate index (Wang *et al.*, 2018).

CONCLUSIONS

The physiological and endocrine relationships in face of climatic variations were reflected in the HLI, describing that the latter presents a correlation with the physiological parameters, although its value was low (0.03–0.55; $P \leq 0.05$). The assessment of associations when cortisol was determined and closely related to the time and degree of the stress conditioner that is present and to which the animals respond (0.06–0.24; $P \leq 0.05$). The multiple linear regression showed that 3 variables T3 (P<0.0502), heart rate (P<0.0102), and quadratic ruminal frequency (P<0.0167) were equally significant for HLI and ITH indexes. In the case of the present experiment, no acute and severe challenge could have generated an elevation in the concentration of cortisol (126.6–233.8 nmol L^{-1}), nor extreme changes in the values registered of the physiological parameters (Heart rate, 43.1-87.7 beats min^{-1}; respiratory rate, 29.9–65.7, mov min^{-1}; ruminal movements, 1.9–3.0 min^{-1}; and rectal temperature, 39.3–39.8 °C). Studies in physiology such as the present one, are essential to know the biological mechanisms of adaptation of Colombian Creole cattle.

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