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# Use of thermography to assess heat tolerance in cattle breeds

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#### **Research Article**

Keywords: air temperature, bovine breed, broken line, logistic regression, eye temperature, respiration rate

Posted Date: November 11th, 2022

DOI: https://doi.org/10.21203/rs.3.rs-2198859/v1

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**Version of Record:** A version of this preprint was published at Tropical Animal Health and Production on May 24th, 2023. See the published version at https://doi.org/10.1007/s11250-023-03613-4.

# Abstract

Thermography has grown in use in recent years. It is a valuable tool for measuring animal heat tolerance under heat stress conditions since it is a non-invasive, safe and practical methodology. Physiological variables such as respiration rate and eye temperature, and environmental variables such as air temperature and wet bulb temperature were analysed in animals from nine cattle breeds (Angus, Braford, Brangus, Canchim, Charolais, Hereford, Nelore, Simmental and Santa Gertrudis) and one bubaline (Mediterranean) from the Expointer event in Rio Grande do Sul, Brazil. Positive correlations were observed between air temperature and respiration rate and eye temperature. Furthermore, the breed strongly influenced the eye temperature and respiration rate of the animals. The inflexion points in the broken line analysis indicated the environmental temperature limits at which breeds begin to change their respiration to compensate for environmental variation. Logistic regression analysis allows us to observe how each breed behaves with the temperature change. Using respiration rates and eye temperatures it was possible to identify physiological limits for comfort in different breeds of bovine.

# Introduction

Cattle production in tropical regions accounts for more than half of all cattle raised worldwide (Rubio Lozano et al., 2021), despite being challenging in terms of body thermal control. In addition to increased solar radiation, these high-temperature regions greatly influence animal welfare and production, especially in terms of heat stress.

Most species of domestic animals we know today in Brazil were brought by European settlers during Brazilian colonisation (mainly lberian), with the arrival of Zebu cattle in the early 20th century and British and continental breeds more recently (Ferraz and Felício, 2010; Santana et al., 2016). For this reason, improvement programs are carried out with breeds that are not native to Brazil. Many European breeds, frequently selected over numerous years in milder temperature conditions, have had to adapt to the Brazilian climate. Some have adapted better than others, and their physiological processes of temperature control, production and reproduction are more efficient (McManus et al., 2009). The physiology of body temperature control in mammals is analysed using parameters such as rectal temperature, respiratory and heart rate, sweating rate and body temperature. In addition to these factors, climatic conditions such as ambient temperature, humidity, wind speed, solar radiation influence the animals' response (Silva, 2000).

Rectal temperature is considered a sensitive indicator of thermal balance - commonly used to check the animal's body temperature and health - and has been used for years to assess the effects of the environment on animal development, production, and reproduction (Rejeb et al., 2016). However, the whole process surrounding this measurement - from the animal immobilisation to the introduction of the thermometer - can be very stressful (Rubio Lozano et al., 2021). Regarding eye temperature, Daltro et al. (2017) found positive correlations between physiological parameters (rectal temperature, respiratory rate, heart rate, and panting score) and infrared thermography. The rise in temperature of the areas

photographed with infrared thermography corresponds to the increase in physiological parameters and for Knizkova et al. (2007), the temperature variations on the body surface can be reliably monitored with thermography. Therefore, infrared thermography (IRT) use has grown in recent years (McManus et al., 2016b) and is a valuable tool for measuring heat tolerance in sheep and cattle under tropical conditions since it is non-invasive, safe and effective (Cardoso et al., 2015; Daltro et al., 2017; McManus et al., 2015; Stumpf et al., 2021). IRT can be used to measure eye temperature as an indicator of internal temperature and has proven to be a relatively easy technique, especially when compared to rectal thermometers, tympanic thermometers, and other thermometers with microchips and rumen boluses (Church et al., 2014). Furthermore, this measurement has shown a high correlation with rectal temperature (Stumpf et al., 2021).

In recent years, animal research evaluating the physiological mechanisms responsible for adjusting cold and heat tolerance has received more attention due to global warming (McManus et al., 2016a). Also, most studies evaluating animal heat stress tolerance use one or a few breeds and few studies are looking at responses of a large number of breeds and even less using several breeds and many animals simultaneously. This is especially important with climate change where production environments are essential sources of variations for the success of breeding programs.

The identification of physiological and environmental parameters is important because it helps breeders and researchers to determine the best conditions for raising productive livestock. Currently, the development of research and studies that evaluate the behaviour of the breeds according to certain environmental conditions are being sought. McManus et al. (2016a) used thermography and rectal temperature to identify suitable production regions for sheep breeds in Brazil. In the current paper, we extend this analysis to cattle and bubaline breeds, using respiration rates and eye temperatures to identify physiological limits for comfort.

# **Material And Methods**

Data were collected during the Expointer event in Rio Grande do Sul State, Brazil (29°51'40"S, 51°10'51"W and altitude of 12 meters). Expointer is the largest agriculture and livestock fair in Latin America and a traditional event, dating back 120 years when the first event occurred in 1901. The animals presented at the fair are obligatorily purebred.

Observations (1046) were collected over five days (August) on ten bovine breeds (9 cattle and one buffalo) on 438 animals of both sexes. The cattle breeds were Angus (109 observations), Braford (110), Brangus (110), Canchim (80), Charolais (110), Hereford (111), Nelore (111), Simmental (109), and Santa Gertrudis (108) while the buffalo breed was Mediterranean (88). Data was collected from 7:30am to 6:00 p.m. All animals were housed in individual stalls and were exposed to the presence of the fair visitors, however, the data was collected at times when the animals were not in the presence of the public. Air temperature (TA), wet bulb temperature (WBT), and relative humidity (RH%), were collected with the

use of a black globe thermometer (Extech Instruments, Model HT30). The temperature and humidity index (THI) was calculated using the equation model cited by NRC (1971):

THI1 = (1.8 × TA + 32) - [(0.55 - 0.0055 × RH) × (1.8 × TA - 26.8)]

TA: dry bulb temperature (°C) and

RH: relative humidity of the air (%/100).

The animal's respiration rate (RR) was measured through observing the animal's flank for one minute (repeated twice) and eye temperature (ET) was measured using thermography. Thermographic images were obtained with the use of an infrared camera (FLIR®System T300 emissivity at  $\varepsilon$  = 0.98, as recommended by the camera manufacturer for biological tissues). Three images were taken of each animal at each time point, at a distance of 1.5 m and at a 180° angle between the camera and animal (Lateral). QuickReport® software was used to analyse data from the photos.

Coat colour was registered on a scale of 0 (white) to 100 (black), according to Silva (2000). Respiration rate above 36 breaths/min was defined as "altered" (1) and below this value was defined as "normal" (0) according to Radostits et al. (1983). ET physiological limits were defined by calculating 95% upper confidence limits (PROC UNIVARIATE CIBASIC) for animals with THI below 70. In this case the upper confidence limit was 34.96 °C, so temperatures above this value were considered not normal and classified as "1" for logistic regression. ET and RR were submitted to logistic regression analysis after definition. Animals were weighed on collection days.

Data were analysed using SAS v 9.4 (SAS Institute, Cary, North Carolina). General linear models of the response of eye temperature and respiration rate due to linear and quadratic effects of air temperature and humidity and fixed effects of breed, sex, and coat colour were examined. Correlations and principal component analyses were performed as well as cluster analyses. Logistic regression (PROC LOGISTIC) and broken line analyses (PROC NLIN) were used respectively to determine the relationship between AT and THI with RR and ET. A cluster analysis was also performed (PROC CLUSTER).

# Results

Air temperature ranged from 10.7 °C to 39.9 °C, and relative humidity from 41.8 to 92.3% during the experimental period. Figure 1 shows the positive correlation between the variables. As expected, air temperature and THI index affected eye temperature and respiration rate.

Coat colour and animal sex showed no effect on respiration rate (RR) or eye temperature (ET). Simmental showed the highest values for ET and RR; however, there was no statistically significant difference between Nelore with respect to ET, nor compared to Canchim, Charolais, and Hereford with respect to RR. Santa Gertrudis had the lowest ET - although, there was no statistically significant difference between Santa Gertrudis, Angus and Mediterranean (Table 1). Eye temperature was strongly positively related to air temperature and wet bulb temperature (WBT) (Table 2; Figure 2). RR also increased with increased AT and decreased humidity. As expected, air temperature showed a strong positive correlation with dry bulb temperature and a negative correlation with humidity. Coat colour and body weight did not affect the first component, but animals with darker coats and heavier weights showed lower respiration rates in the second.

The broken-line regression showed the limits of RR and ET for THI. The inflection point indicates this limit (Table 3 ).

In the logistic regression, RR above 36 breaths/min was considered "altered" and defined as (1) and below these values were defined as "normal" (0). The relationships between RR and AT and between RR and THI were analysed. The result can be seen in figures 4a and 4b. The relationships between eye temperature and air temperature and THI can be seen in figures 4c and 4d.

Regarding the increase in AT, the Simmental breed showed altered RR before the other breeds and Nelore was the last breed to show a change in RR (Figure 3a). The analysis made with the THI index showed that Simmental, Santa Gertrudis and Charolais showed an increased RR response before the other breeds, and Nelore was again the last to show a change in RR (Figure 3b).

When analysing the relationship between AT and ET, the Hereford showed changes in ET first, while Braford was the last breed to show changes (Figure 3c). For THI and ET, again Hereford showed changes first and Nelore was the last breed to show any response (Figure 3d).

Table 4 shows odd ratios for breed-by-breed comparisons for reference values outside normal limits. Values above 1 mean that the breed in the column has a higher chance of being outside physiological limits than that in the row (Significant in yellow). Nelore showed better heat tolerance for RR than all other breeds except buffalo. Braford and Brangus also showed superior regulation. Few significant differences were seen for ET (P<0.05), but showing Hereford with poorer ET reactions compared to the four breeds mentioned above.

The cluster analysis (Figure 4) showed Nelore and buffalo group, together with Braford. Simmental, Santa Gertrudis and Charolais formed a second group and then Brangus, Canchim, Hereford and Angus.

## Discussion

The use of infrared thermography is growing in animal research. This non-invasive method has been used to indicate thermal changes in animal metabolism resulting from increased body temperature in response to environmental or physiological conditions. McManus et al. (2016b) used respiration rate and eye temperature to determine Brazilian regions suitable for rearing different breeds of sheep. Cardoso et al. (2015) and Stumpf et al. (2021) used infrared thermography to recognise heat stress in dairy cows and to identify the best part of the animal to be evaluated for this.

Air temperature (AT) and temperature and humidity index (THI) have been shown to affect the variation in eye temperature and respiration rate of animals in general according to the regression analysis (Fig. 1). Vieira et al. (2022) found similar results in a path analysis study that evaluated the relationship between THI index and respiration rate. Air temperature and humidity (combined in the THI index) have a positive relationship with respiration (Habibu et al., 2019; Yan et al., 2021), corroborating the results found in the present study. Some climatic factors, such as the increase in environmental temperature and air humidity, are capable of promoting a physiological response in the animals. They seek to maintain the balance of body temperature and therefore it is possible to observe an increase in respiratory rate and eye temperature. For McManus et al. (2020), respiration rate has been considered the physiological variable most sensitive to heat stress and the precursor of changes in other physiological variables. Stumpf et al. (2021) also found a positive correlation when they analysed the lateral udder thermography method with rectal temperature. This result is explained by the fact that infrared thermography scans the surface temperature (Hoffmann et al., 2013), which varies according to the blood flow that fills the tissues and also the amount of heat lost to the environment. Since the udder is a highly vascularized organ, it is possible to have good results with the use of this technique

Bovine breeds respond differently to heat stress (McManus et al., 2021). The buffalo breed was more resistant in terms of RR mean than the cattle breeds as can be seen in Table 1. According to Naveena and Kiran (2014), buffalo are known for their high adaptability to extreme environments, such as tropical regions. In addition, buffaloes also have dietary flexibility, simple management, high resistance to disease, and acceptability to various types of housing and feeding (Wanapat and Kang, 2013).

The Nelore breed had the second highest value for eye temperature (ET), despite being considered a breed well adapted to the Brazilian climate. This may be a reflection of recent selection pressure on Nelore for increased production, which may have affected its heat tolerance, remembering that only purebred animals can be at the Expointer event. Angus and Santa Gertrudis showed a different pattern between ET and RR, showing an inverse response, where the animals showed lower ET and high RR. In the other breeds, a similar behaviour can be seen, in which an increase in RR accompanies an increase in ET. This result may explain how the physiological responses happen., in which the change in respiratory rate is a response that precedes the increased temperature of the animal. Simmental was the breed that presented the highest values of RR and ET. The Simmental breed (Bos taurus taurus) originated in Switzerland and was brought to Brazil in 1904. According to the official website of Simmental breeders in Brazil, over the years, it has been sought to form lineages through genetic material imported from European countries, Canada and the United States (Associação Brasileira dos Criadores das Raças Simental e Simbrasil, n.d.). Despite being relatively well adapted to the climatic conditions of some Brazilian regions, this breed still show physiological responses characteristic of European animals because they are purebreds. It is important to note that the Simmental breed showed the second highest average value for body weight. This characteristic could be one of the answers for the RR and ET values. According to Vieira et al. (2022), larger animals have a lower efficiency in controlling body temperature. For these authors, the larger the animal, the greater is the requirement on the lungs and heart that allow the conduction of blood

through the body. Cardoso et al. (2015) also found that chest circumference can influence RR by limiting expansion, which also indicates the influence of animal size on thermal control.

Eye temperature was strongly related to air temperature and wet bulb temperature, as shown in in the principal components analysis. RR also increased with increased AT and WBT and showed a negative correlation with humidity. High temperature combined with low air humidity is characteristic of the tropical region of Brazil, with RR increasing with increased AT and RH.

The broken line analysis was performed to determine the inflection point that indicates the moment the animal changes its breathing to adjust its body to environmental variations and the point whereby eye temperature starts to increase as RR no longer controls internal body temperature. Inflexion points were observed for each breed, analysing respiratory rate and eye temperature as a function of THI index. Analysing the variation of RR as a function of THI, the Nelore breed had the lowest inflexion point value, while Hereford and Canchim had the highest values. This indicates that when the THI is close to 65, Nelore animals have already shown a change in RR in an attempt to control their body temperature, while Hereford and Canchim start this control at a THI of approximately 77. Considering ET as a function of THI, Braford had the lowest inflection point value (66.1), while Santa Gertrudis had the highest (79). This means that at THI 66, Braford animals already showed an increase in eye temperature, while Santa Gertrudis animals did not show this increase until later, at THI 79. The use of thermography for eye temperature analysis has proven to be an applicable and efficient technique for evaluating the temperature of animals.

The increase in air temperature directly affects the respiratory rate of the animals. In the logistic regression analysis, it was possible to observe how this reaction occurs for each breed. Using AT as a variable, the results indicate that Simmental had a priority response, showing changes in RR faster than the other breeds, while Nelore had a later response at higher air temperature. This delayed response in Nelore is expected. *Bos indicus* breeds are considered adapted to the conditions of the Brazilian climate in general, and so expected to have a greater tolerance to high environmental temperature. According to McManus et al. (2020), the increased adaptation of *Bos indicus* cattle to tropical environments is also related to the higher transpiration rates due to the higher density, closer to the surface and larger volume of sweat glands in these breeds when compared to *Bos taurus* cattle. There is also a rapid transfer of metabolic heat to the skin due to the lower resistance of the tissues and the coat to heat loss. These authors also found that the density of hair follicles is also greater in B. *indicus* than in B. *taurus*, and this denser coat results in greater protection against radiant heat gain. Analysing the variables AT and ET, Hereford was the first breed to show a response in the variation of ET and Braford the last.

The Charolais, Santa Gertrudis and Simmental breeds showed responses for RR before the other breeds considering the increase THI, with Nelore being the last. The altered response of these animals is considered when the respiratory rate is above 36 breaths/minute. Regarding ET, temperatures above 34.96 °C were considered not normal for logistic regression. Hereford and Nelore stood out as the first and last, respectively, to show response to increase ET according to THI.

The heat tolerance of Nelore can also be observed in the odds ratio analysis. Nelore showed better heat tolerance for RR compared to all other breeds except buffalo. However, when analyzing ET, Nelore did not have an advantage over the other breeds. The good RR response and the poor ET response confirm the other results that we presented throughout this study. Nelore presented itself as a breed with good respiratory rate control, but eye temperature has shown a more rapid change.

The cluster analysis shows Nelore, Mediterranean and Braford forming a group with similar characteristics in terms of thermal control. This result complements the logistic regression analysis, in which Nelore, Mediterranean and Braford breeds always appear close and with similar response values of RR and ET to the variation of AT and THI. Thus, in the cluster analysis, we can observe these three breeds forming a similar group. Simmental, Santa Gertrudis and Charolais formed a second group and the answers are similar to those found in the logistic regression analysis when we analysed variation of RR in relation to AT and THI. Brangus, Canchim, Hereford and Angus formed the last group. These limits can be used to map regions of thermal comfort for breeds under changing climatic conditions (McManus et al., 2016a).

## Declarations

## Funding

The authors declare that no funds, grants, or other support were received during the preparation of this manuscript.

#### **Competing Interests**

The authors have no relevant financial or non-financial interests to disclose.

#### Author Contributions

All authors contributed to the study conception and design.

Renata A. Vieira: Formal analysis, Investigation, Writing – original draft, Writing – review & editing, Visualization.

Eduardo A. Dias: Methodology, Investigation, Writing – review & editing, Validation, Visualization.

Marcelo T. Stumpf: Methodology, Investigation, Writing – review & editing, Validation, Visualization.

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Julio O. J. Barcellos: Conceptualization, Methodology, Investigation, Writing – review & editing.

Giovani J. Kolling: Methodology, Investigation, Writing – review & editing, Validation, Visualization.

Concepta McManus: Conceptualization, Methodology, Formal analysis, Investigation, Writing – original draft, Writing – review & editing, Visualization, Supervision.

## Data Availability

The datasets generated during and/or analyzed during the current study are not publicly available because they are data that were collected manually during the experiment and stored by the researchers in their personal files but are available from the corresponding author upon reasonable request.

#### Ethics Approval

The Ethics Committee approved the present study for Animal Use of the Federal University of Rio Grande do Sul, number 22773.

#### Acknowledgements

To CAPES for scholarships.

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## Tables

Table 1 Effect of breed on thermographic eye temperature (ET), respiration rate (RR), body weight and coat colour.

	ET	SD	RR	SD	Body Weight (kg)	SD	Coat Colour	SD	
Simmental	36.38 <sup>a</sup>	1.48	43.05 <sup>a</sup>	8.94	720.82 <sup>ab</sup>	172.62	72.64 <sup>d</sup>	11.33	
Nelore	35.29 <sup>ab</sup>	1.95	25.40 <sup>bc</sup>	11.28	599.23 <sup>bcd</sup>	194.77	16.06 <sup>e</sup>	10.57	
Canchim	34.87 <sup>b</sup>	1.11	36.04 <sup>ab</sup>	10.04	691.22 <sup>ab</sup>	227.23	10.00 <sup>f</sup>	0.0	
Charolais	34.71 <sup>b</sup>	1.27	33.18 <sup>abc</sup>	10.87	656.18 <sup>abc</sup>	180.87	10.00 <sup>f</sup>	0.0	
Brangus	34.39 <sup>b</sup>	1.20	28.07 <sup>bc</sup>	10.77	713.91 <sup>ab</sup>	200.20	93.79 <sup>b</sup>	7.26	
Hereford	34.34 <sup>b</sup>	1.31	32.11 <sup>abc</sup>	11.42	804.41 <sup>a</sup>	214.09	87.50 <sup>c</sup>	6.66	
Braford	34.32 <sup>bc</sup>	1.52	27.00 <sup>bc</sup>	9.51	713.54 <sup>ab</sup>	240.07	89.59 <sup>c</sup>	6.10	
Mediterranean	33.94 <sup>bcd</sup>	1.36	23.17 <sup>c</sup>	10.18	496.32 <sup>d</sup>	70.17	100.00 <sup>a</sup>	0.0	
Angus	32.83 <sup>cd</sup>	1.49	29.30 <sup>bc</sup>	11.85	650.90 <sup>bcd</sup>	109.42	96.38 <sup>b</sup>	6.71	
Santa Gertrudis	32.63 <sup>d</sup>	1.51	36.36 <sup>ab</sup>	10.97	522.82 <sup>cd</sup>	57.25	94.05 <sup>b</sup>	7.41	
SD = Standard Deviation. Means in the same column followed by different letters are significantly different by the Tukey test (P < 0.05)									

Correlations between environmental variables and physiological variables in cattle									
	ET	RR	AT	WBT	Humidity	Body Weight			
RR	0.36								
ТА	0.72	0.49							
WBT	0.71	0.45	0.93						
Humidity	-0.69	-0.41	-0.90	-0.83					
Body Weight	0.14	0.08	0.08	0.07	-0.18				
Coat Colour	0.07	0.01	0.04	0.01	-0.05	0.02			
ET: Eye Temperature; RR: Respiration Rate; AT: Air Temperature; WBT: Wet Bulb Temperature.									

Table 2

Table 3 Inflexion points for Temperature Humidity Index from broken line regression by breed for respiration rate and thermographic eye temperature in bovine breeds.

	Respira	ation Ra	te		Thermographic Eye Temperature				
Breed	Mean	SD	Lower	Upper	Mean	SD	Lower	Upper	
Angus	74.87	3.25	68.41	81.33	66.44	5.02	56.47	76.41	
Charolais	66.07	1.87	62.34	69.78	70.86	4.57	61.79	79.93	
Hereford	77.61	0.47	76.69	78.54	69.04	6.80	55.55	82.52	
Mediterranean*	70.34	4.22	61.92	78.74					
Nelore*	65.99	0.59	64.83	67.17					
Simmental	75.70	3.33	69.07	82.34	75.35	5.71	64.02	86.65	
Braford	69.85	1.60	66.66	73.03	66.16	1.61	62.96	69.36	
St G	67.73	1.51	64.72	70.74	79.06	0.66	77.75	80.36	
Brangus	83.10	1.87	81.34	85.72	77.24	1.17	74.93	79.56	
Canchim	77.62	1.93	75.17	79.99	71.62	2.37	68.52	76.34	
*Mediterranean and Nelore did not converge for eye temperature. SD – standard deviation.									

**Table 4** Oddratios in breed-by-breed comparisons for respiratory rate and eye temperature in bovine

 breeds

	Angus	Braford	Brangus	Canchim	Charoles	Hereford	Mediterranean	Nelore	Simmental
Respiratory Rate									
Braford	5.66								
Brangus	2.09	0.37							
Canchim	0.90	0.16	0.43						
Charoles	0.55	0.10	0.27	0.61					
Hereford	1.15	0.20	0.55	1.27	2.07				
Mediterranean	6.99	1.24	3.35	7.73	12.61	6.08			
Nelore	31.94	5.65	15.30	35.32	57.62	27.79	4.57		
Simmental	0.55	0.10	0.26	0.60	0.98	0.47	0.08	0.02	
Sta Gert	0.54	0.10	0.26	0.60	0.98	0.47	0.08	0.02	0.99

#### Thermographic Eye Temperature

Braford	1.61								
Brangus	1.38	0.86							
Canchim	0.98	0.61	0.71						
Charoles	1.11	0.69	0.81	1.13					
Hereford	0.51	0.31	0.37	0.51	0.45				
Mediterranean	1.93	1.20	1.40	1.96	1.74	3.83			
Nelore	2.01	1.25	1.46	2.04	1.81	3.98	1.04		
Simmental	0.80	0.50	0.58	0.81	0.72	1.58	0.41	0.40	
Sta Gert	0.62	0.39	0.45	0.63	0.56	1.23	0.32	0.31	0.78

# Figures



## Figure 1

Effect of air temperature (a) and THI (b) on eye temperature (ET) and respiration rate (RR)



## Figure 2

First two principal components of bovine breeds data. ET: Eye Temperature; RR: Respiration Rate; AT: Air Temperature; WBT: Wet Bulb Temperature

## Air Temperature



#### Figure 3

Probabilities of response outside normal conditions (ranked as 1) of breeds for respiratory rate (a and b) and Eye Temperature (c and d) according to increasing Air Temperature and THI\_NRC index



## Figure 4

Bovine breed Cluster analysis