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Maycon Rodrigues da Silva

Índices de conforto e características adaptativas de ovinos nativos mantidos em ambiente de estresse térmico em câmara climática

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Tese submetida ao Programa de Pós-Graduação em Ciência e Saúde Animal, da Universidade Federal de Campina Grande, como requisito parcial para obtenção do Título de Doutor em Ciência e Saúde Animal.

Orientador: Prof. Dr. Bonifácio Benicio de Souza

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MAYCON RODRIGUES DA SILVA

ÍNDICES DE CONFORTO E CARACTERÍSTICAS ADAPTATIVAS DE OVINOS NATIVOS MANTIDOS EM AMBIENTEDE ESTRESSE TÉRMICO EM CÂMARA CLIMÁTICA

Tese apresentada ao Programa de Pós- Graduação em Ciência e Saúde Animal como pré-requisito para obtenção do título de Doutor em Ciência e Saúde Animal.

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SUMÁRIO

RESUMO

Objetivou-se avaliar os índices conforto e características adaptativas de ovinos nativos dos grupos genéticos Soinga e SPRD e das raças Morada Nova e Santa Inês em câmara climática. Essa Tese é composta de três capítulos, sendo uma revisão sistemática e dois artigos técnicos. A revisão sistemática aborda os índices de conforto térmico usados nas avaliações da termorregulação de ovinos em regiões tropicais. Este trabalho foi desenvolvido no laboratório de Bioclimatologia da UFCG/CSTR/Patos-PB. Foram obtidos os estudos a partir da busca em bases de dados renomadas, como "Scopus", "Scielo", "Pubmed", "Web of Science" e "Embase". Com base nos estudos selecionados para compor a revisão, foi evidenciada uma semelhança dos critérios metodológicos para obtenção dos índices ambientais e parâmetros fisiológicos, sendo a FR e TR mais relevantes para o estudo da capacidade de termorregulação. Mais estudos são necessários sobre a relação dos índices ambientais e os níveis de estresse térmico, para uma adequação de índices específicos para ovinos, considerando o alto grau de adaptação e tolerância ao calor dos grupos genéticos criados em regiões de clima quente. O primeiro artigo técnico que compõe o segundo capítulo da tese, trata-se da avaliação dos parâmetros fisiológicos e temperaturas superficiais de ovinos nativos de diferentes genótipos submetidos a estresse por calor em câmara climática. O terceiro capítulo trata-se dos efeitos do estresse por calor induzido em câmara climática sobre os parâmetros bioquímicos e hormonais de ovinos nativos de diferentes genótipos. Foram usados vinte e quatro ovinos machos não castrados, com peso vivo médio de 20 ± 5 kg e idade média de cinco meses, consumindo a mesma dieta e água *ad libitum*, foram alocados em 3 baias coletivas, contendo 8 animais em cada baia (2 de cada grupo genético), submetidos a 3 ambientes térmicos distintos (TA = 28 ºC, 32 ºC e 36°C). Na fase experimental, foram registrados dados climatológicos, assim como frequência respiratória (FR), frequência cardíaca (FC), temperatura retal (TR), temperatura superficial geral (TS), temperaturas superficiais das regiões corporais, parâmetros bioquímicos e hormonais em função das variações do ambiente térmico no interior da câmara climática. As variações do ambiente na câmara climática, demonstraram ambiente indicativo de estresse térmico leve aos 28°C e grave aos 32°C e 36°C, afetando as médias da frequência respiratória, frequência cardíaca, temperatura retal e temperatura superficial (geral e das regiões corporais). Houve influência dos ambientes térmicos sobre os parâmetros metabólicos e hormonais. As alterações significativas da FR, FC e TS representaram uma eficiente termorregulação dos animais em ambos os experimentos, fato comprovado pela manutenção da TR dentro dos níveis normais para ovinos, mesmo quando expostos a estresse térmico muito elevado (ITGU>88). A TS média do globo ocular (GO) e timpânica (TP) apresentaram maior sensibilidade às variações do ambiente que as outras regiões corporais. A influência do ambiente sobre os parâmetros metabólicos não ocasionou alterações fora da normalidade para espécie, considerando a adaptação dos animais. A elevação da FR e dos níveis de cortisol (P<0,05), a manutenção da TR em níveis normais, bem como os baixos valores de T3 e T4, indicaram uma resposta termorregulatória eficiente de todos os genótipos. Todos os animais conseguiram manter a homeotermia mesmo em ambiente de estresse térmico, evidenciando a equivalência adaptativa entre os ovinos Soinga, Morava Nova, Santa Inês, e os sem padrão racial definido (SRD).

PALAVRAS-CHAVE: Ovinos Soinga, respostas termorreguladoras, endocrinologia, adaptação ao calor.

ABSTRACT

The objective was to evaluate the comfort indices and adaptive characteristics of native sheep of the Soinga and SPRD genetic groups and of the Morada Nova and Santa Inês breeds in a climatic chamber. This thesis is composed of three chapters, one systematic review and two technical articles. A systematic review addresses the thermal comfort indices used to estimate the thermoregulation of sheep in tropical regions. This work was developed in the Bioclimatology laboratory of UFCG/CSTR/Patos-PB. The studies were obtained from the search in renamed databases, such as “Scopus”, “Scielo”, “Pubmed”, “Web of Science” and “Embase”. Based on the studies selected for review, compatibility of methodological criteria for obtaining environmental indices and physiological parameters was evidenced, with RR and TR being more relevant for the study of thermoregulation capacity. More studies are needed on the relationship between environmental indices and heat stress levels, for the evolution of specific indices for sheep, considering the high degree of adaptation and heat tolerance of genetic groups raised in hot climate regions. The first technical article that makes up the second chapter of the thesis, deals with the evaluation of the physiological parameters and temperatures suffered by native sheep of different genotypes manifested by heat stress in a climatic chamber. The third chapter deals with the effects of heat stress induced in a climatic chamber on the biochemical and hormonal parameters of native sheep of different genotypes. Twenty-four uncastrated male sheep, with an average live weight of 20 ± 5 kg and an average age of five months, consuming the same diet and water ad libitum, were allocated in 3 collective pens, containing 8 animals in each pen (2 of each genetic group), tolerated in 3 different thermal environments (TA = 28 ºC, 32 ºC and 36°C). In the experimental phase, climatological data were recorded, as well as respiratory rate (RR), heart rate (HR), rectal temperature (TR), general Surface temperature (TS), tolerated temperatures of body regions, biochemical and hormonal parameters depending on variations of the thermal environment inside the climatic chamber. The variations of the environment in the climatic chamber, showed an environment indicative of mild thermal stress at 28°C and severe at 32°C and 36°C, affecting the mean respiratory rate, heart rate, rectal temperature and surface temperature (general and regional) body). There was influence of thermal environments on metabolic and hormonal parameters. The observed changes in RR, HR and TS represented an efficient thermoregulation of the animals in both experiments, a fact controlled by the maintenance of TR within normal levels for sheep, even when exposed to very high thermal stress (ITGU>88). The average TS of the eyeball (GO) and tympanic (TP) showed greater sensitivity to environmental variations than the other body regions. The influence of the environment on the metabolic parameters did not cause abnormal changes for the species, considering the adaptation of the animals. The elevation of RR and cortisol levels (P<0.05), the maintenance of TR at normal levels, as well as the low values of T3 and T4, indicated an efficient thermoregulatory response of all genotypes. All animals were able to maintain homeothermy even in a thermally stressed environment, evidencing the adaptive equivalence between Soinga, Morava Nova and Santa Inês sheep, and those with no defined racial pattern (SRD).

KEYWORDS: Soinga sheep, thermoregulatory responses, endocrinology, heat adaptation.

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LISTA DE ABREVIATURAS E SIGLAS

ALB	Albumina
ANOVA	Análise de Variância
Bat/min	Batimento por Minuto
CEUA	Comissão de Ética no Uso de Animais
CONCEA	Conselho Nacional de Controle de Experimento Animal
COL	Colesterol
CRE	Creatinina
CSTR	Centro de Saúde e Tecnologia Rural
CTC	Coeficiente de Tolerância ao Calor
CTR	Carga Térmica de Radiação
CV	Coeficiente de Variação
CE	Cervical
FR	Frequência Respiratória
FC	Frequência Cardíaca
GLI	Glicose
GL	Glúteo
GO	Globo Ocular
ITC	Índice de Tolerância ao Calor
ITGU	Índice de Temperatura do Globo Negro e Umidade
ITU	Índice de Temperatura e Umidade
Mov/min	Movimento por Minuto
PT	Proteínas Totais
SAEG	Sistema para Análises Estatísticas
SAS	Statistical Analysis System
TA	Temperatura Ambiente
TGN	Temperatura de Globo Negro
TR	Temperatura Retal
TRI	Triglicerídeos
TS	Temperatura Superficial
TIV	Termografia de infravermelho
TPO	Temperatura do Ponto de Orvalho

T3	Triiodotironina
T4	Tiroxina
TO	Torácica
TP	Timpânica
UFCG	Universidade Federal de Campina Grande
UR	Umidade Relativa
URE	Ureia
ZCT	Zona de Conforto Térmico

LISTA DE SÍMBOLOS

%	Percentual
<	Menor que
>	Maior que
≤	Menor ou igual que
±	Mais ou menos
-	Menos
°C	Grau Célsius
Cm	Centímetro
g/dL	Grama por decilitro
H	Hora
Kg	Quilograma
m^2	Metro quadrado
mg/dL	Miligrama por decilitro
mg/kg	Miligrama por quilograma
µg/kg	Micrograma por quilograma
mg/kg/h	Miligrama por quilograma por hora
µg/kg/h	Micrograma por quilograma por hora
Min	Minutos
mL	Mililitro
Mm	Milímetros
mmol/L	Milimol por litro
ng/mL	Nanograma por mililitro
S	Segundos

INTRODUÇÃO GERAL

A evolução constante da ovinocultura no Brasil evidencia o potencial dessa atividade nas diversas regiões do país. O rebanho ovino atingiu 19,7 milhões de animais em 2018, e teve um aumento para 20,7 milhões de cabeças em 2020, com 70,6% desse total presentes na região Nordeste (IBGE, 2020). A concentração de rebanhos na região semiárida é resultado da variabilidade genética e da capacidade adaptativa desses animais. A maioria das criações, nessa região, são de maneira extensiva, se alimentando da vegetação da Caatinga, com predominância de animais sem padrão racial definido (SPRD) ou raças nativas.

A região semiárida é caracterizada pela condição seca e excessivamente quente, com flutuações intensas dos recursos hídricos e alimentar (LEROY et al., 2018). A localização geográfica dessa região promove um ângulo de declinação solar, cujo resultado é um padrão de radiação solar intensa, altas temperatura do ar e fotoperíodo (SILVA, 2006; AMORIM et al., 2019). Em virtude disso, os efeitos diretos do estresse por calor ocorrerão principalmente devido ao aumento das temperaturas ambientais e à frequência e intensidade das ondas de calor (LACETERA, 2019; LEITE et al., 2021).

As consequências das mudanças nos padrões climáticos irão impactar consideravelmente as atividades agrícolas e pecuárias, devido a sensibilidade desses sistemas produtivos as mudanças atmosféricas (SERRANO et al., 2022). A Organização Meteorológica Mundial (OMM) descreve o estado atual das mudanças climáticas provocadas por humanos, em um nível considerado preocupante (IPCC, 2021). Tendo em vista esse cenário climático, pesquisas têm sido realizadas para entender os efeitos do ambiente térmico sobre os parâmetros fisiológicos dos animais, bem como seu desempenho produtivo em condições de estresse por calor (POLOSKY e KEYSERLIN, 2017; BROWN-BRANDL, 2018; LEITE et al., 2021).

Nas épocas de maior carga térmica, baixo gradiente térmico e alta temperatura média radiante, fica prejudicada a eliminação de calor pelos animais (FONSECA et al., 2017). Os mecanismos de transferência de calor, tais como condução, convecção e radiação tornam-se insuficientes, de maneira que os meios evaporativos atuam como mecanismos mais eficientes para a dissipação do calor do animal, evitando o aumento do calor endógeno, o que acarretaria em sérios prejuízos as funções fisiológicas dos animais (DANTAS et al., 2019).

Avaliações da temperatura retal e frequência respiratória, perfis endocrinológicos e produção de calor metabólico estão entre os principais aspectos avaliados para um melhor entendimento da capacidade de termorregulação em pequenos ruminantes (BERIHULAY et al., 2019). Aliado a isso, existem altas correlações entre índices ambientais e as respostas fisiológicas em ovinos (DANTAS et al., 2019). Por isso, é importante estabelecer quais valores são indicativos de desconforto térmico para adotar medidas para mitigar o estresse térmico e não comprometer o desempenho produtivo das raças (SEIXAS et al., 2017).

A manutenção da temperatura corporal fora da zona de conforto térmico exige gasto energético devido as alterações fisiológicas e comportamentais para sobrevivência dos animais, e quanto mais tolerante ao calor, maior a chance de obter melhores índices produtivos sob condições ambientais adversas (MARQUES et al. 2018). Em vista disso, a partir da observação dos níveis de estresse térmico e capacidade de termorregulação, pode-se verificar características adaptativas específicas de diferentes raças ou grupos genéticos que são considerados promissores para serem perpetuados na região semiárida.

Em toda extensão da região nordestina, são criados ovinos de raças exóticas, nativas e sem padrão racial definido (SPRD), e os animais das raças Santa Inês, Morada Nova e Soinga, são exemplos de animais nativos e que vem ganhando destaque pelo elevado grau de adaptação, apresentando vantagem competitiva. Devido a essa capacidade de tolerância ao calor, e por se tratarem de animais bem adaptados, a faixa de temperatura de zona de conforto pode ser mais ampla, podendo ser explorados em ambientes de altas temperaturas, com mínimas perdas em sua produção.

Vale destacar o ovino Soinga e sua potencialidade para o semiárido, originado do cruzamento entre Bergamacia, originária da Itália, Morada Nova Branca, selecionada no Nordeste do Brasil, e Somalis Brasileira, da África do Sul. São bastante resistentes às condições da região semiárida, e apresentam rusticidade, são precoces, pesados e prolíferos (MEDEIROS et al., 2019). São animais que possuem carne marmorizada, o que significa que a gordura entremeada nas fibras da carne pode ser vista no corte, considerada por especialistas como uma carne de excelente sabor. Além disso, apresentam autossuficiência de leite, mortalidade reduzida e excelentes habilidades maternas (CAVALCANTE, 2018).

Esse genótipo ainda não é considerado como raça pelo Ministério da Agricultura Pecuária e Abastecimento (MAPA). Em vista disso, devido à escassez de estudos sobre esses animais, são necessárias validações científicas consistentes sobre suas características adaptativas e produtivas, que reafirmem a potencialidade desse genótipo, bem como a viabilidade de sua criação dentro das imposições climáticas do semiárido.

Para isso, estudos usando câmaras climáticas permitem controlar algumas variáveis ambientais, como a temperatura e a umidade relativa, de modo a proporcionar uma avaliação precisa da influência do ambiente sobre os parâmetros fisiológicos dos animais (ARAÚJO et al., 2017; MIRANDA et al., 2018; MARQUES et al., 2021). Desta forma, pode-se avaliar as perdas e ganhos, e criar parâmetros de produção bem elaborados, que poderão ser utilizados pelos produtores para melhorar as técnicas de manejo e a sua lucratividade.

Diante disto, o objetivo desta tese é descrever os índices de conforto e métodos que vêm sendo utilizados para avaliar as características termorregulatórias de ovinos em regiões tropicais e avaliar as características adaptativas de ovinos nativos de diferentes genótipos, mantidos em diferentes temperaturas em câmara climática.

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CAPÍTULO I:

Use of thermal comfort indices in thermoregulation assessments of sheep in tropical regions: a systematic review

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Use of thermal comfort indices in thermoregulation assessments of sheep in tropical regions: a systematic review

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Abstract – The objective of this work was to describe the methods that have been used to evaluate thermoregulation in sheep, emphasizing the relationship between thermal comfort indices (ITGU and ITU) and variables in tropical climate regions. The data survey was carried out through a search in renowned databases, such as "Scopus", "Scielo", "Pubmed", "Web of Science", "Embase" and "Directory of open access journals", on the premises from the Bioclimatology Laboratory of the Federal University of Campina Grande (UFCG - CSTR). They were selected with the following descriptors: "sheep" (sheep), "thermal stress" (thermal stress) and "physiological responses" (physiological responses), considering studies published in English and Portuguese from the year 2011 according to the inclusion criteria and pre-established exclusion. From a total of 66 articles, 18 were selected because they met the inclusion and exclusion criteria, which take into account: year of publication, sheep species, environment with high temperatures and the use of ITGU and UTI indexes in association with thermoregulatory response. The use of similar methods to relate thermal comfort indices to physiological responses was observed, the ITGU being the most used by the authors, followed by the ITU. There was a relationship between the high indices and physiological changes, especially increases in RR indicating thermal stress. In other situations, even though the environment was indicated as being highly stressful, the sheep managed to maintain the homeotherm. The need for further research to establish and adapt specific thermal comfort indices for sheep raised in tropical climate regions was highlighted.

Index terms: sheep, heat stress, physiological responses

1 INTRODUCTION

Over the next few decades, humanity will face successive climate changes, in particular the rise in global surface temperature, and this will make the effects of thermal stress due to changes in climate become more severe and frequent (Habibu et al., 2018; Serrano et al., 2022; IPCC, 2021). Changes in Arctic and Antarctic ice temperatures, heat waves, fluctuations in rainfall patterns, ocean acidification, extreme weather events such as drought are some of the hostile effects of climate change (UNFCCC, 2014; Sejian et al., 2019).

In tropical and subtropical regions, there has been an increase in agricultural activity in terms of production and productivity, however, due to global warming, yields tend to decrease by up to 20% (Jones and Thornton, 2013) by the year 2050, which will cause negative effects on agricultural productivity, which is directly affected by meteorological changes (Serrano et al., 2021).

The susceptibility of sheep to climate change is lower when compared to other herds (Titto et al. 2016). They are animals that can resist the stresses imposed by the increase in temperature, poor pasture availability, diseases and water shortages (Sejian et al. 2017). But when continuously exposed to these environmental conditions, as the heat load increases, there is the possibility of significantly reduced production and reproduction capacity.

In research carried out with sheep, the use of indices that allow the evaluation of interactions between ambient temperature, relative humidity, and factors such as radiation and wind speed are crucial for understanding the impacts of climate on these animals (Baêta and Souza, 2010; Buffington et al., 1981). The Black Globe Temperature and Humidity Index (ITGU), Temperature and Humidity Index (ITU) and Radiation Thermal Load (CTR) have been used and analyzed together with physiological responses in several works in the field of Bioclimatology and Ambience (Souza et al. al., 2012; Dantas et al., 2015; Furtado et al., 2017; Torres et al., 2017; Mascarenhas, 2018).

This type of evaluation has a great contribution in the search for efficient solutions for sheep farming in hot climate regions, deepening the knowledge about the factors involved in the ability of these animals to withstand high heat loads and other environmental stressors. In addition, it contributes to the identification, improvement and dissemination of breeds and genetic groups of sheep with different skills, linked to adaptation to heat, aiming at greater genetic variability of sheep resistant to the weather present in hot climate regions.

Given the existence of several works relating the use of thermal comfort indices to the thermoregulatory response of animals, the making of a systematic review allows a better understanding of how these indices have been applied, and how they can help in the understanding of changes in the biological functions of animals. animals raised in tropical regions.

Systematic review is a form of research that brings together studies to identify, select and critically evaluate a previously formulated question obeying methodological criteria, which makes it less prone to bias (Oliveira et al., 2010). From this, this work aimed to describe the methods that have been used to evaluate the thermoregulatory characteristics of sheep, emphasizing the relationship between the thermal comfort indices and the main physiological variables measured in studies carried out in tropical regions.

2 MATERIAL AND METHODS

2.1 Place

The data collection consisted of four evaluation phases, and the data collection was carried out from June 25 to 28, 2021 at the Bioclimatology Laboratory of the Federal University of Campina Grande (UFCG - CSTR) using the "search bases" tool available on the CAPES Periódicos page, a fact that constituted the first phase of this review.

In the second phase, the articles were selected through a search in renowned databases, namely "Scopus", "Scielo", "Pubmed", "Web of Science", "Embase" and "Directory of open access journals". The following descriptors and their combinations in Portuguese and English were used as search terms: "sheep", "thermal stress" and "physiological responses", as well as their combinations.

2.2 Inclusion and exclusion criteria

To be included in the work in question, the grouped studies had to meet the following criteria: (I) articles published in English or Portuguese; (II) Using sheep breeds or genetic groups; (III) Studies carried out in regions with a tropical climate, including those carried out in a controlled environment (climatic chamber); (IV) works that analyzed the results along with at least one of the environmental indices: black globe temperature and humidity index (ITGU) and temperature and humidity index (ITU).

In the third phase, literature reviews, course completion work, theses and dissertations were excluded, as well as duplicate articles and those that did not meet the established criteria, listing only applied research articles, leaving 66 copies.

2.3 Study evaluations and choices

The articles were evaluated by two evaluators and based on the inclusion and exclusion criteria, 18 studies, published in the period from 2015 to 2020, were selected to compose the review. Data from the periods or seasons most likely to cause heat stress in sheep, such as afternoon shifts, summer and periods of low rainfall were preferably considered for discussion and critical evaluation of the selected works, based on the temperature indexes of the black globe and humidity (ITGU) and temperature and humidity indices (ITU).

As criteria for evaluating thermal comfort, based on the dew point temperature and the temperature of the black globe, the studies that calculated the ITGU followed the model indicated for dairy cows based on the equation proposed by Buffington et al., 1981:

$$BGHI = T_{gn} + 0{,}36\ T_{po} + 41{,}5$$

Em que:

BGHI: black globe temperature and humidity indices, °C;
T_{gn}: Black globe temperature, °C;
T_{po}: Dew point temperature, °C.

To estimate the severity of heat stress based on air temperature and relative humidity, the studies used the THI (THI), calculated according to Marai et al. (2007), using the following equation when TA was obtained in (ºC):

$$THI = TA - \{(0{,}31 - 0{,}31 * UR) * (TA - 14{,}4)\}$$

Likewise, when the TA was obtained in (°F), the THI was calculated following the equation proposed by the Livestock and Poultry Heat Stress Indices, from the University of Clemson, USA (LPHSI, 1990):

$$ITU = TA - \{(0{,}55 - 0{,}55 * UR) * (TA - 58)\}$$

Em que:

THI: temperature and humidity index,

TA: air temperature, °C
HR: relative air humidity,%.

The studies that considered calculating the CTR did so according to Esmay's proposal (1969) using the Stefan-Boltzmann equation:

$$CTR = \sigma(TMR)^4$$

Em que:

CTR: Radiant Thermal Load $W.m^{-2}$

σ = constant de Stefan-Boltzmann, $5,67x10^{-8}$, $W.m^{-2}\ K^{-4}$

TRM = Average Radiant Temperature, K.

Average Radiant Temperature was obtained through the following equation:

$$TMR = 100\sqrt[4]{2,51} \cdot \sqrt{V} \cdot (Tgn - Tbs) + \left(\frac{Tgn}{100}\right)^4$$

V = air speed ($m.s^{-1}$);

Tgn = black globe temperature, em K;

Tbs = dry bulb temperature, em K.

3 RESULTS

The geographic regions of origin of the selected surveys are within the tropical, arid and semi-arid climate zones. The dry climates of these regions (arid and semi-arid) are characterized by low rainfall and high temperatures that predominate throughout the year (Köppen, 1884). Regarding these regions, it should be noted that, although the descriptors used in the research did not specify any country, that is, which makes the analysis worldwide, the selected articles were mostly developed in Brazil.

The other studies were carried out in the United States, India, Venezuela and Australia, and of the total number of articles selected, 61.2% were accepted for publication in foreign journals and the other 38.8% in national journals. In Brazil, most of these studies were concentrated in the Northeast of the country, totaling 77%.

The works carried out in Brazil were published between 2015 and 2020, and it was the only country that published studies considering the heat tolerance index (ITC), the radiant thermal load (CTR) together with the ITGU and the heart rate (HR) and tympanic temperature (TT) among the evaluated physiological responses. Research from India and Australia considered calculating only the THI temperature and humidity index, with Indian studies being the only ones that verified the Pulse Rate (PF) in the years 2017 and 2019. The study carried out in the United States calculated the THI and the heat load index (HLI). The heat tolerance coefficient (CTC) was considered only by the work developed in Venezuela that was accepted for publication in 2018 (Table 1).

It should be noted that the selected articles used hairless and partially haired sheep as the object of study and the breeds of animals used in the studies were variable, with a predominance of specimens of the “Morada Nova” and “Santa Inês” breeds. The N sample of each experiment ranged from 6 animals to 383 animals among the experimental designs used by the authors, the different statistical analyzes can be considered at a later time to verify the feasibility of performing a meta-analytical analysis of the data.

As for sex, most of the evaluations found were obtained in non-pregnant females and outside the lactation period, and in the works that used males, all chose to use non-castrated animals. The experiments were organized for data collection in herds in their natural conditions, as well as in different systems, mainly the intensive system, which was the most found in the works, and the extensive and semi-extensive soon after. Only 2 of the studies were developed in a climatic chamber (controlled environment).

Environmental data were obtained from meteorological stations installed at the experimental sites, using various meteorological devices such as data loggers, thermometers, thermohygrometers, portable digital anemometers and electronic sensors. These, installed in the center of the sheds or places of study and positioned at the level of the back of the animals, recording the data during the experimental periods.

The physiological responses evaluated were rectal temperature (TR), respiratory rate (RR), heart rate (HR), in addition to superficial skin temperature (TS) and tympanic temperature (TT). Endocrinological profiles and morphological characteristics were evaluated, such as blood, biochemical and hormonal parameters; and coat morphology from coat thickness (CT), length (HL), diameter (HD) and density (D) of hairs.

Among the studies described, all analyzed rectal temperature (TR) and respiratory rate (RR), although only three included heart rate (HR) in the measurements. The verification of the surface temperature (TS) was performed in 11 works, and only the work by Souza et al (2015) included the tympanic temperature (TT). In 4 articles, behavioral responses were evaluated, involving ingestive behavior and water consumption.

Regarding the thermal comfort indices, the calculation of at least one of the indices described in the inclusion criteria for the selection of works was evidenced, and the CTR, ITC and CTC, used separately, were present in addition to the pre-established ones (ITGU and ITU). or associated, to estimate the degree of stress that the environment can cause to animals, through unique variables represented for each index. A single value represents the association of the effects of radiant energy, air temperature and wind speed, allowing to conclude on the level of comfort or discomfort that a given environment provides to the animals.

4 DISCUSSION

Studies have evaluated the thermoregulation of sheep under high temperatures in regions with a semi-arid tropical climate in different ways, both in natural conditions and in a controlled environment with the use of climate chambers (Leite et al., 2019; Joy et al., 2020). The studies obtained the thermal comfort indices: ITGU, THI and CTR, which were calculated separately or associated from the proposals found in the reference literature (Buffington et al., 1981; Marai et al. 2007; Esmay, 1969). According to the results of the indices, the environments were analyzed according to the time of day (including morning and afternoon), times of the year (dry and rainy), as well as seasons (summer, autumn, spring and winter).

From 2015 to 2020 works such as Costa et al., 2015; Noble., 2016; Pantoja et al., 2017; Rathwa et al., 2017; Nobre et al., 2018; Da silva et al., 2019; Machado et al., 2020 considered behavioral variables among their analyses; dietary supplementation levels; endocrine profiles; morphological characteristics, and blood, biochemical and hormonal parameters.

About the ITGU, 12 studies calculated this variable, which represents 66.67% of the selected articles. Of these, 4 (33%) considered this index alone, 2 (16.66%) used it together with the UTI and 6 (50%) obtained it together with the CTR. According to Buffington et al., 1981, ITGU values up to 74 indicate a situation of comfort for the animals, from 74 to 78 mild stress; between 79 and 84 a dangerous situation and above 84 indicate an emergency situation. Such reference values are not specific for the sheep species, especially the authors adopt these parameters as the basis for their studies.

Based on this evidence, it is noteworthy that of the 12 studies that calculated the ITGU, only 3 (25%) reported values below 74, and 7 (58.33%) found values above 84. Demonstrating that in most cases of studies the environment in which the animals were found was from mild stress to emergency. Which does not mean that the animals suffered heat stress due to these results. Costa et al (2015) found a mean UGTI value of 85.5 in the summer, however, RR values below 60 mov/min and TR less than 40.5°C indicated that the animals did not present high stress, being well adapted to local conditions. Variations in respiratory rate allow the quantitative assessment of thermal stress in ruminants, so that respiratory rate of 40-60; 60-80 and 80-120 mov./min characterize low, medium-high and high stress, respectively, and above 200 mov./min would characterize severe stress in sheep (Silanikove 2000).

A situation similar to what was previously mentioned was reported in a study with Morada Nova and Santa Inês sheep evaluated in the summer, in which even the ITGU demonstrating an alert situation, the animals maintained respiratory movements between 40 and 60 mov/min, indicating low need loss of sensible heat through the respiratory tract (Pantoja et al., 2017). Even though this index is high to the point of indicating severe conditions (94.16 and 90.46), Morada Nova sheep managed to maintain the RR and TR in the dry season within physiological limits and outside the values considered stressful for the species (Leite et al. al., 2018a; Leite et al., 2018b; Costa et al., 2018).

Nobre et al (2016) showed medium-high and high stress in the afternoon, confirmed by the maximum RR elevation values of the animals in the afternoon at 79.98 and 86.18 mov/min., being in an emergency situation according to the ITGU value of 84.65 (Silanikove 2000; Buffington et al., 1981). The same author reported an average ITGU of 78.7 in the afternoon shift and RR elevations reaching 105 mov/min (Nobre et al., 2018).

THI values were obtained in 8 (44%) studies, half of which were used in isolation and 2 (25%) were calculated and analyzed together with the ITGU. This index, according to Marai et al., 2007, represents the degree of thermal stress according to the values in ºC: < 22.2 = absence of thermal stress; 22.2 to 23.3 = moderate heat stress; 23.3 to 25.6 = severe thermal stress; and ≥ 25.6 = severe extreme heat stress.

It was reported that the THI data in the study by Pantoja et al. (2017) were above 30, indicating the possibility of severe stress, however the evaluated sheep did not raise the RR above normal levels, not indicating stress conditions according to Silanikove 2000. In the same way, the evaluated data behaved by Mohapatra et al (2019), who reported a mean THI value of 37, although the animals did not present RR greater than 60 mov/min., being within the physiological limits that do not present thermal discomfort.

There was an increase of 51.1% (29.46 to 45.57) in the RR when the THI reached a value of 30 in a climatic chamber at a temperature of 32°C, even though the changes in RR and TR did not reach values considered stressful , even with high such responses to maintain homeothermia (Leite et al., 2019). As in the previous study, Joy et al (2020), evaluating sheep in a climatic chamber, found a THI of 34.4 at the highest temperatures (>35°C), which caused an increase in RF in the genetic groups studied and evidenced the Dorper breed being more tolerant to heat, even with its RR reaching 164 mov/min.

Among studies that calculated the THI based on the dry bulb temperature in °F, according to the LPHSI (1990), the highest THI values ranging from 80.1 to 85.7 were reported (Rathwa et al., 2017; Reyes et al., 2018; Tadesse et al., 2019). The values obtained suggest the following: <82 = absence of thermal stress; 82 to 84 = moderate heat stress; 84 to 86 = severe heat stress and over 86 = severe extreme heat stress (LPHSI, 1990). However, these findings do not imply that there were alterations in the physiological responses that represented high stress, considering the values of 82.9 and 85.7 found by Tadesse et al (2019) for the UTI not to have caused excessive stress in the evaluated animals.

Upon obtaining a THI of 82.55 in the summer season, Rathwa et al. (2017) reported changes in RR and TR, as well as other variables that indicated that the animals were under thermal stress, showing that other factors must be considered to verify the degree of stress that the environment caused to the animals. In a study in environments with and without shade, Reyes et al (2018) concluded that THI values greater than or equal to 80 are related to thermal discomfort in sheep.

5 CONCLUSION

The effects of climate on animal production and the interaction between physiological responses and adaptation traits of breeds have driven several studies involving heat tolerance indices.

The physiological parameters FR and TR associated with environmental indices are the main indicators of stress or thermal discomfort for sheep raised in tropical regions.

Studies are needed to establish environmental indices as specific parameters for the sheep species, considering the high degree of adaptation and heat tolerance of genotypes raised in tropical regions.

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Tabela 1. País de origem, índices ambientais e de conforto térmico, respostas fisiológicas, e ano de publicação dos estudos.

País de origem do estudo	**Índices calculados**	**Respostas fisiológicas**	**Ano de publicação**
BRAZIL	ITGU, THI, CTR, ITC	FR, TR, FC, TS, TT	2015 a 2020
ÍNDIA	THI	FR, TR, TS E FP	2017 e 2019
U.S	THI e HLI	FR, TR	2019
VENEZUELA	THI, CTC	FR, TR, TS	2018
AUSTRÁLIA	THI	FR, TR, TS	2020

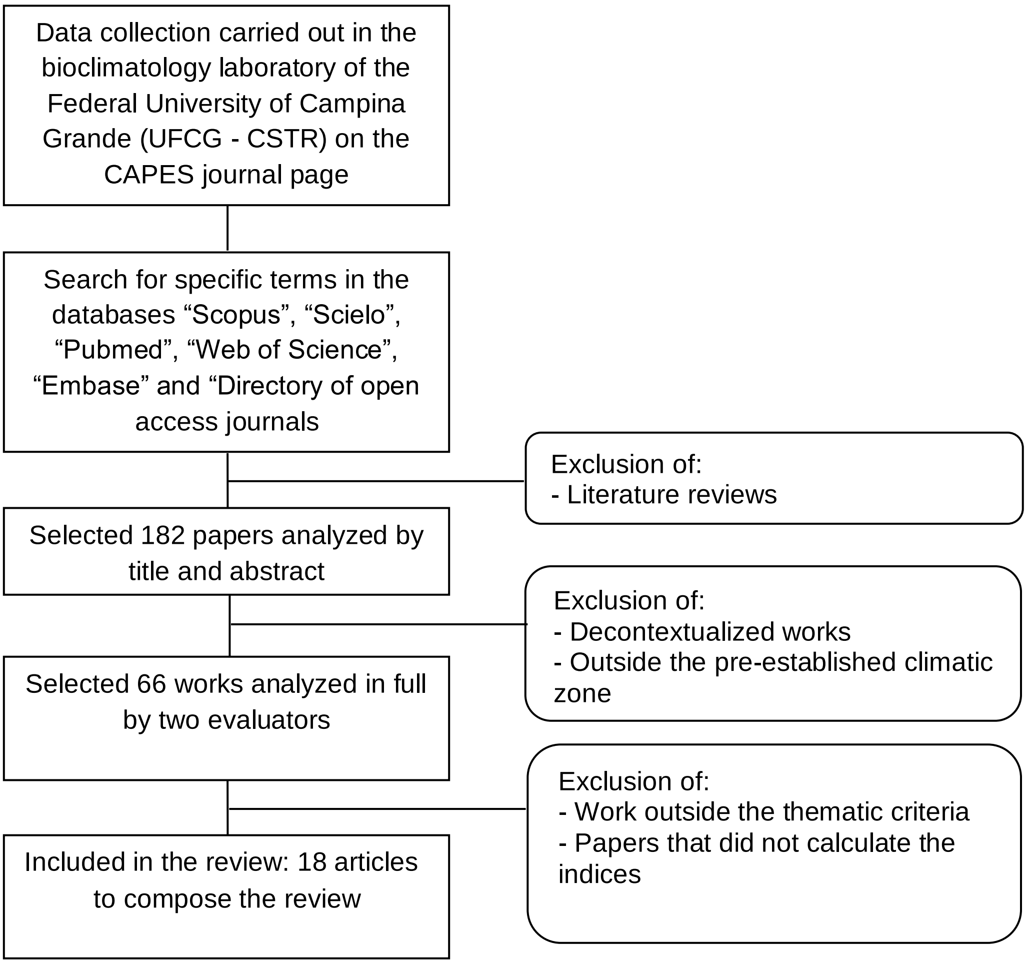

Figure 1 Article search flowchart.

CAPÍTULO II:

Physiological parameters and surface temperatures of sheep of different genotypes subjected to heat stress in a climatic chamber

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Physiological parameters and surface temperatures of sheep of different genotypes subjected to heat stress in a climatic chamber

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Declaration of Conflict of Interest

The authors declare no conflict of interest.

ABSTRACT

The objective of this study was to evaluate the effect of the thermal environment of heat stress on the physiological parameters and surface temperatures of sheep Soinga, Morada Nova, Santa Inês and without defined breed standard (SPRD) in a climatic chamber. 24 uncastrated male sheep were allocated in 3 individual pens, containing 8 animals in each pen (2 from each genetic group), submitted to 3 levels of ambient temperature (TA = 28 ºC, 32 ºC and 36°C) for a period of 15 days, 10 hours a day, considering the environmental data, physiological parameters (TR, FR and HR) and surface temperatures (TS) of the cervical (CE), thoracic (TO), gluteal (GL), eyeball (GO) regions.) and tympanic membrane (TP) of the afternoon shift of the first 3 days in each temperature cycle. The ITGU averages indicated that from 32°C the environment was considered to be of severe thermal stress, which added to a relative humidity greater than 80%, placed the animals outside the thermal comfort zone (ZCT). The variations in TA affected the physiological parameters, mainly at 36°C, raising the averages of FR and FC of all genotypes above the reference values for the species. However, these elevations were the main thermoregulatory mechanisms that the animals use to maintain the RT within its physiological limits. TS varied similarly between genotypes according to temperature changes. The variations of the thermal environment affected the body surface temperatures, and the mean values of TS followed a similar pattern in all genotypes, showing an effect only of treatments ($P<0.05$). For the body regions where the TS were obtained, there was a statistical difference (< 0.05) between the TS of the regions during the experimental period. The mean TS of the eyeball (GO) and tympanic (TP) showed greater sensitivity to variations in the environment, being higher ($P<0.05$) than the other body regions. The 4 groups were equally tolerant to heat, managing to maintain homeothermy, even in environments considered to be of severe stress. The correlation between the TS of the GO and TP regions and the temperature of the central core of sheep needs to be further studied, reaffirming the importance of infrared thermography (IRT) to diagnose situations of thermal discomfort in native sheep, in a practical and non-invasive way.

Keywords: Native sheep, infrared thermography, heat stress, adaptability.

1 INTRODUCTION

It is important to note that the effects of heat stress due to changes in climate are becoming more severe and frequent (Habibu et al., 2018, IPCC, 2021), making continuous monitoring of changes in the physiological state and adaptive capacity of sheep essential. in the face of climate change, especially in semi-arid tropical regions, due to their climatic and environmental characteristics.

Although they are considered adapted animals, it is known that climatic adversities can cause changes in the physiological variables of sheep, often beyond what the species is able to tolerate under normal conditions, causing thermal discomfort, a decline in welfare and productivity. Physiological responses such as rectal temperature, surface temperature, respiratory and heart rate, may be influenced by the time of year, day shift, ambient temperature, relative humidity and solar radiation (Furtado et al., 2017 and Torres et al., 2017).

Several studies have shown that a stressful environment can generate respiratory and hormonal problems, lack of appetite, increased incidence of diseases and decreased meat production (Sousa Júnior et al., 2008; Freitas et al., 2017; Furtado et al., 2020). Technological advances for obtaining biological indicators of heat stress in sheep are still a major challenge, and need to be further studied and developed.

Infrared thermography (IVT) has been used, as it is a non-invasive technique that shows surface temperature in real time (Roberto et al., 2014; Pulido-Rodríguez et al., 2017 and 2021; Hooper et al., 2018; Dantas, 2019). This technique, when used in climatic chambers (controlled environment), can detect with great precision, temperature variations in specific body regions, which can be correlated with physiological changes that indicate a state of thermal stress.

Therefore, this study aimed to evaluate the effect of the thermal environment on the physiological parameters and surface temperatures of sheep Soinga, Morada Nova, Santa Inês and without defined racial pattern (SPRD) in a climatic chamber.

2 MATERIAL AND METHODS

2.1 Experiment site, animals and management

The procedures performed in this study were approved by the Research Ethics Committee (CEP) of the Federal University of Campina Grande, Paraíba, Brazil, Protocol CEP Nº. 097.2019.O experimento foi conduzid em câmara climática, entre os meses de setembro e

November 2021, at the Laboratory of Rural Constructions and Ambience (LACRA) of the Federal University of Campina Grande (UFCG), located in the municipality of Campina Grande-PB (7° 13′ 51″ South, 35° 52′ 54″ West). The climatic chamber used is 6.14 m long and 2.77 m wide, with a constructed area of 17.00 m2 (Figure 1), made of laminated steel sheets with anti-corrosion protection and filled with styrofoam, allowing isolation thermal with the external environment.

Twenty-four male sheep of the Soinga, Morada Nova, Santa Inês breeds without defined racial pattern (SPRD) were used, six of each breed/genetic group, with an average age of five months and an average weight of 20 kg. All animals were examined and dewormed in the pre-experimental phase.

The ration provided consisted of Tifton hay - Cynodon dactylon, (L) Weight (50.54 kg), corn bran (34.69 kg), soybean meal (13.32 kg), limestone (0.45 kg) and mineral supplement (1.0 kg). The animals were fed ad libitum and provided twice a day, at 8:00 am and 4:00 pm, with daily adjustment of consumption to allow 20% of leftovers, and quantified by the total supplied minus leftovers in the 24-hour period. Water consumption was ad libitum and provided once a day, in a portion of 3 kg per animal/day.

2.2 Experimental design and environmental data

The animals were kept in 3 collective pens of about 4.00 m2 each, 8 animals were randomly distributed in each pen, 2 from each genetic group. The floor of the climatic chamber was lined with a 10 cm layer of wood sawdust (figure 2), which was changed weekly so that there was good absorption of the animals' feces and urine.

The sheep were exposed to temperatures of 28 ºC, 32 ºC and 36ºC, with average air humidity of 79.70%, 83.09% and 85.28%, and a period of 15 consecutive days of exposure to the controlled environment was established of the climate chamber. Of this total, measurements of physiological parameters and surface temperatures of the initial 3 days of exposure to each pre-established temperature were considered for this research.

The animals were maintained for 10 hours of continuous exposure per day at each temperature (from 7:00 am to 5:00 pm), alternating with 14 hours of room temperature with the climatic chamber turned off and the doors open. Between each treatment, the animals remained at the experimental site with the equipment turned off and doors open, without the influence of controlled temperature, for 5 consecutive days, to eliminate the residual effect.Durante todo

During the experimental period, climatological data were recorded using 3 HOBO-type dataloggers, with an external cable and sensors attached to the black globe.), and were programmed to record data every hour for 24 hours, considering the hours from 7:00 am to 5:00 pm, the time when the chamber was closed with temperature and humidity controlled (figure 5), and the remaining hours in which the climatic chamber had its doors open and the air conditioning system turned off (figure 6).

To check the wind speed, a portable AD-250 digital anemometer was used, with a measurement range of 0.4 to 30 m/s, positioned at the height of the animals' center of mass so that it could record air movement. caused by exhaust fans, air conditioners and the entry and exit of people inside the chamber, when they are providing food or collecting experimental data.

The values of the thermal comfort indices were determined from the observed data, in the period from 7:00 am to 5:00 pm, time of controlled temperature with the chamber closed, at intervals of one hour. To calculate the Black Globe Temperature and Humidity Index (BTI), the formula suggested by Buffington et al (1981) was used, expressed as follows:

$$BGHI = T_{gn} + 0,36\ T_{po} + 41,5$$

Em que:

BGHI: black globe temperature and humidity indices, °C;
T_{gn}: Black globe temperature, °C;
T_{po}: Dew point temperature, °C.

2.3 Physiological variables and surface temperatures

2.3.1 Rectal temperature, respiratory and heart rate

The physiological variables were measured in the afternoon shift in all animals in the experiment, between 2:30 pm and 3:30 pm, during the first three days of each experimental phase (28 ºC, 32 ºC and 36ºC), and rectal temperature (TR), respiratory rate (RR) and heart rate (HR). The rectal temperature was obtained by introducing a clinical veterinary thermometer, with a scale of up to 44 °C, inserted into the rectum in contact with the animal's rectal mucosa, at a depth of 5 cm, remaining there for a period of 2 min.

The respiratory rate was measured by means of indirect auscultation of the sounds, with the aid of a flexible stethoscope, at the level of the thoracic region, counting the number of movements during one minute, thus obtaining the number of movements per minute (mov min-

[1]). The heart rate was quantified with the aid of a stethoscope in the region of the third rib of the animal, in the lateral region of the thorax, during 1 minute, thus obtaining the number of beats per minute (bat min-1).

2.3.2 Surface temperatures

Surface temperatures (TS) were obtained by infrared thermography during the collection of physiological parameters (between 2:30 pm and 3:30 pm), when the animals remained immobile, with little manipulation, using a thermographic camera, model Fluke® Ti55FT, with a precision of ± 2 °C or 2% of actual temperature, thermal sensitivity (NETD) <50 mK, infrared resolution of 320 × 240 pixels, and 5.7 inch (14.4 cm) screen with 320 × 240 pixel resolution. To measure the tympanic temperature (TT), an infrared digital laser thermometer (model HP 8580) with a precision of 1.5°C was used, pointing the laser directly at the intra-auricular region of the sheep.

Thermograms were analyzed using Smartview software version 4.1, obtaining the mean in degrees Celsius (°C) considering an emissivity of 0.98. A circular design was made delimiting the area of the eye between the upper, middle and lower edges and the lacrimal caruncle to determine the surface temperature in the animal's eye region (GO), recording the maximum temperature of the delimited region (figure 4) (Hoffmann et al., 2013; Pulido-Rodríguez et al., 2017 and 2021; Hooper et al., 2018). The mean surface temperatures were obtained from the maximum temperature points (°C) of the following regions: cervical (CE), thoracic (TO), gluteal (GL), eyeball (GO) and tympanic (TP) (figure 4) obtained in each treatment (28 °C, 32 °C and 36 °C).

2.4 Statistical analysis

A completely randomized design (CID) was used with a 4 x 3 x 8 factorial arrangement (four genetic groups, three ambient temperatures and 8 surfaces temperature points). The data of the physiological parameters and superficial temperatures were submitted to analysis of variance, through the statistical program SAEG 9.1 and the averages compared by the Tukey Test test at the significance level of 5% of probability.

3 RESULTS AND DISCUSSIONS

3.1 Environmental variables

The results of the environmental variables expressed in figure 5 shows that the ambient temperatures (TA °C) obtained by the dataloggers were always in accordance with the pre-established ones for the experiment. The relative humidity (HR%) and the black globe temperature (TGN) increased according to the conditions of increasing temperatures.

An ITGU value equal to 83 can already be considered a situation of medium-high stress for sheep (Souza, 2010). In this study, the indices of temperature of the black globe and humidity (ITGU) obtained at temperatures 28°C, 32°C and 36°C were 77.87, 84.39 and 88.12 respectively (Figure 5), which made the environment from 32°C onwards more likely to cause a situation of high heat stress, capable of causing changes in the physiological parameters of the animals under study. According to Buffington et al (1981), for high production dairy cows, ITGU values up to 74 indicate a situation of comfort for the animals, from 74 to 78 mild stress; between 79 and 84 a dangerous situation and above 84 indicate an emergency situation.

For sheep, the recommended thermal comfort zone (TCC) is found at temperatures between 15 and 30 °C and relative humidity between 50 and 70% (Baêta and Souza, 2010; Eustáquio Filho et al., 2011), with wind speed between 1.3 and 1.9 m s-1 (McDowell, 1989). In the present study, the sheep were exposed to a temperature of 36°C, with relative humidity above 80% in all treatments, which places the animals outside the ZCT recommended by the aforementioned authors.

Air temperature together with relative humidity are the two main environmental variables with regard to the animal thermal comfort zone, because in environments with high temperatures, the thermal gradient between the surface of the animal and the environment is reduced, the which makes it difficult to dissipate heat in sensible form (through radiation, conduction and convection).

It is important to highlight that, in this work, the thermal energy absorbed came from the facilities, objects and surfaces surrounding the animals, including the thermal exchanges between them, since they were under an intensive rearing system in collective pens in a closed environment, without exposure to direct sunlight.

If, in addition to the air temperature, the humidity is also high, heat losses will be limited, both in sensible form (by non-evaporative means) and insensible form (by meios evaporativos, como a respiração e sudorese). Nesses casos, devido ao efeito da umidade, o animal elimina calor with difficulty due to cutaneous evaporation (sweating) and, as a compensation, it increases the respiratory movements for the process of thermolysis. The increase in RR results in heat loss because the inspired air, when in contact with the respiratory tract, is humidified; on expiration, the air is saturated with water, which changes from a liquid state to a vapor state (respiratory evaporation).

The average values of ITG at 28 °C (figure 5) and when the climatic chamber was turned off (figure 6) were between 75 and 78, not indicating a situation of thermal stress for sheep according to Souza (2010). The values of the environmental variables for the period in which the climate camera was turned off and the doors open are described in figure 6, with average values showing an environment incapable of providing thermal stress to the sheep under study. reference for the ITGU are not specific for the sheep species, especially several authors adopt these parameters, associated or not with other indices, as a basis for their studies in small ruminants (Costa et al., 2015; Pantoja et al., 2017; Nobre et al., 2018; Dantas et al., 2019).

3.2 Physiological parameters

Table 1 shows that there was a significant effect (P<0.05) of treatments (28°C, 32°C and 36°C) on the physiological parameters (TR, FR, and HR), represented by increases in averages proportionally to the increase in temperature (°C) throughout the experimental cycles, except for HR, which had the highest value at 36°C, but did not differ (P>0.05) between treatments 28°C and 32°C (Table 1).

Table 2 shows the mean values of the physiological parameters (TR, RR and HR) corresponding to the total days of exposure to temperatures 28°C, 32°C and 36°C, where a statistical difference was observed between the genetic groups for HR, and the Soinga group had a higher mean value (P<0.05) and the other groups studied did not differ from each other (P>0.05) for this same physiological parameter

The increase in AT resulted in an increase in heart rate, especially at 36°C (Table 1). This increase occurs due to peripheral vasodilation, which, by increasing the caliber of blood vessels, promotes greater blood circulation in the animal's body, which, when reaching the external tissues, raises the surface temperature proportionally to the increase in AT (Souza et al., 2012 ; Dantas et al., 2019).

In this way, heat dissipation occurs by conduction from the central core to the animal's extremities and from these to the environment, by the blood flow being redirected to the superficial areas of the body in order to eliminate more heat by sensitive mechanisms (conduction, convection and radiation).) and insensitive (skin water diffusion) (Marai et al., 2007). This mechanism justifies the increase in the TS of sheep seen in this study, which increased according to variations in the thermal environment (Figure 7).

The environmental temperature, in addition to other physiological variables, can alter the vagal tone by intensifying the activity of the cardioaccelerator and vasoconstrictor center, thus increasing the heart rate (Torres et al., 2017). In this sense, it is known that HR can be used as a parameter for evaluating thermal stress in animals (Kolb, 1981), in the case of sheep, the normal heart rate for sheep is considered, ranging from 70 to 80 beats.min -1 (Reece, 1996).

Although it differed statistically ($P<0.05$) between the genetic groups, and Soinga demonstrated a greater increase in HR at 36° C, it can be considered that in this treatment the increase in this parameter was common to all groups, which altered blood flow as a function of the need to dissipate heat to the environment, consequently reducing the thermal gradient between TS and AT.

For small ruminants, ideally there should be a thermal gradient of around 6°C between the core temperature and the surface temperature, and the latter with the ambient temperature (Medeiros et al., 2015), generating less energy expenditure to maintain the core temperature within the normal range, where metabolic and physiological reactions can be carried out without damage by heating or cooling (Sejian et al., 2019).

According to Silanikove, (2000) respiratory rates of 40-60; 60-80 and 80-120 mov min-1 characterize, respectively, low, medium-high and high stress and above 200 mov min-1 characterize severe stress in sheep (Silanikove, 2000). In the study in question, all sheep had mean RR representing medium-high and high stress according to the referred author. The increase in RR occurred due to the gradual increase in air temperature, resulting in a decrease in the thermal gradient between the animal and the environment, with a lower efficiency in heat dissipation by the sensitive form when compared to the insensitive form (Dantas et al., 2019).

For TR, it is observed that the mean values of the genetic groups under study were within the normal range for the species in all treatments (table 1), which according to Swenson and Reece (1996) the mean baseline limit should be 39, 1°C and for Cunninghan (1999), this variable in sheep varies between 38.5 and 39.9 ºC. The observed TR values denote the efficiency of the genetic groups in dissipating heat to the environment and in maintaining thermal balance using other physiological mechanisms of adaptation, preserving body temperature (TR) within normal limits, even in a stressful thermal environment (Costa et al., 2015).

It is important to mention that TR is one of the main variables indicative of adaptability to the environment (TR), since animals that present a smaller increase in TR are considered more tolerant to heat (Baccari Jr. et al., 1986). The temperature representing that of the central core of animals represents a good measure of heat tolerance, and its variations demonstrate the result of heat loss and gain processes (Henry et al., 2018).

Sheep are able to tolerate high temperatures, as they reduce their metabolism, to avoid an excessive increase in body heat during heat stress, reducing the risk of death. animals (Pulido-Rodriguez et al., 2021). Therefore, the importance of searching for resistant genotypes that present an economically viable performance and considered “optimal”, considering the climatic reality of the environment where it is inserted and the creation system used.

3.3 Surface temperatures by body regions

Variations in the thermal environment affected body surface temperatures (Figure 7), and the mean values of TS followed a similar pattern in all genotypes, showing an effect of treatments only ($P < 0.05$). For the body regions where the TS were obtained (Figure 8), there was a statistical difference (< 0.05) between the TS of the regions during the experimental period. The surface temperature of the eyeball (GO) and tympanic temperature (TP) demonstrated a greater sensitivity of these regions to variations in the thermal environment, which may indicate both a greater absorptivity of heat inside the chamber, and a greater production of heat generated by the increase blood flow in these regions, in an attempt to facilitate heat dissipation to the environment.

Surface temperatures, when obtained by infrared thermography (TIV) are able to show different responses to the environment depending on the genetic group and are highly correlated with phenotypic traits linked to heat tolerance (Paim et al., 2013).

IVT is a non-invasive remote sensing method used to measure changes in heat transfer and blood flow by detecting small changes in body temperature (Roberto et al., 2014; Sturion et al., 2020). Thus, when animals undergo a situation of heat stress, as a response to this condition, they promote sympathetic stimulation and the hypothalamic-pituitary-adrenocortical axis is activated, and heat is produced by increased concentrations of catecholamines and cortisol (Joy et al., 2020).

In table 3, significant differences can be observed by the analysis of variance ($P<0.05$) between the TS of the regions and between the treatments (28°C, 32°C and 36°C). There was a progressive increase in means over exposures at each temperature, with the exception of TP where the mean increased at 32°C and decreased at 36°C. Among the evaluated TS, it is noted that the surface of the eyeball (GO) presented the highest mean already at the initial temperature, at 28°C. In the exposure at 32 °C, the GO presented 37.91 °C, being below ($P<0.05$) the TP that reached 39.01 °C. The general means of the experimental period did not reveal differences ($P>0.05$) between the TS of the regions: cervical, thoracic, gluteal, ventral and general TS (Figure 8).

As the environments varied, the thermal gradient between the body surface of the animals and the environment decreased, and this caused changes in the physiological parameters (Table 1) due to the difficulty in losing heat by convection, conduction and radiation. This stressful condition promotes activation of the hypothalamic-pituitary-adrenal axis, increased levels of cortisol and changes in blood flow that generate the heat detected by IVT.

Although the mean GO in this study was the highest when considering the entire experimental period (Figure 8), this region did not differ from the others ($P<0.05$) after exposure to the highest temperature (36°C), demonstrating an increase uniformity of the TS of the body regions in this treatment. It is known that the increase in eyeball temperature can be mediated by a sympathetic component of the autonomic nervous system, an activity related to one of the first phases of the stress response (Stewart et al., 2010).

Due to their anatomical position, the eye and the back of the ears are considered reliable points for measuring body surface temperature using infrared thermography (Schmidt et al., 2013). In the study by Pulido-Rodriguez et al (2021), a correlation was observed between rectal and ocular temperatures, allowing the use of IVT as a non-invasive methodology to detect changes in the animals' core temperature. Thus, the surface temperature of the ocular and tympanic regions may be associated with the temperature of the body core due to its proximity to the brain, assuming that these regions are more sensitive to environmental variations than other body regions.

4 CONCLUSION

The 4 genetic groups showed similar heat tolerances, evidenced by the efficiency in dissipating heat through the increase in heart rate and respiratory movements, maintaining the normal RT for the species, even in an environment of alarming stress.

The Soinga showed a greater increase in heart rate as a means of adaptive compensation. Even so, HR, RR and TS elevations were means of heat dissipation common to all groups as the thermal environment changed.

Surface temperatures of the eyeball and tympanic membrane were more sensitive to temperature variations. However, more research is needed on the relationship between the TS of these regions and the core temperature, as well as other physiological changes in native sheep, which can be identified through TIV.

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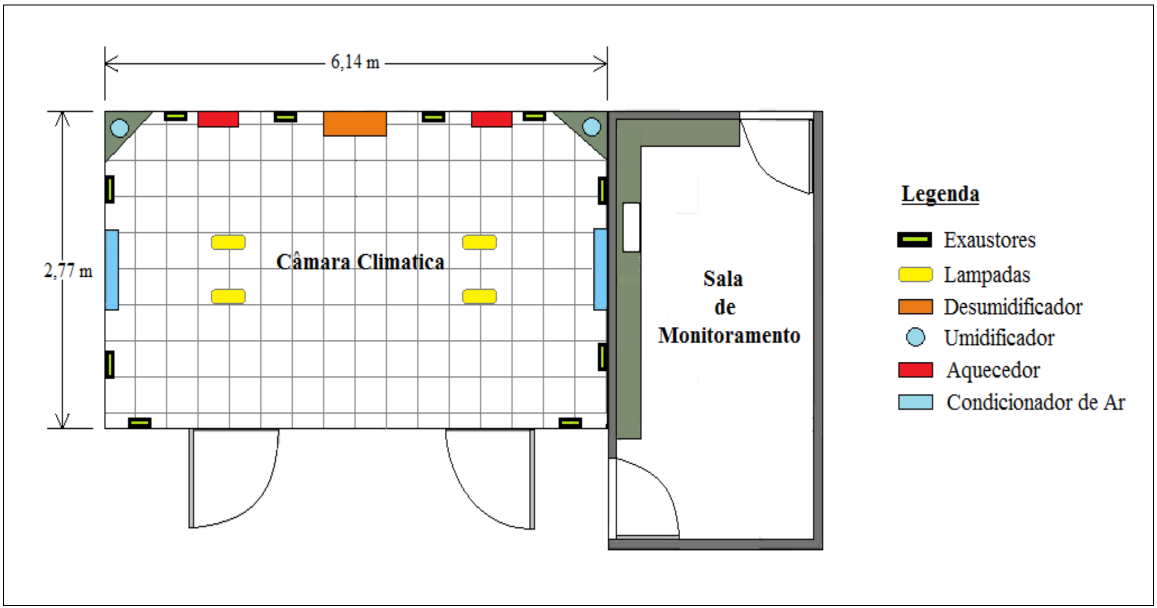

Figure 01: Internal layout of the climatic chamber and monitoring room with the description of the respective equipment.

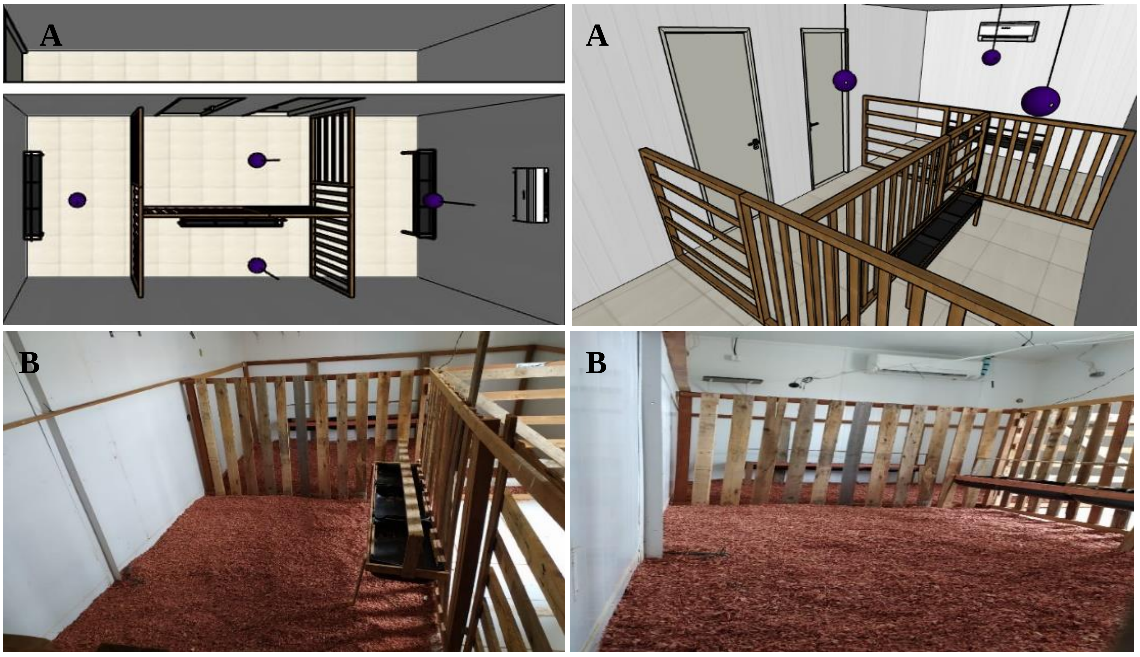

Figure 02: Schematic representation of the stalls (A) and photo of the interior of the climatic chamber after making the stalls (B). (Source: personal archive, 2021).

Figure 03: HOBO-type datalogger (A), and external cable with the sensors attached to the black globe, close to the height of the animals in the central area of the stall (B). (Source: personal archive, 2021).

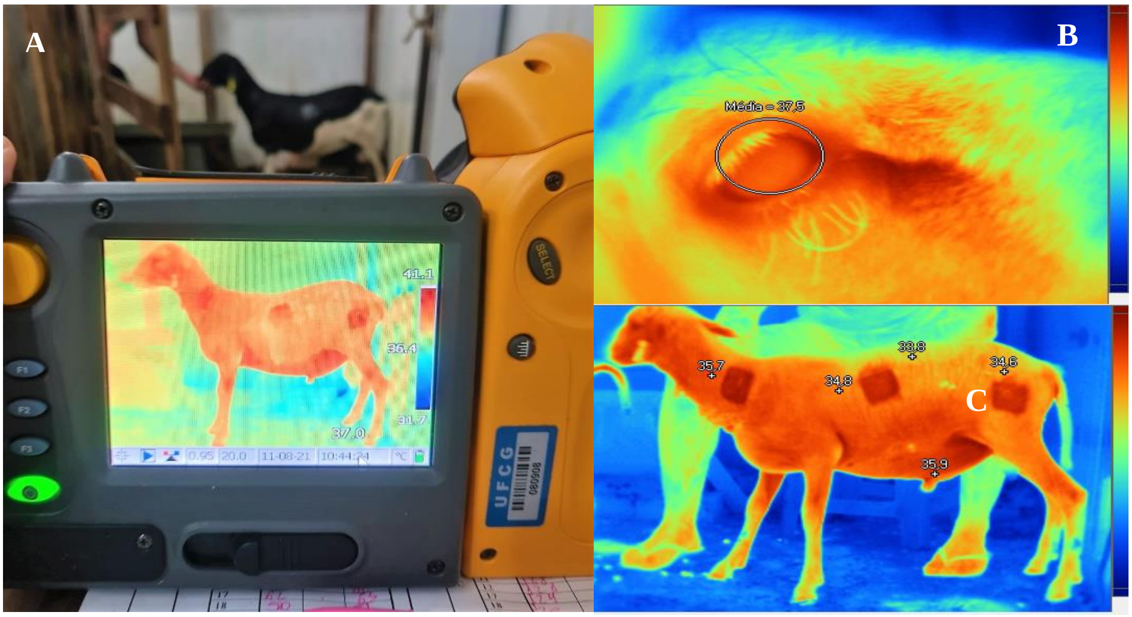

Figure 04: Infrared thermography of sheep in a climatic chamber (Fluke® Ti55FT thermographic camera) (A), thermograms of the eyeball (B) and of the cervical, thoracic, dorsal, ventral and gluteal regions analyzed in Smartview version 4.1 (C) (Source: personal archive, 2021).

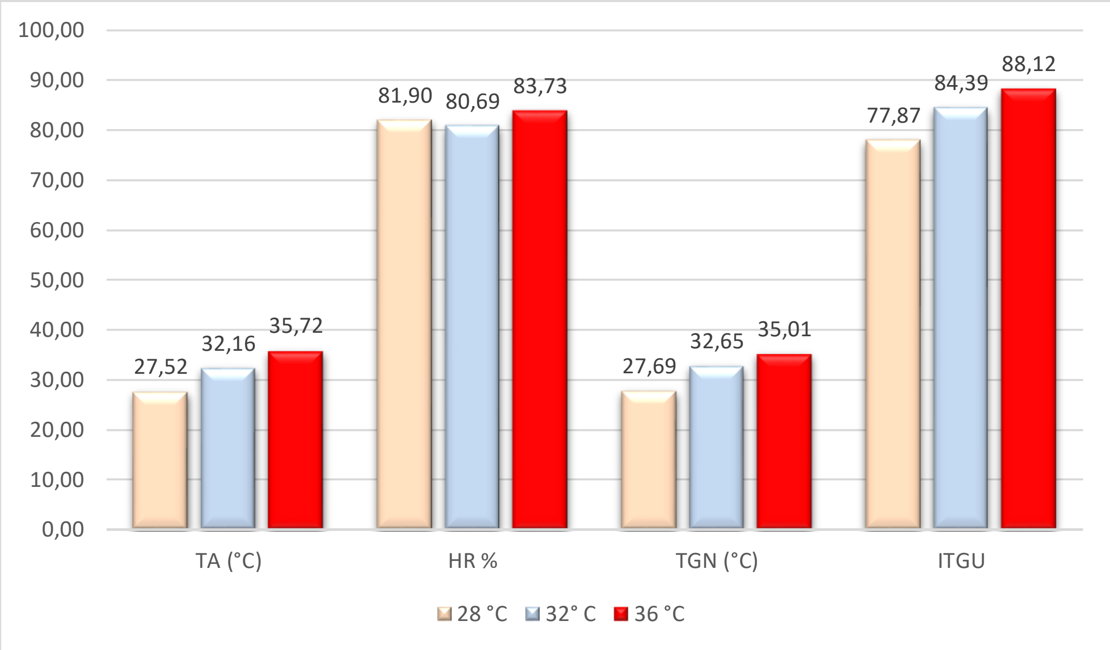

Figure 5. Averages of meteorological data: ambient temperature (TA), relative humidity (RH), black globe temperature (TGN) and black globe temperature and humidity index (ITGU) inside the climate chamber, as a function of temperatures 28°C, 32°C and 36°C.

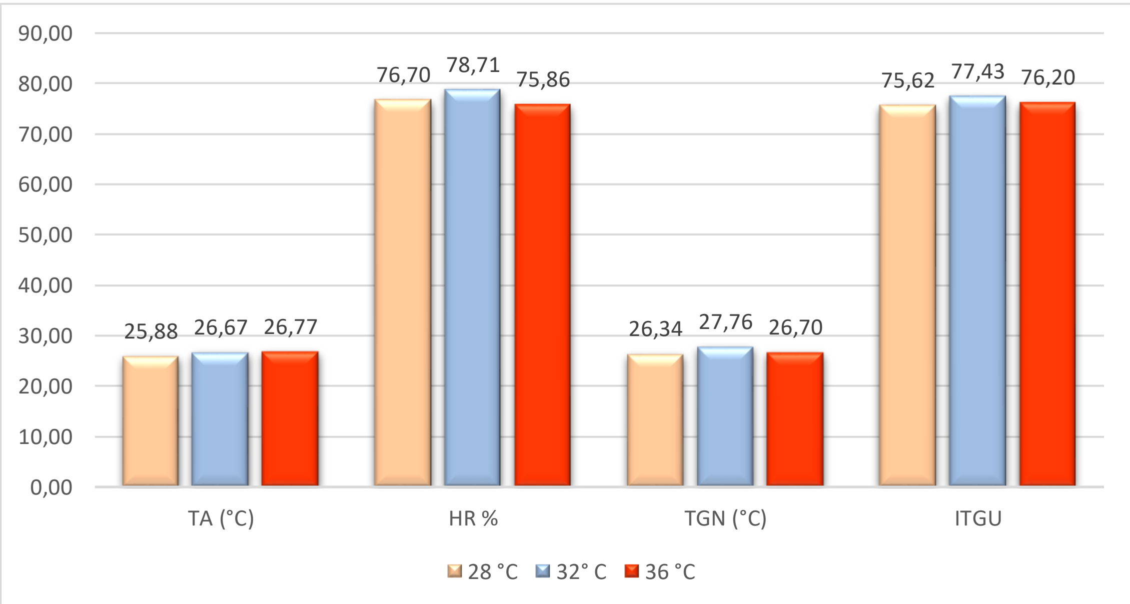

Figure 6. Averages of meteorological data: ambient temperature (TA), relative humidity (RH), black globe temperature (TGN) and black globe temperature and humidity index (ITGU) without temperature control, with air conditioners off and climatic chamber doors open

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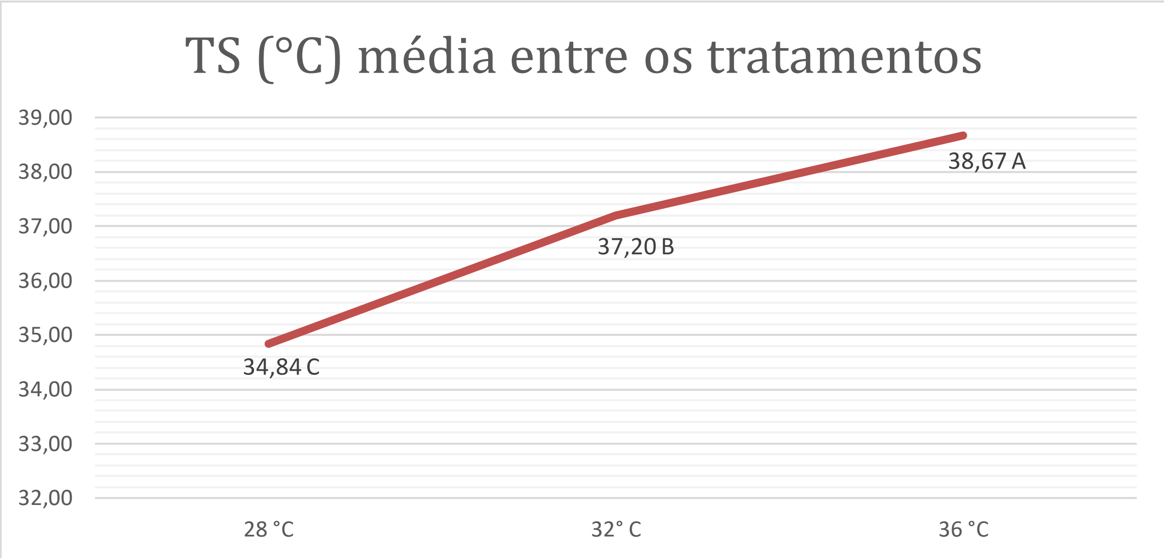

Figure 7. Overall average of surface temperatures of sheep from different genetic groups in a climatic chamber as a function of temperatures 28 °C, 32 °C and 36 °C. Different letters between data labels indicate statistical difference (P<0.05) by Tukey's test.

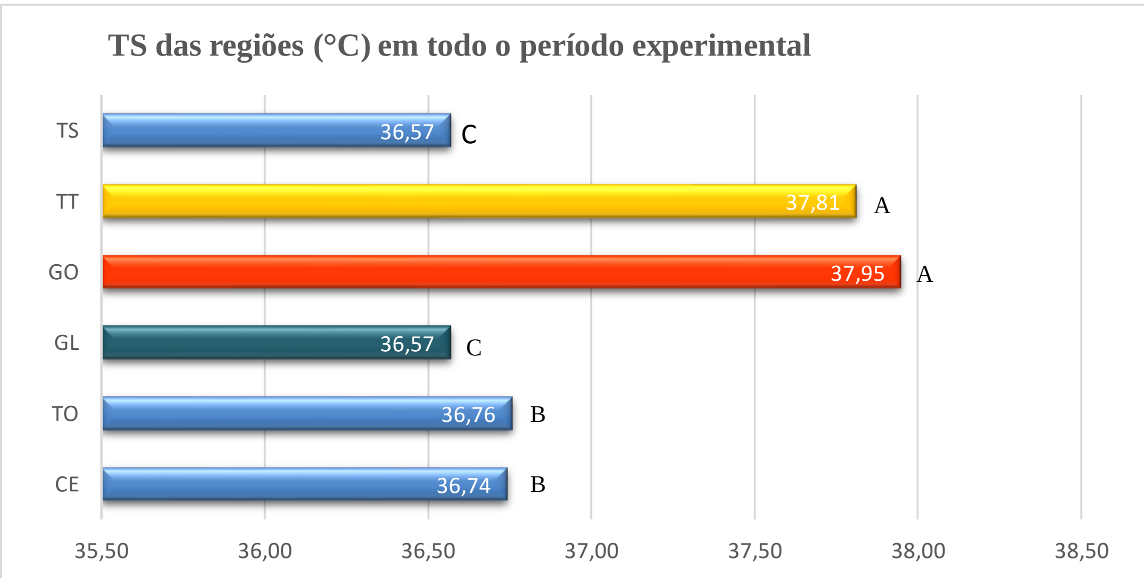

Figure 8. Average surface temperatures by body regions: cervical (CE), thoracic (TO), gluteal (GL), eyeball (GO), tympanic (TP) and General Superficial (TS) of sheep from different genetic groups in a climatic chamber. Different letters between data labels indicate statistical difference (P<0.05) by Tukey's test.

Table 1 – Overall average of physiological parameters: rectal temperature (TR), respiratory rate (RR) and heart rate (HR) of sheep of different genotypes as a function of temperatures 28 °C, 32 °C and 36 °C in a climatic chamber.

Ambient temperature (°C)	**Physiological variables**		
	TR (°C)	RR (mov min^{-1})	HR (bat min^{-1})
28 °C	38,56 C	30,05 C	93,00 B
32 °C	38,78 B	71,33 B	92,66 B
36 °C	39,23 A	179,88 A	102,00 A
CV (%)	0,71	22,35	10,35

Means followed by different letters in the column indicate difference for the factor (P<0.05) by Tukey's test.

Table 2 – General average of physiological parameters: rectal temperature (TR), respiratory rate (RR) and heart rate (HR) of sheep Soinga, Morada Nova, Santa Inês and SRD, in a climatic chamber, considering the entire experimental period.

Physiological parameters	**Genetic groups**				
	Soinga	Morada Nova	Santa Inês	SRD	CV (%)
TR (°C)	38,88 ± 0,32	38,74 ± 0,32	38,91 ± 0,24	38,92 ± 0,15	0,21
RR (mov min^{-1})	96,59 ± 14,48	89,63 ± 13,94	95,70 ± 20,89	93,11 ± 17,81	3,33
HR (bat min^{-1})	105,78 ± 7,49 A	90,66 ± 10,72 B	96,37± 6,52 B	90,74 ± 4,13 B	7,42

Means followed by different letters in the column indicate difference for the factor (P<0.05) by Tukey's test.

Table 3 – Mean surface temperatures by body regions: cervical (CE), thoracic (TO), gluteal (GL), eyeball (GO), tympanic (TP) and General Superficial (TS) of sheep from different genetic groups as a function of temperatures 28, 32 and 36 (°C) in a climatic chamber.

Surface Temperatures by body regions (°C)	**Temperatura ambiente (°C)**		
	28 °C	**32 °C**	**36 °C**
Cervical (CE)	34,71Cc	36,90 Cb	38,62 Aa
Thoracic (TO)	34,49 Cc	37,01 Cb	38,77 Aa
Gluteal (GL)	34,25 Dc	36,70 Cb	38,76 Aa
Eyeball (GO)	36,86 Ac	37,91 Bb	39,07 Aa
Tympanic (TP)	35,56 Bc	39,01 Aa	38,86 Ab
TS general (TS)	34,38 Dc	36,77 Cb	38,57 Aa
CV (%)	2,92	2,32	0,68

Means followed by different uppercase letters in the column and lowercase letters in the row indicate difference between treatments and surface temperatures by regions respectively (P<0.05) by Tukey's test.

CAPÍTULO III:

Effects of induced heat stress on the physiological, biochemical and hormonal parameters of native sheep of different genotypes in a climatic chamber

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Effects of induced heat stress on the physiological, biochemical and hormonal parameters of native sheep of different genotypes in a climatic chamber

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Declaration of Conflict of Interest

The authors declare no conflict of interest.

ABSTRACT

This study aimed to evaluate the effect of different thermal environments on the physiological, biochemical and hormonal parameters of Soinga, Morada Nova, Santa Inês and SRD sheep, in a climatic chamber. 24 uncastrated male sheep were allocated in 3 individual pens, containing 8 animals in each pen (2 from each genetic group). The sheep were exposed to 3 thermal environments (TA = 28 °C, 32 °C and 36 °C) for a period of 15 days, 10 hours a day, and environmental data were obtained throughout the experiment. Rectal temperature (TR), respiratory rate (RR) and surface temperature (TS) were measured and hormonal (Cortisol, T3 and T4) and biochemical parameters were evaluated after exposure to each temperature. The variations of the thermal environment influenced ($p<0.05$) the averages of TR, FR and TS, mainly at 36°C. The ITGU indicated severe stress from 32°C (>84), with peak elevation at 36°C (>88). All genotypes changed physiological parameters as the environment varied, but there was no significant difference ($P>0.05$) between the genetic groups. The concentrations of T3 and T4 were within the reference for the species in all treatments, with no statistical differences ($P>0.05$). Only Cortisol differed ($P<0.05$) between treatments and genetic groups, with the lowest averages at 28°C. At 32°C, Soinga and Morada Nova presented the lowest means compared to Santa Inês and SRD, and at 36°C, cortisol levels increased, but without differences ($P>0.05$) between the genetic groups. There were differences ($P<0.05$) in mean cholesterol (COL) between treatments and genetic and triglyceride (TRI) groups only between treatment groups with TA= 36°C. For glucose (GLI) there were differences ($P<0.05$) only between treatments; and there was no significant effect ($P>0.05$) of the metabolites: total protein, albumin, creatinine and urea. Elevation of RR and cortisol levels, maintenance of RT, as well as low values of T3 and T4, indicated an efficient thermoregulatory response of all genotypes. All animals were able to maintain homeothermy even in a heat stress environment, evidencing the adaptive equivalence between Soinga, Morava Nova, Santa Inês sheep and those without a defined racial pattern (SRD), due to high heat tolerance in extreme environments.

Keywords: Soinga, blood metabolites, thermoregulation, heat tolerance.

1 INTRODUCTION

Sheep farming has established itself in recent decades as an important animal production activity expanding around the world, serving as a source of income and as a means of settling producers and their families in the countryside. Although there is great potential, sheep farming systems express productive and reproductive rates below their real potential. Among the main causes related to this fact, climatic conditions stand out, with regard to high temperatures and variations in relative humidity (Berihulay et al., 2019).

The World Meteorological Organization (WMO) has released the new landmark report from the Intergovernmental Panel on Climate Change (IPCC) - Climate Change 2021: The Basis of Physical Science - which describes the current state of human-caused climate change, at a level considered to be worrying (IPCC, 2021). As a result, the direct effects of heat stress will mainly occur due to the increase in environmental temperatures and the frequency and intensity of heat waves (Lacetera, 2019), considerably influencing the sustainability of animal production worldwide.

In tropical regions, several breeds of native sheep are bred with a high capacity for heat tolerance, considered genetic resources of great importance due to their high adaptability to the climatic constraints of the region. The Soinga genetic group has stood out for being able to live in the semi-arid climate, with good productivity rates, including weight gain and excellent quality meat (Cavalcante 2018; Medeiros et al., 2019). Even so, there are few publications of scientific studies on this genotype, which has the potential to be improved and disseminated as a genetic resource for coping with climate change in tropical semi-arid regions.

The evaluation of physiological parameters of animals raised in hot environments is important to identify the capacity for thermal regulation based on homeostatic changes, such as changes in body temperature, metabolism and thermogenic hormones (Costa et al., 2015; Costa et al., 2018). Along with this, physiological variables and homeostatic indicators related to blood biochemistry are important within the context of adaptation to the environment (Helal et al., 2010).

Hormonal evaluations, mainly those produced by the hypophysis (prolactin), adrenal (cortisol) and thyroid (triiodothyronine-T3 and thyroxine-T4), are important due to the determinant role of these hormones in thermoregulation and metabolic adjustments in animals in hot environments (Joy et al. al., 2020). Cortisol is considered the main stress hormone in ruminants, although with regard to heat stress, more studies are needed. validate this hormone as a reliable heat stress indicator for sheep (Das et al., 2016).

Experiments in climatic chambers allow controlling environmental variables, such as temperature and relative humidity, in order to provide an accurate assessment of the influence of the thermal environment on the thermoregulatory capacity of animals. This information is essential to identify the degree of adaptation to the heat of genotypes native to the semi-arid region, enabling improvements and adjustments in management practices, mitigating possible losses within production systems (Torres et al., 2017; Polli et al., 2020).

Thus, the objective of this work was to evaluate the effect of different thermal environments (28°C, 32°C and 36°C) in a climatic chamber, on the physiological, biochemical and hormonal parameters of native sheep from different genetic groups.

2 MATERIAL AND METHODS

2.1 Site and experimental design

The procedures performed in this study were approved by the Research Ethics Committee (CEP) of the Federal University of Campina Grande, Paraíba, Brazil, Protocol CEP Nº. 097.2019.

The experiment was conducted in a climate chamber, between September and November 2021, at the Laboratory of Rural Constructions and Ambience (LACRA) of the Federal University of Campina Grande, located in the municipality of Campina Grande-PB 7° 13′ 51″ South, 35° 52′ 54″ West). The climatic chamber used is 6.14 m long and 2.77 m wide, with a constructed area of 17.00 m2 (Figure 1), made of laminated steel sheets with anti-corrosion protection and filled with styrofoam, allowing isolation thermal with the external environment.

Twenty-four male sheep of the Santa Inês, Morada Nova, Soinga breeds with no defined racial pattern (SPRD) were used, six of each breed/genetic group, with an average age of five months and an average weight of 20 kg. All animals were examined and dewormed in the pre-experimental phase.

The ration provided consisted of Tifton hay - Cynodon dactylon, (L) Weight (50.54 kg), corn bran (34.69 kg), soybean meal (13.32 kg), limestone (0.45 kg) and mineral supplement (1.0 kg). Animals were fed ad libitum and provided twice a day, at 8:00 am and 4:00 pm, with daily adjustment and water consumption was ad libitum.

The animals were kept in 3 collective stalls of approximately 4.00m2 each, with 8 animals randomly distributed in each stall. The floor of the climatic chamber was lined with a 10 cm layer of wood sawdust (figure 2), which was changed weekly so that there was good absorption of the animals' feces and urine.

The sheep were exposed to temperatures of 28 ºC, 32 ºC and 36ºC, with an average air humidity of 79.70%, 83.09% and 85.28%, and for each temperature, a period of 15 consecutive days was established exposure in a controlled environment (climatic chamber). On the 11th day of exposure to each temperature, blood was collected for laboratory analysis of biochemical and hormonal parameters.

The animals were maintained for 10 hours of continuous exposure per day at each temperature, alternating with 14 hours at room temperature with the climatic chamber turned off and the doors open. Between each treatment, the animals remained at the experimental site with the equipment turned off and doors open, without the influence of controlled temperature, for 5 consecutive days, to eliminate the residual effect.

2.2 Data collect

2.2.1 Environmental data

During the entire experimental period, climatological data were recorded using 3 HOBO-type dataloggers, with an external cable with sensors attached to the black globe, these equipment were installed in the central area of each pen, allocated at the height of the center of mass of the animals (figure 3), and were programmed to record data every hour for 24 hours, being used for statistical analysis, the hours from 7:00 am to 5:00 pm (time when the chamber was closed with controlled temperature and humidity).

To check the wind speed, a portable AD-250 digital anemometer was used, with a measurement range of 0.4 to 30 m/s, positioned at the height of the animals' center of mass so that it could record air movement. caused by exhaust fans, air conditioners and the entry and exit of people inside the chamber, when they are providing food or collecting experimental data.

The values of the thermal comfort indices were determined from the observed data, in the period from 7:00 am to 5:00 pm, time of controlled temperature with the chamber closed, at intervals of one hour. To calculate the Black Globe Temperature and Humidity Index (BGII), the formula suggested by Buffington et al. (1981), expressed as follows:

$$BGHI = T_{gn} + 0{,}36\ T_{po} + 41{,}5$$

Em que:

BGHI: black globe temperature and humidity indices, °C;
T_{gn}: Black globe temperature, °C;
T_{po}: Dew point temperature, °C.

2.2.2 Rectal and surface temperature and respiratory rate

The physiological variables were measured in the afternoon shift in all the animals in the experiment, between 2:30 pm and 3:30 pm, during the days of collection of each experimental treatment (28 ºC, 32 ºC and 36ºC), and the rectal temperature (TR), respiratory rate (RR), and surface temperature (ST). Rectal temperature was obtained by introducing a veterinary clinical thermometer, with a scale of up to 44 °C, directly into the rectum, in contact with the animal's rectal mucosa, at a depth of 5 cm, remaining for a period of 2 min.

The respiratory rate was measured by means of indirect auscultation of the sounds, with the aid of a flexible stethoscope, at the level of the thoracic region, counting the number of movements during one minute, thus obtaining the amount of mov min-1.

The surface temperature (TS) was obtained by infrared thermography, when the animals remained immobile, without any restriction and with little manipulation, using a thermographic camera model Fluke® Ti55FT, with precision of ± 2 °C or 2% of the real temperature, sensitivity thermal (NETD) <50 mK, infrared resolution of 320 × 240 pixels, and 5.7 inch (14.4 cm) screen with 320 × 240 pixel resolution.

The thermograms were analyzed using the Smartview software, version 4.1, considering an emissivity of 0.98. The TS were obtained from the average of the maximum temperature points (°C) of the regions: cervical, thoracic, dorsal, ventral and gluteal (figure 4) obtained in the afternoon shift during the days of measurements of physiological parameters in each experimental phase and their respective temperatures (28 °C, 32 °C and 36 °C).

2.2.3 Biochemical parameters

For the evaluation of serum parameters, on the 11th day of exposure to each temperature (28 ºC, 32 ºC and 36ºC), 4 ml of blood were collected by puncture of the jugular vein in the animals. Blood collection was performed in the morning, before feeding, using 5 ml syringes, which were transferred to tubes without anticoagulant, previously identified, and then passed through a centrifugation process (centrifuge model 90-1, Coleman Equipamentos para

Laboratório Comp. E Imp. Ltda, Brazil) at 3000 rpm/15 min, in which after obtaining the serum, the material was stored in identified microtubes and frozen at -20 °C.

Then, plasmatic concentrations were measured at the Laboratory of Clinical Pathology of the Veterinary Hospital, Campus de Patos-PB, in an Automatic Biochemical Analyzer (Cobas C111, Roche). The values were determined through enzymatic or colorimetric assays in specific kits of urea (URE) kinetic UV (urease and glutamate dehydrogenase), creatinine (CRE) kinetic (modified Jaffé reaction), total proteins (PT) colorimetric (biuret reaction), albumin (ALB) colorimetric (bromocresol green - BCG), cholesterol (COL) kinetic (cholesterol oxidase (CHOD)-PAP), triglycerides (TRI) kinetic (glycerol-3-phosphate oxidase -GPO-PAP).

2.2.4 Thyroid hormones (T3 and T4) and cortisol

Blood samples were collected on the 11th day of each experimental treatment (28 ºC, 32 ºC and 36ºC), through jugular vein puncture after asepsis with iodized alcohol. In the collections, 0.8x25mm gauge needles and 5ml vacuum tubes containing 10% sodium ethylene diamine tetraacetate (EDTA) anticoagulant were used. Blood samples were centrifuged at 4°C at 3,000 x rpm (1,100XG) for 15 minutes. The supernatant resulting from centrifugation was separated into 1.5mL aliquots and stored at -20ºC.

Plasma concentrations of cortisol, thyroxine (T4) and triiodothyronine (T3) were analyzed in duplicates by means of the ELISA technique, using commercial laboratory kits (INVITRO), developed for the quantitative evaluation of hormones.

2.3 Statistical analysis

A completely randomized design (CID) with a 4 x 3 factorial arrangement (four genetic groups and three temperatures) was used. The data of environmental variables and physiological parameters were submitted to analysis of variance, using the statistical program SAEG 9.1 and the averages compared using the Tukey test at a significance level of 5% of probability.

For the blood parameters data (Biochemical, T3, T4 and Cortisol), analysis was performed for two independent variables (breeds and heat treatments) through ANOVA (Dual-way) followed by post hoc Bonferroni test in GraphPrism® (GraphPad Software Inc ., San Diego, CA, USA) for Windons, and statistical significance was set at $P \leq 0.05$.

3 RESULTS

In figure 5 are the variables that characterize the thermal environment, air temperature (TA), black globe temperature (TGN), relative humidity (RH) and the indices of black globe temperature and humidity (ITGU). In each experimental phase the TA values were in accordance with the pre-established (28 ºC, 32 ºC and 36ºC). The average values of UR, TGN and ITGU increased proportionally with the increase in temperature, reaching maximum values of 85.28%, 35.19 °C and 88.44 at 36 °C respectively, demonstrating an environment outside the thermal comfort zone of the animals (Figure 5).

When the climatic chamber was turned off and the doors were open, the environmental variables (TA, HR, TGN), as well as the ITGU, demonstrated that there were no conditions that represented thermal discomfort for the animals under study (Figure 6).

During the experimental period, exposure to different thermal environments significantly ($P<0.05$) affected rectal temperatures (TR), surface temperature (TS) and respiratory rate (RR) of sheep (Table 1). For the genetic group factor, similar variations were observed and the mean values of the mentioned physiological parameters increased as the exposure temperature was high, differing only between treatments ($P<0.05$) with the highest values in exposure to 36 °C (Table 1).

Plasma concentrations of T3 and T4 were within the reference values for the species in all treatments, with no statistically significant ($P>0.05$) differences (Table 2). Only Cortisol levels differed significantly ($P<0.05$) between treatments and genetic groups, with all groups having the lowest means at 28°C. At 32°C, Soinga and Morada Nova sheep had the lowest means compared to Santa Inês and SRD, and at 36°C cortisol was significantly higher ($P<0.05$) between treatments, with no significant difference ($P> 0.05$) between genetic groups (Table 2).

For the metabolic parameters shown in table 3, statistical differences ($P<0.05$) were found in the means of cholesterol (COL) between treatments and genetic groups and triglycerides (TRI) only between treatment groups with TA= 36° Ç. For glucose (GLI) there were differences ($P<0.05$) only between treatments; and there was no significant effect ($P>0.05$) of the metabolites: total protein, albumin, creatinine and urea.

The Soinga genetic group showed a smaller variation in the COL means as a function of the experimental temperatures, unlike the other genotypes in which the values increased from the 28 °C to the 32 °C treatment and decreased significantly ($P<0.05$) after exposure to the treatment 36 °C (Table 3).

4 DISCUSSION

Inside the facilities, the average maximum temperature (TA) (35.98°C) (figure 5) and the minimum TA (26.25°C) (figure 6) are, respectively, above the thermal comfort zone (ZCT) for the species, which must be below 25.0°C with relative humidity of 65.0% according to Eustáquio Filho et al (2011). The amplitude between AT was 9.73 °C, which requires a quick physiological adaptation to thermal variations, a situation similar to what occurs in the semi-arid region.

Figure 5 shows that the relative humidity (RH) exceeded 80% at 32°C and 36°C, being above the ideal values for sheep according to Baêta and Souza (2010), which is between 50 and 80%. The increase in RH, associated with higher temperatures, promote an increase in the metabolic rate of animals, increasing water consumption and consequent elimination through sweat and urine, which may have contributed to the increase in RH, as additional sources of humidity in the chamber (Miranda et al., 2018).

Relative air humidity significantly affects caloric balance in a hot environment, where heat loss through evaporation is essential for maintaining body temperature. As RH and TA exceed the ZCT, there is an increase in the sensitivity of sheep to thermal stress, making it difficult to dissipate heat and increasing body temperature (Borges et al., 2018).

ITGU values up to 74 indicate a situation of comfort for the animals, from 74 to 78 mild stress; between 79 and 84 a dangerous situation and above 84 indicate an emergency situation for dairy cows (Buffington et al., 1981). For the sheep species, according to Souza (2010), an ITGU value equal to 83 is enough to indicate a condition of medium-high stress.

Although the values of this index have been established for dairy cows by Buffington et al. (1981), studies concluded that this same scale can be appropriately used for sheep (Furtado et al., 2017; Torres et al., 2017). Therefore, already in the first experimental phase (28°C), a representative condition of mild stress was seen, and as the thermal environment varied, emergency situations were observed at 32°C and 36°C with an average ITGU of 84.80 and 88.44 respectively (figure 5). When the climatic chamber was open and without temperature control and exposure at 28°C, this index showed a mild stress condition (ITGU<78).

Mean TGN values were above what is considered critical only at 36°C, as according to Motta (2001), the TGN seen as regular is 27°C and 34°C, and in the latter treatment, it reached 35.19. Along with this, from the physiological changes found in the studied thermal environment variations, it was observed that the sheep were under significant thermal stress only from 32°C (ITGU>84).

When subjected to environments with air temperatures above the ZCT, homeothermic animals compensate for heat gain using thermoregulation mechanisms to maintain internal body temperature in a range that allows thermal balance (Mckinley et al., 2017). In this study, there were similar physiological alterations between the genetic groups according to the environmental variations, and all groups were efficient in dissipating the absorbed heat, without raising the RT above the physiological limits, even in a thermally stressed environment (Table 1).

Physiological parameters such as rectal temperature, heart rate and respiratory rate of sheep are relevant indicators of their comfort during thermal stress and the related environment, with rectal temperature being the one that reflects the core body temperature of animals for a practical purpose (De et al., 2017). For Rashamol et al (2018) changes in physiological parameters are alternatives that animals adopt to deal with situations of thermal stress.

At high ambient temperature, the thermal gradient between the animal and the surrounding environment is reduced, which leads to failure to eliminate heat in sensible form, increasing its effort to dissipate body heat through a higher rate of respiration (De et al. ., 2017; Dantas et al., 2019). This fact was verified in this research from the exposure of the animals to 32°C and 36°C, in which all genotypes presented a high respiratory rate.

The average FR of the 32°C and 36°C treatments were characterized as high heat stress for ruminants according to the Silanikove scale (2000), in which a FR of 40-60, 60-80, 80-120 mov/min characterizes a low, medium-high and high stress for ruminants, respectively; and above 200 for sheep, the stress is classified as severe.

Although the RF values were elevated above the species standard, this was the main way that sheep of all groups used to dissipate heat to the environment. The elevation of this parameter is the first control mechanism for sheep in heat stress environments (Habeeb et al., 2018; Marai et al., 2007; McManus et al., 2009). Therefore, the fact that the RR was high does not necessarily characterize a stress condition, but rather a physiological mechanism functioning properly to prevent the increase in body temperature (TR), which was, in all treatments, within the parameters for the species according to the interval proposed by Cunningham (1999).

Surface temperature depends, above all, on environmental conditions (room temperature, relative air humidity and ventilation) and physiological conditions (such as vascularization and sweat evaporation). Nascimento (2019), evaluating the adaptability of Soinga and Santa Inês sheep as a function of diet and thermal environment, found that the surface temperature of the Santa Inês breed exceeded that of the Soinga genotype. In the present study, the TS did not differ ($P>0.05$) between the genetic groups, even though they were high according to the variations in the environment and the decrease in the thermal gradient, this demonstrates that all groups were efficient in directing the blood flow to the skin surface, to facilitate heat dissipation.

It is known that cortisol is a biomarker that determines the physiological reaction and emotional state of the animal in response to stressful stimuli, and its level can vary by different factors, including temperature, humidity, handling and pathologies (Nejad et al., 2014). The plasma concentration of this hormone increases in direct proportion to the increase in ambient temperature (Cardoso et al., 2021).

When the animal is exposed to high temperatures, activation of the hypothalamic-pituitary-adrenal axis (HPA axis) occurs, allowing increased production of corticotropin-releasing hormone (CRH) in the hypothalamus, which stimulates adrenocorticotropic hormone (ACTH) from the anterior pituitary gland. , causing increased adrenal secretion of cortisol (Engler et al., 1989). In such cases, antidiuretic hormone (ADH) expression is also increased, reducing water excretion (Engler et al., 1989).

Sheep breeds native to semi-arid tropical regions show greater thermotolerance at temperatures of 40◦C and 42◦C, increasing cortisol production (Indu et al., 2015). In research with goats subjected to a controlled environment, Cardoso et al., (2021), observed elevations of cortisol at 32°C, which despite indicating a thermoregulatory response, plasma levels were within the range found by other authors (3 to 15 ng /ml) (Costa et al., 2015; Ribeiro et al., 2015).

According to McDonald and Pineda (1989), cortisol levels for small ruminants range from 8–19 ng/mL. According to this variation, it is observed that at 28°C the cortisol levels of all groups were below the reference interval. When exposed to 32°C, Soinga and Morada Nova kept cortisol levels below the reference level, and Santa Inês and SRD showed the highest averages.

Although differences ($P<0.05$) were shown between groups at 32°C, the cortisol elevations found did not exceed the reference limit for small ruminants (McDonald and Pineda, 1989). This demonstrates that the thermal environment did not affect cortisol levels in a way that would indicate thermal stress for the animals, which reinforces the high thermotolerance of the evaluated genetic groups, which maintained a stable TR, below the limit for the species, even in a stressful environment. severe (ITGU = 88.44).

T4 and T3 concentrations can decrease as air temperature increases (Helal et al., 2010; Costa et al., 2015). In this research, at all temperatures, the values of these hormones were within the reference range for the species according to Gonzáles and Silva (2017), and there was no distinction in the means of these hormones between the genetic groups ($P>0.05$), regardless of thermal variation (table 2). This suggests that the genetic groups under study maintained low levels of T3 and T4, due to the efficient adaptive mechanism of these animals to avoid internal heat production due to the calorigenic effect of these hormones on metabolic activity.

The pattern of metabolic rate in a mammal at rest can provide a means for researchers to assess how well animals can tolerate environmental temperature changes, through the determination of the thermoneutral zone (Fonseca et al., 2019). When evaluating the thermal regulation of Morada Nova sheep in a semi-arid tropical environment, Fonseca et al., (2019) observed that the animals managed to eliminate heat by evaporation, without a significant increase in metabolic activity, which had an evident change only at 37°C.

In a study of goats subjected to controlled temperature in a climate chamber, a decrease in T3 and T4 hormones was demonstrated when subjected to 32°C (Cardoso et al., 2021), a result similar to that of Praga et al (2018) who, when evaluating native goats under high ambient temperatures (THI =86.5), reported lower plasma concentrations of T3 and T4. This shows the efficient thermoregulation of these animals to avoid the production of additional metabolic heat, preventing a thermal stress condition.

The sheep in this study were not exposed to solar radiation, which is an important factor that influences the thermal balance of free-ranging animals that are raised in the field (Mitchell et al., 2018). Even so, the animals increased respiratory evaporation as the AT increased (with a thermal variation of 9.73°C). However, analysis of thermal balance as a function of air temperature revealed that sheep regulated body temperature within narrow limits through evaporative and non-evaporative heat loss, without significant changes in T3, T4 and Cortisol levels, even at the extremes (TA=36°C).

Among climatic factors, air temperature is the most relevant condition on the metabolic and physiological changes that make up thermal stress (Dias and Silva et al., 2016; Joy et al., 2020). Biochemical parameters change within the same species due to factors such as age, physiological state, breed, production level, management and especially climatic stress (Ribeiro et al. 2016). Even so, in the present study, no significant differences were found in most of the evaluated metabolites (PT, ALB, CRE, URE, TRI) between genotypes and treatments, although some of these values were out of the reference range for the sheep species (Kaneko et al., 2008).

The cholesterol levels found in this study were above the reference values for the species according to Kaneko et al (2008) at the two initial temperatures (TA = 28°C and 32°C). Only at 36°C did Morada Nova, Santa Inês and SRD show values slightly lower than the standard for the species, and Soinga maintained values above the range, according to the aforementioned author. Even so, COL means are within the range (14 – 126 mg dL-1), described by Silva et al (2020), who evaluated several studies with sheep and found a range of 112 mg dL-1 for cholesterol levels

, while the amplitude seen by Kaneko et al (2008) was 24 mg dL-1 with a range between 52 – 76 mg dL-1.

Although increases in TA did not cause a decrease in GLI and COL levels in Soinga, the decrease in COL observed in other genotypes can be explained by the influence that the thermal environment (TA=36°C, ITUG >88) caused in these animals. Because in a stressful situation, cholesterol levels decrease and cortisol synthesis increases in response to thermal stress (Cardoso et al., 2021). Cortisol limits the use of glucose and, under these conditions, the animal mobilizes other energy reserves, such as triglycerides and proteins (Sejian et al., 2010).

Triglycerides are the main form of energy storage in the animal organism and are synthesized in almost all tissues, especially in the liver and adipose tissue. Although they differed statistically between the genetic groups of the 36 °C treatment, the TRI values were within the reference range for the species in all treatments. The values obtained in this research were within the range established by Kaneko et al (2008), which is between 9-30 mg dL-1. However, the TRI values defined by Silva et al, (2020) present a broader reference range (5-71 mgdL-1), obtained from studies using sheep raised in different regions of Brazil.

In ruminants, the maintenance of plasma glucose concentration is related to glycemic stability, since dietary carbohydrates are almost completely used in the rumen (Gressler et al., 2015). Physiological changes due to stressful environmental factors can alter the hormonal dynamics that regulate gluconeogenesis and cellular utilization of glucose, which can increase levels in the bloodstream (Kozloski, 2017, Libardi et al., 2018). In this study, at temperatures of 32°C and 36°C, sheep similarly increased blood GLI levels, even so, the values were within the standard of the species (Kaneko et al., 2008; Silva et al., 2020).

Total protein blood concentration demonstrates protein nutritional status very reliably. They are important for the transport of nutrients, metabolites, hormones, maintenance of osmotic pressure and blood viscosity, regulation of blood pH and blood clotting (Varanis, 2018). Although they did not differ statistically (P>0.05) for treatments and genetic groups, in this study PT levels were slightly below the standard for the species at all experimental stages (Kaneko et al., 2008), yet they remain within of the range for sheep described by Silva et al (2020).

According to González (2018) The exacerbated increase of this metabolite in the blood can be the result of dehydration, infections, tumors, shock, fluid loss and even advanced age of the animal. The PT values can be explained by the reduction of food consumption, which is a behavioral strategy of animals to deal with the increase in temperature, avoiding the increase in heat generated by the digestion process.

Albumin, according to Cardoso (2021), may vary due to several factors such as physiological and genetic adaptation. In this study, the ALB was slightly below the reference values at 28°C and 36°C (Kaneko, 2008). Blood proteins are synthesized mainly by the liver, and the synthesis rate is directly related to the nutritional status of the animal, therefore, animals that have suffered some type of stress that caused food restriction, have a decrease in the protein profile (Libardi et al., 2018).

CRE was slightly below the range (0.4 – 1.7 mg dL-1) described by (Silva et al., 2020), but according to Kaneko et al (2008), the means found varied between 0.30 to 0.42 mg dL-1 are below normal for sheep which is between 1.2 to 1.9 mg dL-1 (Kaneko et al., 2008). Creatinine is excreted only via the kidneys, therefore its plasmatic concentration reflects the glomerular filtration rate. High levels of this metabolite may indicate impaired kidney function (Varanis, 2018; Satake et al., 2018).

In general, urea is a sensitive and immediate indicator of protein intake, while albumin is a long-term indicator of protein status (González, 2018). In this research, the animals presented ERU levels within the normal range for the species (Kaneko et al., 2008). This represents the little influence of thermal variations on the protein metabolism of the genetic groups under study, associated with an ideal consumption of the energy/protein ratio in the diet.

The concentration of blood metabolites undergoes changes due to the influence of several factors intrinsic to the animal and the environment, mainly the diet. The physiological adaptation of the animal to a certain thermal environment directly influences the functioning of the metabolic pathways, which may alter the levels considered normal. However, these variations do not necessarily mean pathological changes, or metabolic dysfunctions, and should be analyzed taking into account the environment in which the animal is found (Neto et al., 2017; Varanis, 2018; Silva et al., 2020).

Furthermore, the absence of reference values that are representative of the environmental reality of the place where the animals are raised is perceived, mainly in the Northeast region, which has a large part of the Brazilian sheep herd. Therefore, it is essential to carry out new studies, which, when analyzed together with the existing ones, can contribute to the determination of reference values of blood metabolites for sheep, mainly considering the semi-arid climate and locally adapted native animals.

5 CONCLUSION

Exposure to different thermal environments influenced the physiological, hormonal and biochemical parameters of the studied sheep. However, the significant increase in RR and cortisol levels, the maintenance of TR within the ideal range for the species, as well as the low values of T3 and T4, indicated an efficient thermoregulatory response of all studied genotypes.

All genotypes were able to maintain homeothermy with changes in physiological dynamics, even in heat stress environments. This fact exposes the adaptive equivalence between sheep of the Soinga genetic group, the Morava Nova and Santa Inês breeds, and animals without a defined breed pattern (SRD).

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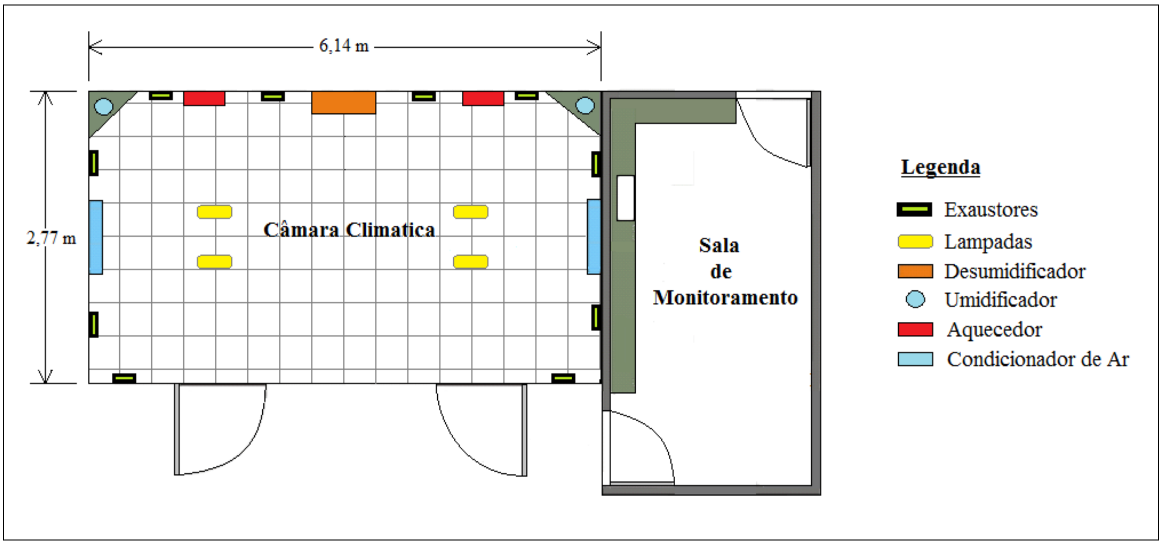

Figure 01: Internal layout of the climatic chamber and monitoring room with the description of the respective equipment.

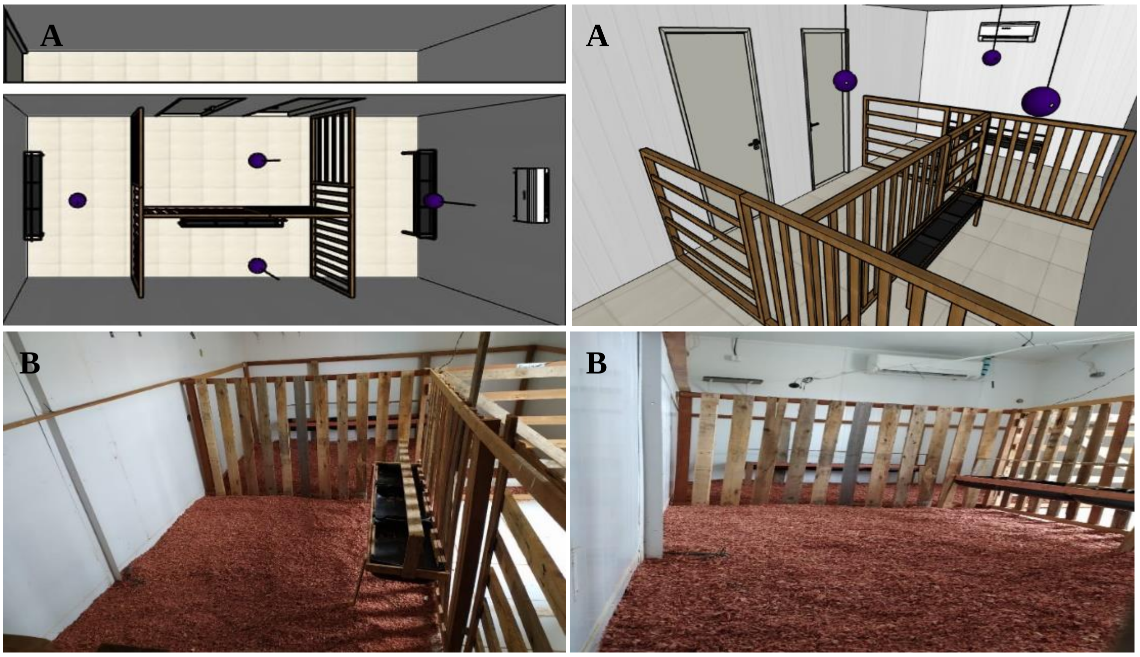

Figure 02: Schematic representation of the stalls (A) and photo of the interior of the climatic chamber after making the stalls (B). (Source: personal archive, 2021).

Figure 03: HOBO-type datalogger (A), and external cable with the sensors attached to the black globe near the height of the animals in the central area of the stall (B). (Source: personal archive, 2021).

Figure 04: Infrared thermography of sheep in a climatic chamber (Fluke®Ti55FT thermographic camera) (A) and thermogram analyzed in Smartview version 4.1 (B) (Source: personal archive, 2021).

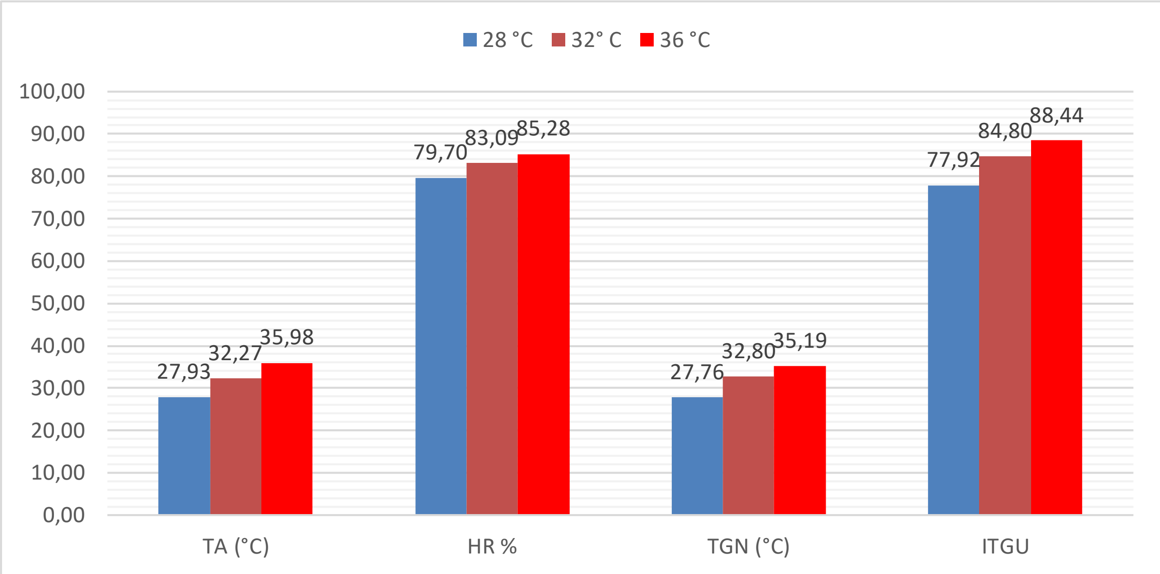

Figure 5. Averages of meteorological data: ambient temperature (TA), relative humidity (RH), black globe temperature (TGN) and black globe temperature and humidity index (ITGU) inside the climate chamber, as a function of temperatures 28°C, 32°C and 36°C.

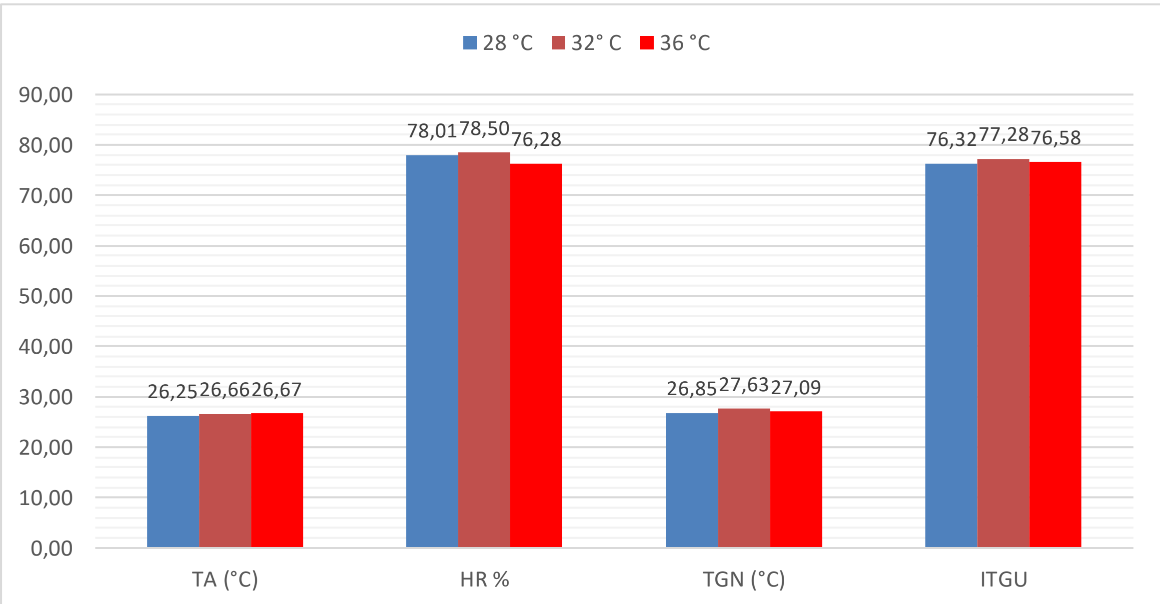

Figure 6. Averages of meteorological data: ambient temperature (TA), relative humidity (RH), black globe temperature (TGN) and the black globe temperature and humidity index (ITGU) without temperature control, with air conditioners turned off and climatic chamber doors open.

Table 1 – Overall average of physiological parameters: rectal temperature (TR), respiratory rate (RR) and surface temperature (ST) of sheep of different genotypes as a function of temperatures 28 °C, 32 °C and 36 °C in a climatic chamber.

Ambient temperature (°C)	**Physiological variables**		
	TR (°C)	RR (mov min^{-}	TS (°C)
28 °C	38,63 C	32,00 C	33,54 C
32 °C	38,84 B	89,16 B	36,78 B
36 °C	39,34 A	190,61 A	38,76 A
CV (%)	0,53	11,09	1,32

Means followed by different letters in the column indicate difference for the factor (P<0.05) by Tukey's test.

Table 2 – Mean plasma concentrations of cortisol, thyroxine (T4) and triiodothyronine (T3) of different genetic groups as a function of temperatures 28, 32 and 36 (°C).

Parameters hormonal	Genetic groups				Reference values for sheep
	Soinga	Morada Nova	Santa Inês	SRD	
28°C					
Cortisol (ng/ml)	7,69 ± 1,00 B	7,11 ± 0,48 C	7,15 ± 1,23 B	7,37 ± 0,59 C	6,00 ± 1,00
T4 (ng/ml)	1,23 ± 0,06	1,23 ± 0,06	1,16 ± 0,06	1,24 ± 0,02	-
T3 (ng/ml)	0,95 ± 0,08	0,99 ± 0,16	0,93 ± 0,19	1,04 ± 0,15	0,63 - 1,5
32°C					
Cortisol (ng/ml)	7,72 ± 1,19 Bc	7,76 ± 0,58 Bc	9,35 ± 0,69 Aa	8,45 ± 0,40 Bb	6,00 ± 1,00
T4 (ng/ml)	1,22 ± 0,04	1,22 ± 0,03	1,17 ± 0,20	0,99 ± 0,17	-
T3 (ng/ml)	0,93 ± 0,05	0,91 ± 0,11	0,85 ± 0,16	0,88 ± 0,09	0,63 - 1,5
36°C					
Cortisol (ng/ml)	9,00 ± 0,85 A	9,77 ± 0,16 A	9,82 ± 0,17 A	9,57 ± 0,57 A	6,00 ± 1,00
T4 (ng/ml)	0,93 ± 0,19	1,04 ± 0,15	0,93 ± 0,05	0,91 ± 0,11	-
T3 (ng/ml)	0,79 ± 0,13	0,90 ± 0,10	0,76 ± 0,06	0,76 ± 0,08	0,63 - 1,5

Means followed by different uppercase letters in the column and lowercase letters in the row differ statistically by the ANOVA test (Dual-way) followed by the post hoc Bonferroni test (P<0.05).

Table 3 – Means of metabolic parameters: total protein (PT), albumin (ALB), creatinine (CRE), urea (URE), glucose (GLI), triglycerides (TRI) and cholesterol (COL) from sheep of different genotypes as a function of temperature 28 , 32 and 36 (°C) in a climatic chamber.

Metabolic parameters	Genetic groups				Reference values for sheep
	Soinga	Morada Nova	Santa Inês	SRD	
28°C					
PT (g/dL)	3,97±0,12	4,00±0,14	4,00±0,22	4,18±0,13	6,0 – 7,9
ALB (g/dL)	2,36±0,10	2,28±0,11	2,37±0,15	2,34±0,18	2,4 – 3,0
CRE (mg/dL)	0,33±0,05	0,40±0,10	0,37±0,10	0,37±0,08	1,2 – 1,9
URE (mg/dL)	21,05±4,92	19,13±1,74	20,82±2,16	21,09±4,13	17,12 – 42,8
GLI (mg/dL)	40,55±4,93 B	38,43±2,73 B	39,53±1,79 B	36,64±5,62 B	50 -80
TRI (mg/dL)	29,19±3,39	27,64±4,11	28,61±6,45	27,68±8,87	9 - 30
COL (mg/dL)	87,71±11,5 Ba	86,51±12,67 Ba	92,26±17,04 B	76,96±14,8Bb	52 - 76
32°C					
PT (g/dL)	3,97±0,27	3,82±0,13	3,73±0,25	3,93±0,15	6,0 – 7,9
ALB (g/dL)	2,44±0,23	2,48±0,10	2,49±0,08	2,54±0,13	2,4 – 3,0
CRE (mg/dL)	0,40±0,08	0,40±0,06	0,42±0,07	0,37±0,10	1,2 – 1,9
URE (mg/dL)	17,14±2,39	19,34±3,33	20,73±1,68	17,93±4,05	17,12 – 42,8
GLI (mg/dL)	65,55±4,76 A	65,92±4,20 A	72,94±1,96 A	65,71±3,93 A	50 -80
TRI (mg/dL)	27,56±8,90	22,60±3,03	18,83±4,37	22,04±4,70	9 - 30
COL (mg/dL)	89,03±11,9Bb	106,20±14,8 Aa	114,01±39,8 A	93,38±13,6 Ab	52 - 76
36°C					
PT (g/dL)	3,65±0,13	3,67±0,08	3,68±0,31	3,83±0,19	6,0 – 7,9
ALB (g/dL)	2,19±0,10	2,05±0,10	2,26±0,31	2,21±0,10	2,4 – 3,0
CRE (mg/dL)	0,35±0,10	0,35±0,08	0,30±0,08	0,35±0,08	1,2 – 1,9
URE (mg/dL)	19,03±4,99	18,49±3,99	18,52±4,51	18,43±3,18	17,12 – 42,8
GLI (mg/dL)	66,82±3,57 A	63,72±4,24 A	64,67±6,45 A	62,28±5,76 A	50 -80
TRI (mg/dL)	20,62±2,99 c	27,96±1,41a	24,89±5,62 b	24,64±5,67 b	9 - 30
COL (mg/dL)	96,41±11,52 Aa	48,89±8,43 Cb	46,31±6,86 Cb	45,18±9,05 Cb	52 - 76

A, B, C Within-column means are significantly different (P < 0.05); a, b, c Means within the line are significantly different (P <0.05) by ANOVA (Dual-way) test followed by post hoc Bonferroni test.

CONCLUSÃO GERAL

Conforme os estudos recentes, contatou-se que a FR e TR são os parâmetros considerados mais relevantes para expor a capacidade termorreguladora de ovinos criados em regiões tropicais. Os autores ainda não utilizam, como base para seus estudos, índices ambientais específicos para ovinos dessas regiões. Isso deixa claro a necessidade de adequação de índices que indiquem as condições de estresse e desconforto térmico, considerando o alto grau adaptativo e tolerância ao calor de raças nativas de regiões de clima quente.

O globo ocular e região timpânica recebem um maior fluxo sanguíneo em ambientes de estresse térmico. Mesmo assim, são necessários mais estudos sobre a relação entre a temperatura do núcleo central e os pontos de temperatura máxima da superfície de regiões corporais de ovinos nativos do semiárido.

Todos os animais conseguiram manter a homeotermia mesmo em ambiente de estresse térmico, demonstrado pelas alterações dos parâmetros fisiológicos e sanguíneos (bioquímicos e hormonais) comum a todos os animais, evidenciando a equivalência adaptativa entre os ovinos Soinga, Morava Nova, Santa Inês, e os sem padrão racial definido (SRD).