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Dissertação

Avaliação de características associadas a termotolerância em vacas da raça Holandês de variedade preta ou vermelha

Jéssica Lazzari

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Dissertação apresentada ao Programa de Pós-Graduação em Veterinária da Faculdade de Veterinária da Universidade Federal de Pelotas, como requisito parcial à obtenção do título de Mestre em Ciências (área de concentração: Patologia e Reprodução animal).

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Resumo

LAZZARI, Jéssica. **Avaliação de características associadas a termotolerância em vacas da raça Holandês de variedade preta ou vermelha.** 2023. 54f. Dissertação (Mestrado em Ciências) - Programa de Pós-Graduação em Veterinária, Faculdade de Veterinária, Universidade Federal de Pelotas, Pelotas, 2023.

O estresse térmico é um dos principais problemas que acometem a atividade leiteira e ocorre quando há o desbalanço entre calor produzido pelo organismo e absorvido do ambiente e a capacidade de dissipação, influenciada pela conformação da camada de pelos e da pele. As colorações da pelagem diferem na absorbância, que em manchas pretas é de 90%, em manchas vermelhas de 60-70% e em manchas brancas 40%. Geralmente os estudos que comparam a termorregulação na raça Holandês, incluem animais majoritariamente pretos ou brancos e intermediários e não os vermelhos. Dessa forma, o estudo teve por objetivo comparar a capacidade termorregulatória de vacas da raça Holandês de pelagem vermelha e branca ou preta e branca criadas em sistema semiextensivo, em condições de termoneutralidade e estresse térmico. Vacas da raça Holandês, criadas em sistema semiextensivo, foram alocadas nos grupos: grupo HVB, de pelagem vermelha e branca, e grupo HPB, de pelagem preta e branca. Para cada vaca HVB, buscou-se uma vaca HPB equivalente quanto a porcentagem de manchas, escore de condição corporal, peso, produção de leite, dias em lactação, ordem de parição e status reprodutivo. As condições ambientais foram registradas a cada hora e calculado o índice de temperatura e umidade (ITU), considerando-se ausência de estresse quando o ITU < 68. A temperatura interna foi registrada a cada 30min por termômetros intravaginais fixados em implantes de progesterona novos (n=18). As temperaturas superficial e da carúncula lacrimal foram obtidas através de imagens termográficas (n=26 na estação fria; n=34 na estação quente). A taxa de sudação foi estimada por teste colorimétrico de SCHLEGER e TURNER. Na estação fria, as condições ambientais se mantiveram na classificação ausência de estresse e a temperatura interna, horas em hipertermia, taxa de sudação e temperatura da carúncula lacrimal não diferiram entre os grupos. Apenas a temperatura superficial média (HPB: 30,9±0,3 ºC; HVB: 29,6±0,3ºC; p=0,02) e máxima (HPB: 32,0±0,3ºC; HVB: 30,9±0,3ºC; p=0,05) foram inferiores no grupo HVB. Na estação quente, nas horas mais quentes do dia, o ITU foi de estresse leve e moderado e em alguns momentos atingiu estresse severo, enquanto nas horas mais frias, a classificação foi de ausência de estresse. A temperatura superficial, da carúncula lacrimal e interna, horas em hipertermia e taxa de sudação não diferiram entre os grupos. A temperatura interna média foi discretamente inferior no grupo HVB (HPB: 38,75±0,01 ºC; HVB: 38,62±0,1 ºC; p=<0,0001). Quando a avaliação foi segmentada em quartis, observou-se efeito do grupo e do ITU (P<0,001) e não houve interação (p=0,62). Dessa forma, em estresse térmico leve a moderado, vacas Holandês vermelhas e brancas não demonstraram ser mais termotolerantes que vacas pretas e brancas. As alterações estatisticamente significantes não traduzem vantagens fisiológicas aos animais vermelhos nas condições testadas.

Palavras-chave: atividade leiteira, características fenotípicas, coloração da pelagem, dissipação do calor, estresse térmico.

Abstract

LAZZARI, Jéssica. **Evaluation of characteristics associated with thermotolerance in black or red Holstein cows.** 2023. 54f. Dissertation (Master degree in Sciences) - Programa de Pós-Graduação em Veterinária, Faculdade de Veterinária, Universidade Federal de Pelotas, Pelotas, 2023.

Heat stress is among the main problems that affect dairy farming and occurs when there is an imbalance between heat produced by the body and absorbed from the environment and the dissipation capacity, influenced by the conformation of the layer of hair and skin. The coat colors differ in absorbance, in black spots is 90%, in red spots is 60-70% and in white spots is 40%. Generally, studies that compare thermoregulation in the Holstein breed include mostly black or white and intermediate animals and not the red ones. Thus, the study aimed to compare the thermoregulatory capacity of Holstein cows with red and white or black and white, managed in semi-extensive system, under thermoneutrality and heat stress. Holstein cows created in semi-extensive system were allocated in the groups: HRW group, with red and white coats, and HBW group, with black and white coats. For each HRW cow, an equivalent HBW cow was selected for percentage of spots, body condition score, weight, milk production, days in milk, parity and reproductive status. Environmental conditions were registered every hour and the temperature and humidity index (THI) was calculated, considering the absence of stress when the THI < 68. Internal body temperature was recorded every 30 minutes using intravaginal thermometers fixed in new progesterone implants (n=18). The surface and lacrimal caruncle temperatures were obtained through thermographic images (n=26 cold season; n=34 hot season). The sweating rate was estimated using the SCHLEGER and TURNER colorimetric test. In the cold season, environmental conditions of absence of stress were observed throughout the period. Internal body temperature, time in hyperthermia, sweating rate and lacrimal caruncle temperature did not differ between groups. Only the average surface temperature (HBW: 30.9±0.3 ºC; HRW: 29.6±0.3ºC; p=0.02) and maximum (HBW: 32.0±0.3ºC; HRW: 30, 9±0.3ºC; p=0.05) were lower in the HRW group. In the hot season, in the hottest hours of the day, the THI was mild and moderate stress and in few moments was observed severe stress. In the coldest hours, the classification was of absence of stress. Surface, lacrimal caruncle and internal body temperature, time in hyperthermia and sweating rate did not differ between groups. The mean internal body temperature was slightly lower in the HRW group (HBW: 38.75±0.01 ºC; HRW: 38.62±0.1 ºC; p=<0.0001). When the evaluation was segmented into quartiles, there was an effect of the group and the THI (P<0.001), there was no interaction (p=0.62). Thus, under mild to moderate heat stress, red and white Holstein cows did not prove to be more thermotolerant than black and white cows. The statistically significant alterations may not represent physiological advantages to the red animals under the tested conditions.

Keywords: coat color, dairy cattle, heat dissipation, phenotypic characteristics, thermal stress.

Lista de Figuras

Lista de Abreviaturas e Siglas

cm	Centímetros
CTE	Condutividade Térmica Efetiva
D1	Dia 1
D2	Dia 2
D3	Dia 3
DEL	Dias Em Lactação
ECC	Escore de Condição Corporal
EF	Estação Fria
EQ	Estação Quente
g	Gramas
h	Horas
HPB	Holandês Preto e Branco
HVB	Holandês Vermelho e Branco
ITU	Índice de Temperatura e Umidade
kg	Quilogramas
mg	Miligrama
min	Minutos
T	Temperatura ambiental
TI	Temperatura Interna
ton	Toneladas
TS	Temperatura superficial
UR	Umidade Relativa

Lista de Símbolos

US$	Dólares
°C	Grau Celsius
>	Maior que
<	Menor que
≤	Menor e igual a
%	Porcentagem

Sumário

1 Introdução

O leite é a quarta commodity mais produzida no Brasil e confere a quarta posição entre os maiores produtores de leite cru a nível mundial (FAO, 2022), com pouco mais de 35 bilhões de litros produzidos e aproximadamente 16 milhões de vacas ordenhadas (IBGE, 2022). A região Sul destaca-se nacionalmente, pois os três estados estão entre os maiores produtores, atrás apenas de Minas Gerais. Todavia, a região detém a maior produtividade com 3700 litros/vaca/ano (IBGE, 2022). Apesar disso, em 2022, 133 mil ton de laticínios foram importados, totalizando US$ 514 mi, sendo que Rio Grande do Sul e Santa Catarina estão entre os maiores importadores (COMEX STAT, 2021). Portanto, o conjunto de indicadores aponta a necessidade do aumento da produtividade para atingir a autossuficiência e a questão climática é um desafio (NEIVA, 2022).

Cerca de dois terços do leite produzido no Brasil advêm de regiões de clima tropical, caracterizadas por temperaturas médias mais elevadas (IBGE, 2022). O restante, no clima subtropical, mesmo que em menor grau, tem sido afetado também por condições climáticas adversas, cuja projeção indica agravamento nos próximos anos (CARVAJAL et al., 2021). Dessa forma, há a necessidade de criação de animais termotolerantes, fato refletido na cadeia nacional, em que 80% do leite é produzido por vacas Girolando (SILVA et al., 2022).

Nos bovinos leiteiros em estresse térmico há um desbalanço entre termogênese e termólise, em que a condição ambiental adversa (frio ou calor) leva a alteração da temperatura interna, o que desencadeia a ativação de mecanismos termorregulatórios para manutenção da homeotermia (LEE, 1965). Geralmente o desafio envolve temperaturas elevadas que culminam principalmente na queda de produção, reflexo da diminuição da ingestão de matéria seca (GORNIAK et al., 2014) e subfertilidade devido ao impacto no balanço endócrino (ROTH, 2000; WILSON et al., 1998) na dominância folicular (WOLFENSON et al., 1995), na competência oocitária (AL-KATANANI; PAULA-LOPES; HANSEN, 2002) e na mortalidade embrionária (GARCÍA-ISPIERTO et al., 2006; RYAN et al., 1993).

Para a raça Holandês a zona de termoneutralidade gira em torno de -5º a 21ºC (JOHNSON, 1986), podendo variar de acordo com o nível de produção. Quando a temperatura ambiental se encontra fora dela são ativadas respostas fisiológicas: vasodilatação periférica, taquicardia e taquipneia (BEAKLEY; FINDLAY, 1955a, 1955b; NEUWIRTH et al., 1979). Juntamente, desde que haja diferença de gradiente térmico entre a temperatura superficial do animal e do ambiente, ocorre a dissipação do calor através das vias não evaporativas: radiação, condução e convecção (KIBLER; BRODY, 1949). Acima do limite crítico superior, assume a transferência térmica por evaporação, representada inicialmente pela via respiratória, que, a partir de 27ºC, é assumida principalmente pela via cutânea, com o aumento da taxa de sudação (MAIA; DASILVA; BATTISTON LOUREIRO, 2005; SANTOS et al., 2016). Associado a temperatura ambiental, a eficiência das trocas térmicas é influenciada pela umidade relativa do ar, velocidade do vento e nível de radiação (LEE, 1965).

Além das condições climáticas, a eficiência da termólise, evaporativa ou não, é influenciada pela configuração da capa que reveste o animal, no qual inclui a espessura da pele, comprimento, espessura, angulação, densidade e coloração dos pelos (MAIA; SILVA; BERTIPAGLIA, 2003). O conjunto de fibras atua como uma barreira a passagem da energia térmica e dependendo de sua organização, contribuem para facilitar ou dificultar ainda mais a troca do ar saturado de vapor e intensidade da penetração da radiação solar (ALFONZO et al., 2016; DIKMEN et al., 2008; MAIA et al., 2009).

A quantidade de calor absorvida varia conforme a coloração da pelagem, ou seja, pela quantidade de pigmento depositado nos pelos e pele. Isso porque, a absorbância de manchas brancas é de 40%, de 90% para manchas pretas e de 60-70% para a pelagem vermelha (DA SILVA; LA SCALA JR; TONHATI, 2003; FINCH; BENNETT; HOLMES, 1984; STEWART, 1953). Apesar das características favoráveis da pelagem branca, como a coloração da pele acompanha a do pelo, a epiderme despigmentada torna-se suscetível aos danos da radiação ultravioleta (WALSBERG, 1988). Dessa forma, para sistemas baseados a pasto, a pelagem vermelha seria a mais adequada, visto que a absorbância é intermediária, ao mesmo tempo que a epiderme fica protegida.

Os estudos que avaliam o desempenho de animais da raça Holandês de diferentes colorações de pelagem são limitados. Quase sua totalidade compara animais majoritariamente pretos ou brancos e geralmente criados em sistemas confinados dotados de refrigeração, o que não produz um desafio térmico ideal para medir o efeito da característica. De modo geral, o desencadeamento de resposta termorregulatória foi mais presente em vacas majoritariamente pretas, visto que apresentam maior temperatura superficial e interna (ANZURES et al., 2019; DA SILVA; MAIA, 2011; HANSEN, 1990; SANTOS et al., 2016; TUCKER; ROGERS; SCHÜTZ, 2008). Recentemente, Isola et al. (2020) demonstraram que fêmeas Holandês vermelhas e brancas apresentam menor temperatura superficial em manchas pigmentadas ou brancas, e menor temperatura interna, se comparadas com pretas e brancas. Entretanto, o referido estudo apresentou desafios no pareamento dos animais conforme o nível de produção, dias em lactação e paridade, devido ao limitado número de fêmeas do fenótipo vermelho. Além disso, a medição da temperatura interna ocorreu apenas em momentos pontuais.

Dessa forma, há indícios de que animais de pelagem vermelha e branca sejam mais termotolerantes. Para isso, o documento foi organizado para inicialmente revisar de que forma as características fenotípicas da raça Holandês, juntamente aos mecanismos termorregulatórios, modulam a resposta ao estresse térmico e impactam no desempenho. Na sequência, é apresentado e discutido os resultados do experimento de mestrado que objetivou comparar a capacidade termorregulatória de vacas Holandês de pelagem vermelha e branca ou preta e branca criadas em sistema semiextensivo, em condições de termoneutralidade e estresse térmico.

2 Revisão de literatura

A efetividade da dissipação do calor está correlacionada positivamente com a condutividade térmica da camada de pelos e pele, dependente do comprimento, espessura, densidade e cor. Vacas Holandês majoritariamente brancas apresentam maior diâmetro, comprimento e densidade de pelos e maior espessura da pele, o que favorece a condutividade térmica (MAIA et al., 2009). Todavia, de acordo com o comprimento do pelo, a condutividade térmica efetiva (CTE, na ausência de convecção) se comporta de maneira diferente. Isso porque, animais com alta densidade e pelos maiores que 1,8 cm, apresentam maior CTE; enquanto animais com alta densidade e pelos menores que 0,5cm, a relação é inversa e a dissipação dificultada (MAIA et al., 2009).

Animais com pele despigmentada, apresentam maior penetração da radiação solar. Por isso, como mecanismo de proteção, possuem maior número de pelos e mais longos (MAIA; SILVA; BERTIPAGLIA, 2003). A melanina presente nos pelos e pele protege, mas absorve maior radiação. A absorbância para manchas pretas é de 90%, de 40% para brancas e de 70% para pelagem vermelha (DA SILVA; LA SCALA JR; TONHATI, 2003; STEWART, 1953). Dessa forma, para diminuir a resistência à termólise, é favorável que o animal apresente pelos mais curtos, grossos e menos numerosos, pois a menor densidade de pelos favorece a penetração do vento (MAIA; SILVA; BERTIPAGLIA, 2003).

Assim como observado por Lee, Baek e Parkhurst (2016), o pelo branco de vacas Holandês confinadas é mais grosso e longo, enquanto o pelo preto de menor diâmetro, é mais curto. Somado a isso, o maior aquecimento da superfície preta é compensado pelo aumento da perda de calor evaporativa (DA SILVA et al., 2012; DA SILVA; MAIA, 2011; MAIA; DASILVA; BATTISTON LOUREIRO, 2005; SANTOS et al., 2016). Neste mesmo princípio, vacas majoritariamente bancas apresentam menor temperatura superficial e por conseguinte, menor taxa de sudação (DA SILVA; MAIA, 2011).

A temperatura superficial, perda de calor por evaporação e taxa de sudação são correlacionadas positivamente com o ITU (LIM et al., 2021; MAIA; DASILVA; BATTISTON LOUREIRO, 2005). A temperatura superficial, aferida principalmente pela termografia a partir da radiação emitida pelo animal, é um método não invasivo e diferentes regiões do corpo têm sido estudadas para determinar qual o melhor preditor da temperatura interna, sendo apontada principalmente a carúncula lacrimal (DALTRO et al., 2017; ISOLA et al., 2020; PENG et al., 2019). Nos estudos de comparação de pelagens, a temperatura superficial da lateral do animal é utilizada para avaliar a capacidade termorregulatória (SANTOS et al., 2016). A mensuração da perda de calor por evaporação e a taxa de sudação são parâmetros estudados com menor frequência, por depender de métodos mais laborais ou de custo elevado, se comparado com outros marcadores de estresse térmico, como a frequência respiratória e temperatura interna (DA SILVA; MAIA, 2011; DIKMEN et al., 2008, 2014; SCHARF et al., 2008). A avaliação da taxa de sudação mais frequente é o método de Schleger e Turner. Este teste colorimétrico é de baixo custo e fácil preparo; todavia, o tempo dispendido para execução do teste limita o número de coletas.

O índice de temperatura e umidade (ITU) é uma ferramenta amplamente utilizada para avaliação da condição ambiental e identificação de estresse térmico a partir de variáveis ambientais – temperatura do ar (T) e umidade relativa (R). Foi criado para humanos por Thom (1958) e adaptado por NOAA (1976), o qual descreve o cálculo como: $ITU = (1.8 \times T + 32) - (0.55 - 0.0055 \times R) \times [(1.8 \times T + 32) - 58)]$. O índice classifica como ausência de estresse térmico ITU < 68, estresse leve para ITU entre 68 e 72, estresse moderado quando 72 > ITU ≤ 80 e em estresse severo quando o ITU ultrapassava 80 (ZIMBELMAN; COLLIER; EASTRIDGE, 2011).

A dificuldade de eliminação do calor latente pela via evaporativa cutânea devido à alta densidade do pelo e maior espessura do pelo e pele, culmina no aumento da temperatura interna, diminuição da frequência respiratória e da frequência cardíaca e aumento da temperatura superficial (ALFONZO et al., 2016; ANZURES et al., 2019; TUCKER; ROGERS; SCHÜTZ, 2008). A taxa de respiração é um bom indicador para avaliação de estresse térmico, bem como a taxa de ofegação (ARIAS et al., 2021), já que existe uma relação linear entre o agravamento da taxa de ofegação e o aumento do índice de temperatura e umidade (VEISSIER et al., 2018).

Anzures-Olvera et al. (2019), em vacas Holandês confinadas, observaram maior temperatura interna para vacas pretas, sem diferir na frequência respiratória, escore de ofegação e termografia. Isola et al., (2020) ao compararem vacas Holandês vermelhas e brancas e pretas e brancas, observaram que na estação quente, a temperatura tanto das manchas pigmentadas como de não pigmentadas e a temperatura retal foram inferiores em vacas vermelhas. Alfonzo et al., (2016) avaliaram vacas ½ Holandês e ½ Gir e observaram maior espessura de pele, menor diâmetro e comprimento do pelo e maior densidade de pelos, em animais cruzados se comparado com animais puros Holandês. Além disso, a temperatura interna, temperatura superficial e frequência respiratória foram inferiores no período mais quente do dia nos animais cruzados.

A mensuração da temperatura interna por meio de termômetros intravaginais demanda pouca manipulação do animal, não perturba seu comportamento natural e possibilidade maior número de registros, se comparado com a temperatura retal (KAUFMAN; SAXTON; RÍUS, 2018). Ademais, a temperatura vaginal apresenta correlações fortes com o ITU (KAUFMAN; SAXTON; RÍUS, 2018; PENG et al., 2019) e é mais sensível a mudanças na temperatura corporal do que a temperatura retal (BURDICK et al., 2012). Para bovinos leiteiros, o intervalo fisiológico de temperatura interna gira em torno de 38,0ºC a 39,2ºC (CUNNINGHAM; KLEIN, 2009) e temperaturas acima de 39,2ºC (quadro de hipertermia) são bons indicativos de estresse por calor (WEST, 2003).

Além dessas colorações preta e vermelha, há pouco tempo foi adicionado um novo fenótipo ao gado Holandês, o *Larson Blue*, em que as manchas pigmentadas são de coloração acinzentada (DIKMEN et al., 2017), de absorbância semelhante a coloração vermelha (DA SILVA; LA SCALA JR; TONHATI, 2003). Nestes animais não foram observadas diferenças na temperatura interna e produção de leite, visto que o sistema de criação confinado contava com sistema de resfriamento e não produziu desafio térmico (DIKMEN et al., 2017). Recentemente, através de edição genômica, o fenótipo foi obtido a partir de deleções no gene da proteína pré-melanossomal 17 (LAIBLE et al., 2021). No estudo, uma gestação falhou, um bezerro nasceu com hidropsia e o outro nasceu saudável, mas veio a óbito com quatro semanas de vida após ser acometido por infecção não identificada. Apesar dos resultados iniciais, a mutação mostra-se promissora.

Outro fenótipo termotolerante são os animais portadores do gene Slick (vacas Holandês cruzadas com touros Senepol) que apresentam pelos curtos e brilhantes, características que diminuem a resistência a perda de calor pela superfície corporal. Isso porque, a menor absorção da radiação solar, culmina na maior dissipação do calor via condução e convecção. Ademais, a termotolerância do genótipo é justificada principalmente pela maior taxa de sudação, no qual reflete, assim como nas demais características fenotípicas, em menor temperatura interna e superficial e menor dependência da frequência respiratória como mecanismo de termorregulação (DIKMEN et al., 2008, 2014; SÁNCHEZ-RODRÍGUEZ et al., 2019).

Quanto ao desempenho reprodutivo, quanto maior espessura de pele, maior é número de inseminações por concepção (BERTIPAGLIA; SILVA; MAIA, 2005). Para King et al., (1988) vacas brancas (<40% de manchas pretas) que pariram na estação fria apresentam menos dias em aberto e menor número de serviços por concepção. Na mesma linha, Godfrey e Hansen (1994) observaram menor intervalo entre partos para vacas com porcentagem de manchas pretas menor ou igual a 25%. Ao passo que Anzures-Olvera et al. (2019) não observaram diferença na taxa de concepção, serviços por concepção, intervalo entre partos e perdas fetais. Isso porque, provavelmente, pelos animais estarem confinados, não houve desafio térmico suficiente para se observar o efeito. Da mesma forma, Becerril et al. (1993) não observaram diferenças na idade ao primeiro parto, dias em aberto e intervalo entre partos. Para vacas portadoras do gene Slick, o intervalo entre partos foi inferior (ORTIZ-URIARTE et al., 2020).

Quanto ao desempenho lactacional, para Anzures-Olvera et al. (2019) a composição do leite não diferiu entre vacas brancas e pretas, mas a produção de leite foi superior em 394kg de leite para vacas brancas em uma lactação de 305 dias. Becerril et al. (1993) observaram uma correlação linear entre porcentagem de manchas brancas e produção de leite e Hansen (1990) menor queda de produção para vacas brancas no verão. Por outro lado, para Godfrey e Hansen (1994) a porcentagem de manchas pretas não afetou a produção, mas reduziu o tempo em lactação. Já Anzures et al. (2019) concluíram que as características do pelo não afetaram a produção. Quanto às portadoras do gene Slick, há menor queda de produção de leite na estação quente (CONTRERAS-CORREA et al., 2016; DIKMEN et al., 2014; SÁNCHEZ-RODRÍGUEZ et al., 2019).

3 Artigo

Thermoregulatory response of black and white or red and white Holstein cows in the hot and cold season

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Thermoregulatory response of black and white or red and white Holstein cows in the hot and cold season

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Abstract
Dairy cows in pasture-based systems are more susceptible to the effects of heat stress. The Holstein breed has the black or red phenotypes, the latter having lower absorbance of solar radiation. Therefore, the objective of the study was to evaluate whether animals with red and white coats are more resistant to heat when raised in a subtropical climate. Red-and-white (RW) and black-and-white (BW) Holstein lactating cows were evaluated during the cold and hot seasons for internal, surface, and lacrimal caruncle temperature and the sweating rate. In the cold season, the body temperature (n=9/group) did not differ between the groups, but the average superficial temperature (n=13/group) was lower in the red group (BW: 30.9±0.3 ºC; RW: 29.6±0.3ºC; p=0.02). In the hot season, under mild to moderate stress, the mean body temperature (n=9/group) of RW cows was lower (BW: 38.75±0.01 ºC; RW: 38.62±0.1 ºC; p=<0 .0001) whereas no significant difference was observed on superficial temperature (n=17/group). The maximum internal temperature and sweating rate (n=11/group), measured in the hot season, did not differ, as well as the number of evaluations in hyperthermia and lacrimal caruncle temperature in both seasons. Therefore, under mild to moderate stress conditions, the results indicate differences in thermoregulation between phenotypes. However, considering that only discrete differences were observed, it is unlikely that the red and white coat benefits cow’s welfare under mild to moderate thermal stress.

Keywords: coat color, internal temperature, pasture systems, surface temperature, thermal stress, thermoregulation

1. Introduction

With the worsening of climatic conditions and negative impacts of heat stress on dairy farming, in terms of production, reproduction and health (reviewed by Bagath et al., 2019; Roth, 2021; Tao et al., 2020), there is a growing need to seek alternatives to mitigate heat stress. This is particularly relevant to pasture-based systems in which animals are exposed to direct solar radiation (Da Silva et al., 2010). An inherent characteristic of the animal, the color of the coat, affects the absorbance of solar radiation, which is 90% for black spots, 70% for red spots and 40% for white spots (Da Silva et al., 2003; Stewart, 1953). Thus, it would be more interesting to breed animals with white fur; however, as in the Holstein breed the hair color follows the skin color, the epidermis is depigmented (Walsberg, 1988). Under direct sunlight exposure, the lack of pigmentation in the epidermis can result in tissue damage due to a greater absorbance of UV radiation, predisposing to photosensitization (Hussain et al., 2018). Thus, for extensive systems, in theory, the ideal would be to use animals with red coats, because they have intermediate characteristics: they absorb less radiation while the epidermis is protected.

Studies comparing the thermoregulatory capacity and performance of Holstein cattle with different coat colors under heat stress are limited. The vast majority segment the groups according to the percentage of black spots in relation to white spots. In general, mostly black cows have a higher internal temperature (IT) and surface temperature (ST) when compared to mostly white cows (Anzures-Olvera et al., 2019; Da Silva and Maia, 2011; Hillman et al., 2001). Recently, our group compared red and white (RW) Holstein cows with black and white (BW) and observed lower ST of pigmented and non-pigmented spots and lower IT of red and white animals (Isola et al., 2020). However, in that study, cutaneous evaporative loss was not considered. In addition, the IT was measured in only two moments in each season, which may not be representative of the real condition of the animals.

Based on the above mentioned, there are indications that the color of the coat can influence how the animals are affected by thermal stress. Therefore, the study was developed with the objective of comparing the thermoregulatory capacity of Holstein cows with RW or BW coats raised in a semi-extensive system, under conditions of thermoneutrality and thermal stress. The hypothesis is that RW cows are more thermotolerant than BW cows.

2. Materials and methods

The Institutional Committee for Ethics in Animal Experiments at the Federal University of Pelotas (Protocol 020479/2021-91) approved all procedures.

2.1 Location and experimental design

The experiment was carried out on a commercial farm located in southern Brazil (30.983727639875234, 55.41404559838045), where the Köppen-Geiger climate classification is subtropical humid. Holstein lactating cows were evaluated for three days under two environmental conditions: cold season (CS, n = 26; August 2021) and hot season (HS, n = 34; February 2022). In each season, the animals were equally allocated into two groups: RW, cows with red and white coats; and BW, cows with black and white coats. To reduce bias, a BW cow was chosen to match each RW cow, by having similar pigmented spots percentage, parity, days in milking (DIM), milk production, weight, body condition score (BCS: scale from 1 to 5, being 1 very thin and 5 very fat) and reproductive status (inseminated, first/second/third third of pregnancy) (Table 1). No significant differences were observed between groups within each season. The percentage of spots was measured by an adaptation of the method proposed by Becerril et al., 1994 using photos of the left side of the animals, in which a rectangle was delimited with vertical lines drawn from the insertion of the tail and the dorsal edge of the scapula towards the ground and along the dorsal and ventral lines of the animal. The percentage of pigmented spot was determined by ImageJ software by dividing the pigmented area by the total area of the rectangle.

2.2 Animal handling

The animals were reared in a semi-extensive system, where they remained on pasture (oats/ryegrass in CS; Tifton in HS) most of the time, except during milking (two daily milkings: 4am and 4pm in CS; 5am and 5pm in the HS). After each milking, they received a ration in total mixture in the CS composed of 7.5 kg of corn silage, 3.2 kg of wet corn grain silage, 1.5 kg of soybean meal, and 300 mg of mineral salt with buffer; and in the HS: 8kg of corn silage, 4 kg of 18% protein concentrate and 100mg of mineral salt. For primiparous, recently calved, high-yielding cows and/or receiving recombinant bovine somatotropin, 1 kg of wet corn grain silage or commercial concentrate was supplemented. The animals had free access to the rest paddock, located next to the milking parlor, with natural shading and drinking fountains and which was connected to all the pasture paddocks.

2.3 Data collection

Variables studied included environmental conditions, IT, ST, lacrimal caruncle temperature, and sweating rate.

Environmental conditions

Within each season, three consecutive sunny days representative of the season were selected. Environmental conditions were recorded every hour by a meteorological station (National Institute of Meteorology - INMET) located 24.85 km in a straight line from the farm. The Temperature and Humidity Index (Thom, 1958 adapted by NOAA, 1976) was calculated by the formula: $THI = (1.8 \times T + 32) - (0.55 - 0.0055 \times RH) \times [(1.8 \times T + 32) - 58)]$; where T is the temperature in degrees Celsius and RH the relative humidity given in percentage. The animals were in an environment free of heat stress when $THI < 68$, mild stress for THI between 68 and 72, moderate stress when $72 > THI \leq 80$, and severe stress when THI exceeded 80 (Zimbelman et al., 2011 adapted by Armstrong, 1994).

Internal temperature (IT)

On the day before the beginning of evaluations, 18 cows (RW: n=9; BW: n=9), which were not in the final third of pregnancy (in order to avoid negative effects on pregnancy), received an intravaginal thermometer (Ibutton®, Thermochron, Whitewater, USA) fixed in a new intravaginal progesterone device (1g, Primer, Tecnopec) (Suthar et al., 2013). The thermometer was configured in the Thermo Data Viewer software (version 3.2.6) to record the IT every 30 min. Data were recovered after the collection period by the same software. Temperature above 39.2°C indicated hyperthermia (West, 2003). The experiment was performed in CS and HS.

Surface temperature (ST) and lacrimal caruncle temperature

Thermographic images were recorded in all animals (CS: n=26; HS: n=34) on the three days, starting at 2 pm, after the animals remained in the shade for two hours. Thermography was performed in the waiting room of the milking parlor, on the right side of the animals, alternating between the two groups. The cows were kept free and sheltered from the sun and wind. Body surface thermography was performed 4 m away from the animal (Figure 1) with Testo 870-1 Termovisor, which has the following characteristics: infrared resolution: 320 × 240 pixels; infrared spectral interval: 7.5–14 μm; accuracy: ± 2 ºC; thermal sensitivity: 100 mK at +30 ºC; fixed focus 34º lens; and refresh rate: 9 Hz. The color pallet used to analyze the images was the one entitled "cold-hot" and the value of emissivity of 0.98 was used for the processing of the images, as previously described by Isola et al. (2020). The Testo IRSoft 4.7 software as used for analysis of the surface temperature, considering the dry bulb temperature and relative humidity at the moment when each picture was taken. A rectangle was delimited with vertical lines drawn from the caudal angle of the scapula and the coxal tuberosity; and horizontal lines drawn at the height of the transverse processes and distal epiphysis of the femur (Figure 1). Afterwards, the histogram tool was chosen to obtain the average ST, minimum ST and maximum ST. To measure the temperature of the lacrimal caruncle, the thermographic image was captured under the same conditions mentioned above, but at 2 m away from the corresponding anatomical location (near the ocular medial commissure).

Sweating rate

In the HS, after the afternoon milking (5 pm), in 22 animals (RW: n=11; BW: n=11), the sweating rate was evaluated through the Berman (1957) method, modified by Schleger and Turner (1965). Trichotomy was performed in the pigmented region close to the dorsal margin of the scapula on the day before the start of the experiment, together with the placement of vaginal implants. To obtain the sweating rate, three discs of filter paper impregnated with 10% cobalt chloride on adhesive tape were fixed on the previously cleaned, trichotomized region. Afterwards, the time required for the color change of the three discs from blue violet (dry) to light pink (wet) was measured. The mean time, in seconds, when applied to the equation 22 * 3600 / 2.06 * t, provided the sweating rate expressed in $g/m^2/h$.

2.4 Statistical analysis

The effect of THI on IT was analyzed by covariance model using as fixed effects group, THI, interaction of the group with THI and the effect of the animal within each group. The effects of the distinct factors on the other continuous variables were analyzed by analysis of variance followed by the Tukey-HSD post hoc test. The effect of THI on the occurrence of hyperthermia (>39.2°C) was determined by logistic regression. The ROC curve (receiver operating characteristic curve) was used to determine the THI in which the probability of cows being in hyperthermia increases. Sweating data over time were analyzed by mixed linear models for repeated data, including in the model the fixed effects of group, percentage of spots, day, production as well as the interactions of the group with the percentage of spots and group with the day. Analyzes were performed using the JMP software (JMP Statistical Discovery LLC), using a significance level of P<0.05.

3. Results

In the CS (Fig. 2), weather conditions remained within the no-stress classification (THI less than 68) throughout the period. In the HS, during hot hours (9 am to 7 pm), on day 1 and day 2 stress was mild to moderate (THI: 68 to 76) and on day 3 stress was moderate to severe (THI: 72 to 80); while in the coldest hours, it remained in the no-stress.

The mean IT (Fig. 3a) in CS did not differ between groups (BW: 38.62±0.01ºC; RW: 38.62±0.01ºC; p=0.6). In HS, the RW group had a lower mean IT (BW: 38.75±0.01 ºC; RW: 38.62±0.1 ºC; p=<0.0001) and therefore, the evaluation was segmented into quartiles (Fig. 3b). Despite the observed effect of group and THI (p<0.001), there was no interaction (p=0.62). The maximum IT in the HS did not differ between the days of the experiment (Table 2). There was no difference between the groups in the number of evaluations in hyperthermia (>39.2 ºC) in the CS (BW: 7.5±2.2; RW: 8.7±2.2; p=0.7) and in the HS (BW: 30.1±7.3; RW: 21.3±6.3; p=0.37) (Fig. 3c). Through analysis of the ROC curve, it was observed that with THI above 70.2, the probability of cows being in hyperthermia increases (data not shown). Still on the HS, there was no difference in the sweating rate (Fig. 3d) between the groups over the days.

ST was higher in the HS and did not differ between the groups in the same season (Fig. 4). In the CS, RW animals showed a lower average ST (BW: 30.9±0.3 ºC; RW: 29.6±0.3ºC; p=0.02) and maximum temperature (BW: 32.0±0.3ºC; RW: 30.9±0.3ºC; p=0.05) and did not differ from the BW group in minimum surface temperature (BW: 28.7±0.4ºC; RW: 27.6±0.4ºC; p=0.06). No significant difference was observed in the temperature of the lacrimal caruncle (Fig. 4).

4. Discussion

This study evaluates some thermotolerance characteristics of RW and BW Holstein cows in both heat stress and thermoneutral environments. The hypothesis was that RW Holstein cows are more thermotolerant than BW Holstein cows. In the thermoneutral environment, in the absence of stress, there was no difference in internal temperature, but the mean ST of RW cows was lower. However, in the heat stress environment, under mild to moderate stress, the internal temperature was lower in the RW group, with no difference in ST.

The climatic condition is widely evaluated using the THI, which considers air temperature and relative humidity (NOAA, 1976). Over time, the equations have been optimized for different production systems, such as pasture-based systems, where additional variables that influence thermal exchange, such as radiation and wind speed, have been included (reviewed by Herbut et al., 2018). Although it may not be the most suitable methodology, the THI used in this study proved to be a viable alternative, as it was identified that starting from THI 70 (the beginning of mild stress), the probability of hyperthermia increased in the herd, following the classification proposed by Zimbelman et al. (2011). In a subtropical climate with high-producing cows, Pinto et al. (2020) also observed changes in rectal temperature when the THI reached the threshold of 70. However, changes in respiratory rate were observed earlier at THI 65 if the animals were lying down, increasing to 70 when they were standing. These variations demonstrate that the THI is a useful tool, but it is essential to consider the relationship with the physiological and individual responses of each herd.

The imbalance between the heat produced by the animal and received from the environment, and the capacity for heat dissipation, results in the accumulation of heat reflected in an increase in IT (Berman and Morag, 1971). This fact can be observed in our results as the average IT during winter did not differ between groups, whereas in summer, in the first quartile (nighttime THI) with the same THI as in the thermoneutral environment, the average IT of the BW group was higher. Therefore, RW cows absorbed a lower amount of heat or dissipated it more efficiently, given that with equalized groups, heat production would be similar. The lower nighttime temperature allows for the dissipation of accumulated heat during the day, contributing to the stabilization of IT, which supports this proposition (Garcia et al., 2015).

In the CS, there was no difference in IT between groups, and most of the time, the animals remained in the thermoneutral range (38.0°C to 39.2°C; Cunningham and Klein, 2009) due to the absence of stress (THI < 68), with the air temperature within the thermoneutral zone (-5°C to 21°C; Johnson, 1986). In the HS, there was a slight overall lower mean IT for RW cows, which remained more pronounced when THI was divided into quartiles. The means for both groups remained within the physiological limits, with the BW group closer to the threshold for hyperthermia in the fourth quartile. IT above 39.2°C is the main indicator of heat stress (West, 2003). Measurement through intravaginal thermometers requires minimal animal manipulation and does not disturb their natural behavior (Kaufman et al., 2018). However, the results cannot be obtained at any time. Therefore, other approaches have been sought to predict IT, such as the caruncular ocular temperature. The method shows moderate correlations with IT, heart rate, and respiratory rate of cows under heat stress (Daltro et al., 2017). Nevertheless, in the present study, there was no difference between groups and seasons on IT. Furthermore, the number of observations in hyperthermia was higher in the HS, but no significant difference was observed between groups. In this scenario, thermoregulatory mechanisms such as increased sweating and peripheral circulation activation (inferred by ST) occur.

Sweating is the primary mechanism for heat dissipation when there is a decrease in the thermal gradient between the animal and the environment, and there is a positive correlation between heat loss through evaporation and air temperature (Maia et al., 2005). When the air temperature ranges from 27°C to 30°C, heat dissipation through sweating accounts for 85% to 88% (Maia et al., 2005; Santos et al., 2016). Considering that such conditions are observed in summer, sweat rate measurement was only conducted in the HS and did not differ between groups over the three days of evaluation, even under severe THI.

The colorimetric test of Schleger and Turner (1965) is low-cost and easy to prepare. However, it requires the animals to be restrained for a relatively long period, which further limits the number of samples, considering the need to minimize interference with the animals' routine. BW Holstein cows or those crossbred with Gir, predominantly black, have a higher sweating rate and ST than white cows (Da Silva and Maia, 2011; Santos et al., 2016). The greater heating of black spots is compensated by greater heat loss through cutaneous evaporation (Da Silva and Maia, 2011; Santos et al., 2016), which is why the analysis was added to the experiment.

The results from the precursor study by Isola et al., (2020) were replicated in the present study. IT did not differ in the thermoneutral environment (THI 59) and was lower in RW group in the heat stress environment by 0.3°C (THI 79). Although the IT of both groups remained within the physiological range, the BW group approached the threshold for hyperthermia. Additionally, the ST of pigmented spots was lower in the RW group in the thermoneutral environment and tended to be lower in the heat stress environment, although it did not differ significantly in the present study. In addition to the lower absorbance of red spots, the opposite behavior of IT and ST between seasons may indicate a difference between the groups in heat transfer from the central body core to the periphery. Dikmen et al., (2008) observed that the sweating rate of animals carrying the slick gene is higher than that of non-carrier animals, suggesting that the ST of carrier animals would be lower if heat transfer were similar. However, ST did not differ between groups, highlighting the difference in heat transfer.

In addition to coat color, other characteristics of the coat influence thermal conductivity, such as length, thickness, hair density, and skin thickness. The interaction of these characteristics favors or hinders the flow of heat from the skin to the coat's surface and the elimination of latent heat through cutaneous evaporation (Alfonzo et al., 2016; Bertipaglia et al., 2005). In depigmented skin, there is greater penetration of solar radiation and, therefore, a higher hair density and length provide protection. In pigmented skin, melanin in the hair and skin enhances radiation absorption, leading to shorter, thicker, and more numerous hairs to decrease resistance to thermolysis (Lee et al., 2016; Maia et al., 2003). In the present study, such characteristics were not evaluated, which could have provided more insights about whether red coats have differences in their composition that influence heat dissipation capacity.

Since the 1990s, the performance and behavior of Holstein cows of different coat colors under heat stress have been studied, usually comparing predominantly white or black animals. More recently, a mutation that originated the Larson Blue coat color (gray and white) has been identified (Dikmen et al., 2017), which can be obtained from deletions in the pre-melanosomal protein 17 gene (Laible et al., 2021). In these animals, Dikmen et al. (2017) observed that coat color did not affect IT and production levels. However, as the animals were kept in confinement with sprinklers and fans, the authors attribute the absence of differences to this fact and suggest that they may be more thermotolerant when exposed to direct solar radiation.

5. Conclusions

Collectively, the results do not support the hypothesis that the intermediate coat color is more thermotolerant under mild to moderate thermal challenge. Although some statistically significant differences were detected, they may not be physiologically relevant, considering that the number of observations in hyperthermy did not differ between groups. Further evaluations are needed under severe environmental conditions.

Abbreviations

BCS – Body Condition Score
BW – Black and White
CS – Cold Season
DIM – Days in milking
HS – Hot Season
IT – Internal Temperature
RW – Red and White
ST – Surface Temperature
THI – Temperature and Humidity Index

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CRediT authorship contribution statement

J. Lazzari: conceptualization, methodology, investigation, writing - original draft, writing - review and editing and project administration. **J.V. V. Isola:** conceptualization, methodology, investigation, resources, writing - original draft, writing - review and editing, supervision and project administration. **V. L. Szambelan:** investigation and resources. **G. Menegazzi:** methodology. **M. Busanello:** methodology and formal analysis. **M. T. Rovani:** formal analysis. **J. Sarubbi:** methodology, resources and supervision. **E. Schmitt:** methodology and resources. **R. Ferreira:** formal analysis. **P. B. D. Gonçalves** - project administration and funding acquisition. **B. G. Gasperin:**

conceptualization, writing - original draft, writing - review and editing, supervision, project administration and funding acquisition. **R. G. Mondadori:** conceptualization, methodology, writing - original draft, writing - review and editing, methodology, supervision and funding acquisition.

Conflict of Interest Statement

The authors declare no conflicts of interest.

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Figure captions

Fig. 1. Thermographic image for measuring surface temperature (left) and lacrimal caruncle temperature (right).

Fig. 2. Climatic conditions evaluated by the Temperature and Humidity Index (THI) in the three days of the experiment, in the cold and hot season, obtained from the air temperature and relative humidity data from the meteorological station located approximately 25km from the study site. The red line indicates the threshold of no thermal stress (THI = 68).

Fig. 3. A) Mean internal temperature obtained from intravaginal thermometers of the Red-White (RW) and Black-White (BW) groups in the cold and hot season; B) Mean internal temperature of the RW and BW groups in the hot season according to the THI arranged in quartiles (1st quartile: 52.00-60.70; 2nd quartile: 60.71-68.00; 3rd quartile: 68.01-72.50; 4th quartile: 72.51-80.50); C) Hours of hyperthermia (internal temperature greater than 39.2°C) of the RW and BW groups in the cold and hot season; D) Sweating rate of the RW and BW groups in the hot season. * $p=<0.0001$

Fig. 4. (A) Maximum, (B) average and (C) minimum surface temperature (ST) and (D) lacrimal caruncle temperature of the Red-White (RW) and Black-White (BW) groups in the cold and hot season. The ST was obtained from histograms of thermographic images of the right side of cows and the temperature of the lacrimal caruncle from the fixation of a point in the anatomical place. * $p \leq 0.05$.

Table 1: Mean ± SE of pigmented spots percentage, days in milking (DIM), milk production, weight, body condition score (BCS) of red-and-white (RW) and black-and-white (BW) Holstein cows in the cold and hot season.

		Pigmented spots (%)	**DIM (days)**	**Production (L)**	**Weight (kg)**	**BCS**
Season	**Group**	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE	Median (Min-Max)
Cold	BW	65.5 ± 5.4	247.5 ± 29.1	27.8 ± 2.6	568.5 ± 24.2	3.5 (3 – 4)
	RW	52.5 ± 6.6	244.6 ± 30.8	25.3 ± 2.1	561 ± 16	3.5 (3– 4.5)
Hot	BW	62.6 ± 5.3	160 ± 23.8	23.4 ± 1.3	554.9 ± 25.9	3 (3 – 4)
	RW	49.9 ± 5.7	179.5 ± 22.9	24.3 ± 1.6	560.6 ± 21.2	3.5 (3– 3.5)

No significant differences were observed between groups within each season.

Table 2: Maximum internal temperature (Mean±SE) of Black-White and Red-White Holstein cows in the hot season for three days.

Day	Black and White (°C)	Red and White(°C)	P value
1	39.3±0.08	39.1±0.07	0.15
2	39.4±0.10	39.3±0.08	0.2
3	39.5±0.09	39.4±0.08	0.6

Figure 1

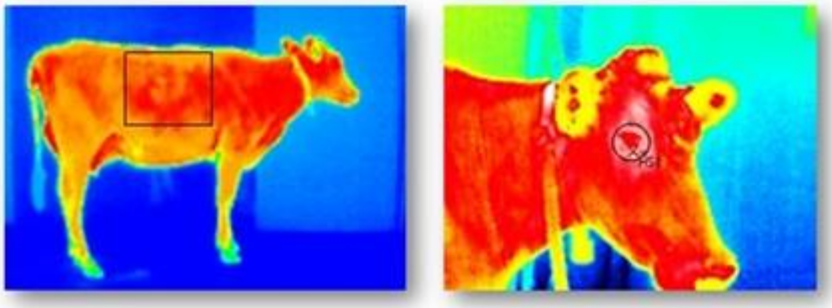


Figure 2

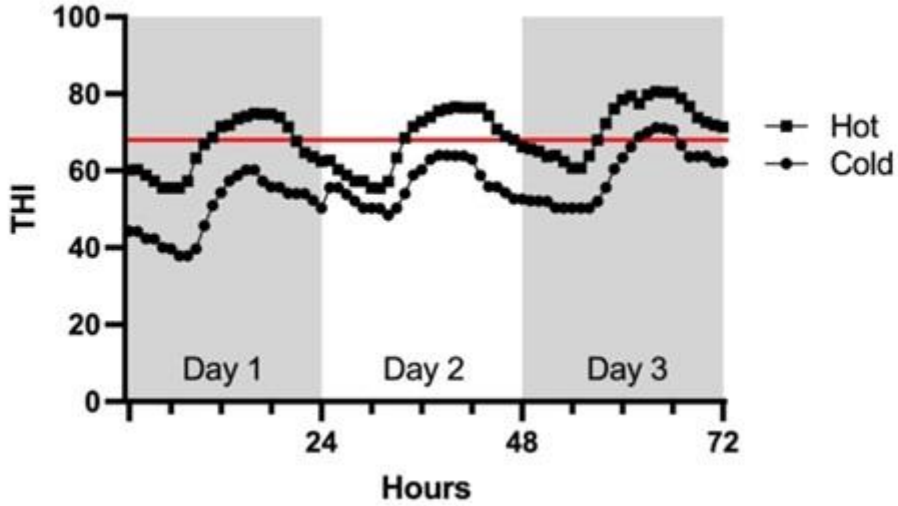


Figure 3

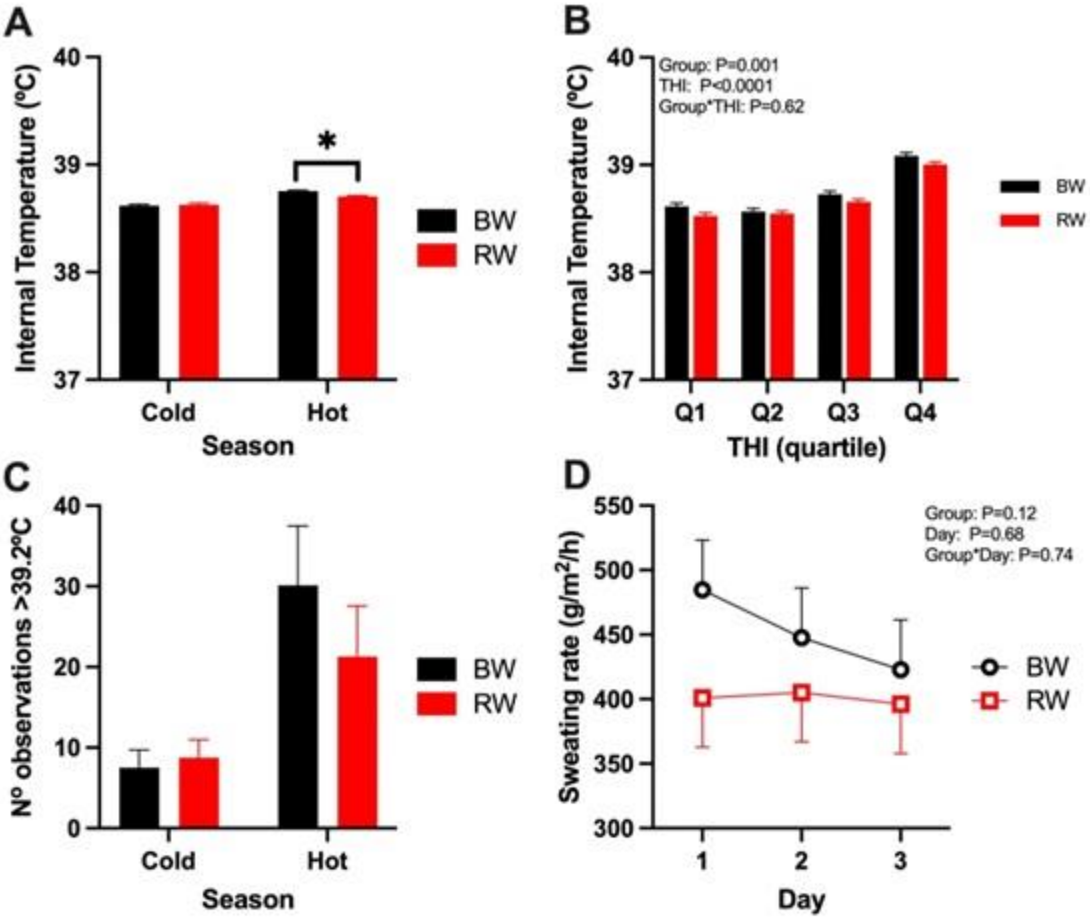
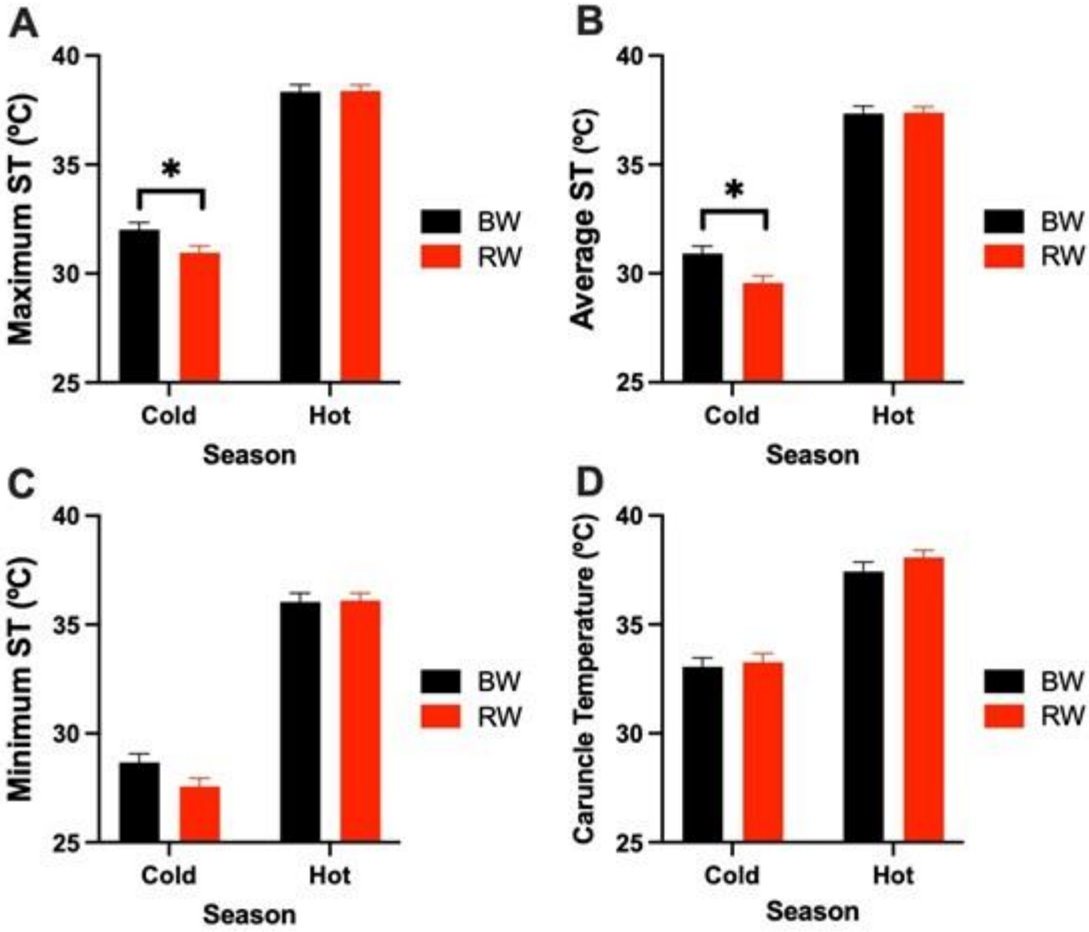

A
Internal Temperature (ºC)
40
39
38
37
*
Cold
Hot
Season
BW
RW
B
Group: P=0.001
THI: P<0.0001
Group*THI: P=0.62
Internal Temperature (ºC)
Q1
Q2
Q3
Q4
THI (quartile)
BW
RW
C
Nº observations >39.2ºC
40
30
20
10
0
Cold
Hot
Season
BW
RW
D
Group: P=0.12
Day: P=0.68
Group*Day: P=0.74
Sweating rate (g/m2/h)
550
500
450
400
350
300
1
2
3
Day
BW
RW

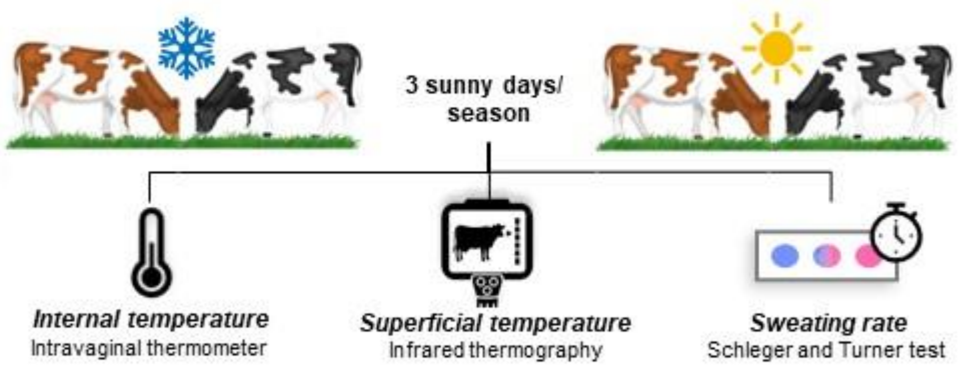
Figure 4


A
40
35
30
25
Maximum ST (ºC)
*
Cold
Hot
Season
BW
RW
B
Average ST (ºC)
C
Minimum ST (ºC)
D
Caruncle Temperature (ºC)

Highlights

- Two Holstein phenotypes (red and black) were evaluated in the hot and cold seasons.
- The body temperature of the red phenotype was lower in the hot season.
- The surface temperature of the black phenotype was higher in the cold season.
- Sweating rate in the hot season did not differ between phenotypes.

Graphical abstract



4 Considerações finais

A seleção de animais com revestimento corporal que favoreça a dissipação e reduza a absorção de energia térmica é uma alternativa para sistemas de criação cujo ambiente seja desfavorável nos períodos quentes. Vacas Holandês de pelagem vermelha e branca demonstraram discretamente diferença na capacidade de dissipação de calor, através da inversão do comportamento das variáveis entre as estações. Durante a estação fria, em ambiente de termoneutralidade, não houve diferença na temperatura interna, mas a temperatura superficial média de vacas vermelhas e brancas foi inferior. Durante a estação quente, em estresse leve a moderado, a temperatura interna foi inferior no grupo vermelho e branco, sem diferir na temperatura superficial.

O clima subtropical, caracterizado por temperaturas ambientais mais amenas, se comparado ao clima tropical, e maior gradiente de temperatura entre o dia e a noite, favorece a dissipação do calor acumulado e estabilização da temperatura interna à noite. Ainda assim, como as projeções indicam piora das condições climáticas, os animais seguem reféns nesses ambientes. Mesmo que estatisticamente significantes, em condições de estresse leve e moderado, as alterações encontradas não exprimem claramente que animais de pelagem intermediária são mais tolerantes ao calor, hipótese que deve ser testada em condições mais severas.

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COMEX STAT. **COMEXVIS - Leite**.

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Anexo

Anexo I - Documento da Comissão de Ética e Experimentação Animal

PARECER Nº 134/2021/CEUA/REITORIA
PROCESSO Nº 23110.020479/2021-91

Certificado

Certificamos que a proposta intitulada **"Comparação de parâmetros relacionados ao estresse térmico entre vacas de pelagem preta ou vermelha das raças Holandês e Angus"**, registrada com o n° 23110.020479/2021-91**,** sob a responsabilidade de **Rafael Gianella Mondadori -** que envolve a produção, manutenção ou utilização de animais pertencentes ao filo Chordata, subfilo Vertebrata (exceto humanos), para fins de pesquisa científica (ou ensino) – encontra-se de acordo com os preceitos da Lei nº 11.794, de 8 de outubro de 2008, do Decreto nº 6.899, de 15 de julho de 2009, e com as normas editadas pelo Conselho Nacional de Controle de Experimentação Animal (CONCEA), e recebeu parecer **FAVORÁVEL** a sua execução pela Comissão de Ética no Uso de Animais, em reunião de 11 de novembro de 2021.

Finalidade	(x) Pesquisa ( ) Ensino
Vigência da autorização	Início = **01/12/2021** Término = **01/12/2024**
Espécie/linhagem/raça	*Bos taurus* / Holandês e Angus
N° de animais	**26 Holandês e 316 Angus**
Idade	2 a 10 anos
Sexo	Fêmeas
Origem	Fazendas localizadas no Rio Grande do Sul

Código para cadastro nº **CEUA 020479/2021-91**

Priscila Marques Moura de Leon

Coordenadora da CEUA

Documento assinado eletronicamente por **PRISCILA MARQUES MOURA DE LEON**, **Professor do Magistério Superior/Adjunto**, em 11/11/2021, às 16:11, conforme horário oficial de Brasília, com fundamento no art. 4º, § 3º, do Decreto nº 10.543, de 13 de novembro de 2020.

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