UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL
FACULDADE DE AGRONOMIA
PROGRAMA DE PÓS-GRADUAÇÃO EM ZOOTECNIA

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INCLUSÃO DE VARIÁVEIS BIOCLIMATOLÓGICAS NA AVALIAÇÃO GENÉTICA DE BOVINOS LEITEIROS: ABORDAGEM DO ESTRESSE TÉRMICO NA SELEÇÃO ANIMAL

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"A tarefa não é tanto ver aquilo que ninguém viu, mas pensar o que ninguém ainda pensou sobre aquilo que todo mundo vê."

Arthur Schopenhauer

INCLUSÃO DE VARIÁVEIS BIOCLIMATOLÓGICAS NA AVALIAÇÃO GENÉTICA DE BOVINOS LEITEIROS: ABORDAGEM DO ESTRESSE TÉRMICO NA SELEÇÃO ANIMAL[1]

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RESUMO

Em todo o mundo, principalmente em regiões de clima tropical, o estresse térmico causa grandes perdas econômicas e tem importantes implicações no bem-estar animal. Para mitigar os efeitos do estresse térmico, modificações estruturais do ambiente têm sido adotadas com frequência. No entanto, estas mudanças não são herdáveis e requer alto potencial de investimento. Nesse contexto, para superar os desafios provocados pelo estresse térmico, a seleção genética para melhorar a termotolerância é a estratégia de mitigação mais viável. Assim, os principais objetivos deste estudo foram identificar o limite crítico do estresse térmico para características de produção e saúde em bovinos da raça Holandesa, investigar abordagens para ajustar dados fenotípicos para indicadores de estresse térmico, além de quantificar o impacto na reclassificação de touros e na confiabilidade das predições, utilizando dados bioclimatológicos como o índice de temperatura e umidade (THI) e a amplitude térmica (DTV) via modelos de regressão aleatória. O limiar do conforto térmico identificado foi de THI = 74 e DTV = 13 para produção de leite no dia do controle (TDMY), THI = 70 e DTV = 9 para escore de células somáticas (SCS) e THI = 74 e DTV = 16 para rendimento de gordura (FAT) e proteína (PROT). As médias das variáveis bioclimatológicas de dois dias anteriores ao controle leiteiro são indicadas para correção dos dados. Houve significativa reclassificação dos touros e aumento na confiabilidade das predições dos valores genéticos quando THI e DTV forma considerados nos modelos. Ao incluir de forma pioneira o nível de estresse térmico na modelagem da regressão fixa do modelo, observaram-se melhorias ainda mais expressivas no processo de avaliação genética. Contudo, há variabilidade genética suficiente para realizar seleção concomitante para aumento da eficiência produtiva e termotolerância nos animais. Adicionalmente, foi possível inferir que o SCS pode ser considerado um indicador precoce de estresse térmico nos animais. Em síntese, conclui-se que não corrigir os dados fenotípicos para os indicadores de estresse térmico no processo a avaliação genética resulta em predições viesadas, em que os animais apontados como detentores dos melhores méritos genéticos não são verdadeiramente os melhores. Sendo assim, recomendamos adoção do THI e DTV nos procedimentos de seleção animal, não somente visando melhorias futuras nos aspectos produtivos/saúde, mas como forma de melhorar o bem-estar animal frente a mudanças climáticas.

Palavras chave: bem–estar animal; dados longitudinais; interação genótipo–ambiente; regressão aleatória; termotolerância

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INCLUSION OF BIOCLIMATOLOGICAL VARIABLES IN GENETIC EVALUATION OF DAIRY CATTLE: APPROACH TO HEAT STRESS IN ANIMAL SELECTION[2]

Author: Renata Negri
Adviser: Jaime Araújo Cobuci

ABSTRACT:
Throughout the world, especially in tropical regions, heat stress is seen as a source of major economic losses and has important implications on animal welfare. To mitigate the heat stress effects, structural changes in the environment have been frequently adopted. However, these changes are not inheritable and require high investment potential. In this context, to overcome the challenges caused by heat stress, the genetic selection to improve thermotolerance is the most viable mitigation strategy. Therefore, the main objectives of this study were to identify the critical threshold of heat stress for production and health traits in Holstein cattle, investigate approaches to adjust phenotypic data for heat stress indicators, and quantify the impact on the reranking of sires and on the reliability of predictions, using bioclimatological data such as temperature and humidity index (THI) and diurnal variation temperature (DTV) via random regression models. The heat comfort threshold identified was THI = 74 and DTV = 13 for test-day milk yield (TDMY), THI = 70 and DTV = 9 for somatic cell score (SCS), and THI = 74 and DTV = 16 for fat (FAT) and protein (PROT) yield. The averages of bioclimatological variables of two days before the test-day are indicated to correct the data. There was a significant sires reranking and an increase in the reliability of predictions of breeding values when THI and DTV were included in the models. By including, in a pioneering way, the heat stress level in the fixed regression of the model, there were even more significant improvements in the genetic evaluation process. However, there is sufficient genetic variability to perform concomitant selection for an increase production efficiency and thermotolerance in animals. Additionally, it was possible to infer that SCS can be considered an early indicator of heat stress in animals. In summary, we concluded that by not correcting phenotypic data for heat stress indicators in the genetic evaluation process results in biased predictions, in which the animals identified as having the best genetic merits are not truly the best. Thus, we recommend the adoption of THI and DTV in selection procedures, not only future improvements in the productive/health aspects, but as a way to improve animal welfare in face of climate change.

Keywords: animal welfare; longitudinal data; genotype–by–environment interaction; random regression; thermotolerance

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[2] Doctoral thesis in Animal Science, Faculdade de Agronomia, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brazil. (166 p.) March, 2021.

SUMÁRIO

LISTA DE TABELAS

CAPÍTULO II

CAPÍTULO III

CAPÍTULO IV

CAPÍTULO V

LISTA DE FIGURAS

CAPÍTULO IV

CAPÍTULO V

LISTA DE ABREVIATURAS E SIGLAS

G×E	Interação genótipo ambiente
THI	Índice de temperatura e umidade/ *temperature-humidity index*
NRC	*National Research Council*
DTV	Amplitude térmica/ *diurnal temperature variation*
RRM	Modelo de regressão aleatória/ *random regression models*
DIM	Dias em lactação/ *days in milk*
SM	*Standard model*
–2logL	*Log-likelihood function*
AIC	*Akaike's information criterion*
BIC	*Schwarz's Bayesian information criterion*
EBV	*Estimated breeding value*
TD	*Test-day*
DTB	*Dry bulb temperature*
RH	*Relative humidity*
0DB	*On TD record*
1DB	*One day before TD*
2DB	*Two days before TD*
3DB	*Three days before TD*
2DM	*Mean between the two last days before each TD (1DB and 2DB)*
M1	*Model 1*
M2	*Model 2*
M3	*Model 3*
M4	*Model 4*
M5	*Model 5*
M6	*Model 6*
M7	*Model 7*
M8	*Model 8*
HYM	*Herd-year-month of the test*
TDMY	*Test-day milk yield*
HS	*Heat stress*

CG	*Contemporary group*
SD	*Standard deviation*
WL	*Wilmink parametric function*
LS	*Linear splines*
LP	*Legendre orthogonal polynomial*
REML	*Restricted maximum likelihood*
EBV_TDMY	*Estimated breeding value for test-day milk yield*
EBV_HS	*Estimated breeding value for heat stress*
SCS	*Somatic cell score*
SCC	*Somatic cell count*
FAT	*Fat yield*
PROT	*Protein yield*

CAPÍTULO I

1. Introdução

Independente do sistema de produção da pecuária leiteira, as mudanças climáticas bruscas causam prejuízos à cadeia produtiva (Misztal, 2017). Tradicionalmente, os programas de melhoramento genético de bovinos de leite se concentram na seleção para a produção de leite, desconsiderando a sensibilidade ambiental. Dessa forma, com a seleção intensa para produção, houve um comprometimento da termotolerância dos animais quanto às variações ambientais (correlação genética -0,30, Ravagnolo & Misztal, 2000).

Nos últimos anos, o estresse térmico passou a ser considerado um problema caro para a pecuária leiteira. Foram estimadas perdas de 5,5 kg de leite/dia e em torno de 400 euros/vaca/ano em função do estresse térmico (Pyron & Malynyn, 2015). Além das perdas econômicas relacionadas à produtividade, a exposição à fatores estressores incidem em impactos negativos na saúde e bem-estar animal (Polsky & Von Keyserlingk, 2017).

Todavia, os fatores bioclimáticos que são potenciais indicadores de conforto e estresse térmico, não são rotineiramente considerados nas avaliações genéticas. Adicionalmente pode-se ressaltar que os ambientes futuros podem não ser bem representados pelos ambientes atuais, comprometendo a médio e longo prazo, os ganhos em seleção (cumulativa) a qual vem priorizando a produção de leite e sólidos. Mesmo diante da importância econômica da pecuária leiteira e apesar da miríade de pesquisas na área, existe uma lacuna em relação ao entendimento da interação genótipo-ambiente e os efeitos do estresse térmico para seleção de reprodutores (Rekwotet al., 2016), visando o equilíbrio entre produção e termotolerância nas avaliações genéticas nacionais.

Nessa acepção, Bohmanova et al. (2008a) enfatizaram que as variáveis bioclimáticas desempenham papel expressivo na definição de diferentes ambientes produtivos, não devendo o pesquisador, negligenciar a interação genótipo-ambiente. Sendo assim, se faz necessária a investigação de métodos e metodologias para compreender em âmbito genético os efeitos do estresse térmico e consequentemente melhorar os mecanismos de avaliação, a fim de permitir uma seleção ponderada dos animais para termotolerância e características produtivas.

Duas possíveis estratégias são passíveis de aplicação na avaliação genética de bovinos leiteiros: a correção de dados de produção para os indicadores de estresse térmico e/ou a seleção concomitante para produção de leite e termotolerância. Acredita-se que o maior impacto entre o modelo tradicionalmente utilizado e as estratégias propostas está na predição dos valores genéticos e confiabilidades, e consequentemente na alteração da classificação dos animais (*rank*). Sendo assim, a não consideração do efeito do estresse térmico poderia resultar em uma seleção equivocada que comprometeria os ganhos genéticos a longo prazo.

Diante do contexto, serão descritos e discutidos quatro estudos. O primeiro aborda a temática de correção dos dados longitudinais de produção de leite para as variáveis bioclimatológicas indicadoras de estresse térmico. O segundo consiste em caracterizar a sensibilidade ambiental e analisar a possibilidade de seleção para termotolerância e produção de leite. O terceiro refere-se à investigação de um indicador precoce de estresse térmico utilizando informações do escore de células somáticas. E o quarto estudo, aborda a utilização do nível de estresse térmico como uma alternativa à modelagem de regressão fixa para características de produção de gordura e proteína em bovinos da raça Holandesa.

2. Revisão Bibliográfica

2.1 Estresse térmico

O estresse térmico pode ser definido como o somatório de forças externas ao animal homeotérmico, afetando a homeostase, causando impactos negativos no organismo, como o aumento da temperatura corporal (Dikmen & Hansen, 2009) e desencadeando respostas comportamentais, fisiológicas e metabólicas (Kolmodin et al., 2002), que podem levar à redução da produção, comprometimento reprodutivo e, deterioração das condições de vida (saúde e bem estar), em casos extremos, à morte (Mader et al., 2006).

A teoria evolutiva da seleção natural proposta por Darwin defende que os indivíduos mais adaptados têm maiores chances de sobrevivência e por isso deixam maior número de descendentes. Dentro de uma mesma raça, com o passar das gerações, alguns animais podem desenvolver mecanismos adaptativos para aumentar o fluxo de energia líquida e formas de ativação (Polsky & Von Keyserlingk, 2017) como mitigação aos efeitos do estresse térmico. Por isso, é possível observar diferenças na termotolerância não só entre as raças (tamanho, cor da pele, cor do pelame, produção, etc.), mas também dentro da raça. A adaptabilidade pode ser avaliada por meio da habilidade do animal em se ajustar às condições médias ambientais de clima adverso, com perdas mínimas de desempenho e conservando taxa para manutenção reprodutiva, resistência a doenças, longevidade e baixa taxa de mortalidade (Baêta & Souza, 1997).

Fisiologicamente, os mecanismos para evitar o colapso do organismo, ocorrem em uma sequência de eventos, de modo que é o sistema nervoso que desempenha a função de controlar as alterações orgânicas, metabólicas e enzimáticas. A termorregulação ocorre via termorreceptores centrais e periféricos, de condução aferente culminado em respostas compensatórias dos impulsos térmicos (Braz, 2005), e é o hipotálamo o regulador corporal (Collier et al., 2017).

Primeiramente, a vasodilatação seguida da sudorese e aumento da frequência respiratória (Polsky & Von Keyserlingk 2017). Quando tais mecanismos não são suficientes para a termólise, ocorre aumento da temperatura corporal, intensificando os mecanismos de defesa descritos anteriormente (Collier et al., 2017). Na sequência, é observado aumento do consumo de água e ocorre diminuição de outras atividades: redução de ingestão de matéria seca (frequência e volume), alteração do comportamento de pastejo (quando animais a pasto) e diminuição do tempo em ruminação (Collier et al., 2017). Em consequência, é possível observar a diminuição da produção de leite, redução na produção e porcentagem de gordura no leite, redução nos sinais de cio, queda da imunidade e aumento da contagem de células somáticas (Collier et al., 2017).

As perdas decorrentes do estresse térmico causam prejuízos aos animais, produtores e a toda indústria de laticínios. St-Pierre et al. (2003) avaliaram em até 1,5 bilhões de dólares por ano o prejuízo na indústria do leite nos EUA em virtude do estresse térmico. Pyron & Malynyn (2015) estimaram perdas de 5,5 kg de leite por dia, ou seja, em torno de 400 euros/vaca/ano devido ao estresse térmico em países Europeus. Enquanto que Pragna et al. (2017), calcularam perdas de 900 milhões de dólares/ano na indústria de laticínios.

Os fatores ambientais são um dos principais vetores para alterar a condição de equilíbrio do animal, resultando em prejuízos produtivos e econômicos (Ravagnolo & Misztal, 2000), principalmente em vacas de alta produção, que necessitam de maior ingestão de nutrientes, gerando como consequência, maior quantidade de calor metabólico (Pegorer, 2006). Assim, o estresse térmico é um típico problema da bovinocultura leiteira nos trópicos e subtrópicos, principalmente em regiões de clima predominantemente tropical, como é o caso do Brasil.

Além disso, embora as variações climáticas geralmente remetam ao aquecimento, também se refere a maiores flutuações ambientais, mudanças bruscas, na disponibilidade de água e menor qualidade dos alimentos (Tilio Neto, 2010). Ou seja, os ambientes futuros podem não ser bem representados pelos ambientes atuais, demonstrando a importância na investigação de seleção concomitante para produção de leite e termotolerância para a continuidade do progresso genético dos rebanhos.

O problema se agrava quando abordamos o estresse térmico sob o ponto de vista genético. O estresse térmico sob o ponto de vista genético caracteriza uma importante interação entre o genótipo e ambiente em que as informações de porte físico, velocidade da taxa metabólica e nível produtivo têm sido associados à termotolerância (Polsky & Von Keyserlingk, 2017).

Nas últimas seis décadas, a produção animal tornou-se mais eficiente (Misztal, 2017), principalmente para características de produção. Como mencionado anteriormente, animais altamente produtivos tendem a sofrer mais por questões ambientais, consequentemente manifestando reações comportamentais, e variações na produção (Zimbelman et al., 2013), na composição do leite (Gonzalez et al., 2009) e a reprodução (Haile-Mariam et al., 2008).

A importância em desenvolver estudos sobre o efeito do estresse térmico no Brasil se dá pela ampla diversidade climática, devido a grande extensão territorial, com diferenças de relevo, altitude e dinâmica de massas de ar, correntes marítimas, variações longitudinais, latitudinais e altimétricas (Bresolin et al., 2015). De acordo com a classificação climática de Köppen-Geiger, a maior parte da área do Brasil está na Zona Intertropical, ou seja, áreas de baixa latitude, com climas quentes e úmidos (Alvares et al., 2013), além do fator geográfico e da distância da linha do equador.

No contexto produtivo, a produção de leite no Brasil ocorre predominantemente em sistemas de produção caracterizados pelo uso de pastagens, com suplementação de ração concentrada (Meyer & Rodrigues, 2014), os quais podem sofrer alterações conforme estação do ano.

Outro fator agravante, é que entre as raças exploradas no país, o destaque é para o gado da raça Holandesa, a qual desempenha um papel importante na indústria de laticínios, sendo utilizada como raça pura e como a principal raça nos cruzamentos (Santana-Jr et al., 2017). No entanto, por ser uma raça de origem Europeia, a adaptação destes animais é considerado um grande problema para os produtores brasileiros, sendo necessários grandes investimentos na estrutura ambiental dos sistemas de criação, o que não é a realidade da maioria dos produtores (poder de investimento). Tornando este, o principal fator restritivo ao uso de bovinos da raça pura Holandesa em muitos sistemas de produção (Peixoto et al., 2011).

Devido à dimensionalidade continental do Brasil, não é possível afirmar que o material genético de apenas um reprodutor é melhor em todo o país e para todos os sistemas de produção. Até por que, muitos animais podem ser penalizados por suas filhas serem criadas em ambientes mais susceptíveis ao desconforto térmico. Diante disso, é reiterada a importância da inclusão dos indicadores de estresse térmico para correção dos dados ou realização da seleção para termotolerância.

2.2 Avaliação da Termotolerância

2.2.1 Interação genótipo-ambiente

A interação genótipo ambiente (G×E) pode ser definida como uma mudança no desempenho relativo do genótipo em ambientes diferentes (Bowman, 1972). Apesar de sua grande importância na produção animal, a G×E é negligenciada nas avaliações genéticas atuais, os quais assumem homogeneidade das variâncias entre os níveis dos efeitos fixos do modelo estatístico proposto (Reverter et al., 1997).

A importância desse componente de variação foi incialmente destacado por Lush (1945), o qual afirmava que os animais deveriam ser mantidos e avaliados nos locais no qual suas progênies seriam criadas. Posteriormente, Haldane (1946) chamou atenção para que a G×E pode apresentar diversas formas e que deveria corresponder a um componente de variância e que a ordenação dos genótipos em cada ambiente poderia ser utilizada para indicar a presença dessa interação.

Em 1947, Hammond (1947) aventou a possibilidade dos animais serem selecionados nos melhores ambientes, de modo a expressar seu potencial genético máximo. Com um enfoque sistêmico, Falconer (1952) sugeriu que o conjunto epigenético responsável pela expressão de determinada característica podia variar de acordo com o ambiente de criação, pois na otimização da trajetória fisiológica, muitos genes inativos podem ser alterados.

Em 1959, Robertson (1959) sugeriu o uso da correlação genética para relacionar o desempenho em dois ambientes diferentes, abordando não como a mesma característica, mas como características diferentes. Quando a correlação for maior que 0,80 não há interação significativa, ou seja, os genes ativos nos dois ambientes são parcialmente idênticos. Quando a correlação for menor que 0,80 a interação passa a assumir importante papel e as características devem ser consideradas distintas. A equação utilizada por Robertson (1959) segue abaixo:

$$r_g = \frac{\sigma_G^2 - \sigma_{GxE}^2}{\sigma_G^2 + \sigma_{GxE}^2 - 2\sigma_E^2}$$

em que, σ^2_G é a variância genética aditiva; σ^2_{GxE} é a variância da interação genótipo ambiente; e σ^2_E é a variância do erro amostral.

A equação proposta por Robertson foi reformulada por Falconer em 1989:

$$r_g = \frac{\sigma_{H_1H_2}}{\left[(\sigma^2_{S_1} - R_1)(\sigma^2_{S_2} - R_2)\right]}$$

em que, $\sigma_{H_1H_2}$ é a covariância das filhas nos ambientes 1 e 2; R_1 e R_2 são iguais a $1/s \sum \left(\frac{1}{N_{ij}}\right)\sigma^2_{e_j}$ e estimam a quantidade de variância dentro de reprodutor; s é número de reprodutores com filhas em ambos os ambientes; N_{ij} é número de filhas do reprodutor *i,* no ambiente *j*; e $\sigma^2_{e_j}$ é a variância do erro no ambiente *j*.

Um componente de alta variação de G×E resultará em uma herdabilidade baixa (Kang, 2002). Em 1963, Van Vleck (1963) já mencionava que a diferença da herdabilidade em dois ambientes poderia indicar presença de G×E. Assim, nos casos de alta herdabilidade em um dos dois ambientes, seria possível a seleção indireta para o ambiente com menor herdabilidade. Mas para isso, segundo Pani, Krause & Lasley (1971) e Reis & Lôbo (1991), é necessário à existência de uma correlação genética alta entre as características ou ambientes.

Além disso, outro complicador para os programas de melhoramento, é que poderão ser observadas graves reduções na eficiência dos programas quando a correlação genética entre ambientes for menor que 0,80 (Carabaño et al., 2014). Uma consequência adicional das baixas correlações, é que nas avaliações genéticas se tenderia a indicar como superiores os genótipos cuja capacidade adaptativa fosse mais estável (Kolmodin et al., 2002), o que não significa, obrigatoriamente, que tais genótipos sejam superiores em todos os ambientes.

Genótipos semelhantes respondem diferentemente às mudanças ambientais, e essa interação se tornou um componente crítico na produção pecuária. Tem sido observadas alterações dos valores genéticos dos indivíduos e reclassificação dos animais, dependendo das áreas geográficas e sistemas de manejo. Ou seja, o ambiente não modifica a constituição genética do indivíduo, e sim determina a extensão na qual o genótipo será expresso (Baye et al., 2011), por isso há a possibilidade de que o melhor genótipo em um ambiente não o seja em outro. Isso de deve a três possíveis efeitos:

I) Heterogeneidade de variâncias genéticas entre ambientes (também conhecido como efeito de escala);

II) Reclassificação (*re-ranking*) dos animais entre ambientes baseado em valores genéticos preditos;

III) Heterogeneidade de correlações entre duas ou mais características entre ambientes.

2.2.2 Indicadores de estresse térmico

Quando se trata de G×E envolvendo estresse térmico, as variáveis bioclimáticas apresentam grande potencial para serem incluídas nos modelos estatísticos para correção dos dados ou para seleção da termotolerância em animais (Bohmanova et al., 2008a). Na área vegetal, há muito tempo utilizam as variáveis bioclimatológicas em virtude da expressão dos genótipos em diferentes ambientes (Jarquín et al., 2014; Lopez-Cruz et al., 2015).

Em bovinos, Ravagnolo et al. (2000) sugeriram associar registros da produção de leite no dia do controle às medições meteorológicas, como potenciais indicadores de estresse térmico nas avaliações genéticas. No entanto, há muitas variáveis bioclimáticas com potencial investigativo para definição dos indicadores de estresse térmico: temperatura média (Mulim et al., 2021), amplitude térmica (Sae-Tiao et al., 2017), umidade relativa do ar (West, 2003), radiação solar (Tucker et al., 2008) e a combinação de fatores, como por exemplo, índice de temperatura e umidade (THI) (Ravagnolo & Misztal, 2000; Bohmanova et al., 2007; Aguilar et al., 2009; Bernabucci et al., 2014; Tiezzi et al., 2015). Deve-se ressaltar que as variáveis bioclimáticas além de serem utilizadas para a correção dos dados fenotípicos, podem ser úteis para o delineamento do gradiente ambiental, por meio de estudos de norma de reação.

2.2.2.1 Índice de temperatura e umidade (THI)

Entre as variáveis bioclimáticas utilizadas como indicadores de estresse térmico, o THI é amplamente adotado. Originalmente desenvolvido por Thom (1958) para detectar estresse térmico em seres humanos, o THI é um índice de valor único desenvolvido para avaliar o desconforto relacionado à alta temperatura ambiente combinada à alta umidade relativa. Posteriormente, a proposta inicial para calcular o THI foi melhorada por Berry et al. (1964), tornando-se o indicador mundial de estresse térmico em bovinos. Depois, muitos pesquisadores apresentaram propostas para a equação, sendo que a metodologia sugerida pelo National Research Council (NRC, 1971) a mais utilizada (Bohmanova et al., 2007; Bohmanova et al., 2008b; Dikemen & Hansen, 2009; Brügemann et al., 2012; Hammami et al., 2013), a qual relaciona a temperatura e a umidade do ar por ajuste das medidas de termômetro de bulbo seco.

O conhecimento sobre um limiar específico (crítico superior) do THI é o primeiro passo e talvez o mais importante para a compreensão do estresse térmico e do nível de adaptação em animais (Dikmen & Hansen, 2009; Collier et al., 2017). Esse limiar pode ser definido como o ponto em que é possível observar a queda de produção ou aumento significativo das células somáticas.

Os impactos na produção estão descritos na literatura. É relatado por Bouraoui et al. (2002) a redução do consumo de matéria seca da ração (9,6%) e diminuição na produção de leite (21%) com um aumento THI de 68 para 78. Segundo Spiers et al. (2004), o aumento no THI por unidade acima de 69 foi o motivo principal da redução da produção de leite nas vacas em 0,41 kg por dia. Conforme relatos de Burjakov et al. (2016) em bovinos de leite são suficientes quatro horas em condições de estresse térmico moderado para gerar uma perda de 1 kg de leite por dia. Nesta ordem, Vasilenko et al. (2018) afirmaram que bovinos expostos ao estresse térmico por longos períodos (13-18 horas) podem deixar de produzir 5,5 kg de leite por dia. Além da produção de leite ser prejudicada, a alta temperatura e umidade está relacionada à redução no rendimento de gordura e proteína do leite em 39,7 e 16,9%, respectivamente (López-Gatius & Hunter, 2017; Vasilenko et al., 2018).

Ravagnolo & Misztal (2000) encontraram uma variação moderada devido ao estresse térmico (via THI) e correlações genéticas negativas entre a produção em condições mais amenas e a taxa de declínio sob estresse térmico. Bohmanova et al. (2008a) criou um perfil de um touro tolerante ao calor e constataram que tal touro teria avaliações genéticas que eram mais baixas para leite e sólidos, mas maiores para fertilidade, longevidade e escore de úbere.

Aguilar et al. (2009) analisaram os efeitos do estresse por calor por meio do THI conforme a ordem de parto e concluíram que a variância genética do estresse térmico dobrou aproximadamente da primeira para a segunda parição e dobrou novamente da segunda para a terceira parição. Sendo assim, identificar animais termotolerantes precocemente diminuiria os prejuízos no setor leiteiro devido a G×E. Além disso, os níveis críticos de THI poderiam fornecer subsídios aos produtores para adoção de técnicas de manejo que minimizem os problemas decorrentes do estresse térmico em seus rebanhos (Azevedo et al., 2005).

2.2.2.2 Amplitude térmica (DTV)

Outro importante indicador de estresse térmico é a amplitude térmica. Definida como a diferença entre a temperatura máxima e mínima registradas em um período determinado (Alvares et al., 2013), representa a variação da temperatura em graus Celsius (°C).

É relevante notar que a maioria dos estudos e análises atuais das mudanças globais têm se concentrado em valores médios e índices, dedicando menos atenção às flutuações das variáveis climáticas. Nesse sentido, estudos que investiguem mudanças na variabilidade da temperatura e seus efeitos nas características fenotípicas podem ser importantes em diferentes escalas evolutivas, ecológicas e fisiológicas (Inchausti & Halley, 2003; Folguera et al., 2009).

Acredita-se que as espécies animais exploradas em atividade pecuária não conseguem se adaptar as mudanças bruscas da temperatura, resultando em perdas significativas na produção. Sae-Tiao et al. (2017), estimaram diminuições de 0,029 kg/vaca/dia devido a aumento excessivo da DTV na Tailândia. Como pode ser observada, a DTV também afeta negativamente a produção de leite, mas em menor grau quando comparado ao THI, no entanto, a variável merece atenção na gestão diária para minimizar os efeitos estressores.

2.3 Metodologia para detectar e quantificar os efeitos da interação genótipo-ambiente

Os animais são avaliados geneticamente para uma variedade de características usando metodologia de modelos mistos (Schaeffer, 2014) e isso permite a seleção de animais mais adequados para um ambiente específico ou local. Com a incorporação das metodologias adequadas é possível corrigir problemas de reclassificação dos animais e melhorar a confiabilidade das estimativas (Tiezzi et al., 2015). A metodologia mais utilizada para detectar e quantificar os efeitos da G×E via avaliações genéticas em bovinos é o modelo de regressão aleatória.

2.3.1 Modelos de regressão aleatória (RRM)

O conceito geral de uso de RRM para análise de covariância em um contexto de criação animal foi sugerido por Henderson (1982), o qual apresenta uma suposição adequada referente aos coeficientes de regressão associados às covariáveis, que cada membro possui distribuição aleatória. Kirkpatrick e Heckman, (1989), Kirkpatrick et al. (1990) e kirkpatrick et al. (1994) introduziram o modelo de dimensão infinita para características repetidamente mensuradas no indivíduo, e sugeriu modelos de covariâncias genéticas de trajetórias através de funções de covariância. Assim, a utilização de RRM vem se tornando mais rotineira na avaliação genética de bovinos leiteiros, por representar de forma mais realista os fenômenos associados a dados longitudinais do que os modelos de repetibilidade e de dimensão finita (Oliveira et al., 2019).

Em análises onde as características são associadas a curvas, o RRM deve ser considerado em dois conjuntos de regressão. O primeiro conjunto refere-se à regressão fixa (para indivíduos que pertencem à mesma classe de efeitos fixos) e o segundo conjunto abrange os efeitos aleatórios que descrevem o desvio de cada individuo em relação à regressão fixa (Resende et al., 2012). Ou seja, a regressão fixa tem um importante papel na predição dos valores genéticos.

Em bovinos, tradicionalmente a modelagem da regressão fixa está associada à classe de idade (em meses) e estação de parto (Strabel et al., 2004; Muir et al., 2007; Costa et al. 2008; Cobuci et al., 2011). Porém, outros autores já testaram diferentes fatores, como a média de produção da população (Herrera et al., 2008) ou subpopulações (Cobuci & Costa, 2012), características lineares da vaca, idade em anos, ano de nascimento e códigos de gestação-lactação (Arango et al., 2004).

Os efeitos genéticos aditivos dos animais são ajustados em função do tempo, como o número de dias em lactação (DIM) e podem ser facilmente modeladas na estrutura do modelo misto (Henderson, 1982). As covariáveis são geralmente funções não lineares, tais como polinômios, que relacionam o tempo com as características de produção de leite, gordura ou proteína e contagem de células somáticas (Dzomba et al., 2010).

Um RRM é dado matricialmente por:

$$y = \mathbf{X}b + \phi_g g + \phi_p p + e$$

em que, y é o vetor da variável resposta; $\mathbf{X}$ é a matriz de incidência de efeitos fixos; b é o vetor de efeitos fixos; ϕ_g é a matriz de incidência para os coeficientes de regressão aleatória dos efeitos genéticos aditivos; g é o vetor de efeitos genéticos aditivos; ϕ_p é a matriz de incidência para os coeficientes de regressão aleatória dos efeitos permanentes de ambiente; p é o vetor de efeitos de ambiente permanente; e é o erro associado a cada observação.

As distribuições dos coeficientes de regressão aleatória são dadas por:

$$g \sim N(0, \mathbf{A} \otimes \mathbf{K}_g)$$

em que, A é a matriz de parentesco entre os indivíduos e $\mathbf{K}_g$ é uma matriz de dimensão $(\mathbf{K}_g + \mathbf{I})(\mathbf{K}_g + \mathbf{I})$ de covariâncias entre os coeficientes de regressão aleatória para os efeitos genéticos aditivos.

$$p \sim N(0, \mathbf{I}_n \otimes \mathbf{K}_p)$$

sendo $\mathbf{I}_n$ uma matriz identidade de ordem n, e $\mathbf{K}_p$ uma matriz de dimensão $(\mathbf{K}_p + \mathbf{I})(\mathbf{K}_p + \mathbf{I})$ de covariâncias entre os coeficientes de regressão aleatória para os efeitos de ambiente permanente.

Estatisticamente,

$$y_{ij} = F_{ij} + \sum_{m=0}^{k_\beta - 1} \beta_m \phi_m (a_{ij}^*) + \sum_{m=0}^{k_g - 1} g_{im} \phi_m (a_{ij}^*) + \sum_{m=0}^{k_p - 1} \rho_{im} \phi_m (a_{ij}^*) + e_{ij}$$

em que, y_{ij} é a característica observada no *j*-ésimo THI do *i*-ésimo indivíduo; F_{ij} refere-se ao conjunto de efeitos fixos (Ex: grupo de contemporâneos); β_m *m*-ésimo coeficiente de regressão de efeito fixo da curva média da população; g_{im} e ρ_{im} são os *m*-ésimos coeficientes de regressão aleatória referentes aos efeitos genéticos aditivos e de ambiente permanente, respectivamente, para o *i*-ésimo indivíduo; k_g e k_p são as ordens das funções matemáticas utilizadas para descrever os efeitos genéticos aditivos e de ambiente permanente, respectivamente; a^*_{ij} é o THI *j* do indivíduo *i*; $\phi_m(a^*_{ij})$ refere-se à função matemática avaliada (Polinômios de Legendre, Wilmink, Splines, etc.); e e_{ij} é o efeito aleatório residual associado a cada observação.

Segundo Ravagnolo & Misztal (2000), o RRM é uma das alternativas para avaliar a interação genótipo ambiente (G×E) em avaliações genéticas. A correção dos dados para os indicadores de estresse térmico ou a seleção concomitante para tolerância ao calor e produção de leite são possíveis, pois a metodologia permite quantificar a sensibilidade animal em relação aos indicadores de estresse térmico. Segundo Misztal (2006), o RRM permite implementações simples e numericamente estáveis para avaliações genéticas.

Alguns autores já iniciaram investigações dos efeitos bioclimáticos na produção animal via RRM. Estudos são descritos por Kirkpatrick & Heckman (1989), Meyer (1998), Van Der Werf et al. (1998), Gomulkiewicz & Kirkpatrick (1992), Ravagnolo & Misztal (2000), Calus & Veerkamp (2003), Schaeffer (2004), Santana-Jr et al. (2016). O RRM tem sido apontado como um método útil para avaliar o impacto de parâmetros ambientais que têm distribuições contínuas (por exemplo, temperatura ambiente e umidade). Portanto, aplicar o RRM para estudar a trajetória fenotípica e genética de caracteres fisiológicos na dependência do regressor ambiental é uma alternativa para classes ambientais distintas combinadas com modelos multivariados (Wakchaure et al., 2016).

Estudos abordando o componente genético do estresse térmico identificaram que os modelos RRM são superiores aos demais modelos, especialmente quando se objetiva analisar a dinâmica do estresse térmico, a fim de identificar os limiares de THI e a G×E em bovinos leiteiros (Ravagnolo et al., 2000; Ravagnolo & Misztal, 2000; Brügmann et al., 2011; Brügmann et al., 2012; Brügmann et al., 2013; Hammani et al., 2013; Bernabucci et al., 2014; Hammani et al., 2015).

Vasilenko et al. (2018) enfatizam a necessidade de estudos para predizer a influência das variáveis bioclimatológicas na produtividade de leite de vacas, por meio de modelos de regressão aleatória. A vantagem do RRM é que a curva de regressão aleatória pode ser modelada dentro de cada efeito aleatório (por exemplo, variáveis bioclimáticas indicadoras de estresse térmico) e, portanto, permite a estimativa de herdabilidades, repetições e variações genéticas para o regressor ambiental específico. Além disso, permite modelar curvas individualmente e a característica é descrita em todos os pontos do gradiente.

2.3.1.1 Funções matemáticas

Muitas funções matemáticas podem ser aplicadas a dados fenotípicos em análises que utilizam os modelos de regressão aleatória. No entanto, não existe consenso sobre o melhor modelo para ajustar os dados de produção e saúde. As estimativas dos parâmetros genéticos obtidas com RRM geralmente dependem das funções de regressão utilizadas e da estrutura de covariância para efeitos genéticos aditivos, ambientais permanentes e residuais. Segundo Brotherstone et al. (2000), a função utilizada exerce uma influência marcante nas estimativas dos parâmetros genéticos.

A função exponencial de Wilmink (1987), foi desenvolvida especificamente para modelar curvas de lactação (Wilmink 1987; Schaeffer et al., 2000). Apesar de proporcionar estimativas de parâmetros genéticos que diferem daquelas obtidas por meio de funções matemáticas generalistas como polinômios ortogonais de Legendre (Bignardi et al., 2011), a função tem sido utilizada em muitos estudos (Cobuci et al., 2007; Dorneles et al., 2016; Daltro et al., 2021; Santos et al., 2021). A função pode ser descrita como:

$$y_t = a + be^{-kt} + ct$$

em que, *y* é a produção de leite no dia do controle, *t* é o dia de lactação (DIM), *a* é a produção inicial de leite, *b* é o acréscimo de produção até o pico de lactação e *c* o declínio da produção de leite após o pico. O parâmetro *k* é relacionado ao tempo de pico da lactação e geralmente assume um valor fixo, derivado de uma avaliação preliminar análise feita na produção média.

Outra função muito utilizada são os polinômios ortogonais de Legendre. A regressão aleatória baseada em polinômios ortogonais de Legendre foi usada na maioria dos estudos para modelar a estrutura de covariâncias para efeitos genéticos aditivos aleatórios e efeitos ambientais permanentes (Strabel & Misztal, 1999; Bohmanova et al., 2008b). Desenvolvida por Kirkpatrick et al. (1994), apresentam como vantagem a utilização de funções ortogonais, o que é útil para analisar padrões de variação genética (Kirkpatrick et al. (1990). Além disso, registros ausentes podem ser previstos com mais precisão do que com a curva de Wilmink e podem ser utilizados quando polinômios convencionais falhar (Pool & Meuwissen (1999). A função pode ser descrita como:

$$y_t = \sum_{i=0}^{n} \alpha_i \Phi_i (d_t^*)$$

Em que, d_t^* é unidade de tempo padronizada (DIM padronizado) que varia de -1 a +1: $d_t^* = -1 + 2\left(\frac{d_t - d_{min}}{d_{max} - d_{min}}\right)$, em que, d_{min} e d_{max} são os dias mais baixos e mais altos da lactação que podem ser encontrados no conjunto de dados, respectivamente; e d_t é o DIM. Para o DIM padronizado, o polinômio ortogonalizado é dado pela expressão:

$$\Phi_{(d_i^*)j} = \frac{1}{2^j}\sqrt{\frac{2j+1}{2}} \sum_{m=0}^{[j/2]} (-1)^m \binom{j}{m} \binom{2j-2m}{j} (d_i^*)^{j-2m}$$

em que, d_i^* é i-ésimo DIM, j é a ordem da função de Legendre e *m* é o número de índices necessários para determinar o polinômio e $[j/2]$ indica que os valores da fração são arredondados para baixo, assumindo o valor inteiro mais próximo..

Outra função em crescente utilização é o Linear Spline (Bohmanova et al., 2008b). Proposta por Misztal (2006), a função de Linear Spline em RRM são semelhantes aos dos modelos de características múltiplas, com características correspondendo a pontos em nós. Segundo Misztal (2006), a vantagem do método é devido às propriedades numéricas geralmente superiores de Splines em comparação com polinômios e sistema de equações mais esparso. Além disso, na prática, permite ajustar os nós de forma que cobrem toda a trajetória, apresentem variância aproximadamente linear no intervalo entre os nós e as correlações entre os nós adjacentes são de pelo menos 0,60 (Misztal, 2006). A função pode ser descrita como:

Seja *T* um vetor de nós "n", então as covariáveis do linear Splines para DIM $t(\Phi_i(t))$ localizado entre os nós T_i e T_{i+1}, pode ser calculado como:

$$\Phi_i(t) = \frac{t - T_i}{T_{i+1} - T_i},$$

$$\Phi_{i+1}(t) = \frac{T_{i+1} - t}{T_{i+1} - T_i} = 1 - \Phi_i(t), \text{ e } \Phi_{1...i-1,i+2...n} = 0$$

Se $t = T_i$, $\Phi_i(t) = 1$ e $\Phi_{1...i-1,i+2...n} = 0$. O vetor Φ de DIM *t* tem no máximo dois elementos diferentes de zero e a soma de todos os elementos do vetor é igual a um. A fórmula acima assume que $T_i \leq t < T_n$.

2.3.2 Modelos de norma de reação

Analisando graficamente os resultados da regressão aleatória sob o indicador do estresse térmico (gradiente ambiental), temos um modelo de norma de reação. Onde é possível detectar a presença de G×E e modelar a trajetória do desempenho do animal em função do gradiente ambiental.

Quando um animal produz uma resposta que varia como uma função contínua e gradativa em relação ao ambiente, esta relação é chamada de norma de reação (Woltereck, 1909). As normas de reação podem ser definidas como um conjunto completo de trajetórias ontogênicas multivariadas, produzidas por um único genótipo, exposto a vários ambientes biologicamente diferentes (Schlichting & Pigliucci, 1998). Essa variação dos fenótipos em função de uma variação contínua ambiental é normalmente representada por uma linha ou curva num gráfico de mensuração de uma característica fenotípica sob um fator ambiental (Stearns, 1989). Dessa forma, é possível observar que o genótipo não determina apenas o fenótipo, mas sim, a representatividade da sensibilidade do animal (capacidade adaptativa) frente a ambientes variantes (Rauw & Gomez-Raya, 2015).

A aplicabilidade dos modelos de norma de reação está em permitir a exploração de características (medidas repetidas ou dados longitudinais) em escalas fenotípicas e genéticas na dependência de descritores ambientais contínuos (Meyer, 2005; Mrode, 2014).

2.4 Seleção genética para termotolerância em bovinos leiteiros

Os fatores climáticos exercem grande influência sobre os animais domésticos, estejam eles em um ambiente artificial ou habitat natural. Animais altamente produtivos tendem a sofrer mais por questões ambientais, consequentemente manifestando reações comportamentais, indicando também a adaptabilidade ou não, no ambiente o qual está exposto. Todavia, aspectos ambientais podem causar variações na produção (Zimbelman et al., 2013), na composição do leite (Gonzalez, 2004), podem afetar a reprodução (Haile-Mariam et al., 2008), e por fim afetam a seleção dos animais com base no mérito genético.

A seleção genética para termotolerância em bovinos leiteiros é possível, uma vez que há fortes indícios de variabilidade genética substancial para ser explorada (Carabaño et al., 2014; Carabaño et al., 2019). Nesse sentido, a inclinação de resposta de cada indivíduo pode ser usada como critério de seleção.

Apesar da correlação genética desfavorável entre a produção de leite e a resistência ao calor, segundo Carabaño et al. (2019) é possível que a seleção genética seja uma ferramenta econômica para melhorar a termotolerância de animais. No entanto, para isso, é necessário um profundo conhecimento sobre a base genética da resposta do animal ao estresse por calor. Nesse quesito, a genética quantitativa permitiria auxiliar a identificação do limiar para a população no ambiente de criação e a partir deste ponto, adotar medidas de gestão e manejo para minimizar os efeitos negativos na produção de leite, além da opção de selecionar linhagem mais termotolerantes.

De modo geral, até agora, as tentativas de estimação genética para selecionar animais tolerantes foram com base em análises de desempenho sob estresse térmico. Muitos autores mencionam a alteração nos valores genéticos estimados, reclassificação e melhoria das confiabilidades (Carabaño et al., 2019; Osei-Amponsah et al., 2019) após corrigir os dados para os indicadores de estresse térmico.

Bryant et al. (2007), quantificaram a extensão da reclassificação dos touros dentro da raça para características de produção de leite e observaram benefícios na seleção de touros específicos para determinados ambientes. Hammami et al. (2008) encontraram considerável reclassificação de touros da raça Holandesa, para produção de leite, entre Luxemburgo (clima temperado) e Tunísia (clima quente e úmido).

Bernabucci et al. (2014) observaram considerável alteração no rank quando incluíram o THI como covariável no modelo de avaliação genética. Carabaño et al. (2014) demonstram o desvio genético estimado conforme o aumento das temperaturas para produção de leite, gordura, proteína e contagem de células somáticas, onde é possível observar a variabilidade na resposta genética e reclassificação de animais, o que indica certo grau de genótipo por interação com o ambiente. Lee et al. (2019) observaram que a herdabilidade diminuía ligeiramente conforme o THI aumentou (acima do limiar) e a classificação dos touros variou com o THI.

Cheruiyoy et al. (2020) afirmam que o nível de G×E está aumentando com o passar das gerações e devem ser monitorados regularmente, especialmente considerando o aumento previsto das mudanças climáticas. Além disso, os autores sugerem que variações genéticas existentes, podem ser utilizadas para selecionar animais que apresentam um desempenho ótimo em diferentes ambientes.

Na Austrália, Nguyen et al. (2017) já relatavam iniciativas de implementação da seleção para termotolerância para gado Holandês e Jersey. Os autores afirmam que embora esforços tenham sido feitos em nível de gerenciamento de rebanho para fornecer um ambiente adequado para vacas, o melhoramento genético para tolerância ao calor não foi praticado. Neste caso, utilizaram genótipos de milhares de vacas e touros já disponíveis e descreveram um plano de implementação dos valores genéticos genômico para estresse térmico.

2.4.1 Impacto do estresse térmico nas características de produção e saúde

Quando consultada a literatura quanto ao impacto do estresse térmico na produção de leite em bovinos da raça Holandesa, é possível observar variações quanto na definição do limiar. Na Geórgia, Ravagnolo et al. (2000) estimaram um limiar para THI de 72. Na Tunísia, Bouraouri et al. (2002) realizaram um estudo de dois anos e encontraram correlação negativa entre produção de leite e THI diário, com uma diminuição de 0,41 kg/vaca/dia/ THI que excedeu 69. No Arizona, Bohmanova et al. (2007), identificaram um limiar de THI = 74 e Zimbelman et al. (2009) estabeleceram um valor THI = 68 para vacas Holandesas de alta produção.

Brügemann et al. (2011) em vacas Holandesas na Alemanha, estimaram limiar de THI 72, enquanto que Hammami et al. (2013) estimaram o limiar de 62. Em um estudo com vacas Holandesas Iranianas, Bohlouli et al. (2013) definiram limiar de 72 para THI. Bernabucci et al. (2014) usando informações de vacas Holandesas na Itália, também estimaram limiar de 72 para THI. Carabaño et al. (2014) estimaram o limiar em 73 THI na Espanha. Em 2015, Nguyen et al. (2015), identificaram que o declínio na produção de leite, gordura e proteína ocorriam em THI que excede o limite de 60.

No Brasil, Santana-Jr et al. (2016) identificaram um limiar de THI = 66, com queda de 0.230 kg/dia/THI que excedeu 66. Na Tailândia, Sae-Tiao et al. (2017) identificaram queda na produção de -0,029 kg/vaca/dia devido à alta amplitude térmica. Enquanto que Lee et al. (2019), na Correia do Sul, identificaram um limiar de 72.

Para a característica de produção de gordura, Bryant et al. (2007), na Nova Zelândia, relataram limiar de 50 para rendimento de gordura. Brügemann et al. (2011), na Alemanha, não estimaram um limiar válido. Na Espanha, Carabaño et al. (2014) estimaram o limiar em 59 THI. Bernabucci et al. (2014) não conseguiram identificar um limiar para porcentagem de gordura (ausente), mas para rendimento de gordura variou de 71 a 72 THI. Ravagnolo et al. (2000) estimaram um limiar de 72 THI. Na Correia do Sul, Lee et al. (2019), não identificaram um limiar para produção de gordura, pois está continuou diminuindo com o aumento do THI.

Enquanto que para produção de proteína, na Nova Zelândia, Bryant et al. (2007), relataram limiar de 60 para rendimento de proteína. Brügemann et al. (2011), na Alemanha, não estimaram um limiar válido. Na Espanha, Carabaño et al. (2014) estimaram o limiar em 62 THI. Bernabucci et al. (2014) identificaram um limiar de 65 a 71 THI para porcentagem de proteína e de 72 a 73 para rendimento de proteína. Enquanto que Lee et al. (2019), na Correia do Sul, não identificaram um limiar para produção de proteína, pois está continuou diminuindo com o aumento do THI.

Em características relacionadas a saúde, Bouraoui et al. (2002) afirmam que o aumento da contagem de células somáticas da primavera para o verão, está atrelado às altas temperaturas, confirmando as tendências já observadas por outros autores como Collier et al. (1982) e Muller et al. (1994), onde o aumento das células somáticas está aninhado aos mecanismos de defesa mamária.

Brügemann et al. (2011) estimaram limiar de THI 60 para escore de células somáticas (SCS) em vacas Holandesas na Alemanha, enquanto que Hammami et al. (2013) estimaram o limiar de 66. Na Espanha, Carabaño et al. (2014) estimaram o limiar em 59 THI, como aumento de 0,0024 U/°C acima do limiar.

3. Hipóteses

1. Existe forte presença da interação genótipo-ambiente nos sistemas de produção leiteira.

2. Há diferenciação das predições de valores genéticos dos animais para características de produção conforme o dia de inclusão das variáveis bioclimatológicas.

3. A incorporação de indicadores de estresse térmico nos modelos melhora as predições dos valores genéticos dos animais, assim como de suas confiabilidades.

4. Há uma forma equilibrada de selecionar os animais simultaneamente para produção de leite sem penalizar a tolerância ao calor.

5. Há um indicador biológico precoce de identificação de sinais de estresse térmico pelos animais antes que a produção de leite diária seja afetada significativamente.

6. A incorporação das classes de estresse térmico na curva fixa de regressão permite melhoria do processo de avaliação genética da raça Holandesa via modelo de regressão aleatória.

7. O estresse térmico é capaz de afetar a predição dos valores genéticos dos animais a ponto de provocar uma expressiva reclassificação dos touros.

4. Objetivos

1. Quantificar as perdas devido à interação genótipo-ambiente, com ênfase no estresse térmico e identificar o limite do conforto térmico das vacas.

2. Determinar a influência do dia de coleta das informações bioclimatológicas sobre a predição dos valores genéticos dos animais para características de produção e saúde.

3. Verificar e quantificar o aumento na confiabilidade dos valores genéticos ao corrigir os dados para os indicadores de estresse térmico.

4. Investigar a viabilidade da seleção genética de animais da raça Holandesa para termotolerância e produção de leite.

5. Verificar a existência de um indicador biológico precoce do estresse térmico em vacas antes que a produção de leite diária seja fortemente afetada.

6. Determinar uma forma alternativa de corrigir os dados para os indicadores de estresse térmico por meio da regressão fixa via metodologia de regressão aleatória.

7. Verificar a magnitude de reclassificação dos touros ao incluir as variáveis bioclimatológicas indicadoras de estresse térmico na avaliação genética.

CAPÍTULO II

Inclusion of bioclimatic variables in genetic evaluations of dairy cattle[3]

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Inclusion of bioclimatic variables in genetic evaluations of dairy cattle

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ABSTRACT

Objective: Considering the importance of dairy farming and the negative effects of heat stress, more tolerant genotypes need to be identified. The objective of this study was to investigate the effect of heat stress via temperature-humidity index (THI) and diurnal temperature variation (DTV) in the genetic evaluations for daily milk yield of Holstein dairy cattle, using random regression models.

Methods: The data comprised 94,549 test-day records of 11,294 first parity Holstein cows from Brazil, collected from 1997 to 2013, and bioclimatic data (THI and DTV) from 18 weather stations. Least square linear regression models were used to determine the THI and DTV thresholds for milk yield losses caused by heat stress. In addition to the standard model (SM, without bioclimatic variables), THI and DTV were combined in various ways and tested for different days, totaling 41 models.

Results: The THI and DTV thresholds for milk yield losses was THI = 74 (−0.106 kg/day/ THI) and DTV = 13 (−0.045 kg/day/ DTV). The model that included THI and DTV as fixed effects, considering the two-day average, presented better fit (−2logL, AIC and BIC). The estimated breeding values (EBVs) and the reliabilities of the EBVs improved when using this model.

Conclusion: Sires are re-ranking when heat stress indicators are included in the model. Genetic evaluation using the mean of two days of THI and DTV as fixed effect, improved EBVs and EBVs reliability.

Keywords: Heat stress; Random regression; Temperature-humidity index, Diurnal temperature variation

INTRODUCTION

Brazil is the fourth largest milk producer in the world, with an annual milk production of 33.5 billion liters [1]. Minas Gerais is historically the largest dairy region in the country, accounting for 27% of the national production. However, the production potential may be threatened due to heat stress that negatively impacts livestock production, especially in tropical regions [2,3].

Traditionally, dairy cattle breeding programs have focused on intense selection to increase milk yield. However, it has already been proven that milk yield and heat tolerance are antagonistically correlated [4,5,6,7], and intense selection for milk production increases sensitivity to heat stress [7]. To minimize this effect, it is necessary to quantify heat stress, correct the data for the effect and, than carry out the genetic evaluation. Failure to include heat stress indicators can affect the estimation of breeding values (EBVs), compromising selection.

The methodology for including bioclimatic variables to quantify the level of heat stress has shown positive results in animal selection in subtropical regions [2,3,5,7]. Heat stress is diagnosed by decreasing the daily milk production after a specific limit of the bioclimatic indicator. The most used indicators to determine the degree of heat stress in dairy cattle are temperature-humidity index (THI) and diurnal temperature variation (DTV). THI is a unitary bioclimatic index that represents a combination of temperature and air humidity, while DTV is obtained by the difference between maximum and minimum daily temperatures.

Despite many researches on dairy cattle, little is known about the implication of data correcting considering bioclimatic indicators of heat stress and what is the best way to correct the data for this effect and its implications for the re-ranking of animals. Considering the importance of dairy farming in Brazil and the negative effects of heat stress on almost all livestock activities, investigating factors that affect yields is necessary to producers to improve their herd quality and become competitive in the market.

In this context, the objective of this study was to investigate the effect of heat stress via temperature-humidity index and diurnal temperature variation in the genetic evaluations for daily milk yield of Holstein dairy cattle, using random regression models.

MATERIAL AND METHODS

Data

Data consisted of test-day (TD) milk yield collected by the Service of the Minas Gerais Association of Holstein Breeders (ACGHMG), accredited by the Ministry of Agriculture, Livestock and Supply (MAPA). Data of Holstein cows of the state of Minas Gerais – Brazil (19º55' S – 43º57' W), from 1996 to 2015 were used. For this study, only information from the first lactation was considered. We excluded records with extreme age at calving (<18 or >48 months), days in milk (DIM, <5 or >305 days) and milk yield (<4 or >44.8 kg) from the data set. Only healthy animals with at least four individual TD records during lactation were retained for analysis. The minimum size of each contemporary group (described in each model) was three animals. Records of daughters of sires with at least one daughter in at least three herds were accepted to the evaluation.

Following these criteria, a total of 94,549 TD records from 11,294 first lactations of Holstein cows (average calving age: 29 months) from 129 farms, collected from 1997 to 2013, were analyzed. The same database was used for all models evaluated. The pedigree file included 32,409 animals. The edited data are described in Table 1.

Minas Gerais is characterized by three predominant climate types (according to the Köppen-Geiger climate classification): subtropical of altitude; subtropical with dry winter; and tropical with dry winter. Seasonal factors influence the herd management, in warmer conditions the animals tend to be kept in pastures, while in colder periods animals are semi-confined and supplemented with silage [8].

The climate variables used were average daily dry bulb temperature (DBT; °C), maximum temperature (°C), minimum temperature (°C), and average daily relative humidity (RH; %) (Figure 1), as recorded by the National Institute of Meteorology through 18 weather stations (representing 86 municipalities) located less than 60 km away from the evaluated farms, using the nearest station information [9]. The temperature-humidity index (THI) was evaluated according to equation described by the National Research Council – NRC [10]:

$$THI = (1.8 \times DBT + 32) - [(0.55 - 0.0055 \times RH) \times (1.8 \times DBT - 26)]$$

The diurnal temperature variation (DTV) was calculated by the difference between daily maximum and minimum temperatures, in degrees Celsius (ºC).

Models

The daily averages of THI and DTV were tested up to three days before each TD record - THI and DTV on the TD record (0DB); one day before (1DB); two days before (2DB); three days before (3DB), and mean between the two last days before each TD (1DB and 2DB) (2DM) (Figure 2). It was not possible to test more days before the control because of the lack of THI and DTV for some herds, which would increase the elimination of data in approximately 40% due to the need to use the same data file for all models.

To delimit the heat comfort zone, the average loss of daily milk yield per THI and DTV unit was estimated by linear regression of milk yield on the THI and DTV values as deviation from the threshold limit. Fixed effects of the threshold model were considered: contemporary group, milking frequency, and DIM. The age of cows at calving was considered covariate (linear effect). The average daily loss of milk yield was estimated considering the mean THI and DTV values (only 2DM).

For genetic evaluation, a random regression model was used for the test-day milk yield analysis using the Wilmink parametric function [11]. The additive genetic and permanent environmental covariance functions were estimated by the random regression model of the DIM for 9 models, which will be defined later (SM, M1, M2, M3, M4, M5, M6, M7, and M8).

The standard random regression model does not consider bioclimatic variables for correction of the data, as shown by the equation:

$$\text{(SM)}\quad y_{ijklm} = HYM_l + \sum_{m=0}^{df} \phi_{jkm}\beta_m + \sum_{m=0}^{dr} \phi_{jkm}\mu_{jm} + \sum_{m=0}^{dr} \phi_{jkm} pe_{jm} + e_{ijklm}$$

where y_{ijklm} is the *i*th TD record of the *j*th cow on the *k*th DIM within the *l*th subclass herd-year-month of the test (HYM); β_m is the *m*th fixed regression coefficient, defined as the age classes: 1 (18 to 25 months), 2 (26 to 27 months), 3 (28 to 29 months), and 4 (30 to 48 months), combined with the calving season subclasses: 1 (rainy: October to March) and 2 (dry: April to September), totaling eight fixed curves; μ_{jm} is the *m*th random regression coefficient for the additive genetic effect of the *j*th cow; pe_{jm} is the *m*th random regression coefficient for the permanent environmental effect the *j*th cow; ϕ_{jlm} is the *m*th Wilmink function corresponding to the TD record of the *k*th DIM of the *j*th cow; df and dr are orders of fixed and random regression coefficients; and e_{ijklm} is the random residual effect.

The M1 to M8 models were used to verify the best way to include bioclimatic data for the correction of the genetic evaluation model, according to the day included with THI and DTV data: 0DB, 1DB, 2DB, 3DB and 2DM, totaling 40 models.

Model 1 (M1): Contemporary group and THI class (21 classes, every two units of THI was considered a class: THI 51 and 52 = class 1, THI 53 and 54 = class 2,…, THI 91 and 92 = class 21) (fixed effects).

Model 2 (M2): Contemporary group (fixed effect), and THI (linear covariate).

Model 3 (M3): Contemporary group (fixed effect), and THI (linear and quadratic covariates).

Model 4 (M4): Contemporary group (fixed effect), and DTV (linear covariate).

Model 5 (M5): Contemporary group is defined as herd-THI class (twenty-seven THI classes) (fixed effect). Only model with a contemporary group different from that defined in SM.

Model 6 (M6): Contemporary group (fixed effect), THI and DTV (linear covariates).

Model 7 (M7): Contemporary group, THI and DTV (fixed effects).

Model 8 (M8): Contemporary group (fixed effect). The fixed curve is defined as age classes: 1 (18 to 25 months), 2 (26 to 27 months), 3 (28 to 29 months), and 4 (30 to 48 months), combined with the THI subclasses (seven classes, with five THI each class: THI 51 to 56 = class 1, THI 57 to 62 = class 2, …, THI 87 to 92 = class 7), totaling twenty-eight fixed curves. Only model with a fixed curve different from that defined in SM.

For all models, the fixed curve, additive genetics and permanent environmental covariance functions were estimated by random regression used Wilmink of DIM. The residual variance was considered homogeneous for all models.

Genetic evaluation and statistical analysis

All genetic analyses were performed with an animal model, using the REMLF90 program [12]. The quality of the adjustment was carried out through comparison tests between non-nested models and penalties according to the number of parameters to be estimated. The following criteria were used: log-likelihood function (−2logL); Akaike's information criterion ($AIC = -2\log L + 2p$, where *p* is the number of parameters in the model); Schwarz's Bayesian information criterion ($BIC = -2\log L + p\log(\lambda)$, where log (λ) is the natural logarithm of the sample size (or dimension of *y*) and p is the number of parameters in the model), BIC is more rigid than AIC. The model with the lowest value, for both criteria, is considered the best fit. To check the re-ranking of the estimated breeding values (EBVs) of the sires, Spearman rank correlation coefficient (*p*) for 1% and 10% of the upper sires EBVs was used. The reliabilities of the EBVs were calculated using the triangular matrices of prediction error (co)variances for random regression effects, from the inverse of the mixed model equations obtained in the BLUPF90 program [12].

RESULTS AND DISCUSSION

Milk yield

Climatic conditions were found to exert an influence on milk production in dairy farms in Minas Gerais State, Brazil. The bioclimatic variables used as indicators of heat stress play an important role in animal production, predicting the critical limit between comfort and stress. The identified thresholds provide the essential pre-requisites for identification of genetic components of heat stress and allow promote solutions and the development of improvement strategies.

THI is the most widely used environmental indicator of heat stress effects in literature. However, DTV also has the potential to correct data and minimize the stressor effect on genetic evaluations.

The THI threshold for milk yield losses was THI = 74, considering the complete lactation (5 to 305 day) (Figure 3). The decreases in milk production were –0.106 kg/cow/day/THI unit above 74. The effects of THI stratified according to lactation phase (initial – DIM 5 to 60; intermediate – DIM 61 to 180; and final phase – DIM 181 to 305) showed a significant effect of THI on milk yield, with decreases of –0.092 kg/day/THI (DIM 5 to 60, p-value <0.0001), –0.108 kg/day/THI (DIM 61 to 180, p-value <0.0001), and –0.114 kg/day/THI (DIM 181 to 305, p-value <0.0001).

The DTV threshold was DTV = 13. The average annual temperature of the state of Minas Gerais is approximately 16.3 ºC, with average daytime temperature variation of 5.8 to 7.6 ºC [9]. This thermal amplitude above 13 °C causes decreases in milk yield of cows by up to –0.045 kg/DTV unit (p <0.001) increased above 13. The effects of DTV stratified according to the lactation phase were significant for milk yield, with decreases of −0.002 kg/day/DTV (DIM 5 to 60, p-value = 0.002), −0.056 kg/day/ DTV (DIM 61 to 180, p-value = 0.004), and −0.005 kg/day/DTV (DIM 181 to 305, p-value = 0.003).

This study, most cows are daughters of sires that imported semen (especially from the United States, Canada and the European Union). Approximately 90% of lactating cows were exposed to heat stress conditions in at least five TD records; and 69% of the TD records were in conditions of heat stress. This result is concerning due to potential of economic impacts. The loss of 1.28 ± 0.31 kg of milk at the peak of lactation by heat stress reflects a loss of production of about 221 ± 2.2 kg at the end of complete lactation; however, this result can reach 2000 kg of loss. In addition, cows that calved in the summer (average THI = 82 and DTV = 14) produced an average of 6% less milk when compared to cows calved in the beginning of winter (average THI = 73 and DTV = 10).

The magnitude of production losses shows the importance of evaluating heat stress through bioclimatic variables, especially when considering the economic factor. The impact on reducing milk production is very expensive, and this scenario can be extrapolated to Brazil and world, because the climate of Minas Gerais represents a large part of the Brazilian climate and of several regions of the world.

Listed as one of the major concerns in dairy cattle, heat stress affects production potential almost worldwide and it is believed that the financial impact due to heat stress probably outweighs the impact due to mastitis and reproductive parameters. In addition, the combination of elevated temperature and humidity negatively affects quality milk, food intake [10], and reproductive potential [13]. When it comes to Holstein cattle, the values quoted in the literature are similar to the result obtained in these studies, in Missouri the estimated threshold was THI = 70 [14], in Georgia THI = 72 [15] and, in Arizona THI = 74 [5]. In Thailand, Sea-Tiao et al. [16] estimated decreases of -0.029 kg/cow/day due to DVT (ºC). DTV also negatively affects milk production, but to a lesser extent, when compared to THI, however, the variable deserves attention in daily management to minimize the effects.

Adjustment of models

Most models that consider bioclimatological variables present better fit than the standard model (SM) (Figure 4). M7 included THI and DTV as a fixed effect and presented the best overall fit for correcting milk yield data, regardless of the day included with THI and DTV data. The M7-2DM was the best adjustment model, denoting that the mean between 1DB and 2DB better explains the milk yield loss due to heat stress.

Biologically, the use of 2DM of stress-causing factors (THI and DTV) can better explain the animal performance due to the amount of circulating cortisol in the body. Result that agrees with that obtained by West et al. [17], who stated that the use of weather information, as in the 2DM, better shows the effect of heat stress on milk yield. The response to heat stress is not immediate, but cumulative [13]. The plasma cortisol concentration in animals exposed to heat stress shows a peak in the first 12 hours after the onset of heat stress and tends to return to normal values within two days [18]. Thus, the environment is important for metabolism and affects the cardiovascular system and absorption of nutrients in the mammary gland, directly affecting milk production [19].

To check the impact of heat stress on the estimation of sires EBVs, in addition to the SM (routinely used), the best model for each day tested was chosen to verify the change in the sires' rank: M7-0DB, M7-1DB, M7-2DB, M7-3DB, and M7-2DM.

Estimated breeding values, ranking and reliability

The magnitude of the estimated Spearman rank correlations coefficient, especially for the SM, confirmed the reranking of the sires when including the bioclimatic variables in the models (Figure 5). The EBVs of the animals changed when correcting the data for the stress indicators, so the selection process may be compromised and the observed genetic gains may not be equal to the expected genetic gains. Genetic superiority animals may be being eliminated from the mating selection due to the fact that their daughters are more penalized for disregarding heat stress in the evaluation.

This probably affects not only the producer, but the entire dairy industry. Research shows significant economic losses of around $ 900 million dollars per year [20].St-Pierre et al. [21] estimated an annual economic loss of $897 million to $1.5 billion dollars for the USA dairy industry due to the heat stress of dairy cattle.

In Brazil, the strategy of farmers in the search for productivity improvement is the use of imported semen. It is genetic material selected in other countries under different environmental conditions. However, the milk production of these animals in Brazil is not expected to correlate with those in the environments in which they were originally bred. In addition to milk yield, other traits and parameters of milk quality are also highly affected by bioclimatic features [22].

According to Zwald et al. [23], the genotype-environment interaction can significantly affect productive and reproductive characteristics, causing re-rankings; and variables, such as temperature, can be used to group herds into similar production environments. Robertson [24] reported that the re-ranking can be aggravated by combining different characteristics into a selection index, since genetic correlations below 0.8 may result in re-ranking of the animals. The same was found in South Korean dairy cattle by Lee et al [25], where selection for high milk yield decreased thermotolerance and when the THI incorporated, the sires ranking was changed. The re-ranking and changes in the magnitude of differences of the genetic merit of animals can affect important productive and economic aspects.

The comparison of the reliability of the EBVs of the ten best sires and the top 1% for milk yield in the TD records in the SM with the reliability of the EBVs of these sires in the other models evaluated, showed no changes, although there was a re-ranking (Table 2). However, when extrapolating to the top 5%, top 10%, and to all sires evaluated, a significant difference (p <0.05) was found when considering the fixed effects in the M7-2DM model to estimate the reliability of the EBVs of the sires (Table 2). The mean reliability of the EBVs of the sires for the milk yield in TD record was 10 to 25% higher for the top 5%, 18 to 35% higher for the top 10%, and 75 to 100% for all sires evaluated, when using the M7-2DM model.

Sires with few daughters had lower reliability of the EBVs when using the SM (Table 3). When included the two day averages of THI and DTV as a fixed effect in the genetic evaluation model, it's possible to observe an increase in reliability of the EBV of sires with less than 30 daughters: a 27% increase for sires with up to 10 daughters, 8% for sires with 11 to 20 daughters, and 5% for sires with 21 to 30 daughters. This fact contributed to the large re-ranking of the sires and the low Spearman rank correlation coefficient found.

Considering that the choice of sires to be used for breeding is the EBVs, the use of some sires may be misleading, and others should not be used. The intense use of some sires is evidenced by the low number of sires with more daughters. Therefore, without the inclusion of bioclimatic variables in the model, some sires may be penalized because their daughters are conditioned to environmental stress factors.

CONCLUSION

The inclusion of bioclimatic variables in the genetic evaluation of Holstein dairy cattle directly affects sires selection. The ranking of sires changes severely when THI and DTV are included as fixed effects in the model, changing the estimated breeding value for milk yield and significantly improving the reliability the of EBVs of the sires.

The best way to include THI and DTV as fixed effects in the model is to consider the mean between the two last days before each TD. Considering the great diversity of environmental conditions in Brazil and that certain sires are not penalized by the environment (THI and DTV) in which their daughters are bred, it is essential to include bioclimatic variables in the genetic evaluation models to avoid compromising the genetic progress of the herds.

CONFLICT OF INTEREST

We certify that there is no conflict of interest with any financial organization regarding the material discussed in the manuscript.

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Table 1. Summary of the standard model data structure.

Item	Statistics
Animals in the pedigree file	32,409
Animals with records	11,294
Dams in the pedigree file	8,639
Sires in the pedigree file	641
Test-day records	94,549
Mean records/animal	8.37
Milk yield mean (kg/day)	25.81 ±7.21
Contemporary groups	5257

Table 2. Reliability of estimated breeding values of the best ten sires, Top 1%, Top 5%, Top 10%, and all sires for milk yield in the test-day (TD) records, according to the standard model (SM) and best adjusted model (M7) for the days included with THI and DTV data—on the TD record (0DB); one day before (1DB); two days before (2DB); three days before (3DB), and mean between the two last days before (2DM)

	Models					
	SM	M7-0DB	M7-1DB	M7-2DB	M7-3DB	M7-2DM
Sire a	0.57	0.58	0.56	0.53	0.56	0.56
Sire b	0.58	0.61	0.59	0.54	0.59	0.59
Sire c	0.58	0.60	0.58	0.55	0.58	0.58
Sire d	0.54	0.54	0.51	0.48	0.51	0.51
Sire e	0.79	0.81	0.78	0.73	0.78	0.78
Sire f	0.56	0.57	0.55	0.51	0.55	0.55
Sire g	0.37	0.39	0.38	0.36	0.38	0.38
Sire h	0.86	0.91	0.85	0.78	0.85	0.85
Sire i	0.67	0.69	0.67	0.62	0.67	0.67
Sire j	0.40	0.41	0.39	0.38	0.39	0.39
Means	0.59	0.61	0.59	0.55	0.59	0.59
	Groups of selected sires					
Top 1%	0.52[a] (0.27 to 0.83)*	0.48[a] (0.27 to 0.83)	0.47[a] (0.25 to 0.80)	0.47[a] (0.26 to 0.80)	0.47[a] (0.25 to 0.80)	0.56[a] (0.38 to 0.83)
Top 5%	0.46[b] (0.11 to 0.86)	0.42[b] (0.11 to 0.86)	0.41[b] (0.08 to 0.83)	0.42[b] (0.11 to 0.84)	0.42[b] (0.08 to 0.83)	0.51[a] (0.24 to 0.86)
Top 10%	0.39[b] (0.10 to 0.88)	0.36[b] (0.10 to 0.86)	0.35[b] (0.10 to 0.79)	0.35[b] (0.10 to 0.84)	0.34[b] (0.10 to 0.86)	0.46[a] (0.11 to 0.88)
All	0.16[b] (0.01 to 0.92)	0.15[b] (0.01 to 0.92)	0.14[b] (0.01 to 0.90)	0.15[b] (0.01 to 0.90)	0.14[b] (0.01 to 0.90)	0.28[a] (0.05 to 0.90)

Median followed by different letters in the rows are significantly different (p <0.05) by the Kruskal-Wallis test. *Minimum and maximum.

Table 3. Average reliability of estimated breeding values (EBVs) of the sires, according to their number of daughters; comparison between the traditional standard model (SM) and the model that includes the mean of two days of bioclimatic variables as fixed effects (M7-2DM)

N Daughters	N Sires	Reliability of EBVs	
		SM	M7-2DM
< 10	364	0.34 (0.01 to 0.85)*	0.43 (0.05 to 0.86)
11 to 20	127	0.52 (0.34 to 0.81)	0.56 (0.35 to 0.82)
21 to 30	59	0.63 (0.51 to 0.82)	0.66 (0.51 to 0.83)
31 to 40	23	0.68 (0.58 to 0.75)	0.69 (0.61 to 0.76)
41 to 50	12	0.73 (0.67 to 0.81)	0.74 (0.69 to 0.82)
51 to 100	43	0.78 (0.68 to 0.85)	0.79 (0.69 to 0.86)
> 101	13	0.86 (0.83 to 0.92)	0.87 (0.83 to 0.92)

* Minimum and maximum.

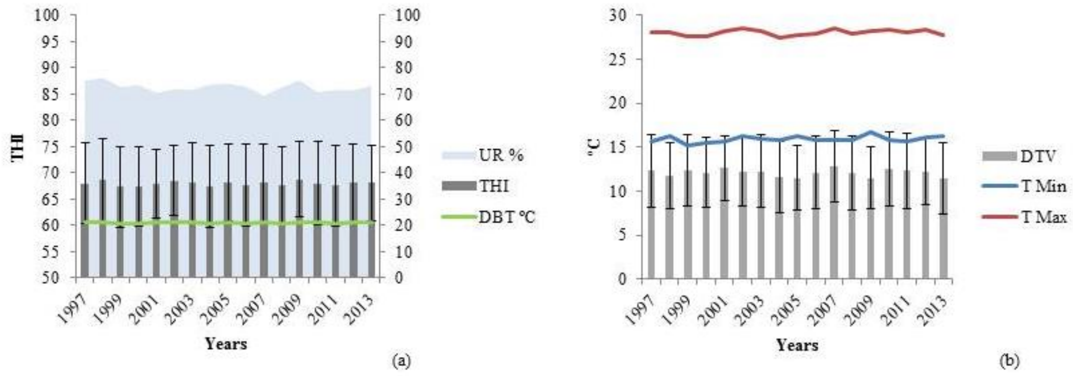
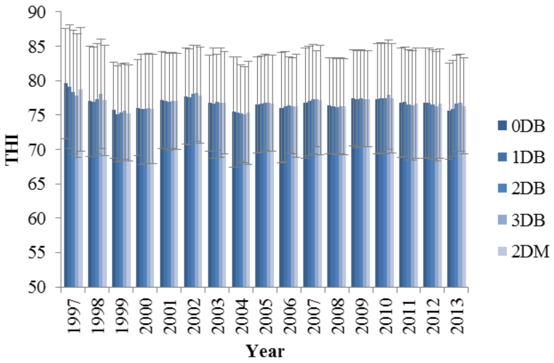


Figure 1. Annual average bioclimatic information: Dry bulb temperature (DBT, °C), relative humidity (UR, %) and temperature-humidity index (THI) (a); diurnal temperature variation (DTV, °C), maximum temperature (T Max, °C) and minimum temperature (T Min, °C) (b).



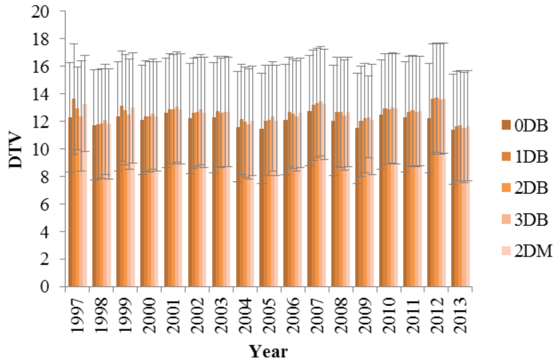


Figure 2. Average estimates of the temperature-humidity index (THI) and diurnal temperature variation (DTV) according to the evaluation day: THI and DTV on the TD record (0DB); one day before (1DB); two days before (2DB); three days before (3DB), and mean between the two last days before each TD (2DM).

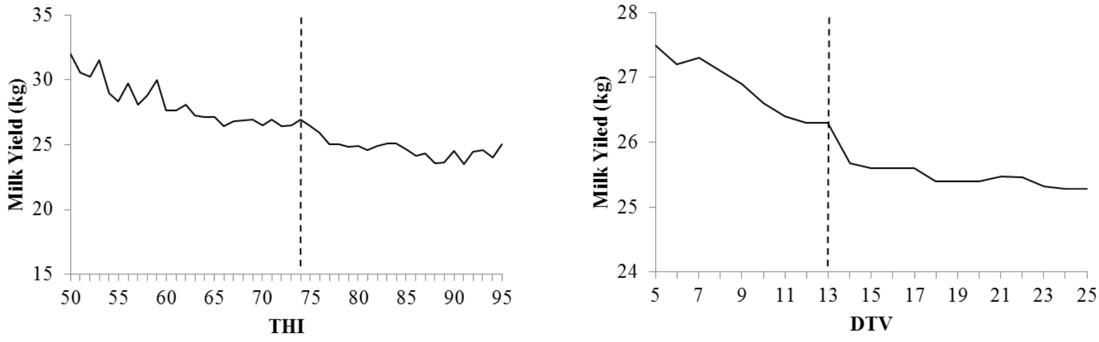


Figure 3. Heat stress threshold (dotted line) and average daily milk yield corrected according to temperature-humidity index (THI = 74) and diurnal temperature variation (DTV =13).

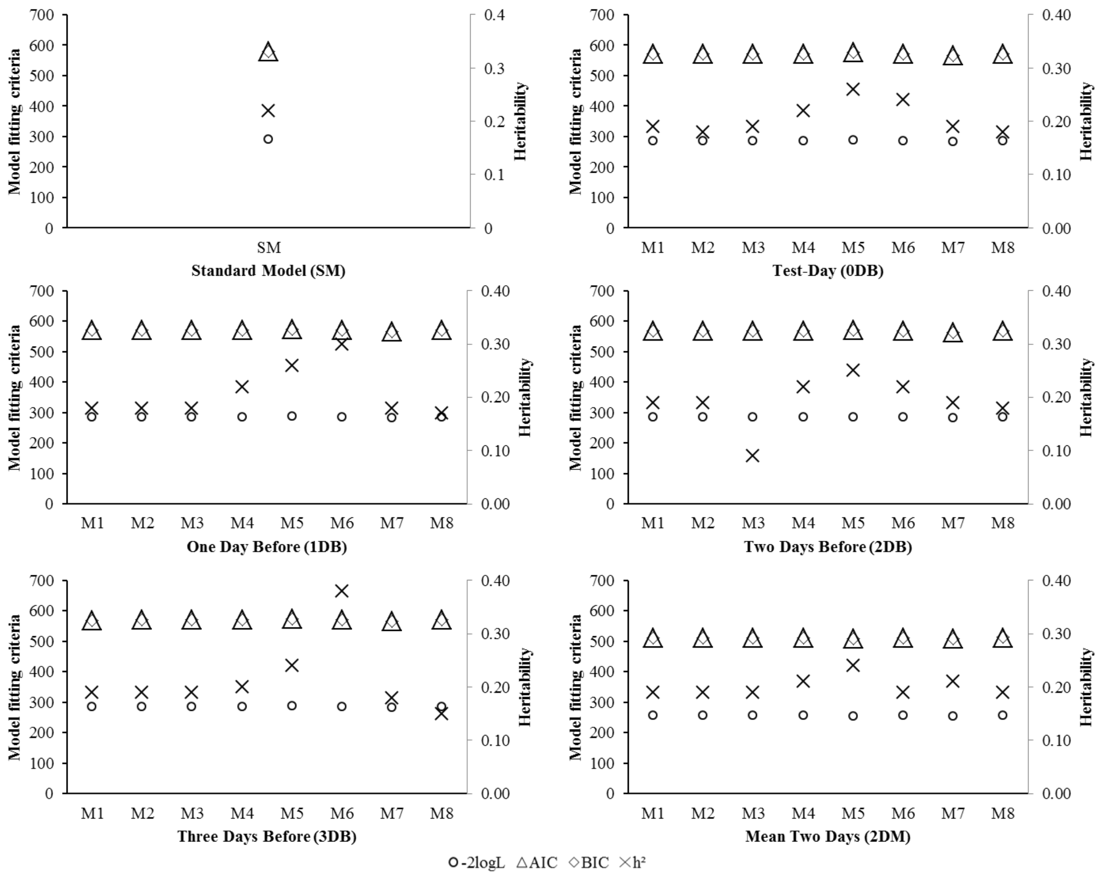


Figure 4. Estimates of models fitting criteria: log-likelihood function (-2logL), Akaike information criterion (AIC) and Schwarz Bayesian information criterion (BIC); and heritability estimates for 305 days of milk production (h^2), for each model (M1 to M8) and day of inclusion of bioclimatic variables.

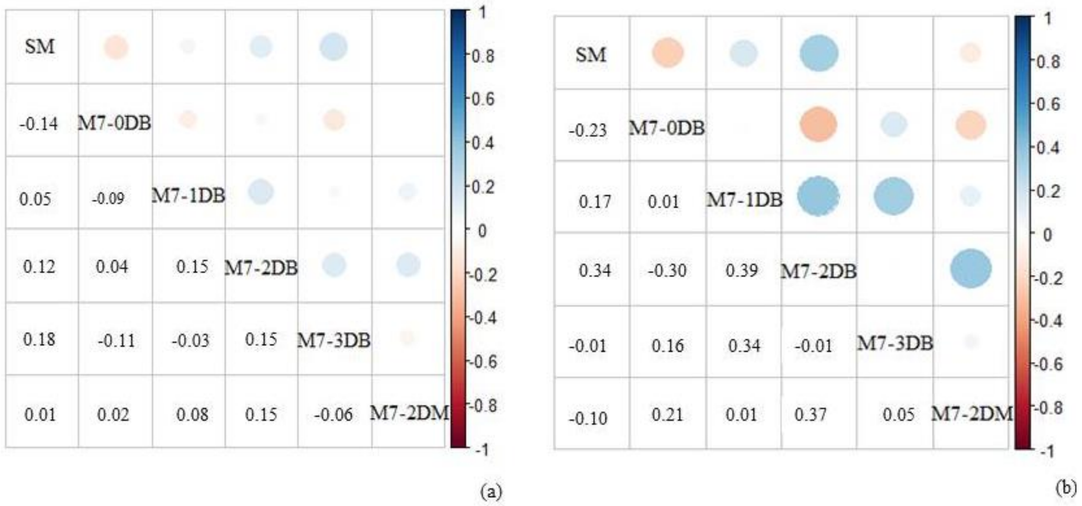


Figure 5. Spearman rank correlations coefficient for the top 10% (a) and top 1% (b) best sires (estimated breeding values - EBVs) for milk yield, according to the standard model (SM).

Standard model (SM), and best adjusted model (M7) for the days included with THI and DTV data — on TD record day (M7-0DB); one day before (M7-1DB); two days before (M7-2DB); three days before (M7-3DB), and mean between the two last days before (M7-2DM).

Positive correlations are displayed in blue and negative correlations in red color. Color intensity and the size of the circle are proportional to the correlation coefficients.

CAPÍTULO III

Selection for test-day milk yield and thermotolerance in Brazilian Holstein cattle[4]

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Selection for test-day milk yield and thermotolerance in Brazilian Holstein cattle

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Simple Summary

Interest in selection for milk yield and thermotolerance in cattle has grown, since heat stress has caused great losses in milk yield. However, few studies on how to carry out concurrent selection are available. Milk yield was analyzed by traditional methods, including heat stress indicators, in genetic evaluation. The results showed that the best sires for milk yield are not the best for heat tolerance, and only a small proportion of individuals have the aptitude for joint selection. Despite a small population fraction allowed for joint selection, sufficient genetic variability for selecting more resilient sires was found, which promoted concomitant genetic gains in milk yield and thermotolerance.

Abstract

Intense selection for milk yield has increased environmental sensitivity in animals, and currently, heat stress is an expensive problem in dairy farming.

The objectives were to identify the best model for characterizing environmental sensitivity in Holstein cattle, using the test-day milk yield (TDMY) combined with the temperature–humidity index (THI), and identify sires genetically superior for heat-stress (HS) tolerance and milk yield, through random regression. The data comprised 94,549 TDMYs of 11,294 first-parity Holstein cows in Brazil, collected from 1997 to 2013. The yield data were fitted to Legendre orthogonal polynomials, linear splines and the Wilmink function. The THI (the average of two days before the dairy control) was used as an environmental gradient. An animal model that fitted production using Legendre polynomials of quartic order for the days in milk and quadratic equations for the THI presented a better quality of fit (Akaike's information criterion (AIC) and Bayesian information criterion (BIC)). The Spearman correlation coefficient of greatest impact was 0.54, between the top 1% for TDMY and top 1% for HS. Only 9% of the sires showed plasticity and an aptitude for joint selection. Thus, despite the small population fraction allowed for joint selection, sufficient genetic variability for selecting more resilient sires was found, which promoted concomitant genetic gains in milk yield and thermotolerance.

Keywords: animal resilience; genotype by environment interaction; longitudinal data

Introduction

Dairy breeding programs have traditionally focused on selection for milk yield. This intense selection has increased sensitivity to environmental changes in animals. Climate and variability in climate have negatively affect milk yield due to impacts on metabolic efficiency and immune responses [1]. Currently, heat stress is an expensive problem in dairy farming.

Garcia et al. [2] observed a 21% milk yield loss in a commercial herd of Holstein cows in southern Brazil caused by heat stress. According to Pegorer et al. [3], approximately 60% of dairy farms in the world are in heat-stress environments. Heat stress decreases milk yield by 30% to 40% [4], which represents approximately 600 to 900 kg of milk per lactation per cow [5], and can exceed 1300 kg of milk per cow [6]. The impact of heat stress on the dairy cattle industry resulted in an economic loss of USD 900 million in 2012 [4], and the estimated loss in 2014 was USD 1.2 billion for the US dairy sector [7].

According to Sigdel et al. [8] and Ansari-Mahyari et al. [9], a possible strategy for reducing the effects of heat stress on dairy cattle is the selection of genetically more thermotolerant animals. However, it requires methodology that allows for the identification and subsequent selection of animals according to specific regions and climates.

Brazil has a diverse climate, from warm and dry/humid to cold and humid climates, due to a wide variation in longitude, latitude, and altitude in its territory and the effects of coastal, continental vegetation [10]. In addition, it is affected by seasonal factors, which influence the management of herds. In warmer conditions, animals tend to be kept on pasture (to use the grass cycle), while in colder periods, animals are semi-confined and supplemented with silage [11]. Thus, each region of the country is more suitable and productive for specific dairy cattle genotypes, which requires a combined selection for heat tolerance and milk yield for each region.

Different thermotolerances are found between and within dairy cattle breeds. The selection of animals within a breed is an alternative when crossbreeding is not feasible. Physical sizes, metabolic rates and productive levels have been associated with thermotolerance [12].

Ravagnolo and Misztal [13] described a method to identify the most resilient animals regarding heat tolerance, proposing a random regression method that quantifies the heat stress level based on climate information (the temperature–humidity index) on the test day. This methodology allows for the detection of the presence of genotype-by-environment interactions (GxEs), and the modeling of animal performance as a function of the environmental gradient.

The evaluation of decreases in milk yield per THI-unit increase from a determined threshold is a method for predicting the relationship between production and climate conditions [5]. The THI is a bioclimatic index commonly used to determine heat stress in cattle [14]. Diurnal temperature variation (DTV) also has potential for use as an environmental indicator of heat stress [15].

Several hypothetical models and fitting equations have been proposed to estimate the effect of heat stress. A combination of methods allows the identification of the best methodology for explaining the variation in genetic and non-genetic components within an environmental gradient. This enables critical genetic analysis and the reduction of the negative effects of heat stress on dairy cattle performance and impacts the dairy production chain.

In this context, the objective of this study was to identify the best model for characterizing environmental sensitivity in Holstein cattle in Brazil, using the test-day milk yield combined with temperature–humidity index data from public weather stations, and identify sires genetically superior in terms of heat tolerance and milk yield through random regression models.

Materials and Methods

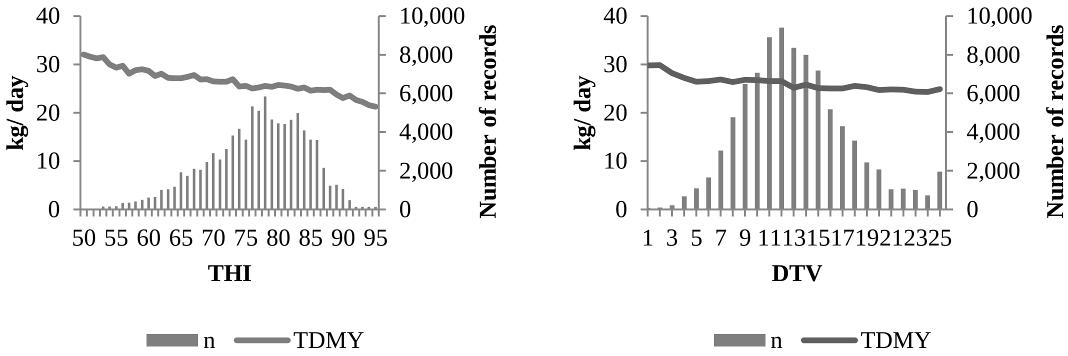
Climate Data

Bioclimatic data from 18 weather stations (representing 86 municipalities) were obtained from the Instituto Nacional de Meteorologia. The stations were located within 60 km of the evaluated farms. The temperature–humidity index (THI) was calculated according to the equation described by the National Research Council—NRC [16]:

$$THI = [(1.8 \times DBT + 32) - (0.55 - (0.0055 \times RH) \times (1.8 \times DBT - 26))]$$

where DBT is the dry bulb temperature (°C) and RH is the relative humidity (%). The THI was found to range from 50 to 95.

The diurnal temperature variation (DTV) was calculated by the difference between the daily maximum and minimum temperatures (°C). The DTV was found to range from 2 to 25. The numbers of test-day milk yield records by the THI and DTV are shown in Figure 1.



(a) **(b)**

Figure 1. Number of records (n) and means of test-day milk yield (TDMY) per temperature–humidity index (THI) (a) and diurnal temperature variation (DTV) (b) in Brazilian Holsteins cattle.

The THI and DTV daily averages used were calculated based on averages of two days (one and two days before the test-day milk yield records), as described by Negri et al. [17].

Climate Data

The test-day milk yield (TDMY) data, provided by the Associação de Criadores de Gado Holandês de Minas Gerais (ACGHMG), consisted of records of Holstein cows at first lactation in the state of Minas Gerais, Brazil (19º55’ S – 43º57’ W), from 1996 to 2015.

Records of animals with ages at calving <18 or >48 months, days in milk (DIM) <5 or >305 days, and milk yields <4 or >44.8 kg were excluded from the data set. Only healthy animals with at least four TDMY records during lactation were used for analysis. The minimum size of each contemporary group (CG; based on the herd, years and months of the TDMY records) was three animals. Records of daughters of sires with at least one daughter in at least three herds were included in evaluation.

Considering these criteria, a total of 94,549 TDMY records from 11,294 first lactations of Holstein cows, with a mean test-day milk yield of 25.81 kg, from 129 herds, collected from 1997 to 2013, were analyzed. This database was fitted to all the evaluated models. The pedigree file included 32,409 animals. The structure of the dataset after editing is shown in Table 1.

Table 1. Overall traits of first-parity of Brazilian Holstein cattle (SD in parentheses).

Item	Statistics
Number of test-day records	94,549
Number of animals with records	11,294
Number of animals in pedigree file	32,409
Number of dams in pedigree file	8,639
Number of sires in pedigree file	641
Number of contemporary groups	5,257
Number of herds	129
Mean test-day milk (kg)	25.81(7.21)
Mean records/animal	8.37

Models

Five models (M1 to M5) and three fitting equations were used to analyze the TDMY records: the Wilmink parametric function (WL) [18], linear splines (LS) [19], and Legendre orthogonal polynomial (LP) [20]. Additive genetic and permanent environmental (co)variance functions were regressed to the THI, DTV and DIM, according to the models described below.

Model 1 (M1): The contemporary group, milking frequency (two and three times a day), variable t (described below the models), and DIM with 60 classes (every five units of DIM was considered a class: DIM 5 to 10 = class 1, DIM 11 to 15 = class 2 . . . DIM 300 and 305 = class 60) were fixed effects. The fixed curve (described below the models), additive genetic and permanent environmental functions were regressed to the THI, using the LP (4th order), LS (4 knots) and WL.

Model 2 (M2): The contemporary group, milking frequency and variable *t* were fixed effects. The fixed curve, additive genetic and permanent environmental functions were regressed: the DIM using the LP (4th order), and to the THI using the LP (2nd order); to the DIM using LS (4 knots) and to the THI using LS (3 knots); to the DIM and THI using the WL.

Model 3 (M3): The contemporary group, milking frequency and variable *t* were fixed effects. The fixed curve, additive genetic and permanent environmental functions were regressed: to the DIM using the LP (4th order), and to the THI and DTV using the LP (2nd order); to the DIM using LS (4 knots), and to the THI and DTV using LS (3 knots); to the DIM, THI and DTV using the WL.

Model 4 (M4): The contemporary group, milking frequency, variable *t* and DTV (with5 classes: DTV 2 to 6 = class 1, DTV 7 to 11 = class 2 . . . DTV 22 to 25 = class 5) were fixed effects. The fixed curve, additive genetic and permanent environmental functions were regressed: to the DIM using the LP (4th order), and to the THI using the LP (2nd order); to the DIM using LS (4 knots), and to the THI using LS (3 knots); to the DIM and THI using the WL.

Model 5 (M5): The contemporary group, milking frequency, variable *t* and DIM with 60 classes (every five units of DIM was considered a class: DIM 5 to 10 = class 1, DIM 11 to 15 = class 2 . . . DIM 300 and 305 = class 60) were fixed effects. The fixed curve, additive genetic and permanent environmental functions were regressed: to the THI and DTV using the LP (2nd order); to the THI and DTV using LS (3 knots); to the THI and DTV using the WL.

A dummy variable *t* was defined to estimate the decreases in milk yield caused by heat stress (HS). The threshold for HS used was a THI of 74, based on Negri et al. [17]. Therefore,

if THI ≤ 74, *t* = 0 (no heat stress); *else if THI > 74 then t* = THI - 74

The fixed curves considered in all the models were defined by the age classes—1 (18 to 25 months), 2 (26 to 27 months), 3 (28 to 29 months) and 4 (30 to 48 months)—combined with calving season subclasses—1 (rainy: October to March) and 2 (dry: April to September)—totaling eight fixed curves. The residual variance was considered homogeneous in all the models (Table 2).

Table 2. Model layout.

Models	Fixed Effects					Regressor		
	Contemporary group	Milking Frequency	Variable t	DIM	DTV	DIM	THI	DTV
M1	*	*	*	*	-	-	○○○○	-
	*	*	*	*	-	-	+ + + +	-
	*	*	*	*	-	-	◊ ◊ ◊	-
M2	*	*	*	-	-	○○○○	○○	-
	*	*	*	-	-	+ + + +	+ + +	-
	*	*	*	-	-	◊ ◊ ◊	◊ ◊ ◊	-
M3	*	*	*	-	-	○○○○	○○	○○
	*	*	*	-	-	+ + + +	+ + +	+ + +
	*	*	*	-	-	◊ ◊ ◊	◊ ◊ ◊	◊ ◊ ◊
M4	*	*	*	-	*	○○○○	○○	-
	*	*	*	-	*	+ + + +	+ + +	-
	*	*	*	-	*	◊ ◊ ◊	◊ ◊ ◊	-
M5	*	*	*	*	-	-	○○	○○
	*	*	*	*	-	-	+ + +	+ + +
	*	*	*	*	-	-	◊ ◊ ◊	◊ ◊ ◊

DIM: Days in milk; DTV: Diurnal temperature variation; THI: Temperature–humidity index; * Considered; - not considered; ○○ Legendre orthogonal polynomial LP (2nd order); ○○○○ LP (4th order); + + + Linear splines LS (3 knots); + + + + LS (4 knots); ◊ ◊ ◊ Wilmink parametric function WL.

Analysis of Models

Random regression models (RRM) were used for analysis. Henderson's mixed model equations [21] for RRM can be described as follows:

$$\begin{bmatrix} X'R^{-1}X & X'R^{-1}Z & X'R^{-1}W \\ Z'R^{-1}X & Z'R^{-1}Z + A^{-1} \otimes G_0^{-1} & Z'R^{-1}W \\ W'R^{-1}X & W'R^{-1}Z & W'R^{-1}W + I \otimes P_0^{-1} \end{bmatrix} \begin{bmatrix} \hat{b} \\ \hat{a} \\ \hat{p} \end{bmatrix} = \begin{bmatrix} X'R^{-1}y \\ Z'R^{-1}y \\ W'R^{-1}y \end{bmatrix}$$

where **y** is the vector of observations; **X**, **Z** and **W** are the incidence matrices for the fixed effects (**b**), additive genetic random regression coefficients (**a**), and permanent environmental random regression coefficients (**p**), respectively; **A** is the additive genetic numerator relationship matrix based on pedigree information; and **I** is an identity matrix. **G**0 and **P**0 are the (co)variance matrices of the additive genetic and permanent environmental random regression coefficients, respectively, and **R** is the (co)variance matrix of the residual.

All the genetic analyses were performed with an animal model, using the REMLF90 program [22]. Considering the REML estimation method, the model assumptions can be described as:

$$\begin{bmatrix} y \\ a \\ p \\ e \end{bmatrix} \sim N\left(\begin{bmatrix} X\beta \\ 0 \\ 0 \\ 0 \end{bmatrix}; \begin{bmatrix} ZGZ'+WPW'+R & ZG & WP & R \\ & A\otimes G_0 & \phi & \phi \\ & & I\otimes P_0 & \phi \\ Sim. & & & R \end{bmatrix} \right)$$

where **e** is the vector of the residuals, and all the other terms were previously defined. The genetic (Σ) and environmental (Φ) (co)variance matrices for time points can be obtained as follows (assuming the same function for factors):

$$\boldsymbol{\Sigma} = \boldsymbol{T}\boldsymbol{G_0}\boldsymbol{T}' \text{ and } \boldsymbol{\Phi} = \mathbf{T}\mathbf{P_0}\mathbf{T}'$$

where **T** is a matrix of independent covariates for all time points (DIM, THI or DTV) associated with the model and function used.

The quality of fit was evaluated considering non-nested models and penalties, according to the number of parameters to be estimated. The following criteria were used: the maximum likelihood estimation (—2logL), Akaike's information criterion (AIC = —2logL + 2p, where p is the number of parameters in the model), and Schwarz's Bayesian information criterion (BIC = —2logL + p log (λ), where log (λ) is the natural logarithm of the sample size (or dimension of *y*) and p is the number of parameters in the model). The BIC is more rigid than AIC. The model with the lowest value for both criteria was the one with the best fit.

The estimated breeding value (EBV) of an animal i obtained with M4 was computed using DIM (EBV_TDMY) and environmental gradient (THI values, EBV_HS) information, according to the equation

$$EBV_l^{j,k} = \phi_{(j)k}\hat{a}_i^{'}$$

where $\hat{a}_i^{'}$ the vector of the estimated additive is genetic values for the orthogonal regression coefficients of animal *i* (coefficients corresponding to DIM and THI) and $\phi_{(j)k}$ is a vector of the orthogonal coefficients evaluated in THI *j* and DIM *k*.

The top 1% and 5% dairy Holstein sires (with at least 20 daughters) for EBV_TDMY and EBV_HS were sampled to represent the resilience of the animals, considering the DIM and THI scale (i.e., reaction norms).

Results

Loss in Milk Yield and Adjustment of Models

Considering only the THI, approximately 31% of all the TDMY records were obtained under heat-comfort conditions (<THI 74) and 69% were under HS conditions. The data show that the milk yield tended to decrease as the THI was increasing. The mean and standard deviation of the TDMY were 28.57 ± 1.8 kg for THI < 74, and 23.95 ± 1.9 kg for THI > 74.

Approximately 46% of all the TDMY records were obtained at DTV within heat comfort conditions (<DTV 13), and 54% were in HS conditions. The mean and standard deviation of the TDMY records were 27.33 ± 1.3 kg for DTV < 13 and 24.94 ± 0.4 kg for DTV > 13. The phenotypic value tended to decrease as the DTV increased. However, slope is more representative for assessing the THI effect.

Five models that used the LP to fit fixed and random curves showed better quality of fit (Figure 2). The best model according to the information criteria (AIC and BIC) was M4, with AIC = 508,170 and BIC = 508,264, which regresses data to the DIM and THI, and included the DTV as a fixed effect. Thus, in addition to the model minimizing the Kullback–Leibler divergence (related to missing information), the probability of fit of the true model is maximized.

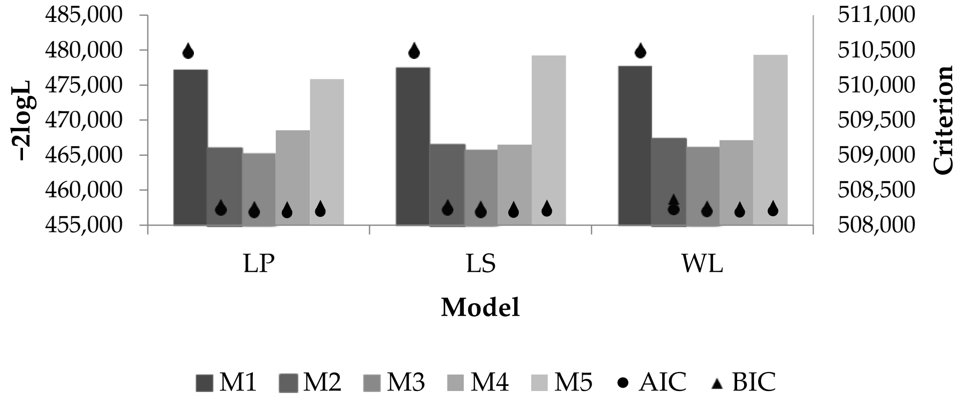


Figure 2. Estimates of maximum of likelihood function (—2logL), Akaike's information criterion (AIC) and Schwarz's Bayesian information criterion (BIC) according to evaluation model and random effects adjustment equation of test-day milk yield (TDMY) in Brazilian Holsteins.

AIC includes the complexity and predicted ability of the data to fit the model, and is linked to the BIC due to the probability function. However, the BIC penalizes models more because of the number of parameters. Thus, the quality of fit (AIC and BIC) indicates the same model, confirming that the chosen model is the most appropriate.

Heritability

The overall heritability estimate for the TDMY regressed to the THI ranged from 0.15 to 0.21 when using the LP equation (Figure 3), while for the WL, it ranged from 0.11 to 0.19, and when using LS, it varied from 0.07 to 0.22.

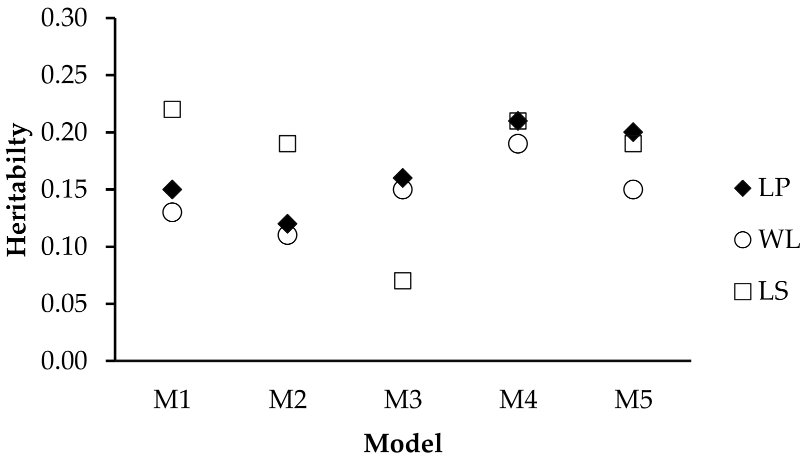


Figure 3. Estimated average heritability for test-day milk yield (TDMY) according to temperature– humidity index (THI) according to model and using Legendre polynomials (LP), linear splines (LS) and Wilmink (WL) in Brazilian Holsteins.

The heritability estimated as a function of lactation in M4 using the LP (best fit) weighted by the THI and DIM, after the THI threshold (THI = 74), showed a decrease (Figure 4). The environmental variation increased, and the additive genetic variation decreased, directly interfering with the heritability of the trait. Thus, the highest selection responses can be expected for the thermal-comfort range. The extrapolation of estimates close to the beginning (DIM 5) and end (DIM 305) of lactation was due to the low number of registered TDMYs.

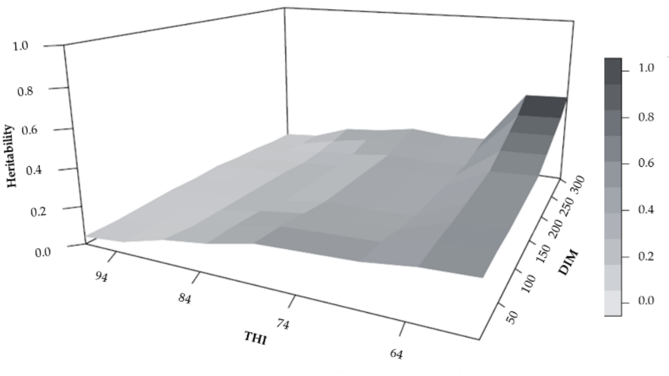


Figure 4. Estimated heritability for test-day milk yield, TDMY, according to temperature–humidity index (THI) and days in milk (DIM) according to model 4 (M4) using Legendre polynomials.

The fixed effects solution (BLUE) for DTV shows that an increase in the variation temperature is reflected in a decrease in milk yield (Figure 5). The fixed effect considered in model that regresses to the DIM and THI (M4) better explains the behavior of the lactation curve, since the temperature variation needs to be nested at some thermal reference point in order to define the magnitude and importance of its effect. Thus, the use of DTV should always be nested in a heat-stress indicator, in order to anchor the inferred heat comfort.

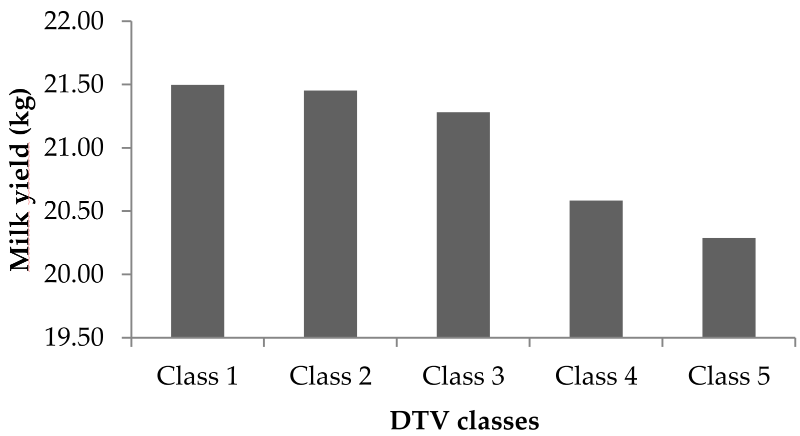


Figure 5. Solution of fixed effect (BLUE) of temperature variation (DTV) according to classes considered in the model 4 (M4) model using Legendre polynomials. Classes: DTV 2 to 6 = class 1; DTV 7 to 11 = class 2; DTV 12 to 16 = class 3; DTV 17 to 21 = class 4; DTV 22 to 25 = class 5.

The existence of additive genetic variability for the slope of the environmental gradient indicated the presence of G x E interaction (Figure 6). Some of the best sires (EBV_TDMY) (Figure 6 a,b) had interesting EBV_HS (Figure 6 c,d).

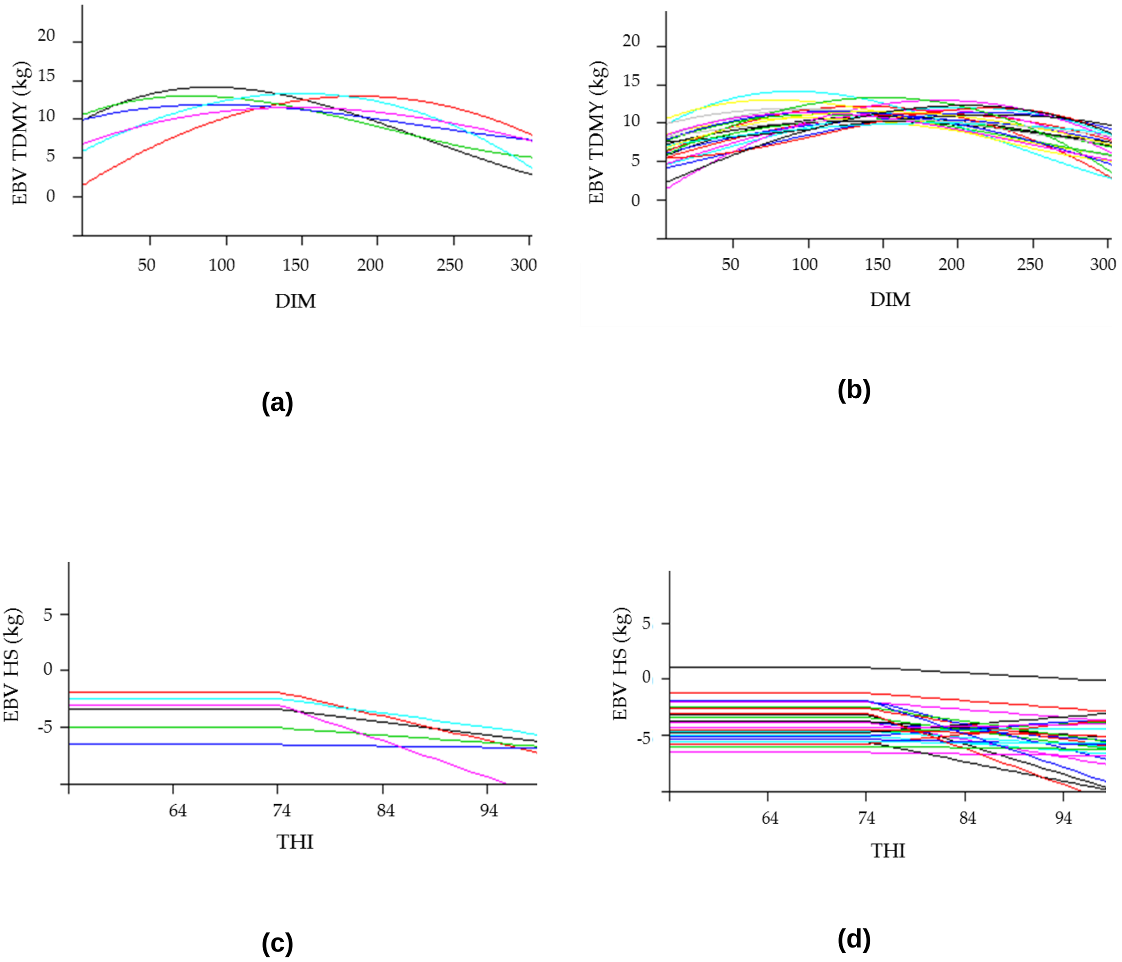


Figure 6. Estimated breeding value gradient (i.e., reaction norm model) for best sires: top 1% (**a**) and top 5% (**b**) for test-day milk yield (EBV_TDMY) and for tolerance to heat stress (EBV_HS) of the same sires (**c,d**).

Approximately 9% of the sires were resilient to changes in the THI, considering their EBV for TDMY and HS tolerance; however, approximately 30% showed probable plasticity. This allows the selection and formation of a resilient lineage with good productivity.

The reranking of sires was confirmed by the Spearman rank correlation coefficients, comparing the genetic evaluation for TDMY and thermotolerance (Figure 7). Therefore, the Holstein sires with the best EBV_TDMY records in favorable environments may not be the best under EBV_HS conditions. Moreover, the impact of reranking is higher when the selection pressure is the strongest (Top 1%).

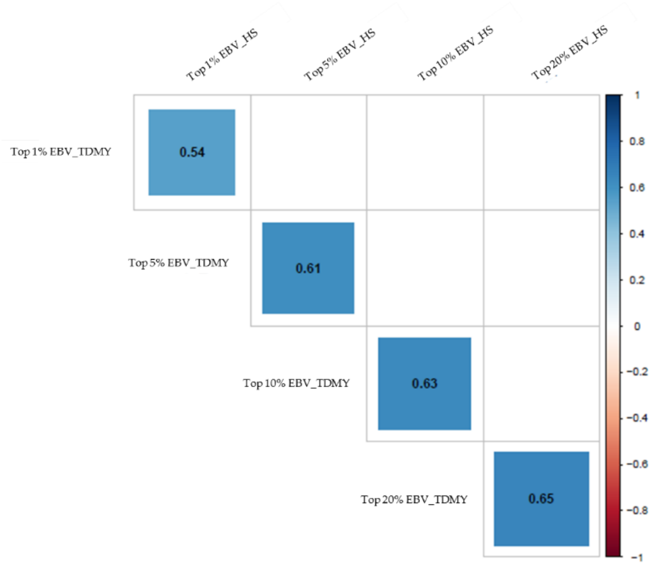


Figure 7. Spearman rank correlation coefficients for top 1%, top 5%, top 10% and top 20% best
sires (estimated breeding values—EBVs) regarding test-day milk yield (TDMY) and heat stress (HS), according to model 4 (M4) using Legendre polynomials. The colors inside the squares indicate the magnitudes and directions of the associations.

The database, in which 90% of the records were measured under heat-stress conditions, showed that the traditional model (which does not consider heat stress) is inefficient for estimating the EBV when animals are under heat stress. Thus, considering two situations, when sires are considered equally in heat comfort (THI = 74) and in heat stress (THI = 84), sires with less than 40 daughters are the most penalized, and their EBVs are underestimated (Table 3).

Table 3. Efficiency in estimating breeding value for test-day milk yield (EBV_TDMY) of Brazilian Holstein cattle under conditions of homeostasis and heat stress.

		EBV_TDMY	
N Daughters	**Sires**	**THI 74**	**THI 84**
>101	13	100%	91%
51 to 100	43	100%	84%
41 to 50	12	100%	84%

31 to 40	23	100%	75%
21 to 30	59	100%	74%
11 to 20	127	100%	73%
<10	364	100%	71%

The effect of the genotype-by-environment interaction is minimized and the traditional methodology is not biased when a sire has more than 101 daughters in several herds. However, some breeders intensively use high EBV_TDMY, and others use them less often. This unequal contribution to adaptive values makes the estimates biased.

Discussion

Most dairy cows in Brazil are kept in open barns and in grazing systems [23–25]. Minas Gerais has three predominant climate types—subtropical at altitude, subtropical with dry winters, and tropical with dry winters—according to the Köppen—Geiger classification. Thus, environmental factors such as the THI and DTV have a direct impact on cow productivity. Lactating dairy cows must be in favorable environments because stresses negatively affect cow maintenance, milk yield, growth, the preservation of body condition (health) and reproduction.

Climate changes may increase heat-stress levels in dairy cows; thus, these environmental phenomena, which frequently cause droughts, heat waves, storms and floods, should be considered [26]. Increases in climate variability have negatively impacted livestock production, especially dairy farming [15,27]. Temperatures in tropical regions increased by 0.1 to 0.3 °C per decade between 1951 and 2000 because of increases in greenhouse gases [26], and the variations in temperatures have increased by 0.7 to 0.8 °C because of the El Niño Southern Oscillation (ENSO) over the past century [28]. However, the selection of animals for milk yield has not considered animals' tolerance to heat stress, as shown in the present study.

These changes justify and foster research that evaluates the effects of climate variables on animal production, mainly regarding genetic breeding, in which the effects are cumulative and long term. In addition, the use of heat stress indicators collected from public weather stations, rather than directly from farms, has been widely explored for inclusion in genetic evaluation. According to Lee et al. [29], the evaluation should not be affected by this substitution, because the use of contemporary groups compensates for effects at the farm, management, nutrition and technological levels.

Legendre polynomials are more widely used for these evaluations [30–33]. Despite the complexity of these assessments, better results can be obtained when considering the two-day average of climate variables for test-day milk yield, including the DTV as a fixed effect in the model and regressing data to the THI and DIM.

A low number of observations of extremes of lactation may affect the prediction coefficients of functions fitted through random regression [34]. The same database was fitted to LP, WL and LS functions, and the LP was the more robust model, considering possible biases caused by the low number of observations at the ends of the gradient.

The sooner HS is detected, the greater the chances of keeping more resilient animals in production and, consequently, the more productive they are in different heat conditions. According to Aguilar et al. [35], the genetic variance of heat stress for milk yield increases significantly from the first lactation. Thus, cows become more sensitive to heat stress as the number of parities is increased. Thus, detecting susceptibility to heat stress in the first lactation allows the prediction of losses in subsequent lactations.

Brügemann et al. [36] emphasize that the effect of heat stress can suppress the expression of animals' genetic potential, and reported that a random regression model showed a trend of higher heritability in the THI range corresponding to the comfort zone of cows, as found in the present study. Thus, the selection of these superior environments can contribute to accurate genetic differentiation among candidates for selection.

The use of random regression to detect G x E fits a variance–covariance structure of repeated measures along a gradient for traits such as the TDMY [37]. Similarly, the model proposed by Kolmodin et al. [34], using covariance functions, is a good G x E indicator.

Selecting sires only by EBV_TDMY, disregarding the herd rearing system, technological level and bioclimatic conditions of a region, may compromise the genetic gain of herds. Moreover, investments in high-merit genetic material from animals evaluated in highly technological environments should not be recommended for farmers in environments with low technological investments.

An ideal curve would be a high and constant EBV_TDMY under different THIs; however, few animals presented this desired profile. Thus, considering the needs for breeding of each herd is important for more quicky increasing the TDMY or tolerance to HS. Each sire has a thermotolerance slope limit, as shown by the difference between the curves, which allows the better targeting of sires for different uses, according to farmers' demands.

The results shown in Figure 6 denote the possibility of using individual random slopes as a selection criterion for resilience (animals that withstand variations that can cause heat stress). Animals with a subtle slope are less sensitive to environmental variation. The results indicate high genetic variations in sires within different environments.

This high variability indicates that the apparent genetic merit of sires for milk yield may change depending on the environment, generating great concern about genetic evaluation and the choice of sires, which can be affected by this dependence. Phenotypic and genetic parameters are dependent on the population and environment, and can have different magnitudes, resulting in heterogeneous variations. Thus, changes in variances and the covariance would promote changes in important parameters, such as heritability, repeatability and correlations, which may result in incorrect choices of selection methods adopted in a breeding program.

According to Santana et al. [31], the flattening of the slope is particularly important for countries with a hot climate when focusing on selecting animals with high production levels and tolerant to heat stress. In tropical climate regions, the effects of environmental variations can be addressed by different methods of genetic evaluation. Thus, some applicable options can be used to simultaneously select animals for TDMY and HS *in loco*: the creation of a selection index for the simultaneous selection and targeting of mating to develop a tolerant lineage. However, it requires attention to avoid estimation biases. Negri et al. [17] pointed out the importance of correcting test-day milk yield data using heat-stress indicators and reported significant increases in estimates of repeatability and the reranking of sires, especially for sires with fewer daughters.

Conclusions

The existence of genetic variation for sensitivity to heat stress allows for the selection of genetically resilient animals.

The most adequate selection methodology for improving heat tolerance without decreasing productivity includes diurnal temperature variation as a fixed effect and regresses data to the temperature–humidity index and days in milk.

Legendre polynomials should be used to ensure better predictions of the estimated breeding value and determine the genetic effect of heat stress through random regression models.

Antagonism between the test-day milk yield and heat stress was confirmed, and enabled the use of heat tolerance as a selection criterion for improving animal thermotolerance and productivity simultaneously. The development of selection indexes weighted by technological levels or sire summaries that include information on the environmental gradient could be viable solutions. The selection and development of more thermotolerant lineages is a more attractive option than the development of selection indexes.

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CAPÍTULO IV

Heat stress effects on somatic cell score of Holstein cattle in tropical environment[5]

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Heat stress effects on somatic cell score of Holstein cattle in tropical environment

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ABSTRACT

Considering the importance of dairy farming and negative effects of heat stress, the objective of this study was to investigate the effect of heat stress via temperature-humidity index (THI) and diurnal temperature variation (DTV) for somatic cell score (SCS) of Holstein dairy cattle, using random regression models. Data were a total of 52,012 test-day records for SCS of 9,765 first parity Holstein cows from Brazil, collected from 1997 to 2013, along with weather records (THI and DTV) from 18 weather stations. Least square linear regression models were used to determine THI and DTV thresholds for SCS increase caused by heat stress. In addition to the standard model (SM; without bioclimatic variables), THI and DTV were combined in various ways and tested for different days, totaling 21 models. Thresholds of THI and DTV for SCS increase was 70 (0.09 score unit/THI) and 9 (0.03 score unit/DTV), respectively. The model that included THI and DTV as fixed effects, considering the two days average, presented better fit (AIC, BIC and -2logL). Estimated breeding values (EBVs) and reliability of EBVs improved when using this model. Changes on SCS may be an early indicator of heat stress in Holstein cattle reared in tropical conditions. The sires are re-ranked when bioclimatic variables are included in the model. More significant reclassifications of sires were observed when we increased selection pressure. This study provides strong evidence of a genotype by environment interaction on SCS. Genetic evaluation using average of two days of THI and DTV as fixed effects, improves EBVs and reliability of EBVs.

Keywords: diurnal temperature variation, random regression, somatic cell count, temperature-humidity index.

1. Introduction

Negative effects of heat stress in dairy cattle have been widely investigated because of economic losses in milk chain worldwide (Summer et al., 2019). At first signs of heat stress, animal physiology triggers specific neuroendocrine defense mechanisms to prevent a collapse of body system, seeking to preserve body health (thermoregulation).

To minimize the effects of heat stress, animals increase their respiratory rate (causing respiratory alkalosis) (Benjamin, 1981), reduce their dry matter intake and rumination (Collier and Zimbelman, 2007) as measures of thermoregulatory function. Consequently there is an increase in somatic cell count (SCC) (Harmon, 1994), drop in milk production (Perissinotto et al., 2007), and reduction of reproductive signals (Hansen, 2007).

Reduced immunity of animals due to heat stress increases SCC of milk of animals (Roma Jr et al., 2009). Somatic cell count in milk can be used as a measure of mammary gland health and milk quality (Quintao et al, 2017). It quantitatively indicates the degree of mammary gland infection, usually in response to invasive agents, which can be characterized by an increase SCC (Habbeb et al., 2018). This trait or a logarithmic transform, called somatic cell score (SCS), is used in some genetic evaluation schemes, aiming to lower prevalence of mastitis by indirect selection for SCC (Sadeghi-Sefidmazgi and Rayatdoost-Baghal, 2014).

Investigative hypothesis is that changes in mammary gland health occur before a decrease of milk yield, as a body defense mechanism. Detecting this indicator early, could assist in decision making to minimize the effects of heat stress.

Temperature-humidity index (THI), widely used to detect heat stress in cattle, and is a single value that incorporates the effects of both temperature and relative humidity (Collier et al. 2012). A high THI has been associated with increased SCS in several studies (Bouraoui et al., 2002; Hammami et al., 2013; Lambertz et al., 2014).

Considering that SCS can be a good indicator of heat stress at an early stage and that it is considered a criterion for payment of milk to farmers, the objective of work was to investigate, at a genetic level, the effects of heat stress in SCS of Holsteins in a tropical environment.

2. Material and methods

2.1 Data

Score cell count (SCC) in test-day data, provided by the Service of Associação dos Criadores de Gado Holandês de Minas Gerais, consisted of records of Brazilian first-lactation Holstein cows, and collected between 1997 and 2013. We excluded records with extreme age at calving (<18 or >48 months), days in milk (DIM, <5 or >305 days), and SCC (± 3standard deviation) from the data set. Somatic cell score was calculated by taking the logarithm of SCC, SCS = $\log_2$(SCC/100) + 3 (Ali and Shook, 1980).

Only healthy animals with at least four records during lactation were used in analysis. Minimum size of each contemporary group (herd-year- month of records) was three animals. Records of daughters of sires with at least one daughter in at least three herds were accepted for evaluation.

Considering these criteria, a total of 52,012 test-day records from 9,765 first-lactation Holstein cows, were analyzed. This database fitted to all evaluated models. The pedigree file included 30,807 animals. The dataset structure after editing is shown in Table 1.

2.2 Bioclimatic variables

Bioclimatic data from 18 weather stations (representing 86 municipalities) were obtained from Instituto Nacional de Meteorologia. Stations were located within 60 km from evaluated farms. Temperature-humidity index (THI) was evaluated according to equation described by National Research Council (NRC, 1971):

$$THI = (1.8 \times DBT + 32) - [(0.55 - 0.0055 \times RH) \times (1.8 \times DBT - 26)]$$

In which DBT is dry bulb temperature (°C) and RH is relative humidity (%).

Diurnal temperature variation (DTV) was calculated by the difference between daily maximum and minimum temperatures (ºC) (see Negri et al. 2021).

2.3 Models

Daily averages of THI and DTV were tested up to three days before each test-day record: THI and DTV in test-day record (0DB), one day before (1DB), two days before (2DB), three days before (3DB), and average between the last two days before each test-day (1DB and 2DB) (2DM).

To detection the heat stress threshold for SCS, we used a 2-phase linear regression procedure applied to least squares means. Fixed effects of model were considered: contemporary group, milking frequency, and days in milk (DIM). Age of cows at calving was considered as covariate (linear effect). Significant increase in SCS was estimated considering average THI and DTV values (only 2DM, see Negri et al., 2021).

Random regression models (RRM) were used for analysis of test-day SCS, using fourth-order Legendre polynomial regression coefficients (Kirkpatrick et al., 1994). Additive genetic and permanent environmental covariance functions were estimated by RRM of DIM.

Standard RRM does not consider bioclimatic variables for correction of the data, as shown below:

Standard model (SM): Contemporary group and milking frequency (two or three) as fixed effects.

M2 to M5 models were used to verify best way to include bioclimatic data for correction of genetic evaluation model for SCS, according to day included with THI and DTV data: 0DB, 1DB, 2DB, 3DB and 2DM, totaling 20 models each.

Model 2 (M2): Contemporary group, milking frequency (two or three), and THI as fixed effects.

Model 3 (M3): Contemporary group, and milking frequency (two or three) as fixed effects; THI is linear covariate.

Model 4 (M4): Contemporary group, milking frequency (two or three), THI and DTV as fixed effects.

Model 5 (M5): Contemporary group and milking frequency (two or three) as fixed effects; THI and DTV are linear covariates.

Residual variance was considered homogeneous for all models. Models are summarized in Table 2.

2.4 Genetic evaluation and statistical analysis

All genetic analyses were performed with an animal model, using REMLF90 program (Misztal et al., 2002). Quality of fit was evaluated considering non-nested models and penalties, according to the number of parameters to be estimated. Following criteria were used: log-likelihood function

(-2logL); Akaike's information criterion ($AIC = -2\log L + 2p$, in which *p* is the number of parameters in model); and Schwarz's Bayesian information criterion ($BIC = -2\log L + p \cdot \log(\lambda)$, in which log (*λ*) is natural logarithm of sample size (or dimension of *y*) and *p* is the number of parameters in model). Schwarz's Bayesian information criterion is more rigid than AIC. The model with lowest value for both criteria was one with best fit.

To check re-ranking of estimated breeding values (EBVs) of sires, Spearman rank correlation coefficient for Top 1% and Top 10% EBV sires for SCS was used. It was also verified the proportion of common animals among the Top 10% and Top 1% sires with more than 30 daughters. Reliability of EBVs was calculated using triangular matrices of prediction error (co)variances for random regression effects, from inverse of mixed model equations obtained in BLUPF90 program (Misztal et al., 2002).

3. Results

Climatic conditions were found to exert an influence on SCS in dairy farms in Minas Gerais State, Brazil. The THI threshold for SCS elevation is THI = 70, considering complete lactation (5 to 305 days) (Figure 1). Increase on SCS was 0.09 score unit/THI unit above 70, considering average between the last two days before each test-day (2DM).

Effects of THI stratified according to lactation phase (initial – DIM 5 to 60; intermediate – DIM 61 to 180; and final phase – DIM 181 to 305) showed a significant effect of THI on SCS, with increases of 0.16 (DIM 5 to 60, p-value <0.0001), 0.16 (DIM 61 to 180, p-value <0.0001), and 0.01 (DIM 181 to 305, not significant). That is, a high impact of heat stress on SCS was observed in initial phase of lactation and peak production, periods of high milk yield.

The DTV threshold for SCS increase was 9, considering complete lactation (5 to 305 days) (Figure 1). Increase on SCS was 0.03 score unit//DTV unit above 9. Effects of DTV stratified according to lactation phase also showed a significant effect on SCS only initial and intermediate phases, with increases of 0.04 score unit in initial, and 0.05 score unit in intermediate phase.

All models that considered bioclimatological variables presented better fit than SM (Figure 2). The best overall fit was for M4 model, considering average between the two last days before each test-day (2DM) (M4-2DM), denoting better quality of fit. That is, average between 1DB and 2DB better explains the SCS increase due to heat stress.

Magnitude of estimated Spearman rank correlations coefficient, confirmed reranking of sires when including bioclimatic variables in models (Figure 3). In Top 1%, EBVs of animals changed when correcting the data for stress indicators (range 0.49 to 0.54 with SM), so selection process may be compromised and observed genetic gains may not be equal to expected genetic gains. Animals of genetic superiority may be being eliminated from mating selection because their daughters are more penalized for disregarding heat stress in evaluation. For Top 10%, no significant reranking was observed (correlation > 0.80).

Comparison between reliability of EBVs of Top 1% for SCS in test-day records in the SM and reliability of EBVs of these sires in other models evaluated showed a significant change (Table 3) in this population, an increase of 0.11 (approximately 20%). However, from Top 5%, Top 10%, and all sires, it was not possible to observe improvements in reliability estimates. There were also no significant improvements in estimation of reliability according to the number of daughters of sires, when comparing reliability of the SM and M4-2DM models for SCS (Table 4).

4. Discussion

When it comes to heat stress, farmers are concerned about losses in milk yield. However, heat stress can also cause cows to have a high SCS. This is because heat stress can weaken immune system of cattle which will eventually facilitate mastitogenic udder infection (Giesecke, 1985; Pragna et al., 2017). Nickerson (2014) states that high temperature and humidity are very much favorable for development of mastitis causing bacteria such as streptococci and coliforms.

Somatic cell score tends to increase with temperature and humidity levels or unexpected variations. In these situations, in search of thermal comfort, cows tend to change their behavior, standing or lying where it is cooler and spending less time feeding (Hillman et al. 2005). During severe heat stress, cows wallow in mud to regulate body temperature and usually their muddy udders are washed, and milking such wet udders makes animal more susceptible to inter-mammary diseases (Vermunt and Tranter, 2011). As result, there is an increased exposure of the tip of udders to bacteria and body no longer responds with full immunity. Somatic cell score was established to be a good indicator of udder health (Pragna et al., 2017).

According to Christison and Johnson (1972), high circulating levels of stress hormones (such as cortisol) interfere with ability of immune system to fight bacteria, but it is possible to detect an immune response via somatic cells. Stress hormones have a depressive effect on somatic cells, which, in turn, limits their function of total protection against organisms that cause mastitis (Olde Riekerink et al., 2006). That is, SCS is not only an indicator trait for mastitis (Philipsson et al. 1995), but also reflects a cow's immune response to general stress situations (Coffey et al. 1986).

Change in SCS has an extremely damaging influence on composition and physicochemical characteristics of milk, such as shelf life of dairy products (Li et al. 2014). Once heat stress threshold is identified, it is easier to direct management and promote methods of mitigating heat stress to decrease SCS and prioritize integrity of animal organism. In this way, it is possible to improve management practices around heat stress, and reduce incidence of mastitis, but it also increases the production and overall profitability of farm. Further, it was also established in dairy cattle that udders are very sensitive to variations in THI (Hammami et al., 2015).

Hammami et al. (2013) identified THI threshold = 66 for SCS in Luxembourg Holstein under temperate climate conditions and the authors claim that the thresholds in this environment may be lower compared to estimates in tropical, subtropical and Mediterranean climatic conditions. Brügemann et al. (2012) estimated a THI threshold = 60 for German Holsteins in continental temperate conditions. Carabaño et al. (2014), identified heat comfort limits of THI = 59 for SCS in Spanish Holstein, and highlight that the impact of heat stress is even greater in animals that already have mastitis. Bertocchi et al. (2014) reported a 0.0003 slope of SCS before THI threshold (THI = 72.8) and 0.0133 after THI breakpoint in Italian Holstein, and claim that low quality due to SCS leads to decrease in the milk price paid to farmer.

According to Hagiya et al. (2019), for Japanese Holstein, when heat stress effect was assumed to be linear, THI cutoff estimates were 68.5 for SCS. That is, response to heat stress depends on region and environments and evolution in adaptability of Holstein cattle. Higher frequency of mastitis in dairy cows during heat stress could be a reason that high temperatures facilitate survival and multiplication of pathogenic vectors populations associated with hot-humid conditions (Das et al., 2016).

In Brazil, it is possible to observe that effect of heat stress on SCS (THI = 70 and DTV = 9) before that for milk yield, According to Negri et al. (2021) threshold for test-day milk yield for same population was observed at THI = 74 and DTV = 13. That is, at 4ºC less, it is already possible to detect heat stress on SCS. So, monitoring SCS can be an early indicator of heat stress, assisting in decision making regarding management to reduce effects of heat stress and consequently reducing financial losses.

The model with best fit observed in this study was the same as that observed for milk yield trait for the same population, described by Negri et al. (2021), who considered the average of two days before test-day for THI and DTV as fixed effects. In other words, applicability of weighing via heat stress indicators in national genetic evaluation, considering effects as fixed and using average of two days before test-day records is adequate for production traits and SCS, thus promoting improvements in evaluation methodology and providing a more accurate choice of sires.

It is important to note that SCS represents SCC, whose selection objective is to reduce SCC occurrence in herd, as it is closely related to animal health. It is known that heat stress has harmful effects on animal health, and SCS can be an early indicator of heat stress. Therefore, for SCS, lowest (negative) values identify animals with best EBVs for tolerance to heat stress. This would imply less loss of milk yield, milk quality and greater permanence in herd (longevity) (Kern et al., 2018).

A commonly observed management is that many cows are discarded after their first lactation. Therefore, it is very important to correct phenotypic data adequately in relation to environmental effects to avoid exclusion of genetically superior animals that can be penalized due to fact that their daughters are evaluated under heat stress). According to Oliveira et al. (2019), SCS has a more quantitative nature when compared with other milk traits (yield, fat and protein). This reflects a low proportion of additive genetic variance which, associated with thermal events, makes SCS a potential indicator of onset of heat stress in Holstein cattle. According to Carabaño et al. (2014), genotype-environment interaction is expected to be larger for SCS than for milk yield.

In this sense, when an animal is exposed to heat stress conditions, health challenges are increased. The living organism, when triggering the thermoregulation mechanisms, ends up becoming more susceptible to udder infection by microorganisms (Bueno et al. 2005), reflecting issue of animal's adaptability and demonstrating an interaction of its genotype with environment.

In addition to fluctuation observed in EBVs of individuals, when correcting data for bioclimatological variables that indicate heat stress, it was also possible to verify that the number of daughters did not affect average reliability of sires, but it did affect reliability of those sires identified as better by models that included bioclimatic variables (in comparison with SM). Thus, it affected reliability of sires that would be most used in selection (Top 1%), providing a greater reliability that these individuals are truly most suitable for use in reproduction (commonly via artificial insemination).

Bouraoui et al. (2002) claimed that heat stress impaired breast defense mechanisms of cattle with high SCS, and Lambertz et al. (2014) confirmed increased prevalence and incidence of udder inflammation due to heat stress. Further, heat stress can inhibit flow of glucose to mammary glands (Wheelock et al., 2010) and influence oxidative glucose metabolism fluctuations, which controls secretory cell number, level of secretory activities, and secretory epithelium integrity of udder (Pragna et al., 2017).

Hammami et al. (2008) estimated considerable reranking of Holstein sires between temperate and hot and humid climate. In Brazil (tropical climate), genetic material of most used Holstein sires comes from United States and Canada (temperate climate) (Silva et al., 2016). This means that cows, daughters of these sires, will be more challenged in terms of adaptability, thermotolerance and health (including SCS). Holstein cattle selected in different environments may not perform well when exposed to another climate or lower technological level of production. Hammami et al (2008) stated that low-to-medium production systems, if they cannot select from their own populations, should consider using semen from selected sires in regions with low-to-medium production environment from countries with leading dairy industries to reduce negative impacts of genotype-environment interaction. Authors also advise that when estimates of genetic correlations between countries are less than 0.60, paying premium prices for elite animals/semen in temperate environments is not necessarily a good strategy, highlighting importance of importing countries, such as Brazil, to select animals in their populations to meet elementary needs of high-yield breeds.

If we take into account that, in Brazil, SCS has direct (dairy industries generally pay premiums under SCS) and indirect (expenses with mastitis treatment) economic values, besides a potential indicator of early heat stress, selection within existing populations in Brazil, would allow not only genetic gains, but also economic gains in entire production chain.

5. Conclusion

Changes in somatic cell score can be an early indicator of heat stress in Holstein cattle reared in tropical conditions, even before decrease in milk yield. Identifying evidence of heat stress early allows the farmers to adopt techniques that minimize production losses in the farm even before reaching an inflated level in production traits. The increase in SCS is observed above the threshold of 70 for temperature-humidity index and above 9ºC for temperature variation.

This study provides strong evidence of a genotype by environment interaction in somatic cell score. It is recommended to include in the genetic evaluation the temperature-humidity index and the temperature variation as fixed effects, considering the average between two days before each test-day to minimize the bias of heat stress in reclassification of best sires. The wrong choice of the best sires can compromise the genetic progress of herds pure and their cross.

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Table 1 – Overall traits of first-parity (SD in parentheses) of Holstein cattle.

Item	Statistics
Animals in pedigree file	30,807
Animals with records	9,765
Dams in pedigree file	8,607
Sires in pedigree file	612
Test-day records	52,012
Mean records/animal	5.33
SCS mean	3.30 (1.9)
Contemporary groups	3,279

Table 2 – Models layout.

Models	Fixed Effects				Covariate		Regressor
	Contemporary group	Milking Frequency	THI	DTV	THI	DTV	DIM
SM	*	*	-	-	-	-	○○○○
M2-0DB	*	*	+	-	-	-	○○○○
M2-1DB	*	*	□	-	-	-	○○○○
M2-2DB	*	*	⌂	-	-	-	○○○○
M2-3DB	*	*	◊	-	-	-	○○○○
M2-2DM	*	*	Δ	-	-	-	○○○○
M3-0DB	*	*	-	-	+	-	○○○○
M3-1DB	*	*	-	-	□	-	○○○○
M3-2DB	*	*	-	-	⌂	-	○○○○
M3-3DB	*	*	-	-	◊	-	○○○○
M3-2DM	*	*	-	-	Δ	-	○○○○
M4-0DB	*	*	+	+	-	-	○○○○
M4-1DB	*	*	□	□	-	-	○○○○
M4-2DB	*	*	⌂	⌂	-	-	○○○○
M4-3DB	*	*	◊	◊	-	-	○○○○
M4-2DM	*	*	Δ	Δ	-	-	○○○○
M5-0DB	*	*	-	-	+	+	○○○○
M5-1DB	*	*	-	-	□	□	○○○○
M5-2DB	*	*	-	-	⌂	⌂	○○○○
M5-3DB	*	*	-	-	◊	◊	○○○○
M5-2DM	*	*	-	-	Δ	Δ	○○○○

* Considered; - no considered; $^{○○○○}$ LP (4-order); $^{+}$ 0DB; $^{□}$ 1DB; $^{⌂}$ 2DB; $^{◊}$ 3DB; ^{Δ} 2DM.

Table 3 – Reliability average of estimated breeding values (EBVs) of best Top 1%, Top 5%, Top 10%, and all sires for somatic cell score (SCS) in test-day records, according to the standard model (SM) and day best fit (2DM) for evaluated models (M2 to M5).

	Models				
Sires	SM	M2-2DM	M3-2DM	M4-2DM	M5-2DM
Top 1%	0.56[b] (0.30 to 0.76)*	0.67[a] (0.48 to 0.77)	0.67[a] (0.48 to 0.85)	0.67[a] (0.48 to 0.85)	0.67[a] (0.48 to 0.85)
Top 5%	0.49[a] (0.18 to 0.83)	0.48[a] (0.18 to 0.84)	0.48[a] (0.18 to 0.84)	0.49[a] (0.18 to 0.84)	0.48[a] (0.18 to 0.84)
Top 10%	0.48[a] (0.18 to 0.83)	0.48[a] (0.17 to 0.86)	0.48[a] (0.18 to 0.86)	0.48[a] (0.17 to 0.86)	0.48[a] (0.18 to 0.86)
All	0.13[a] (0.01 to 0.88)	0.13[a] (0.05 to 0.88)	0.13[a] (0.05 to 0.88)	0.13[a] (0.05 to 0.88)	0.13[a] (0.05 to 0.88)

Median followed by different letters in rows are significantly different ($p < 0.05$) by Kruskal-Wallis test. *Minimum and maximum.

Table 4 – Reliability average of estimated breeding values (EBVs) of sires, according to their number of daughters; comparison between traditional standard model (SM) and model that includes the average of two days of bioclimatic variables as fixed effects (M4-2DM).

N Daughters	N Sires	Reliability of EBVs	
		SM	M4-2DM
< 10	345	0.30 (0.01 to 0.82)*	0.31 (0.05 to 0.83)
11 to 20	130	0.46 (0.31 to 0.78)	0.47 (0.31 to 0.77)
21 to 30	58	0.58 (0.41 to 0.79)	0.59 (0.43 to 0.79)
31 to 40	23	0.63 (0.54 to 0.69)	0.64 (0.56 to 0.70)
41 to 50	12	0.69 (0.63 to 0.77)	0.70 (0.63 to 0.77)
51 to 100	31	0.75 (0.61 to 0.83)	0.76 (0.62 to 0.83)
> 101	13	0.85 (0.82 to 0.88)	0.86 (0.83 to 0.88)

* Minimum and maximum.

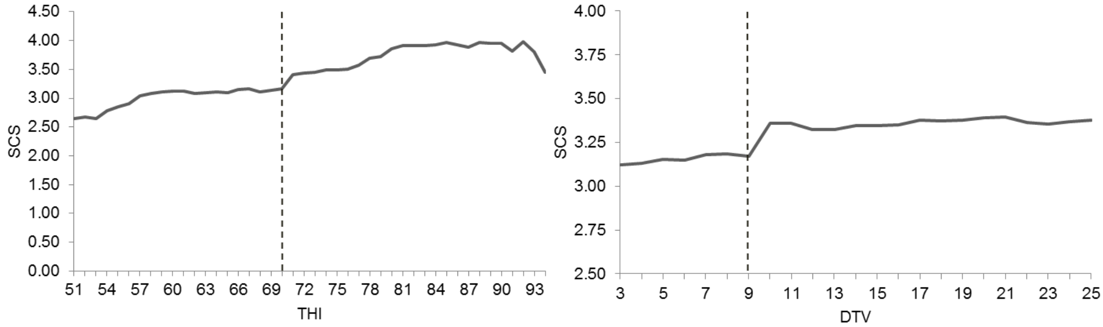
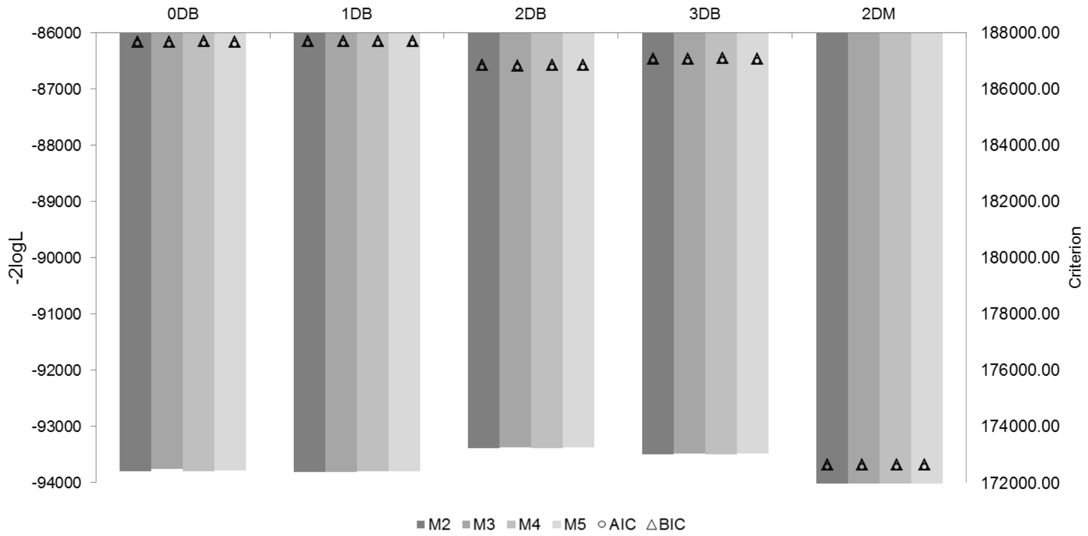
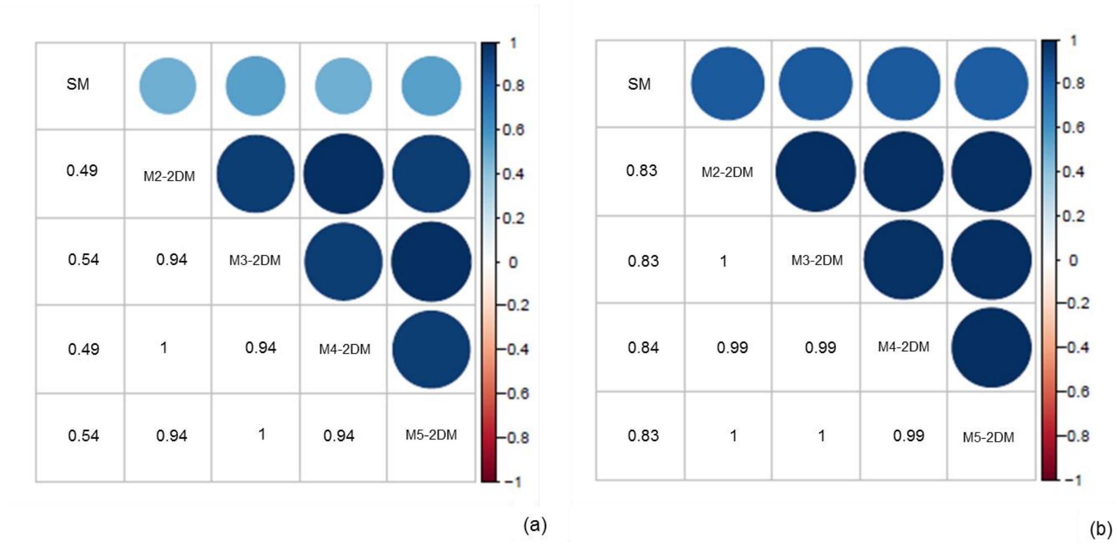


Figure 1 - Heat stress threshold (dotted line) and average somatic cell score (SCS) corrected according to temperature-humidity index (THI = 70) and diurnal temperature variation (DTV = 9).



Standard model (SM) estimates: -2logL= -97360.29; AIC= 194734.59; BIC= 194796.60.

Figure 2 - Estimates of models fitting criteria: log-likelihood function (-2logL), Akaike information criterion (AIC) and Schwarz Bayesian information criterion (BIC); for each day of inclusion of bioclimatic variables (0DB, 1DB, 2DB, 3DB and 2DM) and model (M2 to M5).



Colors inside squares indicate the magnitude and direction of associations.

Figure 3 - Spearman rank correlations coefficient for Top 1% (a) and Top 10% (b) best sires (estimated breeding values - EBVs) for somatic cell score (SCS), according to standard model (SM) and day best fit (2DM) for evaluated models (M2 to M5).

CAPÍTULO V

Heat stress level as an alternative to fixed regression modeling for fat and protein yield traits in Holstein cattle[6]

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Heat stress level as an alternative to fixed regression modeling for fat and protein yield traits in Holstein cattle

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ABSTRACT

To date, no study has tested the inclusion of bioclimatic variables or heat stress indicators in the formation of fixed regression for the genetic evaluation of dairy cattle. The objective of the present study was to investigate the inclusion of heat stress level in modeling of fixed regression in random regression models to predict population response and individual genetic components for fat and protein yield in test-day, using data from Holstein cattle in tropical environment. The data comprised 52,012 test-day fat and protein records of 9,858 first-parity Holstein cows from Brazil, collected from 1997 to 2013, and bioclimatic data (temperature-humidity index – THI, and diurnal temperature variation – DTV) from 18 weather stations. Least square linear regression models were used to determine THI and DTV thresholds for fat and protein yield losses caused by heat stress. In addition to the standard model (SM, without bioclimatic variables), THI and DTV were analyzed as fixed effects (considering the average of two days before the test-day control), and heat stress level was considered in formation of fixed regression, totaling three models for each trait. THI and DTV thresholds for fat and protein losses was THI = 74 (−0.030 kg/day/ THI) and DTV = 16 (−0.020 kg/day/ DTV), for both traits. The model that included THI and DTV as fixed effects, and used heat stress level in formation of fixed regression (fixed curve), presented a better fit (AIC, BIC, and residual variance). Estimated breeding values (EBV) are improved when using this model, and there is an increase in reliability of estimates. There is an important reranking of sires when heat stress indicators are included in the model to evaluate fat and protein yield in test-day. The increase in reliability of EBV by including heat stress level in the fixed regression shows a better fit of model to the data. It is possible to conclude that the inclusion of heat stress level in the formation of fixed regression is an important factor to be considered in dairy cattle reared in tropical regions to minimize environmental effects and optimize the process of evaluation and genetic selection.

Keywords: diurnal temperature variation, fixed curve, random regression, temperature-humidity index.

1. Introduction

Despite fact that dairy cattle selection programs around the world have traditionally selected to increase milk production and milk solids (Sigdel et al. 2019), production can be strongly affected due to negative effects of heat stress. According to Aguilar et al. (2010), intense genetic selection for production, resulted in greater sensitivity to environmental changes in animals and the damage caused in chain is of great representativeness. In addition to decrease in production, a major impact is also in reduction of milk composition, which moves a large part of the dairy sector financially.

Elevated temperature and humidity can reduce the ability of cattle to dissipate excess heat, which can ultimately lead to associated physiological alternations (Novak et al., 2007), through direct and indirect mechanisms. That is, declined fat and protein concentration could be attributed to specific down thermoregulation activity of mammary synthesis (Cowley et al., 2015), and not just to feed intake reduction, as is normally understood.

Fat and protein yield demands research in relation to heat stress to improve their understanding, as they have great economic interest in dairy industry. Many dairy companies pay (bonuses and penalties) for fat and protein yield, as they are components with greater added value. Impact on herds specialized in high production of conditioned milk in tropical regions can suffer great damage in all links of the chain.

There are studies in literature that indicate the inclusion of heat stress indicators (bioclimatic variables) as fixed effects in the models of genetic evaluation to improve the estimated breeding values in dairy cattle (Bernabucci et al., 2014; Negri et al. 2021a). However, random regression models allow to split the shape of production curve into two parts: a fixed part (fixed regression/fixed curve) to assess similarities or critical issues of the curves within specific groups of animals (i.e., age, calving season, lactation stage, parity, and influence of an environmental effect), which could be an alternative way of using heat stress information in genetic assessments; and a second, random part (random regression), specific for each animal (Bormann et al., 2003; Cobuci et al., 2011). But none of them has tested the inclusion of bioclimatic variables or heat stress indicators in the fixed regression.

Given the above, the present study aimed to investigate in a pioneering way, the inclusion of heat stress level in the modeling of fixed regression in random regression models to predict population response and individual genetic components for fat and protein yield in test-day, using data from Holstein cattle in a tropical environment.

2. Material and methods

Data

Data consisted of test-day (TD) records for fat (FAT, kg) and protein (PROT, kg) yield collected by the service of the Associação dos Criadores de Gado Holandês de Minas Gerais, Brazil. Data of first-lactation Holstein cow's collected between 1997 and 2013 were used. We excluded records with extreme age at calving (<18 or >48 months), days in milk (DIM, <5 or >305 days), FAT yield (± 3 standard deviation) and PROT yield (± 3 standard deviation) from the data set.

Cows were required to have at least four records. Minimum size of each contemporary group (herd-year- month of TD records) was three animals. Records of daughters of sires with at least one daughter in at least three herds were accepted to evaluation.

Following these criteria, a total of 52,012 TD records from 9,858 first lactations of Holstein cows, were analyzed. The same database was used for all models evaluated. Pedigree file included 30,289 animals. Description data file is shown in Table 1.

Bioclimatic variables

Climate variables used were average daily dry bulb temperature (DBT; °C), maximum temperature (°C), minimum temperature (°C), and average daily relative humidity (RH; %), as recorded by Instituto Nacional de Meteorologia, through 18 weather stations located less than 60 km away from evaluated farms, using nearest station information. Temperature-humidity index (THI) was evaluated according to equation described by National Research Council (1971):

$$THI = (1.8 \times DBT + 32) - [(0.55 - 0.0055 \times RH) \times (1.8 \times DBT - 26)]$$

Diurnal temperature variation (DTV) was calculated by the difference between daily maximum and minimum temperatures (ºC) (see Negri et al. 2021a).

Models

THI and DTV were considered as the average between the last two days before each TD (2DM) (see Negri et al. 2021a).

To detect the heat stress threshold for each trait, two-phase linear regression procedure was applied to least squares means. Fixed effects of model considered were: contemporary group, milking frequency, and DIM. Age of cows at calving was considered as a covariate (linear effect). Significant decrease in FAT and PROT was estimated considering mean THI and DTV values.

For genetic evaluation, a random regression model was used for test-day FAT and PROT analyses using Legendre polynomial regression coefficients (4th order) (Kirkpatrick et al., 1994). Additive genetic and permanent environmental covariance functions were estimated by random regression model of DIM. Models were used to verify the best way to include bioclimatic data for correction of genetic evaluation model for FAT and PROT, considered as fixed effects or in the formation of the fixed regression, totaling three models each.

Standard random regression model (SM) does not consider bioclimatic variables for correction of the data, as shown below:

$$\text{(SM)} \quad y_{ijklmn} = HYM_l + MF_n + \sum_{m=0}^{df} \phi_{jkm}\beta_m + \sum_{m=0}^{dr} \phi_{jkm}\mu_{jm} + \sum_{m=0}^{dr} \phi_{jkm}pe_{jm} + e_{ijklmn}$$

where y_{ijklmn} is the *i*-th TD record of the *j*-th cow on the *k*-th DIM within the *l*-th subclass herd-year-month of the test (HYM) and *n*th milking frequency (MF); β_m is the *m*-th fixed regression coefficient, defined as the age classes: 1 (18 to 25 months), 2 (26 to 27 months), 3 (28 to 29 months), and 4 (30 to 48 months), combined with the calving season subclasses: 1 (rainy: October to March) and 2 (dry: April to September), totaling eight fixed curves; μ_{jm} is the *m*-th random regression coefficient for the additive genetic effect of the *j*-th cow; pe_{jm} is the *m*-th random regression coefficient for the permanent environmental effect the *j*-th cow; ϕ_{jlm} is the *m*-th Legendre polynomial coefficients corresponding to the

TD record of the k-th DIM of the j-th cow; df and dr are orders of fixed and random regression coefficients; and e_{ijklmn} is the random residual effect.

Model 2 (M2): Contemporary group, milking frequency, THI and DTV as fixed effects. The fixed regression coefficient (fixed curve), defined as the age classes: 1 (18 to 25 months), 2 (26 to 27 months), 3 (28 to 29 months) and 4 (30 to 48 months), combined with the calving season subclasses: 1 (rainy: October to March) and 2 (dry: April to September), totaling eight fixed curves (fixed regression coefficient equal to SM).

Model 3 (M3): Contemporary group, milking frequency, THI and DTV as fixed effects. The fixed regression coefficient (fixed curve), defined as the age classes: 1 (18 to 25 months), 2 (26 to 27 months), 3 (28 to 29 months) and 4 (30 to 48 months), combined with the calving season subclasses: 1 (rainy: October to March) and 2 (dry: April to September), and heat stress level: 0 (homeostasis, if THI <74), 1 (heat stress, if THI >74 and THI <84), 2 (severe heat stress, if THI >84), totaling twenty-four fixed curves.

Residual variance was considered homogeneous for all models.

Genetic evaluation and statistical analyses

All genetic analyses were performed with an animal model, using REMLF90 program (Misztal et al., 2002). Quality of fit was evaluated considering non-nested models and penalties, according to the number of parameters to be estimated. The following criteria were used: Akaike's information criterion ($AIC = -2\log L + 2p$, in which p is the number of parameters in the model); and Schwarz's Bayesian information criterion (

$BIC = -2\log L + p \cdot \log(\lambda)$, in which log ($\lambda$) is the natural logarithm of sample size (or dimension of *y*) and *p* is the number of parameters in the model). Schwarz's Bayesian information criterion is more rigid than AIC. The model with the lowest value, for both criteria, was the one with best fit.

To check reranking of estimated breeding values (EBVs) of sires, Spearman's rank correlation coefficient for 1% (Top 1%) and 10% (Top 10%) of superior sires EBVs was used. It was also verified the proportion of common animals among the Top 10% and Top 1% sires with more than 30 daughters. Reliability of EBVs was calculated using triangular matrices of prediction error (co)variances for random regression effects, from inverse of mixed model equations obtained in BLUPF90 program (Misztal et al., 2002).

3. Results

Heat stress influences of fat and protein yield in dairy farms in Minas Gerais State, Brazil. THI threshold for FAT and PROT is THI = 74, considering complete lactation (5 to 305 days). Decrease was 30 g/ THI unit above 74, for both traits.

For FAT, effects of THI stratified according to lactation phase (DIM 5 to 60; DIM 61 to 120; DIM 121 to 180; DIM 181 to 240; and DIM 241 to 305). Showed a significant effect of THI, with decreases of 0.010 kg for 241 to 305 DIM (p-value < 0.05). That is, a representative impact of heat stress on fat yield was observed in the final phase of lactation. While for PROT, it was not possible to detect a significant difference in established phases.

DTV threshold for FAT and PROT decrease is 16ºC. The decrease of FAT and PROT was of 20 g/ DTV unit, which exceeded 16ºC, for both traits. For FAT, effects of DTV stratified according to lactation phase showed a significant effect of DTV, with decreases of 0.010 kg, starting from 181-days of lactation (p-value < 0.05). While for PROT, fall is 0.010 kg, after 120-days of lactation, thus confirming that the greatest effect of heat stress occurs between intermediate and final stages of lactation.

All models that considered bioclimatological variables presented better fit than the SM (Figure 1). It was observed in the M3, lower values of AIC and BIC, denoting better quality of fit. That is, an option to perform genetic evaluation via random regression models for fat and protein yield is to use heat stress level indicated by THI in the formation of fixed regression.

The heritability estimate showed a slight increase, due to reduction of residual variance when including bioclimatic variables in the model. The best result was observed when we included the heat stress in formation of fixed curve.

The fixed regression solution shows a difference according to heat stress level for FAT (Figure 2). The SM and M2 solutions are the same, as the curve formation consists of the same factors.

It is possible to observe that animals with parity registered in the rainy season (Figure 2: a, b, c, and d), from October to March, are less affected in homeothermia conditions (absence of heat stress) and moderate heat stress, being very close to the models that disregard heat stress level in fixed regression (SM and M2). In the same way, it is possible to observe that older animals (over 28 months) are more affected when compared with young animals, which can be verified by the behavior and distance of the curve. While for animals delivered during the dry season (Figure 2: e, f, g, and h), from April to September, the biggest health challenges for animals are clear.

In general, an interesting result is the proximity of the heat stress curve (M3 - heat stress) with the SM and M2 curves, which may be due to the large number of animals facing thermal stress conditions, which pull the average of the SM and M2 curves in order to be close, demonstrating that animals in homeostasis and severe stress are penalized or benefited in genetic evaluations. An emphasis is placed on the severe heat stress level, which distances and changes the behavior of the fixed curve in relation to the others, clearly demonstrating that it is not possible to compare fairly animals that are in different heat stress conditions, masking the prediction of the breeding value of individuals and relatives due to effect of heat stress that can cause a possible collapse of the phenotypic trajectory or animal performance, differing their productive behavior from that expected for the category based only on age class and calving season.

For PROT, the fixed regression solution (Figure 3) also shows that there is a difference between the curves according to heat stress level, keeping the sharp differentiation of animals that are in conditions of severe heat stress, once again highlighting the importance of incorporating this vector in genetic analysis.

In general, animals with calving registered in the dry season (Figure 3: e, f, g and h), have a greater impact on the phenotypic trajectory and a more atypical behavior of the curve. Despite the not-so-big differences (distance from the lines), the behavior is different and has a strong influence on the estimation of breeding values and, consequently on the selection.

The most significant impact of Spearman's correlation coefficients was observed between SM and M3 in Top 1% (0.62) (Figure 4), demonstrating an important reranking of sires when the bioclimatic variables are included in genetic evaluation, especially when heat stress level is added and used to model the fixed curve. In other words, the correction of data for THI strongly influences the ranking of sires for fat yield.

The same behavior was observed for Top 10%. Correlation below 0.80 indicates strong reranking of sires. Associations between M2 and M3 for FAT suggest that the ranking of sires is not significant.

For PROT, Spearman's correlation coefficients also confirm a strong reranking of sires between M2 and M3 with SM (0.65, and 0.59, respectively) in Top 1% (Figure 5). For Top 10%, the same behavior was observed, with a significant impact between the SM and the other models (correlation below 0.80). However, the impact on PROT seems to be slightly more expressive when compared with FAT.

The inclusion of variables that indicate heat stress led to an increase in the reliability of EBV (Figure 6). The greatest increase can be seen in the protein yield trait. However, although subtle, the increase in the estimation of reliability is particularly important in the practice of animal selection, as it increases certainty at the moment of choice, based on the intensity of use of the sires.

Among the top sires (Top 10% and Top 1%) with more than 30 daughters with phenotypes, the number of those overlapping across the three models reflects the magnitude of the reranking of sires (Table 2). The number of common sires is lower, when comparing models that include bioclimatic variables (M2 and M3) with SM. The smallest divergence observed is between M2 and M3, especially in the Top 10%. The magnitude of the reranking increases when we disregard the effects of heat stress and bioclimatic variables in the model for the two traits (FAT and PROT), with a greater reranking effect in the Top 1%.

4. Discussion

Impact observed in composition of milk reflects direct and indirect effect of heat stress on production and composition, since in a country with a predominantly tropical climate, dairy cattle in Brazil are, in most cases, managed in a grazing system; thus, under heat stress conditions, animals drastically reduce their feed intake. The impacts of THI and DTV (climate components) differ in terms of their influence, and when they do, the effect differs over the lactation (or DIM) phases.

According to Bernabucci and Calamari (2008), a possible explanation for lower levels of fat observed in milk of cows in heat stress conditions would be the variation in forage intake by animals.

This fact corroborates the statement by Collier et al. (1985), who identified that the lower intake of roughages causes a change in acetate/propionate ratio, thus changing milk composition, and that in most cases, protein yield is negatively affected by heat stress, with decrease in casein contents. According to Summer et al. (2019), bovine milk is rich in caseins and comprises about 77% of total protein yield, which results in an excessively big impact on dairy industry, as it affects cheese-making process, and milk coagulation.

As for direct effect, Cowley et al. (2015) found that maintaining the same feed as cows, animals exposed to heat stress produced milk with less protein than cows housed in comfortable temperature conditions, showing a direct response to heat stress instead of a reduction in feed intake.

Several authors have confirmed the negative effect of heat stress on fat and protein yield. Hammami et al. (2013) failed to identify a threshold for fat yield in Luxembourg Holstein under temperate climate conditions. The authors noted that fat yield tends to decrease steadily with increasing THI. While for protein, they observed that milk protein yield remains almost constant before specific THI threshold. However, authors such as Bouraoui et al. (2002) and Gantner et al. (2011) observed a reduction in milk protein levels due to heat stress after higher critical point. Brügemann et al. (2012) identified no universally valid thresholds for daily fat and protein percentage in German Holsteins. Carabaño et al. (2014), identified heat comfort limits of THI = 59 for fat yield and THI = 62 for protein yield in Spanish Holstein. Bertocchi et al. (2014) in Italian Holstein, reported a negative correlation between THI and fat (THI = 50) and protein percentage (THI = 65).

As for the fit of the models, the applicability of weighting via heat stress indicators in the national genetic evaluation, considering effects THI and DTV as fixed, using average of two days before the test-day control, has been reported in the literature as an appropriate strategy for milk yield (Negri et al., 2021a, Negri et al. 2021c) and somatic cell score (Negri et al., 2021b) using random regression models. Promoting improvements in methodology of traits and allowing most accurate selection of individuals, weighted by bioclimatic variables that indicate heat stress.

When heat stress level was included in the formation of the fixed curve, results showed a subtle reduction in residual variance, which can be considered a good biological indicator of fit of models. Because it demonstrates that the model can describe data more fully, reducing unidentifiable effects in the model. In turn, the residual variance influences the quality of estimated genetic parameters and breeding values (Cavalcante et al., 2020).

As for the fixed curve, other authors have tested different factors to form fixed regression, in additional to those traditionally used (age and calving season) (Strabel et al., 2004; Muir et al., 2007; Costa et al. 2008; Cobuci et al., 2011). Other authors tested different factors, such as the average production of the population (Herrera et al., 2008) or subpopulations (Cobuci and Costa, 2012), cow lineage, age in years, season of measurement, and their interactions, year of birth, and pregnancy-lactation codes (Arango et al., 2004). But none have tested the inclusion of bioclimatic variables or heat stress indicators.

It is possible to observe that, in general, the phenotypic trajectory tends to increase as animals face heat stress conditions (no heat stress, heat stress, severe heat stress). This fact may be related to the approach of residual variance in random regression models. Therefore, fixed regression modeling, considering heat stress level, in addition to age class and calving season, can improve the partition of phenotypic variance.

It is evident the importance of including heat stress level in the fixed curve to allow a fairer comparison of the animals, given the conditions that strongly influence the production gradient. Emphasizing that by including heat stress level in the fixed regression, it is possible to improve the reliability of the EBV of selected individuals.

It is worth remembering that in Brazil; most of the semen used comes from imports from the United States, Canada, and the European Union. This strategy culminates in greater concerns in the understanding of the genotype by environment interaction, with the reclassification, selection, and possibly smaller genetic gains. Hammami et al. (2008) already warned of strategies for choosing genetic material and having a negative impact on the genotype by environment interaction, especially among countries with a genetic correlation below 0.60.

The importance of using more appropriate fixed regression, that is, that includes heat stress level, allows improving the estimation of the variance components, genetic parameters, breeding values and reliability. In analyses that use random regression models, the breeding value is obtained through the difference between the random and the fixed regression.

However, the number of fixed curve classes should be increased carefully, since it increases the number of parameters to be estimated, which can make the analysis more difficult (Cavalcante et al., 2020). The use of heat stress level instead of pure THI value solves the problem that it is ideal to have few curves to compare animals, that is, few fixed curves. Therefore, the use of stress level is an applicable alternative in the routine of genetic evaluations.

As for the magnitude of the reranking sires, the impact on the two traits was similar when comparing SM and those that include bioclimatic variables, and very close, when we compare between models that consider THI.

Ranking of sires for protein yield is more impacted by heat stress than for fat. The greatest impact observed in PROT may be related to early impact of heat stress described by Negri et al. (2021b) in somatic cell score (SCS), in which the heat stress threshold for SCS is reached at THI = 70 and DTV = 9. Guo et al. (2010) drew attention to a highly significant negative correlation coefficient in SCS and protein in a dairy farming program. This change may be related to immune response of cows to infection. Samoré et al. (2007) also identified an antagonistic association between SCS and protein production. A relevant biological interpretation, according to Haile-Mariam et al. (2001), is that the occurrence of mastitis events can cause damage to the texture of the udder and lead to decreased protein production. A fact that must be considered, as selection of cows by farmers usually occurs after first lactation, is that after collecting production data, animals with low production and high SCS are often discarded, without a genetic evaluation. That is, it also suggested that different mechanisms were involved in relationships between fat and protein yield. Thus, the fact that SCS presents a threshold that occurs before protein yield, could affect production of proteins and consequently estimation of EBV of individuals, resulting in an important reranking of sires.

According to Hill and Wall (2015), the best metric (best fit day) for each climate element significantly influences traits of milk (protein and fat) when tested individually using REML. This may justify the most significant change in rank for PROT. According to Hill and Wall (2015) longer-term metrics (more distant from TD) are generally more effective in explaining effects on protein content. Despite this, the authors suggested that bioclimatic effects have a more sustained impact on milk production and fat content than when compared with protein yield. Whereas for fat, Hill and Wall (2015) pointed out that more distant metrics from TD (close to 7-days earlier) are needed to visualize significant differences.

Biologically, the comparison of animals when we include heat stress level in the fixed curve, becomes more appropriate, which probably reflects in the increase in the reliability of the estimates, since it removes the non-genetic environmental effects, improving the process. That is, when we used three variables (age class, calving season and heat stress level) in the fixed regression instead of two variables (age class and calving season), it allowed a better explanation of the data behavior, reflecting in improvement of the evaluation process and increase of reliability of EBV by the model.

5. Conclusion

To date, no study has tested the inclusion of bioclimatic variables or heat stress indicators in the formation of fixed regression for the genetic evaluation of dairy cattle. In this sense, the present research pioneers an alternative to improve the modeling of fixed regression, including the indicators of the heat stress level.

To achieve the objective of selection on fat and protein yield in milk, the genetic evaluation should include the temperature-humidity index and temperature variation, considering the average between two days before each test-day as fixed effects, and include the heat stress level in fixed regression. The solution of fixed regression and reclassification of sires points to importance of correcting phenotypic data for bioclimatological variables to correctly perform genetic selection of animals in tropical environment, and the increase in reliability reflects the best fit of the model. Therefore, parameters estimated in this study are likely to be useful as a preliminary step in developing of weighted genetic evaluation for heat stress.

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Table 1 – Overall traits of first-parity of Holstein cattle (SD in parentheses).

Item	Statistics
Animals in pedigree file	30,289
Animals with records	9,858
Dams in pedigree file	8,602
Sires in pedigree file	609
Test-day records	52,012
Mean records/animal	5.28
Fat yield, kg/day	0.873(0.273)
Protein yield, kg/day	0.832(0.220)
Contemporary groups	3,274

Table 2 – Proportion of common animals among the Top 10% and Top 1% sires with more than 30 daughters with phenotypes according to models in Holstein dairy cattle in a tropical environment.

Models	Trait	Top 10%	Top 1%
SM – M2	FAT	27%	15%
SM – M3		21%	12%
M2 – M3		71%	53%
SM – M2	PROT	24%	12%
SM – M3		33%	9%
M2 – M3		77%	54%

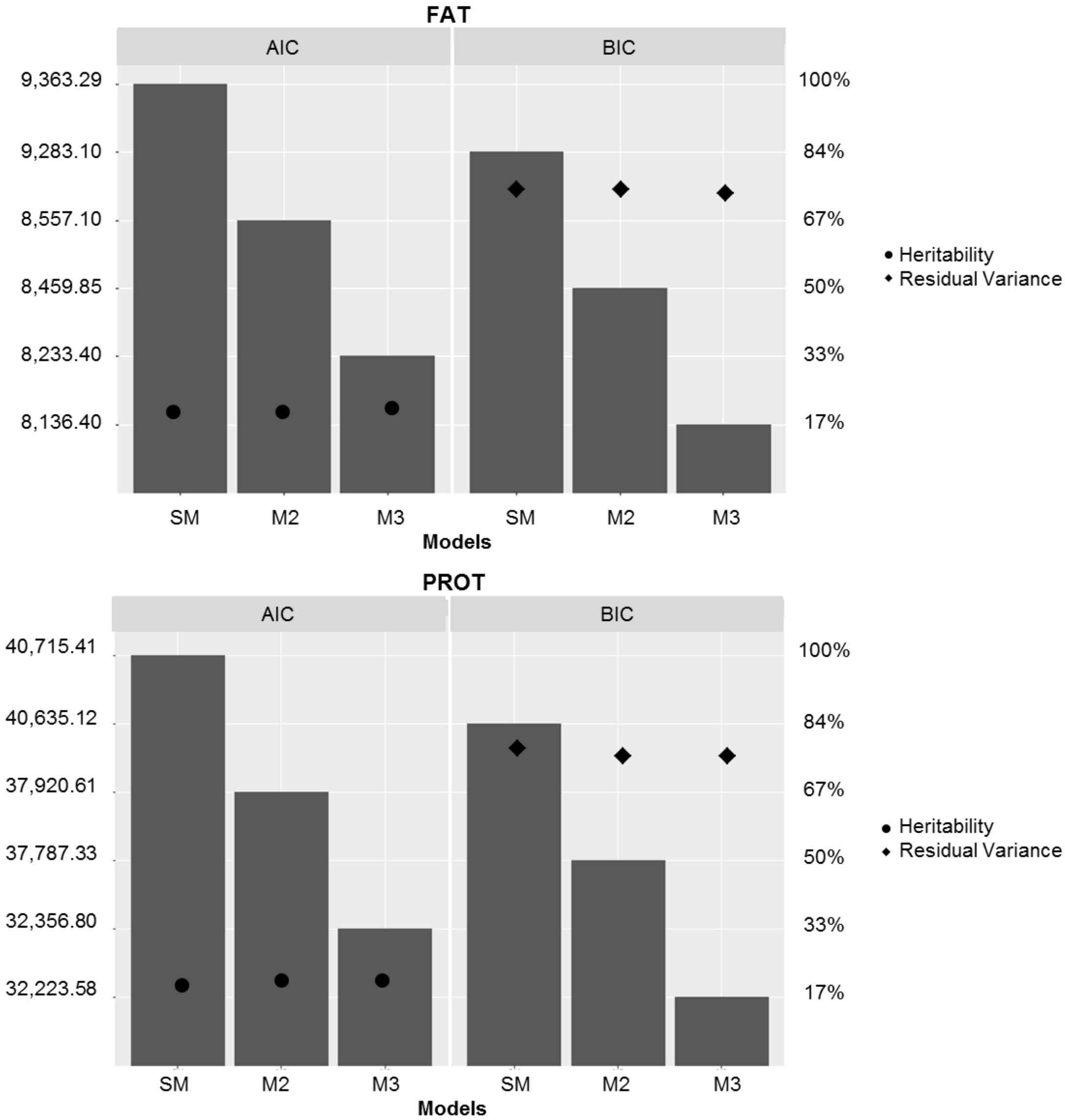
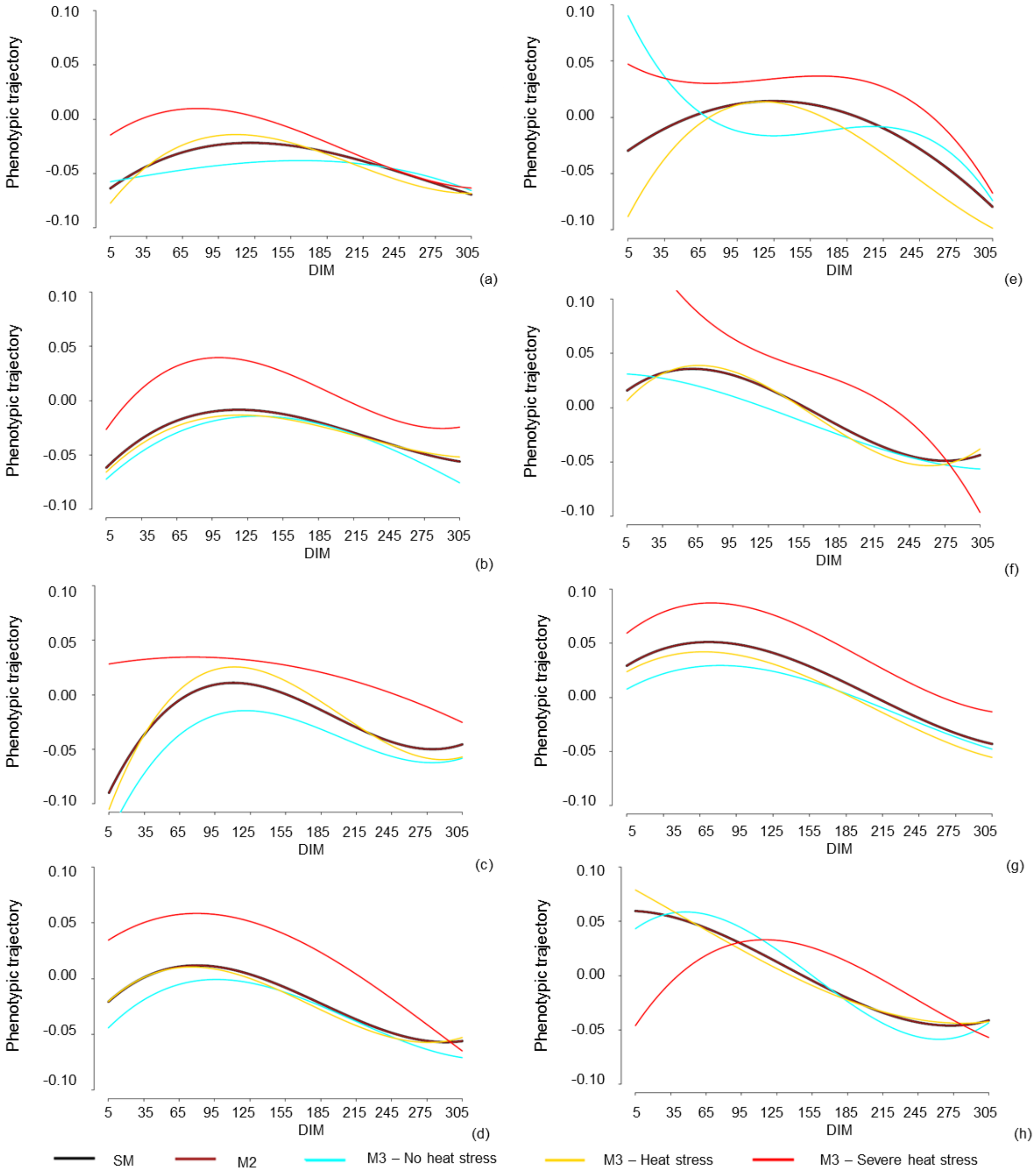


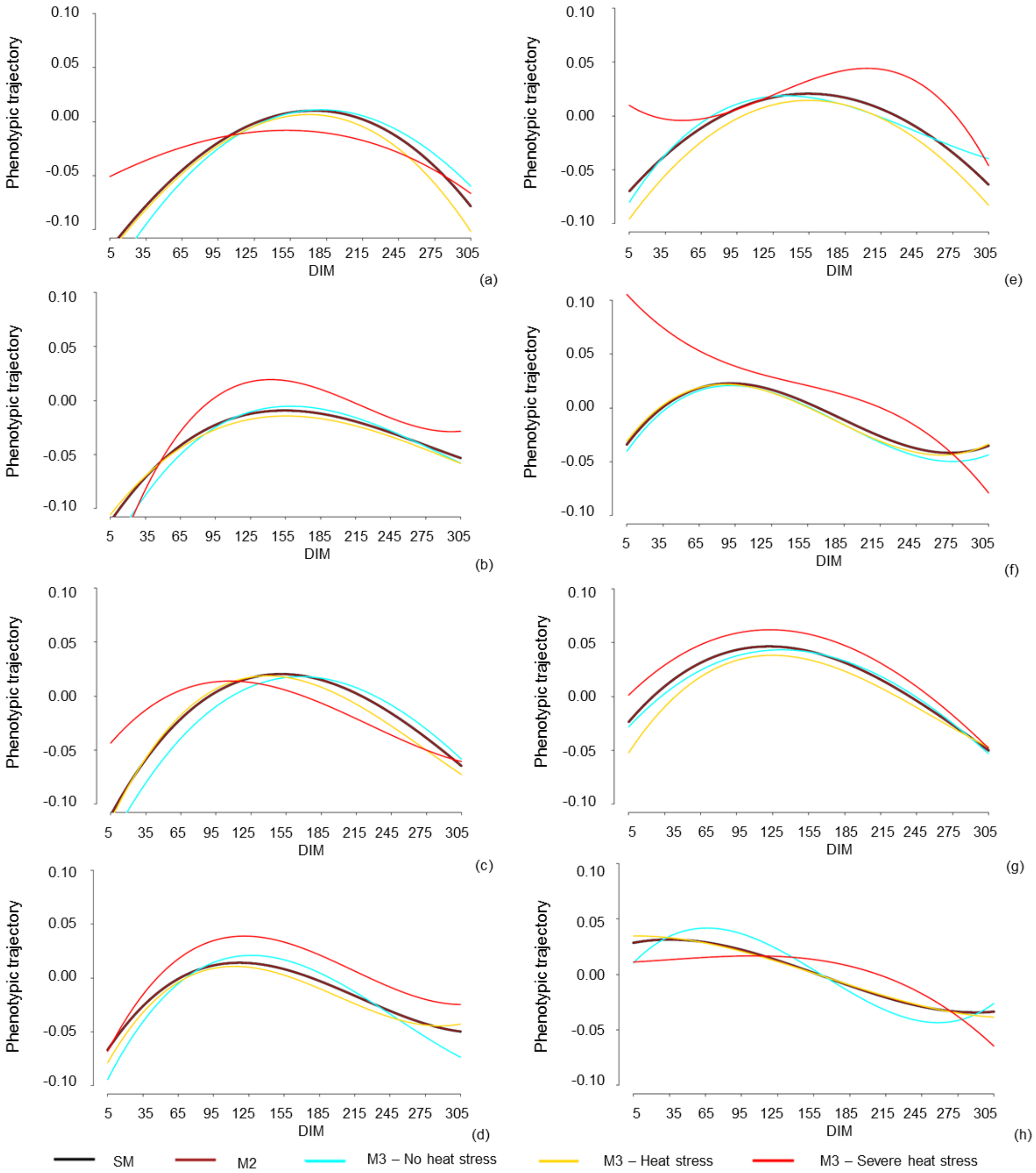
Figure 1 - Estimates of models fitting criteria: Akaike information criterion (AIC) and Schwarz Bayesian information criterion (BIC); heritability and residual variance for each model.



Standard model (SM), Model 2 (M2); Model 3 (M3).

a: rainy calving season/ age 18 to 25 months; b: rainy season/ age 26 to 27 months; c: rainy season/ age 28 to 29 months; d: rainy season/ age 30 to 48 months; e: dry calving season/ age 18 to 25 months; f: dry season/ age 26 to 27 months; g: dry season/ age 28 to 29 months; h: dry season/ age 30 to 48 months.

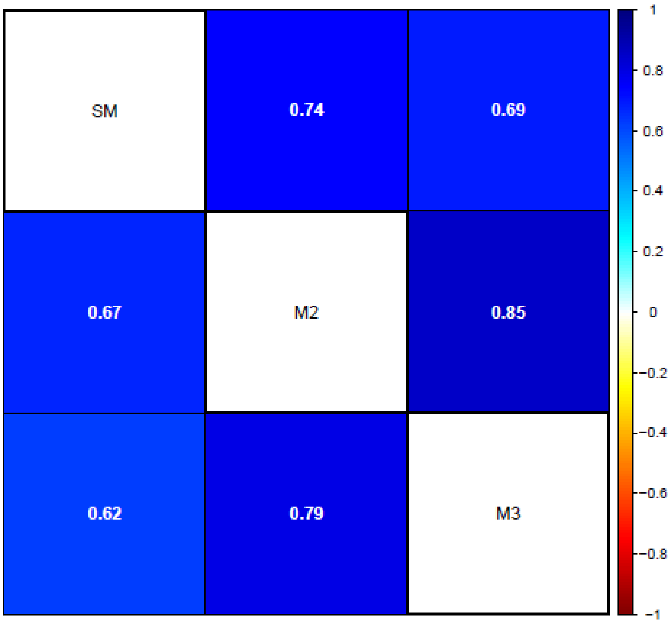
Figure 2 – Fixed regression solutions according to models and heat stress level for fat yield (FAT) in Holstein dairy cattle in a tropical environment.



Standard model (SM), Model 2 (M2); Model 3 (M3).

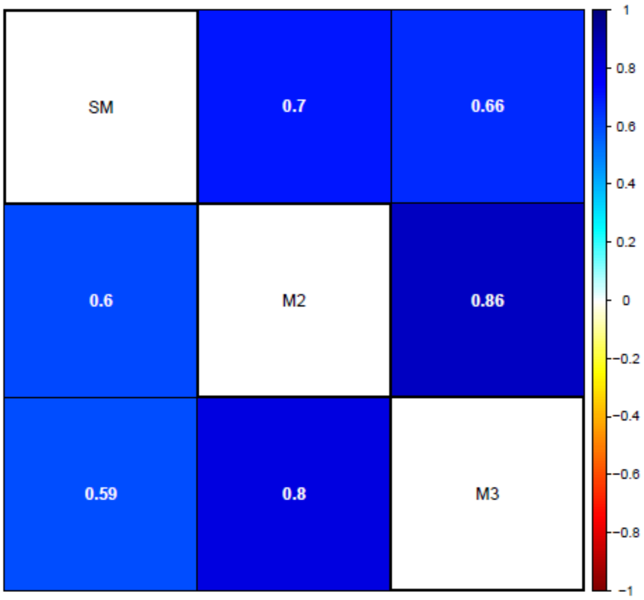
a: rainy calving season/ age 18 to 25 months; b: rainy season/ age 26 to 27 months; c: rainy season/ age 28 to 29 months; d: rainy season/ age 30 to 48 months; e: dry calving season/ age 18 to 25 months; f: dry season/ age 26 to 27 months; g: dry season/ age 28 to 29 months; h: dry season/ age 30 to 48 months.

Figure 3 – Fixed regression solutions according to models and heat stress level for protein yield (PROT) in Holstein dairy cattle in a tropical environment.



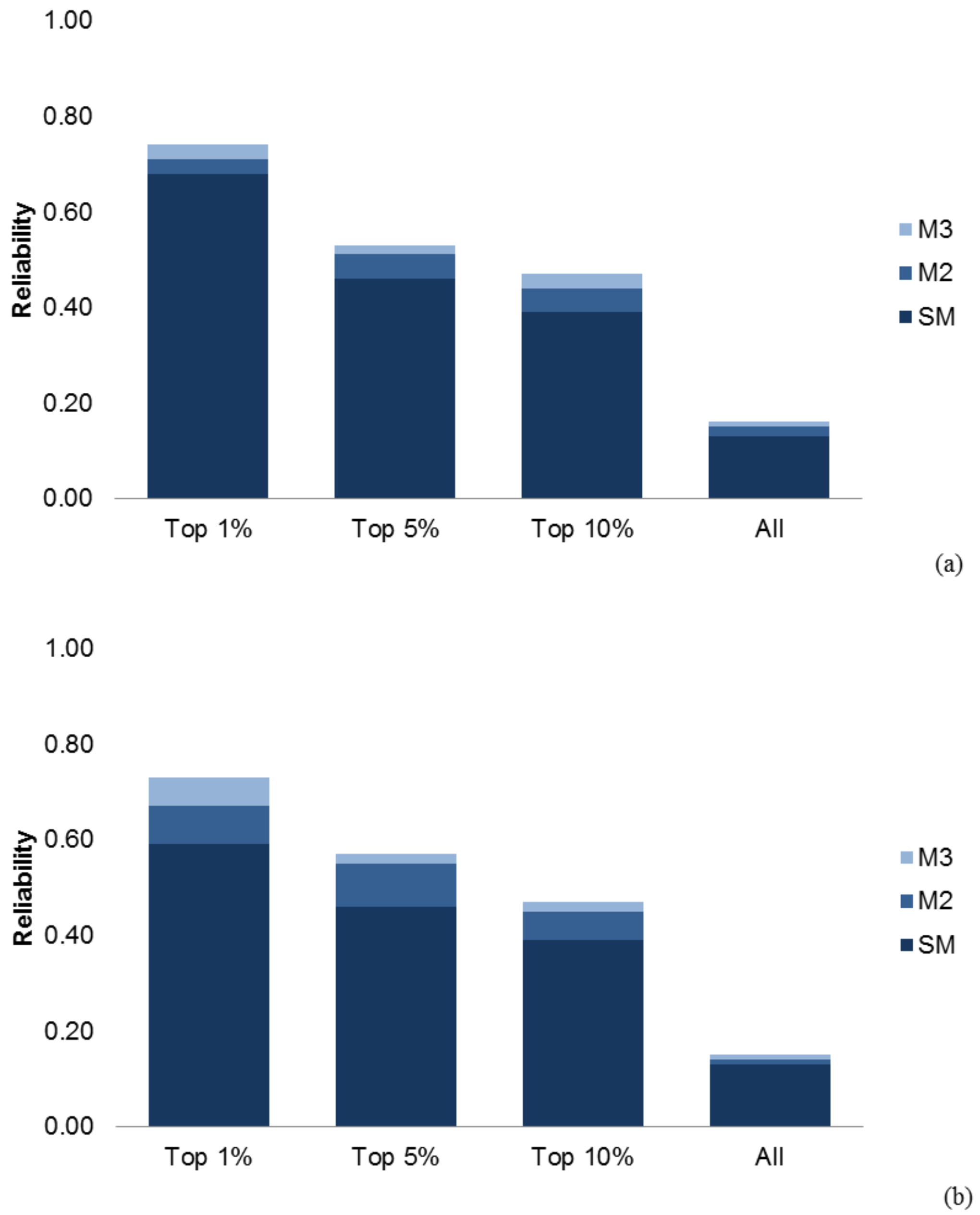
Size and colors indicate the magnitude and direction of associations.

Figure 4 – Spearman's rank correlations coefficient for Top 10% (above the diagonal) and Top 1% (below the diagonal) best sires (estimated breeding values - EBVs) for fat yield (FAT) in Holstein dairy cattle in a tropical environment.



Size and colors indicate the magnitude and direction of associations.

Figure 5 – Spearman's rank correlations coefficient for Top 10% (above the diagonal) and Top 1% (below the diagonal) best sires (estimated breeding values - EBVs) for protein yield (PROT) in Holstein dairy cattle in a tropical environment.



Standard model (SM), Model 2 (M2); Model 3 (M3).

Figure 6 - Reliability increased of estimated breeding values of the Top 1%, Top 5%, Top 10%, and all sires for fat (a) and protein (b) yield in the test-day (TD) records in Holstein dairy cattle in a tropical environment.

5. Considerações finais

Os estudos realizados identificaram os limiares de conforto térmico em bovinos da raça Holandesa para produção de leite (THI= 74 e DTV=13), escore de células somáticas (THI= 70 e DTV=9) e produção de gordura e proteína do leite (THI= 74 e DTV=16), com perdas significativas em produtividade animal e bem estar animal, ao ultrapassar estes limites. A investigação revela que o impacto do estresse térmico no escore de células somáticas ocorre previamente à produção de leite, permitindo adoção de técnicas, por parte dos criadores, que minimizem as perdas produtivas no campo antes mesmo de atingir um patamar inflacionado nas características produtivas.

Também são destacadas três vias para minimizar os efeitos do estresse térmico na avaliação, reclassificação e seleção dos animais: incluir os THI e DTV como efeitos fixos no modelo, considerando sua média obtida dois dias que antecedem o controle leiteiro; incorporar o nível de estresse térmico na porção de regressão fixa, que constitui o modelo de regressão aleatória, além dos efeitos rotineiramente já considerados; realizar a seleção para termotolerância, concomitantemente com as características de produção.

Com a alteração no mérito genético predito e na confiabilidade dessas predições, fica comprovado o forte e positivo efeito que a correção dos dados fenotípicos para as variáveis bioclimáticas indicadoras de estresse térmico é de extrema importância para garantir o contínuo progresso genético dos rebanhos nacionais. O maior conhecimento da intensidade dessa interação genótipo ambiente permitirá a orientação de manejos e acasalamentos de forma a sustentar a produção animal frente às mudanças climáticas.

Para sistemas de produção onde o estresse térmico possui efeito protagonista na produção animal, algumas formas de apresentar os resultados em sumários podem ser adotadas, como a apropriação de um selo para os animais termotolerantes ou até mesmo, apresentar as normas de reação com base no gradiente ambiental (THI) que permitem a escolha adequada para cada panorama de produção além da classificação dos animais conforme sua aptidão: extremamente robusto, robusto, plástico e extremamente plástico.

O setor de laticínios reconhece a grandeza da influência dos fatores bioclimáticos no desempenho animal. Embora haja iniciativas que investiguem os efeitos do estresse térmico e as formas de minimizá-los nas propriedades, ainda existem poucos estudos que exploram como as variáveis bioclimáticas podem ser incorporadas nas avaliações genéticas.

Para auxiliar nas tomadas de decisões futuras, se tornam necessários estudos que abordam a temática da variância residual homogênea x heterogênea dentro do contexto do estresse térmico na seleção animal e a busca por novas variáveis indicadoras de estresse térmico, onde além de explorar novas variáveis bioclimáticas seja possível investigar novos fenótipos como a resposta animal ao ambiente (por exemplo, temperatura vaginal). Além da investigação por meio de normas de reação vinculadas a origem dos touros utilizados nos rebanhos nacionais e a incorporação de avaliação genômicas dentro do contexto e a abrangência de outras ordens de lactação nas análises futuras.

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Vita

Renata Negri, filha de Valdecir Negri e Neli Dutra Negri, nasceu em Itapiranga – Santa Catarina, no dia 24 de Junho de 1991. Em 2010 ingressou no curso de graduação em Zootecnia na Universidade Tecnológica Federal do Paraná – UTFPR/ Dois Vizinhos. Bolsista do Programa de Educação Tutorial – PET desenvolveu atividades de ensino, pesquisa e extensão. Recebeu o prêmio de Acadêmico Destaque, obtendo seu título de Bacharel em Zootecnia em Março de 2015. Na mesma instituição, obteve seu título de Mestra em Zootecnia (2015-2017) junto ao Programa de Pós-Graduação em Zootecnia, na área de concentração de Produção Animal, sendo bolsista da Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – CAPES. Em abril de 2017 ingressou no curso de Doutorado junto ao Programa de Pós-Graduação em Zootecnia da Universidade Federal do Rio Grande do Sul – UFRGS, na área de concentração de Produção Animal, com bolsa do Conselho Nacional de Desenvolvimento Científico e Tecnológico – CNPq. O projeto foi aprovado junto ao CNPq na chamada MCTIC/CNPq Nº 28/2018 (Universal 2018) - Universal/Faixa C - De R$ 0,00 a R$ 120.000,00. Foi submetida à banca examinadora de defesa de tese em 19 de março de 2021.