**UNIVERSIDADE ESTADUAL PAULISTA – UNESP**

**CÂMPUS DE JABOTICABAL**

# GENETICS OF TOLERANCE TO HEAT STRESS IN MILK YIELD OF DAIRY BUFFALOES ASSESSED BY A RANDOM REGRESSION MODEL

**Gabriela Stefani Fernandez**

**Zootecnista**

**2020**

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**Tese apresentada à Faculdade de Ciências Agrárias e Veterinárias – UNESP, Câmpus de Jaboticabal, como parte das exigências para a obtenção do título de Doutor em Genética e Melhoramento Animal**

**2020**

S816g

Stefani, Gabriela

Genetics of tolerance to heat stress in milk yield of dairy buffaloes assessed by a random regression model / Gabriela Stefani. -- Jaboticabal, 2020

36 p. : il., tabs.

Tese (doutorado) - Universidade Estadual Paulista (Unesp), Faculdade de Ciências Agrárias e Veterinárias, Jaboticabal

Orientador: Humberto Tonhati

Coorientador: Mário Luiz Santana Júnior

1. Melhoramento Genético. 2. Genética animal. 3. Interação genótipo-ambiente. I. Título.

Sistema de geração automática de fichas catalográficas da Unesp. Biblioteca da Faculdade de Ciências Agrárias e Veterinárias, Jaboticabal. Dados fornecidos pelo autor(a).

UNIVERSIDADE ESTADUAL PAULISTA

Câmpus de Jaboticabal

## CERTIFICADO DE APROVAÇÃO

TÍTULO DA TESE: GENETICS OF TOLERANCE TO HEAT STRESS IN MILK YIELD OF DAIRY BUFFALOES ASSESSED BY A REACTION NORM MODEL

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Jaboticabal, 17 de dezembro de 2020

# AGRADECIMENTOS

O presente trabalho foi realizado com apoio da Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Código de Financiamento 001.

Agradeço a todos que contribuíram para o desenvolvimento deste trabalho, especialmente:

Ao prof. Dr. Humberto Tonhati, pela amizade, confiança e orientação.

Ao Dr. Mário Luiz Santana Júnior, por todo seu empenho e ajuda, que foi essencial para a realização deste trabalho.

À Dra. Lenira El Faro, minha eterna mentora.

Aos membros da banca examinadora, pelas excelentes e valiosas sugestões que contribuíram para aprimorar este trabalho.

À UNESP Jaboticabal, pelas condições proporcionadas para a realização deste curso, e ao Departamento de Zootecnia, pelo fornecimento dos dados.

À minha família, que sempre esteve me apoiando e torcendo por mim.

# DADOS CURRICULARES DO AUTOR

**Gabriela Stefani Fernandez -** nascida em 9 de abril de 1990, na cidade de Sertãozinho – SP, Brasil, filha de Paulo Roberto Stefani e Maria Helena Tiengo Stefani. Iniciou em 2007 o curso de graduação em Zootecnia na Universidade Estadual Paulista "Júlio de Mesquita Filho" – UNESP – Câmpus de Jaboticabal. Durante a graduação, foi bolsista de iniciação científica pela FAPESP, com projeto de pesquisa na área de Genética Molecular. Obteve o título de Zootecnista ao término de 2011. Em Janeiro de 2012 iniciou sua jornada profissional na empresa multinacional CRV Lagoa, na qual atuou por 5 anos na área de melhoramento genético de bovinos de leite. Em agosto de 2014, iniciou o curso de Mestrado do Programa de Pós-Graduação em Genética e Melhoramento Animal na Faculdade de Ciências Agrárias e Veterinárias – UNESP – Câmpus de Jaboticabal, obtendo o grau de Mestre em 2016 sob orientação do Prof. Dr. Humberto Tonhati. Em Agosto de 2017, iniciou o curso de Doutorado no Programa de Pós-Graduação em Genética e Melhoramento Animal na Faculdade de Ciências Agrárias e Veterinárias – UNESP – Câmpus de Jaboticabal, obtendo o grau de Doutora em 2020 sob orientação do Prof. Dr. Humberto Tonhati.

# SUMÁRIO

**Página**

# GENETICS OF TOLERANCE TO HEAT STRESS IN MILK YIELD OF DAIRY BUFFALOES ASSESSED BY A RANDOM REGRESSION MODEL

**ABSTRACT -** Buffaloes are recognizably rustic and well adapted to adverse tropical climates. However, there are reports in the literature that these animals show signs of suffering when exposed to high temperatures and solar radiation. Despite being an important issue, the effect of heat stress on milk yield in buffaloes has never been studied in Brazil. The objectives of this study were to assess the effects of heat stress on the milk yield and investigate the presence of genotype x environment interaction (G×E) in Brazilian dairy buffaloes reared under tropical conditions. With this, 53,113 test-day (TD) records for milk yield from 3,179 first-lactations of dairy buffaloes, collected between 1987 and 2018 were evaluated. A mixed model considering days in milk (DIM) and temperature-humidity index (THI) was applied to quantify milk yield losses due to heat stress. The most detrimental effect of THI on TD milk yield was observed in the intermediate stages of lactation, after lactation peak, in DIM 105-154 and 155-204 days (-0.002 and -0.014kg/day per THI, respectively). The least squares means of TD milk yield was used to identify a heat stress threshold by a piecewise linear regression model. A substantial reduction in TD milk yield due to heat stress was observed for THI values above 76.8 (-0.26 kg/day per increase of 1 THI unit). An analysis using a single-trait random regression animal model was carried out to estimate variance components and genetic parameters for TD milk yield over THI and DIM values. The additive genetic variance and heritability estimates were higher for lower THI values and earlier DIM. Lower genetic correlations between TD records were observed between opposite extremes of THI scale (THI=60 vs THI=80), reaching zero value. The genetic trends observed for the regression coefficients related to general yield level (0.02) and specific ability to respond to heat stress (-0.006) indicated that selection to increase milk yield did not affect the specific ability to respond to heat stress until the present moment. These trends reflect the low genetic correlation between these components (0.085±0.157), and are indicative of GxE with reordering of the estimated breeding values across the environment classes. Thus, the best animals for milk yield in the comfort zone are not necessarily the best in the zone of heat stress. Therefore, actions for monitoring trends of genetic components related to response to heat stress are recommended.

**Keywords:** buffaloes, genotype by environment interaction, heat stress, random regression, temperature-humidity index

# GENÉTICA DA TOLERÂNCIA AO ESTRESSE CALÓRICO NA PRODUÇÃO DE LEITE DE BÚFALAS LEITEIRAS AVALIADA POR UM MODELO DE REGRESSÃO ALEATÓRIA

**RESUMO** - Búfalos são animais reconhecidamente rústicos e bem adaptados à climas tropicais adversos. No entanto, há relatos na literatura de que esses animais mostram sinais de sofrimento quando expostos à elevadas temperaturas e radiação solar. Apesar de ser um assunto importante, o efeito do estresse calórico sobre a produção de leite em bubalinos nunca foi estudado no Brasil. Os objetivos deste estudo foram avaliar os efeitos do estresse calórico sobre a produção de leite e investigar a presença da interação genótipo x ambiente (G×E) em bubalinos brasileiros criados em condições tropicais. Com isso, foram avaliados registros de produção de leite de 53.113 controles leiteiros de 3.179 primeiras lactações de búfalas leiteiras, coletadas entre 1987 e 2018. Foi aplicado um modelo misto considerando dias em lactação (DIM) e índice de temperatura-umidade (THI) para quantificar as perdas na produção de leite devido ao estresse calórico. O efeito mais prejudicial do THI sobre a produção de leite no dia do controle foi observado nos estágios intermediários da lactação, após o pico da lactação, nos DIM 105-154 e 155-204 dias (-0,002 e -0,014kg/dia por THI, respectivamente). A média dos mínimos quadrados da produção de leite no dia do controle foi usada para identificar um limiar de estresse calórico por meio de um modelo de regressão linear por partes. Uma redução substancial na produção de leite no dia do controle devido ao estresse calórico foi observada para valores de THI acima de 76,8 (-0,26kg/dia por cada aumento de 1 unidade de THI). Uma análise utilizando um modelo animal unicaracterística de regressão aleatória foi realizada para estimar os componentes de variância e os parâmetros genéticos para a produção de leite no dia do controle sobre os valores de THI e DIM. A variação genética aditiva e as estimativas de herdabilidade foram maiores para valores mais baixos de THI e menores DIM. Menores correlações genéticas entre as produções de leite no dia do controle foram observadas entre os extremos opostos da escala THI (THI=60 vs THI=80), alcançando valor zero. As tendências genéticas observadas para os coeficientes de regressão relacionados ao nível de produção geral (0,02) e capacidade específica de responder ao estresse calórico (-0,006) indicaram que a seleção para aumentar a produção de leite não afetou a capacidade específica de responder ao estresse térmico até o momento presente. Essas tendências refletem a baixa correlação genética entre esses componentes (0,085±0,157), e são um indicativo de GxE com reordenamento dos valores genéticos estimados ao longo da escala ambiental. Isso significa que os melhores animais para produção de leite na zona de conforto não são necessariamente os melhores na zona de estresse calórico. Portanto, ações para monitorar tendências de componentes genéticos relacionados à resposta ao estresse calórico são recomendadas.

**Palavras-chave:** búfalos, interação genótipo-ambiente, estresse calórico, regressão aleatória, índice de temperatura-umidade

# 1. INTRODUCTION

The domestic Asian water buffalo (*Bubalus bubalis*) are distributed worldwide, with a a global population of some 207 million (FAO, 2007). The introduction of buffaloes in Brazil occurred at the end of the XIX century and throughout the XX century, coming mainly from Italy and India (ABCB, 2020). Its great adaptability to the several environments of the country, allowed the specie to show a significant evolution (Bernardes, 2007), and currently Brazil has the largest herd of buffalo among western countries, estimated at about 1 million heads (IBGE, 2017). Among the several purposes of buffaloes, in Brazil they are primarily used for milk production. The growing interest in buffalo milk is a result of the greatest milk industrial performance of the buffalo milk and bigger added value of buffaloes dairy products (Bernardes, 2007; Guo & Hendricks, 2010), such as the “Mozzarella”, one of the most valued cheeses by the local population (Mariante et al., 2002).

In addition to the quality of dairy by-products, another important factor that contributes to increased use of buffaloes in various regions of the world is its great rusticity, as they are well adapted to harsh environments. Thereby, buffaloes are considered a suitable option in areas where bovines do not adapt (Damasceno et al.,2010), as well as an interesting option for the occupation of the areas rejected by the agriculture (Bernardes, 2007). In Brazil, buffaloes are raised predominantly on pasture and in adverse climatic conditions. However, there is evidence in the literature that buffaloes exhibit signs of suffering when exposed to direct solar radiation, as they absorb a large amount of solar radiation through their dark skin and sparse coat, in addition to their reduced perspiration capacity (Marai and Haeeb, 2010; Hussain, 2016).

According to Dash et al. (2016), heat stress is defined as the state that causes imbalance in the animal's body in response to unfavorable environmental conditions. Animals under heat stress show a depression in feed intake, disturbances in metabolism of water, hormonal secretions, among other biological changes in their metabolism which cause a reduction in the efficiency of milk yield (Habeeb et al., 2018; Hussain, 2016; Pragna et al., 2017). Marai & Haeeb (2010) highlight that the negative effect of heat stress on productive and reproductive performance is aggravated when the heat is accompanied by high relative humidity (Marai & Haeeb, 2010).

Several indices have been developed as predictors of the negative influence of thermal stress on the production traits of dairy cows and buffaloes in tropical environments, and the most widespread is the temperature-humidity index (THI), initially developed for human comfort, and later adapted for cattle (NRC, 1971). This index incorporates the two most important climatic elements and is easily measured in a single value: temperature and relative humidity. THI has been widely used to quantify performance losses due to heat stress (Ravagnolo et al., 2000; Freitas et al., 2006; Hill and Wall, 2015). Furthermore, the THI, in conjunction with reaction norm models, has proven useful to infer on the genetic merit of animals for heat tolerance (Ravagnolo and Misztal, 2000; Aguilar et al., 2009).

Due to the importance of the effect of heat stress on the milk yield, some studies have assessed the decline in the performance of milk yield in dairy buffaloes with respect to an increase in THI. In India, Pawar et al. (2013) reported a decrease in daily milk yield from 4.46 to 3.65 kg or 18.2% due to heat stress. In the same country, Choudhary and Sirohi (2019) found a decline in milk yield greater than 1% for each increase of a THI unit above the critical level. Nasr (2017) studied productive indices of crossed dairy buffaloes in Egypt under warm weather and stated that high THI dropped significantly daily milk yield and peak milk yield in all studied breed compositions. The threshold level of maximum THI for buffaloes from the literature findings was identified with values ranging between 74 and 75, for the reason that with an increase in average THI above the threshold, the decline in production and reproduction rates was observed (Dash, 2016; Behera et al., 2018; Choudhary and Sirohi, 2019). In Brazil, despite several studies highlighting the importance of studying the influence of heat stress on buffalo farming (Garcia, 2013; Barros et al., 2015; da Silva et al., 2015), no study has effectively assessed its impact on milk yield.

Another important factor related to heat stress is the identification and selection of genetically tolerant animals. The selection for improved heat tolerance aims to maintain both high productivity and survivability of buffaloes when exposed to heat stress conditions and would have cumulative and permanent effects. In dairy cattle, several studies have identified genotype by environment interaction (G×E) due to heat stress for milk yield, and concluded that the selection to increase milk yield might result in reduced heat stress tolerance (Aguilar et al., 2010; Hammami et al., 2013; Bernabucci et al., 2014; Carabaño et al., 2014; Santana et al., 2015; Santana et al., 2017). For dairy buffaloes, however, this important subject remains unexplored worldwide since there are no previous studies that have modeled this effect in order to estimate the genetic parameters of milk yield in response to heat stress.

## 2. LITERATURE REVIEW

Milk yield is a typical example of repeated measurement or longitudinal data. There are various methods to study the milk yield in the lactation. The repeatability model has been employed for the analysis of data when multiple measurements on the same trait are recorded on an individual. The simplest approach is where lactation 305-d yields were considered as repeated measures of the same trait to a system where each lactation was considered to be a separate trait and the analyses were on test-day yields (Schaeffer et al., 2000). The main advantages of this model are its simplicity, fewer computation requirements and fewer parameters compared to a multivariate model (Mrode and Thompson, 2005). The problem is that this approach assumes that there is no variability in the shape of the lactation curve between animals, thus eliminating some genetic variation in milk yield (Mrode and Thompson, 2005). In addition, exclusion of short or incomplete lactation records might cause bias due to the pre-selection of data. The use of total yields or yields accumulated in long lactation periods implies not only the impossibility of considering environmental effects that may influence milk yield during certain periods but also of including in the analysis incomplete data from young animals (Tonhati et al., 2008).

Another alternative is the use of fixed regression models. The model involves the use of individual test-day records, and it allows the inclusion of incomplete lactation data without the need for projections, thus allowing more frequent evaluations and reducing generation intervals (Swalve, 2000). Some benefits attributed to the adoption of test-day models, as: a better definition of contemporary groups and it removes environmental effects more accurately, it models the shape of the lactation curve and the variability of yields around some general shapes and, therefore, more accurate genetic evaluations of cows and bulls than evaluations from 305-d yields (Mrode and Thompson, 2005; Schaeffer et al., 2000). The only major disadvantage is that the volume of data to be analysed is much larger. Schaeffer and Dekkers (1994) extended the fixed regression model for genetic evaluation by considering the regression coefficients on the same covariables as random, thus allowing for between-animal variation in the shape of the curve. Thus the genetic differences among animals could be modelled as deviations from the fixed lactation curves by means of random parametric curves (Guo and Swalve, 1997) or orthogonal polynomials, such as Legendre polynomials (Brotherstone et al., 2000).

The random regression models (RRM) are intended to model the deviations around the phenotypic trajectories. Orthogonal Legendre polynomials have been frequently used to model the covariance structure between test-day records by RRM (Bignardi et al., 2009; Bohmanova et al., 2008; El Faro et al., 2008). The main advantage of using Orthogonal Legendre polynomials is the reduced correlations among the estimated coefficients (Jamrozik and Schaeffer, 1997). According to Meyer (2005), changes in variance along a continuous scale are generally well modeled by high-order Legendre polynomials, but these polynomials may provide implausible parameter estimates for the beginning and the end of the trajectory by overemphasizing observations at the extremes. One alternative to high-order Legendre polynomials are segmented polynomials (splines). Splines are defined as curves that consist of individual segments of lower-degree polynomials joined at specific points, called knots (Meyer, 2005).

RRM have been shown to be very useful to model the effects of heat stress in dairy cattle (Misztal 1999; Ravagnolo and Misztal 2000). Using a linear reaction norm model, it is possible to obtain two genetic components with important biological interpretation for each animal; one is the overall production capacity and the other is the specific capacity to respond to heat stress (Menéndez-Buxadera et al. 2014). In most studies on heat stress using RRM, the trajectory of genetic (co)variance components for a given trait is described across a widely used environmental covariate, the temperature-humidity index (Bohmanova et al. 2008; Bernabucci et al. 2014). Brügemann et al. (2011) showed that RRM can accommodate both a time-dependent (days in milk, DIM) and temperature × humidity-dependent covariate. According to these authors, the application of such RRM permits the identification of genotype by environment interaction (G×E) for combinations of DIM and THI.

The GxE indicates that the performance of the different genotypes is not equally influenced by the different environments, which might cause a reclassification or changes in ranking genetic values (Falconer and Mackay, 1996). Distinguishing between genotypes that are more or less sensitive to environmental changes called plasticity (Bradshaw, 1965). Therefore, the presence of G×E may also be characterized as the difference in plasticity among individuals (Falconer and Mackay, 1996). According to Robertson (1959) estimates of genetic correlations between different environments <0.80 suggests that the genotype environment interaction is biologically important, then GxE needs to be considered in genetic-statistical models used for the genetic evaluation and selection of animals. According to Santana et al. (2015), GxE may lead to an important error in selection, and may affect the efficiency of selection programs by reducing the response in the performance traits and making importation of improved genotype economically less efficient (Montaldo, 2001).

Buffalo are raised in Brazil under different production systems, and are exposed to heterogeneous tropical climates, which generates numerous environmental challenges. In addition, the utilization of genotypes selected in regions with other climatic conditions is very common. According to Montaldo (2001), GxE is a potential source of reduced efficiency in genetic improvement programs involving animals in tropical areas and developing countries. These facts emphasize the importance of verify the existence of GxE for milk yield in buffaloes.

# 3. OBJECTIVES

The present study was based on data representing the first lactation of buffaloes reared in a tropical environment and the objectives were to: (1) quantify losses in milk yield due to increase in THI; (2) detect the THI value (heat stress threshold) at which milk yields started to decline due to heat stress (3) analyze genetic parameters of milk yield across the THI scale; (4) estimate genetic components of tolerance to heat stress.

# 4. MATERIAL AND METHODS

## 4.1. Data

The data used in this study were provided by Dairy Control Program maintained by the Animal Science Department of the School of Agricultural and Veterinarian Sciences of the São Paulo State University (UNESP) in Jaboticabal, SP, Brazil. The final data set consisted of 53,113 test-day (TD) records for milk yield from 3,179 first-lactations of dairy buffaloes, collected between 1987 and 2018, belonging to 6 herds located in 6 municipalities of 3 Brazilian states (Rio Grande do Norte, 43.5%; São Paulo, 42.8%; and Ceará, 13.7% of the TD records). The animals are raised predominantly on pasture and in regions characterized by humid tropical and semi-arid climates.

Restrictions were applied on the data as suggested by Aspilcueta-Borquis et al. (2013). The TD records were obtained between days 5 and 305 of the first lactation. The lactations shorter than 90 days were disregarded. Only lactations with the first TD record measured before 45 days after calving and with at least four TD during lactation were considered in the analyses. Animals without any pedigree information, and with age at first calving outside the range 22-60 months were discarded. The contemporary groups were defined as herd-year-month of TD, with the restriction that each group should contain at least four animals. Records outside 3.0 standard deviations from the mean of the contemporary group were discarded. The days in milk (DIM) classes (DIMc) were defined by preliminary analysis as 1 class for every 50 days, resulting in 6 classes with intervals of 5-54, 55-104, 105-154, 155-204, 205-254, 255-305 days. The pedigree file contained 4,522 animals, and it was constructed considering seven generations.

The climate variables were daily dry bulb temperature (T; in °C) and relative humidity (RH; in %) provided by the Instituto Nacional de Meteorologia (INMET, Brasília-DF, Brazil) at four weather stations located less than 70 km away from the farms. T and RH were recorded at three standardized times every day (0900, 1500, and 2100 h) in each weather station. The data recording procedure in the weather stations is standardized throughout the country according to the recommendation of the World Meteorological Organization. T and RH were combined in an index (THI) using the equation described by NRC (1971):

$$THI = (1.8 \times T + 32) - (0.55 - 0.0055 \times RH) \times (1.8 \times T - 26)$$

This formula was adopted because it is suited to the climatic variables available in Brazilian weather stations (T and RH). In addition, several studies show the usefulness of this formula for this type of study (Bohmanova et al., 2008; Brügemann et al., 2011; Brügemann et al., 2012; Hammami et al., 2013). The night time conditions are important for helping animals recover from the effects of heat stress during the day, but the only information available for this study was obtained in the three standard times mentioned above. The structure of the data set after editing is summarized in Table 1. The number of TD milk yield records according to THI is shown in Figure 1. Figure 2 shows the evolution of the THI over the months.

Table 1. Summary of data structure (standard deviation in parentheses).

| Item | Statistics |
|---|---|
| Animals in the pedigree (7 generations) | 4,522 |
| Number of animals with records | 3,179 |
| Number of parents without records | 1,343 |
| Test-day (TD) milk yield records | 53,113 |
| Mean number of TD per animal | 16.7 |
| Mean of TD milk yield (kg) | 7.63 (3.01) |
| Contemporary groups | 940 |
| Mean number of cows per contemporary group | 25.17 |
| Mean age at first calving | 38.58(6,09) |
| Mean temperature-humidity index (units) | 74.6 (4.86) |
| Mean number of different levels of THI per cow | 4.12 |
| Mean distance between farms and weather stations (km) | 46.74 |

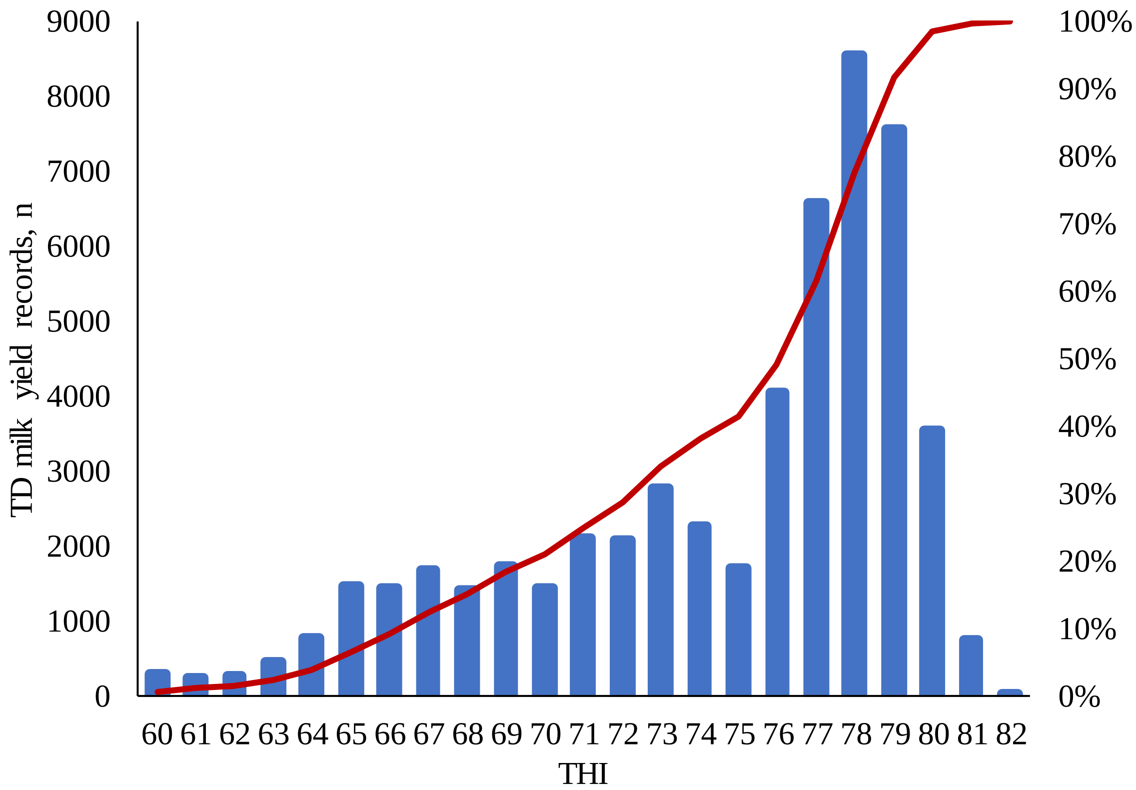


Figure 1. Frequency of test-day (TD) milk yield records (bars, left axis) and cumulative frequency (line, right axis) according to the average daily temperature–humidity index (THI) 5 days before the test date.

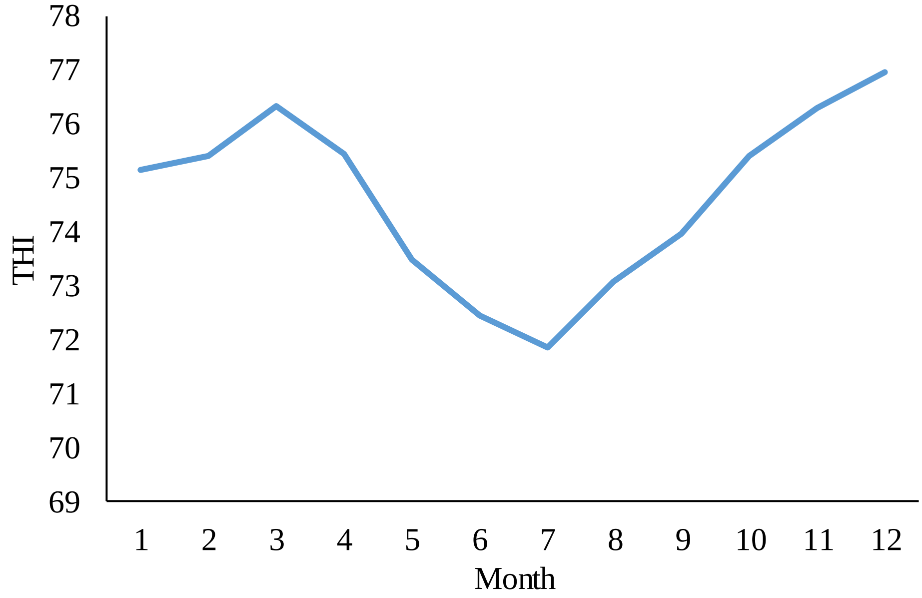


Figure 2. Evolution of the average daily temperature–humidity index (THI) 5 days before the test date according to the month.

### 4.2. The choice of best average daily THI

Previous studies found that weather measured prior to the TD date explained milk yield better than weather measured on the TD date (Bouraoui et al. 2002, Bertocchi et al., 2014). Based on this, the choice of average daily THI to be used as an environmental descriptor in the following analyses was performed using the procedure MIXED of the SAS 9.1 statistical program (SAS Institute Inc, 2009). Averages daily THI over 1, 2, 3, 4, 5, 6 and 7 days before the TD date (1 day is the TD date itself) were tested and the criteria used to select the best model was the maximum-likelihood function. A similar procedure was also adopted by Bohmanova et al. (2008) and Santana et al. (2016). The following model was used:

$$y_{ijklmn} = CG_i + MF_j \times DIMc_k + AFC_l + THI_m \times DIMc_k + \gamma_n + e_{ijklmn}$$

where $y_{ijklmn}$ is the TD milk yield record; $CG_j$ is the fixed effect of the *i*th contemporary group (defined as above); $MF_j \times DIMc_k$ is the fixed effect of the *j*th milking frequency (*j* = 1 or 2 times/day) by the *k*th (*k* = 1 to 6) class of days in milk; $AFC_l$ is the fixed effect of *l*th age at first calving (in months); $THI_m \times DIMc_k$ is the fixed effect of *m*th average daily THI to be tested by the *k*th class of days in milk; $\gamma_n$ is the random effect of the *n*th buffalo cow (ignoring the genetic relationship); and $e_{ijklmn}$ is the random effect of residual.

### 4.3. The effect of heat stress

The effect of increasing a THI unit on TD milk yield according to the DIMc was obtained by using the model described just above with the most appropriated average daily THI. In addition, least square means (LSM) of TD milk yield were estimated as a function of THI. The LSM of TD milk yield of DIMc most affected by the heat stress were analyzed separately using a broken piecewise linear regression with 2 phases (Ryan and Porth, 2007). This procedure was adopted to detect the THI value (heat stress threshold) at which milk yields started to decline due to heat stress. The following models were used:

$\mathrm{y} = \mathrm{a} + \mathrm{b}_1\mathrm{THI}$, if THI ≤ c, and

$\mathrm{y} = [\mathrm{a} + \mathrm{c}(\mathrm{b}_1 - \mathrm{b}_2)] + \mathrm{b}_2\mathrm{THI}$, if THI > c,

where $\mathrm{y}$ was the LSM of TD milk yield; $\mathrm{a}$ was the intercept; $\mathrm{b}_1$was the regression coefficients of TD milk yield over THI values below the heat stress threshold; $\mathrm{b}_2$ was the regression coefficients of TD milk yield over THI values above the heat stress threshold; and $\mathrm{c}$ was the heat stress threshold. The PROC NLIN of

SAS 9.1 statistical program (SAS Institute Inc, 2009) was used to fit the models to the data.

### 4.4. Genetic analysis

Because the number of TD records were relatively limited in the present study, in this step THI values were grouped every 2 units, with the first class beginning at THI=58 and last class at THI=82, totaling 13 classes. Variance components and genetic parameters for TD milk yield were estimated by a random regression animal model that included regression of the animal's EBV over the values of DIMc and THI, which were modeled using Legendre polynomials. The random regression model (model 1, M1) can be described in matrix form as:

$$y = FIXED_k + \sum_{n=1}^{q} \beta_{ln}\, \omega_n(d) + \sum_{n=1}^{q} \gamma_{ln}\, \omega_n(d) + \sum_{n=1}^{q} \delta_{ln}\, \omega_n(t) + \sum_{n=1}^{q} \varepsilon_{ln}\, \omega_n(t) + \ e_{ijklm}$$

where $y_{jklmn}$ is the mth TD milk yield record of the *l*th animal; $FIXED_k$ is the *k*th combination of fixed effects of GC (defined as above), classes of combination between milking frequency and DIMc, regression coefficient for the linear and quadratic effects of cow's age at calving in months, regression coefficients for the linear, quadratic and cubic effects of DIMc nested within classes of herd-2 years of calving, and regression coefficient for the linear effect of THI nested within DIMc; $\beta_{ln}$ the *n*th random regression coefficient for the additive genetic effect of animal *l* by DIMc; $\gamma_{ln}$the *n*th random regression coeffiecient for the permanent environmental effect of animal *l* by DIMc; $\delta_{ln}$ the *n*th random regression coefficient for the additive genetic effect of animal *l* by THI; $\varepsilon_{ln}$ the *n*th random regression coefficient for the permanent environmental effect of animal *l* by THI; *q* the number of regression coefficients; $\omega_n(d)$ the *n*th orthogonal Legendre polynomial corresponding to DIMc *d*;

$\omega_n(t)$ the *n*th orthogonal Legendre polynomial corresponding to THI *t*; and $e_{ijklm}$ the random residual effect.

The fixed and random regressions on DIMc were modeled with Legendre polynomials of order 3 (intercept, linear, quadratic, cubic), based on the results found by Aspilcueta-Borquis et al. (2012). The linear fixed and random regressions were modeled with Legendre polynomials of order 1, equivalent to a classical reaction norm model (intercept and linear terms). The residual variance was assumed to be heterogeneous across lactation (DIMc: 1, 2-4 and 5-6) and THI values (THI: 58-62, 64-82), resulting in 6 different combinations. The classes of residual variance were defined in a previous analysis by similarity of residual variances estimated with models of individual values of DIM and THI. The (co)variance structure follows:

$$\begin{bmatrix} \beta \\ \delta \\ \gamma \\ \varepsilon \\ e \end{bmatrix} = \begin{bmatrix} A \otimes G_{\beta} & A \otimes G_{\beta\delta} & 0 & 0 & 0 \\ A \otimes G_{\delta\beta} & \mathrm{A} \otimes G_{\delta} & 0 & 0 & 0 \\ 0 & 0 & I_l \otimes P_{\gamma} & I_l \otimes P_{\gamma\varepsilon} & 0 \\ 0 & 0 & I_l \otimes P_{\varepsilon\gamma} & I_l \otimes P_{\varepsilon} & 0 \\ 0 & 0 & 0 & 0 & I_m\sigma^2_{e_o} \end{bmatrix}$$

where $G_{\beta}$ and $G_{\delta}$ are (co)variance matrices of the random regression coefficients for additive genetic effects by DIMc and THI, respectively; $G_{\beta\delta}$ and $G_{\delta\beta}$ the covariance matrices for additive genetic effects for combinations of DIMc and THI; *A* the additive genetic relationship matrix; $\otimes$ the Kronecker product; $P_{\gamma}$ and $P_{\varepsilon}$ the (co)variance matrices of the random regression coefficients for permanent environmental effects by DIMc and THI, respectively; $P_{\gamma\varepsilon}$ and $P_{\varepsilon\gamma}$ the covariance matrices for permanent environmental effects for combinations of DIMc and THI; and $\sigma^2_{e_o}$ the residual variance, with *o* ranging from 1 to 6 according to the number of residual class; $I_l$ is an identity matrix of appropriate size for the permanent environmental effect (*l* is the number of animals with records) and $I_m$ an identity matrix of appropriate size for the residual (*m* is the number of TD records).

The analysis was performed under a Bayesian approach in a single-trait animal model using the GIBBS3F90 program (Misztal et al., 2002), that supports heterogeneous residual variances defined by classes. The prior distributions for all random effects were inverse Wishart distributions. The analysis consisted of a single chain of 500,000 cycles, with a conservative burn-in period of 150,000 cycles and a thinning interval of 50 cycles. Convergence was determined by Geweke's diagnostic (1991), visual inspection of the posterior chains of the parameters, and assessing the autocorrelation and effective sample size of the posterior parameter samples.

### 4.5. Calculation of genetic parameters

As indicated above, the G or P matrices models the genetic or permanent environment covariance structure of TD milk yield over THI and DIMc values. Thus, the additive genetic and permanent environmental (co)variances matrices as a function of DIMc or THI were calculated as $\Phi G \Phi'$ and $\Phi P \Phi'$ respectively; where, Φ is a matrix of Legendre polynomial function of DIMc or THI of order *t* (the number of DIM or THI classes) by *k* (where *k* is the order of fit); the diagonal of these (co)variances matrices were additive genetic variances ($\sigma_a^2$) and permanent environmental variances ($\sigma_{pe}^2$) for each DIMc or THI. The covariance's between DIMc *i* and *j* were calculated from $\Phi_i G_\beta \Phi'_j$ and $\Phi_i P_\gamma \Phi'_j$; covariance's between THI *i* and *j* from $\Phi_i G_\delta \Phi'_j$ and $\Phi_i P_\varepsilon \Phi'_j$. The covariance's between DIMc *i* and THI *j* were calculated as $\Phi_i G_{\beta\delta} \Phi'_j$ and $\Phi_i P_{\gamma\varepsilon} \Phi'_j$. Therefore, the genetic correlation between *i*th DIMc in j*th* level of THI ($r_{a(ij)}$) was calculated as:

$$r_{a(ij)} = \frac{\sigma_{a\beta\delta(ij)}}{\sqrt{\sigma_{a\beta(i)}^2 * \sigma_{a\delta(j)}^2}},$$

and heritability for *i*th DIMc in j*th* level of THI ($h_{(ij)}^2$) was calculated as:

$$h_{(ij)}^2 = \frac{\sigma_{a\beta(i)}^2 + \sigma_{a\delta(j)}^2 + 2\sigma_{a\beta\delta(ij)}}{\sigma_{a\beta(i)}^2 + \sigma_{a\delta(j)}^2 + 2\sigma_{a\beta\delta(ij)} + \sigma_{p\gamma(i)}^2 + \sigma_{p\varepsilon(j)}^2 + 2\sigma_{p\gamma\varepsilon(ij)} + \sigma_e^2}$$

where $\sigma_{a\beta\delta(ij)}$ and $\sigma_{p\gamma\varepsilon(ij)}$ are covariances of *i*th DIMc and *j*th level of THI for additive genetic and permanent environmental effects, respectively; $\sigma^2_{a\beta(i)}$ and $\sigma^2_{a\delta(j)}$ are the additive genetic variances of *i*th DIMc and *j*th level of THI, respectively; $\sigma^2_{p\gamma(i)}$ and $\sigma^2_{p\varepsilon(j)}$ are permanent variances of *i*th DIMc and *j*th level of THI, respectively; and $\sigma^2_e$ is the matrix of residual variances.

### 4.6. Breeding values and selection implications

The estimated breeding value (EBV) of an animal $i$ obtained with the random regression model described above was computed using the DIMc and THI values as follows: $EBV_l^{j,k} = \phi_{(j)k}\hat{\alpha}_i'$ where $\hat{\alpha}_i'$ is the vector of estimated additive genetic values for the orthogonal regression coefficients of animal $i$ (coefficients corresponding to DIMc and THI) and $\phi_{(j)k}$ is a vector of orthogonal coefficients evaluated in THI $j$ and DIMc $k$. Thus, the EBVs for 305-days cumulative milk yield (EBV305) was calculated as the sum of the EBVs from 5 to 305 days of lactation at a THI value of interest. Were analysed the pattern of response of the EBV305 of 30 bulls (with at least 10 daughters) across the THI scale to assess the existence of differences in environmental sensitivity of these animals.

To assess differences between a standard genetic evaluation versus the genetic evaluation considering environmental sensitivity to heat stress for milk yield, a standard model (model 2, M2) was used to calculate the traditional EBV305. This model is basically the same as M1, but with the random covariates based only on DIMc (without fixed and random regressions on linear Legendre polynomials of THI). So, the additive genetic and permanent environmental covariance functions were estimated by random regression on cubic Legendre polynomials of DIMc. The top 10 sires (with at least 10 daughters, total of 83 sires) were selected by EBV305 using the M1 and M2 models to determine the proportion of sires selected in common, i.e., the agreement of the animals selected between different points of the environmental gradient (THI=60, 72 and 80) and the agreement of the animals selected by the M1 and M2 models.

### 4.7. Genetic trends

Average estimates of all animals for the random regression coefficients intercept and slope for TD milk yield were analised according to the year of birth, generating a genetic trend over the years. The intercept represents the general production level, and slope represents the specific ability to respond to heat stress. In addition, the distribution of individual slopes (animal's ability to respond to heat stress) was evaluated aiming verify the existence of genetic variation.

## 5. RESULTS

The mean TD milk yield was 7.63 ± 3,01 kg (Table 1), similar to other studies that reported values ranging from 5.29 to 8.10 for dairy buffaloes (Aspilcueta-Borquis et al., 2010b; Singh et al., 2016; Choudhary and Sirohi, 2019). The mean THI of the data set was 74.6, with most of TD being recorded under high THI values (Figure 1) in addition to high THI values throughout most of the year (Figure 2), reflecting the harsh climatic conditions in the studied herds. The values of -2 Log Likelihood (subtracting 220,000) obtained in the preliminary analysis were 410, 400, 385, 379, 374, 378, and 383 for the models that consider averages daily THI over 1, 2, 3, 4, 5, 6 and 7 days before the TD date, respectively. It demonstrated that the most appropriate time interval to calculate the average daily THI was obtained as the average over five days (four days before and the TD date). This result indicates that the average over five days explained more variability in milk yield. Therefore, this average daily THI was assigned to each TD record for the subsequent analysis.

The linear effects of THI on TD milk yield, stratified according to DIMc, can be found in Table 2. Significant positive effects of THI were observed in early lactation stages: DIMc=1 and 2 (5-54 and 55-104 days), with an increase of 0.052 kg/day and 0.020 kg/day, respectively. Significant negative effects of THI were observed in mid-lactation stages: DIMc=4 and 5 (155-204 and 205-254 days), with losses of −0.014 kg/day and −0.009 kg/day per THI, respectively. Figure 3 shows the LSM of TD milk yield as a function of THI values for the different DIMc.

Table 2. Linear effects of temperature-humidity index (THI) on test-day milk yield records stratified according to the lactation stage (DIMc).

| DIMc | DIM (days) | Estimate | Standard error | p-value |
|---|---|---|---|---|
| 1 | 5-54 | 0.052 | 0.005 | <0.0001 |
| 2 | 55-104 | 0.020 | 0.005 | 0.0001 |
| 3 | 105-154 | -0.002 | 0.005 | 0.6826 |
| 4 | 155-204 | -0.014 | 0.005 | 0.0025 |
| 5 | 205-254 | -0.009 | 0.005 | 0.0504 |
| 6 | 255-305 | 0.006 | 0.006 | 0.2687 |

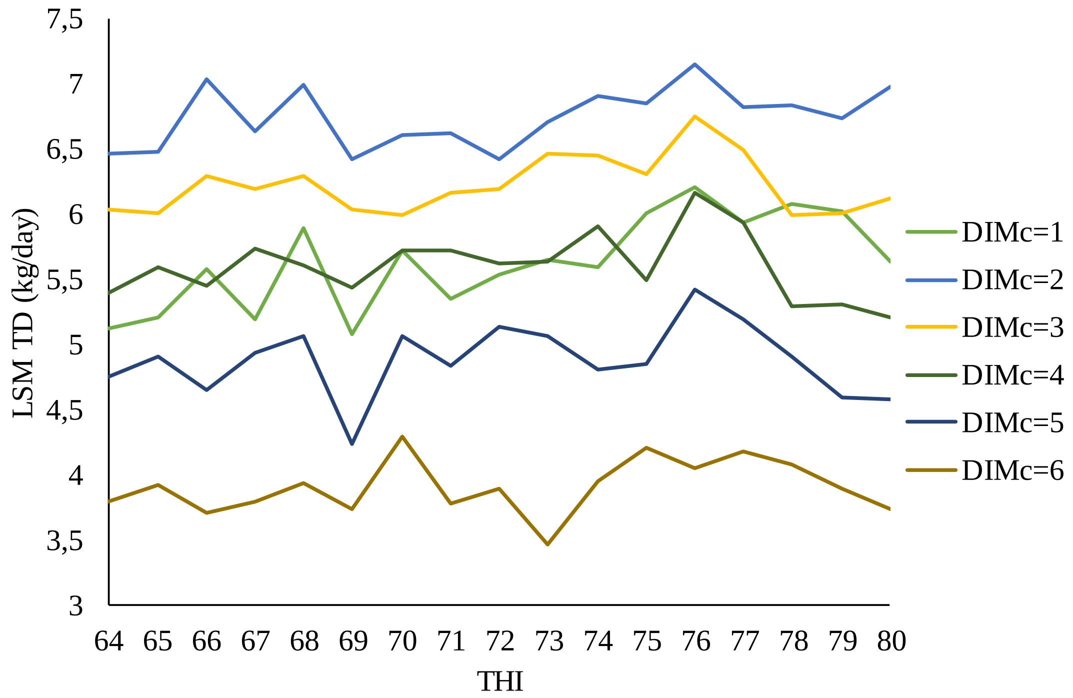


Figure 3. Least squares means (LSM) of test-day (TD) milk yield records as a function of the temperature-humidity index (THI) for different days in milk classes (DIMc).

The piecewise linear regression model was adjusted to identify the heat stress threshold using only the LSM of TD records obtained from the two DIMc significantly and unfavorably affected by heat stress (155-204 and 205-254). The estimated parameters can be found in Table 3. The TD milk yield showed a positive trend below the heat stress threshold (0.032kg/day per THI, $P < 0.01$) and was negatively affected for THI values above 76.8 (−0.262kg/day per THI, $P < 0.01$). The LSM and estimated picewise regression for TD milk yield TD as a function of THI values were shown in Figure 4. Figure 5 illustrates the combinations of T and RH that result in the THI threshold for heat stress.

Table 3. Estimated piecewise linear regression for LSM of test-day (TD) milk yield records (days in milk between 155 and 254) as a function of the temperature-humidity index (THI).

| Parameter | Estimate | Standard Error | 95% Confidence Limits | | |
|---|---|---|---|---|---|
| a | 3.11 | 1.18 | 0.57 | to | 5.66 |
| $b_1$ | 0.03 | 0.02 | 0.00 | to | 0.07 |
| c | 76.8 | 0.83 | 75.01 | to | 78.60 |
| $b_2$ | -0.26 | 0.10 | -0.48 | to | -0.04 |

a=Intercept
$b_1$= The plateau when the TD is not affected by the heat stress
$b_2$= The slope of the decrease in TD when THI increases by 1 unit
c= Estimated threshold of THI

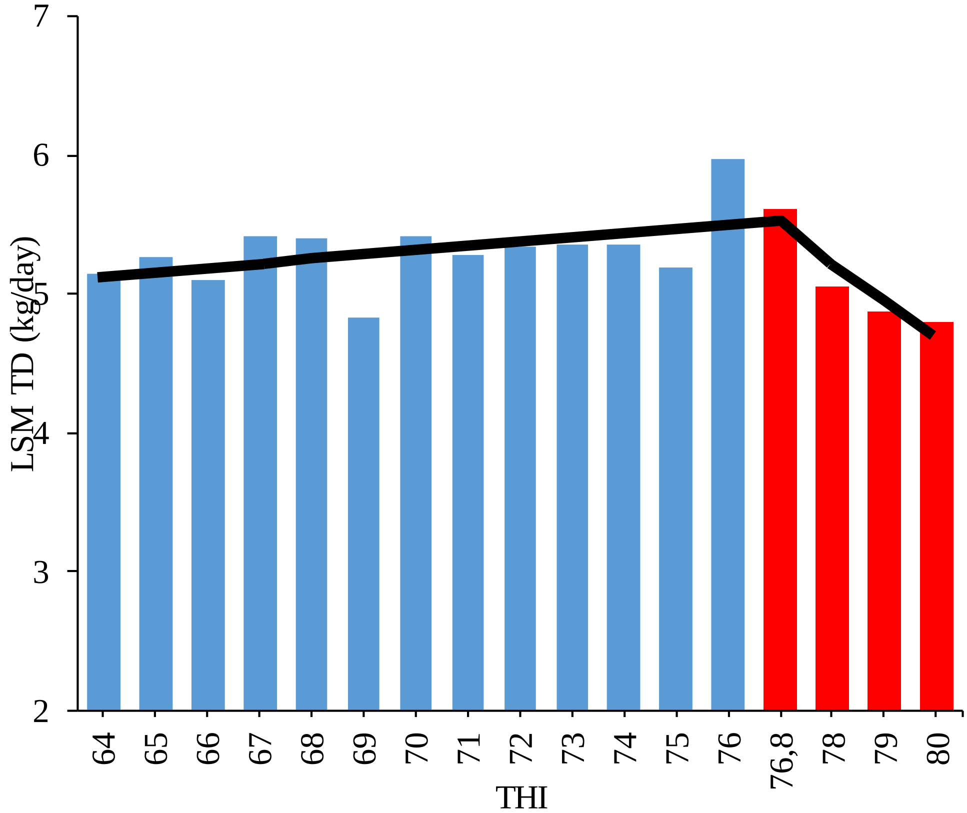


Figure 4. Least squares means (LSM, bars) of test-day (TD) milk yield records (days in milk between 155 and 254) and estimated picewise regression (dotted line) for TD milk yield as a function of the temperature-humidity index (THI).

| | Relative Humidity (%) | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Temperature (°C) | 0 | 5 | 10 | 15 | 20 | 25 | 30 | 35 | 40 | 45 | 50 | 55 | 60 | 65 | 70 | 75 | 80 | 85 | 90 | 95 | 100 |
| 23 | 65 | 65 | 66 | 66 | 67 | 67 | 68 | 68 | 68 | 69 | 69 | 70 | 70 | 70 | 71 | 71 | 72 | 72 | 73 | 73 | 73 |
| 24 | 66 | 66 | 67 | 67 | 68 | 68 | 69 | 69 | 70 | 70 | 71 | 71 | 71 | 72 | 72 | 73 | 73 | 74 | 74 | 75 | 75 |
| 25 | 67 | 67 | 68 | 68 | 69 | 69 | 70 | 70 | 71 | 71 | 72 | 72 | 73 | 73 | 74 | 74 | 75 | 75 | 76 | 77 | 77 |
| 26 | 67 | 68 | 69 | 69 | 70 | 70 | 71 | 71 | 72 | 73 | 73 | 74 | 74 | 75 | 75 | 76 | 77 | 77 | 78 | 78 | 79 |
| 27 | 68 | 69 | 69 | 70 | 71 | 71 | 72 | 73 | 73 | 74 | 74 | 75 | 76 | 76 | 77 | 78 | 78 | 79 | 79 | 80 | 81 |
| 28 | 69 | 70 | 70 | 71 | 72 | 72 | 73 | 74 | 74 | 75 | 76 | 76 | 77 | 78 | 78 | 79 | 80 | 80 | 81 | 82 | 82 |
| 29 | 70 | 71 | 71 | 72 | 73 | 73 | 74 | 75 | 76 | 76 | 77 | 78 | 78 | 79 | 80 | 81 | 81 | 82 | 83 | 84 | 84 |
| 30 | 71 | 71 | 72 | 73 | 74 | 75 | 75 | 76 | 77 | 78 | 78 | 79 | 80 | 81 | 81 | 82 | 83 | 84 | 85 | 85 | 86 |
| 31 | 71 | 72 | 73 | 74 | 75 | 76 | 76 | 77 | 78 | 79 | 80 | 80 | 81 | 82 | 83 | 84 | 85 | 85 | 86 | 87 | 88 |
| 32 | 72 | 73 | 74 | 75 | 76 | 77 | 77 | 78 | 79 | 80 | 81 | 82 | 83 | 84 | 84 | 85 | 86 | 87 | 88 | 89 | 90 |
| 33 | 73 | 74 | 75 | 76 | 77 | 78 | 79 | 80 | 80 | 81 | 82 | 83 | 84 | 85 | 86 | 87 | 88 | 89 | 90 | 91 | 91 |
| 34 | 74 | 75 | 76 | 77 | 78 | 79 | 80 | 81 | 82 | 83 | 84 | 85 | 86 | 86 | 87 | 88 | 89 | 90 | 91 | 92 | 93 |
| 35 | 75 | 76 | 77 | 78 | 79 | 80 | 81 | 82 | 83 | 84 | 85 | 86 | 87 | 88 | 89 | 90 | 91 | 92 | 93 | 94 | 95 |
| 36 | 76 | 77 | 78 | 79 | 80 | 81 | 82 | 83 | 84 | 85 | 86 | 87 | 88 | 89 | 90 | 92 | 93 | 94 | 95 | 96 | 97 |
| 37 | 76 | 77 | 79 | 80 | 81 | 82 | 83 | 84 | 85 | 86 | 87 | 89 | 90 | 91 | 92 | 93 | 94 | 95 | 96 | 98 | 99 |
| 38 | 77 | 78 | 79 | 81 | 82 | 83 | 84 | 85 | 86 | 88 | 89 | 90 | 91 | 92 | 93 | 95 | 96 | 97 | 98 | 99 | 100 |
| 39 | 78 | 79 | 80 | 82 | 83 | 84 | 85 | 86 | 88 | 89 | 90 | 91 | 93 | 94 | 95 | 96 | 97 | 99 | 100 | 101 | 102 |

Under thermal confort    Heat stress Threshold    Under heat stress

Figure 5. The levels of heat stress in dairy buffaloes estimated by a picewise regression according to the temperature-humidity index (THI).

The estimates of variance components obtained for the end of lactation (DIMc=6) were probably overestimated, so the values were not shown. The overestimation of variances at the extremes of the lactation is often explained by Legendre polynomials' lack of asymptotes; poor performance of fitting data at the extremes of lactations (López-Romero et al., 2004), or not accounting for between-herd variation in the shape of the lactation (Gengler and Wiggans, 2001; Jamrozik et al., 2001; de Roos et al., 2004). In general, the additive genetic variance decreased with increasing THI (Figure 6). For any THI, the estimates were higher in early lactation (DIMc=1, 2 and 3). The highest estimates of permanent environmental variance were obtained for combinations of higher THI values and DIMc=2 and 3 (Figure 6). In respect to heritability, all estimates were of low magnitude. However, the highest estimates were obtained for lower THI values and in early lactation stages, with estimates between 0.05 to 0.17 for combinations of THI<64 and DIMc=1, 2 and 3 (Figure 7). The residual variance was relatively constant over the classes of THI and DIMc, except for the combination of DIMc=2-4 and THI=58-63, where estimates were slightly lower (Figure 7).

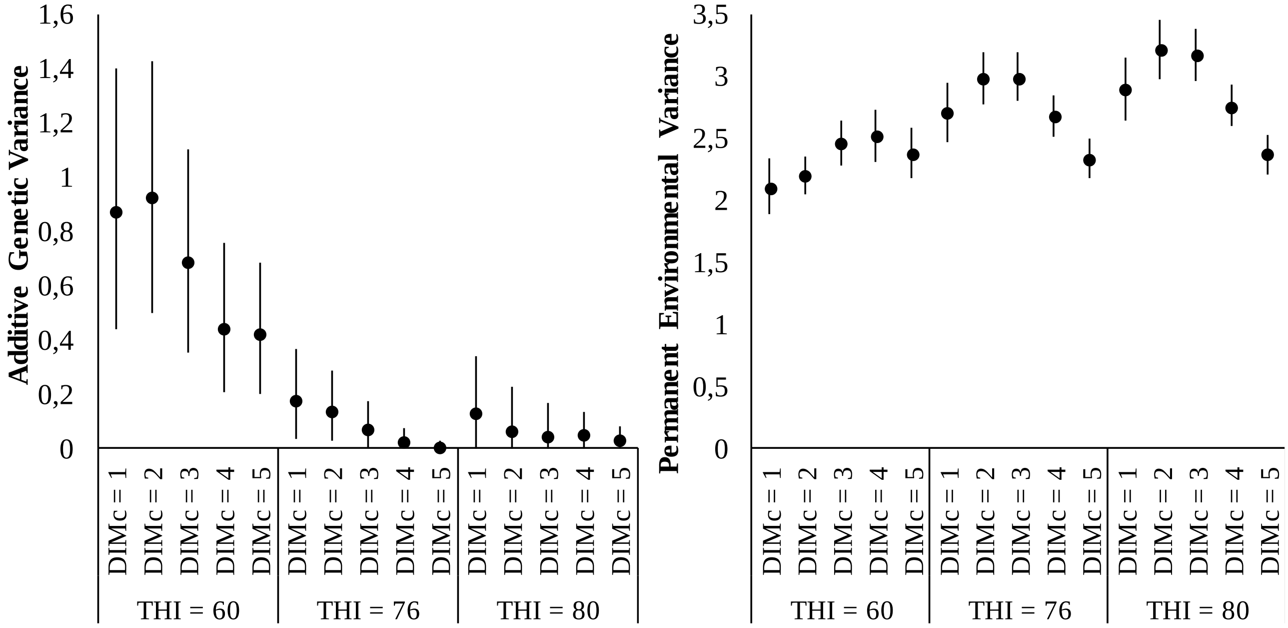


Figure 6. Posterior means (•) and 95% highest posterior density regions (vertical bars) of additive genetic variance (kg²) and permanent environmental variance (kg²) of test-day (TD) records for different combinations of days in milk classes (DIMc) and temperature-humidity index (THI).

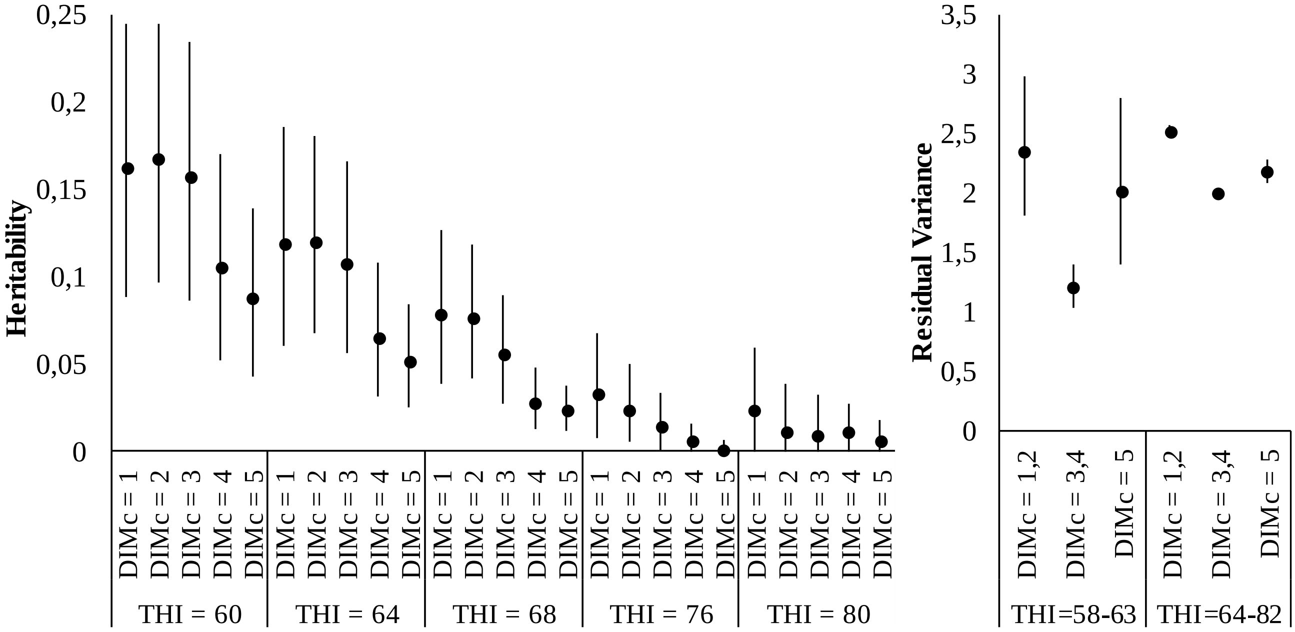


Figure 7. Posterior means (•) and 95% highest posterior density regions (vertical bars) of heritability estimates and residual variance (kg²) of test-day (TD) records for different combinations of days in milk classes (DIMc) and temperature-humidity index (THI).

The posterior means of genetic correlation between TD records at different DIMc were always higher than 0.82 (Figure 8), with higher values between TD records at adjacent DIMc and between the beginning and end of lactation. The lowest genetic correlations between TD records at different combinations of DIMc and THI were observed in higher THI values (Figure 8). The estimates ranged from -0.07 to 0.16 in combinations of DIMc with THI=80 and were higher than 0.90 in combinations of DIMc with THI=60. The genetic correlations between TD records at different THI ranged from 0 to 1 (Figure 8). The highest estimates were between TD records at adjacent THI values, and the lowest estimates were between TD records at the opposite extremes of THI scale (THI=60 vs. THI=80).

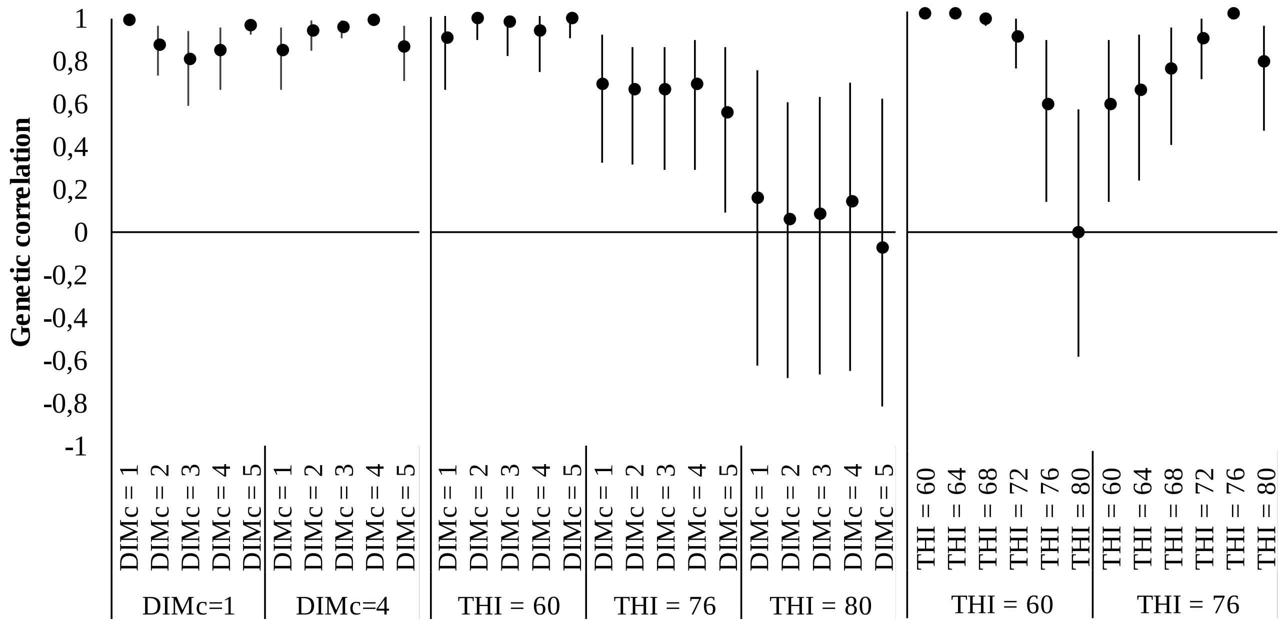


Figure 8. Posterior means (•) and 95% highest posterior density regions (vertical bars) of genetic correlations between test-day (TD) records for different combinations of days in milk classes (DIMc) and temperature-humidity index (THI).

Analysis of the pattern of response of the EBV305 of 30 bulls (with at least 10 daughters) across the THI scale shows differences in the environmental sensitivity of these animals (Figure 9), illustrating G×E. Animals that were sensitive to changes in THI values and animals that were indifferent to these changes were identified. Comparison of the top 10 sires (with at least 10 daughters) selected for EBV305 in different environments (THI=60, 72 and 80) using M1, and the top sires selected by M2 showed important differences in the proportion of bulls selected in common (Table 4).

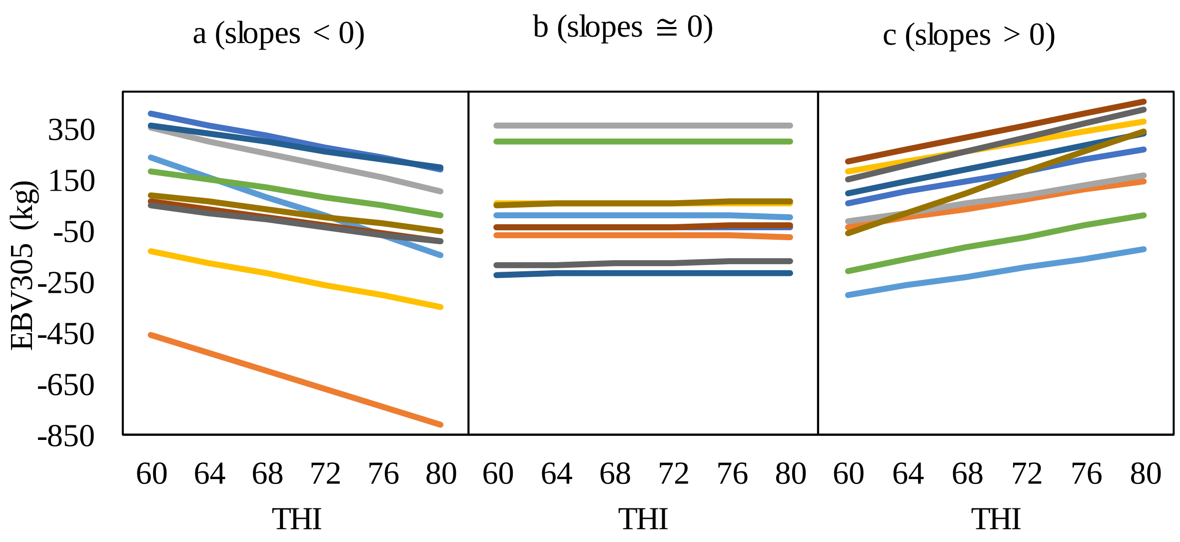


Figure 9. Pattern of response through the temperature–humidity index (THI) scale of the estimated breeding values for 305-days cumulative milk yield (EBV305) of sires (with at least 10 daughters) grouped into "negative" plastic (a), robust (b), and "positive" plastic (c) animals using the model with random regression on days in milk and THI (M1).

Table 4. Proportion of top 10 sires selected in common for estimated breeding value for 305-days cumulative milk yield (EBV305) between different points of temperature–humidity index (THI=60, 72 and 80) by the model with random regression on days in milk and THI (M1), and by the standard model with random regression on days in milk (M2).

| | Proportion of sires selected (%) | | | |
|---|---|---|---|---|
| Selection by | M1 THI=60 | M1 THI=72 | M1 THI=80 | M2 (Standard) |
| M1 THI=60 | 100 | 50 | 30 | 50 |
| M1 THI=72 | 50 | 100 | 80 | 90 |
| M1 THI=80 | 30 | 80 | 100 | 80 |
| M2 (Standard) | 50 | 90 | 80 | 100 |

Figure 10 shows the trends for the average estimated regression coefficients associated with the production level (intercept of the reaction norm) and the specific ability to respond to heat stress (slope of the reaction norm). The trend of the level was 0.02 kg/year (P < 0.001), and the trend of the slope was –0.006 kg/year (P < 0.004). Figure 11 illustrates important variation of the animal’s individual slopes for TD milk yield. The genetic correlation between level and slope was not different from zero (Table 5). The ratio of genetic variances for slope and level of the reaction norms was 0.135, indicating the not negligible G×E due to heat stress (Table 5). The permanent environmental correlations between level and slope were –0.194, and the environmental variance ratio of slope and level was 0.008.

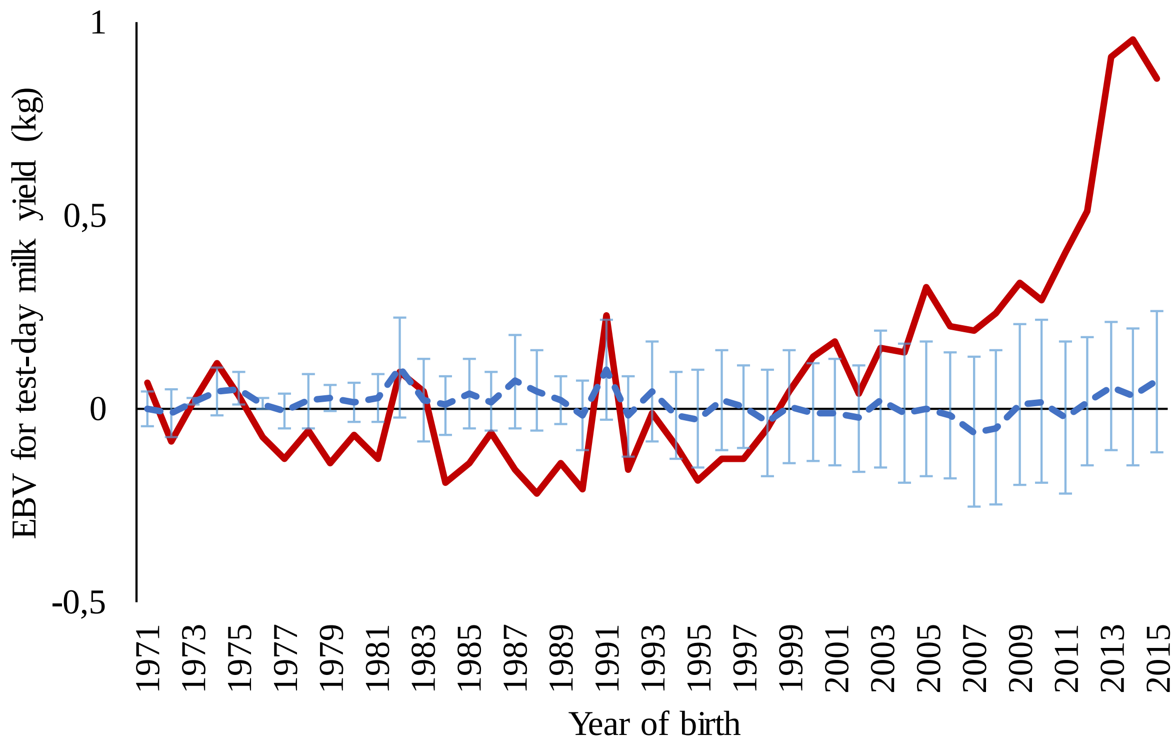


Figure 10. Genetic trends for the posterior mean estimates of random regression coefficients level (red line) and slope (dotted blue line with standard-deviations in vertical bars) for test-day milk yield of dairy buffaloes raised under tropical conditions according to the year of birth.

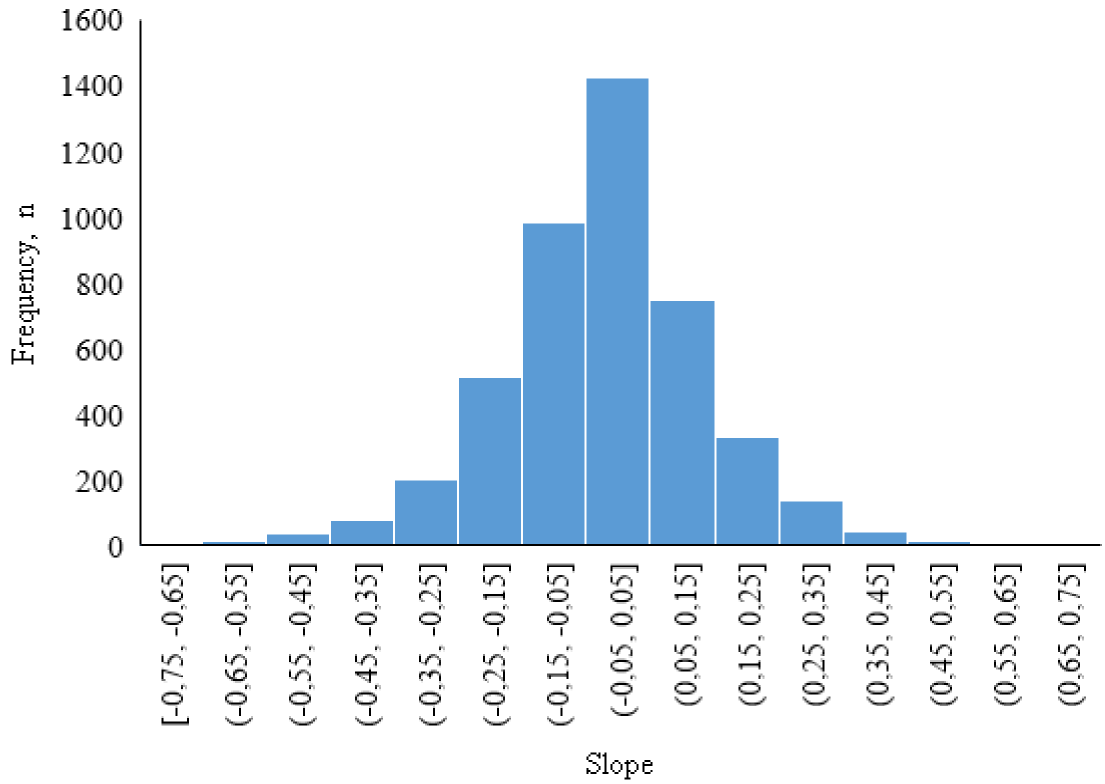


Figure 11. Frequency distribution of the individual slopes (specific ability to respond to heat stress) for test-day milk yield over the temperature–humidity index (THI) scale.

Table 5. Posterior mean, standard deviation (SD), and 95% highest posterior density interval (HPD) of additive genetic and permanent environmental parameters of level (L) and slope (S) of the reaction norm model

| Item | Mean | SD | 95% HPD | | |
|---|---|---|---|---|---|
| Additive genetic effects | | | | | |
| L | 1.556 | 0.293 | 1.021 | to | 2.172 |
| S | 0.203 | 0.068 | 0.082 | to | 0.345 |
| Covariance L- S | 0.042 | 0.079 | -0.126 | to | 0.183 |
| Genetic correlation L - S | 0.093 | 0.157 | -0.189 | to | 0.416 |
| S/L ratio | 0.135 | 0.053 | 0.050 | to | 0.253 |
| Permanent environmental effects | | | | | |
| L | 3.243 | 0.221 | 2.811 | to | 3.675 |
| S | 0.027 | 0.003 | 0.021 | to | 0.032 |
| Covariance L- S | -0.061 | 0.020 | -0.100 | to | -0.022 |
| Genetic correlation L - S | -0.206 | 0.063 | -0.325 | to | -0.078 |
| S/L ratio | 0.008 | 0.001 | 0.006 | to | 0.010 |

## 6. DISCUSSION

The minor importance of buffalos with regard to global milk yield, in addition to lower selection intensity for high productivity and their significant adaptability to hot environments, explains why less heat stress attention has been given to these species (Bernabucci et al., 2010). The present study demonstrated that the performance of dairy buffaloes is strongly influenced by the thermal environment. Both low values as high values of THI were observed to negatively impact the TD milk yield (Figures 3 and 4), however the main challenge for the studied herd is the heat stress, since they are being raised mainly under tropical conditions, with high THI values throughout most of the year (Figures 1 and 2). The effect of THI was dependent on the lactation stage (Table 2), with harmful effects in the intermediate stages, after lactation peak (DIMc=4 and 5). Other authors studied the effects of THI on milk yield of buffalo cows without stratifying according to the lactation stage. They reported close values ranging from -0.015 to -0.082kg/day per unit increase in THI (Upadhyay et al., 2007; Pawar et al., 2013; Choudhary and Sirohi, 2019). In taurine and zebu cattle breeds, studies also reported that the most detrimental effect of THI on milk yield was observed in the intermediate stages of lactation (Bernabucci et al., 2010; Santana et al., 2015; Santana et al., 2020). Bernabucci et al. (2010) reported that the stage of lactation is an important factor affecting how dairy animals respond to heat stress. The nutritional–metabolic conditions of dairy cows during the different lactation stages might explain the higher heat sensitivity of mid-lactating dairy cows.

According to results obtained with the piecewise linear regression model, the heat stress threshold for TD milk yield was THI=76.8 (Table 3; Figure 4). This result indicates that buffaloes have outstanding suitability to hot-humid conditions, with higher critical THI than taurine and zebu cattle breeds (Brügemann et al., 2012; Hammami et al., 2013; Santana et al., 2015; Santana et al., 2017). However, we can conclude that its production performance is susceptible to heat stress, leading to a decline in milk productivity when exposed to a THI above the threshold level. To better illustrate the magnitude of the values found, a supposed animal in THI=82 may have its milk yield reduced by 1.36kg/day (or 25% of the mean) due to heat stress. Furthermore, 51% of TD records of this study were within the stress zone (THI=77 to 82, Figure 2). Despite the differences with respect to methodology, other studies reported heat stress threshold between THI=74 and 75, and a loss of 18.2% in milk yield above the threshold (Pawar et al., 2013; Sigdel et al., 2015; Dash et al., 2016; Choudhary and Sirohi, 2019).

No studies estimating the genetic components of heat stress for dairy buffaloes exist either. In this respect, the present results are not comparable to the literature. The results obtained with a random regression model showed that the genetic parameter estimates varied as a function of DIMc and also across the THI scale. A reduction was observed in additive genetic variance with increasing THI values (Figure 6). It was lower in high THI in combination with a final lactation period. According to Brügemann et al. (2011), the effect of heat stress can suppress the full expression of the genetic potential of animals. The heritability estimates (Figure 7) obtained for the different combinations of DIMc and THI were higher for lower THI values and low DIMc, indicating that the greatest responses to selection can be expected for the thermal comfort zone and at the beginning of lactation. Santana et al. (2016) explain that poorly adapted animals raised in tropical environments may be being selected most of the time within the stress zone. Consequently, there may be less genetic variability of production in this zone. Bohlouli et al. (2013) and Santana et al. (2015, 2020), also reported lower values of additive genetic variance and heritabilities with increasing THI for milk yield of Holstein, Gir, and Dual-purpose Guzerá cattle, respectively.

The genetic correlations between TD milk yield at combinations of THI and DIMc (Figure 8) show that the best animals for TD milk yield at a specific DIMc tend to be also the best in low THI values, but in general, are not the best in high THI values. Genetic correlations that tended to be lowest were found for higher THI values. The genetic correlations of TD milk yield between extreme THI values reached zero. These estimates indicate that the expression of this trait can vary according to THI values, demonstrating G×E due to heat stress. In other words, this result indicates that important changes in the ranking of animals may occur between extreme THI values. these results are in accordance with Robertson (1959), who states that genetic correlations between performances on two environments below 0.8 are indicative of GxE with reordering of the EBVs across the environment classes. Therefore, considerable error in the selection may thus occur due to differences in the genetic sensitivity of animals to heat stress (Santana et al., 2015).

The reaction norms of 30 bulls commonly used in the studied herds are plotted in Figure 9. Several of these animals respond negatively (“negative” plastic animals, slope<0) to an increase in THI, others may respond positively (“positive” plastic animals, slope>0) or even be indifferent in terms of performance to the increase in THI (robust animals, slope ≅ 0). In this sense, the best animals for milk yield in the comfort zone are not necessarily the best in the zone of heat stress. When top 10 sires (with at least 10 daughters) were selected for EBV305 in different environments (THI=60, 72 and 80) using M1 (Table 4), the proportions of animals selected in common varied considerably, mainly between extreme environments (THI=60 vs THI=80), where the proportion of bulls selected in common was only 30%. The selection of the best 10 sires without consideration of the G×E (standard model M2) can lead to errors of up to 50 % compared to M1 at extreme values of THI (THI=60, Table 4). The highest proportion of bulls selected in common by M1 and M2 was under conditions of thermal comfort (THI=72). These results demonstrate the existence of important divergence between the practice of selecting only for milk yield and selection considering heat stress resistance.

The genetic correlation between level and slope was close to zero (Table 5), indicating that high performance for milk yield is not linearly associated with a high or low ability to respond to heat stress. The genetic trends (Figure 10) for level and slope are a reflection of this low genetic correlation, since the increase in the level (selection for milk yield) did not cause a change in the trend of slope. However, it is possible to observe that the selection for milk yield increased dispersion of the slope over the years (Figure 10), which probably causes an increase in the re-ranking of animals across the THI scale. The negative permanent environmental correlation between the level and slope indicates that animal performance under the studied environmental conditions was unsteady (Hammami et al., 2015). Components of the genetic response to heat stress (slope) showed an important variation (Table 5), while environmental components of slope and environmental variance ratio of slope and level were near to zero. This means that the sensitivity to heat stress for milk yield is more genetic than environmental, allowing response to selection. This fact is also supported by the important variantion in the individual slopes of animals illustred in Figure 11. The genetic additive slope-to-intercept variance ratio was 0.135, corresponding to an accentuated G×E, where the THI has a large effect on selection response. (Kolmodin and Bijma, 2004). Lower values have been reported in studies on different breeds of dairy cattle in temperate climates, ranging from 0.003 to 0.005 (Ravagnolo and Misztal, 2000; Bohmanova et al., 2008; Bernabucci et al., 2014), and similar values in tropical climates, ranging from 0.04 to 0.16 (Santana et al., 2016; Santana et al., 2020). The permanent environmental slope-to-intercept variance ratio of 0.008 is an indicative that the individual sensitivity to heat stress for milk yield is more environmental than genetic. This facts support the importance of G×E for milk yield in dairy buffaloes reared under tropical climate.

The genetic progress for the general production level was almost zero in three decades (Figure 10). However, after the beginning of 21th century, the trend of level indicate an increase in the EBV of the population for TD milk yield, a fact probably explained by the accumulation of information collected by the Dairy Control Program in the late 1990s that allowed to estimate genetic values and select to increase milk production, and send bulls to semen collection and storage centres. The trend of slope showed a slight decrease over the years, indicating that the genetic selection for higher milk yield did not cause a big loss in response to heat stress of the buffaloes until the present moment. Similar previous studies with different breeds of dairy cattle found a deterioration of the genetic response to heat stress due to selection for high milk yield, with genetic correlation estimates between production level and the slope term ranging from −0.61 to −0.23 (Ravagnolo and Misztal, 2000; Hammami et al., 2015; Santana et al., 2015; Santana et al., 2020). However, these authors reported that the genetic trends for slope became negative only after the implementation of respective breeding programs. These programs generated great genetic progress for milk yield, reaching a trend of the general production level 20 times higher than the current level trend of the buffalo population studied here. This fact highlights the importance of improving and investing in the genetic improvement program for Brazilian dairy buffaloes with systematic milk recording to collect phenotypic, pedigree, and genomic data. Therefore, monitoring trends of the genetic component of heat stress would be a reasonable measure to avoid a possible deterioration in animal performance in the future.

## 7. CONCLUSION

Important milk yield loss with increasing temperature-humidity index (THI) was observed in the present study. The significant effect of THI on TD milk yield was only observed for the intermediate stages of lactation, after lactation peak. Heterogeneity in the genetic (co)variance components was observed across the THI scale, indicating the existence of genotype by environment interaction (G×E). There is a considerable genetic variation for the animal's ability to respond to heat stress of the present population, allowing the response to selection. Genetic trends indicate that selection to increase milk yield did not lead to a deterioration in the general ability to respond to heat stress until the present moment. However, the GxE may lead to an important error in selection. Therefore, actions for monitoring trends of genetic components related to response to heat stress are recommended.

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