## **ORIGINAL PAPER**



# **Heat stress on breeding value prediction for milk yield and composition of a Brazilian Holstein cattle population**

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#### **Abstract**

Due to the high milk production of Holstein cows, many countries have chosen to import semen to improve local dairy herds. This strategy would be more efective if this semen was used in the same environment conditions in which the bulls were selected. If the effect of genotype by environment  $(G \times E)$  interaction is not considered, the estimated breeding values (EBVs) may vary, potentially reducing the selection response. We evaluate the impact of heat stress on selection for milk yield and composition of Holstein cows using random regression models. To verify the interference of heat stress in milk yield (MY) and composition traits (fat, protein, total saturated, and total unsaturated fatty acids content in milk), temperature–humidity index (THI) on test-day milk records was used. The threshold value to divide the environments using test-day information from Brazilian Holstein cows was 72 units of THI, i.e.,<72 represented no heat stress and>72 represented heat stress. Legendre polynomials of second-order (Leg 2) model and two lactation points (33 and 122 DIM) were used to estimate heritabilities and EBVs for fve important dairy traits. The heritabilities of milk components and fatty acids were low (0.09–0.29), regardless of lactation period and degree of heat stress, with the exception of protein content (0.30–0.35). Fat content was the only milk component that was reduced according to the degree of heat stress and lactation period. The EBVs tended to decrease in heat stress conditions, thus animals with high genetic potential demonstrated evidence of  $G \times E$ interaction. However, acclimatization of dairy cows to heat stress in the farm production systems may have been responsible for the low diferences among genetic parameters and EBVs with and without heat stress found in this study.

**Keywords** Genotype by environment interaction · Genetic parameters · Temperature–humidity index · Random regression

## **Introduction**

The climate changes around the world are considerably increasing the temperatures over the years, especially in tropical regions, causing changes in agriculture and livestock production. Extreme temperatures linked to factors such as relative humidity affect the animals' comfort zone due to the increase of body temperature, which infuences the homeostatic balance (Boettcher et al. [2015](#page-6-0); Vasconcelos et al. [2020](#page-7-0)). Heat stress is the most signifcant impact related to climate change in livestock production, as it reduces feed intake (Kappes et al.

 $2022$ ), reproductive efficiency (Menegassi et al.,  $2016$ ), and increases respiration rate (Ferreira et al. [2021a](#page-7-2)) and peripheral blood (Silveira et al. [2021](#page-7-3)). Many factors contribute to the vulnerability of livestock to heat stress such as species genetic potential, age, physiological, and nutritional status (Thornton et al. [2021;](#page-7-4) Ferreira et al. [2021b](#page-7-5)).

Aiming to increase dairy productivity, developing tropical countries are being encouraged to import genetic material to improve local herds. However, this strategy would be more efective if the imported genetic material was derived from bulls evaluated under the same environmental conditions and herd management. Increasing intensifcation of dairy systems in developing tropical regions with temperate-breed genetic stock could lead to greater vulnerability of animals to rising temperatures. Moreover, it is expected that progenies of animals evaluated in temperate environments will present a diferent performance in tropical climate, not only

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because of the diferences in climate itself but also because the genotypes have diferent responses according to the environment to which they are exposed (Hammami et al. [2008](#page-7-6)).

In that sense, an unfavorable genotype by environment interaction could reduce the benefts of a strategy based on imported semen (Banos and Smith [1991\)](#page-6-1). Milk production losses have already been mentioned if the  $G \times E$  interaction is ignored, especially when using imported semen, as in many cases exporting countries have a diferent production environment compared to importing countries (Payne and Hodges [1997;](#page-7-7) Hayes et al. 2003). For example, Silva et al.  $(2021)$  $(2021)$  demonstrated genotype  $\times$  environment interaction for Holstein cattle milk yield in temperate (Portugal) × tropical environment (Brazil). Furthermore, international bull evaluations showed that there is low genetic correlation between countries using the production system pasture and countries using a more industrialized production system, such as New Zealand and the USA (Strandberg et al. [2009](#page-7-9)).

One of the tools used to measure heat stress in farm animals is the use of the temperature–humidity index (THI; NRC [1971](#page-7-10)), which is already used to estimate genetic parameters related to heat tolerance (Sungkhapreecha et al. [2021](#page-7-11); Carrara et al. [2021;](#page-7-12) Mbuthia et al. [2021](#page-7-13)). Given the above, the aim of this study was to evaluate the impact of heat stress on selection for milk yield and composition of Brazilian Holstein cows using a random regression model.

## **Material and methods**

### **Data set**

We used 49 monthly records of milk yield (MY; kg day<sup>-1</sup>), fat (%), protein (%), total saturated fatty acids (SFA; %), and total unsaturated fatty acids (UFA; %) from 2896 Holstein cows with calving between 1 and 6 from four commercial Brazilian farms (Table [1\)](#page-1-0), and collected between May 2012 and July 2016. All the farms had three-times-a-day milking and nutritional conditions were standardized in farms, such that there were no diferent nutritional conditions within a farm. The farms considered in this study are well established and their productions are stable

<span id="page-1-0"></span>**Table 1** Number of observations (*N*), mean, standard deviation (SD), and range obtained for fat (%), protein (%), total saturated and unsaturated fatty acids in milk, and test-day milk yield (kg)

| Traits                             | N                 |           |      | Mean $\pm$ SD Range |
|------------------------------------|-------------------|-----------|------|---------------------|
| Milk yield (kg day <sup>-1</sup> ) | 20.359 34.71 9.57 |           |      | 5.00 - 55.50        |
| Fat $(\%)$                         | 21.410            | 3.46 0.77 |      | $0.81 - 6.83$       |
| Protein $(\%)$                     | 21.425            | 3.04      | 0.29 | $2.23 - 4.23$       |
| Total saturated fatty acids (%)    | 18.987            | 2.21      | 0.47 | $0.90 - 3.50$       |
| Total unsaturated fatty acids (%)  | 18,820            | 1.03      | 0.27 | $0.30 - 1.80$       |

during the year. This way, the calving is not concentrated in one period of the year. In addition, the selection criteria adopted by the farms are milk production, morphological traits, productive life, somatic cell count, and livability.

The meteorological data recorded during the study are presented in Fig. [1.](#page-2-0) The milk components were determined by mid-infrared spectrometry (Delta Instruments CombiScope™ Filter, Advanced Instruments, Inc., Norwood, USA). Quality control excluded animals with days in milk lower than fve or higher than 305; without valid measurements (mean $\pm$ 3 SD); without calving date or lactation order, and age information; and with age higher than 9 years. The contemporary groups (CGs) were formed by the combination of calving periods: dry — April to September, and rainy — October to March, calving year based on the start of calving periods, farm, and collection month. CGs containing lower than five individuals were eliminated. The pedigree consisted of 8789 animals, 4197 dams, and 576 sires of 7 generations, considering the frst generation as one. All animals were considered in the analyses.

THI was determined from air temperature (AT, °C) and relative humidity (RH, %), following the National Research Council (NRC [1971](#page-7-10)):

$$
THI = [1.8 \times AT + 32] - [0.55 - (0.0055 \times RH)]
$$
  
× [1.8 \times AT - 26] (1)

The AT and RH were obtained from the Space Administration/Prediction of Worldwide Energy Resources database (NASA/POWER; Power [2016](#page-7-14)) based on the geographic coordinates of each dairy farm. The heat stress degree (*t*) was obtained based on the THI and defned as the number of units of mean THI above 72 (NRC [1971;](#page-7-10) Bohmanova et al. [2008](#page-6-2)).

#### **Genetic analysis**

#### **Criterion model settings**

The analyses were carried out by using a random regression model to describe the phenotype expression in relation to genotype based on the environmental changes. In this case, the environments are not classifed into groups. The phenotypes will change gradually and continuously over an environmental gradient, being the regression performance of genotypes in each thermal environment the most used method for this type of analysis (Kolmodin et al. [2002](#page-7-15)).

To model covariance functions, first-degree (linear) Legendre polynomials were used. Studies with dairy cows showed that lower orders for Legendre polynomials could be sufficient to fit the data. In addition, previous studies have shown that lower-order polynomials are preferable (in terms of better ft) to milk yield and components in this same evaluated population (Carrara et al. [2021](#page-7-12)).

<span id="page-2-0"></span>

#### **Model considering the genotype×environment interaction**

To verify the presence of  $G \times E$  interaction for MY, fat, protein, SFA, and UFA, a random regression model was used as suggested by Meyer [\(1998](#page-7-16)) and Bohmanova et al. [\(2008](#page-6-2)).

$$
y_{ij} = \mu + F + \beta_m \phi_m + \sum_{m=1}^{k_a - 1} \alpha_{0im} \phi_m + \sum_{m=1}^{k_a - 1} \alpha_{1im} \phi_m x_t + \sum_{m=1}^{k_p - 1} \rho_{0im} \phi_m + \sum_{m=1}^{k_p - 1} \rho_{1im} \phi_m x_t + \varepsilon_{ij}
$$
(2)

considering the environmental sensitivity of the animal *i* to heat stress *t*;  $k_{\beta}$ ,  $k_{\alpha}$ , and  $k_{\beta}$  are the orders of the polynomials (linear);  $\phi_m$  is the *n*th Legendre polynomial function; and  $\varepsilon_{ii}$  is the random residual effect.

Variance components were obtained using WOMBAT software (Meyer [2006](#page-7-17)). The covariance functions were estimated for the additive genetic and permanent environmental effects using frst-degree (linear) orthogonal Legendre polynomials (Leg 2) taking into account two points of lactation, 33 days in milk (DIM) and 122 DIM. These points were chosen based on the largest number of information in the data set.

The variance covariance structure was:

$$
ar\left[\alpha_0 \; \alpha_1 \; p_0 \; p_1 \; e\right] = \left[A\sigma_a^2 \; A\sigma_{aa} \; 0 \quad 0 \quad 0 \; A\sigma_{aa} \; A\sigma_a^2 \; 0 \quad 0 \quad 0 \; 0 \; 0 \; 0 \; 0 \; 0 \; 0 \; I\sigma_p^2 \; I\sigma_{px} \; 0 \; I\sigma_{px} \; I\sigma_p^2 \; 0 \; 0 \; 0 \; I\sigma_e^2\right] \tag{3}
$$

where  $y_{ij}$  is the vector of the response variable *i* measured at the animal *j*;  $\mu$  is the global average; *F* is the vector of fixed effects, composed by the contemporary groups;  $\beta_m$  is the regression coefficient for the Legendre polynomial  $m$ ;  $\alpha_{0im}$ and  $\rho_{0im}$  are random regression coefficients for the direct additive genetic effect and permanent environment for animal *i* regardless of the level of heat stress *t*;  $\alpha_{lim}$  and  $\rho_{lim}$  are random regression coefficients for the direct additive genetic effect and permanent environment effect, respectively, where *A* is the relationship matrix; *I* is the identity matrix;  $\sigma^2$ <sup>*a*</sup> is the variance of the additive genetic effect;  $\sigma^2$ <sup>*p*</sup> is the variance of the permanent environmental effect;  $\sigma_{\alpha\alpha}$  is the covariance between the intercept and the additive genetic effect; and  $\sigma_{p\pi}$  is the covariance between the intercept and the effect of permanent environment.

The heritability coefficient  $(h^2)$  was estimated for each trait as the ratio of the additive genetic variance  $(\sigma^2_a)$  and the phenotypic variance  $(\sigma_y^2)$ .

$$
h^2 = \frac{\sigma_a^2}{\sigma_y^2} = \frac{\sigma_a^2}{\sigma_a^2 + \sigma_p^2 + \sigma_e^2}
$$
 (4)

where  $\sigma_p^2$  is the permanent environment variance and  $\sigma_e^2$  is the residual variance.

#### **Estimated breeding value**

Breeding values were estimated by the following equation:

$$
EBV_{pj} = \sqrt[2]{\frac{1}{2}} * \alpha_{pi} + \sqrt[2]{\frac{3}{2}} * \beta_{pi} * w
$$
 (5)

where  $EBV_{pi}$  is the breeding value of cow *p* at DIM *j*;  $\alpha_{pi}$  is the regression coefficient of additive breeding value of cow *p* for trait *i*;  $\beta_{ni}$  is the second-order regression coefficient of additive breeding value of cow *p* to trait *i*; and *w* is the lactation stage standardized.

## **Results and discussion**

The threshold value to divide the environments using test-day information from Brazilian Holstein cows was 72 units of THI, i.e.,<72 represented no heat stress and>72 represented heat stress. Leg 2 model and two lactation points (33 and 122 DIM) were used to estimate heritabilities and EBVs for five important dairy traits.

#### **Genetic variance components and heritabilities**

The estimates of variance components and heritability for MY and composition traits obtained for both thermal environments (no heat stress and heat stress) are given in Table [2](#page-3-0). The additive genetic component decreased under heat stress environments for fat and SFA. Furthermore, protein and UFA showed higher additive genetic values in the heat stress environment. Regarding the lactation periods evaluated (33 and 122 DIM), only the additive genetic variance reduced for fat. We believe that this reduction is explained by diferent causes, in which at 33 days, they are more related to heat stress, as milk production is higher, while at 122 days, it may be related to the reduction of natural milk in the lactation curve of the cows. It is also worth mentioning that fat is the component of milk with the greatest variation, being mainly infuenced by diet, breed, feed intake, and milk production (Stürmer et al. [2018](#page-7-18); Silveira et al. [2022\)](#page-7-19).

Greater heritabilities  $(>0.30)$  were observed in both lactation stages (33 and 122 DIM) and in the environment with no heat stress for protein, indicating that this trait can be able to provide a good response to selection. Similar results were obtained by Jamrozik and Schaefer ([1997;](#page-7-20) 0.33 to 0.69), Biassus et al. (2011; 0.10 to 0.35), and Borquis et al. [\(2013](#page-6-3); 0.21 to 0.37) for protein. The heritability for protein was lower than those estimated by Jamrozik and Schaeffer [\(1997\)](#page-7-20) (0.34 to 0.68) and Soyeurt et al. [\(2008](#page-7-21)) (0.23 to 0.44), especially in heat stress environments. Generally, frst-parity cows show higher heritability estimates than higher-order-parity

<span id="page-3-0"></span>**Table 2** Variance components, heritability  $(h^2)$ , and their respective standard errors (in brackets) for fat  $(\%)$ , protein  $(\%)$ , saturated fatty acids (SFA), and unsaturated fatty acids (UFA) for 33 and 122 days in milk in both environments

| Days in milk (DIM) | Environment conditions | Traits         | $\widehat{\widehat{\boldsymbol{o}}}_a^2$ | $\widehat{\acute{o}}_{pe}^2$ | $\widehat{\widehat{o}}_{e}^{2}$ | $\hat{\delta}^2$<br>$\overline{p}$ | h <sup>2</sup> |
|--------------------|------------------------|----------------|--|------------------------------|---------------------------------|------------------------------------|----------------|
| 33 days            | No heat stress         | Fat $(\%)$     | 0.10(0.0115)                             | 0.05(0.0095)                 | 0.35(0.0032)                    | 0.50(0.0073)                       | 0.20(0.021)    |
|                    |                        | Protein $(\%)$ | 0.02(0.0020)                             | 0.01(0.0016)                 | 0.04(0.0003)                    | 0.07(0.0012)                       | 0.30(0.026)    |
|                    |                        | $SFA(\%)$      | 0.05(0.0061)                             | 0.03(0.0052)                 | 0.13(0.0015)                    | 0.21(0.0039)                       | 0.23(0.0270)   |
|                    |                        | UFA $(\%)$     | 0.01(0.0014)                             | 0.01(0.0014)                 | 0.05(0.0006)                    | 0.07(0.0011)                       | 0.10(0.021)    |
|                    | Heat stress            | Fat $(\%)$     | 0.06(0.0062)                             | 0.06(0.0043)                 | 0.38(0.0050)                    | 0.50(0.0090)                       | 0.11(0.0110)   |
|                    |                        | Protein $(\%)$ | 0.02(0.0016)                             | 0.01(0.0010)                 | 0.03(0.0005)                    | 0.07(0.0016)                       | 0.32(0.0150)   |
|                    |                        | $SFA(\%)$      | 0.05(0.0030)                             | 0.02(0.0020)                 | 0.14(0.0024)                    | 0.21(0.0051)                       | 0.22(0.0060)   |
|                    |                        | UFA $(\%)$     | 0.01(0.0006)                             | 0.01(0.0004)                 | 0.06(0.0010)                    | 0.07(0.0017)                       | 0.13(0.0020)   |
| 122 days           | No heat stress         | Fat $(\%)$     | 0.12(0.0099)                             | 0.04(0.0070)                 | 0.35(0.0032)                    | 0.51(0.0061)                       | 0.23(0.0180)   |
|                    |                        | Protein $(\%)$ | 0.02(0.0017)                             | 0.01(0.0012)                 | 0.03(0.0003)                    | 0.07(0.0010)                       | 0.35(0.0220)   |
|                    |                        | $SFA(\%)$      | 0.06(0.0053)                             | 0.02(0.0038)                 | 0.13(0.0015)                    | 0.21(0.0032)                       | 0.29(0.0220)   |
|                    |                        | UFA $(\%)$     | 0.01(0.0008)                             | 0.004(0.0007)                | 0.05(0.0006)                    | 0.06(0.0007)                       | 0.09(0.0130)   |
|                    | Heat stress            | Fat $(\%)$     | 0.08(0.0069)                             | 0.04(0.0044)                 | 0.38(0.0050)                    | 0.49(0.0066)                       | 0.16(0.0130)   |
|                    |                        | Protein $(\%)$ | 0.02(0.0015)                             | 0.01(0.0009)                 | 0.03(0.0005)                    | 0.07(0.0011)                       | 0.34(0.0180)   |
|                    |                        | $SFA(\%)$      | 0.05(0.0037)                             | 0.02(0.0023)                 | 0.14(0.0024)                    | 0.20(0.0036)                       | 0.25(0.0150)   |
|                    |                        | UFA $(%)$      | 0.006(0.0005)                            | 0.003(0.0003)                | 0.06(0.0010)                    | 0.07(0.0011)                       | 0.09(0.0070)   |

 $*\hat{\delta}^2$ <sup>2</sup> additive genetic variance,  $\hat{\phi}^2_{pe}$  permanent environment variance,  $\hat{\phi}^2_{e}$  $\int_{e}^{2}$  residual variance,  $\hat{\hat{o}}_{p}^{2}$  $p_p^2$  phenotypic variance,  $h^2$  heritability cows; therefore, the use of cows with diferent parities in the database may be responsible for causing these diferences in estimates (Bastin et al. [2013](#page-6-4); Petrini et al. [2016](#page-7-22)).

Low to moderate heritabilities (0.10 to 0.28) were estimated for SFA and fat, similar to Bobe et al. [\(2008](#page-6-5)) (0.27), Penasa et al. [\(2015](#page-7-23)) (0.24), and Petrini et al. [\(2016](#page-7-22)) (0.25) indicating that these traits can be able to provide an improvement in response to selection although slow. Higher heritabilities for SFA were reported by Soyeurt et al. ([2007](#page-7-24); 0.36), Soyeurt et al. [\(2008\)](#page-7-21) (0.42), and Bastin et al. ([2011\)](#page-6-6) (0.43). Diferent heritability estimates among studies on the same breed can be explained by diferences in the sample size, type of records used in each study (e.g., frst or multiple lactations), and estimation methods.

In both thermal environments, SFA heritabilities were higher than UFA meaning that SFA are more heritable than UFA. The reason for this diference could be related to fatty acids (FA) origin. Fatty acids in milk are derived from synthesized de novo by the mammary gland and from the blood (Knutsen et al. [2018\)](#page-7-25). SFA are de novo synthesized in the mammary gland from blood precursors and regulated by two enzymes. On the other hand, UFA is provided by diet and is hydrogenated in the rumen by bacteria and transported by the blood (Gion et al.  $2011$ ) then their concentration in milk is close to the absorbed quantities in the intestine. Bastin et al. ([2011\)](#page-6-6) suggest that de novo synthesis FA are under stronger genetic control than FA originating from the diet than from body fat mobilization.

Fat composition of cow milk is infuenced by metabolic status and stage of lactation of the cow, as a negative energy balance directly impacts the presence of UFA. The mobilization of fat reserves has been found to increase the content of UFA in milk. In general, the estimates of heritabilities decreased in the heat stress environment. Therefore, the environment may have infuenced the selection response in an animal breeding program. These diferences in the heritabilities for FA can be justifed by the fact that these traits are highly infuenced by environmental conditions (Penasa et al. [2015](#page-7-23)).

#### **Genotype by environment interaction**

The EBV for MY and composition traits obtained for both environments and DIM (33 and 122 days) are shown in Table [3](#page-4-0). In addition, Fig. [2](#page-6-7) shows the top ten animals for each trait according to the EBV values at diferent environmental conditions.

It is noted that the EBVs varied over the two environments, as well as a re-ranking of individuals, especially for fat and SFA. The EBV for fat and SFA reduced signifcantly with the heat stress interference, evidencing the presence of  $G \times E$ interaction. Low variation of the EBV of animals for protein and UFA shows that the performance of these animals was relatively stable under diferent environmental conditions, indicating that breeding values were more robust during the lactation (Bignardi et al. [2015](#page-6-8)). The cause of this stability is due to high genetic correlations between them, and increasing the stability of genotypes represents a lower environmental sensitivity of these genotypes. Acclimatization of dairy cows to heat stress in the farm production system may have been responsible for low diferences found in this study. Moreover, the THI value used (72) may not be enough to fnd more meaningful G×E interactions. Another important factor to be taken into account is the choice of the day used to obtain the relative humidity and air temperature information to calculate the THI, since heat stress effects can be observed a few days after animal exposure to hot temperature.

For MY, the EBVs were similar in the heat stress environment. The results found in this study difer from a research conducted by Carabaño et al. [\(2014\)](#page-7-27), in which the animals with the best classifcations showed lower EBVs when exposed to environments with higher temperatures, confrming the antagonistic relationship between the level of production and tolerance to heat stress found in other studies (Silva et al. [2021\)](#page-7-8). Milk yield of dairy cows with high genetic merit is reduced when these animals are exposed to an environment with high temperatures (Kadzere et al. [2002\)](#page-7-28), but it is possible that the animals under study have some degree of adaptation. In addition, the

<span id="page-4-0"></span>**Table 3** The variation in estimated breeding values (EBVs) in environments without interference from temperature– humidity index [THI (no heat stress — environment A)] and in environments with interference of THI (with heat stress — environment B) for Holstein cows with 33 and 122 days in milk production





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<span id="page-6-7"></span>**Fig. 2** Estimated breeding values (EBVs) of top ten animals for milk traits ◂according to the environment (A — environment without heat stress and B — environment with heat stress) considering 33 and 122 DIM

high environmental sensitivity can be beneficial in controlled environments; it is possible to maintain the high levels of production of these animals with the aid of physical improvements in the environment, such as radiation, temperature, and humidity control. However, we emphasize that modifying the physics of the thermal environment with the use of refrigerated equipment means increases the production cost per liter of milk, which is often infeasible for dairy farming in tropical conditions.

EBV versus days of lactation  $(33 \times 122$  days) were similar for milk composition, but we observed a reduction in EBV for milk yield at 33 compared to 122 DIM under both heat stress conditions (THI $\leq$ 72 and THI $>$ 72), which is explained by the higher metabolic heat production at the beginning of lactation for milk synthesis, especially in the period of peak lactation (1st phase of the lactation curve), making cows more susceptible to heat stress in this production phase, while dairy cows after peak lactation (2nd and 3rd phases of the lactation curve) produce less metabolic heat and are less susceptible to heat stress.

Finally, animals with lower environmental sensitivity are appropriate to include in an animal breeding program, as they are able to adapt to diferent environments, producing consistently. Although it is not possible to exclude the effects of  $G \times E$ interaction by genetic means, these effects could be minimized by using animal breeding strategies. In this context, studies that include  $G \times E$  in the models allow the evaluation of the phenotypic plasticity of individuals, i.e., to identify robust sires that do not substantially vary their genetic merit in diferent environments. Thus, the selection of robust animals with high breeding value can contribute to obtaining progenies more adapted to tropical conditions and with satisfactory performance in the diferent Brazilian climatic environments. While the exclusion effect of  $G \times E$  interaction by genetic means can be minimized by using animal breeding strategies, in the future sensors, robots may solve the problem using artifcial intelligence (AI). Healthy farm animals/dairy cattle with a high degree of accuracy can be used by a camera with AI to achieve a "smart" cowhouse which could enable early detection of injuries and illnesses that could impact the quantity and quality of milk production.

# **Conclusion**

The heritability of milk components and fatty acids is low, regardless of lactation period and degree of heat stress, with the exception of protein. The EBVs at higher temperatures tended to decrease in heat stress conditions, thus animals with high genetic potential demonstrated evidence for G×E interactions in Brazilian Holstein cattle population.

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**Data availability** Data will be made available on request.

#### **Declarations**

**Ethics approval** All experimental procedures related to animal experimentation employed in the present study were performed in agreement with the protocol N° 2017.5.1197.11.3 approved by Institutional Animal Care and Use Committee Guidelines from "Luiz de Queiroz" College of Agriculture (ESALQ), University of São Paulo (USP) to ensure compliance with international guidelines for animal welfare.

**Conflict of interest** The authors declare no competing interests.

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