Genetic parameters for rectal temperature, respiration rate, and drooling score in Holstein cattle and their relationships with various fertility, production, body conformation, and health traits

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ABSTRACT

Genetic selection for improved climatic resilience is paramount to increase the long-term sustainability of high-producing dairy cattle, especially in face of climate change. Various physiological indicators, such as rectal temperature (RT), respiration rate score (RR), and drooling score (DS), can be used to genetically identify animals with more effective coping mechanisms in response to heat stress events. In this study, we investigated genetic parameters for RT, RR (score from 1–3), and DS (score from 1–3). Furthermore, we assessed the genetic relationship among these indicators and other economically important traits for the dairy cattle industry. After data editing, 59,265 (RT), 30,290 (RR), and 30,421 (DS) records from 13,592 lactating Holstein cows were used for the analyses. Variance components were estimated based on a multiple-trait repeatability animal model. The heritability $\pm$ standard error estimate for RT, RR, and DS was 0.06 ± 0.01 , 0.04 ± 0.01 , and 0.02 ± 0.01 , respectively, whereas their repeatability was 0.19, 0.14, and 0.14, respectively. Moderate genetic correlations of RR with RT and DS (0.26 ± 0.11 and 0.25 ± 0.16) and nonsignificant correlation between RT and DS (-0.11 ± 0.14) were observed. Furthermore, the approximate genetic correlations between RT, RR, and DS with 12 production, 29 conformation, 5 fertility and reproduction, 5 health, and 9 longevity-indicator traits were assessed. In general, the approximate genetic correlations calculated were low to moderate. In summary, 3 physiological indicators of

heat stress response were measured in a large number of animals and shown to be lowly heritable. There is a value in developing a selection index including all the 3 indicators to improve heat tolerance in dairy cattle. All the unfavorable genetic relationships observed between heat tolerance and other economically important traits can be accounted for in a selection index to enable improved climatic resilience while also maintaining or increasing productivity in Holstein cattle.

Key words: animal resilience, Chinese Holstein cattle, drooling score, heat stress, rectal temperature, respiratory rate score

INTRODUCTION

Heat stress causes extensive welfare (Polsky and von Keyserlingk, 2017) and economic losses in the worldwide dairy cattle industry due to its unfavorable correlation with production (Sammad et al., 2020), reproduction (Schüller et al., 2014), health and immunity (Das et al., 2016; Wang et al., 2019), and longevity (Burdick Sanchez et al., 2013). Therefore, in addition to management strategies (Renaudeau et al., 2012), genetic selection for improved climatic resilience in cattle is crucial to improve welfare, productivity, and reproductive efficiency and thus, increase the long-term sustainability of the dairy industry. This is even more important as extreme temperatures and weather events become more frequent (Oleson et al., 2015). One approach to genetically select for improved heat tolerance is through reaction norm models, based on routinely collected data sets (e.g., milk yield) and public weather station records (Ravagnolo and Misztal, 2000; Carabaño et al., 2014). In general, breeding values for the slope of a regression of productive performance on environmental gradient [e.g., temperature, relative humidity, temperature-humidity index (**THI**)] as covariates are

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calculated (Ravagnolo and Misztal, 2000; Bernabucci et al., 2014). This approach has been applied in dairy cattle and various other dairy species (Menéndez-Buxadera et al., 2014; Gourdine et al., 2017; Santana et al., 2017). When dairy cows are exposed to hot conditions, there is a delay in milk yield reduction, whereas their physiological status is immediately changed (Spiers et al., 2004; Tao et al., 2020). Hence, it is crucial to identify early indicators of heat stress response to facilitate cooling management strategies and effective selective breeding. In general, physiological variables more efficiently indicate heat stress response before reduction in milk production is observed (Brown-Brandl et al., 2005). Therefore, physiological responses, such as respiration rate, skin temperature, and rectal temperature (**RT**), might better capture the genetic background of heat tolerance (Amamou et al., 2019).

Maintaining homeothermy in a hot environment essentially depends on the animal's ability to balance thermogenesis and heat dissipation (Carabaño et al., 2019; Becker et al., 2020). Measuring physiological indicators under heat stress conditions enables the identification of animals with better coping mechanisms. Heat stress causes changes in thermal homeostasis, and therefore, in physiological performance (Atrian and Shahryar, 2012), including increased respiration rate (Gaughan et al., 2000), more frequent breathing or panting (Brown-Brandl et al., 2006), excessive salivation (Mader et al., 2006), and higher body temperature (Fournel et al., 2017).

Respiration involves the elimination of carbon dioxide from the body tissues, oxygen intake, evaporation of moisture from the respiratory tract, and prevention of hyperthermia under high ambient temperatures (Atrian and Shahryar, 2012). Increased respiration rate eliminates a greater volume of carbon dioxide, which can lead to a disturbance in acid-base balance (Sammad et al., 2020). Therefore, excessive salivation usually accompanies increased respiration rate (Mader et al., 2006; Gaughan et al., 2010). In turn, this process leads to increased body fluid loss which can further increase maintenance requirements to mitigate dehydration and blood homeostasis (Collier et al., 2006). All detrimental effects induced by heat stress can increase body temperature to various degrees (Kendall and Webster, 2009; Burfeind et al., 2012). Rectal temperature and respiration rate are common physiological indicators of heat stress response (Li et al., 2020; Brito et al., 2020). Despite the usefulness of these indicators to assess heat tolerance, their routine collection can be time-consuming and laborious. Monitoring the degree of panting, respiration rate, or both based on a scoring system could be an alternative for (quantitatively)

measuring body temperature and respiration rate to assess animal heat load (Gaughan et al., 2008; Schütz et al., 2014). For instance, panting score is used to evaluate the extent of elevated respiration rate accompanying excessive salivation when the animals are experiencing heat stress (Mader et al., 2006). However, the respiration and salivation patterns can differ, as they are controlled by different biological mechanisms. A visual scoring system assessing respiration and salivation patterns separately has been proposed by Mader et al. (2006).

In addition to evaluating the genetic parameters of physiological indicators, it is crucial to assess their genetic relationship with other economically important traits in dairy cattle (Hammami et al., 2015). This enables the prediction of indirect genetic responses and contributes to the development of proper selection indices. In this context, the main objectives of this study were to: (1) describe the phenotypic variability of 3 physiological indicators of heat stress in Holstein cattle using large data sets; (2) estimate genetic parameters for RT, respiration rate score (**RR**), and drooling score (**DS**); and (3) assess the genetic relationships between RT, RR, and DS with 12 production, 29 conformation, 5 fertility and reproduction, 5 health, and 9 longevity-indicator traits in Holstein cattle.

MATERIALS AND METHODS

Ethics Committee Approval

The data collection process was carried out in strict accordance with the protocol approved by the Animal Welfare Committee of the China Agricultural University (Protocol Number: DK996).

Phenotypic and Pedigree Data Sets

Data from 13,593 lactating Holstein cows were collected in the summer period (June to August) from 2013 to 2018 at 12 dairy cattle farms in the Beijing region (40°N, 116°E), China. All herds were equipped with fans and sprinklers. Animals were kept in freestall housing systems, fed TMR, and given ad libitum access to water. Cows were milked 3 times daily in a milking parlor system. There were cooling treatments with sprinklers and fans in the milking parlor waiting room (before milking). The pedigree file contained 449,819 animals, spanning over 6 generations.

Rectal temperature was measured using a digital thermometer (Omron MC-347, Omron Corp., Osaka, Japan). The RR and DS measurement protocols can be described as follows. RR score 1: respiration rate is

normal, and it is difficult to visualize the movement of chest and rumen; RR score 2: respiration rate is in the range of 80 to 120 times/min and small visual signs of movement of chest and rumen are observed; and RR score 3: respiration rate is greater than 120 times/min, and there are clear signs of the movement of chest and rumen with joggling of body and open-mouthed respiration. DS score 1: no drooling and normal condition of jaw; DS score 2: some drooling and some filament saliva flow out of the mouth and a wet jaw may be observed; and DS score 3: excessive drooling and large amount of saliva flowing out of the mouth and wet jaw. An illustration of the DS system is also shown in Figure 1. Each lactating cow was recorded twice a day for 2 consecutive days (0700–1100 and 1400–1800 h). A small proportion of cows (4.6%) were measured in more than one lactation (i.e., >4 records/cow). Rectal temperature was measured from 2013 to 2018 ($n = 59,265$ records), whereas RR and DS were collected from 2015 to 2018 ($n = 30,290$ and $30,421$ records for RR and DS, respectively; Supplemental Figure S1, <https://doi.org/10.6084/m9.figshare.13662278>). Environmental temperature and humidity were recorded before and after measuring the cows for the 3 physiological indicators, using a digital thermometer placed at a height of 1.5 to 3.0 m above the floor, and the average environmental temperature and relative humidity from all thermometers installed in each farm were used. The THI was calculated based on the following formula (Mader et al., 2006):

$$THI = (0.8 \times T) + [(RH/100) \times (T - 14.4)] + 46.4,$$

where T is the dry-bulb temperature ($^{\circ}\text{C}$) and RH represents relative humidity (%).

Statistical Model Development

Pearson correlations were calculated to assess the phenotypic relationships among RT, RR, and DS. The PROC MIXED function of the SAS software (version 9.2; SAS Institute Inc., Cary, NC) was used to test the significance of environmental factors potentially affecting RT, RR, or DS. Multiple testing correction was done based on the Bonferroni adjustment ($\alpha = 0.05$). The mixed linear model for each trait had the following general form (model 1):

$$y_{ijklno} = \mu + FY_i + P_j + S_k + M_l + bTHI + PE_n + \varepsilon_{ijklno}, \quad [1]$$

where y_{ijklno} is the phenotypic record for RT, RR, or DS; μ is the overall mean; FY_i is the effect of the farm-year i for RT or farm-year-scoring-person i for RR and DS; P_j is the effect of the parity j (1, 2, or 3+); S_k is the effect of the lactation stage k (DIM 1–50, 51–100, 101–150, 151–200, 201–250, 251–300, or >300); M_l is the effect of the milking status l (data collected before milking, after milking, or unknown); THI is as defined above; b is the fixed regression coefficients for THI; PE_n is the random effect of permanent environment of animal n , and ε_{ijklno} is the random residual term. It was assumed that $PE_n \sim N(0, \mathbf{I}\sigma_{pe}^2)$ and $\varepsilon_{ijklno} \sim N(0, \mathbf{I}\sigma_{\varepsilon}^2)$, where σ_{pe}^2 is the variance of permanent environment effect and σ_{ε}^2 is the residual variance.

Genetic Analyses

Variance components were estimated based on a multiple-trait repeatability animal model, using the aver-

Figure 1. Standard example for scoring systems of drooling condition: 1, 2, and 3 from left to right.

age information (**AI**)-REML procedure implemented in the DMU package (Madsen et al., 2014). The statistical model used can be described in matrix form as:

$$\mathbf{y} = \mathbf{X}\boldsymbol{\beta} + \mathbf{Z}_a\mathbf{a} + \mathbf{Z}_{pe}\mathbf{pe} + \mathbf{e},$$

where $\mathbf{y}$ is the vector of phenotypic records for RT, RR, and DS (RR and DS were treated as continuous traits and estimated by linear model. This was done as there were small differences between estimates of genetic parameters (in the observed scale) from linear and threshold models (results not shown); $\boldsymbol{\beta}$ is the vector of systematic effects, including the same fixed effects described for model 1; $\mathbf{a}$ is the vector of random animal additive genetic effects of cows; $\mathbf{pe}$ is the vector of random permanent environmental effect; $\mathbf{e}$ is the vector of random residual effect; $\mathbf{X}$, $\mathbf{Z}_a$, and $\mathbf{Z}_{pe}$ are the incidence matrices linking phenotypic records to $\boldsymbol{\beta}$, $\mathbf{a}$, and $\mathbf{pe}$, respectively. $\mathbf{A}$ is a pedigree-based relationship matrix and $\mathbf{I}$ is an identity matrix. The model assumptions are:

$$a \sim N \left(0, \mathbf{A} \otimes \begin{bmatrix} \sigma_{a(RT)}^2 & \sigma_{a(RT,RR)} & \sigma_{a(RT,DS)} \\ \sigma_{a(RT,RR)} & \sigma_{a(RR)}^2 & \sigma_{a(RR,DS)} \\ \sigma_{a(RT,DS)} & \sigma_{a(RR,DS)} & \sigma_{a(DS)}^2 \end{bmatrix} \right),$$

$$pe \sim N \left(0, \mathbf{I} \otimes \begin{bmatrix} \sigma_{pe(RT)}^2 & \sigma_{pe(RT,RR)} & \sigma_{pe(RT,DS)} \\ \sigma_{pe(RT,RR)} & \sigma_{pe(RR)}^2 & \sigma_{pe(RR,DS)} \\ \sigma_{pe(RT,DS)} & \sigma_{pe(RR,DS)} & \sigma_{pe(DS)}^2 \end{bmatrix} \right),$$

$$e \sim N \left(0, \mathbf{I} \otimes \begin{bmatrix} \sigma_{e(RT)}^2 & \sigma_{e(RT,RR)} & \sigma_{e(RT,DS)} \\ \sigma_{e(RT,RR)} & \sigma_{e(RR)}^2 & \sigma_{e(RR,DS)} \\ \sigma_{e(RT,DS)} & \sigma_{e(RR,DS)} & \sigma_{e(DS)}^2 \end{bmatrix} \right),$$

where $\sigma_{k(i,j)}^2$ is the variance of the effect k (a , pe , e) of trait i , and $\sigma_{k(i,j)}$ is the covariance between trait i and j for the effect k . The heritability and repeatability parameters were defined as $h^2 = \frac{\sigma_a^2}{\sigma_p^2}$ and $r_e = \frac{\sigma_a^2 + \sigma_{pe}^2}{\sigma_p^2}$,

respectively, where $\sigma_p^2 = \sigma_a^2 + \sigma_{pe}^2 + \sigma_e^2$. The square of the SE for the heritability estimates was calculated as follows (Su et al., 2007):

$$SE^2(h^2) = \left[\frac{\hat{\sigma}_a^2}{\hat{\sigma}_p^2} \right]^2 \left[\frac{Var(\hat{\sigma}_a^2)}{(\hat{\sigma}_a^2)^2} + \frac{Var(\hat{\sigma}_p^2)}{(\hat{\sigma}_p^2)^2} - 2 \frac{Cov(\hat{\sigma}_a^2, \hat{\sigma}_p^2)}{\hat{\sigma}_a^2 \hat{\sigma}_p^2} \right].$$

The genetic correlation coefficient was $r = \frac{s_{ij}}{\sqrt{s_i^2 s_j^2}}$, and

the square of the SE for the genetic correlation coefficient was calculated as follows (Su et al., 2007):

$$SE^2(r) = \left[\frac{\hat{\sigma}_{ij}^2}{\hat{\sigma}_i^2 \hat{\sigma}_j^2} \right] \left[\frac{Var(\hat{\sigma}_{ij}^2)}{(\hat{\sigma}_{ij}^2)^2} + \frac{Var(\hat{\sigma}_i^2)}{4(\hat{\sigma}_i^2)^2} + \frac{Var(\hat{\sigma}_j^2)}{4(\hat{\sigma}_j^2)^2} - \frac{Cov(\hat{\sigma}_{ij}^2, \hat{\sigma}_i^2)}{\hat{\sigma}_{ij}^2 \hat{\sigma}_i^2} - \frac{Cov(\hat{\sigma}_{ij}^2, \hat{\sigma}_j^2)}{\hat{\sigma}_{ij}^2 \hat{\sigma}_j^2} + \frac{Cov(\hat{\sigma}_i^2, \hat{\sigma}_j^2)}{2\hat{\sigma}_i^2 \hat{\sigma}_j^2} \right].$$

The variances and covariances of the estimated parameters (σ_i^2 , σ_{ij}) were obtained from the inverse of the average information matrix. The reliability of the EBV was calculated as:

$$\text{Reliability} = 1 - \frac{SEP^2}{\sigma_a^2},$$

where SEP is the standard error of prediction and σ_a^2 is the direct additive genetic variance.

Alternative data sets (or scenarios) were used to estimate variance components and to compare models based on the SE of the heritability estimates and the Akaike information criterion (based on the same data sets), where lower values indicate better estimation of heritability and better fit of the models, respectively. The data sets created were (1) full data and assuming $\sigma_{pe(i,j)} = 0$; (2) data subset containing only animals with records for RT, RR, and DS and assuming a potential covariance for the random permanent environmental effects [$\sigma_{pe(i,j)}$]; and (3) data subset containing only animals with records for RT, RR, and DS and assuming $\sigma_{pe(i,j)} = 0$.

Calculation of Approximate Genetic Correlations

The approximate genetic relationship between physiological indicators of heat stress and other relevant traits (production, reproduction, fertility, body conformation, and health) is of great importance for the design of dairy cattle breeding programs aiming to improve heat tolerance. The traits included in this

analysis are: 12 milk-related traits (milk yield, fat yield, protein yield, and SCS; each parity was considered as a different trait), 29 body conformation traits [capacity (or frame), rump, feet and legs, mammary system, dairy character, conformation (final score), body size, stature, fore body (termed forepart), chest width, body depth, loin strength, rump width, rump angle, bone quality, foot angle, hoof height, rear legs side view, rear legs rear view, udder depth, udder texture, median suspensory, fore attachment, fore teat placement, average teat length, rear attachment height, rear attachment width, rear teat placement, angularity], 5 fertility and reproduction traits (age at first calving – heifer, age at first service – heifer, interval from calving to first service – cow, interval from first to last insemination – cow, and interval from first to last insemination – heifer), 5 health traits (fertility disorders, udder diseases, feet and legs disease, digestive disorders, and metabolic disorders), and 9 longevity-indicator traits for both heifers and cows (heifer longevity: survival up to 60 d of age, survival up to 365 d of age, and survival until calving; cow longevity: survival at second, third, fourth, fifth, and sixth parity; and productive life). Due to limited access to raw data sets, approximate genetic correlations between RT, RR, and DS with the other traits were calculated through the method proposed by Calo et al. (1973), following multiple other studies in the literature (e.g., Montaldo et al., 2009; Dikmen et al., 2012). Approximate genetic correlations were calculated based on EBV of individuals with reliability greater than 0.35 for both traits (the average reliabilities for each trait pair is presented in Supplemental Table S1; <https://doi.org/10.6084/m9.figshare.13662275>):

$$\hat{r}_{g1,2} = \frac{\sqrt{(\sum RL_1) \times (\sum RL_2)}}{\sum (RL_1 \times RL_2)} \times r_{1,2},$$

where $\hat{r}_{g1,2}$ is the approximate genetic correlation between traits 1 and 2; $\sum RL_1$ and $\sum RL_2$ are the sum of reliabilities of traits 1 and 2; RL_1 and RL_2 are reliabilities of traits 1 and 2; and $r_{1,2}$ is the Pearson correlation between EBV for traits 1 and 2. The SE of the approximate genetic correlations were estimated as:

$$SE = \sqrt{\frac{1 - \hat{r}_{g1,2}^2}{n - 2}},$$

where n is the number of individuals with records (Sokal and Rohlf, 1995). The EBV for all traits included in the analyses were provided by the Beijing Dairy Cattle Center (Beijing, China) in early 2020. Genetic param-

eters for milk yield were estimated based on a test-day animal model, multiple-trait random regression models, and the Gibbs sampling method, as reported by Miglior et al. (2009). The 305-d milk performance was calculated by summing up the daily milk yield from 5 to 305 d based on the random regression coefficients and approximate reliabilities, following de Oliveira et al. (2018). The statistical models used to calculate EBV for the 29 conformation traits are similar to those described by Wu et al. (2013). The single-trait linear animal model with AI-REML were used to estimate genetic parameters and EBV for fertility, reproduction, and health traits (Guo et al., 2014; Liu et al., 2017). The fixed effects included in the models were herd-year of measurement, parity and calving season, and the random effects of animal additive genetic effect, permanent environment, and residual effect. The trait definitions and models used for the longevity traits are described in Zhang et al. (2019). A multivariate animal model was used to estimate survival time up to different parities and a single-trait animal model was fitted for productive life. Age at first calving, herd-year of birth, and birth season were fitted as fixed effect in the model and the random animal additive genetic effect. The analyses for the longevity traits were also done based on AI-REML and single-trait animal models. The data sets used to estimate genetic parameters and EBVs for the traits described above were extracted in late 2019.

RESULTS AND DISCUSSION

Phenotypic Variability

The descriptive statistics of the phenotypic data sets and environmental variables are presented in Table 1. The phenotypic distribution of RT, RR, and DS in a wide range of THI values is shown in Figure 2. The average RT increased, and the percentage of cows with RR and DS equal to 1 (the lowest score) decreased with increasing THI levels. High heat loads beyond the animal's ability to cope is a consequence of elevated ambient climatic conditions and animal factors, which lead to variations in behavior and physiology. Physiological variables, including RT, respiration rate, and panting score, are used to identify more heat-tolerant breeds (Dalcin et al., 2016; McManus et al., 2009,) as well as heat-tolerant animals within a breed or population considered as heat susceptible (Gaughan et al., 2008; Schütz et al., 2014; Brito et al., 2020). Higher milk yield is usually accompanied by greater metabolic activity and thus, additional heat production (Polsky and von Keyserlingk, 2017). All the fixed effects tested were statistically significant ($P < 0.05$) for RT, RR

Figure 2. Distribution of rectal temperature (A: boxplot), proportion of each score category for respiration rate score (B: bar plot) and drooling score (C: bar plot) of milking Holstein cows under different temperature-humidity index conditions (71–74, 75–78, 79–82, and > 82).

(except parity), and DS. The 3 physiological indicators for different parities and lactation stages are significantly different, which is similar with the correlation of milk yield with parity and lactation stage. Aguilar et al. (2009) found that the genetic variance for heat tolerance doubled from the first to second parity and increased by 20 to 100% from the second to third parity and genetic variance for heat tolerance was changed with DIM by using random regression model.

Least squares means (LSM) of milking status are presented in Table 2. The LSM of RT, RR, and DS after milking were significantly lower than that before milking ($P < 0.05$). During the period of measurement in

the current study, THI was above the threshold value of heat stress (THI = 68; Zimbelman et al., 2011). Therefore, it is reasonable to assume that cows were suffering from excessive ambient heat. Furthermore, higher average values of RT (38.83 in the morning and 39.15 in the afternoon) and proportion of scores greater than 1 for RR (42% in the morning and 48% in the afternoon) and DS (50% in the morning and 65% in the afternoon) were observed in the afternoon with significantly higher THI in the afternoon compared with the morning period (independent t -test; $P < 0.05$). A decrease of the thermal load during the night usually results in a body temperature reduction and a circadian rhythm of the

Table 1. Descriptive statistics of phenotypic records and environmental variables used in the analyses¹

Variable	NR	NC	Minimum	Maximum	Median	Mean	SD
Parity	63,091	—	1	10	2	2.15	1.35
Days in milk	63,091	—	0	919	188	195.92	125.89
Rectal temperature (°C)	59,265	13,479	37.2	40.8	38.90	38.99	0.59
Respiration score	30,290	9,148	1	3	2	1.78	0.76
Drooling score	30,421	9,204	1	3	1	1.55	0.67
Temperature-humidity index	63,091	—	70.5	90.2	81.10	80.77	3.74

¹NR = number of records; NC = number of cows; SD.

Table 2. Least squares means ($\pm$ SE) of rectal temperature, respiration rate score, and drooling score of milking Holstein cows in summer in Beijing, China

Fixed effect	Level	Rectal temperature	Respiration rate score	Drooling score
Milking status	Before	39.08 $\pm$ 0.004 ^a	1.90 $\pm$ 0.014 ^a	1.68 $\pm$ 0.014 ^a
	After	38.97 $\pm$ 0.004 ^b	1.78 $\pm$ 0.013 ^b	1.61 $\pm$ 0.014 ^b
	Unknown	38.93 $\pm$ 0.013 ^c	1.93 $\pm$ 0.035 ^c	1.65 $\pm$ 0.034 ^b

^{a-c}Values with different lowercase superscripts indicate significant difference at $P < 0.05$.

body temperature (body temperature in the afternoon is higher than that in the morning) can be observed (Piccione et al., 2003; Shehab-El-Deen et al., 2010). Similar findings were reported in Brazilian crossbred cows, in which the proportion of cows with RT greater than 39.3°C or respiration rate higher than 36 breaths/min were significantly greater in the afternoon than in the morning period (da Costa et al., 2015). Rectal temperature, RR, and DS decreased after milking (Table 2), which might have occurred in response to evaporative cooling treatments with sprinklers and fans before and during milking (Tarazón-Herrera et al., 1999; Gallardo et al., 2005). The use of cooling devices (e.g., sprinklers, fans) in the milking parlor waiting room is expected to alleviate the effect of heat exchange. Thus, milking status was included in the models as cooling devices were used before milking and it could affect the phenotypic measurements.

Genetic Parameters for Physiological Indicators of Heat Stress

We compared various scenarios for the estimation of genetic parameters (Supplemental Table S1, <https://doi.org/10.6084/m9.figshare.13662275>). Considering the amount of data used, accuracy of the estimates, and fitness of the model, the scenario considering the full data set and assuming nonzero covariance between permanent environmental effects was selected. Therefore, the results presented and discussed below were obtained from this scenario, but all the other results are presented in the Supplementary Table S2 (<https://doi.org/10.6084/m9.figshare.13662272>). Variance components and heritability estimates for RT, RR, and DS are presented in Table 3, while approximate genetic

correlations among these 3 heat stress indicators are shown in Table 4. The heritability estimates for RT, RR, and DS are 0.06 ± 0.01 , 0.04 ± 0.01 , and 0.02 ± 0.01 , respectively. The repeatability estimates for RT, RR, and DS are 0.19, 0.14, and 0.14, respectively. The heritability estimates for the 3 traits are low, but are all significant. There are other traits with similar heritability range (e.g., fertility traits), in which substantial genetic progress has been achieved in dairy cattle populations (Toghiani Pozveh et al., 2009; Denholm et al., 2017). Therefore, these heat stress indicator traits can be employed when aiming to genetically improve heat tolerance, but alternative indicators (ideally with higher heritability) should be investigated. In this context, it is expected that phenotypes measured based on precision technologies (instead of scoring systems) is likely to result in traits with higher heritability estimates.

We also compared the use of a linear or threshold model to estimate genetic parameters for RR and DS, but small differences were observed (results not shown). The same findings were reported in other studies (Carlén et al., 2006; Malchiodi et al., 2017). Therefore, we presented only the variance components obtained from the linear model. Dikmen et al. (2012) reported a higher heritability of 0.17 ± 0.13 for RT in American Holstein cattle than our estimate; however, their SE was also much larger than that of the current estimates. Al-Kanaan (2016) focused on physiological traits (RT, skin temperature, vaginal temperature, respiration rate, and pulse rate) in heat stress response for physiological traits in dairy and dual-purpose cattle and reported that the additive genetic variances and heritabilities of those physiological traits were smaller in the heat stress environment (THI >65) than under thermoneutral conditions (THI <65). The low heritability estimates

Table 3. Genetic components and parameters for rectal temperature, respiration rate score, and drooling score in Holstein cattle¹

Trait	σ_a^2 (SE)	σ_{pe}^2 (SE)	σ_e^2 (SE)	Heritability (SE)	Repeatability
Rectal temperature	0.02 (0.002)	0.03 (0.002)	0.20 (0.001)	0.06 (0.01)	0.19
Respiration rate score	0.02 (0.003)	0.04 (0.003)	0.31 (0.003)	0.04 (0.01)	0.14
Drooling score	0.01 (0.002)	0.04 (0.003)	0.31 (0.003)	0.02 (0.01)	0.14

¹Where σ_a^2 , σ_{pe}^2 , and σ_e^2 are estimated additive variance, permanent variance, and residual variance, respectively.

Table 4. Genetic correlations (above the diagonal) and phenotypic correlation (below the diagonal) among rectal temperature, respiration rate score, and drooling score in Holstein cattle¹

Trait	Rectal temperature	Respiration rate score	Drooling score
Rectal temperature		0.26 (0.11)*	−0.11 (0.14)
Respiration rate score	0.47 (0.01)*		0.25 (0.16)
Drooling score	0.24 (0.01)*	0.24 (0.01)*	

¹Values shown are correlation coefficients (SE).*Indicates correlation coefficient being significantly different from 0 ($P < 0.05$).

obtained in this study indicate that genetic progress will be lower compared with higher heritability indicators. As additional heat stress indicators are defined and larger data sets are obtained, higher heritability estimates might be observed. Furthermore, genomic selection might be a feasible alternative to increase the accuracy of breeding values, after a large enough training population is assembled. The low repeatability estimates (0.14 to 0.19) indicate the need to collect multiple records in the same animal to increase the EBV reliability. Low repeatability of physiological traits (RT, vaginal temperature, and respiration rate) ranging from 0.09 to 0.12 during heat stress were also reported by Al-Kanaan (2016). Phenotypic correlations among RT, RR, and DS are positive, moderate (0.24–0.47) and significant ($P < 0.05$). However, significant genetic correlations were observed only between RT and RR (0.26 ± 0.11).

Physiological traits such as body temperature (Srikandakumar and Johnson, 2004), respiration rate (Da Costa et al., 2015), pulse rate (Elayadeth-Meethal et al., 2018), and heart rate were also considered as reliable indicators of heat tolerance in crossbred cows (Cambodian native $\times$ Holstein; Bun et al., 2018). However, their application in large-scale populations are still limited, because they are difficult to measure in a large number of animals under field conditions. Panting scores (measured by visually assessing both RR and DS) have been proposed as a reliable indicator of heat load status in feedlot beef cattle, under commercial and research conditions (Gaughan et al., 2004; Brown-Brandl et al., 2006). The moderate genetic correlation observed between RR and DS (0.25 ± 0.16), indicate that both traits are complementary, and still different traits (i.e., capture different heat stress coping mechanisms). It is also recommended to measure and include both traits in genetic evaluation schemes aiming to improve heat tolerance. The nonsignificant genetic correlation observed between RT and DS indicate that these 2 variables are different indicators of heat stress. However, it is worth noting that 65% of the individuals with RT did not have DS measurements. Our research group continues to measure these physiological indica-

tors and this study will be repeated when a much larger data set becomes available.

When cows are under heat stress, the first visible physiological signs are increase of RR and DS, potentially followed by an increase in RT depending on the severity of the heat stress events (Atrian and Shahryar, 2012). Increased respiration rates with drooling is a biological function of animals for increasing heat loss (Gaughan et al., 2010). RT indicates balance between heat loss and heat load as a consequence of the physiological adaptations of homeotherms (Fournel et al., 2017). As physiological status indicator of cows during heat stress events, RR and DS are more visualized and directly compared with RT. Therefore, for the purpose of evaluating heat tolerance in dairy cows, it is recommended to assess RR and DS separately, as described in the Materials and Methods section. If RR or DS are greater than 1, it means that the cows are suffering from heat stress (or activating biological functions to counteract the negative effects of heat stress). As systematic scores of heat response, it is usually practical for farmers to assess the level of heat stress for a cow by measuring RR and DS, and provide different cooling treatments for the cows under thermal stress.

Approximate Genetic Correlation Between Physiological Indicators of Heat Stress and Other Relevant Traits

The approximate genetic correlations between each of the 3 physiological indicators of heat tolerance and other economically important traits are shown in Tables 5, 6, 7, and 8. The average reliabilities of EBV used to calculate the approximate genetic correlations are shown in Supplemental Table S2 (<https://doi.org/10.6084/m9.figshare.13662275>). The approximate genetic correlations between milk-related traits and RT were positive and ranged from 0.34 ± 0.01 (with 305-d milk yield in the first lactation) to 0.62 ± 0.02 (with 305-d fat yield in the first lactation). Moderate and positive approximate genetic correlations were also observed between milk-related traits and RR, ranging from 0.04 ± 0.07 (with 305-d protein yield in the third lactation) to

0.33 ± 0.07 (with 305-d fat yield in the first lactation). DS was lowly correlated with milk yield and composition, with correlations ranging from -0.14 ± 0.10 (with 305-d protein yield in the third lactation) to 0.22 ± 0.10 (with 305-d milk yield in the second lactation). Only the approximate genetic correlations between DS and 305-d milk yield in the second lactation were statistically significant at the 5% level. These findings indicate that genetic selection for increased productivity will indirectly reduce heat tolerance, if heat tolerance indicators are not considered simultaneously in a selection index. The approximate genetic correlation between SCS and the 3 heat stress indicator traits were positive (favorable) and ranged from 0.03 ± 0.06 (RT) to 0.35 ± 0.12 (DS), except negative (but nonsignificant) values with RT (-0.02 ± 0.06) for primiparous cows. Heat stressed cows activate physiological and biochemical processes to counter stress and to maintain thermal equilibrium. The cow's regulation mechanisms include heat dissipation to the environment and reduced production of metabolic heat (Kadzere et al., 2002). Heat stress negatively affects milk yield (Broucek et al., 2009; Zhu et al., 2016) and physiological parameters (Atrian and Shahryar, 2012). The unfavorable approximate genetic correlations between milk-related traits and 3 physiological indicators of heat stress are consistent with findings reported by Dikmen et al. (2012). This was expected as high-producing animals generate greater amount of metabolic heat during lactation compared with lower-production animals (West, 2003). This might also explain the greater approximate genetic correlation between milk yield and the 3 heat stress indicators in multiparous compared with primiparous cows. Negative effects of heat stress conditions under a temperate climate on SCS was reported in Holstein-Friesian cows (Lambertz et al., 2014). Under thermal

stress, simultaneous increase of SCS and heat loads can activate heat stress response mechanisms.

Unfavorable (but small or nonsignificant) approximate genetic relationships were observed between RT and most of the body conformation traits ranging from -0.03 ± 0.06 (forepart) to -0.34 ± 0.09 (feet and legs), whereas favorable genetic relationships were observed with body capacity traits such as frame, stature, chest width, body depth, and body size (ranging from 0.11 ± 0.06 to 0.20 ± 0.01). Most of the variation found in heat tolerance was explained by differences in body mass, which may impose a severe constraint on adaptive variation in stress tolerance (Woods, 2013; Klockmann et al., 2016). Both RR and DS had positive (unfavorable) and moderate correlation with udder traits (e.g., milking system, dairy character, udder depth, median suspensory, and fore attachment, ranging from 0.04 ± 0.08 to 0.30 ± 0.09). Positive approximate genetic correlations between RT and the majority of body size traits indicate that bigger cows tend to have higher RT (likely due to great metabolic heat production). Respiration and salivation are key physiological regulators for heat loss and RT is a consequence of the physiological adaptations. Genetic relationships for RR and DS and the majority of body size traits were of opposite sign (positive for RR). So, when increasing body size, a decreased capacity of heat loss and more heat accumulation (higher RT) would be expected. A similar result was obtained by Elayadeth-Meethal et al. (2018) when studying the evidence of parallel evolution in adaptive tolerance to heat stress in dwarf cattle breeds (*Bos taurus indicus*), comparing their thermoregulatory responses with those in standard-size cattle breeds (crossbred animals of *Bos taurus indicus* × *Bos taurus taurus*) and observed that smaller body size enhances thermal tolerance. In this context, Holstein animals are

Table 5. Approximate genetic correlations between 3 physiological indicators of heat stress indicators (rectal temperature, respiration rate score, and drooling score) with milk-related traits in Holstein cattle¹

Trait	Rectal temperature		Respiration rate score		Drooling score	
	N	$r_G \pm SE$	N	$r_G \pm SE$	N	$r_G \pm SE$
305-d milk yield in first parity	4,358	0.34 ± 0.01	242	0.22 ± 0.06	103	0.06 ± 0.10
305-d milk yield in second parity	3,885	0.41 ± 0.01	179	0.29 ± 0.07	100	0.22 ± 0.10
305-d milk yield in third parity	1,568	0.39 ± 0.02	242	0.28 ± 0.06	90	0.04 ± 0.11
305-d fat yield in first parity	2,224	0.62 ± 0.02	202	0.33 ± 0.07	103	-0.06 ± 0.10
305-d fat yield in second parity	1,475	0.54 ± 0.02	163	0.32 ± 0.07	100	0.07 ± 0.10
305-d fat yield in third parity	1,045	0.50 ± 0.03	259	0.19 ± 0.06	90	-0.08 ± 0.11
305-d protein yield in first parity	3,898	0.50 ± 0.01	242	0.21 ± 0.06	103	-0.11 ± 0.10
305-d protein yield in second parity	3,930	0.51 ± 0.01	199	0.23 ± 0.07	100	0.08 ± 0.10
305-d protein yield in third parity	2,238	0.45 ± 0.02	180	0.04 ± 0.07	91	-0.14 ± 0.10
305-d SCS in first parity	315	-0.02 ± 0.06	152	0.05 ± 0.08	101	0.18 ± 0.10
305-d SCS in second parity	242	0.03 ± 0.06	106	0.38 ± 0.09	94	0.12 ± 0.10
305-d SCS in third parity	169	0.23 ± 0.08	242	0.22 ± 0.06	66	0.35 ± 0.12

¹N = number of animals with reliability greater than 0.35 that were used to calculate the approximate genetic correlations; r_G = approximate genetic correlation.

Table 6. Approximate genetic correlations between 3 physiological indicators of heat stress indicators (rectal temperature, respiration rate score, and drooling score) with body conformation traits in Holstein cattle¹

Trait	Rectal temperature		Respiration rate score		Drooling score	
	N	$r_G \pm SE$	N	$r_G \pm SE$	N	$r_G \pm SE$
Capacity	291	0.14 ± 0.06	176	-0.05 ± 0.08	101	-0.49 ± 0.09
Rump	252	0.00 ± 0.06	167	-0.32 ± 0.07	98	-0.41 ± 0.09
Feet and legs	123	-0.34 ± 0.09	101	-0.14 ± 0.10	81	0.15 ± 0.11
Mammary system	273	-0.14 ± 0.06	172	0.04 ± 0.08	100	0.18 ± 0.10
Dairy character	166	-0.25 ± 0.08	128	0.10 ± 0.09	89	0.10 ± 0.11
Conformation (overall score)	243	-0.09 ± 0.06	165	-0.06 ± 0.08	98	-0.06 ± 0.10
Body size	4,233	0.20 ± 0.02	260	-0.02 ± 0.06	103	-0.28 ± 0.10
Stature	4,545	0.20 ± 0.01	261	-0.02 ± 0.06	103	-0.16 ± 0.10
Forepart	302	-0.03 ± 0.06	179	0.05 ± 0.08	102	-0.28 ± 0.10
Chest width	295	0.11 ± 0.06	177	-0.02 ± 0.08	102	-0.47 ± 0.09
Body depth	318	0.12 ± 0.06	184	0.04 ± 0.07	102	-0.01 ± 0.10
Loin strength	373	-0.02 ± 0.05	191	-0.29 ± 0.07	103	-0.34 ± 0.09
Rump width	1,984	0.05 ± 0.02	244	-0.25 ± 0.06	103	-0.02 ± 0.10
Rump angle	151	0.11 ± 0.08	117	-0.18 ± 0.09	86	-0.51 ± 0.09
Bone quality	244	-0.01 ± 0.06	165	0.02 ± 0.08	98	0.04 ± 0.10
Foot angle	212	-0.33 ± 0.07	155	-0.09 ± 0.08	97	0.28 ± 0.10
Hoof height	311	-0.11 ± 0.06	182	-0.06 ± 0.07	102	-0.07 ± 0.10
Rear legs side view	212	0.15 ± 0.07	155	-0.10 ± 0.08	97	-0.09 ± 0.10
Rear legs rear view	303	-0.15 ± 0.06	179	-0.10 ± 0.07	102	-0.12 ± 0.10
Udder depth	204	-0.15 ± 0.07	152	0.10 ± 0.08	95	0.31 ± 0.10
Udder texture	330	0.00 ± 0.06	185	-0.03 ± 0.07	102	-0.13 ± 0.10
Median suspensory	315	-0.15 ± 0.06	184	0.07 ± 0.07	102	0.23 ± 0.10
Fore attachment	2,961	-0.05 ± 0.02	254	0.12 ± 0.06	103	0.3 ± 0.09
Fore teat placement	212	-0.07 ± 0.07	155	0.00 ± 0.08	97	0.16 ± 0.10
Teat length	180	0.20 ± 0.07	137	-0.45 ± 0.08	92	-0.28 ± 0.10
Rear attachment height	373	-0.21 ± 0.05	191	-0.08 ± 0.07	103	-0.05 ± 0.10
Rear attachment width	810	-0.16 ± 0.03	216	-0.05 ± 0.07	103	-0.03 ± 0.10
Rear teat placement	223	0.35 ± 0.06	161	-0.14 ± 0.08	97	-0.56 ± 0.09
Angularity	276	-0.19 ± 0.06	173	0.13 ± 0.08	100	0.08 ± 0.10

¹N = number of animals with reliability greater than 0.35 that were used to calculate the approximate genetic correlations; r_G = approximate genetic correlation.

less tolerant to heat stress than crossbred (Girolando), partially because of the difficulty in dissipating heat due to the larger body size, as well as thicker and longer hair (Alfonzo et al., 2016).

The estimated approximate genetic correlation between reproductive traits in heifers (age at first calving and first service) and RT, RR, and DS were negative

and ranged from -0.02 ± 0.06 to -0.26 ± 0.02 , except for a significantly positive approximate genetic correlation between RT and age at first service (0.05 ± 0.01). Rectal temperature was moderately and positively associated with interval from calving to first service in cows (0.30 ± 0.03). Both RR and DS exhibited positive approximate genetic correlation with interval from first

Table 7. Approximate genetic correlations between 3 physiological indicators of heat stress indicators (rectal temperature, respiration rate score, and drooling score) with fertility, reproduction, and health traits in Holstein cattle¹

Trait	Rectal temperature		Respiration rate score		Drooling score	
	N	$r_G \pm SE$	N	$r_G \pm SE$	N	$r_G \pm SE$
Age at first calving – heifer	1,641	-0.26 ± 0.02	231	-0.22 ± 0.06	100	-0.14 ± 0.10
Age at first service – heifer	5,603	0.05 ± 0.01	274	-0.02 ± 0.06	100	-0.05 ± 0.10
Interval from calving to first service – cow	1,403	0.30 ± 0.03	230	0.09 ± 0.07	100	-0.02 ± 0.10
Interval from first to last insemination – cow	649	0.19 ± 0.04	197	0.29 ± 0.07	100	0.29 ± 0.10
Interval from first to last insemination – heifer	168	-0.23 ± 0.08	130	-0.20 ± 0.09	88	0.06 ± 0.11
Fertility disorders	1,305	0.01 ± 0.03	232	0.11 ± 0.07	101	0.05 ± 0.10
Udder health	1,965	-0.14 ± 0.02	252	0.30 ± 0.06	101	0.39 ± 0.09
Feet and leg diseases	248	0.17 ± 0.06	163	-0.06 ± 0.08	97	-0.04 ± 0.1
Digestive disorders	274	-0.11 ± 0.06	170	-0.02 ± 0.08	98	0.10 ± 0.10
Metabolic diseases	751	-0.38 ± 0.03	209	0.22 ± 0.07	101	0.36 ± 0.09

¹N = number of animals with reliability greater than 0.35 that were used to calculate the approximate genetic correlations; r_G = approximate genetic correlation.

Table 8. Approximate genetic correlations between 3 physiological indicators of heat stress indicators (rectal temperature, respiration rate score, and drooling score) with longevity traits in Holstein cattle¹

Trait	Rectal temperature		Respiration rate score		Drooling score	
	N	$r_G \pm SE$	N	$r_G \pm SE$	N	$r_G \pm SE$
Survival up to 60 d	2,837	0.01 ± 0.02	272	-0.08 ± 0.06	101	-0.21 ± 0.10
Survival up to 365 d	5,622	-0.11 ± 0.01	289	-0.06 ± 0.06	101	-0.07 ± 0.10
Survival until calving	5,555	0.04 ± 0.01	289	0.07 ± 0.06	101	0.02 ± 0.10
Survival up to the second parity	3,785	-0.01 ± 0.02	262	-0.05 ± 0.06	103	0.09 ± 0.10
Survival up to the third parity	898	-0.21 ± 0.03	208	-0.06 ± 0.07	102	0.02 ± 0.10
Survival up to the fourth parity	382	-0.16 ± 0.05	186	-0.07 ± 0.07	102	-0.05 ± 0.10
Survival up to the fifth parity	329	-0.15 ± 0.05	181	-0.06 ± 0.07	101	-0.01 ± 0.10
Survival up to the sixth parity	360	-0.23 ± 0.05	184	0.00 ± 0.07	101	0.04 ± 0.10
Productive life	2,151	-0.31 ± 0.02	213	0.09 ± 0.07	99	0.14 ± 0.10

¹N = number of animals with reliability greater than 0.35 that were used to calculate the approximate genetic correlations; r_G = approximate genetic correlation.

to last insemination in cows (0.29 ± 0.07 and 0.29 ± 0.10 respectively). The magnitude and direction of the genetic relationships between 3 heat stress indicators and functional traits (fertility, diseases, and longevity) obtained in this study are similar to those reported in the literature (Dikmen et al., 2012; Morrell, 2020; Ouellet et al., 2020). In general, heat-susceptible cows have poorer fertility, are more prone to diseases, and have shorter service life. Genetic strategies that increase cow thermotolerance are desirable as genetic gains are cumulative and permanent over generations (Hansen, 2019).

Unfavorable approximate genetic correlations, ranging from -0.11 ± 0.06 to -0.38 ± 0.03 were obtained between health traits and RT, with the exception of feet and leg disorders (0.17 ± 0.06) and fertility disorders (0.01 ± 0.03). Among all the disease traits, udder health had the highest approximate genetic correlation with DS (0.39 ± 0.09). The approximate genetic correlations between RT and longevity-indicators or productive life were mostly negative and ranged from -0.01 ± 0.02 to -0.31 ± 0.02 . The approximate genetic correlations between RR or DS and longevity or productive life were low and ranged from -0.21 ± 0.10 to 0.14 ± 0.10 . Heat stress affects the health of dairy animals by influencing physiology, metabolism, hormonal, and immunity system mechanisms (Das et al., 2016; Sammad et al., 2020). Sanders et al. (2009) observed that lameness incidence increases with greater air temperature, which might be related to increase in standing time (Provolo and Riva, 2009). High-producing cows are usually at a higher risk of diseases compared with lower-production cows (Dobson et al., 2007). Excess heat load in extreme cases not only compromises animal welfare but also results in involuntary culling or death (Das et al., 2016).

Various indicators of thermotolerance have been proposed and used to quantify heat stress response in cattle (Duff and Galyean, 2007; Burdick et al., 2012;

Hoffmann et al., 2019). The rapid technological advancements in the area of livestock production poses a promising opportunity to measure heat stress response on a timely and efficient manner, including automatic monitors for measuring body temperature (Koltes et al., 2018), respiration rate (Milan et al., 2016), and related variables that capture more continuous phenotypic variations in heat stress response and enable improving accuracy of genetic and genomic evaluations (as reviewed by Brito et al., 2020). Furthermore, the use of precision technologies is an opportunity to better understand the biological mechanisms underlying heat stress response with minimal disturbance to the animals. Variations in milk production have been used in genomic selection schemes to improve heat tolerance in Australian dairy cattle (Nguyen et al., 2016). However, physiological indicators were also used to validate heat tolerance genomic predictions (Garner et al., 2016). The approximate genetic correlations of the 3 physiological indicators of heat stress with other routinely-recorded and functional traits reported in this study, provided direct evidences for the importance of establishing selection indexes incorporating heat stress indicators to enable simultaneous progress in heat tolerance as well as other economically important traits. The incorporation of genomic information in the evaluation of these physiological indicators of heat stress is expected to improve the accuracy of breeding values, especially considering the heritability estimates observed.

CONCLUSIONS

Respiration rate score, rectal temperature, and drooling score are lowly heritable traits and moderately correlated at the genetic and phenotypic level. Heat stress response in dairy cows can be quantified based on rectal temperature or 2 independent scoring systems based on respiration rate and drooling condition separately. In

general, the approximate genetic correlations between heat tolerance indicators and routinely-evaluated traits were low to moderate, but unfavorable in some cases. This indicates that genetic selection for improved heat tolerance can be achieved by including a selection sub-index for heat stress response in the routine genetic evaluations and accounting for the relationship among all traits. Future studies should investigate the incorporation of genomic information in similar analyses and the feasibility of using precision technologies for more accurately assessing heat stress response in dairy cattle.

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