Genetic analyses of blood β -hydroxybutyrate predicted from milk infrared spectra and its association with longevity and female reproductive traits in Holstein cattle

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ABSTRACT

Ketosis is one of the most prevalent and complex metabolic disorders in high-producing dairy cows and usually detected through analyses of β -hydroxybutyrate (BHB) concentration in blood. Our main objectives were to evaluate genetic parameters for blood BHB predicted based on Fourier-transform mid-infrared spectra from 5 to 305 d in milk, and estimate the genetic relationships of blood BHB with 7 reproduction traits and 6 longevity traits in Holstein cattle. Predicted blood BHB records of 11,609 Holstein cows (after quality control) were collected from 2016 to 2019 and used to derive 4 traits based on parity number, including predicted blood BHB in all parities (BHBp), parity 1 (BHB1), parity 2 (BHB2), and parity 3+ (BHB3). Single- and multitrait repeatability models were used for estimating genetic parameters for the 4 BHB traits. Random regression test-day models implemented via Bayesian inference were used to evaluate the daily genetic feature of BHB variability. In addition, genetic correlations were calculated for the 4 BHB traits with reproduction and longevity traits. The heritability estimates of BHBp, BHB1, BHB2, and BHB3 ranged from 0.100 ± 0.026 ($\pm$ standard error) to 0.131 ± 0.023 . The BHB in parities 1 to 3+ were highly genetically correlated and ranged from 0.788 (BHB1 and BHB2) to 0.911 (BHB1 and BHB3). The daily heritability of BHBp ranged from 0.069 to 0.195, higher for the early and lower for the later lactation periods. A similar trend was observed for BHB1, BHB2, and BHB3. There are low direct genetic correlations between BHBp and selected

reproductive performance and longevity traits, which ranged from -0.168 ± 0.019 (BHBp and production life) to 0.157 ± 0.019 (BHBp and age at first calving) for the early lactation stage (5 to 65 d). These direct genetic correlations indicate that cows with higher BHBp (greater likelihood of having ketosis) in blood usually have shorter production life (-0.168 ± 0.019). Cows with higher fertility and postpartum recovery, such as younger age at first calving (0.157 ± 0.019) and shorter interval from calving to first insemination in heifer (0.111 ± 0.006), usually have lower BHB concentration in the blood. Furthermore, the direct genetic correlations change across parity and lactation stage. In general, our results suggest that selection for lower predicted BHB in early lactation could be an efficient strategy for reducing the incidence of ketosis as well as indirectly improving reproductive and longevity performance in Holstein cattle.

Key words: β -hydroxybutyrate, Chinese Holstein, metabolic disease, random regression model

INTRODUCTION

Ketosis, also known as hyperketonemia and ketonemia, is one of the most frequent metabolic diseases caused by an energy metabolism disorder in postpartum dairy cows (Overton et al., 2017; Benedet et al., 2019). For instance, the prevalence rate of subclinical ketosis in Holstein cows in 10 European countries ranged from 11.2% to 36.6%, with an average of 21.8% (Suthar et al., 2013). The median incidence of clinical ketosis in Holstein cows was 3.1% based on 11 studies (Pryce et al., 2016). Previous reports indicated that the within-herd incidence of postpartum hyperketonemia in dairy cows ranges from 23% to 60% (McArt et al., 2013a; Mahrt et al., 2015; Vanholder et al., 2015). Such

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high incidence of ketosis not only affects welfare, milk yield and composition, and reproductive performance in current parity, but can also considerably affect the future performance of dairy cows (McArt et al., 2013b; Raboisson et al., 2014; Mostert et al., 2018). In Canadian dairy cattle, every 1% increase in the incidence of subclinical ketosis has been estimated to cause an economic loss of \$0.015 per cow per day (McLaren et al., 2006), and reproductive failure was the main component responsible for these losses (~34%; McArt et al., 2015). Thus, the negative effect of ketosis on the reproductive performance of dairy cattle needs to be further investigated for defining more effective mitigation strategies.

As the main pathological mechanism of ketosis, insufficient adaptation of an individual's metabolism can result in negative energy balance (**NEB**), which leads to excessive body fat mobilization (Esposito et al., 2014). Therefore, high concentrations of nonesterified fatty acids and ketones including BHB, acetone, and acetoacetate appear in the blood, milk, and other body environments (Herdt, 2000). β -Hydroxybutyrate is the major and most stable circulating ketone in body fluids; accordingly, the concentration of BHB is generally used as a reference for ketosis, and BHB can more precisely represent the biological variation in susceptibility than other ketone bodies (Duffield et al., 2009; van der Drift et al., 2012a; Denis-Robichaud et al., 2014). Nonetheless, the cost of common methods, such as commercially available kits (Leroy et al., 2004), makes it difficult to regularly test all the herd cows at risk. A BHB detection program based on routine milk analysis may be a more practical and less labor-intensive approach (Pralle et al., 2018). In this context, BHB in blood or milk can be accurately predicted through analysis of Fourier-transform mid-infrared (**FT-MIR**) spectra of milk with low cost and high throughput (Heuer et al., 2001; van Knegsel et al., 2010; Grelet et al., 2016). The BHB-predicted phenotypes provide an opportunity to implement genetic and genomic selection for lower ketosis incidence in dairy cattle.

Reproductive performance traits are key functional traits as they directly affect productive efficiency in the dairy industry (Lucy, 2001; Stevenson and Britt, 2017). The relationship between ketosis and reproductive performance was first reported in Swedish dairy cattle (Andersson et al., 1991). In general, there is a negative phenotypic correlation between the concentration of BHB in the blood and pregnancy rate at the first service (Walsh et al., 2007). Furthermore, uterine diseases and delayed luteal activity have been associated with elevated BHB concentrations (Reist et al., 2003; Hammon et al., 2006). Many of these reproductive disorders in high-producing dairy cows often result

from metabolic diseases (Dobson et al., 2007; Sheldon et al., 2008). Poor reproductive and health performance often leads to premature culling and decreased life span of dairy cows (Oltenu and Broom, 2010). Therefore, the genetic relationship of BHB with female reproductive performance and longevity is of great importance. To our best knowledge, most studies reported genetic parameters of BHB in milk or blood using linear animal models and explored the relationships of BHB with metabolic diseases, energy balance, and milk production in different populations such as Greek (Oikonomou et al., 2008), Canadian (Koeck et al., 2014, 2016), and Dutch (van der Drift et al., 2012b) Holsteins, and Norwegian Red cattle (Belay et al., 2017). However, the genetic relationships for BHB with reproduction and longevity traits in dairy cattle have not yet been thoroughly investigated. Therefore, this study aimed to (1) estimate genetic parameters for predicted blood BHB using both random regression test-day models through Bayesian inference and linear repeatability models, and (2) calculate the genetic correlations of blood BHB traits with reproductive performance and longevity indicators in Holstein cattle.

MATERIALS AND METHODS

Ethics Committee Approval

The data used in this study were collected in strict adherence to the protocol approved by the Animal Welfare Committee of the China Agricultural University, Beijing, China (protocol number: DK996).

Data Sets

Test-day predicted blood BHB (**BHB_p**) records from 12,157 Holstein cows were obtained from December 2016 to March 2019 in 12 dairy farms located in Beijing, China. The freestall barn system and TMR were used in all herds and the average numbers of cows and records per herd were 967 ± 595 and $2,861 \pm 2,636$, respectively. The average test-day milk yield per cow is about 35 ± 9 kg. Individual milk samples were tested monthly at the Beijing Dairy Cattle Center (Beijing, China), and the pedigree data were also provided. Blood BHB (mmol/L) was predicted from milk spectra data via internal inbuilt modules in FTS machines (Bentley Instruments Inc.).

The 7 reproduction traits (measured in ~140,000 cows) and 6 longevity-indicator traits (measured in ~130,000 cows) were provided by the Independent Innovation League of Dairy Breeding (Beijing, China). The reproduction traits included age at first calving in heifers (**AFC**, d), age at first insemination in heifers

(**AFS**, d), interval from calving to first insemination in first-parity (**ICF1**, d) and multiparous (**ICF2**, d) cows, interval from first to last insemination for first-parity (**IFLC1**, d) and multiparous (**IFLC2**, d) cows, and interval from first to last insemination of heifers (**IFLH**, d). Additional details about the definition of the reproductive performance traits can be found in Guo et al. (2014) and Liu et al. (2017). Furthermore, 6 longevity-indicator traits included the period from the first calving to the end of the first (**LS1**, d), second (**LS2**, d), third (**LS3**, d), fourth (**LS4**, d), and fifth (**LS5**, d) parity, and productive life (**PL**, d). The trait definitions used for the longevity traits are described in Zhang et al. (2019, 2021). The traits described above were recorded until December 2019. All cows with BHB records were also recorded for the fertility and longevity indicators and additional details about the data sets are shown in Supplemental Table S1 (https://figshare.com/articles/dataset/Supplemental_Table_S1_docx/18133721).

Only BHBp records ranging from 0.004 to 4.818 mmol/L were kept in the analyses. The records of cows with DIM out of the range from 5 to 305 d were removed, while 10 DIM stages can be obtained with the interval of 30 d. A total of 34,338 test-day BHBp records from 11,609 Holstein cows were retained in the data set, whereas the concentrations of BHBp in different parities (1, 2, and 3+) were treated as separate traits (**BHB1** for first-parity, **BHB2** for second parity, and **BHB3** for third or greater parity cows) and the numbers of animals were 6,420, 4,395, and 3,925, respectively. In addition, the pedigree used in the genetic analysis was traced back up to 6 generations and included 449,819 animals.

Genetic Analyses of Predicted Blood BHB

Mixed model analyses were performed using the PROC MIXED procedure implemented in SAS 9.2 (SAS Institute Inc.) with BHBp, BHB1, BHB2, and BHB3 as the dependent variables to identify the systematic effects that should be included in the genetic models. Genetic analyses of BHBp were performed using the AI-REML procedure implemented in the DMU software (Madsen et al., 2006). A linear repeatability model (model 1) was used to estimate the heritability and repeatability for BHB. Model 1 can be described as follows:

$$y_{ijklm} = \text{HYS}_i + P_j + \beta_1 \text{DIM}_k + \beta_2 e_k^{-0.05 \text{DIM}} + a_l + pe_l + \varepsilon_{ijklm}, \quad [\text{model 1}]$$

where y_{ijklm} are the phenotypic records for BHBp, BHB1, BHB2, or BHB3; HYS_i is the fixed effect of the herd-year-season i (71 levels); P_j is the fixed effect of parity j (1, 2, or 3+); DIM_k and $e_k^{-0.05 \text{DIM}}$ are the covariate effects of DIM (interval of 5 to 305 d), which modified the lactation curve function (Cobby and Le Du, 1978; Wilmlink, 1987; Zaalberg et al., 2019); β_1 and β_2 are the regression coefficients of DIM; a_l is the random additive genetic effect; pe_l is the random permanent environmental effect; and ε_{ijklm} is the random residual effects. It was assumed that $a \sim N(\mathbf{0}, \mathbf{A}\sigma_a^2)$, $pe \sim N(\mathbf{0}, \mathbf{I}\sigma_{pe}^2)$, and $\varepsilon \sim N(\mathbf{0}, \mathbf{I}\sigma_e^2)$, where $\mathbf{A}$ is the additive pedigree-based relationship matrix, σ_a^2 is the additive genetic variance, σ_{pe}^2 is the permanent environment variance, σ_e^2 is the random residual variance, and $\mathbf{I}$ is an identity matrix. To investigate the genetic relationships among BHB recorded across parities, a multitrait repeatability model (model 2) was fitted and included the same effects as described in model 1.

A single-trait random regression test-day model (model 3) was used to estimate the daily additive genetic variance components, permanent environmental effects, and residual effect for the 4 BHB traits across DIM based on the RJMC module in the DMU software (Madsen et al., 2006). Bayesian methods based on the Gibbs sampler and Markov Chain Monte Carlo (MCMC) algorithm were used to estimate the variance components for all traits. A MCMC chain length of 500,000 cycles, considering a burn-in period of 100,000, and a sampling interval (thin) of 50 cycles were used. The convergence of the chain was assessed using the package “coda” (Plummer et al., 2016) in the R software according to the Geweke (1991) criteria. Model 3 can be described as follows:

$$y_{ijlmno} = \text{HYS}_i + P_j + \sum_{n=0}^3 \text{DIM}_n a_{mn} + \sum_{n=0}^3 \text{DIM}_n pe_{mn} + \varepsilon_{ijlmno}, \quad [\text{model 3}]$$

where y_{ijlmno} is the response variable of BHBp, BHB1, BHB2, or BHB3; DIM_n is the covariate of the n th Legendre orthogonal polynomial ($n = 0, 1, 2, 3$; Kirkpatrick et al., 1990); a_{mn} and pe_{mn} are the additive genetic and permanent environmental random regression coefficients of DIM, respectively. The continuous time point (DIM) was normalized in the range of -1 to 1 and then converted to different orders of Legendre polynomials. As shown in Supplemental Table S2 (https://figshare.com/articles/dataset/Supplemental_Table_S2_docx/18133727), the optimal orthogonal polynomials (**T**) were added into model 3. For the random effects, the

following prior distributions were assumed: $a \sim N(\mathbf{0}, \mathbf{G} \otimes \mathbf{A})$, $pe \sim N(\mathbf{0}, \mathbf{P} \otimes \mathbf{I})$, and $\varepsilon \sim N(\mathbf{0}, \mathbf{I}\sigma_e^2)$, where $\mathbf{G}$ and $\mathbf{P}$ are the covariance matrices associated with the random regression polynomial coefficients for animal additive genetic and permanent environmental effects, respectively. All the other parameters were previously defined. Furthermore, we assumed that $\mathbf{G}$ and $\mathbf{P}$ followed the inverted Wishart ($\mathbf{IW}$) distribution, $\mathbf{IW}(v_G, \mathbf{V}_G)$, and $\mathbf{IW}(v_{PE}, \mathbf{V}_{PE})$, where v is the degrees of freedom ($v = 3$) and $\mathbf{V}$ is the scale matrix. σ_e^2 was assumed to follow a scaled inverted chi-squared distribution.

For model 1 and 2, 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, and $\sigma_p^2 = \sigma_a^2 + \sigma_{pe}^2 + \sigma_e^2$. The genetic correlation coefficient was calculated as $r = \frac{\sigma_{ij}}{\sqrt{\sigma_i^2 \sigma_j^2}}$. The squares of the standard errors (**SE**) for the heritability and genetic correlation coefficient were calculated according to Su et al. (2007):

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

$$SE^2(r) = \left[\frac{\hat{\sigma}_{ij}^2}{\hat{\sigma}_i^2 \hat{\sigma}_j^2} \right]$$

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

The (co)variances of the estimated parameters (σ_i^2, σ_{ij}) between trait i and trait j are obtained from the inverse of the average information matrix of model 2.

For model 3, the additive and permanent environmental (co)variances for all DIM were calculated using the posterior mean of the (co)variance components estimated for the random regression coefficients of these effects (i.e., $\lambda = \mathbf{TGT}'$, $\xi = \mathbf{TPT}'$), where λ and ξ are the additive genetic and permanent environmental (co)variance matrices for the DIM, and $\mathbf{T}$, $\mathbf{G}$, and $\mathbf{P}$ are as previously mentioned. Residual variances were also obtained from the posterior marginal distribution samples. Therefore, the daily variance components and

genetic parameters can be calculated. The breeding value for all DIM (5–305 d) of the animal $\mathbf{i}$ were obtained as $\mathbf{EBV}_i = \mathbf{T}\mathbf{a}_i$, where $\mathbf{a}_i$ is the vector of breeding values for the regression coefficients of the animal $\mathbf{i}$.

Genetic Correlations for BHB with Reproduction and Longevity Traits

Genetic correlations and SE of the whole (5–305 d), early (5–65 d), and late (245–305 d) lactations for the 4 BHB traits with reproductive and longevity traits were calculated based on a bivariate animal model. β -Hydroxybutyrate is a longitudinal trait in each lactation, whereas most of the reproduction and longevity traits are single-measurement traits in each lactation and even in lifetime. Therefore, the adjusted phenotypes for the BHB traits were obtained from model 3 and used as pseudo-phenotypes in the bivariate analyses. For the 7 reproductive traits, the animal models include the fixed effects of herd-year of measurement, calving season, and the random effects of animal additive genetic effect and residual effect. The fixed effects (age at first calving, herd-year of birth, and birth season) and the random effect such as additive genetic were fitted in longevity animal model. The more detailed definition of the statistical models can be found in Guo et al. (2014), Liu et al. (2017), and Zhang et al. (2019, 2021). These analyses were implemented based on the average information-restricted maximum likelihood (**AI-REML**) procedure in DMU software (Madsen et al., 2006).

RESULTS

Descriptive Statistics

The descriptive statistics for the 4 BHB traits are presented in Table 1. The BHBp concentration ranged from 0.004 to 4.818 mmol/L, with an average of 1.450 mmol/L and a standard deviation of 0.468 mmol/L. The average concentration of BHBp is highest (1.686 mmol/L) in early lactation (5–65 DIM) and lowest (1.361 mmol/L) in later lactation (245–305 DIM). The density distribution of BHB is presented in Figure 1, in which the differences in BHB concentration across multiple parities are statistically significant ($P < 0.01$). The BHB least squares means decreased from 1.498 mmol/L (parity 1) to 1.396 mmol/L (parity 3+), but the coefficient of variation (**CV**) ranged from 30.213 to 34.417%. In addition, the proportion of records with BHB values within >1.2 to <3.0 mmol/L (subclinical ketosis) and ≥ 3.0 mmol/L (clinical ketosis) groups for different lactation stages are shown in

Figure 1. The density distribution of predicted blood BHB concentration (mmol/L) in different parities (1, 2, 3+) in Holstein cattle.

Figure 2. The frequencies of subclinical and clinical ketosis are highest in the early lactation, and the proportion of healthy individuals gradually increases with the progression of DIM. Compared with BHBp, BHB1, and BHB2, the curve of BHB3 had a faster slope in Figure 2(a). In general, greater BHB levels are associated with higher incidence of ketosis (Benedet et al., 2019).

Genetic Parameter Estimates

Estimates of variance components, heritability, and repeatability for predicted blood BHB and correlations across multiple parities are presented in Tables 2 and 3. The heritability estimate based on records from all parities was 0.116 ± 0.018 , with a repeatability of 0.241. The heritability of predicted blood BHB was the highest (0.131 ± 0.023) in the first parity and the lowest (0.100 ± 0.026) in the second parity. We found small differences in the repeatability of predicted blood BHB across parities, ranging from 0.236 (BHB2) to 0.262 (BHB1). Thus, predicted blood BHB traits had low to moderate heritability and repeatability estimates. Meanwhile, we observed high genetic correlations among different pari-

ties based on model 2, ranging from 0.788 (BHB2 and BHB3) to 0.911 (BHB1 and BHB3).

The trends of variance components and heritability for BHBp with DIM are shown in Figure 3. From 5 to 305 DIM, the additive genetic variance ranged from 0.011 to 0.097 and the permanent environmental variance ranged from 0.006 to 0.274. These estimates were greater at the beginning of the lactation, and declined rapidly from 5 to 100 DIM. Over the whole lactation period, the highest (0.195) and lowest (0.069) heritability for BHBp was observed at the beginning and end of lactation, respectively, whereas the average heritability estimate is 0.101. The BHBp heritability rapidly decreased from calving to 100 DIM as well as the genetic additive effect. The BHBp repeatability ranged from 0.743 to 0.138, with an average of 0.218.

Overall, similar genetic parameter trends were observed for BHB across parities. However, there are some differences in certain lactation stages. For instance, BHB1 has the highest heritability at the beginning of lactation, whereas BHB2 has significant fluctuation of heritability estimates at the middle of lactation. The BHB1 and BHB2 have higher repeatability at the end of the lactation compared with BHB3.

Table 1. Descriptive statistics of predicted blood BHB traits in Chinese Holstein cattle¹

Trait	n	No. of cows	Mean (mmol/L)	SD	Minimum	Maximum	CV $\times$ 100%
BHBp	34,338	11,609	1.450	0.468	0.004	4.818	32.272
BHB1	15,401	6,420	1.499	0.453	0.025	4.363	30.213
BHB2	9,467	4,395	1.432	0.474	0.011	4.818	33.079
BHB3	9,470	3,925	1.390	0.478	0.004	4.711	34.417

¹n = number of records; BHBp = predicted blood BHB in all parities; BHB1 = predicted blood BHB in first parity; BHB2 = predicted blood BHB in second parity; BHB3 = predicted blood BHB in parity 3+.

Figure 2. The proportion of predicted blood BHB records within the range of subclinical (>1.2 to <3.0 mmol/L; a) and clinical (≥ 3.0 mmol/L; b) ketosis groups for different lactation stages (30 d per stage) during the period from 5 to 305 d in all parities (BHBp), parity 1 (BHB1), parity 2 (BHB2), and parity 3+ (BHB3).

Table 2. Estimates of genetic parameters and variance components for predicted blood BHB in Chinese Holstein cattle¹

Trait	$\sigma_a^2 \pm \text{SE}$	$\sigma_{pe}^2 \pm \text{SE}$	$\sigma_e^2 \pm \text{SE}$	$h^2 \pm \text{SE}$	r_e
BHBp	0.013 $\pm$ 0.002	0.014 $\pm$ 0.002	0.085 $\pm$ 0.007	0.116 $\pm$ 0.018	0.241
BHB1	0.014 $\pm$ 0.003	0.014 $\pm$ 0.003	0.079 $\pm$ 0.002	0.131 $\pm$ 0.023	0.262
BHB2	0.011 $\pm$ 0.003	0.015 $\pm$ 0.002	0.084 $\pm$ 0.006	0.100 $\pm$ 0.026	0.236
BHB3	0.013 $\pm$ 0.003	0.015 $\pm$ 0.003	0.085 $\pm$ 0.003	0.115 $\pm$ 0.025	0.248

¹ σ_a^2 = additive genetic variance; σ_{pe}^2 = permanent environmental variance; σ_e^2 = residual variance; r_e = repeatability; BHBp = predicted blood BHB in all parities; BHB1 = predicted blood BHB in parity 1; BHB2 = predicted blood BHB in parity 2; BHB3 = predicted blood BHB in parity 3+.

Table 3. The genetic correlation (above the diagonal) and its SE (below the diagonal) for predicted blood BHB during different parities in Chinese Holstein cattle¹

Trait	BHB1	BHB2	BHB3
BHB1	—	0.788	0.911
BHB2	0.144	—	0.930
BHB3	0.148	0.173	—

¹BHB1 = predicted blood BHB in parity 1; BHB2 = predicted blood BHB in parity 2; BHB3 = predicted blood BHB in parity 3+.

Genetic Correlations for BHB with Reproduction and Longevity Traits

Genetic correlations for the BHBp with reproduction and longevity traits during the whole (5–305 DIM), early (5–65 DIM), and late (245–305 DIM) lactation are presented in Tables 4 and 5. For the entire lactation, the genetic correlations between reproduction traits and BHBp ranged from -0.084 ± 0.017 (AFS) to 0.141 ± 0.032 (AFC). There were low and positive genetic correlations for BHBp with AFC (0.141 ± 0.032), IFLC2 (0.138 ± 0.018), and IFLH (0.104 ± 0.026). The PL is negatively genetically correlated

(-0.126 ± 0.021) with BHBp, whereas the other estimates are small or not different from zero. Otherwise, the genetic correlations are similar in early (5–65 DIM) and late lactations (245–305 DIM) compared with the whole lactation, especially for longevity traits. Supplemental Tables S3 (https://figshare.com/articles/dataset/Supplemental_Table_S3_docx/18133736), S4 (https://figshare.com/articles/dataset/Supplemental_Table_S4_docx/18133757), and S5 (https://figshare.com/articles/dataset/Supplemental_Table_S5_docx/18133742) show the different relationship for BHB measured in different parities with reproduction and longevity traits. Most of the genetic correlations are negligible, except for AFC, IFLC2, IFLH, and PL.

DISCUSSION

Intensive selection for production traits over the past 50 years has led cows to adjust their metabolism to distribute more nutrients and energy toward milk synthesis (Rauw et al., 1998; Miglior et al., 2017). Thus, high-producing cows rely more on body reserves to support milking, and most of them suffer from metabolic

Figure 3. The trends in genetic variance components (σ_a^2 = additive genetic variance; σ_{pe}^2 = permanent environmental variance; r_e = repeatability) for predicted blood BHB (BHBp = predicted blood BHB in all parities; BHB1 = predicted blood BHB in parity 1; BHB2 = predicted blood BHB in parity 2; BHB3 = predicted blood BHB in parity 3+) with the increase in DIM based on a random regression test-day model in Chinese Holstein cattle.

Table 4. The genetic correlations between predicted blood BHB and reproduction traits in Chinese Holstein cattle based on bivariate animal models¹

Trait	$h^2 \pm SE$	BHBp (5–305 d)	BHBp (5–65 d)	BHBp (245–305 d)
AFC	0.068 $\pm$ 0.005	0.141 $\pm$ 0.032	0.157 $\pm$ 0.019	0.041 $\pm$ 0.009
AFS	0.266 $\pm$ 0.013	–0.084 $\pm$ 0.017	–0.063 $\pm$ 0.003	–0.004 $\pm$ 0.002
ICF1	0.034 $\pm$ 0.004	–0.069 $\pm$ 0.003	–0.078 $\pm$ 0.006	–0.002 $\pm$ 0.001
ICF2	0.016 $\pm$ 0.002	–0.089 $\pm$ 0.011	–0.071 $\pm$ 0.005	–0.064 $\pm$ 0.012
IFLC1	0.021 $\pm$ 0.005	0.090 $\pm$ 0.004	–0.068 $\pm$ 0.011	0.063 $\pm$ 0.014
IFLC2	0.013 $\pm$ 0.002	0.138 $\pm$ 0.018	0.044 $\pm$ 0.002	0.121 $\pm$ 0.032
IFLH	0.008 $\pm$ 0.001	0.104 $\pm$ 0.026	0.111 $\pm$ 0.006	0.078 $\pm$ 0.013

¹BHBp (5–305 d) = predicted blood BHB in all parities during the period of 5 to 305 DIM; BHBp (5–65 d) = predicted blood BHB in all parities during the period of 5 to 65 DIM; BHBp (245–305 d) = predicted blood BHB in all parities during the period of 245 to 305 DIM; AFC = age at first calving (d); AFS = age at first insemination (d); ICF1 = interval from calving to first insemination in first-parity cows (d); ICF2 = interval from calving to first insemination in multiparous cows (d); IFLC1 = interval from first to last insemination of first-parity cows (d); IFLC2 = interval from first to last insemination of multiparous cows (d); IFLH = interval from first to last insemination of heifers (d).

disorders in the transition period (Grummer, 1995; Lean et al., 2013), which tend to result in increased ketosis incidence and decline in reproductive performance and longevity (Lucy, 2001; De Vries and Marcondes, 2020). Therefore, we estimated genetic parameters for predicted blood BHB using linear repeatability, multiple-trait, and random regression test-day models, and calculated genetic correlations for BHB indicators with reproductive and longevity traits in Chinese Holstein cattle.

Descriptive Statistics

The measures of predicted blood BHB in this study are different from traditional test results such as the enzymatic kinetic method (0.19–4.42 mmol/L; Oikonomou et al., 2008), in which few cows tend to have measures lower than zero. This may be associated with the low BHB content in milk (Benedet et al., 2019). A similar finding was also reported in Norwegian Red cattle, in which predicted blood BHB ranged from –4.05

to 6.32 mmol/L with a mean of 1.19 mmol/L (Belay et al., 2017). The average concentrations measured with continuous flow analyzer in Canadian Holstein cattle and spectrophotometric kinetic method in Greek Holstein cattle were 1.14 and 0.829 mmol/L, respectively (Denis-Robichaud et al., 2014; Tsiamadis et al., 2016). The average value (1.45 mmol/L) of Chinese Holstein cattle presented in this research is higher compared with these reported results. Such differences in blood BHB among populations might be due to differences in breed, production level, physiological stage, feeding management, and detection device (e.g., Foss or Bentley). In this context, it has been reported that the incidence of hyperketonemia differs across parity, lactation, and season (van der Drift et al., 2012b). In addition, the incidences of subclinical and clinical ketosis are higher in the early lactation, and their sum is over 80%. Cows with high milk yield usually have higher incidence of ketosis. In this study, the average daily milk yield is 35 $\pm$ 9 kg. Inaccurate BHB predictions could be another reason causing error classification at

Table 5. The genetic correlations between predicted blood BHB and longevity traits in Chinese Holstein cattle based on bivariate animal models¹

Trait	$h^2 \pm SE$	BHBp (5–305 d)	BHBp (5–65 d)	BHBp (245–305 d)
PL	0.034 $\pm$ 0.005	–0.126 $\pm$ 0.011	–0.168 $\pm$ 0.019	–0.134 $\pm$ 0.009
LS1	0.076 $\pm$ 0.008	–0.035 $\pm$ 0.008	–0.039 $\pm$ 0.002	–0.055 $\pm$ 0.006
LS2	0.054 $\pm$ 0.007	–0.079 $\pm$ 0.005	–0.067 $\pm$ 0.007	–0.031 $\pm$ 0.006
LS3	0.048 $\pm$ 0.004	–0.080 $\pm$ 0.009	–0.083 $\pm$ 0.010	–0.091 $\pm$ 0.019
LS4	0.046 $\pm$ 0.003	–0.083 $\pm$ 0.008	–0.056 $\pm$ 0.007	–0.101 $\pm$ 0.020
LS5	0.048 $\pm$ 0.006	–0.059 $\pm$ 0.006	–0.014 $\pm$ 0.004	–0.061 $\pm$ 0.012

¹BHBp (5–305 d) = predicted blood BHB in all parities during the period of 5 to 305 DIM; BHBp (5–65 d) = predicted blood BHB in all parities during the period of 5 to 65 DIM; BHBp (245–305 d) = predicted blood BHB in all parities during the period of 245 to 305 DIM; LS1 = first calving to the end of the first parity (d); LS2 = first calving to the end of the second parity (d); LS3 = first calving to the end of the third parity (d); LS4 = first calving to the end of the fourth parity (d); LS5 = first calving to the end of the fifth parity (d); PL = days from the first calving to culling or death (d).

different levels, which means the predicted blood BHB is more suitable to present a relative value between different cows instead of the exact level.

The trend in blood BHB along with DIM in Chinese Holstein cows is consistent with other studies in the literature where the peak BHB concentration is observed during the first 3 to 4 wk after calving (Oetzel, 2007; Koeck et al., 2014; Belay et al., 2017). Due to the variation of physiological status in a cow's lifetime, previous reports indicated that first-parity cows have lower odds of ketosis compared with cows in parity 3 to 7 (e.g., Berge and Vertenten, 2014), which is contrary to the reduction in blood BHB and lower incidence of subclinical ketosis for higher parities in this study. The proportion curve of different parity in Figure 2 revealed that older cows respond and regulate more quickly to mitigate or eliminate ketosis symptoms.

Heritability Estimates

The BHB heritability estimate in this study was low to moderate based on a linear repeatability model, ranging from 0.100 to 0.131, as reported in previous studies (van der Drift et al., 2012b; Tsiamadis et al., 2016), and higher than the values (0.01–0.10) for ketosis and hyperketonemia evaluated on a categorical scale (Van Dorp et al., 1998; Kadarmideen et al., 2000; Koeck et al., 2012; Häggman et al., 2019). It seems that continuous phenotypes, such as blood BHB concentration, are better indicators of ketosis susceptibility to be used in breeding programs. The heritability estimates were lower when compared with previous studies based on milk BHB in Korean Holstein cattle, in which the BHB heritability estimates ranged from 0.09 to 0.19 (Ranaraja et al., 2018). The population, data set structure, and statistical models used are likely the main reasons for the differences observed in the aforementioned studies. Although low heritability estimates can indicate slower genetic progress, higher estimates may be observed by using larger (and more representative) data sets obtained through other techniques such as FT-MIR spectra of milk or alternative ketosis indicators. The high and positive genetic correlation between BHB indicators across parities indicate that genetic and genomic selection for BHB1 is also expected to reduce the incidence of ketosis in later parities. The moderate repeatability estimates (0.236–0.262) observed indicate that these traits are greatly affected by the environment and repeated records are essential to improve the accuracy of breeding values.

The daily genetic heritability for BHBp are similar to those reported by Oikonomou et al. (2008) in Holstein cows. The heritability of predicted blood BHB in

Norwegian Red cattle had an upward trend from 11 to 120 d after calving (Belay et al., 2017). However, daily genetic parameters for BHB1, BHB2, and BHB3 were not found in the literature. Therefore, no direct comparisons were made. Lee et al. (2016) reported similar results for milk BHB, in which the trend of the daily additive genetic variance in first parity was similar. In this study, the third-order Legendre orthogonal polynomials were fitted because higher orders caused overfitting.

The higher heritability estimates in the early lactation stage (first 100 d after calving) may be associated with the greater variability and prevalence of metabolic challenges faced by high-producing cows during this transition period. Dairy cows need to mobilize their reserves to cope with NEB or other disturbances during the transition period (Drackley, 1999; Sundrum, 2015). Cows with higher ketosis resilience better cope with the metabolic stress at the beginning of the lactation period. Thus, genetically selecting for reduced BHB in the early lactation stage is promising. The aim of fitting random regression is to model the deviations around the phenotypic trajectories of BHB concentration. Compared with the repeatability model, the Legendre orthogonal polynomial was found to be the most adequate way of modeling changes in the trait variance over time, which considers the relationship among the measurements over time (Jamrozik et al., 1997; Oliveira et al., 2019). This approach is more consistent with the actual physiological characteristics of BHB in cows. The average daily heritability (0.101) is close to the repeatability model estimate (0.116) in BHBp. We compared the repeatability model and random regression model by calculating the Spearman correlation of the top 300-bull EBVs for the 4 BHB traits. The high Spearman correlations (0.941–0.985) indicate that the models are similar.

Genetic Correlations for BHB with Reproduction and Longevity Traits

In this study, low direct genetic correlations were observed between BHB and the 7 reproduction traits evaluated. During the period from 5 to 305 DIM, the negative relationships between BHBp with AFS (-0.084 ± 0.017), ICF1 (-0.069 ± 0.003), and ICF2 (-0.089 ± 0.011) indicate that individuals with better reproductive performance (early insemination and less time for involution of uterus) may have slightly higher BHB concentrations in the blood. This might be due to greater energy requirements to maintain high reproductive performance, whereas excessive magnitude or rate of mobilization of fat reserves will cause higher

concentration of BHB in blood. In Finnish dairy cattle, a genetic correlation of -0.17 ± 0.042 between BHB concentration and interval from calving to the first heat (similar to ICF) was also observed (Häggman et al., 2019), which corroborates with our findings. We found positive genetic correlations between BHBp and IFL for heifers (0.104 ± 0.026) and multiparous cows (0.138 ± 0.018), whereas the genetic correlation between BHBp and AFC was 0.141 ± 0.032 . These results indicate that animals with higher BHB concentration in the blood often need to be inseminated more times to get a successful pregnancy. The blood BHB concentration of nonpregnant Canadian Holstein cows after the first AI was higher than that of pregnant individuals between 3 wk before and 9 wk after calving (Walsh et al., 2007). This indicates that high concentration of blood BHB will increase the risk of pregnancy. Impaired reproduction was related to the brain's regulation of reproduction and metabolism (Rodriguez-Martinez, 2010), because the NEB hinders communication along the hypothalamic-pituitary-ovarian axis and ultimately influences the recovery of reproductive function (Butler and Smith, 1989).

During the period from 5 to 305 DIM, low genetic correlations were observed between longevity indicators and BHBp, with the highest correlation with PL (-0.126 ± 0.011) and small genetic association with the other indicators. In general, PL is more representative than other longevity traits; the negative trend of the low genetic correlation still indicated that individuals may be culled early due to the higher concentration of blood BHB. In addition to the lower milk production, other traits such as mastitis, foot and leg injury, fertility performance, and other metabolic diseases also affect the individual life span (De Vries and Marcondes, 2020). Thus, culling rate is influenced by the combination of various factors and ketosis accounts for only a small proportion of culling reasons.

The genetic correlations for BHBp with some reproductive (AFC, ICF1, IFLH) and longevity (PL) traits have slight increase at the beginning of lactation (5–65 d) compared with the whole lactation (5–305 d) results, which indicates that selecting animals with lower BHB concentration in early lactation leads to greater genetic progress in other traits. In addition, Supplemental Tables S3, S4, and S5 revealed that the 13 traits have different genetic correlations with BHB1 to BHB3 in the whole, early, and late lactation stage. Even though the estimates are small, these findings indicated that the genetic correlations change with age and lactation stage. The data size and statistical models affect the genetic correlation estimates. This result should be interpreted with caution, while providing a great reference for reproductive and longevity study in the future.

Therefore, additional studies should be performed to validate these results in larger populations.

Perspectives

With the help of an automated detection system such as FT-MIR spectroscopy, predicted blood BHB records from a large population can be obtained and used for genetic (and genomic) selection in dairy cattle breeding programs. As predicted blood BHB in early lactation has higher heritability but there are low genetic correlations for predicted blood BHB with reproductive and longevity traits, we expect that selection of the predicted blood BHB can not only provide a choice for selecting for ketosis resistance in early lactation but also be used as a complementary indicator trait in the selection index of Chinese Holstein cattle in the future. Based on the above results, these genetic correlations and heritability estimates will be validated based on clinical and subclinical ketosis records in future studies also incorporating genomic information. Furthermore, genomic analyses of predicted blood BHB will be conducted to identify genomic markers associated with ketosis resistance in Chinese Holstein cattle.

CONCLUSIONS

Predicted blood BHB traits are low to moderately heritable and repeatable (0.100 – 0.131 ; 0.236 – 0.262 , respectively), with genetic correlations across parities ranging from 0.788 to 0.911 . This indicates that genetic progress for reduced BHB concentration can be achieved through direct selection and selection for BHB1 (first parity) is expected to indirectly reduce BHB in higher lactations. The 4 BHB traits (BHBp, BHB1, BHB2, and BHB3) evaluated have similar genetic parameters throughout lactation and high heritability in early lactation, which means that selecting low BHB concentration after calving is more effective. The 4 BHB traits have low direct genetic correlations with selected reproduction and longevity traits and they change with parity and lactation stage. During the period from 5 to 65 DIM, a higher BHBp present in blood was genetically associated with older age at first calving (0.157 ± 0.019), longer IFLH (0.111 ± 0.006), and shorter production life (-0.168 ± 0.019). As more predicted BHB data accumulate, additional studies will be performed.

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