Genetic analysis of milk β -hydroxybutyrate and its association with fat-to-protein ratio, body condition score, clinical ketosis, and displaced abomasum in early first lactation of Canadian Holsteins

A. Koeck,*¹ J. Jamrozik,*[†] F. S. Schenkel,* R. K. Moore,‡ D. M. Lefebvre,‡ D. F. Kelton,§ and F. Miglior*[†]

*Centre for Genetic Improvement of Livestock, University of Guelph, Guelph, Ontario, Canada, N1G 2W1

†Canadian Dairy Network, Guelph, Ontario, Canada, N1K 1E5

‡Valacta, Sainte-Anne-de-Bellevue, Québec, Canada, H9X 3R4

§Department of Population Medicine, Ontario Veterinary College, University of Guelph, Guelph, Ontario, Canada, N1G 2W1

ABSTRACT

The aim of this study was to estimate genetic parameters for milk β -hydroxybutyrate (BHBA) in early first lactation of Canadian Holstein cows and to examine its genetic association with indicators of energy balance (fat-to-protein ratio and body condition score) and metabolic diseases (clinical ketosis and displaced abomasum). Data for milk BHBA recorded between 5 and 100 d in milk was obtained from Valacta (Sainte-Anne-de-Bellevue, Québec, Canada), the Canadian Dairy Herd Improvement organization responsible for Québec and Atlantic provinces. Test-day milk samples were analyzed by mid-infrared spectrometry using previously developed calibration equations for milk BHBA. Test-day records of fat-to-protein ratio were obtained from the routine milk recording scheme. Body condition score records were available from the routine type classification system. Data on clinical ketosis and displaced abomasum recorded by producers were available from the national dairy cattle health system in Canada. Data were analyzed using linear animal models. Heritability estimates for milk BHBA at different stages of early lactation were between 0.14 and 0.29. Genetic correlations between milk BHBA were higher between adjacent lactation intervals and decreased as intervals were further apart. Correlations between breeding values for milk BHBA and routinely evaluated traits revealed that selection for lower milk BHBA in early lactation would lead to an improvement of several health and fertility traits, including SCS, calving to first service, number of services, first service to conception, and days open. Also, lower milk BHBA was associated with a longer herd life, better conformation, and better feet and legs. A higher genetic merit for milk yield was associated with higher milk BHBA, and, therefore, a greater susceptibility to hyperketonemia. Milk BHBA

at the first test-day was moderately genetically correlated with fat-to-protein ratio (0.49), body condition score (−0.35), and clinical ketosis (0.48), whereas the genetic correlation with displaced abomasum was near zero (0.07). Milk BHBA can be routinely analyzed in milk samples at test days, and, therefore, provides a practical tool for breeding cows less susceptible to hyperketonemia.

Key words: β -hydroxybutyrate, fat-to-protein ratio, body condition score, metabolic disease

INTRODUCTION

Hyperketonemia or ketosis is one of the most frequent diseases in dairy cattle, and it is caused by a severe negative energy balance in early lactation. The decreased DMI that occurs prepartum causes negative energy balance and increases NEFA and ketone body concentrations. Essentially all dairy cows experience a period of insulin resistance, reduced feed intake, negative energy balance, hypocalcemia, reduced immune function, and bacterial contamination of the uterus shortly before or in the weeks after calving (LeBlanc, 2010; Gumen et al., 2011). Ketosis can manifest clinically as a decrease in feed intake, weight loss, and a decline in milk production, but cows are more likely to suffer from subclinical ketosis. Subclinical ketosis is characterized by increased concentrations of the ketone bodies acetoacetate, BHBA, and acetone in blood, milk, and urine, without any clinical signs. Observed incidences for subclinical ketosis range from 12 to 43% (Duffield et al., 1997; Geishauser et al., 2000; McArt et al., 2012), which are much higher than the 0.5 to 5.1% incidence rates found for clinical ketosis (van Dorp et al., 1999; LeBlanc et al., 2005; Koeck et al., 2012).

The reference test to diagnose hyperketonemia is the determination of BHBA concentration in blood (Duffield, 2000); however, because of practical limitations, this sampling procedure is not suitable for routine analysis. Alternatively, concentrations of ketone

Received May 26, 2014.

Accepted August 8, 2014.

¹Corresponding author: akoeck@uoguelph.ca

bodies in milk are highly correlated with blood levels (Denis-Robichaud et al., 2014) and can be analyzed in milk samples by mid infrared (MIR) spectrometry, as shown in earlier research by Hansen (1999) and Heuer et al. (2001). More recently, de Roos et al. (2007) presented MIR predictions for acetone and BHBA using milk samples from regular milk recording. Milk BHBA determined by MIR and acetone had a sensitivity of 70 and 69%, respectively, to detect hyperketonemia as determined by reference test. Specificity was 95% for both tests.

Since October 2011, screening for hyperketonemia based on a BHBA analysis by MIR of test-day milk samples has been offered in Canada by Valacta (Sainte-Anne-de-Bellevue, QC, Canada), the Canadian DHI organization responsible for Québec and Atlantic provinces. For the use and implementation of milk BHBA in dairy cattle breeding programs, the knowledge of genetic parameters is essential. In the literature, only one genetic study reports heritability estimates for milk BHBA (van der Drift et al., 2012). Therefore, the objectives of this study were (1) to estimate genetic parameters for milk BHBA; (2) to obtain breeding values for milk BHBA and examine relationships with routinely evaluated traits; and (3) to determine genetic correlations between milk BHBA, indicators of energy balance [fat-to-protein ratio (**F:P**) and BCS], and metabolic diseases [clinical ketosis (**KET**) and displaced abomasum (**DA**)] in Canadian Holstein cows.

MATERIALS AND METHODS

Data

Data Set 1. A total of 466,330 test-day records for milk BHBA recorded between 5 and 100 DIM from October 2011 to November 2012 was obtained from Valacta (Sainte-Anne-de-Bellevue, QC, Canada). Test-day milk samples were analyzed by using a MIR spectrometer (MilkoScan FT+, Foss, Hillerød, Denmark) with previously developed calibration equations for milk BHBA from Foss. Only first-lactation Holstein cows between

19 and 43 mo of age were considered. Milk BHBA test-day records with missing milk test-day measurements were excluded from further analyses. The final data set consisted of 129,164 BHBA test-day records from 61,331 first-lactation cows. The percentages of cows with 1, 2, 3, and 4 test-days for milk BHBA were 29.6, 33.0, 34.5, and 2.9%, respectively. As the majority of the cows had only 1 or 2 test-day records, a multiple-trait model was applied instead of a random regression model. Statistical analyses were carried out for milk BHBA at different times of early lactation: (1) 5–20 DIM, (2) 21–40 DIM, (3) 41–60 DIM, (4) 61–80 DIM, and (5) 81–100 DIM. For genetic analyses, the milk BHBA concentrations were $\log_e$ -transformed to normalize their distribution. To allow log transformation of the data, a constant of 1.00 was added to milk BHBA concentrations to prevent negative and zero values, for which $\log_e$ is not defined. A summary of statistics of the analyzed data set is given in Table 1. An animal pedigree file was generated by tracing the pedigrees of cows with records 7 generations back and contained 300,812 animals.

Data Set 2. For the subsequent analysis, test-day records of F:P and type records for BCS and health data were obtained from the Canadian Dairy Network (Guelph, ON, Canada). Body condition score was routinely recorded by professional type classifiers on a scale from 1 (very thin) to 5 (very fat) in increments of 0.25. Only first classifications within 365 DIM were used; reclassification records were not considered. Health data on incidence of KET and DA were recorded by producers on a voluntary basis according to the disease definitions described by Kelton et al. (1998). A more detailed overview about health data recording in Canada, participation, and data quality is given by Neuenschwander (2010) and Koeck et al. (2012). A minimum disease frequency (reported cases per herd and year) of 1% was applied for both diseases to ensure continuous data recording within individual herds. Data editing was applied separately for each disease because not all herds that record KET also record DA and vice versa (Neuenschwander (2010); Koeck et

Table 1. Summary statistics of data set 1¹ and heritability (h^2 ; SE in parentheses) for $\log_e$ -transformed milk BHBA (1) 5–20 DIM, (2) 21–40 DIM, (3) 41–60 DIM, (4) 61–80 DIM, and (5) 81–100 DIM (BHBA₁ to BHBA₅) from univariate analyses

Trait	DIM	Records, no.	Mean	SD	h^2
BHBA ₁	5–20	20,845	0.106	0.071	0.14 (0.02)
BHBA ₂	21–40	26,871	0.087	0.066	0.14 (0.01)
BHBA ₃	41–60	27,404	0.072	0.048	0.17 (0.02)
BHBA ₄	61–80	27,233	0.065	0.040	0.22 (0.02)
BHBA ₅	81–100	26,811	0.064	0.037	0.29 (0.02)

¹Data set 1 consisted of 129,164 BHBA test-day records from 61,331 first-lactation cows obtained from Valacta (Sainte-Anne-de-Bellevue, QC, Canada).

Table 2. Summary statistics of data set 2¹ for log_e-transformed milk BHBA at the first test-day (5–40 DIM), fat-to-protein ratio (F:P) at the first test-day (5–40 DIM), BCS, clinical ketosis (KET), and displaced abomasum (DA)

Trait	Records, no.	Mean	SD
BHBA	7,635	0.096	0.071
F:P	7,635	1.329	0.263
BCS	7,020	2.788	0.327
KET frequency, %	3,437	3.608	—
DA frequency, %	6,894	2.742	—

¹Data set 2 consisted of test-day records of F:P, type records for BCS, and health data obtained from the Canadian Dairy Network (Guelph, Ontario, Canada) merged to milk BHBA records at the first test-day (5–40 DIM) from data set 1. Only cows with information on milk BHBA and health data were considered.

al., 2012). Both KET and DA were defined as binary traits (0 = healthy, 1 = sick) based on whether or not the cow had ≥ 1 case of the respective disease recorded within the first 100 d after calving. Milk BHBA data from data set 1 were merged with data on F:P, BCS, KET, and DA, creating a new joint data set (data set 2). Only first-lactation Holstein cows with information on milk BHBA and health data (KET or DA) were considered. Analysis was carried out for milk BHBA on the first test-day (5–40 DIM), F:P on the first test-day (5–40 DIM), and BCS, KET, and DA. For milk BHBA and F:P, the first test-day was considered, as most metabolic changes and diseases occur in this period. Table 2 gives an overview of the data set 2. An animal pedigree file was generated for this data set by tracing the pedigrees of cows with records 7 generations back and contained 55,725 animals.

Models

Data were analyzed with linear animal models using the average information-restricted maximum likelihood (AI-REML) procedure in the derivative-free approach to multivariate analysis in DMU package (Madsen and Jensen, 2008).

Genetic Parameters for Milk BHBA. Univariate and bivariate linear animal models were applied to milk BHBA at 5–20, 21–40, 41–60, 61–80, and 81–100 DIM using data set 1. In matrix notation, the model was as follows:

$$\mathbf{y} = \mathbf{X}\boldsymbol{\beta} + \mathbf{Z}_h\mathbf{h} + \mathbf{Z}_a\mathbf{a} + \mathbf{e}, \quad [1]$$

where $\mathbf{y}$ is a vector of observations; $\boldsymbol{\beta}$ is a vector of systematic effects, including fixed effects of age at calving, season of calving, and DIM; $\mathbf{h}$ is a vector of random herd of calving effects; $\mathbf{a}$ is a vector of random animal additive genetic effects; $\mathbf{e}$ is a vector of random residuals; and $\mathbf{X}$, $\mathbf{Z}_h$, and $\mathbf{Z}_a$ are the corresponding

cidence matrices. Age at first calving had 16 classes, in which <22 and >35 mo were the first and last classes, respectively, and other classes were single months. Seasons were formed by combining 3 consecutive months (January–March, April–June, July–September, and October–December); DIM was defined in classes, with each day representing a single class. Random effects were assumed to be bivariate normally distributed with means equals to zero, and covariance structure in the bivariate analyses was equal to

$$\text{Var} \begin{bmatrix} \mathbf{h} \\ \mathbf{a} \\ \mathbf{e} \end{bmatrix} = \begin{bmatrix} \mathbf{H}_0 \otimes \mathbf{I} & \mathbf{0} & \mathbf{0} \\ & \mathbf{G}_0 \otimes \mathbf{A} & \mathbf{0} \\ \text{symm.} & & \mathbf{R}_0 \otimes \mathbf{I} \end{bmatrix},$$

where $\mathbf{H}_0$ is the (co)variance (2×2) matrix for herd of calving effects, $\mathbf{G}_0$ is the genetic (co)variance (2×2) matrix, $\mathbf{R}_0$ is the residual (co)variance (2×2) matrix, $\mathbf{I}$ and $\mathbf{A}$ are identity and additive relationship matrices, respectively, and $\otimes$ is the Kronecker product.

Breeding Values for Milk BHBA. Breeding values for milk BHBA at 5–20, 21–40, 41–60, 61–80, and 81–100 DIM were generated from univariate analyses using data set 1 and equation [1]. Pearson correlations between EBV of sires with at least 10 daughters for milk BHBA and official genomic breeding values for routinely evaluated traits were computed.

Associations Between Milk BHBA, F:P, BCS, KET, and DA. A 5-variate linear animal model was fitted for milk BHBA on the first test-day, F:P on the first test-day, BCS, KET, and DA using data set 2. For KET and DA, linear models were applied, although threshold models are, at least in theory, more appropriate to analyze binary traits. In a previous study on Canadian health data, Neuenschwander (2010) found that the use of threshold models did not improve the goodness of fit compared with linear models. Furthermore, genetic correlations are reported to be correct for binary traits using linear models (e.g., Negussie et al., 2008).

In matrix notation, the model fitted was as follows:

$$\mathbf{y} = \mathbf{X}\boldsymbol{\beta} + \mathbf{Z}_h\mathbf{h} + \mathbf{Z}_a\mathbf{a} + \mathbf{e}, \quad [2]$$

where $\mathbf{y}$ is a vector of observations; $\boldsymbol{\beta}$ is a vector of systematic effects, including fixed effects of age at calving for all traits, herd-round-classifier for BCS, DIM for milk BHBA and F:P, stage of lactation for BCS, and season of calving for milk BHBA, F:P, KET, and DA; $\mathbf{h}$ is a vector of random herd of calving effects for milk BHBA, F:P, KET, and DA; $\mathbf{a}$ is a vector of random animal additive genetic effects; $\mathbf{e}$ is a vector of random residuals; and $\mathbf{X}$, $\mathbf{Z}_h$, and $\mathbf{Z}_a$ are the corresponding

Figure 1. Mean milk BHBA (mmol/L) by DIM in first-lactation cows based on data set 1.

Figure 2. Proportion (%) of first-lactation cows with a positive (milk BHBA ≥ 0.20 mmol/L) test result for hyperketonemia, by DIM based on data set 1.

incidence matrices. Stage of lactation was coded in approximately 30-d intervals (1 = 0 to 30 DIM, 2 = 31 to 60 DIM, ..., 10 = 271 to 300 DIM, and 11 = 301 to 365 DIM). Classes for age at first calving, DIM, and season of calving were formed in the same way as for equation [1]. Random effects were assumed to be multivariate normally distributed with means equals to zero and covariance structure was equal to

$$\text{Var} \begin{bmatrix} \mathbf{h} \\ \mathbf{a} \\ \mathbf{e} \end{bmatrix} = \begin{bmatrix} \mathbf{H}_0 \otimes \mathbf{I} & \mathbf{0} & \mathbf{0} \\ & \mathbf{G}_0 \otimes \mathbf{A} & \mathbf{0} \\ \text{symm.} & & \mathbf{R}_0 \otimes \mathbf{I} \end{bmatrix},$$

where $\mathbf{H}_0$ is the (co)variance (4×4) matrix for herd of calving effects, $\mathbf{G}_0$ is the genetic (co)variance (5×5) matrix, $\mathbf{R}_0$ is the residual (co)variance (5×5) matrix, $\mathbf{I}$ and $\mathbf{A}$ are identity and additive relationship matrices, respectively, and $\otimes$ is the Kronecker product.

RESULTS AND DISCUSSION

Phenotypic Description

Mean milk BHBA concentration was the highest at the beginning of lactation between 5 and 40 DIM (Figure 1). The proportion of cows testing positive for

hyperketonemia, which is defined by Valacta as a milk BHBA ≥ 0.20 mmol/L, was 14% at the beginning of lactation and decreased toward zero at the end of the recording period (Figure 2).

Genetic and Phenotypic Parameters for Milk BHBA

Estimates of heritability and genetic and phenotypic correlations for milk BHBA are given in Tables 1 and 3. Heritability estimates for milk BHBA were almost identical in univariate and bivariate analyses and increased with DIM, from 0.14 to 0.29. In agreement with our results, van der Drift et al. (2012) found a heritability of 0.16 for milk BHBA in dairy cows between 5 and 60 DIM.

The genetic correlations between milk BHBA were higher between adjacent DIM intervals and decreased as intervals were further apart. Similar to genetic correlations, the estimates of phenotypic correlations were higher between adjacent intervals.

Correlations Between EBV for Milk BHBA and Routinely Evaluated Traits

Correlations of sire EBV for milk BHBA at different DIM with routinely evaluated traits are shown in Table

Table 3. Heritabilities (in bold on the diagonal) and genetic correlations (above the diagonal) with standard errors (SE) in parentheses, and phenotypic correlations (below diagonal; SE not available) for log_e-transformed milk BHBA at (1) 5–20 DIM, (2) 21–40 DIM, (3) 41–60 DIM, (4) 61–80 DIM, and (5) 81–100 DIM (BHBA₁ to BHBA₅) from bivariate analyses based on data set 1

Trait	BHBA ₁	BHBA ₂	BHBA ₃	BHBA ₄	BHBA ₅
BHBA ₁	0.14 (0.01)	0.95 (0.02)	0.84 (0.04)	0.73 (0.05)	0.64 (0.06)
BHBA ₂	0.42	0.15 (0.01)	0.99 (0.01)	0.86 (0.03)	0.77 (0.04)
BHBA ₃	0.38	0.46	0.18 (0.01)	0.98 (0.01)	0.96 (0.02)
BHBA ₄	0.29	0.41	0.44	0.24 (0.02)	0.99 (0.01)
BHBA ₅	0.25	0.31	0.45	0.47	0.28 (0.02)

Table 4. Pearson correlations between EBV of sires with at least 10 daughters for log_e-transformed milk BHBA at (1) 5–20 DIM, (2) 21–40 DIM, (3) 41–60 DIM, (4) 61–80 DIM, and (5) 81–100 DIM (BHBA₁ to BHBA₅) based on data set 1 and routinely evaluated traits (n = number of sires)

Trait	BHBA ₁ (n = 365)	BHBA ₂ (n = 432)	BHBA ₃ (n = 426)	BHBA ₄ (n = 418)	BHBA ₅ (n = 422)
Milk yield	0.20***	0.22***	0.14**	0.13**	0.06
Protein yield	−0.02	0.06	−0.03	0.03	−0.03
Fat yield	−0.01	0.01	0.01	−0.06	−0.06
SCS	0.19***	0.11*	0.09	0.07	0.02
Calving to first service	0.21***	0.11*	0.05	−0.02	−0.02
56-d nonreturn rate	−0.07	−0.06	−0.05	0.02	0.05
Number of services	0.12*	0.08	0.05	0.01	−0.05
First service to conception	0.17***	0.10*	0.06	−0.01	−0.04
Days open	0.20***	0.11*	0.05	−0.02	−0.05
Direct herd life	−0.24***	−0.21***	−0.22***	−0.18***	−0.08
Overall conformation	−0.19***	−0.25***	−0.22***	−0.16**	−0.06
Overall feet and legs	−0.11*	−0.16***	−0.13*	−0.16**	−0.07

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

4. Please note that none of the EBV were reversed in sign; thus, higher breeding values for milk BHBA, SCS, calving to first service, number of services, first service to conception, and days open are undesirable. Higher genetic merit for milk yield was associated with higher milk BHBA, and therefore a greater susceptibility to hyperketonemia. This association was expected, as genetic selection for milk production has resulted in larger negative energy balance and larger mobilization of body reserves in early lactation (Coffey et al., 2004).

Lower EBV for milk BHBA in early stages of lactation was favorably correlated with several health and fertility measures, which included SCS, calving to first service, number of services, first service to conception, and days open. Negative effects of high postpartum BHBA blood concentrations on probability of pregnancy at first service were previously reported by Walsh et al. (2007). Oikonomou et al. (2008) found moderate genetic correlations between blood BHBA and several fertility traits, with estimates ranging from −0.65 (between blood BHBA and conception rate in the first 305 d of first lactation) to 0.56 (between blood BHBA and number of inseminations per conception).

McArt et al. (2012) reported that cows with subclinical ketosis, which was defined as a blood BHBA concentration of 1.2 to 2.9 mmol/L, were 3.0 times more likely to die or be culled than nonketotic cows.

More interestingly, cows whose first positive blood BHBA concentration was 2.4 mmol/L were 56.7 times more likely to be removed from the herd than cows whose first positive blood BHBA concentration was 1.2 mmol/L. Also, in the present study, longevity (indicated as direct herd life) was favorably associated with milk BHBA EBV, indicating that cows with lower milk BHBA in early lactation stayed longer in the herd.

The overall score for conformation was favorably associated with milk BHBA EBV. A desirable correlation between milk BHBA and the overall score for feet and legs was also found, which is in agreement with earlier phenotypic studies examining lameness. Calderon and Cook (2011) found that moderate and severely lame cows had a significantly higher blood BHBA concentration than slightly lame or nonlame cows. Similar, Suthar et al. (2013) reported that a postpartum blood BHBA threshold of ≥ 1.1 mmol/L increased the odds for lameness in dairy cows 1.8 times.

Phenotypic Associations of Milk BHBA with F:P, BCS, KET, and DA

Phenotypic associations of milk BHBA at the first test-day with F:P, BCS, KET, and DA are shown in Figure 3. Cows were grouped in 3 categories according to their milk BHBA test result: negative (milk BHBA

Table 5. Heritabilities (in bold on the diagonal) and genetic correlations (above the diagonal) with standard errors (SE) in parentheses, and phenotypic correlations (below diagonal; SE not available) for log_e-transformed milk BHBA at the first test-day (5–40 DIM), fat to protein ratio (F:P) at the first test-day (5–40 DIM), BCS, clinical ketosis (KET), and displaced abomasum (DA) based on data set 2

Trait	BHBA	F:P	BCS	KET	DA
BHBA	0.12 (0.02)	0.49 (0.12)	−0.35 (0.14)	0.48 (0.35)	0.07 (0.21)
F:P	0.52	0.12 (0.02)	−0.32 (0.14)	0.56 (0.32)	0.25 (0.20)
BCS	−0.13	−0.11	0.23 (0.04)	−0.29 (0.38)	−0.39 (0.19)
KET	0.15	0.14	−0.05	0.02 (0.02)	0.86 (0.52)
DA	0.03	0.07	−0.04	0.19	0.04 (0.02)

<0.15 mmol/L), suspect ($0.15 \leq$ milk BHBA <0.20 mmol/L), or positive (milk BHBA ≥ 0.20 mmol/L). The thresholds were chosen based on definitions used by Valacta, based on Denis-Robichaud et al. (2014) and the calibration application note (Foss). In early lactation, the mean F:P from cows with a suspect or positive test result for hyperketonemia was higher compared with that from negative cows. In addition, we observed a tendency that cows with a positive BHBA test had a lower BCS throughout lactation compared with negative cows. As expected, frequency of clinical ketosis was the highest among cows testing positive for hyperketonemia (10.8%), followed by cows classified as suspect (5.4%) and negative (2.3%). We also observed a slightly higher frequency of DA among positive cows compared with negative cows (4 vs. 2.5%).

Genetic and Phenotypic Parameters for Milk BHBA, F:P, BCS, KET, and DA

Estimates of heritability and genetic and phenotypic correlations for all traits are given in Table 5. Heritability for milk BHBA on the first test day was 0.12. Heritability estimates for F:P on the first test day, BCS, KET, and DA were 0.12, 0.23, 0.02, and 0.04, respectively, and in agreement with previous studies in Canadian Holsteins (Loker et al., 2011; Jamrozik and Schaeffer, 2012; Koeck et al., 2012).

Milk BHBA was moderately genetically correlated with F:P (0.49), BCS (-0.35), and KET (0.48), whereas the genetic correlation with DA was near zero (0.07). The genetic correlation estimates involving KET and DA had large standard errors and were not statistically significant. However, the magnitude of the point estimate of the genetic correlation between milk BHBA and KET suggests that resistance to clinical ketosis could be improved if milk BHBA were included into future dairy cattle breeding programs.

Metabolic diseases (KET and DA) were highly genetically correlated (0.86). Although the estimate was not statistically significant, a similar result was obtained by Neuenschwander et al. (2012). The remaining genetic correlations ranged from -0.39 (between BCS and DA) to 0.56 (between F:P and KET). These results were in agreement with previous studies in Canadian Holsteins. Loker et al. (2012a) applied random regression models to investigate genetic relationships between F:P and BCS throughout lactation. The genetic correlations between F:P and BCS ranged from -0.08 to -0.24 , with the highest estimates being found in early lactation at 35 to 65 DIM. In a subsequent study, Loker et al. (2012b) found a moderate genetic correlation of -0.44 between BCS and metabolic diseases. Recently, F:P in early lactation was found to be moderately genetically

Figure 3. Mean fat:protein ratio, BCS, and frequency of clinical ketosis and displaced abomasum of first-lactation cows with a negative (milk BHBA <0.15 mmol/L), suspect ($0.15 \leq$ milk BHBA <0.20 mmol/L), or positive (milk BHBA ≥ 0.20 mmol/L) test result for hyperketonemia at the first test-day (5–40 DIM) based on data set 2.

correlated with clinical ketosis ($r = 0.30$) and displaced abomasum ($r = 0.26$; Koeck et al., 2013). Phenotypic correlations were low to moderate, with estimates ranging from -0.13 (between milk BHBA and BCS) to 0.52 (between milk BHBA and F:P).

CONCLUSIONS

Milk BHBA in early first lactation is a heritable trait, with heritability estimates ranging from 0.14 to 0.29 across DIM. Correlations between breeding values for milk BHBA and routinely evaluated traits revealed that selection for lower milk BHBA in the early stages of lactation would lead to an improvement of health and fertility. Also, a lower milk BHBA was genetically associated with a lower F:P, a higher BCS, and a lower frequency of clinical ketosis. Milk BHBA can be routinely analyzed in milk samples on test days, and, therefore, provide a practical tool for breeding cows with a lower susceptibility to hyperketonemia. As more data accumulate, estimation of genetic correlations is warranted to confirm the correlations found between milk BHBA and metabolic disease traits in Canadian Holsteins.

ACKNOWLEDGMENTS

All dairy producers recording health data are gratefully acknowledged. This study was funded by the DairyGen council of Canadian Dairy Network (Guelph, Ontario, Canada) and the Natural Sciences and Engineering Research Council of Canada (Ottawa, Ontario, Canada).

REFERENCES

- Calderon, D. F., and N. B. Cook. 2011. The effect of lameness on the resting behavior and metabolic status of dairy cattle during the transition period in a freestall-housed dairy herd. *J. Dairy Sci.* 94:2883–2894.
- Coffey, M. P., G. Simm, J. D. Oldham, W. G. Hill, and S. Brotherstone. 2004. Genotype and diet effects on energy balance in the first three lactations of dairy cows. *J. Dairy Sci.* 87:4318–4326.
- de Roos, A. P. W., H. J. C. M. van den Bijgaart, J. Hørlyk, and G. de Jong. 2007. Screening for subclinical ketosis in dairy cattle by Fourier transform infrared spectrometry. *J. Dairy Sci.* 90:1761–1766.
- Denis-Robichaud, J., J. Dubuc, D. Lefebvre, and L. DesCôteaux. 2014. Accuracy of milk ketone bodies from flow-injection analysis for the diagnosis of hyperketonemia in dairy cows. *J. Dairy Sci.* 97:3364–3370.
- Duffield, T. 2000. Subclinical ketosis in lactating dairy cattle. *Vet. Clin. North Am. Food Anim. Pract.* 16:231–253.
- Duffield, T. F., D. F. Kelton, K. E. Leslie, K. D. Lissimore, and J. H. Lumsden. 1997. Use of test day milk fat and milk protein to detect subclinical ketosis in dairy cattle in Ontario. *Can. Vet. J.* 38:713–718.
- Geishauser, T., K. Leslie, J. Tenhag, and A. Bashiri. 2000. Evaluation of eight cow-side ketone tests in milk for detection of subclinical ketosis in dairy cows. *J. Dairy Sci.* 83:296–299.
- Gumen, A., A. Keskin, G. Yilmazbas-Mecitoglu, E. Karakaya, and M. C. Wiltbank. 2011. Dry period management and optimization of postpartum reproductive management in dairy cattle. *Reprod. Domest. Anim.* 46:11–17.
- Hansen, P. W. 1999. Screening of dairy cows for ketosis by use of infrared spectroscopy and multivariate calibration. *J. Dairy Sci.* 82:2005–2010.
- Heuer, C., H. J. Luinge, E. T. G. Lutz, Y. H. Schukken, J. H. van der Maas, H. Wilmink, and J. P. T. M. Noordhuizen. 2001. De-termination of acetone in cow milk by Fourier transform infrared spectroscopy for the detection of subclinical ketosis. *J. Dairy Sci.* 84:575–582.
- Jamrozik, J., and L. R. Schaeffer. 2012. Test-day somatic cell score, fat to protein ratio and milk yield as indicator traits for sub-clinical mastitis in dairy cattle. *J. Anim. Breed. Genet.* 129:11–19.
- Kelton, D. F., K. D. Lissimore, and R. E. Martin. 1998. Recommendations for recording and calculating the incidence of selected clinical diseases of dairy cattle. *J. Dairy Sci.* 81:2502–2509.
- Koeck, A., F. Miglior, J. Jamrozik, D. F. Kelton, and F. S. Schenkel. 2013. Genetic associations of ketosis and displaced abomasum with milk production traits in early first lactation of Canadian Holsteins. *J. Dairy Sci.* 96:4688–4696.
- Koeck, A., F. Miglior, D. F. Kelton, and F. S. Schenkel. 2012. Health recording in Canadian Holsteins: Data and genetic parameters. *J. Dairy Sci.* 95:4099–4108.
- LeBlanc, S. 2010. Monitoring metabolic health of dairy cattle in the transition period. *J. Reprod. Dev.* 56:S29–S35.
- LeBlanc, S. J., K. E. Leslie, and T. F. Duffield. 2005. Metabolic predictors of displaced abomasum in dairy cattle. *J. Dairy Sci.* 88:159–170.
- Loker, S., C. Bastin, F. Miglior, A. Sewalem, L. R. Schaeffer, J. Jamrozik, A. Ali, and V. Osborne. 2012a. Genetic and environmental relationships between body condition score and milk production traits in Canadian Holsteins. *J. Dairy Sci.* 95:410–419.
- Loker, S., C. Bastin, F. Miglior, A. Sewalem, L. R. Schaeffer, J. Jamrozik, and V. Osborne. 2011. Short communication: Estimates of genetic parameters of body condition score in the first 3 lactations using a random regression animal model. *J. Dairy Sci.* 94:3693–3699.
- Loker, S., F. Miglior, A. Koeck, T. F. Neuenschwander, C. Bastin, J. Jamrozik, L. R. Schaeffer, and D. Kelton. 2012b. Relationships between body condition score and health traits in first-lactation Canadian Holsteins. *J. Dairy Sci.* 95:6770–6780.
- Madsen, P., and J. Jensen. 2008. An User's Guide to DMU. A package for analyzing multivariate mixed models. Version 6, release 4.7. Danish Institute of Agricultural Sciences, Tjele, Denmark.
- McArt, J. A. A., D. V. Nydam, and G. R. Oetzel. 2012. Epidemiology of subclinical ketosis in early lactation dairy cattle. *J. Dairy Sci.* 95:5056–5066.
- Negussie, E., I. Strandén, and E. A. Mäntysaari. 2008. Genetic analysis of liability to clinical mastitis, with somatic cell score and production traits using bivariate threshold-linear and linear-linear models. *Livest. Sci.* 117:52–59.
- Neuenschwander, T. F.-O. 2010. Studies on disease resistance based on producer-recorded data in Canadian Holsteins. PhD thesis. University of Guelph, Guelph, Canada.
- Neuenschwander, T. F.-O., F. Miglior, J. Jamrozik, O. Berke, D. F. Kelton, and L. R. Schaeffer. 2012. Genetic parameters for producer-recorded health data in Canadian Holstein cattle. *Animal* 6:571–578.
- Oikonomou, G., G. Arsenos, G. E. Valergakis, A. Tsiaras, D. Zygogiannis, and G. Banos. 2008. Genetic relationship of body energy and blood metabolites with reproduction in Holstein cows. *J. Dairy Sci.* 91:4323–4332.
- Suthar, V. S., J. Canelas-Raposo, A. Deniz, and W. Heuwieser. 2013. Prevalence of subclinical ketosis and relationships with postpartum diseases in European dairy cows. *J. Dairy Sci.* 96:2925–2938.
- van der Drift, S. G. A., K. J. E. van Hulzen, T. G. Teweldemedhn, R. Jorritsma, M. Nielen, and H. C. M. Heuven. 2012. Genetic and nongenetic variation in plasma and milk β -hydroxybutyrate and milk acetone concentrations of early-lactation dairy cows. *J. Dairy Sci.* 95:6781–6787.
- van Dorp, R. T., S. W. Martin, M. M. Shoukri, J. P. Noordhuizen, and J. C. Dekkers. 1999. An epidemiologic study of disease in 32 registered Holstein dairy herds in British Columbia. *Can. J. Vet. Res.* 63:185–192.
- Walsh, R. B., J. S. Walton, D. F. Kelton, S. J. LeBlanc, K. E. Leslie, and T. F. Duffield. 2007. The effect of subclinical ketosis in early lactation on reproductive performance of postpartum dairy cows. *J. Dairy Sci.* 90:2788–2796.