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Prediction of whole-genome risk for selection and management of hyperketonemia in Holstein dairy cattle

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Summary

Hyperketonemia (HYK), a common early postpartum health disorder characterized by elevated blood concentrations of β-hydroxybutyrate (BHB), affects millions of dairy cows worldwide and leads to significant economic losses and animal welfare concerns. In this study, blood concentrations of BHB were assessed for 1,453 Holstein cows using electronic handheld meters at four time points between 5 and 18 days postpartum. Incidence rates of subclinical ($1.2 \leq$ maximum BHB ≤ 2.9 mmol/L) and clinical ketosis (maximum BHB ≥ 3.0 mmol/L) were 24.0 and 2.4%, respectively. Variance components, estimated breeding values, and predicted HYK phenotypes were computed on the original, square-root, and binary scales. Heritability estimates for HYK ranged from 0.058 to 0.072 in pedigree-based analyses, as compared to estimates that ranged from 0.071 to 0.093 when pedigrees were augmented with 60,671 single nucleotide polymorphism genotypes of 959 cows and 801 male ancestors. On average, predicted HYK phenotypes from the genome-enhanced analysis ranged from 0.55 mmol/L for first-parity cows in the best contemporary group to 1.40 mmol/L for fourth-parity cows in the worst contemporary group. Genome-enhanced predictions of HYK phenotypes were more closely associated with actual phenotypes than pedigree-based predictions in five-fold cross-validation, and transforming phenotypes to reduce skewness and kurtosis also improved predictive ability. This study demonstrates the feasibility of using repeated cowside measurement of blood BHB concentration in early lactation to construct a reference population that can be used to estimate HYK breeding values for genomic selection programmes and predict HYK phenotypes for genome-guided management decisions.

KEYWORDS
genomic selection, cattle, SNP, variance component, breeding value, animal health

1 | INTRODUCTION

Energetic demands associated with the onset of lactation in high-producing dairy cows typically exceed dry matter intake (DMI) during the early postpartum period, resulting in negative energy balance (NEB) and leading to mobilization of body fat reserves. When non-esterified fatty acids (NEFA) exceed hepatic oxidative capacity, ketogenesis occurs; this results in elevated concentrations of ketone bodies in the bloodstream, which can lead to hyperketonemia (HYK), or ketosis. Cows with untreated HYK produce 5 to 7 kg/d less milk than their healthy contemporaries (McArt, Nydam, & Oetzel, 2012). In addition, cows diagnosed as HYK in the first or second week postpartum have a significantly greater risk of left displaced abomasum (Duffield, Lissemore, McBride, & Leslie, 2009; LeBlanc,

Leslie, & Duffield, 2005; McArt et al., 2012), metritis (Suthar, Canelas-Raposo, Deniz, & Heuwieser, 2013), failure to conceive at first service (McArt et al., 2012), lameness (Suthar et al., 2013), and premature culling (Gröhn, Eicker, Ducrocq, & Hertl, 1998; McArt et al., 2012). Economic losses associated with HYK were estimated by McArt, Nydam, and Overton (2015) as $375 and $256 per case for primiparous and multiparous cows, respectively. When averaged across parities, the cost per case included $117 for direct costs associated with diagnosis, therapy, labour, impaired production, reduced fertility, death, and future culling of cows with HYK, as well as $76 for indirect costs expressed through increased risk of displaced abomasum in cows with HYK, and indirect costs of $95 manifested through increased risk of metritis among cows with HYK.

Blood concentration of β-hydroxybutyrate (BHB) is the reference test for diagnosis of HYK in lactating dairy cows, where HYK is comprised of clinical ketosis (KET) for concentrations of BHB ≥ 3.0 mmol/L and subclinical ketosis (SCK) for concentrations of BHB between 1.2 and 2.9 mmol/L (Duffield et al., 2009). Because laboratory analysis of blood BHB concentration is expensive and time-consuming, electronic handheld devices can be used for cowside testing. These units can provide diagnostic sensitivity and specificity values ranging from 74 to 100%, relative to the gold standard spectrophotometric Randox assay, for detection of SCK and KET (Bach, Heuwieser, & McArt, 2016; Iwersen et al., 2013; Süss et al., 2016). Peak incidence of HYK occurs at approximately 5 days postpartum (McArt et al., 2012), and multiple tests per week during the first 3 weeks postpartum are needed to ensure high sensitivity and specificity. Lactational incidence of SCK ranges from 40 to 60% in herds that implement repeated testing schemes (Duffield et al., 2009; Simensen, Halse, & Gillund, 1990), whereas the lactational incidence of KET ranges from 2 to 15% (Duffield 2000). However, both forms of HYK lead to significant economic losses due to impaired reproduction, lost milk production, and increased risk of involuntary culling.

Genetic selection programmes based on progeny testing have provided large increases in milk yield per cow, significant improvement in udder conformation, and substantial changes in other traits that are easy and inexpensive to measure on hundreds of thousands of cows on commercial farms. However, genetic improvement of traits that are difficult and expensive to measure, such as early postpartum metabolic disorders, calfhood respiratory disease, or feed utilization efficiency, has been elusive. Genomic selection based on dense whole-genome markers (Meuwissen, Hayes, & Goddard, 2001) has revolutionized the dairy cattle breeding industry, while leading to remarkable gains in the response to selection for economically important traits (García-Ruiz et al., 2016). Relative improvements in lowly heritable heath and fertility traits, for which reliabilities (REL) of estimated breeding values (EBV) were a limiting factor in conventional progeny testing schemes, have been particularly impressive (García-Ruiz et al., 2016). Genomic selection also offers possibilities for improving novel health traits (Chesnais et al., 2016). This potentially includes susceptibility to HYK, because it is feasible to carry out multiple measurements of blood BHB concentration per week on several thousand cows. Once genotyped, these cows can form the reference population upon which population-wide genomic selection for reduced disease incidence is based. For this reason, there is renewed interest in identifying novel data sources and developing cost-effective phenotyping strategies for measures of health, fertility, physiology, behaviour, and efficiency that were infeasible and therefore largely ignored in conventional progeny testing schemes (Egger-Danner, Cole, Pryce, & Gengler, 2015). Furthermore, once the phenotypes become available, information regarding EBV for traits such as HYK can be combined with model solutions for herd-year-season, parity, and other environmental effects to facilitate genome-guided management decisions for individual animals, a practice that is analogous to personalized medicine in humans.

Therefore, the objective of this study was to investigate the feasibility of whole-genome selection and genome-guided management for reduced HYK susceptibility using a reference population comprised of genome-tested Holstein cows with phenotypes derived from repeated cowside measurement of blood BHB concentration during the first 3 weeks postpartum.

2 | MATERIALS AND METHODS

All experimental protocols were approved by the Animal Care and Use Committee of the College of Agriculture and Life Sciences at the University of Wisconsin-Madison.

2.1 | Data

Phenotypic data consisted of blood BHB concentrations (mmol/L), for 1,453 Holstein cows at the UW-Madison Emmons Blaine Dairy Cattle Research Center (Arlington, WI) and two nearby commercial dairy farms. Blood samples were collected at the time of the AM feeding two days per week, such that four measurements were available for each cow during the time period from 5 to 18 days postpartum, during the time interval from 31 October 2014 to 27 June 2016. All blood samples were tested with the Precision Xtra meter (Abbott Laboratories, Abbott Park, IL),

an electronic handheld blood glucose and ketone meter. This device has a reported sensitivity of 91% and specificity of 94% when compared with laboratory assays for BHB (Iwersen et al., 2009). Cows that were diagnosed as KET ($BHB_{MAX} \geq 3.0$ mmol/L) or SCK (1.2 mmol/L $\leq BHB_{MAX} \leq 2.9$ mmol/L) were reported to farm staff for treatment of HYK per each farm's standard operating procedure.

The maximum BHB concentration (BHB_{MAX}) for each cow across the four sample time points was considered as the HYK phenotype in subsequent analyses. The BHB_{MAX} phenotype was attractive for two reasons. First, it reflected the worst-case biological phenotype for a given cow, in terms of her HYK status over the four time points. Second, it resolved the problem of biased BHB concentrations for measurements subsequent to the treatment of a cow that was diagnosed as KET or SCK at the first, second, or third time point. To achieve a phenotypic distribution that was more nearly normal, the raw BHB concentration for each cow was transformed to provide an alternative HYK phenotype: BHB_{SQRT} = square root (BHB_{MAX}). Lastly, a binary phenotype describing the presence or absence of HYK for each cow was derived as: $BHB_{BIN} = 1$ if $BHB_{MAX} \geq 1.2$ mmol/L, which included cows diagnosed as SCK or KET, or as $BHB_{BIN} = 0$ if $BHB_{MAX} < 1.2$ mmol/L, which included cows considered as healthy throughout the time period from 5 to 18 days postpartum. Pedigrees were traced for a minimum of four generations using the Council on Dairy Cattle Breeding (Bowie, MD) database. Genotypes consisted of 60,671 single nucleotide polymorphism (SNP) markers distributed throughout the genome. Parent-progeny validation and imputation of missing genotypes were provided by the USDA-ARS Animal Genomics and Improvement Laboratory (Beltsville, MD).

2.2 | Statistical analysis

All analyses were implemented using the BLUPF90 family of programs, as written in Fortran 90/95 (Ignacy Misztal, University of Georgia, Athens). Pedigree-based and genome-enhanced analyses were carried out. The former utilized pedigree data for 1,453 phenotyped cows and 6,441 male and female ancestors. The latter utilized single-step genomic best linear unbiased prediction (ssGBLUP) methodology (Aguilar et al., 2010; Legarra, Christensen, Aguilar, & Misztal, 2014), such that the inverse of the pedigree-based relationship matrix for all animals (A^{-1}) was combined with the inverse of the genomic relationship matrix adjusted for pedigree relationships among 959 genotyped and 801 genotyped male ancestors ($G^{-1} - A_{22}^{-1}$), to obtain the inverse of a genome-enhanced relationship matrix (H^{-1}). The statistical model was as follows:

$$y_{ijk} = HYS_i + parity_j + animal_k + e_{ijk}$$

where y_{ijk} is the BHB_{MAX}, BHB_{SQRT}, or BHB_{BIN} phenotype for a given cow (1,453 observations); HYS_i is the fixed effect of herd-year-season contemporary group (ten levels, with seasons defined as January–March, April–June, July–September, or October–December, resulting in a range of 71 to 416 cows per level), $parity_j$ is the fixed effect of parity (five levels, defined as 1, 2, 3, 4, or 5+, resulting in a range of 107 to 448 cows per level), $animal_k$ is the random additive genetic effect of animal (7,894 levels), and e_{ijk} is the random residual. Variance components were estimated using average information restricted maximum likelihood (AIREML) in a linear model for BHB_{MAX} and BHB_{SQRT} and Gibbs sampling in a threshold model for BHB_{BIN}; the EBV for phenotyped cows and their ancestors were predicted conditionally on these variance components.

Five-fold cross-validation was used to assess the abilities of the pedigree-based and genome-enhanced models to predict hyperketonemia phenotypes. Cows with phenotypes were randomly split into five-folds within each HYS contemporary group, such that 80% of the observations within each HYS were used for training the model, while the remaining 20% of the observations in each HYS were used for testing the predictions. This process was repeated five times, so each cow was included in four training sets and one testing set. The five training and testing samples from within specific contemporary groups were then pooled across HYS to form the five population-wide training and testing sets. Sizes of the population-wide training sets were 1,156, 1,162, 1,164, 1,164, and 1,166 cows, whereas sizes of the corresponding testing sets were 297, 291, 289, 289, and 287 cows. The justification for sampling cows into five-folds within HYS in the initial step was to ensure that the population-wide training and testing sets would be balanced by contemporary group, which is known to be a major source of environmental variation in HYK phenotypes. This sampling strategy also provided training and testing sets that were nearly balanced by parity, another major source of environmental variation in HYK, despite different age distributions within the three herds. The number of primiparous cows per training set ranged from 344 to 355, whereas the corresponding number per testing set ranged from 83 to 94. Conversely, the number of multiparous cows ranged from 809 to 814 per training set and from 201 to 206 per testing set.

Prediction accuracy was measured as the correlation coefficient between predicted and actual BHB_{MAX} and BHB_{SQRT} phenotypes and the percentage of correctly classified BHB_{BIN} phenotypes for cows in the testing sets. It is important to note that predicted phenotypes for BHB_{MAX}, BHB_{SQRT}, and BHB_{BIN} represent the aggregate effects of model solutions for the HYS contemporary group, parity,

and additive genetic breeding value corresponding to each observation. As such, correlations between these predictions and actual HYK phenotypes should be much greater than the correlations between estimated breeding values and individual animal phenotypes that are reported in many genomic analyses. Predicted phenotypes were computed in this manner because the primary intended use of these predictions is not genetic selection, but rather the identification of cows that are genetically and environmentally predisposed to HYK and likely to benefit from intensive monitoring, targeted management, or prophylactic treatment. Threshold model solutions for BHB_{BIN} were transformed back to the original scale, and the proportion of cows in each testing set that were correctly classified for their binary phenotypes based on their aggregated contemporary group, parity, and breeding value estimates was computed, assuming the same HYK incidence rate as the corresponding training set (because the actual incidence rate in the testing set would be unknown in practice).

3 | RESULTS

The distribution of BHB_{MAX} and BHB_{SQRT} phenotypes for 1,453 phenotyped cows in the present study is shown in Figure 1. The mean and standard deviation for BHB_{MAX} were 1.04 and 0.66, respectively, as compared to 0.98 and 0.26, respectively, for BHB_{SQRT}. Phenotypes for BHB_{MAX} on the original scale included many outliers on the right side of the distribution, leading to a skewness value of 3.09, and the kurtosis value of 12.9 for BHB_{MAX} reflects the sharp peak of the distribution. Skewness and kurtosis were improved, but not eliminated, in the transformed BHB_{SQRT} phenotypes, with values of 1.98 and 5.0, respectively. As shown in Figure 1, a total of 1,070 cows (73.6%) were considered as healthy throughout the time period from 5 to 18 days postpartum ($BHB_{MAX} \leq$ 1.1 mmol/L), whereas 383 cows (26.4%) were diagnosed as HYK ($BHB_{MAX} \geq 1.2$ mmol/L). Among the latter, 348 cows (24.0%) were considered as SCK (1.2 mmol/L $\leq BHB_{MAX} \leq 2.9$ mmol/L), while 35 cows (2.4%) were considered as KET ($BHB_{MAX} \geq 3.0$ mmol/L). Means for BHB_{MAX} within HYS contemporary group ranged from 0.82 to 1.21; the range in HYS contemporary group means over time was 0.82 to 0.97 in the first herd, 1.03 to 1.21 in the second herd, and 1.00 in the third herd (which only contained one HYS). The corresponding SD of BHB_{MAX} within HYS contemporary group ranged from 0.40 to 0.80 and tended to increase as mean BHB_{MAX} increased. Mean BHB_{MAX} was greater in multiparous cows, as compared to their primiparous herdmates, with means of 0.88, 1.03, 1.17, 1.20, and 1.08 for first, second, third, fourth, and fifth and later parities, respectively. The SD of BHB_{MAX} within parity showed no obvious trend and ranged from 0.59 to 0.73.

Estimated variance components corresponding to the three HYK phenotypes are shown in Table 1. The 1,453 phenotyped cows in this study were sired by 333 different bulls. The number of phenotyped daughters ranged from 1 to 40 per sire; 41 sires had ten or more daughters, whereas 96 sires had five or more daughters. Genetic and residual variances for BHB_{MAX} were 0.024 and 0.374 in the pedigree-based analysis, resulting in a heritability estimate of 0.059. Incorporation of SNP genotypes for a large proportion of the phenotyped cows and their male ancestors led to a genome-enhanced heritability estimate of 0.074. Transformation of BHB_{MAX} phenotypes to the square-root scale resulted in increased heritability estimates for BHB_{SQRT}, which were 0.072 and 0.093 in the pedigree-based and genome-enhanced analyses, respectively. Transformation of phenotypes to the binary scale, where one group contained cows that were healthy ($BHB_{MAX} \leq 1.1$ mmol/L) and the other group contained cows that had SCK or KET ($BHB_{MAX} \geq 1.2$ mmol/L), led to heritability estimates of 0.058 and 0.071 for the pedigree-based and genome-enhanced analyses, respectively, which were nearly identical to estimates on the original BHB_{MAX} scale. Higher heritability estimates were expected in the genome-based analyses, relative to the pedigree-based analyses, because the additional information provided by SNP genotypes allows greater separation of signal from noise in the HYK phenotypes.

The EBV for HYK from the pedigree-based and genome-enhanced analyses are shown in Figures 2 and 3, respectively, where EBV of phenotyped cows and their ancestors for BHB_{MAX} are plotted against EBV for BHB_{SQRT} and BHB_{BIN}. The correlations between EBV for BHB_{MAX} and BHB_{SQRT} were 0.98 in the pedigree-based and genome-enhanced analyses. This indicates that selection decisions for the vast majority of animals, with the possible exception of those with extremely high or low EBV for BHB_{MAX}, would be affected minimally by a square-root transformation. However, correlations between EBV for BHB_{MAX} and BHB_{BIN} were 0.75 and 0.76 in the pedigree-based and genome-enhanced analyses, respectively, indicating that a binary transformation could have a significant impact on selection decisions, particularly those with extremely high or low EBV for BHB_{MAX}. Normally, one would not consider transforming a continuous trait to the binary scale for genetic or genomic analysis, due to the corresponding loss in precision of phenotypes. However, because cows that exceed the 1.2 mmol/L threshold for SCK diagnosis are subjected to treatment by herd staff immediately, the true BHB_{MAX} and BHB_{SQRT} phenotypes of many cows with HYK are unobserved. In that sense, the BHB_{BIN} phenotypes are

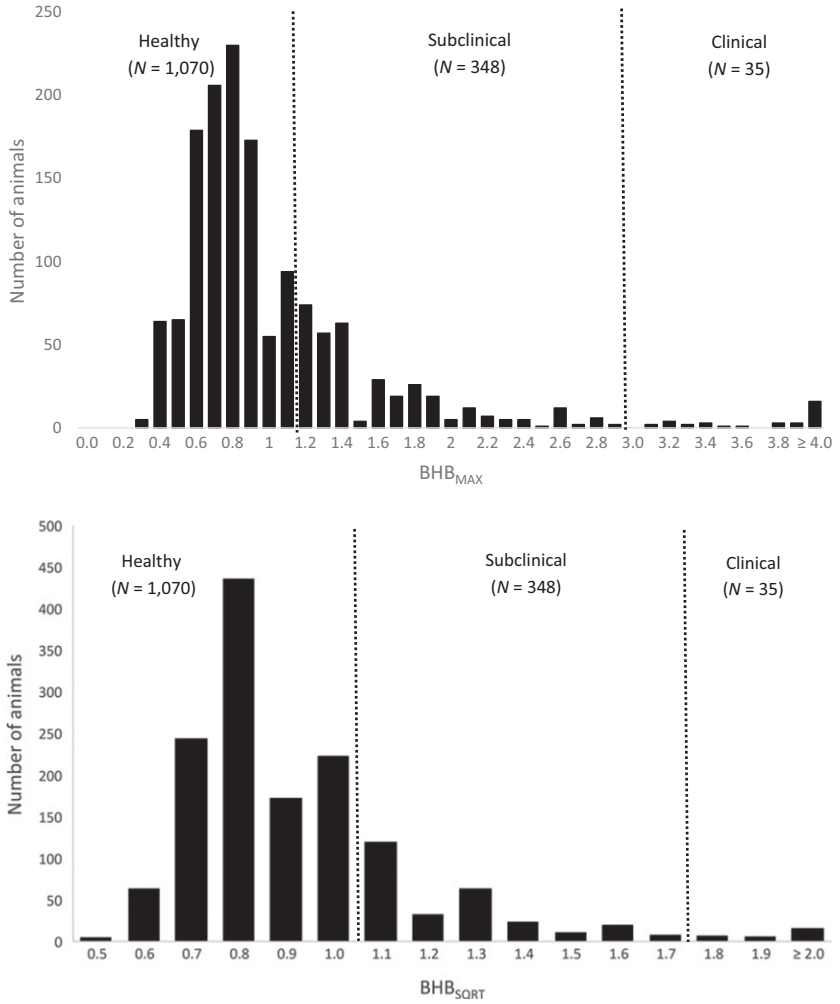

FIGURE 1 Frequency distribution of phenotypes for hyperketonemia, defined as the maximum blood concentration of β-hydroxybutyrate (BHB_{MAX}; mmol/L) from twice weekly sampling between 5 and 18 days postpartum, and its transformation to the square-root scale (BHB_{SQRT} = square root (BHB_{MAX})). Counts for BHB_{MAX} are in the top panel, whereas counts for BHB_{SQRT} are in the bottom panel

TABLE 1 Estimated genetic (σ_g^2) and residual (σ_e^2) variance components (±standard errors) and heritability (h^2) for hyperketonemia, defined as the maximum blood concentration of β-hydroxybutyrate (BHB_{MAX}; mmol/L) from twice weekly sampling between 5 and 18 days postpartum, and for its transformations to the square-root scale (BHB_{SQRT} = square root (BHB_{MAX})) and binary scale (BHB_{BIN} = 1 if $BHB_{MAX} \geq 1.2$, or BHB_{BIN} = 0 if $BHB_{MAX} < 1.2$), from the pedigree-based and genome-enhanced analyses

Phenotype	Relationship type	σ_g^2	σ_e^2	h^2
BHB_{MAX}	Pedigree	0.024 ± 0.019	0.374 ± 0.022	0.059 ± 0.045
	Pedigree + Genomic	0.029 ± 0.017	0.368 ± 0.020	0.074 ± 0.042
BHB_{SQRT}	Pedigree	0.0044 ± 0.0030	0.0567 ± 0.0035	0.072 ± 0.050
	Pedigree + Genomic	0.0056 ± 0.0028	0.0554 ± 0.0032	0.093 ± 0.045
BHB_{BIN}	Pedigree	0.0105 ± 0.0079	0.171 ± 0.0096	0.058 ± 0.043
	Pedigree + Genomic	0.0129 ± 0.0083	0.168 ± 0.0098	0.071 ± 0.044

more objective, despite being less precise. As one would expect, the ranges of EBV from the genome-enhanced analyses were greater than ranges from the corresponding pedigree-based analyses, due to slightly larger estimates of the genetic variance and slightly smaller estimates of the residual variance.

Figures 4 and 5 show the histograms of predicted BHB_{MAX} phenotypes for first- and fourth-parity cows in

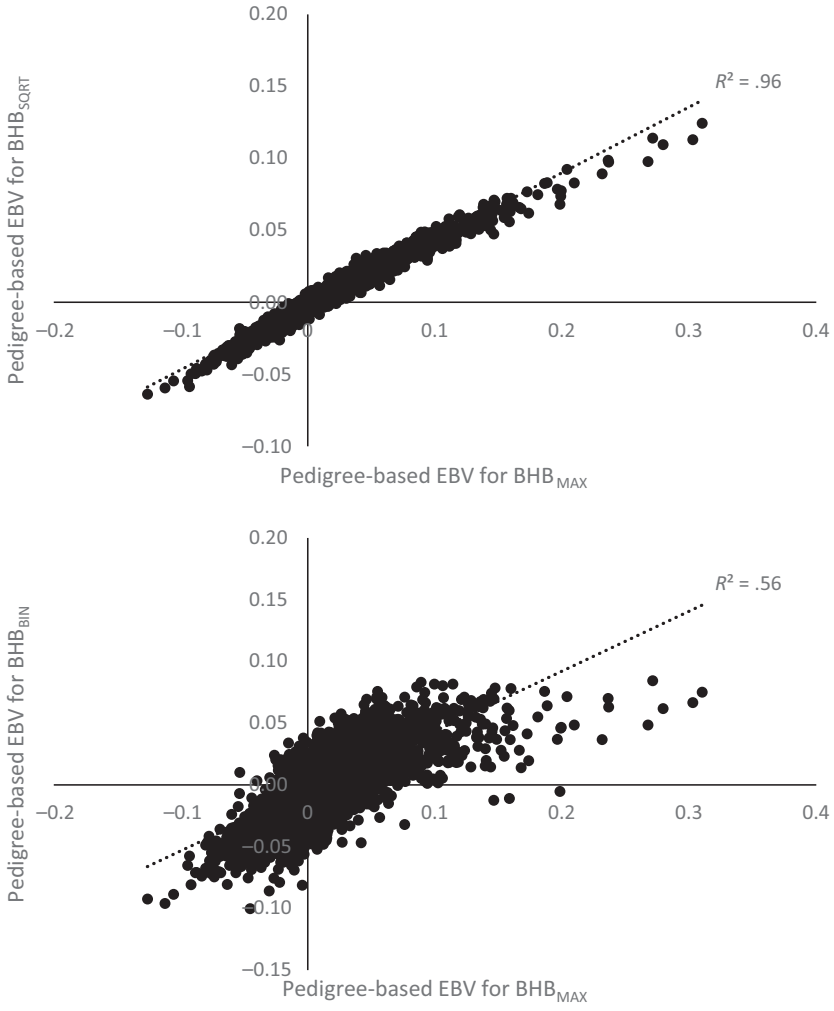

FIGURE 2 Estimated breeding values (EBV) for hyperketonemia, defined as the maximum blood concentration of β-hydroxybutyrate (BHB_{MAX}; mmol/L) from twice weekly sampling between 5 and 18 days postpartum, and its relationships with EBV when transformed to the square-root scale (BHB_{SQRT} = square root (BHB_{MAX})) and binary scale (BHB_{BIN} = 1 if $BHB_{MAX} \geq 1.2$, or BHB_{BIN} = 0 if $BHB_{MAX} < 1.2$), for 7894 phenotyped cows and their ancestors in the pedigree-based analysis. Relationships between EBV for BHB_{MAX} and BHB_{SQRT} are in the top panel, whereas relationships between EBV for BHB_{MAX} and BHB_{BIN} are in the bottom panel

the best, average, and worst HYS contemporary groups from the pedigree-based and genome-enhanced analyses, respectively. Modes of the distributions were 0.55, 0.70, and 1.00 when pedigree-based EBV were combined with solutions for first parity and the best, average, and worst HYS, respectively, whereas modes were 0.95, 1.10, and 1.40 when pedigree-based EBV were matched with solutions for fourth parity and the best, average, and worst HYS. Modes were identical when parity and HYS solutions were combined with EBV in the genome-based analysis. However, the distributions of predicted BHB_{MAX} phenotypes were wider in the genome-enhanced analysis. For example, predicted BHB_{MAX} phenotypes of first-parity cows in an average HYS ranged from 0.60 to 1.00 in the pedigree-based analysis, as compared to the range from 0.55 to 1.10 in the genome-enhanced analysis. Likewise, in the worst-case scenario, predicted BHB_{MAX} phenotypes of fourth-parity cows in the worst HYS ranged from 1.35 to 1.70 in the pedigree-based analysis and 1.25 to 1.80 in the genome-enhanced analysis. Thus, incorporating SNP information allows greater differentiation of cows that are likely to be healthy from those that are likely to suffer from HYK. However, as one would expect from the relatively low heritability estimates, management effects associated with HYS and physiological effects associated with parity appear to play a greater role than genetic predisposition when determining a cow's susceptibility to HYK.

Table 2 shows results of the five-fold cross-validation analysis of the accuracy of predicted phenotypes for BHB_{MAX}, BHB_{SQRT}, and BHB_{BIN} in the pedigree-based and genome-enhanced analyses. Correlations between predicted and actual BHB_{MAX} phenotypes for cows in the testing sets ranged from 0.24 to 0.30 in the pedigree-based analyses, and from 0.25 to 0.31 in the genome-enhanced analyses. Correlations between predicted and actual BHB_{SQRT} phenotypes ranged from 0.29 to 0.36 in the pedigree-based analyses and from 0.28 to 0.37 in the genome-enhanced analyses, indicating that a transformation to reduce skewness and kurtosis may be justified. For BHB_{BIN}, the proportion of correctly classified observations in the training sets after transforming solutions back to the original scale ranged from 0.66 to 0.72 in the pedigree-

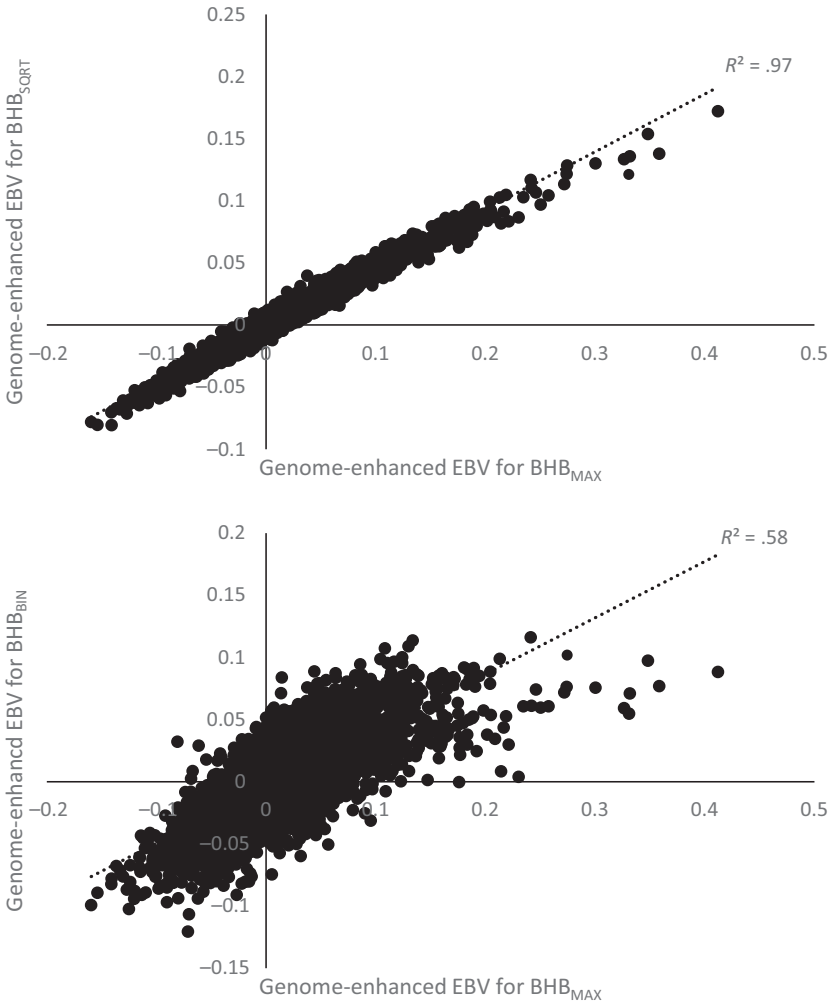

FIGURE 3 Estimated breeding values (EBV) for hyperketonemia, defined as the maximum blood concentration of β-hydroxybutyrate (BHB_{MAX}; mmol/L) from twice weekly sampling between 5 and 18 days postpartum, and its relationships with EBV when transformed to the square-root scale (BHB_{SQRT} = square root (BHB_{MAX})) and binary scale (BHB_{BIN} = 1 if $BHB_{MAX} \geq 1.2$, or BHB_{BIN} = 0 if $BHB_{MAX} < 1.2$), for 7894 phenotyped cows and their ancestors in the genome-enhanced analysis. Relationships between EBV for BHB_{MAX} and BHB_{SQRT} are in the top panel, whereas relationships between EBV for BHB_{MAX} and BHB_{BIN} are in the bottom panel

based analyses and from 0.66 to 0.71 in the genome-enhanced analyses.

4 | DISCUSSION

The 26.4% incidence rate of HYK in the present study was very similar to that of Duffield et al. (2009), who reported that 24.6 and 24.8% of Holstein cows in a sample of 25 Canadian herds had blood BHB concentrations ≥ 1.2 mmol/L in the first and second week postpartum, respectively. However, these incidence rates are considerably lower than those of McArt et al. (2012), who reported that 43.2% of cows in a sample of four New York and Wisconsin dairy herds were diagnosed with SCK, based on blood BHB concentrations of 1.2 to 2.9 mmol/L, the same criteria used in the present study. Incidence rates in the aforementioned studies, which were based on blood concentrations of BHB, far exceed reported incidence rates from studies that utilized farmer-reported incidence data regarding early postpartum health disorders. For example, Zwald, Weigel, Chang, Welper, and Clay (2004) and Vukasinovic, Bacciu, Przybyla, Boddhireddy, and DeNise (2016) reported lactational incidence rates for KET of 10 and 5%, respectively, using farmer-reported incidence data from large US dairy farms. This large difference in incidence rates can be attributed to more precise definition of HYK phenotypes in studies that utilized repeated blood BHB testing of all eligible cows during the first 3 weeks postpartum, because this leads to an objective, sensitive, and consistent HYK detection protocol that, unlike farmer-reported incidence data, is not biased by problems such as missed evaluations of eligible cows, misdiagnosed cases of HYK, subjective treatment of SCK or KET, and errant or incomplete recording of treatment events.

Genetic selection for reduced incidence of early postpartum health disorders in US dairy cattle using farmer-reported incidence data was proposed by Zwald et al. (2004), who reported a heritability estimate of 0.06 for KET that was very similar to the pedigree-based estimate in the present study. Zwald et al. (2004) reported predicted transmitting abilities (PTA) for KET that ranged from 0.063 for the best ten sires to 0.132 for the worst ten sires. Parker Gaddis, Cole, Clay, and Maltecca (2014) extended this concept to genomic selection, by combining genotypic

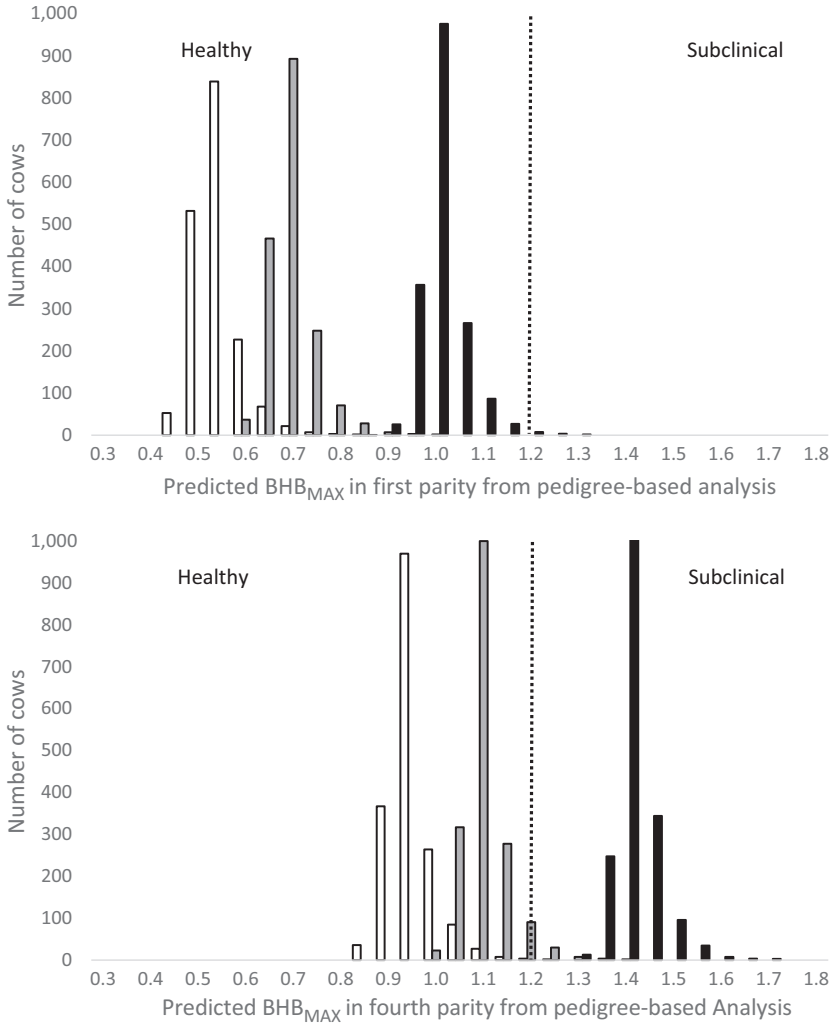

FIGURE 4 Predicted risk values for hyperketonemia, defined as the maximum blood concentration of β-hydroxybutyrate (BHB_{MAX}; mmol/L) from twice weekly sampling between 5 and 18 days postpartum, computed from solutions for herd-year-season (HYS) contemporary group, parity, and animal breeding value for 1453 phenotyped cows in the pedigree-based analysis (white = best HYS, grey = average HYS, black = worst HYS; top panel = first parity, bottom panel = fourth parity)

data of 7,900 Holstein sires with farmer-recorded incidences of clinical ketosis in their progeny, and reported a slightly higher heritability estimate of 0.09 for KET in a genome-enhanced (ssGBLUP) analysis.

Genomic predictions of US dairy cattle for the incidence of clinical ketosis were recently released by Zoetis (Kalamazoo, MI), using 1.8 million farmer-recorded health event phenotypes and 114,000 genotypes of bulls, cows, heifers, and calves in a genome-enhanced (ssGBLUP) analysis, as reported by Vukasinovic et al. (2016), who published a heritability estimate of 0.059 for KET. Mean REL of ketosis PTA for genotyped young sires with no milking progeny were 0.35, whereas mean REL for older sires with milking daughters were 0.61. One might expect heritability estimates from the present study, which ranged from 0.058 to 0.093 in the pedigree-based and genome-enhanced analyses of BHB_{MAX}, BHB_{SQRT}, and BHB_{BIN}, to exceed estimates from studies that relied on farmer-reported incidence data, due to more precise and objective definition of the HYK phenotype. However, it is important to note that the studies of Zwald et al. (2004), Parker Gaddis et al. (2014), and Vukasinovic et al. (2016) utilized data sets that were 35- to 1,200-fold larger than that of the present study.

Selection for reduced incidence of HYK can be justified easily in economic terms, given the costs of $289 per case (McArt et al., 2015), coupled with the fact that the incidence of SCK is several-fold higher than the incidence of KET (Duffield 2000). Parker Gaddis et al. (2014) noted that correlations between ketosis PTA and published PTA for daughter pregnancy rate (−0.48), length of productive life (−0.48), and somatic cell score (0.25) were highly significant, indicating the likelihood of comorbidity between ketosis and other economically important health and fertility traits. This implies that efforts to reduce the incidence of HYK through genetic or genomic selection will be synergistic with efforts to reduce the incidence of other common health disorders, as well as efforts to improve female fertility and length of productive life.

To our knowledge, this is the first study to describe the possibility of genetic selection or genome-guided management of HYK using repeated measurements of blood BHBA concentration during the first 3 weeks postpartum. For all three HYK phenotypes, predictions from the genome-enhanced analyses had greater accuracy than those from the pedigree-based analyses in the majority of folds (4 of 5 for BHB_{MAX} and BHB_{SQRT}, and 3 of 5 for

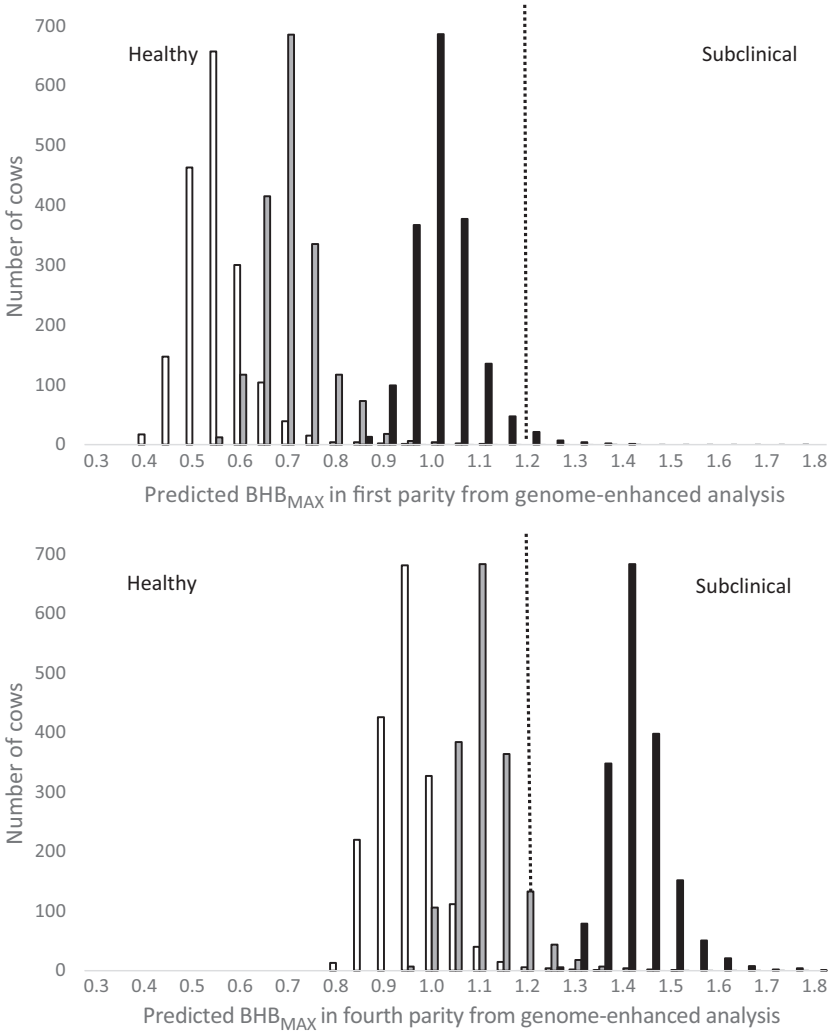

FIGURE 5 Predicted risk values for hyperketonemia, defined as the maximum blood concentration of β-hydroxybutyrate (BHB_{MAX}; mmol/L) from twice weekly sampling between 5 and 18 days postpartum, computed from solutions for herd-year-season (HYS) contemporary group, parity, and animal breeding value for 1453 phenotyped cows in the genome-enhanced analysis (white = best HYS, grey = average HYS, black = worst HYS; top panel = first parity, bottom panel = fourth parity)

TABLE 2 Correlations between predicted and actual phenotypes for hyperketonemia, defined as the maximum blood concentration of β-hydroxybutyrate (BHB_{MAX}; mmol/L) from twice weekly sampling between 5 and 18 days postpartum or its transformation to the square-root scale (BHB_{SQRT} = square root (BHB_{MAX})), as well as proportions of correctly classified observations on the binary scale (BHB_{BIN} = 1 if $BHB_{MAX} \geq 1.2$, or BHB_{BIN} = 0 if $BHB_{MAX} < 1.2$), from five-fold cross-validation applied to the pedigree-based and genome-enhanced analyses

		Cross-validation fold				
Phenotype	**Relationship type**	**1**	**2**	**3**	**4**	**5**
BHB_{MAX}	Pedigree	0.304	0.264	0.293	0.240	0.247
	Pedigree + Genomic	0.310	0.271	0.303	0.247	0.229
BHB_{SQRT}	Pedigree	0.363	0.317	0.355	0.310	0.293
	Pedigree + Genomic	0.368	0.326	0.364	0.320	0.277
BHB_{BIN}	Pedigree	0.705	0.677	0.720	0.685	0.660
	Pedigree + Genomic	0.712	0.682	0.692	0.706	0.658

BHB_{BIN}). While these results are promising, the level of prediction accuracy that was achieved using relatively small training populations of 1,156 to 1,166 cows is not yet sufficient for precise genome-guided management decisions regarding HYK. The number of animals with genotypes and phenotypes in this study falls well short of the ≥10,000 phenotyped cows typically needed to achieve a suitable selection response (Calus, de Haas, Pszczola, & Veerkamp, 2012). However, the sampling protocol described herein could be used to expand the blood BHBA reference population and keep this population up to date, such that elite young bulls and heifers comprising the pool of selection candidates are closely related to cows in HYK reference population. The latter objective could be achieved by allocating sub-elite siblings of the top young selection candidates (i.e., additional females derived from the same

in vitro fertilization matings) to dedicated phenotype farms, where HYK and a broad portfolio of other novel traits could be assessed. An additional concern, as regards routine measurement of blood BHB concentrations on commercial farms (though not on dedicated phenotype farms), is the potential for bias due to preselection of cows to be phenotyped. For example, a farmer may choose to screen cows based on body condition score, milk yield, fat-to-protein ratio, or knowledge gleaned from Fourier transform mid-infrared (FT-MIR) spectrometry of milk samples and invest in blood BHB measurements for only the subset of cows considered to be most susceptible to HYK.

The aforementioned challenge of a small HYK reference population and the potential for bias due to selective phenotyping could be addressed by development of an integrated platform for streaming direct measurements (blood BHB concentrations), subjective assessments (farmer-recorded KET events), indirect indicators (milk-based FT-MIR predictions), and correlated traits (body condition score, milk yield, fat:protein ratio) into a multiple-trait genetic evaluation system. Research activity involving the FT-MIR of milk samples collected routinely in the Dairy Herd Improvement (DHI) milk recording program has flourished in recent years, as reviewed by De Marchi, Toffanin, Cassandro, and Penasa (2014) and Bastin, Theron, Laine, and Gengler (2016), including applications that are relevant to the genetic selection and management of HYK. For example, Grelet et al. (2016) reported R^2 values of 0.63 and 0.67 for predictions of milk BHB and acetone concentrations from FT-MIR calibration equations. Earlier, de Roos, van den Bijgaart, Hørlyk, and de Jong (2007) reported correlations of approximately 0.80 between FT-MIR predictions and milk concentrations of BHB and acetone. Denis-Robichaud, Dubuc, Lefebvre, and DesCoteaux (2014) noted that milk BHB and acetone could provide sensitivities of 84 to 87% and specificities of 95 to 96% for detection of cows with blood BHB $\geq$ 1.4 mmol/L at a given time point, and the correlations of blood BHB with milk BHB (0.89) and milk acetone (0.73) far exceeded correlations with fat yield (0.21), protein yield (0.04), or fat:protein ratio (0.17). While milk-based predictions may be useful for herd-level monitoring of HYK prevalence or selection of families that tend to be less susceptible to HYK, especially when combined with other direct and correlated traits in a multiple-trait genetic evaluation system, their ability to replace direct measurements of blood BHB when making HYK treatment decisions for individual cows is questionable (Mann, Nydham, Lock, Overton, & McArt, 2016). The availability of FT-MIR spectroscopy data from monthly DHI milk samples is improving rapidly, but a major complication is that virtually all DHI milk recording programs are based on a monthly sampling schedule, so approximately half of all cows are not sampled during the period of highest risk (5 to 18 days postpartum), while the other half are sampled at only a single time point during this high-risk period. Implementation of more flexible DHI milk recording schemes on large farms, such as once or twice weekly sampling of pens containing cows that have calved recently (and, conversely, less frequent testing of cows in mid- or late lactation) would facilitate early detection and treatment of common health disorders based on milk compositional analysis.

In summary, this study demonstrates the potential for reducing the incidence of HYK in dairy cattle through genomic selection, as well as the potential for genome-guided management of HYK in the early postpartum period, where the latter represents an application of the bovine equivalent of "personalized medicine." Repeated measurement of blood BHB concentrations during the first 3 weeks postpartum is expensive and time-consuming, but establishment of a reference population of cows with blood BHB phenotypes and SNP genotypes is feasible. Data regarding actual blood BHB concentrations could be augmented with farmer-recorded incidence data regarding SCK or KET, as well as predictions of milk BHB and acetone concentrations from FT-MIR analysis of milk samples and phenotypes for correlated traits such as body condition score. Integration of these data sources would enhance the REL of selection candidates' EBV for HYK, as well as the accuracy of genome-guided management recommendations at the herd or individual cow level. In addition, predicted HYK risk values for individual cows that are derived from genomic data of cows and their contemporaries may have value in herd management troubleshooting, because veterinarians and nutritionists can more precisely evaluate the efficacy of their work if differences in genetic predisposition are considered.

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