



## Heritability estimates of predicted blood $\beta$ -hydroxybutyrate and nonesterified fatty acids and relationships with milk traits in early-lactation Holstein cows

A. Benedet, A. Costa,\* M. De Marchi, and M. Penasa

Department of Agronomy, Food, Natural resources, Animals and Environment, University of Padova, Viale dell'Università 16, 35020 Legnaro (PD), Italy

### ABSTRACT

At the beginning of lactation, high-producing cows commonly experience an unbalanced energy status that is often responsible for the onset of metabolic disorders and impaired health and performance. Blood  $\beta$ -hydroxybutyrate (BHB) and nonesterified fatty acids (NEFA) are indicators of excessive fat mobilization and circulating ketone bodies. Recently, prediction models based on mid-infrared (MIR) spectroscopy have been developed to assess blood BHB and NEFA from routinely collected individual milk samples. This study aimed to estimate genetic parameters of blood BHB and NEFA predicted from milk MIR spectra and to assess their phenotypic and genetic correlations with milk production and composition traits in early-lactation Holstein cows. The data set comprised the first test-day record within lactation and spectra of individual milk samples ( $n = 22,718$ ) of 13,106 Holstein cows collected from 5 to 35 d in milk (DIM). Blood BHB and NEFA were predicted from milk MIR spectra using previously developed prediction models. Genetic parameters of blood metabolites and milk traits were estimated for the whole observational period (5–35 DIM) and within 6 classes of DIM. Blood BHB and NEFA showed similar genetic variation across DIM, with the highest heritability in the first 10 d after calving ( $0.31 \pm 0.06$  and  $0.19 \pm 0.05$  for BHB and NEFA, respectively). The genetic correlation between BHB and NEFA was moderate ( $0.51 \pm 0.05$ ). Genetic correlations of BHB with milk yield, SCS, protein percentage, lactose percentage, and urea nitrogen content were similar to, or at least in the same direction as, the correlations of NEFA with the same traits, whereas opposite correlations were observed with fat percentage and fat-to-protein ratio. Results of the current study suggest that blood BHB and NEFA predicted from milk MIR spectra have genetic variation that is potentially exploitable for breeding

purposes. Therefore, they could be used as indicator traits of hyperketonemia in a selection index aimed to reduce the susceptibility of dairy cows to metabolic disorders in early lactation.

**Key words:** blood metabolite, infrared spectroscopy, bovine milk, genetic correlation

### INTRODUCTION

Early lactation is a critical period for dairy cows, commonly coinciding with an unbalanced energy status due to disequilibrium between energy intake (input) and increased requirements for milk production (output). In particular, the energy demand necessary to support lactogenesis at the beginning of lactation affects body reserves (Pryce et al., 2016) and leads to a negative energy balance that is often responsible for increased incidence of metabolic disorders and reproductive issues (LeBlanc, 2010; McArt et al., 2013; Suthar et al., 2013; Esposito et al., 2014). The excessive mobilization of body reserves involves the increase of circulating BHB and nonesterified fatty acids (NEFA) in dairy cows (Carvalho et al., 2019). The determination of these metabolites in blood is commonly considered the reference test to monitor cow metabolic and nutritional status. For instance, Carvalho et al. (2019) reported that NEFA concentration  $\geq 0.70$  mmol/L is a potential alert for postpartum health problems; moreover, BHB concentration  $\geq 1.2$  mmol/L is used to define hyperketonemia and has been associated with ketosis (Benedet et al., 2019b). Although blood metabolic profile testing relies on laboratory analyses, it requires blood sampling and thus it is expensive, time consuming, and invasive. To limit costs and labor, milk mid-infrared (MIR) spectroscopy has been used to develop prediction models for blood metabolites (Benedet et al., 2019a; Grelet et al., 2019; Luke et al., 2019b). Mid-infrared spectroscopy allows large-scale data collection and has been successfully implemented in the routine milk recording system to determine milk composition (De Marchi et al., 2014). Moreover, phenotypes assessed from routinely collected data could be exploited at both phenotypic and ge-

Received November 15, 2019.

Accepted February 24, 2020.

\*Corresponding author: [angela.costa@unipd.it](mailto:angela.costa@unipd.it)

netic levels. In fact, blood metabolites may be used to monitor and diagnose metabolic issues in dairy farms and could be evaluated as indicator traits in breeding programs to reduce the prevalence of ketosis (Pryce et al., 2016). For instance, in the study of Carvalho et al. (2019), blood BHB was more heritable than ketosis (0.09 to 0.37 vs. 0.02 to 0.08, respectively) and showed moderate genetic correlation with the observed disease (Belay et al., 2017b). Considering that veterinary diagnoses of ketosis are scarce in Italy, an indirect selection based on predicted blood BHB could be effective.

Few studies have estimated genetic parameters of blood BHB measured by reference methods (Oikonomou et al., 2008; van der Drift et al., 2012; Cecchinato et al., 2018) or predicted using milk MIR spectra (Belay et al., 2017b). Therefore, the present study aimed to estimate heritability of blood BHB and NEFA predicted from milk MIR spectra and to assess their genetic correlations with milk production and composition traits in the first month of lactation of Holstein cows. Moreover, genetic parameters of blood metabolites were estimated within classes of DIM to depict the trend of genetic variation of BHB and NEFA in different stages of early lactation.

## MATERIALS AND METHODS

### Data

The initial data comprised 536,685 spectra of individual milk samples of Holstein cows collected during monthly test-day recording procedures in Bolzano province (Italy) between January 2011 and December 2018. The study area is characterized by small farms, with an average herd size of 22 lactating cows present throughout the year (Zuliani et al., 2018), and traditional feeding (forage or hay and concentrates). Also, approximately 15% of herds move their animals to highland pastures in summer season. After milk collection, preservative (Bronysolv; ANA.LI.TIK Austria, Vienna, Austria) was immediately added and samples were processed according to International Committee for Animal Recording recommendations (ICAR, 2019) in the milk laboratory of the South Tyrolean Dairy Association (Sennereiverband Südtirol; Bolzano, Italy). For each milk sample, fat, protein, casein, and lactose percentages and MUN (mg/dL) were determined, and the fat-to-protein ratio ( $F/P$ ) was calculated. Spectral information containing 1,060 infrared transmittance data in the region between 5,000 and 900  $\text{cm}^{-1}$  were stored using MilkoScan FT6000 (Foss Electric A/S, Hillerød, Denmark). Values of SCC (cells/ $\mu\text{L}$ ) were determined using Fossomatic (Foss Electric A/S) and

transformed to SCS through the formula  $\text{SCS} = 3 + \log_2(\text{SCC}/100)$ .

Mid-infrared prediction models previously developed by Benedet et al. (2019a) were applied on the stored spectral data to predict blood BHB and NEFA. Briefly, between December 2017 and June 2018, 295 blood and milk samples were collected from early-lactation dairy cows in 20 herds of northeast Italy. Reference analyses were performed on blood samples for the determination of BHB and NEFA concentrations (mmol/L), and BHB values were  $\log_{10}$ -transformed to achieve normality and homogeneity of variances and to improve the accuracy of prediction (Luke et al., 2019b). Milk spectra were used to develop the prediction models through partial least squares regression after applying backward interval partial least squares algorithm. Coefficients of determination in cross-validation were 0.64 for BHB and 0.53 for NEFA.

Days in milk were restricted to be between 5 and 35, and only the first test day of each lactation of a cow was kept in the data set. Parity ranged from 1 to 10, and 47% of the cows had repeated observations across lactations (i.e., they had 1 test day in more than 1 lactation). Moreover, for each milk trait, values that exceeded 3 standard deviations ( $SD$ ) from the mean were set to missing. No restrictions were imposed to predicted BHB and NEFA to avoid discarding potential diseased animals. Herds were required to be present for at least 4 yr between 2011 and 2018 and have at least 5 cows sampled per year. After editing, 22,718 test-day records of 13,106 cows in 456 herds were available for genetic analyses.

### Estimation of Genetic Parameters

The pedigree of cows with phenotypic information was traced back to 6 generations of ancestors, ending up with 43,943 animals. Variance and covariance components of predicted blood metabolites and milk traits were estimated in ASReml 4.1 software (Gilmour et al., 2015) using univariate and bivariate repeatability animal models, respectively. The general form of the model adopted for the entire data set (5–35 DIM), in matrix notation, was

$$\mathbf{y} = \mathbf{Xb} + \mathbf{Za} + \mathbf{Ww} + \mathbf{e},$$

where  $\mathbf{y}$  is the vector of observations for blood BHB, NEFA, and milk traits;  $\mathbf{b}$  is the vector of fixed effects of parity (4 classes: 1, 2, 3, and  $\geq 4$ ), classes of DIM (6 classes: 5–10, 11–15, 16–20, 21–25, 26–30, and 31–35 d), year of sampling (2011 to 2018), season of calving (4 levels: December to February, March to May, June to

August, and September to November), and herd ( $n = 456$ );  $\mathbf{a}$  is the vector of solutions for the random additive genetic effect of the animal;  $\mathbf{w}$  is the vector of solutions for the random permanent environmental effect of the cow across lactations;  $\mathbf{e}$  is the vector of random residuals; and  $\mathbf{X}$ ,  $\mathbf{Z}$ , and  $\mathbf{W}$  are incidence matrices relating the corresponding effects to the dependent variable. All random effects were assumed to be normally distributed with zero means and variance-covariance structures of additive genetic, permanent environmental, and residual effects in the bivariate models that were  $\mathbf{G} \otimes \mathbf{A}$ ,  $\mathbf{P} \otimes \mathbf{I}$ , and  $\mathbf{R} \otimes \mathbf{I}$ , respectively, where  $\mathbf{G}$ ,  $\mathbf{P}$ , and  $\mathbf{R}$  are  $2 \times 2$  additive genetic, permanent environmental, and residual (co)variance matrices, respectively;  $\otimes$  is the Kronecker product of matrices;  $\mathbf{A}$  is the additive genetic relationship matrix; and  $\mathbf{I}$  is an identity matrix of appropriate order. The above-mentioned model, with the exclusion of the fixed effect of DIM classes, was used to estimate genetic parameters of the traits within each class of DIM.

Heritability ( $h^2$ ), repeatability ( $t$ ), phenotypic correlations ( $r_p$ ), and genetic correlations ( $r_a$ ) were calculated as

$$h^2 = \frac{\sigma_a^2}{\sigma_a^2 + \sigma_{pe}^2 + \sigma_e^2},$$

$$t = \frac{\sigma_a^2 + \sigma_{pe}^2}{\sigma_a^2 + \sigma_{pe}^2 + \sigma_e^2},$$

$$r_p = \frac{\sigma_{p12}}{\sqrt{\sigma_{p1}^2 \times \sigma_{p2}^2}},$$

and

$$r_a = \frac{\sigma_{a12}}{\sqrt{\sigma_{a1}^2 \times \sigma_{a2}^2}},$$

where  $\sigma_a^2$ ,  $\sigma_{pe}^2$ , and  $\sigma_e^2$  are the additive genetic, permanent environmental, and residual variances of the trait, respectively;  $\sigma_{p12}$  and  $\sigma_{a12}$  are the phenotypic and the additive genetic covariances estimated between trait 1 and trait 2, respectively;  $\sigma_{p1}^2$  and  $\sigma_{p2}^2$  are the phenotypic variances of traits 1 and 2, respectively; and  $\sigma_{a1}^2$  and  $\sigma_{a2}^2$  are the additive genetic variances of traits 1 and 2, respectively.

The coefficient of phenotypic variation ( $CV_p$ ) was computed for each trait as the ratio of the phenotypic SD to the mean of the trait, and the coefficient of additive genetic variation ( $CV_a$ ) was calculated as the ratio of the additive genetic SD to the mean of the trait (Houle, 1992). Sires' EBV for the investigated traits were obtained for the whole period (5–35 DIM) using variance components previously assessed through univariate models, but including a larger pedigree file ( $n = 27,557$  sires) than that used to estimate genetic parameters. Pearson correlations between sires' EBV of blood metabolites and EBV of milk traits were assessed without restrictions on reliability.

## RESULTS

### Descriptive Statistics

Concentrations of BHB and NEFA averaged  $0.66 \pm 0.24$  and  $0.41 \pm 0.21$  mmol/L, respectively, with mean values across classes of DIM depicted in Figure 1. Concerning BHB, the greatest values were from 5 to 10 DIM (0.69 mmol/L) followed by a slight decrease until 35 DIM, whereas NEFA showed a linearly decreasing trend moving from the class 5 to 10 DIM ( $0.54 \pm 0.23$  mmol/L) to the class 31 to 35 DIM ( $0.32 \pm 0.17$  mmol/L). The  $CV_p$  between 31 and 35 DIM was the greatest for NEFA and the lowest for BHB (Figure 1). Moving from 5 to 35 DIM, fat percentage, protein percentage, and SCS decreased by 15, 18, and 28%, respectively, whereas milk yield of the last DIM class (31–35 DIM) was 13% higher than milk yield of the first DIM class (5–10 DIM; Table 1). Lactose percentage and MUN content increased with DIM, and, on average, F/P did not show a clear trend, peaking in the DIM class between 21 and 25 DIM and decreasing thereafter (Table 1).

### Genetic Variation and Heritability

Although BHB exhibited lower  $CV_a$  than NEFA, it was more heritable in the first month of lactation; indeed, overall  $h^2$  from 5 to 35 DIM was  $0.21 \pm 0.02$  for BHB and  $0.14 \pm 0.02$  for NEFA (Table 2). For both metabolites the highest  $h^2$  was estimated between 5 and 10 DIM and the lowest between 11 and 15 DIM (Table 2). Moreover, the lowest  $CV_a$  for BHB (6.21%) and NEFA (9.86%) corresponded with the lowest  $h^2$  (11–15 DIM). Repeatabilities of BHB and NEFA estimated for the whole period (5–35 DIM) were  $0.26 \pm 0.01$  and  $0.21 \pm 0.01$ , respectively.

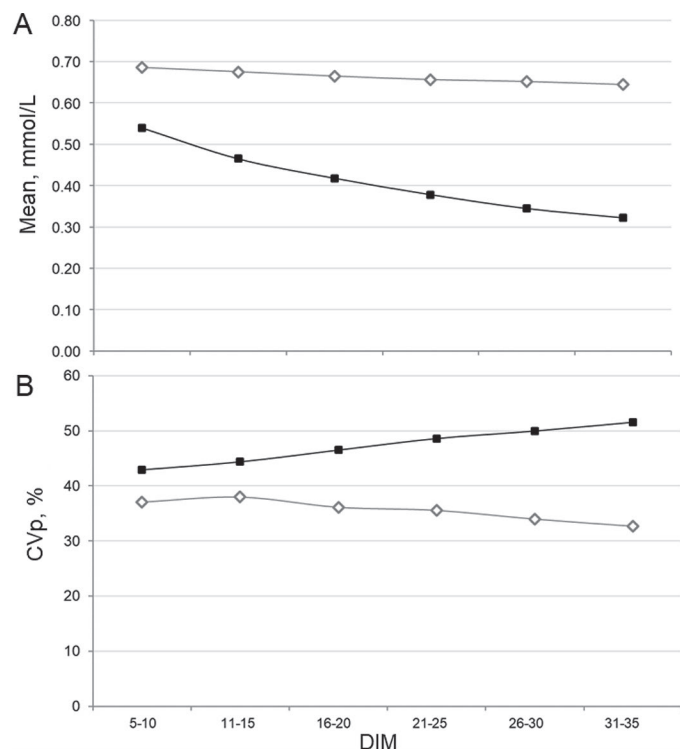
Heritability estimates of milk traits are summarized in Table 2. Focusing on the entire time window (5–35

DIM), the minimum  $h^2$  was observed for SCS ( $0.06 \pm 0.01$ ) and the maximum for lactose percentage ( $0.38 \pm 0.02$ ). On the other hand,  $CV_a$  ranged from 1.93% (lactose percentage) to 20.77% (SCS). In all DIM classes, the highest  $h^2$  were obtained for lactose percentage.

**Correlations of Blood Metabolites**

The  $r_p$  and  $r_a$  between BHB, NEFA, and milk yield and composition traits during the whole observational period (5–35 DIM) are presented in Table 3. In general,  $r_p$  of BHB with NEFA, milk yield, protein percentage, and lactose percentage were similar to  $r_a$  between BHB and the same traits. Conversely,  $r_p$  and  $r_a$  between NEFA and fat percentage ( $0.21 \pm 0.01$  and  $-0.43 \pm 0.07$ , respectively) and between NEFA and F/P ( $0.41 \pm 0.01$  and  $-0.11 \pm 0.08$ , respectively) had opposite directions. Moreover, correlations between NEFA and milk traits were negative except for  $r_p$  and  $r_a$  with milk yield ( $0.16 \pm 0.01$  and  $0.53 \pm 0.07$ , respectively).

The  $r_a$  between BHB and NEFA estimated within each DIM class are summarized in Table 4. The strongest ( $0.78 \pm 0.06$ ) and weakest ( $0.18 \pm 0.26$ )  $r_a$  were assessed from 5 to 10 DIM and 11 to 15 DIM, respectively. The  $r_a$  of BHB and NEFA with milk yield



**Figure 1.** Mean (A) and coefficient of phenotypic variation ( $CV_p$ ; B) of infrared-predicted blood BHB (◇) and nonesterified fatty acids (■) across classes of DIM.

**Table 1.** Mean  $\pm$  SD of infrared-predicted blood metabolites, milk yield, and quality traits across DIM

Trait	DIM						
	5-35 (whole)	5-10	11-15	16-20	21-25	26-30	31-35
Records, no.	22,718	3,456	3,681	3,859	3,918	4,015	3,789
BHB, mmol/L	0.66 $\pm$ 0.24	0.69 $\pm$ 0.25	0.68 $\pm$ 0.26	0.66 $\pm$ 0.24	0.66 $\pm$ 0.23	0.65 $\pm$ 0.22	0.64 $\pm$ 0.21
NEFA, <sup>1</sup> mmol/L	0.41 $\pm$ 0.21	0.54 $\pm$ 0.23	0.47 $\pm$ 0.21	0.42 $\pm$ 0.19	0.38 $\pm$ 0.18	0.34 $\pm$ 0.17	0.32 $\pm$ 0.17
Milk yield, kg/d	34.07 $\pm$ 7.51	31.30 $\pm$ 6.83	32.95 $\pm$ 7.15	34.14 $\pm$ 7.32	34.96 $\pm$ 7.55	35.27 $\pm$ 7.70	35.40 $\pm$ 7.54
Fat, %	4.11 $\pm$ 0.77	4.53 $\pm$ 0.78	4.28 $\pm$ 0.75	4.12 $\pm$ 0.74	4.02 $\pm$ 0.73	3.91 $\pm$ 0.70	3.85 $\pm$ 0.69
Protein, %	3.16 $\pm$ 0.35	3.61 $\pm$ 0.31	3.30 $\pm$ 0.28	3.13 $\pm$ 0.26	3.04 $\pm$ 0.25	2.99 $\pm$ 0.26	2.95 $\pm$ 0.26
Fat-to-protein ratio	1.31 $\pm$ 0.24	1.26 $\pm$ 0.23	1.30 $\pm$ 0.24	1.32 $\pm$ 0.25	1.33 $\pm$ 0.25	1.32 $\pm$ 0.25	1.31 $\pm$ 0.25
Lactose, %	4.78 $\pm$ 0.17	4.65 $\pm$ 0.16	4.76 $\pm$ 0.16	4.81 $\pm$ 0.15	4.82 $\pm$ 0.16	4.83 $\pm$ 0.16	4.83 $\pm$ 0.16
MUN, mg/dL	16.96 $\pm$ 6.53	15.91 $\pm$ 6.63	16.61 $\pm$ 6.38	16.76 $\pm$ 6.39	16.87 $\pm$ 6.53	17.44 $\pm$ 6.55	18.03 $\pm$ 6.48
SCS	2.20 $\pm$ 1.90	2.71 $\pm$ 1.77	2.36 $\pm$ 1.84	2.14 $\pm$ 1.87	2.05 $\pm$ 1.91	2.02 $\pm$ 1.93	1.94 $\pm$ 1.95

<sup>1</sup>Nonesterified fatty acids.

**Table 2.** Heritability ( $h^2$ ; SE in parentheses) and coefficient of additive genetic variation ( $CV_{as}$ , %) of infrared-predicted  $\log_{10}$ -transformed blood BHB, blood nonesterified fatty acids (NEFA), milk yield, and quality traits across DIM

Trait	DIM													
	5-35 (whole)		5-10		11-15		16-20		21-25		26-30		31-35	
	$h^2$	$CV_a$	$h^2$	$CV_a$	$h^2$	$CV_a$	$h^2$	$CV_a$	$h^2$	$CV_a$	$h^2$	$CV_a$	$h^2$	$CV_a$
BHB	0.21 (0.02)	7.53	0.30 (0.06)	9.99	0.13 (0.04)	6.21	0.17 (0.04)	6.89	0.24 (0.04)	7.80	0.22 (0.04)	7.13	0.20 (0.04)	6.42
NEFA, mmol/L	0.14 (0.02)	16.11	0.19 (0.05)	17.37	0.06 (0.03)	9.86	0.16 (0.04)	16.90	0.17 (0.03)	17.74	0.09 (0.04)	13.68	0.06 (0.03)	11.63
Milk yield, kg/d	0.09 (0.01)	5.11	0.05 (0.03)	3.76	0.09 (0.04)	5.26	0.09 (0.03)	4.76	0.06 (0.03)	4.23	0.05 (0.03)	3.83	0.11 (0.04)	5.42
Fat, %	0.17 (0.02)	6.65	0.16 (0.04)	6.33	0.13 (0.06)	5.82	0.17 (0.04)	6.64	0.13 (0.04)	5.84	0.13 (0.04)	5.72	0.08 (0.03)	4.49
Protein, %	0.23 (0.02)	3.98	0.21 (0.05)	3.74	0.26 (0.05)	4.17	0.20 (0.04)	3.52	0.33 (0.05)	4.54	0.21 (0.04)	3.80	0.36 (0.04)	4.96
Fat-to-protein ratio	0.12 (0.01)	5.83	0.12 (0.04)	5.91	0.08 (0.04)	4.67	0.14 (0.04)	6.44	0.11 (0.04)	5.69	0.08 (0.03)	4.75	0.11 (0.04)	5.70
Lactose, %	0.38 (0.02)	1.93	0.23 (0.05)	1.59	0.32 (0.05)	1.75	0.40 (0.06)	1.87	0.43 (0.05)	2.03	0.37 (0.05)	1.82	0.39 (0.05)	1.88
MUN, mg/dL	0.10 (0.01)	11.09	0.15 (0.04)	14.32	0.08 (0.03)	9.93	0.14 (0.04)	12.74	0.11 (0.04)	11.69	0.15 (0.04)	12.92	0.03 (0.02)	5.13
SCS	0.06 (0.01)	20.77	0.09 (0.04)	19.30	0.09 (0.03)	22.56	0.08 (0.03)	24.39	0.07 (0.03)	22.97	0.10 (0.04)	29.70	0.03 (0.03)	15.45

**Table 3.** Phenotypic (above diagonal) and genetic (below diagonal) correlations (SE in parentheses) between infrared-predicted  $\log_{10}$ -transformed blood BHB, blood nonesterified fatty acids (NEFA), milk yield, and quality traits (5-35 DIM)

Trait	BHB	NEFA	Milk yield	Fat	Protein	Fat-to-protein ratio	Lactose	MUN	SCS
BHB	—								
NEFA, mmol/L	0.51 (0.05)	0.63 (0.01)	0.13 (0.01)	0.34 (0.01)	-0.34 (0.01)	0.48 (0.01)	-0.27 (0.01)	-0.03 (0.01)	-0.01 (0.01)
Milk yield, kg/d	0.22 (0.08)	—	0.16 (0.01)	0.21 (0.01)	-0.44 (0.01)	0.41 (0.01)	-0.26 (0.01)	-0.16 (0.01)	0.02 (0.01)
Fat, %	0.04 (0.06)	-0.43 (0.07)	—	-0.10 (0.01)	-0.24 (0.01)	0.01 (0.01)	-0.01 (0.01)	0.03 (0.01)	-0.07 (0.01)
Protein, %	-0.27 (0.05)	-0.54 (0.05)	-0.39 (0.08)	—	0.16 (0.01)	0.87 (0.01)	-0.12 (0.01)	0.04 (0.01)	0.05 (0.01)
Fat-to-protein ratio	0.25 (0.06)	-0.11 (0.08)	-0.46 (0.07)	0.51 (0.05)	—	-0.33 (0.01)	0.07 (0.01)	-0.02 (0.01)	0.03 (0.01)
Lactose, %	-0.30 (0.05)	-0.33 (0.05)	-0.10 (0.09)	0.80 (0.03)	-0.11 (0.07)	—	-0.15 (0.01)	0.04 (0.01)	0.02 (0.01)
MUN, mg/dL	-0.27 (0.07)	-0.28 (0.08)	-0.31 (0.07)	0.05 (0.05)	0.23 (0.05)	-0.12 (0.06)	—	0.03 (0.01)	-0.14 (0.01)
SCS	0.03 (0.09)	0.31 (0.10)	0.08 (0.11)	0.21 (0.08)	0.14 (0.07)	0.13 (0.09)	-0.14 (0.06)	—	-0.04 (0.01)
				-0.07 (0.09)	-0.13 (0.08)	0.01 (0.10)	-0.09 (0.08)	-0.04 (0.10)	—

and composition traits in the different DIM classes are depicted in Figure 2. The pattern of  $r_a$  between BHB and milk yield fluctuated across DIM classes, whereas a decrease was observed between NEFA and milk yield. The  $r_a$  of BHB and NEFA with fat percentage had an erratic trend, with a peak between 16 and 25 DIM. Moreover, the  $r_a$  between NEFA and fat percentage were negative and moderate between 26 and 35 DIM. The patterns of  $r_a$  between BHB and protein percentage was almost identical to the pattern of  $r_a$  between NEFA and protein percentage. Also, the trend of  $r_a$  between BHB and F/P resembled that between NEFA and F/P, but overall, the  $r_a$  between BHB and F/P were positive whereas they fluctuated from positive to negative between NEFA and F/P. Both BHB and NEFA were negatively genetically associated with lactose percentage across DIM; however, the pattern of  $r_a$  with NEFA was more flat. The  $r_a$  between BHB and MUN ranged from  $-0.43 \pm 0.23$  (11–15 DIM) to  $0.12 \pm 0.35$  (31–35 DIM), and the  $r_a$  between NEFA and MUN ranged from  $-0.62 \pm 0.18$  (26–30 DIM) to  $0.15 \pm 0.50$  (31–35 DIM). Regarding SCS, a more erratic trend of  $r_a$  was observed for BHB compared with NEFA; indeed, estimates for BHB varied from  $-0.26$  to  $0.35$  within the observing period, whereas they were positive for NEFA, except for the negative association in the last DIM class (31–35 DIM).

Pearson correlation between sires' EBV of blood NEFA and BHB was moderate ( $0.52$ ;  $P < 0.001$ ), even when only sires with reliability  $\geq 0.50$  ( $n = 218$ ) were selected ( $0.52$ ;  $P < 0.001$ ). Figure 3 depicts Pearson correlations between EBV of blood metabolites and EBV of milk yield, composition traits, MUN, and SCS. The strongest association was between NEFA and lactose percentage ( $-0.49$ ;  $P < 0.001$ ) and the weakest between BHB and SCS ( $0.03$ ;  $P < 0.001$ ). Milk urea nitrogen, protein percentage, and lactose percentage were negatively correlated with NEFA and BHB, whereas the relationships of NEFA and BHB with milk yield, fat percentage, F/P, and SCS were positive (Figure 3).

## DISCUSSION

The objective of the present study was to estimate genetic parameters of blood BHB and NEFA predicted using MIR spectroscopy in a large data set of early-lactation Holstein cows. The coefficients of determination of prediction models were  $0.64$  for BHB and  $0.53$  for NEFA (Benedet et al., 2019a). Such models do not allow precise determination of blood metabolites, but they can be considered sufficiently accurate for screening purposes and for phenotypic and genetic investigations at population level (Belay et al., 2017a,b; Visentin et al., 2017; Wang and Bovenhuis, 2019).

## Descriptive Statistics

The current study focused on early-lactation cows, and a decreasing concentration of blood BHB and NEFA across DIM was somehow expected (Carvalho et al., 2019). However, mean BHB was generally lower and exhibited a smaller decrease across DIM compared with previous studies in Holstein (van der Drift et al., 2012) and Norwegian Red (Belay et al., 2017a) cows. The BHB trend was more similar to that observed by Oikonomou et al. (2008) in primiparous Holsteins. Conversely, NEFA concentrations agreed with results observed by Mäntysaari et al. (2019) in the first 3 wk of lactation of Nordic Red cows.

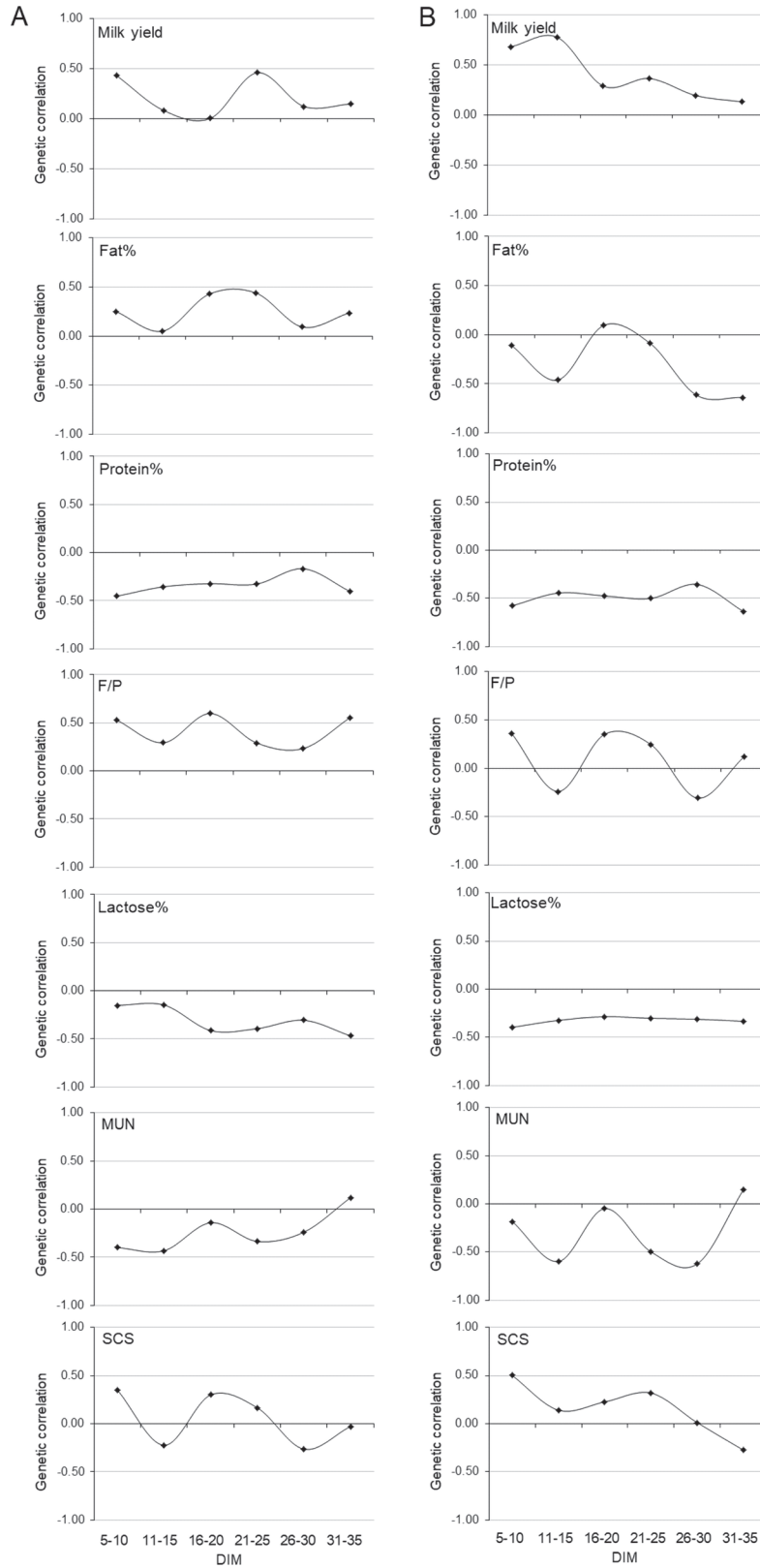
The decrease of fat and protein percentages and the increase of milk yield and lactose percentage from 5 to 35 DIM were also reported in other studies (Miglior et al., 2006; Abdullahpour et al., 2013; Haile-Mariam and Pryce, 2017). The trend for F/P was similar to that observed in Canadian Holsteins by Koeck et al. (2014). Moreover, our results for fat, protein, lactose, MUN, and F/P agreed with those of Ederer et al. (2014) at first test day in early-lactation (8–49 DIM) Austrian Fleckvieh cows.

## Genetic Variance

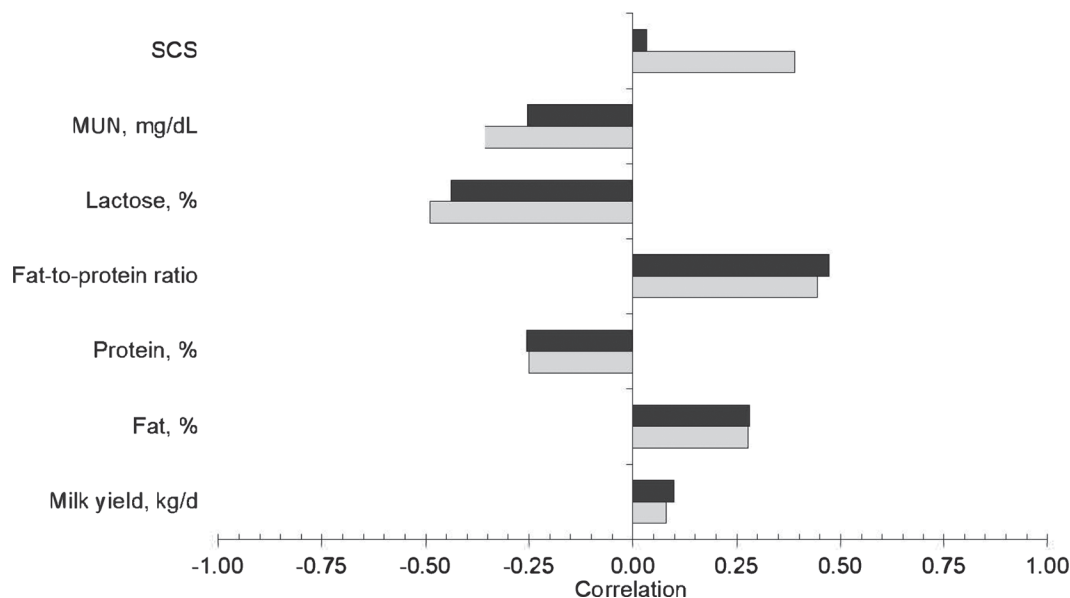
Overall,  $h^2$  estimates of BHB and NEFA were consistent with those reported by Hammami et al. (2017) and obtained from MIR predictions in Holstein cows. Despite this, lower  $h^2$  for BHB and higher  $h^2$  for NEFA have been recently observed in Australian early-lactation cows (Luke et al., 2019a). In agreement with the literature (Oikonomou et al., 2008), the highest  $h^2$  for BHB and NEFA were estimated from 5 to 10 DIM. Then,  $h^2$  of both metabolites slightly decreased in the subsequent weeks in the current study. Repeatabilities of BHB and NEFA were low; overall, this was expected because we estimated across-lactation repeatability. Indeed, the concentrations of BHB and NEFA in blood and milk and the occurrence of hyperketonemia tend to increase with parity (Santschi et al., 2016; Benedet et al., 2019b).

**Table 4.** Genetic correlation (SE in parentheses) between infrared-predicted  $\log_{10}$ -transformed blood BHB and blood nonesterified fatty acids across DIM

DIM	Genetic correlation
5–10	0.78 (0.06)
11–15	0.18 (0.26)
16–20	0.43 (0.14)
21–25	0.63 (0.11)
26–30	0.33 (0.17)
31–35	0.44 (0.19)



**Figure 2.** Genetic correlations of infrared-predicted  $\log_{10}$ -transformed blood BHB (A) and blood nonesterified fatty acids (B) with milk traits across classes of DIM. F/P = fat-to-protein ratio. Standard errors of genetic correlations ranged from 0.10 to 0.35 for  $\log_{10}$ -transformed blood BHB and from 0.11 to 0.50 for blood nonesterified fatty acids.



**Figure 3.** Pearson correlations of sires' EBV ( $n = 27,557$ ) of infrared-predicted  $\log_{10}$ -transformed blood BHB (black bars) and nonesterified fatty acids (mmol/L; gray bars) with milk traits. All correlations were significant at  $P < 0.001$ .

The patterns of  $h^2$  for milk yield and fat and protein percentages were similar to those assessed for Iranian Holsteins by Abdollahpour et al. (2013). Heritability of lactose percentage increased along DIM, as reported by Haile-Mariam and Pryce (2017). In general,  $h^2$  of F/P, MUN, and SCS estimated for the whole period (5–35 DIM) agreed with previous findings in Holstein (Negussie et al., 2008; Koeck et al., 2014; Hammami et al., 2017) and Austrian Fleckvieh (Ederer et al., 2014) cows.

### Correlations

The overall positive correlations of BHB and NEFA with milk yield (Table 3; Figures 2 and 3) indicated that the best individuals for milk yield were those with offspring exhibiting on average greater blood BHB and NEFA in the first 35 DIM, supporting the idea that high-producing cows are more susceptible to metabolic disorders than low-producing animals and that selection for milk yield may have detrimental effects on cows' metabolic status. This also supports the general idea that, in dairy cattle, genetic selection only focusing on milk production has detrimental effects on health and fitness across generations (Stefani et al., 2018; van der Werf et al., 2019), especially in early lactation. In fact, high-producing cows are subjected to homeorhesis, meaning that all metabolic pathways are intended to milk synthesis in the mammary gland (Bauman and Currie, 1980; Costa et al., 2019b). Therefore, the greater the energy requirements for milk synthesis, the greater

the circulating blood NEFA and ketone bodies due to mobilization of fat reserves (Carvalho et al., 2019). The  $r_a$  between BHB and SCS fluctuated from positive to negative within the observing period, and it was almost zero as a whole. However, NEFA were positively genetically associated with SCS in the first 35 DIM (Table 3; Figure 3), suggesting that there may be an indirect (desired) selection for udder health by selecting on resistance to metabolic diseases. In fact, several studies estimated a positive correlation between ketosis and mastitis (Pfeiffer et al., 2015; Pryce et al., 2016; Costa et al., 2019a). However, it is worth highlighting that the relationship of SCS with blood BHB and NEFA in mid and late lactation was not investigated in the current study and may exhibit different directions than in early lactation. As expected, blood BHB and NEFA negatively correlated with lactose percentage (Table 3; Figures 2 and 3), which in turn was negatively genetically related to ketosis in an earlier study (Costa et al., 2019a). The opposite  $r_a$  of fat percentage with NEFA and BHB across DIM (Figure 2) suggested different genetic dependencies of the 2 blood metabolites with this trait in early lactation ( $\leq 35$  DIM). In particular, the difference can be explained by the change of fat synthesis during and after lipomobilization when the peaks of NEFA and BHB occur, respectively. The negative  $r_a$  between BHB and protein percentage confirmed recent findings (Belay et al., 2017b), whereas the negative  $r_p$  between NEFA and protein percentage was in contrast with the estimate (0.12) obtained in Nordic Red cows (Mäntysaari et al., 2019). According to the selection



index theory, our findings support the use of F/P as an indicator of ketosis resistance; in fact, F/P showed enough genetic variation and genetic association with the objective trait to be a potential valid candidate to select against ketosis (Klein et al., 2019). On the contrary, an unexpected negative  $r_a$  between NEFA and F/P from 11 to 15 DIM and from 26 to 30 DIM was observed. These 2 negative peaks might reflect the negative association of NEFA with fat percentage in the same classes of DIM.

The nonlinear trend of  $r_a$  between BHB and NEFA across DIM classes (Table 4) generally reflects the  $h^2$  patterns of the 2 metabolites in the first month of lactation (Table 2). In fact, between 11 and 15 DIM, both metabolites exhibited the lowest  $CV_a$  and  $h^2$  as well as the lowest  $r_a$  between them. The low genetic variation observed between 11 and 15 DIM may suggest that the potential of genetics in reducing susceptibility to ketosis in the Italian Holstein population is not constant, fluctuating in the first 35 DIM. To our knowledge, this is the first study that estimated  $r_a$  of BHB and NEFA with milk traits specifically in the first month of lactation. Thus, the comparison with the literature could be misleading.

## CONCLUSIONS

In this study we estimated  $h^2$  of blood BHB and NEFA predicted from milk MIR spectra as well as their genetic correlations with milk production and composition traits in the first 35 DIM of Italian Holstein cows. The greatest blood concentration and  $h^2$  of BHB and NEFA were observed in the first 10 d after calving. Genetic correlation between blood BHB and NEFA was moderate, suggesting that both traits should be taken into account if selection against metabolic issues is pursued. Blood BHB and NEFA were moderately positively correlated with milk yield and SCS. On average, genetic correlations of BHB and NEFA with MUN content, protein percentage, and lactose percentage were comparable. Data on blood BHB and NEFA concentrations predicted from MIR spectra during routine milk recording may be particularly useful in countries where veterinarian diagnosis is not available on a large scale and selection strategies against ketosis are of interest.

## ACKNOWLEDGMENTS




The authors thank the South Tyrolean Dairy Association (Bolzano, Italy) and the Italian Holstein and Jersey Association (ANAFIJ, Cremona, Italy) for providing data used in the present study. The authors have not stated any conflicts of interest.

## REFERENCES

- Abdollahpour, R., M. M. Shahrabak, A. Nejati-Javaremi, R. Vaez Torshizi, and R. Mrode. 2013. Genetic analysis of milk yield, fat and protein content in Holstein dairy cows in Iran: Legendre polynomials random regression model applied. *Arch. Tierzucht* 6:497–508. <https://doi.org/10.7482/0003-9438-56-048>.
- Bauman, D. E., and W. B. Currie. 1980. Partitioning of nutrients during pregnancy and lactation: Review of mechanisms involving homeostasis and homeorhesis. *J. Dairy Sci.* 63:1514–1529. [https://doi.org/10.3168/jds.s0022-0302\(80\)83111-0](https://doi.org/10.3168/jds.s0022-0302(80)83111-0).
- Belay, T. K., B. S. Dagnachew, Z. M. Kowalski, and T. Ådnøy. 2017a. An attempt at predicting blood  $\beta$ -hydroxybutyrate from Fourier-transform mid-infrared spectra of milk using multivariate mixed models in Polish dairy cattle. *J. Dairy Sci.* 100:6312–6326. <https://doi.org/10.3168/jds.2016-12252>.
- Belay, T. K., M. Svendsen, Z. M. Kowalski, and T. Ådnøy. 2017b. Genetic parameters of blood  $\beta$ -hydroxybutyrate predicted from milk infrared spectra and clinical ketosis, and their associations with milk production traits in Norwegian Red cows. *J. Dairy Sci.* 100:6298–6311. <https://doi.org/10.3168/jds.2016-12458>.
- Benedet, A., M. Franzoi, M. Penasa, E. Pellattiero, and M. De Marchi. 2019a. Prediction of blood metabolites from milk mid-infrared spectra in early lactation cows. *J. Dairy Sci.* 102:11298–11307. <https://doi.org/10.3168/jds.2019-16937>.
- Benedet, A., C. L. Manuelian, A. Zidi, M. Penasa, and M. De Marchi. 2019b. Invited review:  $\beta$ -Hydroxybutyrate concentration in blood and milk and its associations with cow performance. *Animal* 13:1676–1689. <https://doi.org/10.1017/S175173111900034X>.
- Carvalho, M. R., F. Peñagaricano, J. E. P. Santos, T. J. DeVries, B. W. McBride, and E. S. Ribeiro. 2019. Long-term effects of postpartum clinical disease on milk production, reproduction, and culling of dairy cows. *J. Dairy Sci.* 102:11701–11717. <https://doi.org/10.3168/jds.2019-17025>.
- Cecchinato, A., T. Bobbo, P. L. Ruegg, L. Gallo, G. Bittante, and S. Pegolo. 2018. Genetic variation in serum protein pattern and blood  $\beta$ -hydroxybutyrate and their relationships with udder health traits, protein profile, and cheese-making properties in Holstein cows. *J. Dairy Sci.* 101:11108–11119. <https://doi.org/10.3168/jds.2018-14907>.
- Costa, A., C. Egger-Danner, G. Mészáros, C. Fuerst, M. Penasa, J. Sölkner, and B. Fuerst-Waltl. 2019a. Genetic associations of lactose and its ratios to other milk solids with health traits in Austrian Fleckvieh cows. *J. Dairy Sci.* 102:4238–4248. <https://doi.org/10.3168/jds.2018-15883>.
- Costa, A., N. Lopez-Villalobos, N. W. Sneddon, L. Shalloo, M. Franzoi, M. De Marchi, and M. Penasa. 2019b. Invited review: Milk lactose—Current status and future challenges in dairy cattle. *J. Dairy Sci.* 102:5883–5898. <https://doi.org/10.3168/jds.2018-15955>.
- De Marchi, M., V. Toffanin, M. Cassandro, and M. Penasa. 2014. Invited review: Mid-infrared spectroscopy as phenotyping tool for milk traits. *J. Dairy Sci.* 97:1171–1186. <https://doi.org/10.3168/jds.2013-6799>.
- Ederer, S., C. Egger-Danner, W. Zollitsch, and B. Fuerst-Waltl. 2014. Metabolic disorders and their relationships to milk production traits in Austrian Fleckvieh. In Proc. 39th International Committee for Animal Recording (ICAR) Meeting, Berlin, Germany. Accessed Jul. 20, 2019. [https://www.icar.org/wp-content/uploads/2015/09/Fuerst\\_Waltl.pdf](https://www.icar.org/wp-content/uploads/2015/09/Fuerst_Waltl.pdf).
- Esposito, G., P. C. Irons, E. C. Webb, and A. Chapwanya. 2014. Interactions between negative energy balance, metabolic diseases, uterine health and immune response in transition dairy cows. *Anim. Reprod. Sci.* 144:60–71. <https://doi.org/10.1016/j.anireprosci.2013.11.007>.
- Gilmour, A. R., B. J. Gogel, B. R. Cullis, S. J. Welham, and R. Thompson. 2015. ASReml User Guide. VSN International Ltd., Hemel Hempstead, UK.
- Grelet, C., A. Vanlierde, M. Hostens, L. Foldager, M. Salavati, K. L. Ingvarsen, M. Crowe, M. T. Sorensen, E. Froidmont, C. P. Ferris, C. Marchitelli, F. Becker, T. Larsen, F. Carter, and F. Dehareng. 2019. Potential of milk mid-IR spectra to predict meta-

- bolic status of cows through blood components and an innovative clustering approach. *Animal* 13:649–658. <https://doi.org/10.1017/S1751731118001751>.
- Haile-Mariam, M., and J. E. Pryce. 2017. Genetic parameters for lactose and its correlation with other milk production traits and fitness traits in pasture-based production systems. *J. Dairy Sci.* 100:3754–3766. <https://doi.org/10.3168/jds.2016-11952>.
- Hammami, H., F. G. Colinet, C. Bastin, C. Grelet, A. Vanlierde, F. Dehareng, and N. Gengler. 2017. Genetic analysis of milk MIR predicted blood and milk biomarkers linked to the physiological status. Page 403 in *Book of Abstracts of the 68th Annual Meeting of the European Federation of Animal Science*, Tallin, Estonia. Wageningen Academic Publishers, Wageningen, the Netherlands.
- Houle, D. 1992. Comparing evolvability and variability of quantitative traits. *Genetics* 130:195–204.
- ICAR (International Committee for Animal Recording). 2019. Section 12—Guidelines for milk analysis. Accessed Jul. 22, 2019. <https://www.icar.org/Guidelines/12-Milk-Analysis.pdf>.
- Klein, S. L., C. Scheper, K. Brügemann, H. H. Swalve, and S. König. 2019. Phenotypic relationships, genetic parameters, genome-wide associations, and identification of potential candidate genes for ketosis and fat-to-protein ratio in German Holstein cows. *J. Dairy Sci.* 102:6276–6287. <https://doi.org/10.3168/jds.2019-16237>.
- Koeck, A., J. Jamrozik, F. S. Schenkel, R. K. Moore, D. M. Lefebvre, D. F. Kelton, and F. Miglior. 2014. Genetic analysis of milk  $\beta$ -hydroxybutyrate and its association with fat-to-protein ratio, body condition score, clinical ketosis, and displaced abomasum in early first lactation of Canadian Holsteins. *J. Dairy Sci.* 97:7286–7292. <https://doi.org/10.3168/jds.2014-8405>.
- LeBlanc, S. 2010. Monitoring metabolic health of dairy cattle in the transition period. *J. Reprod. Dev.* 56(Suppl.):S29–S35. <https://doi.org/10.1262/jrd.1056s29>.
- Luke, T. D. W., T. T. T. Nguyen, S. Rochfort, W. J. Wales, C. M. Richardson, M. Abdelsayed, and J. E. Pryce. 2019a. Genomic prediction of serum biomarkers of health in early lactation. *J. Dairy Sci.* 102:11142–11152. <https://doi.org/10.3168/jds.2019-17127>.
- Luke, T. D. W., S. Rochfort, W. J. Wales, V. Bonfatti, L. Maret, and J. E. Pryce. 2019b. Metabolic profiling of early lactation dairy cows using milk mid-infrared spectra. *J. Dairy Sci.* 102:1747–1760. <https://doi.org/10.3168/jds.2018-15103>.
- Mäntysaari, P., E. A. Mäntysaari, T. Kokkonen, T. Mehtiö, S. Kajava, C. Grelet, P. Lidauer, and M. H. Lidauer. 2019. Body and milk traits as indicators of dairy cow energy status in early lactation. *J. Dairy Sci.* 102:7904–7916. <https://doi.org/10.3168/jds.2018-15792>.
- McArt, J. A. A., D. V. Nydam, G. R. Oetzel, T. R. Overton, and P. A. Ospina. 2013. Elevated non-esterified fatty acids and  $\beta$ -hydroxybutyrate and their association with transition dairy cow performance. *Vet. J.* 198:560–570. <https://doi.org/10.1016/j.tvjl.2013.08.011>.
- Miglior, F., A. Sewalem, J. Jamrozik, D. M. Lefebvre, and R. K. Moore. 2006. Analysis of milk urea nitrogen and lactose and their effect on longevity in Canadian dairy cattle. *J. Dairy Sci.* 89:4886–4894. [https://doi.org/10.3168/jds.S0022-0302\(06\)72537-1](https://doi.org/10.3168/jds.S0022-0302(06)72537-1).
- Negussie, E., I. Strandén, and E. A. Mäntysaari. 2008. Genetic association of clinical mastitis with test-day somatic cell score and milk yield during first lactation of Finnish Ayrshire cows. *J. Dairy Sci.* 91:1189–1197. <https://doi.org/10.3168/jds.2007-0510>.
- Oikonomou, G., G. E. Valergakis, G. Arsenos, N. Roubies, and G. Banos. 2008. Genetic profile of body energy and blood metabolic traits across lactation in primiparous Holstein cows. *J. Dairy Sci.* 91:2814–2822. <https://doi.org/10.3168/jds.2007-0965>.
- Pfeiffer, C., C. Fuerst, V. Ducrocq, and B. Fuerst-Waltl. 2015. Short communication: Genetic relationships between functional longevity and direct health traits in Austrian Fleckvieh cattle. *J. Dairy Sci.* 98:7380–7383. <https://doi.org/10.3168/jds.2015-9632>.
- Pryce, J. E., K. L. Parker Gaddis, A. Koeck, C. Bastin, M. Abdelsayed, N. Gengler, F. Miglior, B. Heringstad, C. Egger-Danner, K. F. Stock, A. J. Bradley, and J. B. Cole. 2016. Invited review: Opportunities for genetic improvement of metabolic diseases. *J. Dairy Sci.* 99:6855–6873. <https://doi.org/10.3168/jds.2016-10854>.
- Santschi, D. E., R. Lacroix, J. Durocher, M. Duplessis, R. K. Moore, and D. M. Lefebvre. 2016. Prevalence of elevated milk  $\beta$ -hydroxybutyrate concentrations in Holstein cows measured by Fourier-transform infrared analysis in dairy herd improvement milk samples and association with milk yield and components. *J. Dairy Sci.* 99:9263–9270. <https://doi.org/10.3168/jds.2016-11128>.
- Stefani, G., L. El Faro, M. L. Santana Júnior, and H. Tonhati. 2018. Association of longevity with type traits, milk yield and udder health in Holstein cows. *Livest. Sci.* 218:1–7. <https://doi.org/10.1016/j.livsci.2018.10.007>.
- 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. <https://doi.org/10.3168/jds.2012-6035>.
- 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  $\beta$ -hydroxybutyrate and milk acetone concentrations of early-lactation dairy cows. *J. Dairy Sci.* 95:6781–6787. <https://doi.org/10.3168/jds.2012-5640>.
- van der Werf, J., H. U. Graser, R. Frankham, and C. Gondro. 2019. Adaptation and fitness in animal population. Evolutionary and breeding perspectives on genetic resource management. Accessed Aug. 20, 2019. <https://link.springer.com/content/pdf/10.1007%2F978-1-4020-9005-9.pdf>.
- Visentin, G., M. De Marchi, D. P. Berry, A. McDermott, M. A. Fenelon, M. Penasa, and S. McParland. 2017. Factors associated with milk processing characteristics predicted by mid-infrared spectroscopy in a large database of dairy cows. *J. Dairy Sci.* 100:3293–3304. <https://doi.org/10.3168/jds.2016-12028>.
- Wang, Q., and H. Bovenhuis. 2019. Validation strategy can result in an overoptimistic view of the ability of milk infrared spectra to predict methane emission of dairy cattle. *J. Dairy Sci.* 102:6288–6295. <https://doi.org/10.3168/jds.2018-15684>.
- Zuliani, A., M. Mair, M. Kraševc, I. Lora, M. Brscic, G. Cozzi, C. Leeb, M. Zupan, C. Winckler, and S. Bovolenta. 2018. A survey of selected animal-based measures of dairy cattle welfare in the Eastern Alps: Toward context-based thresholds. *J. Dairy Sci.* 101:1428–1436. <https://doi.org/10.3168/jds.2017-13257>.

## ORCIDIS

- A. Benedet  <https://orcid.org/0000-0003-4210-4273>  
 A. Costa  <https://orcid.org/0000-0001-5353-8988>  
 M. De Marchi  <https://orcid.org/0000-0001-7814-2525>  
 M. Penasa  <https://orcid.org/0000-0001-9984-8738>