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The relationships between early lactation energy status indicators and endocrine fertility traits in dairy cows

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ABSTRACT

The relationships between dairy cow milk-based energy status (ES) indicators and fertility traits were studied during periods 8 to 21, 22 to 35, 36 to 49, and 50 to 63 d in milk. Commencement of luteal activity (C-LA) and interval from calving to the first heat (CFH), based on frequent measurements of progesterone by the management tool Herd Navigator (DeLaval), were used as fertility traits. Energy status indicator traits were milk β-hydroxybutyrate (BHB) concentration provided by Herd Navigator and milk fat:protein ratio, concentration of C18:1 *cis*-9, the ratio of fatty acids (FA) C18:1 *cis*-9 and C10:0 in test-day milk samples, and predicted plasma concentration of nonesterified fatty acids (NEFA) on test days. Plasma NEFA predictions were based either directly on milk mid-infrared spectra (MIR) or on milk fatty acids based on MIR spectra ($NEFA_{mir}$ and $NEFA_{fa}$, respectively). The average (standard deviation) C-LA was 39.3 (±16.6) days, and the average CFH was 50.7 (±17.2) days. The correlations between fertility traits and ES indicators tended to be higher for multiparous ($r < 0.28$) than for primiparous ($r < 0.16$) cows. All correlations were lower in the last period than in the other periods. In period 1, correlations of C-LA with $NEFA_{fa}$ and BHB, respectively, were 0.15 and 0.14 for primiparous and 0.26 and 0.22 for multiparous cows. The associations between fertility traits and ES indicators indicated that negative ES during the first weeks postpartum may delay the onset of luteal activity. Milk FPR was not as good an indicator for cow ES as other indicators. According to these findings, predictions of plasma NEFA and milk FA based on milk MIR spectra of routine test-day samples and the frequent measurement of milk BHB by Herd Navigator gave equally good predictions of cow ES during the first weeks of lactation. Our results indicate that routinely measured milk traits can be used for ES evaluation in early lactation.

Key words: dairy cow, fertility, energy status

INTRODUCTION

During early lactation, the energy needs of high-producing cows often exceed the amount of energy the cow can obtain from dietary sources (see, e.g., Mäntysaari et al., 2012, 2019). To fill the energy deficit, cows are forced to mobilize energy from their body reserves, resulting in a negative energy status (**ES**). The detrimental effects of negative ES on health (Esposito et al., 2014) and fertility (Butler, 2000; Walsh et al., 2011) are well known. It has been shown that especially the resumption of ovarian activity is related to ES after calving (Butler, 2000; Reist et al., 2000; Martin et al., 2015).

Adipose tissue mobilization in negative ES results in elevated concentrations of nonesterified fatty acids (**NEFA**) in blood (Dunshea et al., 1989). Excessive mobilization of adipose tissue can lead to inadequate hepatic metabolism of NEFA, which increases blood levels of ketone bodies such as BHB, acetoacetate, and acetone (Esposito et al., 2014). Mammary uptake of NEFA depends on their concentration in blood (Miller et al., 1991). An increased supply of fatty acids (**FA**) for milk fat synthesis leads to an increase in the milk fat content and in the fat:protein ratio (**FPR**) in milk and changes in the milk FA profile (Palmquist et al., 1993; Stoop et al., 2009; Gross et al., 2011). Increased BHB levels in blood are also seen as elevated BHB concentrations in milk (Enjalbert et al., 2001; Denis-Robichaud et al., 2014).

Frequent blood sampling is laborious and stressful for the cow, and the analyses are too expensive as a way to monitor cows' ES. Instead, detailed milk

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composition can be attained from routinely collected test-day milk samples at low cost using mid-infrared spectroscopy (**MIR**). Therefore, milk FPR (Duffield et al., 1997; Mäntysaari and Mäntysaari, 2010; Negussie et al., 2013), milk FA (Stoop et al., 2009; Gross et al., 2011), and the prediction of plasma NEFA concentration based on measures of milk FA concentrations (Jorjong et al., 2014; Mäntysaari et al., 2019) as well as direct prediction of plasma NEFA (Mehtiö et al., 2018; Aernouts et al., 2020; Bach et al., 2021), BHB (Luke et al., 2019), and cows' energy balance (McParland et al., 2011, 2012) from milk MIR spectra have been proposed as ES indicators. Increasing dairy herd sizes have led to increased popularity of automatic analysis systems on farms. Daily measures of milk BHB are provided by the Herd Navigator (**HN**, DeLaval International) management tool, which analyzes several milk constituents automatically during milking.

Although a decrease in fertility as result of energy deficiency during the first weeks after parturition is well documented (Butler, 2000; Reist et al., 2000; Ospina et al., 2010b), very low phenotypic correlations (0.01–0.05) between ES indicators and fertility measures have been reported in previous studies (Negussie et al., 2013; Mehtiö et al., 2020). In their studies, classical fertility measures such as interval from calving to first insemination and days open were used. These traits are highly influenced by management factors. Higher correlations would be expected if the fertility measures were based on the underlying physiological functions of reproduction. For example, the postcalving onset of luteal activity can be detected by changes in reproductive hormone levels, especially progesterone (**P4**), in milk (Friggens et al., 2008). In the HN management system, reproductive performance of cows is monitored by frequent sampling and analysis of milk P4 levels. Changes in milk P4 concentration indicate the commencement of luteal activity (**C-LA**) and days from calving to the first observed heat (**CFH**).

In this study, we estimated correlations between milk-based ES indicators and the underlying physiological fertility traits derived from endocrine functions. Our goal was to assess the associations of postpartum negative energy balance on fertility and to compare the usefulness of different indicators in predicting cows' ES in early lactation.

MATERIALS AND METHODS

Data and Studied Traits

After permission was granted, data from 11 Finnish dairy herds using HN from January 2015 to December 2018 were received from Lattec I/S (Hillerød, Denmark). All herds used automatic milking systems. For the herds, data from 3 different sources were merged: HN management data, typical milk recording test-day data, and milk MIR spectral data from test-day milk samples. All use of animals in scientific experimentation was in line with Directive 2010/63/EU. No invasive research methods were used; therefore a formal license was not required, according to the National Ethics Committee (Hämeenlinna, Finland).

Fertility Traits. Traits C-LA and CFH provided by the HN system were used as fertility measurements. The traits are derived from changes in the concentration of P4 in milk. The HN samples the milk according to a cow-specific biological model starting around 20 d after calving (Friggens et al., 2008) and analyzes the samples using an immunoassay-based dry-stick technique (Samsonova et al., 2015). An extended Kalman filter is used to smooth the raw P4 values. The first increase in P4 concentration (>4 ng/mL) in milk represents the start of cycling (C-LA), and the change from high to low concentration (<5 ng/mL) detects the first heat (CFH). The HN model is not able to reliably detect the first heat event in early lactation because P4 concentrations tend to be constantly low after calving. Thus, CFH represents the first observed heat based on HN P4 measurements (Friggens et al., 2008). Only the C-LA and CFH observations ≤100 DIM were accepted; thus, the fertility observations were between 20 and 100 DIM.

ES Indicator Based on HN Data. The milk BHB concentration provided by HN was studied as an ES indicator. Milk samples for BHB concentration (mmol/L) analyses were automatically taken according to a biological model from every day (during period 1, 1–2 samples per day) to every fourth day and analyzed by HN based on a fluorometric determination (Larsen and Nielsen, 2005). In HN, the raw BHB values were processed to obtain smoothed BHB values, which were then used in our study. Observations between 8 and 63 DIM were used.

ES Indicators Based on Test-Day Data. From the test-day milk samples, FPR, concentration of C18:1 *cis*-9 (g/100 mL), the ratio of fatty acids C18:1 *cis*-9 and C10:0 (C18:1 *cis*-9/C10:0), and predicted plasma concentration of NEFA (mmol/L) were used as ES indicators. Blood plasma NEFA concentration was predicted in 2 ways, either directly from test-day milk MIR spectra (**NEFA**$_{\mathbf{mir}}$) acquired with a MilkoScan FT+ spectrometer (Foss) in the Valio Ltd. milk laboratory (Seinäjoki, Finland) or by a multiple regression equation that included DIM, milk FPR, and milk fatty acids C10:0, C14:0, C18:1 *cis*-9, C14:0 × C18:1 *cis*-9 (**NEFA**$_{\mathbf{fa}}$). The applied prediction equations for NEFA$_{mir}$, based on 1,585 milk spectral readings and 809

NEFA observations, were developed by Mehtiö et al. (2018). They reported a coefficient of determination of cross-validation (R^2_{cv}) of 0.67 and a root mean square error (RMSE) of 0.17 mmol/L for $NEFA_{mir}$ prediction. The milk FA concentrations used in $NEFA_{fa}$ prediction and for C18:1 *cis*-9 and C18:1 *cis*-9/C10:0 traits were predicted from milk MIR spectral readings using calibration equations described by Soyeurt et al. (2011), where the prediction accuracies of the individual FA predictions are reported. The prediction equation for $NEFA_{fa}$, based on 1,525 milk spectral readings and 774 NEFA observations, is described in Mäntysaari et al. (2019). For $NEFA_{fa}$, the R^2_{cv} was 0.62 and RMSE 0.18 mmol/L. The research herds used in development of NEFA prediction equations were not included in the data used in this study. The observation period for ES indicators included lactation d 8 to 63. In the analyses, the study period was further divided into four 2-wk subperiods: period 1 = 8 to 21, period 2 = 22 to 35, period 3 = 36 to 49, and period 4 = 50 to 63 DIM. In calculations, a period-average value of ES traits was used. For ES measures derived from the test-day observation, this average involved only one observation. For BHB, the average included several observations, thus we predicted the test-day value by averaging the BHB measures in the period.

Statistical Analyses

The effects of herd, breed, parity, calving year, and calving season for fertility and ES traits were analyzed with PROC MIXED in SAS (SAS Institute Inc.). The variance component estimation method was REML, and the type of within-subject covariance matrix was compound symmetry. All of the first-order interactions among the effects were first included in the model. Next, the interaction terms found not significant were removed. Because the herd × year interaction was kept in the model, these main effects were removed. Although present in interactions, the main effects of parity and calving season were kept in the model to allow estimation of least squares means (**LSM**). The final model used was

$$Y_{ijklmn} = \mu + B_i + P_j + S_k + (H \times P)_{lj} + (H \times S)_{lk} + (H \times Y)_{lm} + \varepsilon_{ijklmn},$$

where Y_{ijklmn} = dependent variable, μ = overall mean, B_i = breed (Nordic Red or Holstein), P_j = parity (primi- or multiparous), S_k = calving season, $(H \times P)_{lj}$ = interaction between herd and parity, $(H \times S)_{lk}$ = interaction between herd and calving season, $(H \times Y)_{lm}$ = interaction between herd and year, and ε_{ijklmn} = a residual effect of cow n considered as a repeated measure across lactations. The calving seasons were 1 (winter): December, January, February; 2 (spring): March, April, May; 3 (summer): June, July, August; 4 (autumn): September, October, November. When testing the effects of herd, breed, and parity on ES traits, separate analyses were performed for each period.

The associations between different ES indicators at different periods were quantified by using Pearson correlations. The correlations between fertility traits and ES indicators were calculated within herd separately for primiparous and multiparous cows because both fertility traits and all ES indicators in period 1 had significant interactions between the herd and parity. Because any unknown herd effects on fertility that are independent from ES would have masked the association between ES indicators and fertility, the correlations between fertility and ES traits were derived from the between-cow covariances and variances using bivariate models:

$$\begin{bmatrix} Y_{Fij(l)} \\ Y_{Eij(l)} \end{bmatrix} = \begin{bmatrix} h_{Fi} \\ h_{Ei} \end{bmatrix} + \begin{bmatrix} e_{Fij(l)} \\ e_{Eij(l)} \end{bmatrix},$$

where the dependent variables $Y_{Fij(l)}$ are the fertility trait records (F = CL-A or CFH) and $Y_{Eij(l)}$ are ES indicator observations, each in turn, for the cow j in its lth lactation in herd i; h_{Fi} and h_{Ei} are the effects of herd i for the fertility trait F and ES indicator trait E; and $e_{Fij(l)}$ and $e_{Eij(l)}$ are the unexplained correlated residual terms. The residual variance-covariance matrix representing the cow-by-cow variation was estimated with PROC MIXED in SAS (SAS Institute Inc.) pair wise for each fertility and ES indicator combination and separately for each period and for primi- and multiparous cows (i.e., 96 separate variance component analyses), and the covariances were used to derive the correlations between the traits.

RESULTS

The herds in the data set had an average 135 cows, ranging from 69 to 245 cows. Of the cows, 58.4% were Nordic Red dairy cattle and 41.6% were Holstein breed. Data included observations from parities 1 to 9, and primiparous cows formed 34.4% of the observations. Some cows had more than one lactation in the data set. The fertility data included 3,522 C-LA and 3,702 CFH observations from 2,170 and 2,175 cows, respectively (Table 1). The BHB measurements by HN were available for almost all cows with fertility

observations. The number of BHB measurements for each cow based on its sampling schedule resulted in an average 26.4 (±11.2; mean ± SD) samples per cow during lactation d 8 to 63. The test-day milk samples for composition analyses were taken either every month (5 herds) or every other month (6 herds), thus our observation period (8–63 DIM) was expected to cover all cows. However, test-day information and milk spectral readings were not available for all cows with fertility measurements, which led to fewer cows and lactations for the ES traits $NEFA_{mir}$, $NEFA_{fa}$, FPR, C18:1 *cis*-9, and C18:1 *cis*-9/C10:0. The number of cows, lactations, and observations for each ES trait are presented in Table 1.

Table 2 shows the statistics of production and fertility observations during 8 to 63 DIM. The cows milked on average 37.3 kg/d. Because the observation periods were at the beginning of lactation and at a time of increasing milk yields, the variation in the daily milk yield was large: from 6.9 to 69.5 kg/d. Milk fat and milk protein concentrations averaged 4.27 and 3.34%, respectively.

Table 1. The number of cows, lactations, and observations for fertility and energy status traits (8–63 DIM)

Trait[1]	No. of cows	No. of lactations	No. of observations
C-LA	2,170	3,522	3,522
CFH	2,175	3,702	3,702
$NEFA_{mir}$	1,553	2,528	3,491
$NEFA_{fa}$	1,547	2,518	3,433
Milk fat:protein	1,547	2,518	3,433
C18:1 *cis*-9	1,554	2,532	3,501
C18:1 *cis*-9/C10:0	1,554	2,532	3,501
BHB	2,312	4,027	106,358

[1]C-LA = commencement of luteal activity (days; observations from 20 to 100 DIM included); CFH = days from calving to the first heat (days; observations from 20 to 100 DIM included); $NEFA_{mir}$ and $NEFA_{fa}$ = plasma concentration of nonesterified fatty acids (mmol/L) predicted based on milk mid-infrared spectra or milk fatty acids, respectively; C18:1 *cis*-9 = milk C18:1 *cis*-9 concentration (g/100 mL); C18:1 *cis*-9/C10:0 = ratio of fatty acids C18:1 *cis*-9 and C10:0 in milk; BHB = milk BHB concentration (mmol/L).

Fertility Traits

The average C-LA for the cows was 39.3 (±16.6) days and the average CFH was 50.7 (±17.2) days (Table 2). The median values for C-LA and CFH were 35 and 48, respectively. The LSM estimates by parity, breed, and calving season for C-LA and CFH are presented in Table 3. Breed had no significant effect on fertility traits. There was a significant interaction ($P < 0.001$) between herd and parity for both fertility traits, and the interaction between herd and calving season was significant for both fertility traits (C-LA: $P < 0.001$; CFH: $P = 0.02$).

ES Indicator Traits

The statistics of ES indicators are presented in Table 2, and the LSM and standard errors of means (**SEM**) in different periods for primiparous and multiparous cows separately are shown in Figure 1. The LSM and SEM by parity, breed, and calving season for all ES indicators are shown in Supplemental Table S1 (https://osf.io/3fy85; Mäntysaari et al., 2022). The predicted plasma NEFA concentrations ($NEFA_{mir}$ and $NEFA_{fa}$) were highest in period 1 (Figure 1A). In agreement with NEFA predictions, milk C18:1 *cis*-9

Table 2. Statistics of production, fertility, and energy status observations during 8 to 63 DIM

Trait[1]	N	Mean	Median	SD	Minimum	Maximum
Milk, kg/d	3,501	37.3	37.3	10.23	6.9	69.5
ECM, kg/d	3,433	38.0	38.4	9.66	7.3	73.6
Milk composition, %						
Fat	3,433	4.27	4.21	0.667	1.45	8.26
Protein	3,433	3.34	3.32	0.309	2.26	4.94
C-LA, d	3,522	39.3	35.0	16.6	20	100
CFH, d	3,702	50.7	48.0	17.2	21	100
$NEFA_{mir}$, mmol/L	3,491	0.36	0.33	0.18	0.0006	1.57
$NEFA_{fa}$, mmol/L	3,433	0.35	0.32	0.17	0.02	1.51
C18:1 *cis*-9, g/100 mL	3,501	0.94	0.88	0.30	0.20	3.16
C18:1 *cis*-9/C10:0	3,501	9.65	8.12	6.06	2.98	96.24
BHB, mmol/L of milk	106,358	0.121	0.070	0.125	0.0002	1.86
Milk fat:protein	3,433	1.28	1.26	0.18	0.62	2.36

[1]C-LA = commencement of luteal activity (days; observations from 20 to 100 DIM included); CFH = days from calving to the first heat (days; observations from 20 to 100 DIM included); $NEFA_{mir}$ and $NEFA_{fa}$ = plasma nonesterified fatty acids concentration (mmol/L) predicted based on milk mid-infrared spectra or milk fatty acids, respectively; C18:1 *cis*-9 = milk C18:1 *cis*-9 concentration (g/100 mL); C18:1 *cis*-9/C10:0 = ratio of fatty acids C18:1 *cis*-9 and C10:0 in milk; BHB = milk BHB concentration in daily observations.

and C18:1 *cis*-9/C10:0 values were highest in the first period (Figure 1B), whereas the highest milk BHB concentration and FPR were measured in period 2 (Figure 1C). There were small differences between primi- and multiparous cows in all other ES indicators except $NEFA_{mir}$. We detected a significant interaction between herd and parity for all ES indicators in period 1 but only for BHB in the later periods (Figure 1; Mäntysaari et al., 2022).

Pearson correlations between different ES indicators are presented in Table 4. The indicators based on the same milk MIR spectra ($NEFA_{mir}$, $NEFA_{fa}$, C18:1 *cis*-9, and C18:1 *cis*-9/C10:0) had high correlations (0.63–0.95) in all periods. The highest correlations were found between $NEFA_{fa}$ and C18:1 *cis*-9: 0.95, 0.94, 0.91, and 0.89 in periods 1 to 4, respectively. The correlations between milk BHB concentration and predicted plasma NEFA concentrations were moderate (0.22–0.53). The lowest correlation in each period was found between milk FPR and BHB concentration, ranging from 0.17 to 0.26.

Table 3. Least squares means (LSM) and SEM for fertility traits by parity, breed, and calving season

Fertility trait	LSM	SEM	*P*-value[1]
C-LA,[2] d			
Parity			
Primiparous cows	40.9	0.59	
Multiparous cows	39.6	0.50	
Breed			0.36
Nordic Red	40.6	0.48	
Holstein	39.6	0.66	
Calving season[3]			
1	43.7	0.72	
2	39.2	0.70	
3	35.4	0.72	
4	42.7	0.68	
CFH,[4] d			
Parity			
Primiparous cows	50.7	0.57	
Multiparous cows	52.4	0.48	
Breed			0.21
Nordic Red	51.1	0.53	
Holstein	51.9	0.54	
Calving season[3]			
1	54.6	0.72	
2	50.4	0.65	
3	47.3	0.73	
4	53.7	0.66	

[1]The interaction between herd and factor was found significant ($P < 0.05$): C-LA: herd × parity $P < 0.001$, herd × calving season $P < 0.001$; CFH: herd × parity $P < 0.001$, herd × calving season $P < 0.02$. When there is interaction between factors the *P*-value is not presented.

[2]Commencement of luteal activity; only observations from 20 to 100 DIM are included.

[3]Calving season 1 = December, January, February; season 2 = March, April, May; season 3 = June, July, August; season 4 = September, October, November.

[4]Days from calving to the first heat; only observations from 20 to 100 DIM are included.

Associations Between Fertility Traits and ES Indicators

The correlations between fertility and ES indicator traits during different periods are presented in Table 5 for primiparous cows and in Table 6 for multiparous cows. The correlations of all ES indicators with both fertility traits were clearly lower in period 4 than in other periods. The correlations between fertility and ES traits tended to be higher for multiparous than for primiparous cows. The highest correlations between ES indicators and C-LA and CFH, respectively, were 0.155 and 0.156 for primiparous cows and 0.279 and 0.228 for multiparous cows. For primiparous cows, the correlations between ES traits and C-LA decreased with increasing period in most cases. However, the correlations between C-LA and $NEFA_{mir}$ and C18:1 *cis*-9/C10:0 were highest in period 2. For primiparous cows, CFH had the highest correlations with BHB and C18:1 *cis*-9/C10:0 during periods 1 and 2, respectively, and with other ES indicators in period 3. For multiparous cows, correlations were higher in periods 1 and 3 than in periods 2 and 4 between both fertility traits and ES indicators, except BHB, for which the correlation was similar in periods 1 and 2 and lower during later periods. The correlations for multiparous cows between C-LA and ES indicator traits were highest in period 1. The correlations between CFH and ES indicators for multiparous cows were highest in period 1, except for the correlations with $NEFA_{fa}$ and C18:1 *cis*-9/C10:0, which were highest in period 3.

For individual ES indicators, FPR had lower correlations with fertility traits than did milk BHB, $NEFA_{fa}$, or $NEFA_{mir}$. The correlations between fertility traits and NEFA predictions ($NEFA_{fa}$ and $NEFA_{mir}$) were at the same level in all periods except period 1 for primiparous cows. The correlations of C18:1 *cis*-9 and C18:1 *cis*-9/C10:0 with fertility traits were at the same level but, in most cases, a slightly stronger relationship with C18:1 *cis*-9/C10:0 was found. The relationship between $NEFA_{fa}$ prediction and fertility traits was examined using a NEFA concentration of 0.6 mmol/L as the threshold for severe negative ES. In period 1, the average C-LA of cows with a $NEFA_{fa}$ below the threshold was 39.1 d, whereas the average C-LA of cows above the threshold was 46.8 d. Corresponding values for the CFH were 50.5 and 57.7 d.

DISCUSSION

Our data set included observations of cows in Finnish herds using the DeLaval HN management system. The average herd size was 135 cows, whereas the average size of Finnish milk recording herds is much smaller,

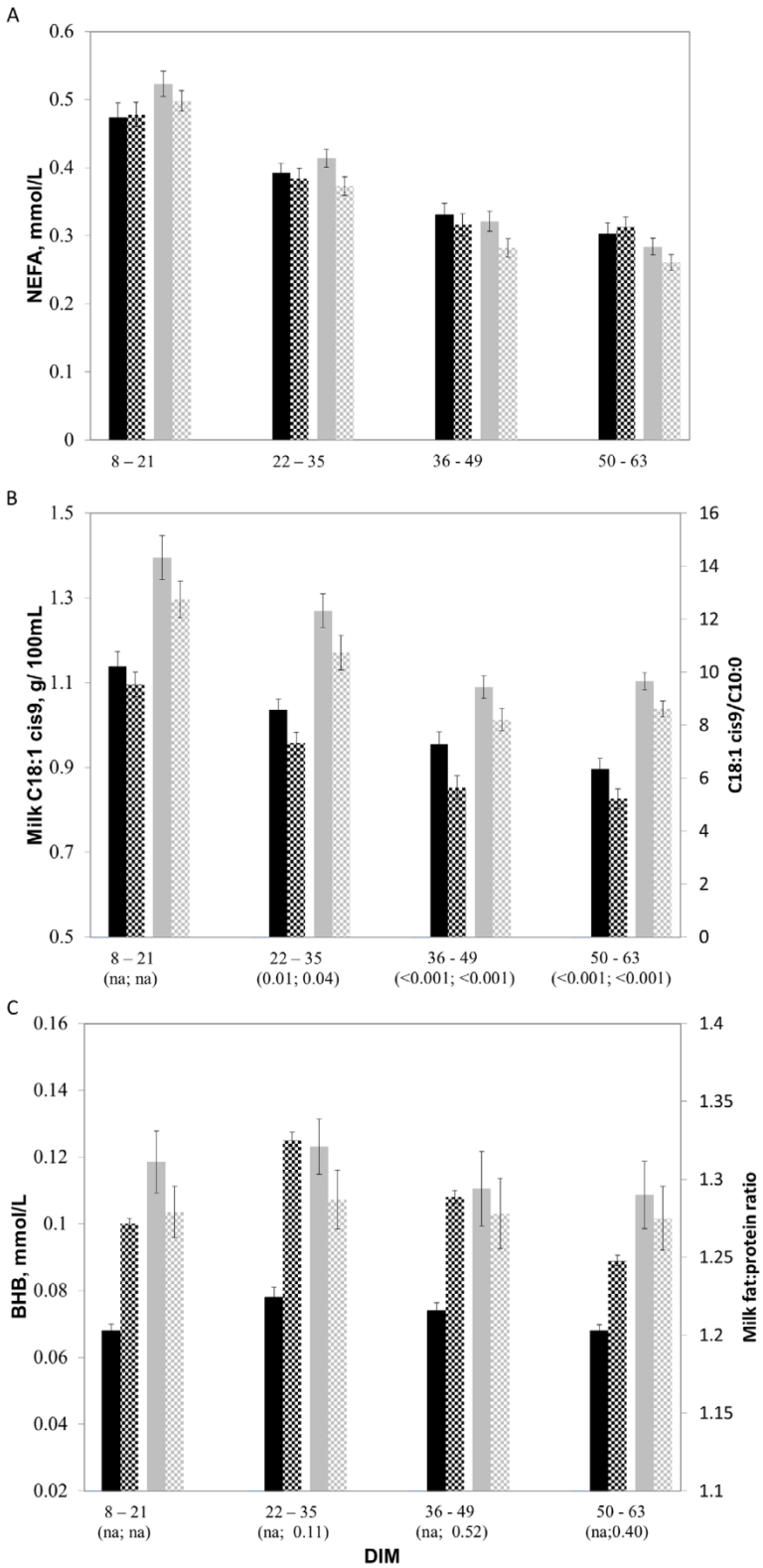

Figure 1. The LSM ± SEM of energy status indicators of primiparous (solid bars) and multiparous (dotted bars) cows during 8 to 63 DIM. (A) Plasma nonesterified fatty acids (NEFA) predicted directly by milk mid-infrared (MIR) spectra ($NEFA_{mir}$; black bars) or by milk fatty acids based on MIR spectra ($NEFA_{fa}$; gray bars); (B) milk C18:1 *cis*-9 (black bars) and milk C18:1 *cis*-9/C10:0 (gray bars); (C) BHB (black bars) and milk fat:protein ratio (gray bars). The significance of the effect of parity is given in parentheses under the DIM period. Two *P*-values are presented, one for each trait; "na" indicates a significant herd × parity interaction.

Table 4. The number of observations (above the diagonal) and Pearson correlations (below the diagonal) between energy status indicators during 8 to 21 (period 1), 22 to 35 (period 2), 36 to 49 (period 3), and 50 to 63 (period 4) DIM

Period and trait[1]	$NEFA_{mir}$	$NEFA_{fa}$	BHB	C18:1 *cis*-9	C18:1 *cis*-9/C10:0	Milk fat:protein
Period 1						
$NEFA_{mir}$, mmol/L		832	823	847	847	833
$NEFA_{fa}$, mmol/L	0.89		811	833	833	833
BHB, mmol/L	0.49	0.42		825	825	813
C18:1 *cis*-9, g/100 mL	0.87	0.95	0.40		849	835
C18:1 *cis*-9/C10:0	0.77	0.82	0.46	0.73		835
Milk fat:protein	0.50	0.65	0.21	0.72	0.36	
Period 2						
$NEFA_{mir}$, mmol/L		852	845	862	862	851
$NEFA_{fa}$, mmol/L	0.86		836	852	852	852
BHB, mmol/L	0.53	0.43		846	846	836
C18:1 *cis*-9, g/100 mL	0.82	0.94	0.41		863	852
C18:1 *cis*-9/C10:0	0.70	0.75	0.46	0.66		852
Milk fat:protein	0.53	0.67	0.26	0.75	0.31	
Period 3						
$NEFA_{mir}$, mmol/L		859	862	875	875	859
$NEFA_{fa}$, mmol/L	0.78		849	862	862	862
BHB, mmol/L	0.45	0.33		865	865	849
C18:1 *cis*-9, g/100 mL	0.74	0.91	0.28		878	862
C18:1 *cis*-9/C10:0	0.68	0.79	0.36	0.63		862
Milk fat:protein	0.46	0.66	0.23	0.77	0.25	
Period 4						
$NEFA_{mir}$, mmol/L		805	791	815	815	805
$NEFA_{fa}$, mmol/L	0.74		783	809	809	809
BHB, mmol/L	0.33	0.22		793	793	783
C18:1 *cis*-9, g/100 mL	0.66	0.89	0.15		819	809
C18:1 *cis*-9/C10:0	0.66	0.83	0.24	0.65		809
Milk fat:protein	0.39	0.17	0.17	0.74	0.27	

[1]$NEFA_{mir}$ and $NEFA_{fa}$ = plasma nonesterified fatty acids concentration predicted based on milk mid-infrared spectra or milk fatty acids, respectively; BHB = milk BHB concentration; C18:1 *cis*-9 = milk C18:1 *cis*-9 concentration; C18:1 *cis*-9/C10:0 = ratio of fatty acids C18:1 *cis*-9 and C10:0 in milk.

at 45.6 cows per herd in 2018 (ProAgria, 2021). The proportion of primiparous cow in our data was 34.4%, which is similar to the average in Finnish herds, which varied from 35.7% to 32.2% from 2015 to 2018 (ProAgria, 2021).

Fertility Traits

The fertility measurements used in this study, C-LA and CFH, are based on changes in the P4 concentration in milk. Compared with classical fertility traits such as calving interval, nonreturn rate, and conception rate, endocrine-based fertility traits give more accurate measures of a cow's fertility status (Petersson et al., 2006b; Tarekegn et al., 2019). The C-LA and CFH are not affected by management bias such as voluntary waiting period or poor heat detection. In our data, average C-LA was 39.3 d and CFH 50.7 d. The interval from calving to C-LA was 4 to 9 d longer than in some previous studies (Veerkamp et al., 2000; Horan et al., 2005; Petersson et al., 2006b; Tarekegn et al., 2019). The differences in C-LA and CFH between studies could be due, in part, to differences in parity distribution of cows. Later C-LA for primiparous cows were recorded by Petersson et al. (2006b) and Nyman et al. (2014), and an increased length of CFH with increasing parity was reported by Häggman et al. (2019). In our data, primiparous cows commenced luteal activity on average later than multiparous cows, but the first heat (CFH) was measured earlier. In our data, however, the herd × parity interaction was significant for the fertility traits. Breed had no effect on endocrine fertility traits. Opsomer et al. (2000) and Petersson et al. (2006a) reported a greater incidence of atypical P4 profiles and delayed cyclicity in winter-calving cows. In the current data, the significant interaction between herd and calving season for fertility traits indicated differences between herds.

ES Indicators

Plasma NEFA concentration can be considered the best indicator of a cow's ES, because its concentration in blood increases with increased fat mobilization (Dunshea et al., 1989). Because blood sampling and plasma analyses are laborious and expensive, we used

Table 5. The number of observations (N) and correlations (r) between fertility traits and energy status indicators of primiparous cows during 8 to 21 (period 1), 22 to 35 (period 2), 36 to 49 (period 3), and 50 to 63 (period 4) DIM

	Fertility trait[2]			
	C-LA		CFH	
Period and energy status trait[1]	N	r	N	r
Period 1				
$NEFA_{mir}$, mmol/L	227	0.071	236	0.025
$NEFA_{fa}$, mmol/L	222	0.148*	232	0.117†
BHB, mmol/L	1,096	0.143***	1,208	0.146***
C18:1 *cis*-9, g/100 mL	228	0.106	237	0.078
C18:1 *cis*-9/C10:0	228	0.148*	237	0.117†
Milk fat:protein	222	0.031	232	0.032
Period 2				
$NEFA_{mir}$, mmol/L	239	0.092	252	0.085
$NEFA_{fa}$, mmol/L	234	0.075	247	0.052
BHB, mmol/L	1,113	0.122***	1,229	0.124***
C18:1 *cis*-9, g/100 mL	240	0.039	253	0.027
C18:1 *cis*-9/C10:0	240	0.155*	253	0.156*
Milk fat:protein	234	−0.036	247	−0.026
Period 3				
$NEFA_{mir}$, mmol/L	242	0.032	250	0.154*
$NEFA_{fa}$, mmol/L	237	0.036	245	0.146*
BHB, mmol/L	1,128	0.074*	1,244	0.066*
C18:1 *cis*-9, g/100 mL	242	0.059	250	0.133*
C18:1 *cis*-9/C10:0	242	0.054	250	0.112†
Milk fat:protein	237	0.002	245	0.050
Period 4				
$NEFA_{mir}$, mmol/L	236	−0.091	251	−0.094
$NEFA_{fa}$, mmol/L	237	−0.015	251	−0.064
BHB, mmol/L	1,112	0.063*	1,239	0.062*
C18:1 *cis*-9, g/100 mL	238	−0.071	253	−0.104
C18:1 *cis*-9/C10:0	238	0.018	253	−0.009
Milk fat:protein	237	0.001	251	−0.013

[1]$NEFA_{mir}$ and $NEFA_{fa}$ = plasma nonesterified fatty acids concentration predicted based on milk mid-infrared spectra or milk fatty acids, respectively; BHB = milk BHB concentration; C18:1 *cis*-9 = milk C18:1 *cis*-9 concentration; C18:1 *cis*-9/C10:0 = ratio of fatty acids C18:1 *cis*-9 and C10:0 in milk.

[2]C-LA = commencement of luteal activity; only observations from 20 to 100 DIM are included. CFH = days from calving to the first heat; only observations from 20 to 100 DIM are included.

***$P < 0.001$; **$P < 0.01$; *$P < 0.05$; †$P < 0.1$.

milk-based ES indicators in this study. As shown in Figure 1, all ES indicators except BHB and FPR in multiparous cows had the highest values during period 1; milk BHB concentration was highest in period 2. The delay in the increase in milk BHB compared with predicted blood NEFA was also reported by Bach et al. (2019), although McArt et al. (2012) measured the peak in blood BHB as early as 5 DIM. This difference in timing could explain, in part, the lower correlation between BHB and NEFA and FA indicators (r = 0.15–0.53) compared with correlations among NEFA and FA indicators (r = 0.66–0.95). In contrast, the high correlations between NEFA predictions and milk FA measures are understandable because they all are based on the same spectral readings from test-day samples. McCarthy et al. (2015) reported a lower correlation (0.26) between NEFA and BHB within the first 21 DIM than we found in period 1 (0.49 and 0.42 for $NEFA_{mir}$ and $NEFA_{fa}$, respectively). In their data, both NEFA and BHB concentrations were measured in blood. The correlation between milk BHB and FPR varied from 0.17 to 0.26 during different periods. This association was lower than the correlations of 0.52 in Koeck et al. (2014) and 0.41 in Mehtiö et al. (2020) between BHB and FPR in test-day samples. In our data, FPR was based on test-day milk samples but BHB was an average of the daily measures during the predefined period, which could explain the lower correlations.

The milk FA profile–based indicators ($NEFA_{fa}$, C18:1 *cis*-9, and C18:1 *cis*-9/C10:0) and milk FPR showed higher values for primiparous cows than for multiparous cows (Figure 1A, B, and C). However, a significant interaction between herd and parity was found for these ES indicators in period 1(Figure 1; Mäntysaari et al., 2022). In periods 2 to 4, we detected significantly higher values for $NEFA_{fa}$, C18:1 *cis*-9, and C18:1 *cis*-

Table 6. The number of observations (N) and correlations (r) between fertility traits and energy status indicators of multiparous cows during 8 to 21 (period 1), 22 to 35 (period 2), 36 to 49 (period 3), and 50 to 63 (period 4) DIM

	Fertility trait[2]			
	C-LA		CFH	
Period and energy status trait[1]	N	r	N	r
Period 1				
$NEFA_{mir}$, mmol/L	557	0.279***	544	0.179***
$NEFA_{fa}$, mmol/L	549	0.260***	536	0.166***
BHB, mmol/L	2,271	0.217***	2,333	0.142***
C18:1 *cis*-9, g/100 mL	558	0.231***	545	0.141**
C18:1 *cis*-9/C10:0	558	0.268***	545	0.191***
Milk fat:protein	551	0.134**	538	0.079†
Period 2				
$NEFA_{mir}$, mmol/L	552	0.141**	554	0.060
$NEFA_{fa}$, mmol/L	548	0.126**	549	0.066
BHB, mmol/L	2,310	0.219***	2,377	0.152***
C18:1 *cis*-9, g/100 mL	552	0.100*	554	0.040
C18:1 *cis*-9/C10:0	552	0.117**	554	0.074†
Milk fat:protein	548	0.015	549	−0.017
Period 3				
$NEFA_{mir}$, mmol/L	576	0.181***	558	0.134**
$NEFA_{fa}$, mmol/L	568	0.240***	551	0.197***
BHB, mmol/L	2,316	0.152***	2,404	0.097***
C18:1 *cis*-9, g/100 mL	579	0.188***	561	0.144**
C18:1 *cis*-9/C10:0	579	0.268***	561	0.228***
Milk fat:protein	568	0.105*	551	0.046
Period 4				
$NEFA_{mir}$, mmol/L	507	0.071	510	−0.007
$NEFA_{fa}$, mmol/L	502	0.107*	502	0.054
BHB, mmol/L	2,293	0.097***	2,395	0.053*
C18:1 *cis*-9, g/100 mL	509	0.058	510	−0.010
C18:1 *cis*-9/C10:0	509	0.117*	510	0.053
Milk fat:protein	502	0.026	502	−0.010

[1]$NEFA_{mir}$ and $NEFA_{fa}$ = plasma nonesterified fatty acids concentration predicted based on milk mid-infrared spectra or milk fatty acids, respectively; BHB = milk BHB concentration; C18:1 *cis*-9 = milk C18:1 *cis*-9 concentration; C18:1 *cis*-9/C10:0 = ratio of fatty acids C18:1 *cis*-9 and C10:0 in milk.

[2]C-LA = commencement of luteal activity; only observations from 20 to 100 DIM are included. CFH = days from calving to the first heat; only observations from 20 to 100 DIM are included.

***$P < 0.001$; **$P < 0.01$; *$P < 0.05$; †$P < 0.1$.

9/C10:0, but not for FPR, in primiparous compared with multiparous cows. The herd × parity interaction during the first weeks of lactation for $NEFA_{fa}$ was also reported by Mäntysaari et al. (2019). The differences between herds can be explained by different feeding and management practices.

Associations Between Fertility Traits and ES Indicators

The correlations between fertility and ES indicators were estimated using a bivariate model to avoid possible large differences in herd-wise management practices affecting the results. For example, feeding diets that systematically affect milk FPR or fat composition could reduce the correlation between fertility traits and ES indicators if the correlations are computed over the herds. However, some herd management decisions may affect both fertility and energy status systematically across herds, and such a systematic effect will be removed by modeled herd effects. Thus, the estimated correlations are likely at the lower bound of the true values.

The correlations with fertility traits varied notably by ES indicator and time period. However, in all cases, we observed a trend for clearly lower correlations in period 4. This is logical because the commonly stated plasma NEFA threshold (0.6–0.7 mmol/L) for severe negative ES and greater risk of metabolic disorders (Ospina et al., 2010a,b) was exceeded only by 2% and 0.5% of the $NEFA_{mir}$ and $NEFA_{fa}$ predictions in period 4, respectively. In periods 1, 2, and 3, the NEFA threshold was exceeded by 25, 11, and 5% of $NEFA_{mir}$ and 23, 7, and 2% of $NEFA_{fa}$ observations. A milk BHB concentration >0.2 mmol/L at the beginning of lactation has been suggested as a threshold for hyperketonemia

(Denis-Robichaud et al., 2014). This was exceeded by 7% of BHB observations during period 4. One reason for the lower correlations between fertility and ES traits in period 4 than in other periods is that most of the cows have started cycling at this stage (C-LA: 39.3 ± 16.6 DIM; CFH 50.7 ± 17.2 DIM).

The correlations between fertility traits and milk-based ES indicators (Tables 5 and 6) during periods 1 to 3 ranged from low (<0.1) to close to moderate (0.2–0.3). They tended to be higher for multiparous than primiparous cows with all ES indicators. From individual ES indicators, only milk FPR differed clearly from other indicators by having a notably lower correlation with both C-LA and CFH. This agrees with the findings of Martin et al. (2015). They measured no relationship between FPR and onset of luteal activity. It seems that milk FPR is not as good predictor of cow's ES as milk FA–based indicators, as also shown in the study of Mäntysaari et al. (2019).

In the current study, plasma NEFA concentration was predicted from the test-day milk samples using MIR spectroscopy. Two developed plasma NEFA predictions—$NEFA_{mir}$ (Mehtiö et al., 2018) and $NEFA_{fa}$ (Mäntysaari et al., 2019)—were tested. According to our results, both NEFA indicators were equally good predictors of a cow's ES when assessed by their correlations with fertility traits. The only difference was found in period 1 for primiparous cows, where the relationships between $NEFA_{fa}$ and fertility traits were higher than those between $NEFA_{mir}$ and fertility traits. The correlations between NEFA predictions and fertility traits in current study were much higher than the phenotypic correlations of $NEFA_{mir}$ and $NEFA_{fa}$ with fertility in the study by Mehtiö et al. (2020). They used interval from calving to first service as fertility trait. The endocrine fertility traits C-LA and CFH have better potential to explain the actual reproductive status of cows than classical traits that are highly influenced by management decisions of farmers (Tarekegn et al., 2019). In our data, cows with $NEFA_{fa}$ below the threshold (using 0.6 mmol/L) in period 1 had an average C-LA of 39.1 d, whereas cows exceeding the threshold had an average C-LA of 46.8 d. Corresponding values for CFH were 50.5 and 57.7 d. These findings indicate the negative effect of predicted energy deficiency on fertility.

The correlations between fertility traits and milk C18:1 *cis*-9 alone or combined with C10:0 were similar to the correlations of plasma NEFA predictions and fertility traits. This indicates that these FA indicators are applicable predictors of a cow's ES. Milk C18:1 *cis*-9/C10:0 seemed to be a more useful predictor, because it was more related to C-LA and CFH than milk C18:1 *cis*-9 alone during periods 1 to 3 in all cases except period 3 for primiparous cows. In agreement with our results, Bastin et al. (2016) suggested that a combination of various FA traits can be used to improve selection for fertility. Also, Martin et al. (2015) found that the proportions of C18:0 and C18:1 *cis*-9 in milk fat were lower for cows with early onset of luteal activity compared with cows with late onset.

Blood BHB concentration is a popular tool for diagnoses of hyperketonemia and subclinical ketosis. The increase in blood BHB concentration can be also seen as an elevated concentration of BHB in milk. Enjalbert et al. (2001) found a correlation of 0.66 and Denis-Robichaud et al. (2014) a correlation of 0.89 between BHB concentrations in blood and milk. In our data, milk BHB concentrations for predicting a cow's ES were measured from the samples taken automatically by HN. In all herds, the samples were taken 1 to 2 times daily during the first period, and every day to every fourth day in later periods. This means that the cow's mean BHB concentration in each period was an average of multiple measures. The other ES indicators based on test-day milk samples were taken once a month or once every 2 mo, so their values in each period represent a single day's measurement. Because of repeated measurements, BHB could be expected to predict ES accurately. However, correlations between BHB and C-LA and CFH did not differ from those between fertility traits and test-day measures of $NEFA_{mir}$, $NEFA_{fa}$, C18:1 *cis*-9, and C18:1 *cis*-9/C10:0. Notably higher correlations were measured only for multiparous cows in period 2. Relatively small gains from more frequent sampling could be in part because BHB can be used in the mammary gland for de novo synthesis of FA (Bauman and Griinari, 2003), which may vary between cows. We also cannot ignore the possible effect of feeding differences in cows on their ruminal production of butyrate, which is absorbed by the rumen wall and released as BHB. Huhtanen et al. (1993) found that a greater proportion of blood BHB is apparently removed in the milk when blood BHB increases as a consequence of increased ruminal butyrate production.

Our results showed some associations between ES indicators and fertility traits during the early weeks of lactation. Because the detrimental effect of negative ES on fertility is well documented, we can conclude that proposed readily available milk-based ES indicators are potential tools for management. They can help farmers identify cows potentially susceptible to metabolic stress and production diseases and to check the appropriateness of feeding practices and timing of insemination.

CONCLUSIONS

The measured relationships between fertility traits (C-LA and CFH) based on frequent measurements of P4 by HN and ES indicators indicated that increases in ES indicators during the first weeks of lactation can be harmful for the fertility of cow. Therefore, monitoring a cow's ES after calving is important. These readily available ES indicators—milk BHB provided by HN and plasma NEFA predictions and milk FA concentrations based on MIR spectra of test-day milk samples—are potentially useful tools for management purposes. Test-day milk FPR was not as good of an indicator of cow's ES as the other indicators.

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