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Body and milk traits as indicators of dairy cow energy status in early lactation

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ABSTRACT

The inclusion of feed intake and efficiency traits in dairy cow breeding goals can lead to increased risk of metabolic stress. An easy and inexpensive way to monitor postpartum energy status (ES) of cows is therefore needed. Cows' ES can be estimated by calculating the energy balance from energy intake and output and predicted by indicator traits such as change in body weight (ΔBW), change in body condition score (ΔBCS), milk fat:protein ratio (FPR), or milk fatty acid (FA) composition. In this study, we used blood plasma nonesterified fatty acids (NEFA) concentration as a biomarker for ES. We determined associations between NEFA concentration and ES indicators and evaluated the usefulness of body and milk traits alone, or together, in predicting ES of the cow. Data were collected from 2 research herds during 2013 to 2016 and included 137 Nordic Red dairy cows, all of which had a first lactation and 59 of which also had a second lactation. The data included daily body weight, milk yield, and feed intake and monthly BCS. Plasma samples for NEFA were collected twice in lactation wk 2 and 3 and once in wk 20. Milk samples for analysis of fat, protein, lactose, and FA concentrations were taken on the blood sampling days. Plasma NEFA concentration was higher in lactation wk 2 and 3 than in wk 20 (0.56 ± 0.30, 0.43 ± 0.22, and 0.13 ± 0.06 mmol/L, respectively; all means ± standard deviation). Among individual indicators, C18:1 *cis*-9 and the sum of C18:1 in milk had the highest correlations (r = 0.73) with NEFA. Seven multiple linear regression models for NEFA prediction were developed using stepwise selection. Of the models that included milk traits (other than milk FA) as well as body traits, the best fit was achieved by a model with milk yield, FPR, ΔBW, ΔBCS, FPR × ΔBW, and days in milk. The model resulted in a cross-validation coefficient of determination (R^2cv) of 0.51 and a root mean squared error (RMSE) of 0.196 mmol/L. When only milk FA concentrations were considered in the model, NEFA prediction was more accurate using measurements from evening milk than from morning milk (R^2cv = 0.61 vs. 0.53). The best model with milk traits contained FPR, C10:0, C14:0, C18:1 *cis*-9, C18:1 *cis*-9 × C14:0, and days in milk (R^2cv = 0.62; RMSE = 0.177 mmol/L). The most advanced model using both milk and body traits gave a slightly better fit than the model with only milk traits (R^2cv = 0.63; RMSE = 0.176 mmol/L). Our findings indicate that ES of cows in early lactation can be monitored with moderately high accuracy by routine milk measurements.
Key words: energy status, indicator, dairy cattle

INTRODUCTION

In the beginning of lactation, the feed intake of high-producing cows seldom fulfills their energy demands (Mäntysaari and Mäntysaari, 2010; Mäntysaari et al., 2012). To fill the energy deficit, cows are forced to mobilize energy from their body reserves, resulting in a negative energy status (**ES**). Even though a negative ES is acceptable for today's high producing cows for a few weeks in early lactation, a deep and long-lasting negative ES can cause health and reproduction problems (de Vries et al., 1999; Collard et al., 2000). One way to cope with increasing metabolic stress (see Knight et al., 1999) and health problems related to a prolonged negative ES is to add postpartum ES into the breeding program. Moreover, as it is expected that future breeding goals will include feed efficiency traits, postpartum ES needs to be monitored to minimize the risk of increasing metabolic stress by selection (Veerkamp and Koenen, 1999).

Cows' ES can be estimated by calculating the energy balance (**EB**) from their energy intake and output. This calculation ($\mathbf{EB_{inout}}$ = energy intake − energy required for milk and maintenance) requires knowledge of the cows' milk production and composition, feed DMI,

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BW, and energy density of the diet. However, because DMI measurements are difficult to carry out at the farm level, it is necessary to develop easier methods for estimating ES of the cows.

Body condition scoring is a technique for estimating the body fat content of dairy cows (Edmonson et al., 1989). Mobilization of body reserves during negative ES will inevitably decrease cows' BCS and BW. Changes in BCS (**ΔBCS**) and changes in BW (**ΔBW**) can therefore be used as indicators of ES. For example, in the studies of Coffey et al. (2001) and Friggens et al. (2007), EB was calculated based on ΔBW and ΔBCS by converting ΔBW into weights of body lipid and protein. Mäntysaari and Mäntysaari (2010) applied a multiple linear regression model including BW and BCS and their changes to predict EB_{inout} on the first test day. The use of ΔBCS and ΔBW as indicators of ES requires frequent measurements of BW and BCS, which has been made possible by the increasing popularity of automated weighing and body condition scoring systems on commercial farms.

It is well known that the milk composition of dairy cows is affected by their ES, especially during early lactation (Stoop et al., 2009; Gross et al., 2011; Jorjong et al., 2014). Cows in a negative ES mobilize their adipose tissue, which elevates the concentration of nonesterified fatty acids (**NEFA**) in blood (Dunshea et al., 1989; Grummer, 1993). This increased supply of fatty acids (**FA**) for milk fat synthesis leads to higher milk fat content and milk fat:protein ratio (**FPR**). The mobilization of adipose tissue increases the supply of long-chain FA (**LCFA**) in particular. The high uptake of LCFA by the mammary gland inhibits de novo synthesis of short-chain FA and medium-chain FA (**MCFA**), causing changes in milk FA composition (Palmquist et al., 1993; Stoop et al., 2009; Gross et al., 2011). Thus, several studies have examined milk composition and especially milk FPR, as well as milk FA concentrations or ratios of individual FA, as ES indicators (de Vries and Veerkamp, 2000; Heuer et al., 2000; Reist et al., 2002; Friggens et al., 2007; Gross et al., 2011; Dórea et al., 2017; Vranković et al., 2017). Milk composition and its changes are good candidates for ES predictors because they can be measured from routinely collected test-day milk samples at low cost by mid-infrared spectroscopy (**MIR**). Prediction of cows' ES directly from the MIR spectrum of milk has also been investigated in a few studies (McParland et al., 2011, 2012; Mehtiö et al., 2018a).

The usefulness and accuracy of ES predictions based on the above indicators depend not only on the correlation between the predictor and ES but also on the precision of the trait estimate used to represent ES of the cow. For example, the estimated EB_{inout} value lacks precision if its calculation is based on standard energy requirements (Chwalibog, 1991; Mäntysaari et al., 2012; Mehtiö et al., 2018b). Because the concentration of NEFA in the blood increases with elevated mobilization of body fat, we used the plasma NEFA concentration as a biomarker to represent cows' negative ES in this study. Thus, the objectives of this study were to examine the associations between plasma NEFA concentration and milk and body traits based on ES indicators and to evaluate the usefulness of them in predicting the ES of dairy cows assessed by plasma NEFA concentration.

MATERIALS AND METHODS

Animals and Feeding

Data were collected during 2013 to 2016 from Luke Jokioinen and University of Helsinki (**UH**) research herds. It included measurements from 137 Nordic Red dairy cows, all of which had a first lactation and 59 of which also had a second lactation. All cows in the Luke Jokioinen herd were housed in a freestall barn, whereas some cows in the UH herd were kept in tiestalls in early lactation before being moved to a freestall barn. Cows in the Luke Jokioinen herd were milked twice a day (0630 and 1600 h) in a 2 × 6 auto-tandem milking parlor. At the UH herd, the cows in tiestalls were milked twice daily at 0630 and 1700 h and those in loose housing were milked using an automatic milking system (Lely Astronaut A3, Lely Industries N.V., Maassluis, the Netherlands). All cows had ad libitum feeding and were fed grass silage and a concentrate mix. At the Luke Jokioinen herd, silage was fed 4 times a day, and concentrate was fed separately using concentrate feeders with 5 feeding periods per day; in addition, each cow received 0.3 kg of concentrate during each milking. At the UH herd, the cows kept in tiestalls were fed grass silage and concentrate separately, whereas cows in the freestall barn received a partial mixed ration of grass silage and concentrate and additional concentrate from the milking robot. The mean intakes of concentrate and silage during the first 180 d of lactation are presented in Table 1.

Measurements and Sampling

Individual daily milk yields and feed intakes were recorded for all cows. The intake of grass silage and partial mixed ration was measured by automatic feed troughs (Insentec BV, Marknesse, the Netherlands). Samples of grass silage for feed analysis were taken twice a week. The subsamples were combined into a maximum 4-wk sample for analysis. Samples were

Table 1. Mean, standard deviation, and range of ECM production, feed intake, BW, and BCS of cow-wise averages during lactation d 2 to 180

Item	Mean	SD	Minimum	Maximum
Primiparous cows (n = 137)				
ECM, kg/d	29.6	3.82	15.7	37.7
Milk fat, %	4.32	0.40	3.26	5.26
Milk protein, %	3.52	0.19	3.09	4.15
Lactose, %	4.59	0.12	3.93	4.86
Intake, kg of DM/d	19.7	1.75	14.5	24.6
Concentrate, kg of DM/d	9.7	0.78	6.4	11.3
Silage, kg of DM/d	10.0	1.14	7.4	13.3
Energy intake, MJ of ME/d	214	17.1	164	261
BW,[1] kg	575	51.0	456	699
Gain, kg/d	−0.04	0.29	−0.77	0.69
BCS	3.21	0.29	2.17	4.36
Energy balance,[2] MJ of ME/d	−4.8	17.1	−42.3	46.6
Energy conversion efficiency[3]	0.141	0.016	0.086	0.172
Multiparous cows (n = 59)				
ECM, kg/d	38.9	4.39	27.3	46.0
Milk fat, %	4.35	0.46	3.12	5.28
Milk protein, %	3.47	0.18	3.15	3.96
Lactose, %	4.49	0.13	3.93	4.73
Intake, kg of DM/d	23.4	2.04	17.2	28.1
Concentrate, kg of DM/d	11.1	1.24	6.3	14.5
Silage, kg of DM/d	12.3	1.29	7.7	14.9
Energy intake, MJ of ME/d	257	25.7	196	330
BW,[1] kg	643	55.6	551	770
Gain, kg/d	−0.03	0.27	−0.67	0.55
BCS	3.02	0.29	2.40	4.05
Energy balance,[2] MJ of ME/d	−8.3	26.2	−78.0	45.0
Energy conversion efficiency[3]	0.153	0.019	0.110	0.212

[1]Smoothed by regression model with fixed function of DIM and random animal effect (Mäntysaari and Mäntysaari, 2015).

[2]Energy intake − energy required for milk and maintenance.

[3]Energy conversion efficiency = daily ECM (kg)/daily ME intake (MJ).

analyzed for DM, ash, CP, NDF, quality of silage fermentation, and in vitro OM digestibility. Concentrate samples were collected once a week and combined to give a 4-wk sample for analysis. These samples were analyzed for DM, ash, CP, ether extract, and NDF. Dry matter was determined by drying at 105°C for 20 h. Silage DM was corrected for loss of volatiles according to Huida et al. (1986). Organic matter of the feeds was determined by ashing at 600°C for 2 h. The analyses of NDF, CP, ether extract, and quality of silage fermentation were performed using procedures described previously by Mäntysaari et al. (2007). Silage was analyzed for pepsin-cellulase solubility (Nousiainen et al., 2003), and the obtained solubility values were converted to digestible OM content in DM (referred to as D-values) using different equations for primary and regrowth grass silages according to Huhtanen et al. (2006).

Milk samples for analyses of fat, protein, lactose, and MIR spectral readings were taken at each milking on 2 d, 3 or 4 d apart from each other, in lactation wk 2 (8–14 DIM) and 3 (15–21 DIM) and on 1 day in lactation wk 20. The MIR spectra of the milk samples were acquired with a MilkoScan FT6000 spectrometer (Foss, Hillerød, Denmark) in the Valio Ltd. milk laboratory (Seinäjoki, Finland). Milk fat, protein, and lactose contents were provided by the predictive models of the manufacturer. However, due to technical problems, milk MIR spectral readings were not available for all cows on all sampling days; thus, the MIR data only included 127 first-lactation and 49 second-lactation cows. As a result, the milk data included 966 milk fat, protein, and lactose measurements from morning and evening samples and 761 and 764 MIR spectral readings for milk FA prediction from morning and evening samples, respectively.

Blood samples for plasma NEFA concentration analyses were taken on milk sampling days in wk 2, 3, and 20 of lactation. The samples were taken from the coccygeal vein once a day after the morning milking. At Luke, herd samples were taken before the day's first forage delivery, and at UH herd samples were taken immediately after the first daily visit to the milking robot. Blood was collected in 10-mL EDTA tubes and stored in ice until centrifuged at −4°C for 15 min at 2,000 × *g*. Plasma samples were frozen and stored at −20°C for later analysis of NEFA. Plasma NEFA concentra-

tion was determined with an enzymatic colorimetric acyl-CoA synthetase–acyl-CoA oxidase method [NEFA-HR(2) kit, Wako Chemicals GmbH, Neuss, Germany]. The data included 963 NEFA measurements in total.

Cows in the Luke Jokioinen herd were automatically weighed and BW recorded using a walk-through static scale (Pellon Group Oy, Ylihärmä, Finland) on their return from morning and evening milkings. At the UH freestall barn, cow BW was obtained during automatic milking. Cows kept in tiestalls were weighed once a week on 2 consecutive days. Body condition scores were assessed within herds by the same evaluator on a scale of 1 to 5 (1 = skinny; 5 = very fat) with intervals of 0.25 (Edmonson et al., 1989) every fourth week.

A small set of data from the Luke Kuopio research herd was used as external validation data. Data collection, milk and blood sampling, and milk and blood sample analyses were done following the same protocol as for the main data; results are presented as means ± standard deviation. The validation data included 48 measurements of daily milk yields (27.2 ± 4.3 kg/d), milk fat (4.71 ± 0.65%), protein (3.32 ± 0.25%), and lactose (4.62 ± 0.15%) concentration and plasma NEFA concentration from 16 Nordic Red dairy cows in lactation wk 2, 3, and 20. The average NEFA concentration in the validation data was 0.445 ± 0.240 mmol/L, varying from 0.087 to 1.256 mmol/L. The corresponding milk MIR spectral-based FA concentrations from morning milk samples were available for all 48 NEFA measurements, whereas milk FA concentrations were available for only 13 evening milk samples.

Calculations and Models

Daily DM and ME intake and ECM yield were calculated for each cow. Grass silage ME content was calculated as 0.016 × D-value (MAFF, 1975, 1984). The ME content of the concentrate was determined from digestible nutrients (MAFF, 1975, 1984). Digestibility coefficients for the concentrate components were obtained from the Finnish feed tables (Luke, 2017). Daily ME intake was corrected using total DMI and concentration of ME and protein in the diet according to the correction equation provided by Luke (2017).

Sampling day-average milk fat, protein, and lactose contents were calculated based on morning and evening milk yields and milk composition. Milk FA concentrations were predicted from milk MIR spectral readings using calibration equations (Soyeurt et al., 2011). Performances of the individual prediction equations used are reported in Soyeurt et al. (2011). Only FA and FA groups with fair ($R^2 \geq 0.89$), good ($R^2 \geq 0.97$), or excellent ($R^2 \geq 0.99$) prediction accuracy (Grelet et al., 2014) were considered in modeling. The considered FA and FA groups are listed in Table 2. The ECM yield was calculated according to Sjaunja et al. (1990). To obtain daily BCS, we assumed a linear change between measurements. The EB_{inout} (MJ of ME/d) was calculated for each cow by subtracting the energy required for milk and maintenance from the cow's total energy intake. The ME used for ECM [ME_{milk} (MJ) = 5.15 × ECM (kg)] and for maintenance [$ME_{maintenance}$ (MJ) = 0.515 × $BW^{0.75}$ (kg)] were based on the Finnish requirements (Luke, 2017). Average daily BW for each cow was determined from daily BW measurements. To correct daily variation in BW, the BW was smoothed by a regression model with a fixed function of DIM and a random animal effect (Mäntysaari and Mäntysaari, 2015).

We used plasma NEFA concentration as a biomarker for ES in this study. First, the relationships between different ES indicators and NEFA were quantified by Pearson correlations. Finally, we developed multiple linear regression models to predict NEFA from milk and body traits using stepwise regression (PROC GLMSELECT in SAS; SAS Institute Inc., Cary, NC). A significance level of $P < 0.05$ was used as a selection criterion. The developed models were validated with a *k*-fold cross-validation method by splitting the data into 137 parts based on the cow ID; thus, the measurements of each cow were excluded in turn. The predictor variables made available for model-specific stepwise regression analyses are listed in Table 2. In the first modeling stage (**M1**), we only considered milk traits (except milk FA concentrations) in model selection. In the second modeling stage (**M2**) we added BW and BCS, and in the third stage (**M3**) we included all body and milk traits (except milk FA concentrations) in model selection. In models **M4** and **M5** we compared the predictive value of the concentrations of selected (Grelet et al., 2014) FA in morning and evening milk samples, respectively. Based on this comparison, the FA concentrations in evening milk were then selected for use in models **M6** and **M7**. All milk traits were made available for selection for model M6, and all milk and body traits were made available for selection for model M7. First-order interactions between milk and body traits and between milk FA or FA groups were examined in the models. All models also considered parity, herd, interactions between herd and parity, and DIM as piecewise variables DIM1 and DIM2; **DIM1** represented DIM less than 60 d, and **DIM2** represented DIM more than 60 d.

A comparison of the goodness of fit of the developed NEFA prediction models was made using the root mean squared error (**RMSE**) of the NEFA estimate as well as the sum of the *k*-predicted residual sum of squares from *k*-fold cross-validation (**CVPRESS**); the CV-

Table 2. Variables made available for selection using stepwise regression in different steps of modeling

Variable for selection[1]	Modeling step[2]						
	M1	M2	M3	M4	M5	M6	M7
Milk yield, kg	x	x	x			x	x
Milk fat, %	x	x	x			x	x
Milk protein, %	x	x	x			x	x
Milk lactose, %	x	x	x			x	x
Milk FPR	x	x	x			x	x
BW, kg		x	x				x
BCS		x	x				x
ΔBW, kg/d			x				x
ΔBCS			x				x
Milk FA in morning milk[3]				x			
Milk FA in evening milk[3]					x	x	x
DIM1	x	x	x	x	x	x	x
DIM2	x	x	x	x	x	x	x
Parity	x	x	x	x	x	x	x
Herd	x	x	x	x	x	x	x
Parity × herd	x	x	x	x	x	x	x

[1]FPR = milk fat:protein ratio; ΔBW = change in BW; ΔBCS = change in BCS; FA = fatty acids; DIM1 = DIM <60 d; DIM2 = DIM ≥60 d.

[2]The modeling stage M1 considered milk traits (except milk FA concentrations) in model selection. In stage M2 BW and BCS were also included, and in stage M3 all body and milk traits (except milk FA concentrations) were included in model selection. In models M4 and M5, FA in morning and evening milk samples were available for selection, respectively. All milk traits were made available for selection for model M6, and all milk and body traits were made available for selection for model M7.

[3]Predicted from mid-infrared spectrometer spectral readings. FA and FA groups: C4:0, C10:0, C12:0, C14:0, C16:0, C18:1 *cis*-9, sum of C18:1 *cis*, sum of C18:1, SFA, MUFA, UFA, short-chain FA, medium-chain FA, and long-chain FA.

PRESS over the 137 validation samples was expressed relative to the total corrected sum of squares (totSS), giving the proportion of variance not explained by the model. The remaining variance [i.e., 1 − (CVPRESS/totSS)] was called the coefficient of determination of cross-validation ($\mathbf{R^2cv}$) of the NEFA prediction for cows not used in estimating the prediction equation. We further evaluated the goodness of fit using the external validation data set. The plasma NEFA concentration of the cows in that data set was predicted using the developed models. The prediction accuracy of the models was described by the correlation of observed and predicted NEFA concentrations. Multicollinearity was evaluated by calculating the variance inflation factor (**VIF**; PROC REG in SAS) for the predictors in each model.

RESULTS

Indicator Traits

The estimated average ECM yields (± SD) were 29.6 ± 3.82 and 38.9 ± 4.39 kg/d for primiparous and multiparous cows, respectively (Table 1). Primiparous cows ate on average 19.7 ± 1.75 kg of DM/d, and those in second lactation ate 23.4 ± 2.04 kg of DM/d. Energy intake was 214 ± 17.1 and 257 ± 25.7 MJ of ME/d for primiparous and multiparous cows, respectively. First-lactation cows had an average BW of 575 ± 51.0 kg, whereas second-lactation cows weighed 643 ± 55.6 kg on average (Table 1). The average BW of all cows decreased during the first month of lactation, after which it started to increase (Figure 1), leading in both parities to a close-to-zero average daily ΔBW during the first 180 d of lactation (Table 1). Figure 1 shows the lactation week averages for EB_{inout} (MJ of ME/d), which was at its lowest during the first 2 wk of lactation and turned positive in lactation wk 12 and 11 for primiparous and multiparous cows, respectively. In the cows' BCS a noticeable decrease was observed during the first 9 to 10 wk (Figure 2), after which it slowly increased. This increase was greater for cows in second lactation than in first lactation. Milk FPR increased during the first weeks of lactation, reaching its peak in wk 5 and 6 of lactation for first- and second-lactation cows, respectively (Figure 2).

Plasma NEFA concentration was measured twice in lactation wk 2 and 3 and once in lactation wk 20. As expected, the plasma NEFA concentration was clearly higher in wk 2 and 3 than in wk 20 (Figure 3). An interaction was found between parity and herd in plasma NEFA concentration in lactation wk 2 and 3 ($P < 0.001$). In the Luke herd, the NEFA concentration was higher for primiparous than for multiparous

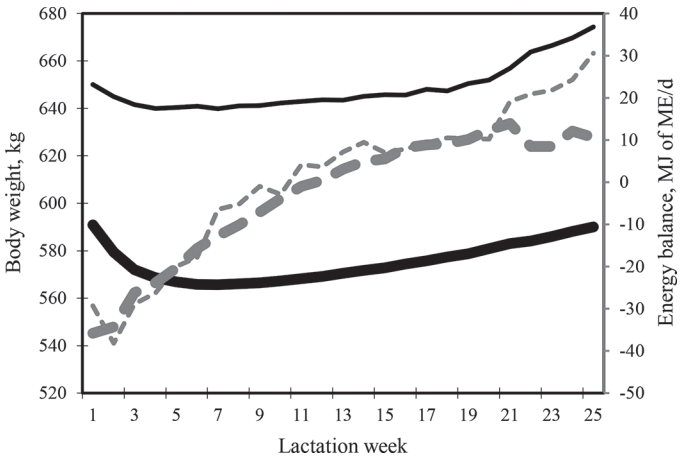

Figure 1. Development of BW (black solid lines) and calculated energy balance (gray dashed lines) of primiparous cows (thick lines) and second-lactation cows (thin lines) during the first 180 d of lactation.

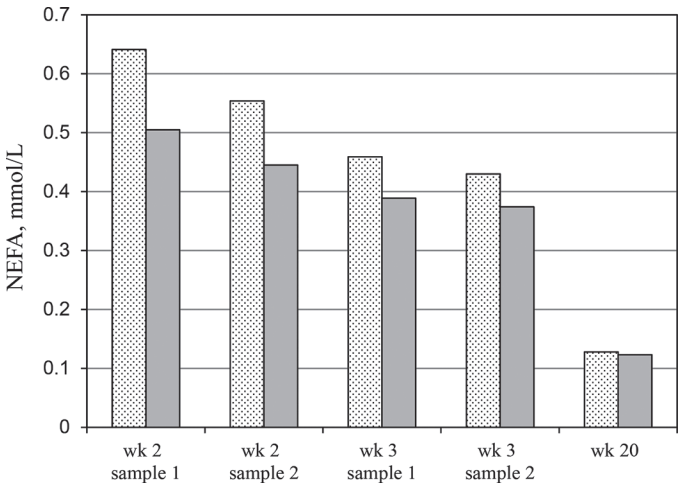

Figure 3. Plasma nonesterified fatty acids (NEFA) concentration (mmol/L) of primiparous cows (dotted bar) and second-lactation cows (solid bar) in lactation wk 2, 3, and 20.

cows (0.582 and 0.409 mmol/L for primiparous and multiparous cows, respectively) and vice versa in the UH herd (0.329 and 0.479 mmol/L for primiparous and multiparous cows, respectively). In lactation wk 20, the concentration was about the same for both parities in both herds.

Figure 4 describes the concentrations of milk FA (g/100 mL of milk) in morning and evening milk based on MIR spectral readings in lactation wk 2, 3, and 20. Only FA and FA groups with fair, good, or excellent accuracy of prediction (Grelet et al., 2014) are presented. The concentration of fat and, thus, of FA was higher in milk samples taken at the evening milking. The LCFA concentration was markedly higher and MCFA concentration was lower in milk samples taken in lactation wk 2 and 3 than in wk 20. In lactation wk 2 the concentration of the sum of C18:1 in milk was the highest, whereas in mid lactation, in wk 20, the concentration of C16:0 was higher than that of the sum of C18:1.

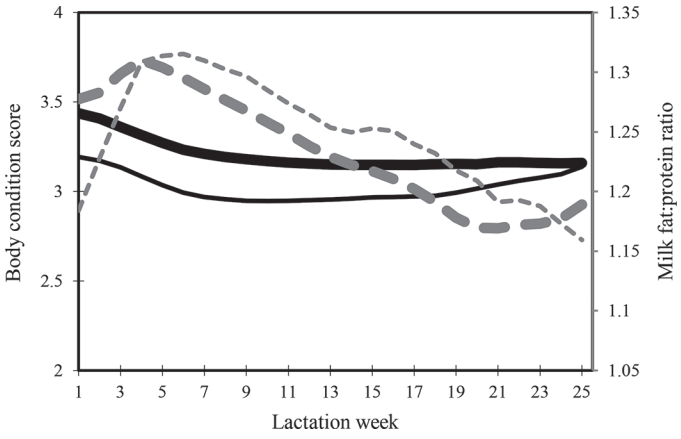

Figure 2. Development of BCS (black solid lines) and milk fat: protein ratio (gray dashed lines) of primiparous cows (thick lines) and second-lactation cows (thin lines) during the first 180 d of lactation.

Correlations of Plasma NEFA with ES Indicators

The Pearson correlations between the cows' plasma NEFA concentration and the considered ES indicators are shown in Table 3. Of the studied body traits, ΔBW had the highest correlation with plasma NEFA concentration (r = −0.51), whereas the correlation between ΔBCS and plasma NEFA was lower (r = −0.29). Moderate correlations between plasma NEFA concentration and milk fat concentration (r = 0.47) and milk FPR (r = 0.41) were also found. Among milk FA and FA groups, the highest correlation was found for the sum of C18:1 and for C18:1 *cis*-9 with plasma NEFA. These correlations were higher for concentrations measured in evening milk (ΣC18:1 r = 0.73 and C18:1 *cis*-9 r = 0.73) than for those measured in morning milk (ΣC18:1 r = 0.64 and C18:1 *cis*-9 r = 0.64).

Prediction Models for Plasma NEFA

The solutions of the developed NEFA prediction models are presented in Table 4. When only milk traits (other than milk FA concentrations) were considered as predictors in stepwise regression modeling (M1), milk yield and milk FPR together with DIM and parity were selected in the model. The R^2cv for model M1 was 0.47, with an RMSE for NEFA of 0.206 mmol/L (Table 4). When BW and BCS were considered as predictors together with milk traits (M2), BCS was included in the model but not BW. This inclusion did not improve the

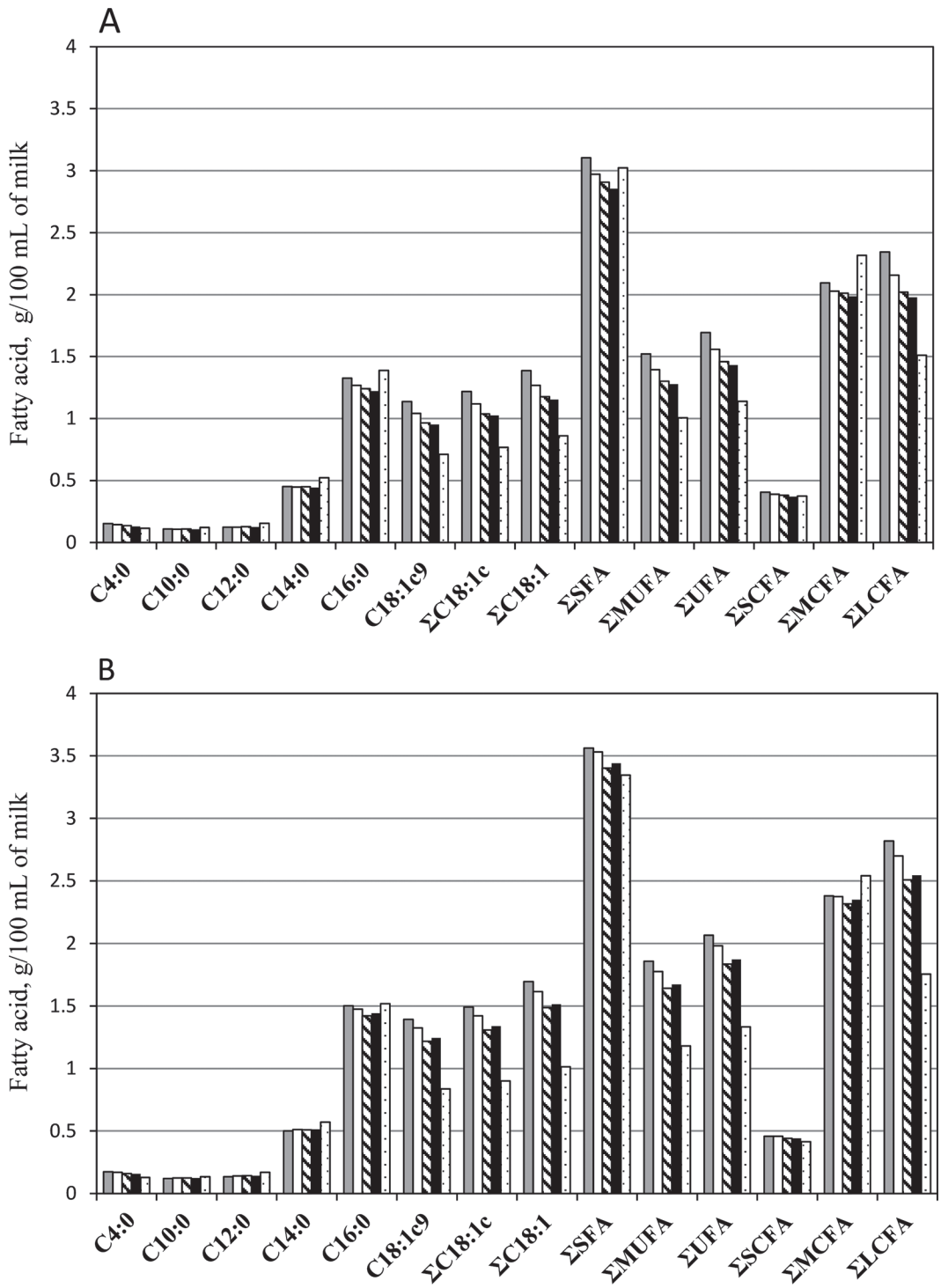

Figure 4. Mean concentrations (based on mid-infrared spectroscopy prediction) of milk fatty acids and group of fatty acids (g/100 mL of milk) considered for modeling. The milk samples were taken at morning (A) and evening (B) milkings in lactation wk 2 (gray = sample 1; white = sample 2), 3 (striped = sample 1; black = sample 2), and 20 (dotted). SCFA = short-chain fatty acids; MCFA = medium-chain fatty acids; LCFA = long-chain fatty acids; c = *cis*.

prediction: R^2cv was 0.47 and RMSE was 0.205 mmol/L (Table 4). However, an improvement was achieved by also considering changes in BW and BCS as NEFA predictors in model M3. Model M3, which included milk yield, milk FPR, ΔBW, ΔBCS, and interaction of ΔBCS and milk FPR, explained 51.3% of the variation in NEFA, with an RMSE of 0.196 mmol/L.

We then considered the concentrations of FA and FA groups in morning milk in model M4 and in evening milk in model M5 as predictors of NEFA. The R^2cv value was higher using FA and FA groups in evening milk (M5: R^2cv = 0.61) instead of morning milk (M4: R^2cv = 0.52; Table 4). With model M5, the RMSE for NEFA prediction was 0.182 mmol/L. The FA predictors selected in models M4 and M5 were different. High associations between plasma NEFA and LCFA concentrations, especially C18 FA, could be expected. The best predictability was attained using sum of C18:1 with morning milk samples (Table 4; M4) and C18:1 *cis*-9 with evening milk samples (Table 4; M5). In addition, in model M4 MUFA and LCFA were selected into the models, and in model M5 MCFA and interaction of C18:1 *cis-9* and MCFA were selected into the models. Changes in these traits were in the opposite direction to changes in NEFA, resulting in negative regression coefficients.

According to models M4 and M5, the FA concentration in evening milk samples was better at predicting the plasma NEFA concentration than the FA concentration in morning samples. Therefore, we used only FA concentrations from evening milk in models M6 and M7. In model M6, besides milk FA concentrations, all other milk traits were also considered. All of these traits are easily available from cows in normal milk recording. The traits selected in model M6 included concentrations of C10:0, C14:0, C18:1 *cis*-9, and interaction between C18:1 *cis*-9 and C14:0 as well as milk FPR as the only other milk trait (Table 4). Model M6 predicted the plasma NEFA concentration slightly better than model M5 (R^2cv = 0.62 vs. 0.61) and decreased the RMSE (0.177 vs. 0.182 mmol/L). In model M7, all body and milk traits including milk FA were considered as predictors. Of milk traits, FPR, C12:0, C14:0, and C18:1 *cis*-9 were selected into the model, and ΔBW as the only body trait, resulting in R^2cv = 0.63 and RMSE = 0.176 mmol/L (Table 4). High multicollinearity (VIF > 10) was observed between the FA groups selected into model M4 as well as between C10:0 and C14:0 in

Table 3. Pearson correlation coefficients between cows' plasma nonesterified fatty acid (mmol/L) concentration and studied energy status indicators

Indicator trait[1]	r	*P*-value		
Milk yield, kg/d	−0.11	0.0004		
Milk fat, %	0.47	<0.0001		
Milk protein, %	0.12	0.0002		
Milk lactose, %	−0.08	0.0161		
Milk FPR	0.41	<0.0001		
BW, kg	−0.03	0.4372		
ΔBW, kg/d	−0.51	<0.0001		
BCS	0.23	<0.0001		
ΔBCS	−0.29	<0.0001		
	Morning milk		Evening milk	
Milk FA or FA groups, g/100 mL of milk	r	*P*-value	r	*P*-value
C4:0	0.40	<0.0001	0.50	<0.0001
C10:0	−0.33	<0.0001	−0.28	<0.0001
C12:0	−0.42	<0.0001	−0.39	<0.0001
C14:0	−0.35	<0.0001	−0.27	<0.0001
C16:0	−0.10	0.0051	0.05	0.1669
C18:1 *cis*-9	0.64	<0.0001	0.73	<0.0001
ΣC18:1 *cis*	0.63	<0.0001	0.73	<0.0001
ΣC18:1	0.64	<0.0001	0.73	<0.0001
ΣSFA	0.00	0.9719	0.17	<0.0001
ΣMUFA	0.61	<0.0001	0.71	<0.0001
ΣUFA	0.61	<0.0001	0.71	<0.0001
ΣSCFA	−0.02	0.6403	0.09	0.0134
ΣMCFA	−0.21	<0.0001	−0.06	0.0828
ΣLCFA	0.61	<0.0001	0.70	<0.0001

[1]FPR = milk fat:protein ratio; ΔBW = change in BW; ΔBCS = change in BCS; FA = fatty acids; SCFA = short-chain FA; MCFA = medium-chain FA; LCFA = long-chain FA.

model M6 and C12:0 and C14:0 in model M7, which could generate illogical regression coefficients. However, this should cause no problem when the models are used only for prediction.

The robustness of the developed prediction equations was tested with a small external validation data set by calculating the correlations between observed and predicted plasma NEFA concentrations. However, because the validation data set was very small, the findings are only indicative. In models M1, M2, and M3, the correlations between observed and predicted NEFA were 0.49 (n = 48), 0.49 (n = 48), and 0.43 (n = 48), respectively. When only morning milk FA composition was used in the prediction (M4), the correlation was 0.53 (n = 48). A marked increase in prediction accuracy was achieved when the evening milk FA concentrations were used as a predictor either alone (M5) or together with milk FPR (M6). In model M5, the correlation between predicted and observed NEFA was 0.93 (n = 13), and in M6 it was 0.95 (n = 13). When only those 13 morning milk measurements that had FA concentrations for both morning and evening milk samples were used in

Table 4. Solutions of different predictor models for cows' plasma nonesterified fatty acid concentration (mmol/L) using milk and body traits as predictor variables and fitting statistics of the built models[1]

	Intercept		Linear coefficient					
Model[2]	Estimate	SE	Variable	Estimate	SE	*P*-value	RMSE, mmol/L	R^2cv
M1	−0.085	0.066	Milk yield	0.006	0.001	<0.0001	0.206	0.47
			Milk FPR	0.474	0.040	<0.0001		
			DIM1	−0.022	0.002	<0.0001		
			DIM2	−0.005	0.000	<0.0001		
			Parity	0.008	0.034	0.0002		
M2	−0.375	0.106	Milk yield	0.007	0.001	<0.0001	0.205	0.47
			Milk FPR	0.476	0.040	<0.0001		
			BCS	0.077	0.022	0.0005		
			DIM1	−0.022	0.002	<0.0001		
			DIM2	−0.005	0.000	<0.0001		
			Parity	0.011	0.034	0.0005		
M3	0.033	0.070	Milk yield	0.004	0.001	0.0016	0.196	0.51
			Milk FPR	0.319	0.045	<0.0001		
			ΔBCS	−2.233	0.649	0.0006		
			ΔBW	0.153	0.043	0.0004		
			FPR × ΔBW	−0.154	0.033	<0.0001		
			DIM1	−0.018	0.002	<0.0001		
			DIM2	−0.004	0.000	<0.0001		
			Parity	0.039	0.033	0.2337		
M4	0.310	0.061	ΣC18:1	3.270	0.479	<0.0001	0.198	0.52
			ΣMUFA	−2.014	0.499	<0.0001		
			ΣLCFA	−0.493	0.111	<0.0001		
			DIM1	−0.009	0.002	<0.0001		
			DIM2	−0.002	0.000	<0.0001		
M5	−0.002	0.087	C18:1 *cis*-9	0.728	0.058	<0.0001	0.182	0.61
			ΣMCFA	−0.010	0.033	0.7642		
			C18:1 *cis*-9 × ΣMCFA	−0.080	0.022	0.0002		
			DIM1	−0.011	0.002	<0.0001		
			DIM2	−0.002	0.000	<0.0001		
M6	0.032	0.086	Milk FPR	0.166	0.054	0.0026	0.177	0.62
			C10:0	2.719	0.753	0.0003		
			C14:0	−1.061	0.272	0.0001		
			C18:1 *cis*-9	0.613	0.069	<0.0001		
			C14:0 × C18:1 *cis*-9	−0.286	0.102	0.0044		
			DIM1	−0.012	0.002	<0.0001		
			DIM2	−0.0023	0.000	<0.0001		
M7	0.164	0.059	Milk FPR	0.244	0.052	<0.0001	0.176	0.63
			ΔBW	−0.034	0.006	<0.0001		
			C12:0	2.529	0.734	0.0006		
			C14:0	−1.576	0.301	<0.0001		
			C18:1 *cis*-9	0.429	0.033	<0.0001		
			DIM1	−0.011	0.002	<0.0001		
			DIM2	−0.002	0.000	<0.0001		

[1]RMSE = root mean squared error; R^2cv = coefficient of determination from *k*-fold cross-validation where the observations for each cow were predicted using data from all the others; FPR = fat:protein ratio; DIM1 = DIM <60 d; DIM2 = DIM ≥60 d; ΔBCS = change in BCS; ΔBW = change in BW; LCFA = long-chain FA; MCFA = medium-chain FA.

[2]Traits considered for stepwise selection for different models are listed in Table 2.

model M4, the correlation between observed and predicted NEFA was 0.62.

DISCUSSION

Monitoring the ES of individual cows is important for management as well as for breeding purposes. It helps farmers to predict which cows are potentially susceptible to metabolic stress and production diseases and to check the appropriateness of current management and nutrition practices. If, as anticipated, feed efficiency will become a part of future breeding goals, selection for this trait can lead to decreased DMI, which in turn may increase the risk of a deep and long-lasting negative ES of dairy cows. Thus, in addition to feed efficiency, it would be important to include cows' ES in the breeding goal as well. An easy and inexpensive way to monitor ES of cows is therefore needed.

Plasma NEFA as Cow ES Biomarker

The aim of our study was to evaluate the usefulness of milk and body traits as predictors of the ES of individual cows. We chose plasma NEFA concentration as an ES marker instead of calculated EB_{inout} because the use of standard estimates for cows' energy requirements can introduce considerable errors in the calculation of EB_{inout}. Differences in energy utilization between cows can arise from differences in digestion (Berry et al., 2007) and utilization of ME for various functions (Chwalibog, 1991; Mäntysaari et al., 2012; Mehtiö et al., 2018b). In contrast to calculated EB_{inout} values, plasma NEFA concentration should be a suitable biomarker for negative ES because its concentration in the blood increases with increased fat mobilization (Dunshea et al., 1989; Grummer, 1993). In the current study, the correlation between observed NEFA and EB_{inout} was only −0.48 when EB_{inout} was based on energy intake on the blood sampling day and −0.55 when intake from the previous day was used. This implies that calculated EB_{inout} deviates somewhat from the ES indicated by NEFA. However, factors other than ES such as stress can affect NEFA plasma concentration (Brickner et al., 2007). One advantage of using plasma NEFA concentration as an ES indicator instead of EB_{inout} trait in modeling is that it avoids dependencies from the use of the same traits as those used in the calculation of EB_{inout} (milk yield and milk fat, protein, and lactose content) as covariables in the prediction models.

Plasma NEFA concentration of more than 0.6 mmol/L in early lactation is often considered as a threshold value for severe negative ES and indicator of a greater risk of developing metabolic disorders (Adewuyi et al., 2005; Ospina et al., 2010). In our data, this threshold was exceeded by 20% of the measurements in lactation wk 2 and 3 (mean ± SD = 0.560 ± 0.300 and 0.425 ± 0.222 mmol/L, respectively), but in lactation wk 20 all measurements were below the threshold. Normal NEFA levels for cows in positive ES are estimated at less than 0.2 mmol/L (Adewuyi et al., 2005). In our study, the average NEFA in wk 20 was 0.126 ± 0.058 mmol/L, indicating that the cows were in positive ES. Only 6.6% of the NEFA concentration measurements in wk 20 exceeded 0.2 mmol/L. When plasma NEFA is used as an ES marker it is good to remember that its concentration can vary diurnally (Sutton et al., 1988; Blum et al., 2000). Sutton et al. (1988) and Blum et al. (2000) reported that the plasma NEFA concentration of cows fed only twice a day tended to increase overnight and then decrease rapidly after morning feeding. Instead, when cows were fed 6 times a day, the concentration of NEFA remained relatively constant over the day (Sutton et al., 1988). Sutton et al. (1988) also observed that NEFA concentration was higher for cows fed twice a day than for those fed 6 times daily, especially with a high-concentrate diet. All cows in our study were fed several times a day, with the feed available 24 h daily. The diet was constant, with an average proportion of 49% (±6.6) of concentrate in diet DM. Also, to diminish the diurnal effect, blood samples were always taken at the same time of day. There were, however, some differences in the herds' feeding schemes, which may have increased variation between herds. Brickner et al. (2007) showed that plasma NEFA concentration was highest 15 min after cows were placed in headlocks and lowest 60 min after lockup. In our study, the handling protocol at sampling was about the same for cows in both herds, blood samples being collected within 15 min after morning milking and headlocking. Based on Brickner et al. (2007), this protocol may yield slightly elevated NEFA concentrations.

Milk and Body Traits as Plasma NEFA Predictors

Milk fat, protein, and lactose contents, or their ratio, were used to predict ES in the studies of Heuer et al. (2000) and Mäntysaari and Mäntysaari (2010). According to their results, milk fat and protein content, FPR ratio, or fat:lactose ratio explained 29.1 to 31.2% of the variation in the predicted variable, EB_{inout}. In the study by Friggens et al. (2007), milk traits explained 39% of EB variation estimated by BW and BCS measurements and 50% of EB variation based on EB_{inout}. In our present study, ES predictors were selected by a forward stepwise method using a significance level of 0.05, which resulted in only milk FPR and milk yield being included in model M1. This model explained 47.1% of the variation in NEFA. The increase in prediction ac-

curacy compared with the mentioned previous studies may be due to our use of NEFA as an ES indicator instead of calculated EB_{inout}.

Plasma NEFA concentration is known to increase with increased fat mobilization. However, in our study, the correlation between plasma NEFA and ΔBW was only moderate (−0.51). This might have to do with inaccuracy in the measurement of ΔBW even though based on smoothed BW data. The inherent inaccuracy of BW during the first weeks of lactation may be due to simultaneous mobilization of tissue reserves and increased mass of the gastrointestinal tract and its content. Nevertheless, with the growing popularity of automated weighing and condition scoring systems on dairy farms, ΔBW and ΔBCS will be potential on-farm indicators of negative ES of the cow (Frigo et al., 2010; Thorup et al., 2012). An upgrade of model M1 with the inclusion of BCS assessment had no effect on the accuracy of NEFA prediction (M2), but when ΔBW and ΔBCS were included as predictors (M3), an increase in prediction accuracy was achieved. Model M3 gave a better fit of prediction (R^2cv = 0.51) than in a previous study (Mäntysaari and Mäntysaari, 2010), where EB_{inout} was predicted using the same traits (R^2 = 0.39). This difference can be partly explained by errors occurring in the calculation of EB_{inout}, as discussed earlier.

Milk FPR was the most informative trait in predicting NEFA, both in model M3 and in models M1 and M2. The effect of DIM was higher (approximately −0.020 vs. −0.005 mmol/L per day) at the beginning of lactation (DIM1) than in mid lactation (DIM2), corresponding to changes in NEFA concentration during lactation. In mid lactation, when cows are most likely to have a positive EB, their plasma NEFA concentration is quite constant. We found an interaction between parity and herd for plasma NEFA concentration in our data, probably resulting from differences in management and feeding schemes between herds. This interaction was found to be significant in models M1, M2, and M3 but not in models M4 to M7, where milk FA concentrations were also included. Thus, the concentrations of milk FA in our data were more robust as ES predictors than other milk traits and body traits against the herd effect.

Milk FA Composition Traits as Plasma NEFA Predictors

Plasma NEFA concentration was predicted more precisely by milk FA composition (M5) than by milk yield, milk FPR, and body traits (M1, M2, and M3). Today, when milk MIR spectral readings can be routinely recorded from test-day milk samples, milk FA composition is a prominent candidate for predicting cow ES. According to our results, NEFA concentration was better predicted from evening (M5) than from morning (M4) milk samples (R^2cv = 0.61 vs. 0.52). Thus, plasma NEFA concentration in the morning seemed to be more related to milk samples taken at the evening milking than to samples taken in the morning just before blood sampling. Proportionally, the MCFA content in milk FA was higher in morning milk samples than in evening samples (46.8 vs. 45.3%), whereas in evening samples, LCFA, originating from ΔBW, were more dominant. This may have to do with cows' diurnal patterns of eating and resting. Similar difference was found between the predictive ability of morning and evening milk samples when morning NEFA concentration was predicted directly from milk MIR spectra points from the same data set (Mehtiö et al., 2018a). There was also a marked difference between models M4 and M5 in their ability to predict the plasma NEFA concentration of cows in the external validation data. However, the high correlation found between observed and predicted NEFA based on model M5 (>0.9) should be viewed with caution due to the very small size of the external validation data set.

Several studies have shown that the concentrations of milk LCFA, especially C18:0 and C18:1 *cis*-9, are effective predictors of cow ES (Stoop et al., 2009; Gross et al., 2011; Jorjong et al., 2014; Vranković et al., 2017). Jorjong et al. (2014) suggested that an elevated concentration of C18:1 *cis*-9 in milk fat in the second lactation week could act as an early warning of a risk of detrimentally high blood NEFA. They found that 64.3% of cows at risk of detrimental blood NEFA content had milk fat C18:1 *cis*-9 concentrations of 24 g/100 g or higher. The high correlation (0.73) between C18:1 *cis*-9 and NEFA in our data agrees with the results of Gross et al. (2011), who reported a correlation of 0.77 between EB and C18:1 *cis*-9. Also in our models, the sum of C18:1 was a significant predictor of NEFA. Concentrations of the sum of C18:1 (M4) and of C18:1 *cis*-9 (M5, M6, M7) with positive coefficients were selected into our prediction models.

Other individual FA besides C18:1 *cis*-9 included in the models were C14:0 in M6 and M7, C10:0 in M6, and C12:0 in M7. An increase in C10:0 and C12:0 but a decrease in C14:0 were associated with elevated NEFA values. However, a detailed interpretation of the effects (i.e., coefficients) of the FA included in our models is not acceptable taking into account the existing multicollinearity (VIF > 10). The effects of DIM1 and DIM2 were selected into all the models containing milk FA (M4–M7), although these effects were smaller than in models M1 to M3.

In developing model M7, we considered all milk and body traits for selection into the model. The inclusion

of ΔBW, milk FPR, and C18:1 *cis*-9, C12:0, and C14:0 decreased the RMSE, but, overall, the improvement was small compared with model M6. Indeed, according to our results, model M6 appears to be the most promising of the developed models. Its particular advantage is that all of the variables are measures from routine milk recording and test-day milk samples and thus available for all recorded cows at no additional cost.

The prediction equations developed in this study were based on data from 2 research herds with Nordic Red cows that were fed a similar diet of grass silage and concentrates with no added fat. Future studies based on more comprehensive data, covering variation in the population, are needed for further equation development.

CONCLUSIONS

According to our results, the plasma NEFA concentration, and consequently ES, of cows can be predicted with moderate accuracy based on their milk yield and milk FPR. The accuracy of prediction increased when changes in BW and BCS were included in the model. However, BW and BCS changes are not often measured on commercial farms, whereas concentrations of FA in milk are today in many countries routinely estimated from test-day milk samples by mid-infrared reflectance spectroscopy. We found that milk FA alone predicted cow ES better than milk yield, milk FPR, and body traits combined. The use of milk FPR together with milk FA concentrations explained 63% of the variation in NEFA. Thus, our findings indicate that the ES of dairy cows during the first months of lactation can be monitored with moderately high accuracy using routine milk measurements.

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