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Fourier transform infrared spectroscopy of milk samples as a tool to estimate energy balance, energy- and dry matter intake in lactating dairy cows

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Research Article

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Abstract

The objective of the study was to evaluate the potential of Fourier transform infrared spectroscopy (FTIR) analysis of milk samples to predict body energy status and related traits (energy balance (EB), dry matter intake (DMI) and efficient energy intake (EEI)) in lactating dairy cows. The data included 2371 milk samples from 63 Norwegian Red dairy cows collected during the first 105 days in milk (DIM). To predict the body energy status traits, calibration models were developed using Partial Least Squares Regression (PLSR). Calibration models were established using split-sample (leave-one cow-out) cross-validation approach and validated using an external test set. The PLSR method was implemented using just the FTIR spectra or using the FTIR together with milk yield (MY) or concentrate intake (CONCTR) as predictors of traits. Analyses were conducted for the entire first 105 DIM and separately for the two lactation periods: $5 \leq \text{DIM} \leq 55$ and $55 < \text{DIM} \leq 105$. To test the models, an external validation using an independent test set was performed. Predictions depending on the parity (1st, 2nd and 3rd-to 6th parities) in early lactation were also investigated. Accuracy of prediction (r) for both cross-validation and external test set was defined as the correlation between the predicted and observed values for body energy status traits. Analyzing FTIR in combination with MY by PLSR, resulted in relatively high r-values to estimate EB ($r = 0.63$), DMI ($r = 0.83$), EEI ($r = 0.84$) using an external validation. Only moderate correlations between FTIR spectra and traits like EB, EEI and dry matter intake (DMI) have so far been published. Our hypothesis was that improvements in the FTIR predictions of EB, EEI and DMI can be obtained by (1) stratification into different stages of lactations and different parities, or (2) by adding additional information on milking and feeding traits. Stratification of the lactation stages improved predictions compared with the analyses including all data $5 \leq \text{DIM} \leq 105$. The accuracy was improved if additional data (MY or CONCTR) were included in the prediction model. Furthermore, stratification into parity groups, improved the predictions of body energy status. Our results show that FTIR spectral data combined with MY or CONCTR can be used to obtain improved estimation of body energy status compared to only using the FTIR spectra in Norwegian Red dairy cattle. The best prediction results were achieved using FTIR spectra together with MY for early lactation. The results obtained in the study suggest that the modeling approach used in this paper can be considered as a viable method for predicting an individual cow's energy status.

Energy balance (EB) in early lactation impacts phenotypic (Martin *et al*., 2015) and genetic (Veerkamp *et al*., 2001) health and fertility traits in cattle. In early lactation, dairy cows are typically not able to ingest sufficient dietary energy to meet the requirements for milk production, resulting in a period of negative EB (NEB) which may compromise health and fertility (Dillon *et al*., 2006). Measuring EB in a cost efficient and accessible way, is thus likely to be useful both for cow management and in breeding programs (Collard *et al*., 2000). Furthermore, such an approach would also have an important environmental and economic role through improved feed utilization because feed is a major variable cost in dairy production (Shalloo *et al*., 2004). The Food and Agricultural Organization (FAO) reports that intensification of dairy and meat production through improved feeding, effective reproduction and good health is the most efficient way of reducing carbon footprint from this sector (Gerber *et al*., 2013).

Energy balance can be calculated by using the difference between energy intake and energy outputs such as milk, maintenance, pregnancy, activity, and growth (Banos and Coffey, 2010). These measurements, and in particular energy intake, are technically challenging and associated with high costs. Reist *et al.* (2002) documented that milk fat-to-protein ratio and milk fatty acid composition are associated with energy balance in cows. Fourier transform infrared spectroscopy (FTIR), i.e. mid-infrared spectroscopy, is a vibrational spectroscopic technique allowing detailed chemical fingerprinting of food and other matrices. The technique is extensively used in the dairy industry to predict variables like fat, protein and lactose concentration in milk (Soyeurt *et al*., 2011; De Marchi *et al*., 2014). Thus, as FTIR analysis is already used globally to determine milk quality variables, a feasible prediction of energy balance and energy intake in lactating cows using these spectra would be cost efficient and more easily implemented at low cost compared to measuring feed intake and milk production by traditional means. Several studies have looked into the possibility of using FTIR of milk for the prediction of EB and efficient energy intake (EEI) in lactating Holstein-Friesian dairy cows (McParland *et al*., 2011, 2012, 2014). However, common for all the studies were low to moderate correlations between FTIR spectra and traits like EB, EEI and dry matter intake (DMI).

In early lactation, EB in most dairy cows is negative as cows cannot consume sufficient energy to meet their requirements of lactation (Bauman and Currie, 1980). Martin *et al.* (2015) documented a correlation between fatty acid composition of milk determined by FTIR spectroscopy and onset of luteal activity post-partum which affects subsequent reproductive performance. Berry *et al.* (2006), Beam and Butler (1999), Buttchereit *et al.* (2010) and McParland *et al.* (2011) documented a strong correlation between milk fat-to-protein ratio and EB in early lactation. It is thus very interesting to investigate if information on stages of lactation, parity (PAR), and milking traits (metadata) can be used to make improvements when predicting EB, EEI and dry matter intake (DMI) using FTIR spectroscopy.

The main objective of our study was to identify potential improvements when FTIR analysis of milk samples was used for the prediction of EB in Norwegian red cattle. Additional objectives were to use FTIR analyses for prediction of EEI and DMI as these parameters are related to EB. Our hypothesis was that stratification of the data into stage of lactation and parity would improve the predictions and that the accuracy of the predictions could be further increased by including data on milk yield (MY) or concentrate intake (CONCTR) in the prediction model. To our knowledge, this is the first time FTIR combined with MY or CONCTR is used for prediction of EB, DMI and EEI within different stages of lactation.

Materials and methods

Production data

This study was performed in the research herd at the Animal Production Experimental Centre (SHF) at the Norwegian University of Life Sciences (Ås, Norway) between September 2016 and February 2017. The experiment followed laws and regulations for animal experiments in Norway and was approved by the Norwegian Animal Research Authority. In total 63 Norwegian Red cows were followed from one week before calving until 105 DIM. The cows were categorized by parity as 1st (PAR1), 2nd (PAR2), and from 3rd to 6th (PAR>2) parities. The cows were milked in an automatic milking system (AMS) (DeLaval International AB, Tumba, Sweden). Milk yield (MY, kg/d) was recorded for each milking and summarized to obtain daily yield using a 7-d rolling average meaning that yield at a specific day is the average of that day and the six previous days. Milk samples were collected three times a week from calving until 105 DIM. All milk samples were conserved with bronopol (2-bromo-2-nitropropane-1,3-diol) and stored at 4 °C for 1–4 d until subsequent analysis by FTIR (Afseth *et al*., 2010). Silage and CONCTR intakes were recorded and calculated on a daily basis, and used to calculate daily DMI following the same 7-d rolling average approach as for milk yield.

Feeding

The nutritional data collected was from a feeding experiment in which cows were fed grass silage *ad libitum* combined with concentrate. Chemical composition of the feeds is shown in Supplementary Table S1 (supplementary file). The grass silage was prepared from timothy (*Phleum pratense*) and meadow fescue (*Festuca pratensis*) dominated swards and conserved in round bales using a formic acid-based additive. The grass was harvested at two stages of maturity. Half of the cows were fed silage harvested just before heading of the timothy (early stage of maturity), whereas the other half of the cows were fed a silage from the same crop, harvested 10 d later (normal stage of maturity). In addition, both groups were fed a commercial compound concentrate (FORMEL Energi Premium 70; Felleskjøpet Agri, Lillestrøm, Norge). Within silage quality, concentrate was provided either according to a standard lactation curve following NorFor recommendations (Volden *et al*., 2011) preset to reach yields of 7500, 8500 and 9000 kg ECM in a 305 d lactation for PAR1, PAR2 and PAR>2 cows, respectively, or after 21 DIM adjusted weekly based on the actual difference between estimated requirements for maintenance and milk production and dietary intake of net energy in feed (MJ NEl/d), using NorFor calculations (Volden, 2011). Silage consumption was automatically recorded through feeding troughs placed on weighing cells (BioControl AS, Rakkestad, Norway) and calculated as described by Kidane *et al.* (2018). Concentrate was provided in the AMS and in separate concentrate feeding stations. Samples for dry matter (DM) determination of the silage were taken twice weekly. Within week, the average DM concentration of these two samples was used to calculate DMI based on the previously described daily recorded consumptions of silage. For concentrate, samples were taken weekly and pooled into a monthly sample for DM determination. Based on the daily recorded concentrate intake previously described, the determined DM concentration was used to calculate DMI of concentrate. For analysis of nutrient composition, the monthly samples of silages and concentrate were analyzed as described by Kidane *et al.* (2018).

Computation of body energy status

Energy balance (MJ NEl/d) was defined as the difference between energy input and energy output. Energy input includes energy from concentrate and roughage intake as described in the previous section. Energy output was computed as described in detail by Volden (2011), and includes energy expenditure for maintenance, milk production and for first lactation cows also growth. Energy for gestation constituted less than 1 MJ NEl/d even at

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105 DIM and was not included in EB. In addition to EB, the EEI summing up daily intake of energy from silage and concentrate (MJ NEl/d) (Banos and Coffey, 2010) was also calculated. Based on DMI of silage and concentrate and subsequent analyses of nutrients, energy intake was calculated using the NorFor feed rationing system (Volden, 2011), which gives energy in MJ net energy of lactation (NEl).

FTIR analysis

Prior to dry film FTIR analysis, all milk samples were removed from refrigeration and stored at room temperature for approximately 30 min. The samples were shaken in a vortex mixer (Whirlimixer, Scientific Industries) for 10 s. Subsequently, the milk samples were diluted with water (75% milk, 25% water) before being shaken in a vortex mixer (Whirlimixer, Scientific Industries) for 5 additional seconds. Samples (2.5 µl) were then transferred to sample well plates (silicon, 96 wells) and dried at room temperature for approximately 1 h. Dry film FTIR was performed using a high throughput screening eXTension (HTS-XT) unit coupled to a Tensor 27 spectrometer (both Bruker Optik GmbH, Germany), equipped with a DLaTGS detector. Spectra were recorded in transmission mode in the spectral region from 4000 to 500/cm with a resolution of 6/cm and an aperture of 5.0 mm. Background spectra of the silicon substrate were collected before each sample measurement to account for variation in water vapor and CO_2. All samples were measured in triplicates (technical replicates).

Calibration analysis

In the current study, FTIR spectral data were composed of 1867 infrared frequencies (wavenumbers) which represented infrared light absorption through the milk sample at wavenumber regions ranging from 399 to 3998/cm. Due to high water absorption, the O-H stretching, and bending regions were omitted and only the regions between 700 and 1805/cm and between 2750 and 3020/cm were maintained for further analyses. We applied Savitzky-Golay (Savitzky and Golay, 1964) first derivatives with window size 9, polynomial order 2, followed by normalization using Extended Multiplicative Scatter Correction (EMSC) (Martens and Stark, 1991) to pre-process the spectral data. Normalization was used to correct variations in the fraction of the transmitted light that hits the detector (e.g. scaling effect due to varying thickness of the dry films). Then the technical replicates were averaged (Afseth *et al*., 2010). When FTIR spectra were combined with metadata such as MY or CONCTR, to establish calibration models for EB, DMI and EEI, normalization of FTIR data and MY or CONCTR was done by their respective Frobenius norms.

The Partial Least Square Regression (PLSR) method (algorithms developed in-house and standard Matlab algorithms, MathWorks, Natick, MA, USA) was used to establish calibration models, and a data set of corresponding trait reference measurements, EB, DMI or EEI, were used as a Y matrix, which was regressed onto an X matrix containing pre-processed FTIR measurements of milk or a combination of FTIR data and MY or CONCTR.

The entire data set was split first into a calibration and an external test set. The validation set was only used to validate the models while the calibration set was used to establish the models. 19 cows were selected as an external test set (for validation) which corresponds to 444 spectra (30%). The calibration set contained 44 cows corresponding to 1927 spectra (70%). Thus, samples of cows of the calibration set were not present in the test set which allows correct validation. Calibration models were established using split-sample (leave-one cow-out) cross-validation. The cross-validation is done to optimize the models, namely the number of latent variables (factors): the optimal number of factors (Fac) is the one which does not yield a significantly larger Root-Mean-Square-Error (RMSE) than the factor corresponding to the minimum RMSE.

The accuracy of prediction (r) both for cross-validation and the external validation was defined as the square root of the coefficient of determination, and it represents the absolute value of the correlation between the true and predicted values of the observed traits (EB, DMI and EEI). In addition to r, the RMSE was calculated as a measure of models' performances. Both the accuracy and RMSE were calculated for cross-validation and for the validation using the external test set.

In order to achieve the main objectives, the following was performed: (1) Analyses were conducted separately for the entire 105 DIM ($5 \leq \text{DIM} \leq 105$) and for two lactation stages of DIM $5 \leq \text{DIM} \leq 55$ and $55 < \text{DIM} \leq 105$ using FTIR; (2) Models depending on parity (PAR) (1^{st}, 2^{nd} and $>2^{nd}$ parities) within first lactation stage of DIM were established using FTIR; (3) Predictions of EB, DMI and EEI for the entire 105 DIM ($5 \leq \text{DIM} \leq 105$) and for two lactation stages $5 \leq \text{DIM} \leq 55$ and $55 < \text{DIM} \leq 105$, separately using FTIR combined with milk yield (MY) or concentrate intake (CONCTR) were investigated. Partial Least Squares Regression was used to develop calibration models. An external validation on an independent test set was done to validate the model.

Results

Data

The mean phenotypic values for the different performance traits EB, DMI and EEI for the whole data set representing 63 cows are summarized in Supplementary Table S2 (supplementary file) (within $5 \leq \text{DIM} \leq 105$), in Supplementary Table S3 (supplementary file) (split into $5 \leq \text{DIM} \leq 55$ and $55 < \text{DIM} \leq 105$), and in Supplementary Table S4 (supplementary file) (for different parities in $5 \leq \text{DIM} \leq 55$). The average EB and DMI profiles from 5 to 105 DIM are shown in Supplementary Fig. 1 (supplementary file) and Supplementary Fig. 2 (supplementary file), respectively.

Accuracy of prediction based on lactation stage using FTIR

The prediction accuracy of the PLSR models for EB, DMI and EEI for the entire 105 DIM, $5 \leq \text{DIM} \leq 105$, and for the two lactation stages, $5 \leq \text{DIM} \leq 55$ and $55 < \text{DIM} \leq 105$, including all parities are presented in Table 1. Depending on the trait, models with different number of factors were obtained. The number of factors (Fac) shows the complexity of a model: the more factors, the more complex is the model. However, to avoid overfitting, it is important to optimize the number and keep it modest. In all models presented in this study, the number of factors is optimized in a range of modest values for PLSR models. Fac of the PLSR models ranged from 4 to 8 for $5 \leq \text{DIM} \leq 105$ and $5 \leq \text{DIM} \leq 55$, and from 3 to 6 for $55 < \text{DIM} \leq 105$. The greatest external validation accuracy of calibration models for EB (0.56), DMI (0.62)

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Table 1. Partial Least Squares Regression (PLSR) results (optimal number of factors (Fac), root mean square error (RMSE), correlation coefficient (*r*)), obtained from prediction of energy balance (EB), dry matter intake (DMI) and efficient energy intake (EEI) in entire first 105 d in milk (DIM) ($5 \leq DIM \leq 105$) and in 2 lactation stages, ($5 \leq DIM \leq 55$) and ($55 < DIM \leq 105$)

	Split-sample cross-validation			External validation		
Trait	Fac	RMSE	*r*	Fac	RMSE	*r*
$5 \leq DIM \leq 105$						
EB	4	13.1	0.45	4	13.9	0.44
DMI	8	2.5	0.59	8	2.8	0.57
EEI	8	18.2	0.56	8	18.5	0.55
$5 \leq DIM \leq 55$						
EB	4	12.1	0.57	4	12.2	0.56
DMI	8	2.2	0.68	8	2.4	0.62
EEI	8	16.8	0.66	8	17.6	0.64
$55 < DIM \leq 105$						
EB	3	14.6	0.44	3	15.1	0.37
DMI	5	2.9	0.43	5	3.1	0.42
EEI	6	19.7	0.46	6	21.3	0.45

Number of cows = 63; Number of observations in in entire first 105 DIM ($5 \leq DIM \leq 105$) = 2371 observations; Number of observations in lactation stage 1 ($5 \leq DIM \leq 55$) = 1299 observations; Number of observations in lactation stage 2 ($55 < DIM \leq 105$) = 1072 observations.

Table 2. Partial Least Squares Regression (PLSR) results (optimal number of factors (Fac), root mean square error (RMSE), correlation coefficient (*r*)), obtained from predicting energy balance (EB), dry matter intake (DMI) and efficient energy intake (EEI) for different parities (PAR) (PAR≥1 = parities≥1, PAR1 = parity 1, PAR2 = parity 2 and PAR>2 = parities>2) in first lactation stage ($5 \leq DIM \leq 55$)

	Split-sample cross-validation			External validation		
Trait	Fac	RMSE	*r*	Fac	RMSE	*r*
PAR≥1						
EB	4	12.1	0.57	4	12.2	0.56
DMI	8	2.2	0.68	8	2.4	0.62
EEI	8	16.8	0.66	8	17.6	0.64
PAR1						
EB	10	13.1	0.57	10	13.2	0.52
DMI	3	2.4	0.44	3	2.7	0.42
EEI	3	17.0	0.46	3	17.5	0.45
PAR2						
EB	3	12.0	0.59	3	12.1	0.58
DMI	7	1.5	0.69	7	2.0	0.68
EEI	6	16.4	0.68	6	16.8	0.67
PAR>2						
EB	4	13.6	0.55	4	13.8	0.49
DMI	9	2.1	0.68	9	2.4	0.66
EEI	9	16.0	0.71	9	17.8	0.62

DIM = days in milk. Number of cows = 63; Number of observations in in entire first 105 DIM ($5 \leq DIM \leq 105$) = 2371 observations; Number of observations in lactation stage 1 ($5 \leq DIM \leq 55$) = 1299 observations; Number of observations in lactation stage 2 ($55 < DIM \leq 105$) = 1072 observations.

and EEI (0.64) was achieved for the data of early lactation ($5 \leq DIM \leq 55$) when FTIR spectra were used as predictor (Table 1).

Accuracy of prediction based on parity using FTIR

The prediction accuracy of the PLSR models for the same traits within lactation stage 1 for PAR≥1, PAR1, PAR2 and PAR>2 was also tested. The results are summarized in Table 2. Models with different number of Fac were obtained for different categories of PAR. The optimal number of Fac of the PLSR models ranged from 4 to 8 when all PAR were included (PAR≥1), from 3 to 10 for PAR1, from 3 to 7 for PAR2 and from 4 to 9 for PAR>2. The external validation accuracy of the PLSR models for all the traits in early lactation stage ($5 \leq DIM \leq 55$) was higher in PAR2 compared with PAR≥1, PAR1 and PAR>2: EB (0.58), DMI (0.68) and EEI (0.67) when FTIR spectra were used as predictor (Table 2).

Accuracy of prediction based on lactation stage using FTIR combined with metadata

The prediction accuracy of the PLSR models established on the entire DIM ($5 \leq DIM \leq 105$) and on two lactation stage separately: $5 \leq DIM \leq 55$ and $55 < DIM \leq 105$ for all parities using only FTIR or FTIR combined with MY or CONCTR as predictors are presented for EB in Table 3, DMI in Table 4 and EEI in Table 5.

Using different predictors (FTIR spectra only, FTIR combined with MY and FTIR combined with CONCTR), models with different number of Fac were obtained in predicting EB (Table 3). The optimal number of Fac of the PLSR models ranged from 4 to 5 in combined lactation stage, from 4 to 6 in lactation stage 1 and from 3 to 6 in lactation stage 2 depending on the model.

The validation accuracy of calibration models for EB increased to 0.51 for combined lactation stage, 0.63 in lactation stage 1 and 0.44 in lactation stage 2. We can see that using metadata such as MY or CONCTR for model establishment increased the models' accuracy of the model in all lactation stages (Table 3). Including MY in the calibration increased the external validation accuracy of EB model more compared with including CONCTR and resulted in the same increase by 0.07 units in combined lactation stage, lactation stage 1 and 2.

The Fac of the PLSR models for DMI ranged from 4 to 8 in combined lactation stages, from 6 to 8 for lactation stage 1 and from 4 to 7 in lactation stage 2 (Table 4). Including CONCTR in the calibration increased the external validation accuracy of DMI model by 0.11 units in combined lactation stage, by 0.12 units in lactation stage 1 and by 0.19 units in lactation stage 2. Compared with the model with spectral information only, including MY in the calibration increased the validation accuracy of DMI model by 0.2 units in combined lactation stages, by 0.21 units in lactation stage 1 and by 0.36 units in lactation stage 2. The greatest validation accuracy of the DMI model (0.83) was achieved when MY was included to the calibration for lactation stage 1.

The Fac of the PLSR models for EEI ranged from 7 to 9 in combined lactation stages, from 7 to 8 for lactation stage 1 and

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Table 3. Partial Least Squares Regression (PLSR) results (optimal number of factors (Fac), root mean square error (RMSE), correlation coefficient (*r*)), obtained from prediction of energy balance (EB), in entire first 105 DIM (5 ≤ DIM ≤ 105) and in 2 lactation stages, (5 ≤ DIM ≤ 55) and (55 < DIM ≤ 105) using FTIR spectral information, FTIR combined with milk yield using FTIR spectral information, FTIR combined with milk yield (MY) and FTIR combined with concentrate intake (CONCTR) as predictors

		Split-sample cross-validation			External validation		
Model	Predictors used	Fac	RMSE	*r*	Fac	RMSE	*r*
5 ≤ DIM ≤ 105							
1	FTIR	4	13.1	0.45	4	13.9	0.44
2	FTIR, MY	4	12.4	0.52	4	12.6	0.51
3	FTIR, CONCTR	5	12.8	0.47	5	12.9	0.46
5 ≤ DIM ≤ 55							
1	FTIR	4	12.1	0.57	4	12.2	0.56
2	FTIR, MY	5	11.4	0.65	5	11.5	0.63
3	FTIR, CONCTR	6	11.7	0.59	6	11.8	0.57
55 < DIM ≤ 105							
1	FTIR	3	14.6	0.44	3	15.1	0.37
2	FTIR, MY	4	13.0	0.48	4	13.8	0.44
3	FTIR, CONCTR	6	14.9	0.42	6	15.1	0.41

Model 1 used only FTIR spectral information as a predictor; Model 2 used FTIR spectral information together with milk yield (MY) as predictors; Model 3 used FTIR spectral information together with concentrate intake (CONCTR) as predictors. DIM = days in milk. Number of cows = 63; Number of observations in in entire first 105 DIM (5 ≤ DIM ≤ 105) = 2371 observations; Number of observations in lactation stage 1 (5 ≤ DIM ≤ 55) = 1299 observations; Number of observations in lactation stage 2 (55 < DIM ≤ 105) = 1072 observations.

Table 4. Partial Least Squares Regression (PLSR) results (optimal number of factors (Fac), root mean square error (RMSE), correlation coefficient (*r*)), obtained from prediction of dry matter intake (DMI), in entire first 105 DIM (5 ≤ DIM ≤) and in 2 lactation stages, (5 ≤ DIM ≤ 55) (55 < DIM ≤ 105) using FTIR spectral information, FTIR combined with milk yield (MY) and FTIR combined with concentrate intake (CONCTR) as predictors

		Split-sample cross-validation			External validation		
Model	Predictors used	Fac	RMSE	*r*	Fac	RMSE	*r*
5 ≤ DIM ≤ 105							
1	FTIR	8	2.5	0.59	8	2.8	0.57
2	FTIR, MY	5	1.6	0.82	5	2.7	0.77
3	FTIR, CONCTR	4	2.0	0.70	4	2.2	0.68
5 ≤ DIM ≤ 55							
1	FTIR	8	2.2	0.68	8	2.4	0.62
2	FTIR, MY	6	1.5	0.84	6	2.1	0.83
3	FTIR, CONCTR	8	1.7	0.59	8	1.9	0.74
55 < DIM ≤ 105							
1	FTIR	5	2.9	0.43	5	3.1	0.42
2	FTIR, MY	7	1.8	0.79	7	2.6	0.78
3	FTIR, CONCTR	4	2.4	0.62	4	2.9	0.61

Model 1 used only FTIR spectral information as a predictor; Model 2 used FTIR spectral information together with milk yield (MY) as predictors; Model 3 used FTIR spectral information together with concentrate intake (CONCTR) as predictors. DIM = days in milk. Number of cows = 63; Number of observations in in entire first 105 DIM (5 ≤ DIM ≤ 105) = 2371 observations; Number of observations in lactation stage 1 (5 ≤ DIM ≤ 55) = 1299 observations; Number of observations in lactation stage 2 (55 < DIM ≤ 105) = 1072 observations.

from 4 to 6 in lactation stage 2 (Table 5). Including CONCTR in calibration increased the external validation accuracy of EEI model by 0.16 units in combined lactation stage, by 0.12 units in lactation stage 1 and by 0.15 units in lactation stage 2. Including MY in calibration increased the accuracy of EEI model by 0.27 units in combined lactation stages, by 0.2 units in lactation stage 1 and by 0.35 units in lactation stage 2. The greatest external validation accuracy of the EEI model (0.84) was achieved when MY was included in the calibration for lactation stage 1.

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Table 5. Partial Least Squares Regression (PLSR) results (optimal number of factors (Fac), root mean square error (RMSE), correlation coefficient (*r*)), obtained from prediction of efficient energy intake (EEI), in entire first 105 DIM (5 ≤ DIM ≤ 105) and in 2 lactation stages, (5 ≤ DIM ≤ 55) (55 < DIM ≤ 105) using FTIR spectral information, FTIR combined with milk yield (MY) and FTIR combined with concentrate intake (CONCTR) as predictors

		Split-sample cross-validation			External validation		
Model	Predictors used	Fac	RMSE	*R*	Fac	RMSE	*r*
5 ≤ DIM ≤ 105							
1	FTIR	8	18.2	0.56	8	18.5	0.55
2	FTIR, MY	7	11.6	0.83	7	12.4	0.82
3	FTIR, CONCTR	9	12.9	0.47	9	13.7	0.71
5 ≤ DIM ≤ 55							
1	FTIR	8	16.8	0.66	8	17.6	0.64
2	FTIR, MY	7	11.1	0.86	7	12.2	0.84
3	FTIR, CONCTR	8	12.0	0.80	8	13.4	0.76
55 < DIM ≤ 105							
1	FTIR	6	19.7	0.46	6	21.3	0.45
2	FTIR, MY	4	11.5	0.81	4	12.9	0.80
3	FTIR, CONCTR	4	13.5	0.63	4	13.9	0.60

Model 1 used only FTIR spectral information as a predictor; Model 2 used FTIR spectral information together with milk yield (MY) as predictors; Model 3 used FTIR spectral information together with concentrate intake (CONCTR) as predictors. DIM = days in milk. Number of cows = 63; Number of observations in in entire first 105 DIM (5 ≤ DIM ≤ 105) = 2371 observations; Number of observations in lactation stage 1 (5 ≤ DIM ≤ 55) = 1299 observations; Number of observations in lactation stage 2 (55 < DIM ≤ 105) = 1072 observations.

Discussion

Calibration analysis

In Norwegian Red cow's, daily milk yield peaks at around 55 DIM (Andersen *et al*., 2011), whereas DMI typically is peaking 50 to 60 DIM (Volden *et al*., 2011). In our study, cows had on average 1.9 kg lower daily production of energy corrected milk than aimed for (data not shown). However, peak yield and DMI aligned well with the two cited references and we found it feasible to use 55 d as the cut off when we stratified the lactation stage. This has also been demonstrated previously (Reksen *et al*., 2001). This is further supported by the fact that most dairy cows undergo a period of NEB during the first period of lactation, which affects their metabolism and also the composition of milk (Bauman and Currie, 1980). Apart from milk production the energy requirements differ between cows in different parities because cattle have not reached their full mature weight until 4 to 8 years of age and reaching this weight requires energy (Poncheki *et al*., 2015). The energy requirements for growth are greatest in the first lactation and reduced in the second lactation, after which the energy requirements for growth are not considered to be significant (Villa-Godoy *et al*., 1988). Due to the differences in growth and energy requirements, it is reasonable to split cows of first and second parity from older cows when establishing calibration models to improve the model's performances although energy requirements for growth was considered only in first parity cows in our study. Doing that, we observed that the prediction accuracy of calibration models was affected by the number of explanatory factors permitted in the prediction model. This is in line with the results of McParland *et al.* (2011), who predicted body energy status in Holstein cows. The optimum number of factors in the present study ranged from 3 to 4 when predicting EB based on FTIR spectral data within different lactation stages (using FTIR spectral only). The optimum number of factors in the study by McParland *et al*. (2011) was 20 for EB when predictions were made across lactations. Interesting results were obtained for EB, DMI and EEI models with greater accuracy in lactation stage 1 than in lactation stage 2. Predicting EB, DMI and EEI within lactation stage 1 depending on PAR is novel and has not been discussed before. Although the lower than expected milk yield reduced the period of negative energy balance, potentially this is important as negative energy balance in this period may have severe negative effect on health and fertility of dairy cows (Reksen *et al*., 2002). Thus, by predicting energy balance more accurately in the first 55 DIM, improvements in both management and breeding of dairy cattle should be possible. This may allow for better feed utilization and thereby reduction of the climatic footprint of dairy production (Green *et al*., 2013).

Effect of parity and lactation stage

Stratification of data into lactation stages and parities improved the predictions of EB, EEI and DIM. Most likely the improvements reflect differences in growth and metabolic status between cows of different parities. A mismatch between DMI and MY usually renders cows in NEB in early lactation. However, compared with older cows, first parity cows produce less milk. In addition, milk production in first parity cows increases more slowly and reaches a lower peak than in older cows and despite having higher requirements for growth, it is easier for first parity cows than older cows to meet their energy requirements in early lactation (Wathes *et al*., 2007). Thus, as milk production increases with parity, multiparous cows are more likely to fall into NEB in early lactation, increasing the risk of being subjected to disease, reducing NEB further (Wathes *et al*., 2007).

Division into parities for lactation stage 1 (5 ≤ DIM ≤ 55) improved the prediction of EB, EEI and DMI for parity 2, but not for PAR1 and PAR>2 cows. We hypothesize that the

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differences in lactation and energy curve shape was less pronounced in second parity cows, thereby giving a higher degree of explanation in second parity than in first and older parity cows.

The predictions were better for DMI and EEI than EB. Dry matter intake and EEI include measurement of feed intake and an estimate of energy concentration of that feed, which usually is easily obtained in feeding experiments, whereas energy balance includes several steps in computation, all with some uncertainty. Thus, less error and a strong correlation between energy intake and MY, may explain better predictions by using DIM and EEI.

Effect of using FTIR combined with metadata

Using metadata, combining MY or CONCTR with FTIR spectra, increased the accuracy of EB, DMI and EEI models in combined lactation stages, lactation stage 1 and lactation stage 2. The most accurate models were obtained for lactation stage 1, using CONCTR in addition to FTIR spectra. The external validation accuracy obtained are better than those reported in literature so far (McParland *et al.*, 2014). Since FTIR spectra, MY and CONCTR are stored in many milk recording systems, it would be straightforward to implement prediction models for the prediction of energy balance in lactating cows based in these variables. We suggest to combine FTIR spectra with MY and CONCTR to improve the prediction models for the energy balance of cows. Therefore, we added MY or CONCTR as an additional variable to the FTIR spectra in order to check if the model was improved by the combined information. Correlations between MY, CONCTR and FTIR spectra are expected. However, it is known that PLSR is a versatile tool for handling correlations between predictor variables. Indeed, PLSR was designed for multivariate variables with high levels of correlations between the variables. The resulting prediction models show that MY and CONCTR contribute with additional information and thus result in improved models compared to the models where the energy balance is predicted using only FTIR spectra as predictor variables.

In addition to improving the prediction, stratification of lactation stage and division into parities show how predictions of traits are correlated within each lactation stage and each parity of lactation stage 1. This knowledge should thus be taken into account when developing future potential solutions of FTIR analysis on farms. It should be noted, however, that even though improvements are seen, the obtained prediction models are only describing maximum 84% of the variation in a given trait.

In conclusion, the results obtained in this study suggest that a modeling approach can be considered as a viable method for predicting an individual cow's energy status. The predictive ability of our models was higher in early lactation and second parity, compared with first parity and older cows, which indicates that alternations in the FTIR spectra were more uniformly related to energy requirements in this group. Combining metadata such as MY or CONCTR with FTIR data increased the accuracy of prediction in all PLSR models.

Supplementary material. The supplementary material for this article can be found at https://doi.org/10.1017/S0022029920001004.

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