Developing an indicator for body fat mobilisation using mid-infrared spectrometry of milk samples in dairy cows

T. Mehtiö[1], P. Mäntysaari[1], T. Kokkonen[2], S. Kajava[3], T. Latomäki[4], L. Nyholm[4], C. Grelet[5], T. Pitkänen[1], E. A. Mäntysaari[1] & M.H. Lidauer[1]

[1]Natural Resources Institute Finland (Luke), Humppilantie 14, 31600 Jokioinen, Finland
terhi.mehtio@luke.fi (Corresponding Author)
[2]University of Helsinki, P.O. Box 28, 00014 Helsinki, Finland
[3]Natural Resources Institute Finland (Luke), Halolantie 31 A, 71750 Maaninka, Finland
[4]Valio Ltd., P.O. Box 10, 00039 Valio, Finland
[5]Centre Wallon de Recherche Agronomiques, 24 Chaussée de Namur, 5030 Gembloux, Belgium

Summary

High energy requirement in the initiation of lactation may force dairy cows to mobilize energy from body tissue which leads to negative energy balance (EB). Negative EB can predispose cows to various health and fertility problems, and therefore should be considered in dairy cattle breeding programs especially if feed efficiency traits are included in breeding objectives. However, measuring of EB is difficult and estimates are imprecise. When a cow is in negative EB and mobilizing its fat reserves, concentrations of milk fatty acids and blood non-esterified fatty acids (NEFA) change. Therefore, it is possible to use these changes as biomarkers for energy status. Mid-infrared spectrometry (MIR) is a routinely used tool for milk samples and many milk fatty acids can be predicted with high accuracy by MIR. Our objective was to assess the capability of MIR to predict blood plasma NEFA concentration from milk samples. Milk and corresponding blood samples were collected from Nordic Red dairy cows in three research farms between 2013 and 2016. There were altogether 1585 milk spectral readings and 809 NEFA records from 141 cows in the data set. Partial least squares regression was used to predict NEFA and EB from MIR spectral data. The coefficient of determination of cross validation (R^2cv) for NEFA was 0.64 when leave-one-out cross validation was used for the whole data set. Higher R^2cv values were found when predicting blood plasma NEFA concentration from evening milk samples (0.67), probably because fatty acid concentrations in milk vary slower than NEFA concentration in blood. The robustness of the developed prediction equation was inspected by calibrating the equations with records from the cows from two research herds and then predicting NEFA for cows from the other herd, so that there were differences in the environment of the calibration data (130 NEFA obs) and test data (647 NEFA obs.) sets. Nevertheless, R^2 in the test data was 0.58 and RMSE 0.19 mmol/l, which indicates that the model is robust.

Keywords: mid-infrared spectroscopy, non-esterified fatty acids, energy balance

Introduction

Dairy cows in early lactation have a very high energy requirement for milk production which seldom can be fulfilled by feed intake and which forces a cow to compensate energy deficits by body fat reserves. This stage of a cow, negative energy balance, can predispose cows to various health and fertility problems (Berry & Crowley, 2013; Roche et al., 2009), and therefore it would seem important to consider energy status in dairy cattle breeding programs. However, measuring energy status of a cow is not easy. Estimation of energy balance (EB) is possible, but its accuracy may be low due to measurement errors. However, when a cow is in negative EB and mobilizing its fat reserves, the concentration of non-esterified fatty acids (NEFA) in blood increases, and similarly increases the proportion of fatty acids originating from adipose tissue (especially C16:0, C18:0 and C18:1 *cis*-9) in milk (Stoop et al., 2009, Bastin et al., 2011). NEFA could be a more precise indicator of cow's energy status than calculated EB, and because of body fat mobilization there should be a relationship between blood NEFA and milk fatty acids in the initiation of lactation in dairy cows.

Recently, mid-infrared spectrometry (MIR) based prediction tools have been studied very widely. McParland et al. (2012) already showed that MIR was capable to predict EB, but random errors in the calculation of energy status in field data inhibited high accuracies. With reasonable high accuracies milk MIR could provide a cost and time beneficial tool to measure negative EB, which would help to improve health and fertility in dairy cattle.

Energy balance may be considered as an imprecise predictor of cow's energy status, but blood NEFA, a biomarker for energy status, also has its challenges. Blum et al. (2000) showed that blood plasma concentrations of NEFA had clear diurnal variation; NEFA concentrations were high in the morning and decreased rapidly after morning feed.

The main objectives of this study were to evaluate the potential to predict blood plasma NEFA concentration from milk samples analysed by FT-MIR samples using partial least squares regression and to validate the developed prediction models for robustness. In addition, we wanted to study if blood plasma NEFA concentration measured in the morning is actually more accurately predicted from milk samples collected in the evening, assuming that fatty acid concentrations in milk do not vary as rapidly as NEFA concentration in blood.

Material and methods

Milk and blood samples were collected between 2013 and 2016 from Nordic Red dairy cows in three research farms: Luke Jokioinen, Luke Maaninka and University of Helsinki in Viikki. Milk samples were collected from morning and evening milks twice in weeks 2 and 3 and once in week 20 after calving, which results in 10 milk samples per lactation. Blood samples were collected after collecting morning milk samples before feeding, resulting in 5 blood samples per lactation from every cow. Energy balance was calculated as described by Mäntysaari and Mäntysaari (2015). Altogether the data set consisted of 1585 spectral records and 809 NEFA observations from 141 cows, of which 49 cows had records also from second lactation.

MIR spectral readings of the milk samples, based on two MilkoScan FT6000 spectrometers (Foss, Hillerød, Denmark), which are calibrated and standardised regularly, were provided by Valio Ltd milk laboratory. MIR spectral data consisted of 1060 data points, which represent infrared light absorbance through milk sample in wavelength area of 900 to

5000 cm^{-1}. The first derivative of spectral data observations was used and 212 informative spectral points were utilized in the analyses.

Prediction model development

Partial least squares regression (PROC PLS; SAS Institute Inc., Cary, NC) was used to predict blood plasma NEFA concentration and EB from milk MIR spectral data. Two models were tested, either using only MIR spectra or using MIR spectra and milk yield as predictor variables. Models were validated with a leave-one-out cross validation and by randomly leaving out 20% of the cows for cross validation (5 random replicates).

MIR spectra from morning and evening milks were studied separately to examine if blood plasma NEFA concentration observed in the morning could be most accurately predicted using milk samples collected the same day at morning or evening milking.

The robustness of the developed prediction equation for blood plasma NEFA concentration was studied by calibrating the prediction equations with records (evening MIR spectral readings) from cows of the Viikki and Maaninka herds only and then predicting NEFA for cows of the Minkiö herd.

Results and Discussion

Means of the observations recorded within three different weeks in milk from cows of the three different research herds is presented in Table 1. In lactation weeks 2 and 3 EB was on average highly negative (from -40.2 to -32.4 MJ/d) and NEFA concentration in blood was high (from 0.58 to 0.45 mmol/l). In lactation week 20 the situation was stabilized with positive EB and decreased NEFA concentration (0.13 mmol/l).

Table 1. Means and standard deviations (in parenthesis) of recorded observations

Week in milk	2	3	20
Records	627	661	297
ECM	30.1 (7.3)	32.1 (7.4)	32.0 (5.9)
DMI	16.4 (3.2)	18.0 (3.4)	22.1 (3.0)
EB	-40.2 (31.4)	-32.4 (32.9)	7.0 (22.9)
NEFA	0.58 (0.30)	0.45 (0.23)	0.13 (0.06)

ECM = Energy-corrected milk (kg/d), DMI = Dry matter intake (kg/d), EB = Calculated energy balance (MJ/d), NEFA = Non-esterified fatty acids (mmol/l)

The fitting statistics for two different models for NEFA and EB are presented in Table 2. Following McParland et al. (2012), alternatively, also milk yield was included in the model as predictor variable. This failed to improve the accuracy when predicting NEFA, but clearly improved the accuracy when predicting EB. However, the correlation between observed NEFA and EB predicted with MIR spectra was -0.65 and the correlation between observed NEFA and EB predicted with MIR spectra and milk yield was only -0.56. This indicates that including milk as a prediction variable to predict EB, actually makes a weaker predictor for energy status. Cross validation results from leaving randomly 20% of cows out were slightly lower than from leave-one-out cross validation, probably because observations used for leave-one-out cross validation were not independent.

Table 2. Partial least squares regression fitting statistics[1] for NEFA and energy balance (EB) using MIR spectra (a.m. and p.m.) or MIR spectra and milk yield (my) as prediction variables in the model (809 NEFA observations), validated by leave-one-out or leave 20% cows out cross validation.

		NEFA			EB		
		N fac	R^2cv	RMSE (mmol/l)	N fac	R^2cv	RMSE (MJ/d)
Leave-one-out	Spectra	20	0.64	0.18	18	0.46	25.82
	Spectra+my	20	0.59	0.18	16	0.64	21.24
Leave 20% cows out	Spectra	9	0.58	0.19	11	0.45	25.94
	Spectra+my	10	0.56	0.19	12	0.62	21.22

[1] N fac = No of factors used (restricted to 20), R^2cv = coefficient of determination of cross validation, RMSE = root mean square error

Predictions based on evening milk samples yielded higher coefficient of determination (R^2cv=0.67) than predictions based on morning samples (R^2cv=0.59) (Table 3). This supports Blum et al. (2000) findings on diurnal variation in plasma NEFA concentration, and suggests that prediction of morning NEFA should be based on evening milk sample spectra.

Table 3. Partial least squares regression fitting statistics[1] from predicting morning blood NEFA from morning and evening milk MIR spectral data.

MIR spectra	N rec	N fac	R^2cv	RMSE (mmol/l)
Morning	809	20	0.59	0.18
Evening	778	20	0.67	0.17

[1] N rec = No of records, N fac = No of factors used (restricted to 20), R^2cv = coefficient of determination of cross validation, RMSE = root mean square error

In many studies on utilizing MIR spectral data it is shown that the variation in the validation data set must be represented in the calibration data set (McParland et al., 2012; De Marchi et al., 2014). The three research herds in our study differed in the mean levels of milk yield, dry matter intake, EB and NEFA, and had differences in feeding and management. When we calibrated the equations with leave-one-out cross validation with evening MIR spectral readings and NEFA records from Viikki and Maaninka herds and then predicted NEFA for cows in Minkiö herd, we found a R^2 of 0.58 and a RMSE of 0.19 mmol/l in Minkiö data set. We compared the spectral variability of Minkiö data with the data from two other herds and the spectral variability was higher in Minkiö data. Also the outliers were examined, but no profound outliers either in the predictor or the response space were found.

The overall mean for observed value of NEFA in early lactation was 0.52 (minimum value 0.10 and maximum value 1.95). Dórea et al. (2017) applied a threshold of > 0.60 mmol/l to classify cows with high level of NEFA concentration. Thus, the RMSE of 0.17-0.20 mmol/l seems to be on a reasonable level for distinguishing cows with normal or very high level of NEFA.

Conclusion

These results indicate that the energy status of a cow could be estimated from milk MIR spectral data with reasonable accuracy. The accuracy of prediction was highest when using evening milk MIR spectral data to predict NEFA measured after morning milking.

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List of References

Bastin, C., N. Gengler, & H. Soyeurt, 2011. Phenotypic and genetic variability of production traits and milk fatty acid contents across days in milk for Walloon Holstein first-parity cows. J. Dairy Sci. 94: 4152-4163.

Berry, D.P. & J.J. Crowley, 2013. Cell biology symposium: Genetics of feed efficiency in dairy and beef cattle. J. Anim Sci. 91: 1594-1613.

Blum, J.W., R.M. Bruckmaier, P.-Y. Vacher, A. Münger & F. Jans, 2000. Twenty-four-hour patterns of hormones and metabolites in week 9 and 19 of lactation in high-yielding dairy cows fed triglycerides and free fatty acids. J. Vet. Med. A 47: 43-60.

De Marchi, M., V. Toffanin, M. Cassandro & M. Penasa, 2014. Invited review: Mid-infrared spectroscopy as phenotyping tool for milk traits. J. Dairy Sci. 97: 1171-1186.

Dórea, J.R.R., E.A. French & L.E. Armentano, 2017. Use of milk fatty acids to estimate plasma nonesterified fatty acid concentrations as an indicator of animal energy balance. J. Dairy Sci. 100: 6164-6176.

McParland, S., G. Banos, B. McCarthy, E. Lewis, M.P. Coffey, B. O'Neill, M. O'Donovan, E. Wall & D.P. Berry, 2012. Validation of mid-infrared spectrometry in milk for predicting body energy status in Holstein-Friesian cows. J. Dairy Sci. 95: 7225-7235.

Mäntysaari, P. & E.A. Mäntysaari, 2015. Modeling of daily weights and body weight changes of Nordic Red cows. J. Dairy Sci. 98: 6992-7002.

Roche, J.R., N.C. Friggens, J.K. Kay, M.W. Fisher, K.J. Stafford & D.P. Berry, 2009. Invited review: Body condition score and its association with dairy cow productivity, health and welfare. J. Dairy Sci. 92: 5769-5801.

Stoop, W.M., H. Bovenhuis, J.M.L. Heck & J.A.M. van Arendonk, 2009. Effect of lactation stage and energy status on milk fat composition of Holstein-Friesian cows. J. Dairy Sci. 92: 1469-1478.