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predicting dairy cow life span with milk sampling in early lactation

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Samenvatting NL. De gemiddelde levensduur van melkkoeien in Nederland is 5,8 jaar. De voornaamste redenen voor onvrijwillige afvoer van melkvee zijn onvruchtbaarheid, mastitis, en klauw- en beenproblemen. Deze gezondheidsproblemen hebben vaak ook een relatie met de eerste weken van de lactatie vanwege een moeilijke lactatiestart of problemen bij de koe om zich aan te passen aan de nieuwe lactatie met stofwisselingsstoornissen als gevolg. Het doel van deze studie was om de waarde te bepalen van Fourier-transform infrared spectroscopy (FTIR) profielen van melk in begin lactatie voor 1) indicatie van metabole status van de koe in begin lactatie; en 2) als voorspeller van het succesvol afronden van de lactatie. De resultaten laten zien dat koeien die de volgende lactatie bereikten aan het begin van de lactatie een hogere concentratie vetzuren in het bloed hadden en een hogere melkproductie met minder variatie tussen dagen.

Summary UK. The average life span of dairy cows in the Netherlands is 5.8 years. The main reasons for involuntary culling in dairy cows are infertility, mastitis and locomotion disorders. These disorders often start during the first weeks of lactation due to the inability of the cow to cope with the metabolic changes of the new lactation. The main objective of the present study was to determine the value of the Fourier-transform infrared spectroscopy (FTIR) mid-infrared profile of milk samples taken from dairy cows in the first weeks of lactation 1) as an indicator of the metabolic state in early lactation; and 2) as a predictor of dairy cow's survival to next lactation. Results showed that cows that reach the next lactation have a higher blood NEFA concentration and a higher milk production with less variation between days. In conclusion, survival of the complete lactation was related with milk performance and metabolic status of dairy cows in early lactation.

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Summary for publication

The average life span of dairy cows in the Netherlands is 5.8 years. The main reasons for involuntary culling in dairy cows are infertility, mastitis and locomotion disorders. These disorders often start during the first weeks of lactation due to the inability of the cow to cope with the metabolic changes of the new lactation. Herewith, it can be hypothesized that the metabolic status of dairy in early lactation is related with health and survival of this cow. Metabolic status can be determined by analysis of metabolites in plasma, but also in milk. The main objective of the present study was to determine the value of the Fourier-transform infrared spectroscopy (FTIR) mid-infrared profile of milk samples taken from dairy cows in the first weeks of lactation 1) as an indicator of the metabolic state in early lactation; and 2) as a predictor of dairy cow's survival to next lactation. For this study, a total of 455 Holstein Friesian dairy cows of all ages were monitored at Dairy Campus between April 2018 and June 2020. In the third and fourth week after calving, milk yield was registered daily and averaged per week. Milk samples were taken in both weeks during the morning and evening milking on Thursday and FTIR spectra were determined. On the same day as the milk samples, a plasma sample was taken and analysed for non-esterified fatty acid (NEFA), β-hydroxybutyrate (BHB) and glucose concentration. Furthermore, cows were monitored for survival of this complete lactation till the next calving moment. Values of metabolites analysed in plasma itself were compared with predictions based on milk FTIR spectra when a model estimate was available (NEFA) or compared with predictions based on boosted regression trees on all available milk FTIR variables. The correlation between plasma NEFA concentration and predicted plasma NEFA concentration from FTIR spectra was weak to moderate ($R^2 \leq 0.25$), depending on sampling moment. Models based on boosted regression trees on all available milk FTIR variables had similar to lower performances for prediction of plasma metabolites. Relation between milk yield, milk components as estimated from FTIR spectra and plasma values were related with survival till the next calving using a repeated measurements model. Cows that did survive till the next calving moment had in early lactation a greater milk yield, lower variation in milk yield and a tendency for a greater lactose content in milk. In addition, NEFA estimated from FTIR spectra was greater and plasma NEFA concentration tended to be greater in cows that survived till the next calving moment. In multiparous cows that survived till the next calving moment, plasma glucose was lower, compared with cows that did not survive. In primiparous cows that survived till the next calving moment, plasma glucose was greater, compared with cows that did not survive. In conclusion, survival of the complete lactation was related with milk performance and metabolic status of dairy cows in early lactation.

Samenvatting

De gemiddelde levensduur van melkkoeien in Nederland is 5,8 jaar. De voornaamste redenen voor onvrijwillige afvoer van melkvee zijn onvruchtbaarheid, mastitis, en klauw- en beenproblemen. Deze gezondheidsproblemen hebben vaak ook een relatie met de eerste weken van de lactatie vanwege een moeilijke lactatiestart of problemen bij de koe om zich aan te passen aan de nieuwe lactatie met stofwisselingsstoornissen als gevolg. Men kan veronderstellen dat de stofwisseling van koeien in begin lactatie gerelateerd is aan gezondheid en levensduur van de koe. Stofwisseling, ofwel metabole status, van de koe kan bepaald worden aan de hand van analyse van metabolieten in het bloedplasma, maar ook in de melk. Het doel van deze studie was om de waarde te bepalen van Fourier-transform infrared spectroscopy (FTIR) profielen van melk in begin lactatie voor 1) indicatie van metabole status van de koe in begin lactatie; en 2) als voorspeller van het succesvol afronden van de lactatie. Voor deze studie zijn in totaal 455 Holstein Friesian melkkoeien van alle leeftijden gevolgd op Dairy Campus tussen april 2018 en juni 2020. In de derde en vierde week na afkalven is dagelijkse melkproductie geregistreerd en gemiddeld per week. Melkmonsters zijn genomen in beide weken gedurende de ochtend -en avondmelking waarin FTIR spectra zijn bepaald. Op dezelfde dag als de melkmonsters, is er ook een bloedmonster genomen voor het bepalen van niet-veresterde vetzuren (NEFA), β-hydroxyboterzuur (BHB) en glucose concentratie. Verder zijn de koeien gevolgd tot en met het volgende afkalfmoment of afvoer, wanneer de dieren niet meer een volgende keer afkalfden. Bloedwaarden zijn vergeleken met schattingen op basis van de melk FTIR profielen voor NEFA in plasma. De correlatie tussen plasma NEFA concentratie en geschatte NEFA concentratie was zwak tot matig ($R2 \leq 0.25$), afhankelijk van het monstername moment. Relatie tussen melkproductie, melkcomponenten zoals geschat op basis van de FTIR profielen en plasma waarden werden gerelateerd aan ‘ overleving van de koe tot de volgende lactatie’ met behulp van een herhaalde waarnemingen model. Koeien welke wel weer een volgende keer afkalfden, en dus de huidige lactatie overleefden, hadden in begin lactatie een hogere melkproductie, minder variatie in melkproductie en er was een tendens voor een hoger lactosegehalte in de melk. Ook was NEFA geschat uit FTIR spectra hoger en was er een trend voor een hogere plasma NEFA concentratie voor koeien welke deze lactatie overleefden en opnieuw afkalfden. In meerderekalfs koeien welke de lactatie overleefden was de plasma glucose concentratie lager in begin lactatie in vergelijking met koeien die niet de huidige lactatie overleefden. In eerstekalfs koeien was dit andersom, daar was de plasma glucose concentratie hoger in begin lactatie voor koeien die de lactatie overleefden, vergeleken met eerstekalfs koeien die niet opnieuw afkalven. In de huidige studie was dus overleving van de complete lactatie tot het volgende afkalfmoment gerelateerd aan melkproductie en metabole status van de koe in begin lactatie.

1 Introduction

1.1 Background

The Dutch dairy industry processes 14 billion kg of milk per year, equivalent to an economic value of 7.6 billion euro (ZuivelNL, 2019). This milk volume is produced by 16,260 dairy farmers managing 1.58 million dairy cows (ZuivelNL, 2019; CBS, 2020). The average life span of dairy cows in the Netherlands is 5.8 years (CRV, 2020). A longer lifespan would not only improve the economic result of the dairy farm, but also increase production efficiency. Around 86% of dairy cows leaving the farm are culled due to health disorders (CanWest DHI). The main reasons for involuntary culling are infertility, mastitis and locomotion disorders (Langford and Stott, 2012). These disorders often start during the first weeks of lactation due to the inability of the cow to cope with the metabolic changes of the new lactation (Van Knegsel et al., 2014). The metabolic state in early lactation is characterized by the mobilization of adipose tissue, increased plasma concentrations of non-esterified fatty acids (NEFA), and beta-hydroxy butyric acid (BHB) and a reduced concentration of plasma glucose (Drackley et al., 2001; Ingvartsen et al., 2006). The low glucose concentration and high concentrations of NEFA and BHB are related to ketosis, hepatic lipidosis (Grummer et al., 1993) and reduced fertility (Leroy et al., 2005), but also a disturbed immune function (Sordillo and Aitken, 2009) and a reduced life span of dairy cows (Seifi et al., 2011).

Traditionally, metabolites like NEFA, BHB and glucose are determined in plasma samples in early lactation, as indicators for the metabolic state or the risk for metabolic disorders at that time point. Plasma NEFA also showed to be related to dairy cow life span (Seifi et al., 2011). Other studies have reported the possible use of other metabolites, like galactose-1-phosphate (Lu et al., 2013) or glycine, choline and carnitine (Xu et al., 2018) as biomarkers of energy balance in early lactation or the risk for culling in the current lactation cycle (Huber et al., 2016).

The metabolic state may also be determined in milk instead of plasma, for example by determining BHB in milk (e.g. Van Knegsel et al., 2010) or by determining plasma NEFA based on fatty acid concentrations in milk (Barbano et al. 2018; Jorjong et al., 2015). To our knowledge, no studies relate milk composition in early lactation to dairy cow lifespan or the risk for culling in that lactation. If milk samples could be used to predict lifespan, a dairy farmer may be able to use this information to improve transition cow management and animal health, decreasing the risk for culling and thereby increasing dairy cow lifespan.

1.2 Objectives

The main objectives of the present study are to determine the value of the Fourier-transform infrared spectroscopy (FTIR) mid-infrared (MIR) profile of milk samples taken from high yielding dairy cows in the first weeks of lactation 1) as an indicator of the metabolic state in early lactation; and 2) as a predictor of survival of dairy cows to the next lactation

2 Materials & Methods

To investigate the relationship between milk analysis in early lactation and the metabolic state of the dairy cow in early lactation as well as the future lifespan of the cow, an animal experiment was performed to collect milk and plasma samples as well as cow information.

2.1 Animals and management conditions

All experimental protocols were approved by the Ethics Committee on Animal Experiments of the Animal Sciences Group of Wageningen University and Research Centre, the Netherlands. A total of 466 Holstein Friesian dairy cows (parity 1 to 9) were monitored at Dairy Campus (Boksumerdyk 11, 9084 AA Leeuwarden, the Netherlands) between April 2018 and June 2020. All cows were housed in groups in the various naturally ventilated barns, with straw-pack bedding or a free stall design with cubicles. All cows were milked twice daily in a 40-stand carrousel milking parlour (GEA, Germany) during the entire lactation.

Table 2.1 *Parity distribution.*

Parity	Number of cows
1	136
2	92
3	102
4	72
5	40
6	13
7	8
8	1
9	2

Cows were fed a mixed roughage diet ad libitum, supplemented with concentrates fed individually in concentrate feeders. Diet composition was regularly recalculated to match the requirements for energy and protein according to common Dutch practice (CVB, 2015). Drinking water was available *ad libitum*.

2.2 Data collection

All cows were monitored from calving until the next calving (or culling). If cows did not conceive (no calving), at least 305 days of lactation were included in the monitoring study.

2.2.1 Milk yield

Milk yield was recorded daily and averaged per week.

2.2.2 Milk and plasma sampling

In the third and fourth week after calving, milk samples were taken during the morning and evening milking on Thursday and the milk yields were registered. At least 100 mL of milk was collected with Tru Test Milk Meters (Datamars Livestock, Lamone, Switzerland), sampling small portions of milk throughout the entire milking session. Samples were cooled on ice and collected by Delta Instruments for FTIR analysis.

In the third and fourth week after calving, on Thursday around 11h, blood was sampled from the coccygeal vein or artery into evacuated EDTA tubes (Vacuette, Greiner BioOne, Kremsmunster,

Austria). Plasma samples were analysed at the laboratory of the Adaptation Physiology group of Wageningen University. Concentration of NEFA was determined using kit code 434-91795 or code 436-91995 (FUJIFILM Wako Chemicals GmbH, Neuss, Germany). Concentration of BHB was determined using kit no 2940, Instruchemie, Delfzijl, Netherlands. Concentration glucose was determined using kit no GOD-PAP reagens, code 11491253 (Roche Diagnostics GmbH, Risch-Rotkreuz, Switzerland).

2.3 Calculations and statistical analysis

In total, 891 plasma samples were taken and analysed from 466 unique cows, so 41 cows were only sampled once. Twice the number (1,782) of milk samples were taken with corresponding milk yield recorded. Milk samples were analysed *in triplo* on the FTIR analyser, resulting in three spectra per milk sample. From these spectra Delta Instruments calculated routine variables, like milk fat, protein, lactose and urea contents, and some other variables, like milk-predicted plasma NEFA and groups of fatty acids contents, characterized as de-novo, pre-formed and mixed (Barbano et al., 2018). The three results per sample were averaged to obtain one value per variable per sample. For 1,476 occasions of 455 cows (out of 466 cows), i.e. morning or afternoon milkings, milk samples could be linked to plasma samples, which were collected on a day basis. For 43 plasma samples no corresponding milk samples were available. Based on strongly deviating milk variables, e.g. predicted plasma NEFA > 3000, one record was discarded before further analyses.

From measured daily milk yield and milk fat and protein content estimated from FTIR spectra, fat-and protein corrected milk yield (FPCM) was calculated as follows:

FPCM (kg) = milk (kg) × [0.337 + 0.116 × fat content (%) + 0.06 × protein content (%) (CVB, 2015)

2.3.1 Prediction of plasma metabolites from milk variables

Observed and predicted values were available for NEFA in plasma. The observed values were determined in the plasma samples as taken on the sample days in week 3 and 4 in lactation of each cow. These values were coupled to the FTIR-predicted plasma NEFA concentrations on the milk samples of the morning and afternoon milking. This means that each plasma result was coupled to two FTIR predicted results.
For all metabolic variables, as determined by analysis of plasma samples, models were developed based on all available milk FTIR variables of the same day. Boosted regression trees were used to develop these models, because decision tree induction is one of the basic and most studied machine learning techniques and boosting is an additional technique to overcome one of the main shortcomings of single trees (Mollenhorst et al., 2020; Witten and Frank, 2005). In this study, the Gradient Boosting Machine (GBM; h2o.gbm function (h2o version 3.20.0.2)) was used. Ten times tenfold cross validation was applied to make sure that no random disturbance of splitting the dataset in train and test set could influence the results. Results shown are averages of all iterations.

Performance of the models was evaluated by plotting observed versus predicted values (Piñeiro et al., 2008), including a y = x line and linear regression line, and computing the root-mean-square error (RMSE), coefficient of determination (R^2) and correlation coefficient. Performance metrics were calculated for each milking separately, i.e., morning or afternoon milking in either week 3 and 4. The RMSE was calculated as

$$RMSE = \sqrt[2]{\frac{\sum_1^n (y_{pred} - y_{obs})^2}{n}}$$

With y_{pred} being the predicted value and y_{obs} the corresponding observed value (RMSE metric from h2o.performance function (h2o version 3.20.0.2)). The R^2, calculated relative to the y = x line was calculated as

$$R^2 = 1 - \frac{\sum(y_{obs} - y_{pred})^2}{\sum(y_{obs} - \bar{y}_{obs})^2}$$

with $\bar{y}_{obs}$ being the mean of all y_{obs} corresponding with the predicted values and remainder as defined above (r2 metric from h2o.performance function (h2o version 3.20.0.2)). By calculating the R^2 relative to the y = x line, the performance of the model is evaluated by comparing the predicted values with the observed ones and tells what fraction of the variation in observed values is covered by the predicted values. When R^2 is 1, the model is able to predict the observed values perfectly. When R^2 is 0, the model is just as good a predictor as the mean of the observed values. And when R^2 is negative, the model is a worse predictor than the mean of the observed values. The fit of the regression line is represented by the Pearson correlation coefficient (r), which was calculated using the cor function from R Stats package. This performance parameter, therefore, shows how well the trend in observed values is predicted by the model.

2.3.2 Dairy cow life span

For milk yield (average per day in week 3 and 4) and plasma values (single observation on Thursday in week 3 and 4), two observation were available per cow. For variables based on milk FTIR spectra, i.e. milk components and groups of fatty acids in milk, four observations were available per cow. Since multiple measurements per cow cannot be regarded as independent units of observation, repeated measures analyses of variance (PROC MIXED in SAS version 9.4; SAS Institute, Inc. Cary, NC) was performed for milk yield, plasma values, milk components and groups of fatty acids in milk. Survival till the next calving moment (yes or no), parity class (1,2,3,4, or ≥5), season (re i.e. October, November, December) and interactions were included in the model as fixed effects. Cow was considered the repeated subject. A first order autoregressive structure was the best fit and was used to account for within-cow variation. Values are presented as LSMEANS with their SEM.

Recent studies report that the variation in milk variables is related with sensitivity of cows to disease (Poppe et al., 2019; Kok et al., 2020). Therefore, the standard deviation per cow is calculated for milk yield, milk components and groups of fatty acids in milk. The SD of each of these variables was analysed using analysis of variance model (PROC MIXED in SAS version 9.4; SAS Institute, Inc. Cary, NC). Survival till the next calving moment (yes or no), parity class (1,2,3,4, or ≥5), season (winter, spring, summer or autumn) and interactions were included in the model as fixed effects. Values are presented as LSMEANS with their SEM.

3 Results

The 455 cows monitored in this study calved between 24-MAR-2018 and 30-JAN-2019. Of the 455 cows, 41 cows calved in winter, 139 cows calved in spring, 135 cows calved in summer, and 140 cows calved in autumn.

3.1 Prediction of plasma NEFA from milk variables

3.1.1 NEFA in plasma predicted by FTIR from milk samples

The four graphs in Figure 3.1 show that the used model for predicting NEFA values in plasma from FTIR spectra of milk was not able to predict accurately. Although the correlation coefficients deviated significantly from 0 ($P < 0.01$), which would suggest that the trend could be predicted, the correlation was only weak to moderate ($R^2 \leq 0.25$).

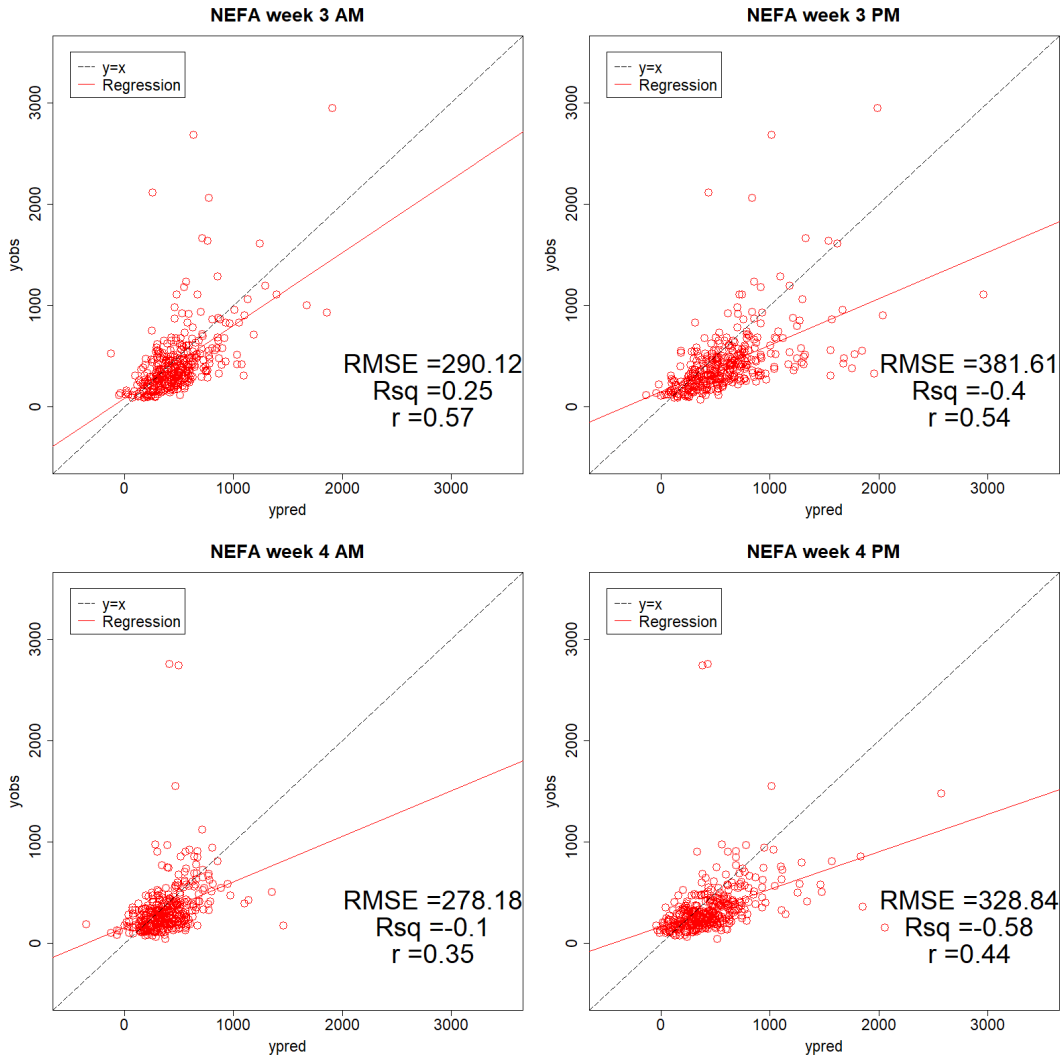

Figure 3.1 *Plots of observed, from plasma analysis, versus predicted, by FTIR on milk samples, values for NEFA in plasma (in µmol/l) of dairy cows in week 3 or 4 in lactation. Plasma samples were collected once per week and related to milk samples from the morning (AM) and afternoon (PM) milking on the same day.*

3.1.2 Prediction of metabolic variables in plasma from all milk variables

Based on the R^2 values of the training of the models (all around 0.80), all three metabolic variables seemed to be predicted rather well. When the models are applied on new instances of the test set, however, model performance was considerably lower with better performance in week 3 than week 4 for all three metabolic variables. Plasma glucose and NEFA were predicted better than plasma BHB. Prediction errors, as represented by the RMSE, doubled between training and testing for all three variables. The large difference in performance on train and test set point at possible overfitting of the model on the training set.

Table 3.1 *Performance metrics of boosted regression tree models predicting metabolic variables from milk variables in week 3 and 4 of lactation*

Metabolic variable	Week	Train R^2	Train RMSE	Test R^2	Test RMSE
BHB	3	0.79	0.26	0.19	0.46
BHB	4	0.82	0.25	-0.09	0.53
Glucose	3	0.82	3.1	0.25	6.1
Glucose	4	0.85	2.6	0.14	6.0
NEFA	3	0.83	128	0.23	245
NEFA	4	0.79	116	0.15	214

The 10 most important milk variables that were included in the different models are shown in Table 3.2. In the boosted regression tree models for BHB and NEFA the proportions of fatty acid groups in total fat were most important. For predicting glucose concentration in plasma, FPCM production and lactose content of milk were most important.

Table 3.2 *Ten most important milk variables, as indicated by the averaged relative importance, in boosted regression tree models predicting BHB, glucose and NEFA concentration in plasma in the 3rd or 4th week of lactation. The three most important milk variables per plasma variable are indicated by bold face values.*

Variable	BHB_3	BHB_4	Glucose_3	Glucose_4	NEFA_3	NEFA_4
FPCM_AM[1]	0.26		0.64	**0.98**		
FPCM_PM			**0.94**	0.36		0.38
Protein_AM	0.15	**0.30**	0.20	0.31		
Protein_PM			0.20		0.11	0.21
Lactose_AM		0.13	0.22	**0.51**		
Lactose_PM	0.16	0.14	**0.75**	**0.80**		
Urea_AM						**0.48**
Urea_PM					0.20	**0.40**
NEFA_AM	0.15	0.28	0.38	0.30		
NEFA_PM	0.27		**0.72**		0.12	0.20
DeNovoPerc_AM[2]	**0.82**	**0.52**		0.28	**0.79**	0.22
DeNovoPerc_PM		0.15		0.32	0.22	**0.94**
MixedPerc_AM	0.13	0.30	0.20		0.17	
MixedPerc_PM				0.29	0.35	0.26
PreFormPerc_AM	**0.78**	**0.88**	0.39	0.36	**0.60**	0.20
PreFormPerc_PM	**0.47**	0.23			**0.61**	0.30
DeNovo_AM		0.11				
PreForm_PM	0.11					

[1] AM / PM indicating morning and afternoon milkings

[2] Variables with "Perc" in the name represent groups of fatty acids expressed in percentage of total fat

3.2 Indicators for dairy cow life span

In total 455 cows calved within this experiment and were sampled in week 3 and 4 after calving. Of these 455 cows, 100 cows did not complete this lactation till the subsequent calving moment (Table 3.3).

Table 3.3 *Number of cows that survived till the next calving moment per parity class.*

	Survival till next calving moment		Total
Parity class	**Yes**	**No**	
1	118	14	132
2	69	21	90
3	77	23	100
4	51	21	72
≥5	40	21	61
Total	355	100	455

3.2.1 Life span related with milk yield and plasma metabolites in early lactation

Cows that survived till the next calving moment had a greater milk yield and fat-and protein corrected milk yield in week 3 and 4 after calving in all seasons, except in winter (Table 3.4).

Cows that survived till the next calving moment tended to have greater plasma NEFA concentration in week 3 and 4 after calving, compared with cows that did not calve again. Primiparous cows that survived till the next calving had a greater plasma glucose concentration in week 3 and 4 of the lactation, while older cows had a lower plasma glucose concentration in week 3 and 4 when they survived till the next calving.

Table 3.4 *Milk yield[1] and plasma values[2] for cows that survived till the next calving moment.*

	Survival till next calving		SEM	*P* -values					
	Yes	**No**		**Survival (Su)**	**Parity[3] (P)**	**Season (Se)**	**Su × P**	**Su × Se**	**P × Se**
Cows, n	355	100							
Milk variables									
Milk yield (in kg/d)	37.9	37.0	0.8	0.38	<0.01	0.09	0.09	<0.01	0.49
FPCM (in kg/d)	40.9	39.7	0.9	0.31	<0.01	<0.01	0.15	<0.01	0.45
Metabolites in plasma									
NEFA[5] (µmol/l)	481	421	28	0.08	<0.01	<0.01	0.57	0.38	<0.01
Glucose (mg/dl)	57.0	57.2	0.7	0.79	<0.01	<0.01	0.03	0.47	0.20
BHB [6] (mmol/l)	0.861	0.917	0.062	0.45	0.06	0.52	0.15	0.95	0.35

[1,2] Measured at two moments in week 3 and 4 after calving; [3] Divided in 5 parity classes (1,2,3,4 and ≥5); [4]Fat-and-protein corrected milk yield; [5] Non-esterified fatty acids; [6] β-Hydroxybutyric acid.

3.2.2 Life span related with FTIR estimates in milk

Cows that survived till the subsequent calving moment tended to have a greater lactose percentage in in week 3 and 4 after calving, compared with cows that did not calve again (Table 3.5). Milk fat percentage, estimated via three different algorithms, protein percentage and urea did not differ between cows that did and cows that did not calve again after the studied lactation.

Cows that survived till the subsequent calving moment had a greater plasma NEFA concentration, as estimated from FTIR spectra, in week 3 and 4 after calving, compared with cows that did not calve again. Fraction of de novo, mixed and preformed did not differ between cows that did and cows that did not calve again after the studied lactation.

Table 3.5 *Estimates based on FTIR spectra[1] for milk components and groups of fatty acids in milk of cows that survived till the next calving moment. Values represent LSMeans ± SEM (maximal standard error).*

	Survival till next calving		SEM	***P* -values**					
	Yes	**No**		**Survival (Su)**	**Parity [2] (P)**	**Season (Se)**	**Su × P**	**Su × Se**	**P × Se**
Cows, n	355	100							
Milk components									
FatA (%)	4.89	4.87	0.09	0.87	<0.01	<0.01	0.80	0.81	0.60
FatB (%)	4.79	4.77	0.09	0.87	<0.01	<0.01	0.71	0.83	0.60
FatPLS (%)	4.65	4.62	0.09	0.78	<0.01	<0.01	0.82	0.85	0.64
Protein (%)	3.44	3.44	0.03	0.89	0.01	0.04	0.16	0.50	<0.01
Lactose (%)	4.72	4.68	0.02	0.07	<0.01	0.17	0.28	0.54	0.68
Urea	17.4	18.4	1.0	0.45	<0.01	<0.01	0.98	0.19	0.44
Groups of fatty acids in milk									
NEFA [3] (µmol/l)	533	451	31	0.03	<0.01	0.52	0.45	0.38	0.52
De novo fatty acids	1.04	1.04	0.03	0.86	<0.01	<0.01	0.89	0.51	0.22
Mixed fatty acids	1.40	1.43	0.02	0.26	<0.01	<0.01	0.20	0.17	0.03
Preformed fatty acids	2.04	1.97	0.07	0.38	<0.01	0.09	0.87	0.96	0.68

[1] Measured at 4 moments in week 3 and 4 after calving; [2] Divided in 5 parity classes (1,2,3,4 and ≥5); [3] Non-esterified fatty acids.

3.2.3 Life span related with variation in milk and milk components in early lactation

Cows that survived till the subsequent calving moment had less variation in milk yield among the 4 sampling moments in week 3 and 4 after calving, compared with cows that did not calve again (Table 3.5). Variation in FPCM, milk components and groups of fatty acids in milk did not differ between cows that did and cows that did not calve again after the studied lactation.

Table 3.6. *Variation (SD) among sampling moments[1] for milk yield, FTIR estimates for milk components and groups of milk fatty acids of cows that survived till the next calving moment. Values represent LSMeans ± SEM (maximal standard error).*

	Survival till next calving		**SEM**	***P* -values**					
	Yes	**No**		**Survival (Su)**	**Parity[2] (P)**	**Season (Se)**	**Su × P**	**Su × Se**	**P × Se**
Cows, n	355	100							
Milk yield (in kg/d)	1.34	1.69	0.14	0.04	0.03	<0.01	0.14	0.27	0.38
FPCM [3](in kg/d)	1.42	1.55	0.16	0.49	0.18	0.08	0.84	0.16	0.69
Milk components									
FatA	0.55	0.58	0.06	0.58	<0.01	0.03	0.61	0.93	0.22
FatB	0.55	0.59	0.05	0.55	<0.01	0.02	0.60	0.95	0.18
FatPLS	0.53	0.57	0.05	0.57	<0.01	0.03	0.59	0.95	0.25
Protein	0.08	0.07	0.01	0.72	0.32	0.51	0.48	0.51	0.79
Lactose	0.060	0.064	0.01	0.64	0.24	<0.01	0.44	0.77	0.56
Urea	2.89	3.29	0.68	0.62	0.78	0.97	0.57	0.95	0.68
Groups of fatty acids in milk									
NEFA[4] (µmol/l)	90.3	82.2	10.2	0.51	<0.01	0.17	0.54	0.56	0.82
De novo fatty acids	0.13	0.14	0.02	0.75	0.11	0.54	0.61	0.81	0.51
Mixed fatty acids	0.13	0.14	0.01	0.41	<0.01	<0.01	0.47	0.62	<0.01
Preformed fatty acids	0.31	0.33	0.04	0.56	<0.01	0.23	0.51	0.99	0.93

[1] Measured at two moments in week 3 and 4 after calving; [2] Divided in 5 parity classes (1,2,3,4 and ≥5); [3]Fat-and-protein corrected milk yield; [4] Non-esterified fatty acids.

4 Discussion and conclusion

Cows that survived till the next calving moment had a greater milk yield, with the exception of cows that calved in winter. This implies that especially high-producing cows had a greater chance for survival. Milk production is an important determinant for survival in dairy cows (Van der Heide et al., 2020). Survival is partly a farmer's decision who is likely to give more credits for high-producing cows when selecting cows for the next lactation. Alternatively, diseased cows can be expected to produce less milk (Hostens et al., 2012), which could also be a reason why cows that did not survive till the next calving moment had on average a lower milk production compared with cows that did survive till the next calving moment. Although it was not part of the focus of the current study, detailed evaluation of type and moment of disease during the lactation in relation with plasma values and milk variables could clarify underlying causes for lack of survival and its relation with milk yield.

Cows that did survive till the next calving moment had in week 3 and 4 of lactation a greater NEFA estimated from FTIR spectra, and a tendency for a greater NEFA and lower glucose concentration in plasma. The greater milk production and greater associated energy requirements for milk production might explain the greater plasma NEFA concentration and lower plasma glucose concentration in early lactation of cows that survived till the next lactation. This is in contrast to earlier work, where cows with elevated NEFA concentration $\geqslant$ 1.0 mmol/L in the first 3 weeks after calving were more likely to be culled (Seifi et al., 2011). Contrast between studies could be related with 1) the extended observation period from the moment of plasma sampling onwards (i.e. complete lactation), compared with Seifi et al. (2011) who monitored the cows for 2 months after plasma sampling, or 2) average NEFA values in the current study were lower, compared with Seifi et al., (2011). Greater average plasma NEFA concentration in Seifi et al., (2011) might be related to sampling earlier in lactation (week 1, 2, and 3) compared with the current study. Moreover the extremely elevated NEFA values for cows that did not survive in Seifi et al. (2011), might represent cows suffering from severe metabolic stress or disease. This in contrast to the current study, where the mildly raised plasma NEFA values for cows that did survive the lactation probably represent the greater energy requirements of high-producing, but mostly healthy, cows.

In the current study, milk lactose was positively related with survival till the next calving moment. Lactose, together with minerals and cells, determine the osmotic value of milk. In addition, the concentration of minerals and cells in milk also depends on integrity of mammary epithelial tissue. Mastitis is characterized with a local inflammatory response in the mammary gland and loss of integrity of epithelial tissue, which both contribute to entering of cells into milk, and consequently a reduction milk lactose content. In the current study, somatic cell count in milk was not measured, but it can be hypothesized that the greater lactose content in cows that survived till the next lactation is associated with a lower incidence of mastitis and lower somatic cell count in milk.

Variation in milk yield among sampling moments within a cow was lower for cows that did survive till the next calving moment. Variation among observations is one of the variables considered to quantify resilience (Scheffer et al., 2018). Resilience of animals is the capacity to cope with short-term perturbations in their environment and to return rapidly to their pre-challenge status (Colditz and Hine, 2016). Reduced resilience in dairy cows can be hypothesized to be related with disease and lack of survival. Indeed in the current study reduced variation in milk yield in early lactation was related with survival of this lactation till the next calving moment. This is in line with other recent studies , which related variation in milk yield within a cow with genetic background for health, longevity and fertility (Poppe et al., 2019) and mastitis (Kok et al., 2020).

The correlation between plasma NEFA concentration and predicted plasma NEFA concentration from FTIR spectra was weak to moderate. This limited relationship, compared with earlier studies at Delta Instruments (R^2 = 0.71) and Cornell University (R^2 = 0.51; Bach et al., 2021), might be related to a wider range in NEFA values, as well as a higher average NEFA value in the earlier studies. Possibly also different feeding strategies, production level and energy status of the cows could contribute to the

difference in accuracy of NEFA predictions based on FTIR profiles. With respect to feeding strategies, differences in ration composition (grass based diets in NL, greater fat content in US), ration management (partial mixed rations and separate concentrate supply in NL, total mixed rations in US) and possible differences in feed intake might result in different contribution of the ration to NEFA levels in plasma, as well as differences in diet effects on milk fat and milk fat composition. Moreover, also absolute concentration of fat in milk is usually greater in NL, which can be hypothesized to result in reduced impact of elevations in NEFA on milk fat yield and milk fat composition.

In conclusion, cows that did survive till the next calving moment had in early lactation (week 3 and 4) a greater milk yield, lower variation in milk yield and a tendency for a greater lactose content in milk. In addition, NEFA estimated from FTIR spectra was greater and plasma NEFA concentration tended to be greater in cows that survived till the next calving moment. In multiparous cows that survived till the next calving moment, plasma glucose was lower, compared with cows that did not survive. In primiparous cows, that survived till the next calving moment, plasma glucose was greater, compared with cows that did not survive.

5 Recommendations for further study

In the current study, the relationship between predicted plasma NEFA and observed plasma NEFA was weak to moderate. This could partly be explained by the relatively low NEFA concentration in the current study compared with earlier work (Barbano et al., 2018). Approaches to increase the number of observations with high plasma NEFA could be: advance the plasma sampling moment relative to calving, or induce a more severe negative energy balance.

The current study focussed on the relation between observed plasma NEFA and predicted NEFA. Further studies could study the value of FTIR spectra to predict BHB, glucose, or metabolic status as a composite trait, combining e.g. BHB, glucose and NEFA predictions.

In the current study, FTIR prediction for milk components, including prediction for plasma NEFA, was related with survival. Survival can be considered a multifactorial trait, related with milk production, health, various types of disease (metabolic health, mastitis, claw health), reproductive success and possibly also behaviour of dairy cows. Detailed evaluation of these underlying factors contributing to (lack of) survival in relation with plasma values and milk variables could clarify underlying causes for lack of survival and its relation with milk yield. Moreover, in a larger dataset it would be interesting to combine disease data with FTIR information to predict specific disease?

In the current study, survival till the next calving moment was related with FTIR predictions for milk components. Prediction power of FTIR spectra for survival could be greater than reported in this study and include other aspects of the milk FTIR profile. Future studies could focus on the value of raw spectra to indicate survival in dairy cows.

In the current study, milk and plasma was sampled on a standardised moment of the day to limit the effect of within-day variation on plasma profiles and possibly also milk composition. For application in herds with robot milking, it can be expected that the within-day variation in metabolic plasma profiles is relevant for interpretation of the data. Moreover, little is known on the consequences of milk sampling moment, for FTIR profiles or prediction accuracy for e.g. fatty acid groups or plasma values of NEFA and BHB. Future studies could focus on identifying the within-day variation of milk FTIR profiles and its consequences for application in herds with milking robots.

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