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Potential of milk mid-infrared spectra to predict nitrogen use efficiency of individual dairy cows in early lactation

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ABSTRACT

Improving nitrogen use efficiency (NUE) at both the individual cow and the herd level has become a key target in dairy production systems, for both environmental and economic reasons. Cost-effective and large-scale phenotyping methods are required to improve NUE through genetic selection and by feeding and management strategies. The aim of this study was to evaluate the possibility of using mid-infrared (MIR) spectra of milk to predict individual dairy cow NUE during early lactation. Data were collected from 129 Holstein cows, from calving until 50 d in milk, in 3 research herds (Denmark, Ireland, and the UK). In 2 of the herds, diets were designed to challenge cows metabolically, whereas a diet reflecting local management practices was offered in the third herd. Nitrogen intake (kg/d) and nitrogen excreted in milk (kg/d) were calculated daily. Nitrogen use efficiency was calculated as the ratio between nitrogen in milk and nitrogen intake, and expressed as a percentage. Individual daily values for NUE ranged from 9.7 to 81.7%, with an average of 36.9% and standard deviation of 10.4%. Milk MIR spectra were recorded twice weekly and were standardized into a common format to avoid bias between apparatus or sampling periods. Regression models predicting NUE using milk MIR spectra were developed on 1,034 observations using partial least squares or support vector machines regression methods. The models were then evaluated through (1) a cross-validation using 10 subsets, (2) a cow validation excluding 25% of the cows to be used as a validation set, and (3) a diet validation excluding each of the diets one by one to be used as validation sets. The best statistical performances were obtained when using the support vector machines method. Inclusion of milk yield and lactation number as predictors, in combination with the spectra, also improved the calibration. In cross-validation, the best model predicted NUE with a coefficient of determination of cross-validation of 0.74 and a relative error of 14%, which is suitable to discriminate between low- and high-NUE cows. When performing the cow validation, the relative error remained at 14%, and during the diet validation the relative error ranged from 12 to 34%. In the diet validation, the models showed a lack of robustness, demonstrating difficulties in predicting NUE for diets and for samples that were not represented in the calibration data set. Hence, a need exists to inte-

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grate more data in the models to cover a maximum of variability regarding breeds, diets, lactation stages, management practices, seasons, MIR instruments, and geographic regions. Although the model needs to be validated and improved for use in routine conditions, these preliminary results showed that it was possible to obtain information on NUE through milk MIR spectra. This could potentially allow large-scale predictions to aid both further genetic and genomic studies, and the development of farm management tools.

Key words: Fourier-transform mid-infrared spectrometry, nutrition, environment, modeling

INTRODUCTION

Improving nitrogen efficiency has become a key target in dairy production systems for several reasons. First, nitrogen losses from dairy systems have a direct effect on the environment and ecosystems (Adenuga et al., 2019). Volatilization of nitrogen from feces and urine as ammonia contributes to acidification of water and soils, and losses as oxides of nitrogen contribute to global climate change (Castillo et al., 2000; Kebreab et al., 2001). In addition, leaching of nitrates into water resources contributes to eutrophication of aquatic environments (Ledgard et al., 1998). Nitrogen efficiency can also affect the economic performance of dairy farms. Although ruminants, including dairy cows, have a unique capacity to transform fibers and pasture-based nitrogen sources into animal proteins, in systems involving high-yielding cows nitrogen from forages are often complemented or replaced by additional feedstuffs to satisfy the nutritional demands of cows (White et al., 2019). In such systems, nitrogen is the most expensive component in the diet (de Freitas et al., 2019), and poor nitrogen efficiency can reduce profitability of dairy farms (Powell et al., 2010). Low nitrogen efficiency can also have a negative effect on reproductive performance (Butler, 2000) and milk processing quality (Hermansen et al., 1999; Castillo et al., 2000). The individual efficiency of dairy cows in transforming feed nitrogen into milk nitrogen is usually expressed as nitrogen use efficiency (**NUE**), which is defined as the ratio of grams of N in milk to grams of N intake (expressed as a percentage; Calsamiglia et al., 2010). The NUE can be highly variable, with values between 8 and 42% reported in the literature (Castillo et al., 2000). This suggests important differences at both the individual animal and the herd levels, and potential opportunities to improve NUE through optimization of diet and improved management, as well as through genetic selection. To achieve this, measurement or prediction of NUE values on a large scale is required. Indeed, previous studies have used milk urea as a biomarker to predict NUE, with Jonker et al. (1998) and Nousiainen et al. (2004) developing models with relative errors of approximately 10% and 7%, respectively. However, milk urea levels have to be measured using chemical methods, which are both time-consuming and expensive. Alternatively, milk urea can be predicted via infrared analysis of milk, but the models are not precise, with a relative error of approximately 20% (Nygaard, et al., 1993), and this combines with the error of the model predicting NUE from milk urea. Low precision of milk urea prediction is due to its low concentration in milk (approximately 0.03%), compared with total nitrogen, which represents approximately 3% of milk (Mathieu, 1998). Methods based on N isotopes have also been investigated. Although Cantalapiedra-Hijar et al. (2016) obtained a model predicting NUE with a coefficient of determination (R^2) of 0.5, Cheng et al. (2011) were unable to validate the link between ^{15}N isotopic fractionation and NUE for the diets used in their experiments. According to Herremans et al. (2019), the ^{15}N isotopic fractionation method would be more appropriate for estimating urinary nitrogen excretion than overall N use efficiency. Additionally, methods involving ^{15}N are both expensive and complex, and are not appropriate for large-scale applications. Fourier-transform mid-infrared (**MIR**) spectra of milk seems a promising proxy (i.e., indicator or indirect trait) to predict NUE, as it contains information on both N in milk and N intake, the 2 parts of the NUE ratio. Nitrogen in milk is routinely predicted using MIR with a high degree of accuracy, with the results used for payment purposes and milk recording. In addition, evidence suggests that diet composition affects milk composition and that, in return, milk MIR spectra can provide some information on diets offered as well (Klaffenböck et al., 2017). Additionally, MIR analysis of milk is fast, cost-effective, and already performed in most of the main dairying countries. Thus the objective of this study is to evaluate the potential of MIR analysis of milk to predict NUE of individual dairy cows.

MATERIALS AND METHODS

Animals and Sampling

The data in this study were collected in the Genotype Plus Environment (**GplusE**) FP7 Project (http://www.gpluse.eu) between October 2014 and May 2015. Common sampling and registration protocols were followed in 3 experimental herds with the goal to sample cows in early lactation. A total of 129 Holstein cows were sampled from 4 to 50 DIM, with parities ranging

Table 1. Overview of diets used in the study

Herd[1]	Diet specifications	Diet[2]	No. of cows
AFBI (UK)	3 isonitrogenous diets comprising mixtures of grass silage and concentrate in different ratios on a DM basis	Low C: 30% Standard C: 50% High C: 70%	18 20 20
AU (DK)	3 isonitrogenous and isocaloric diets comprising grass silage, maize silage, sugar beet pulp pellets, and concentrate including a high level of barley (27%) in the high-starch diet and a high level of dextrose (17%) in the high-sugar diet	High starch: 54% C High sugar: 54% C Standard: 49% C	11 10 14
UCD (IE)	Standard diet comprising grass silage, maize silage, sugar beet pulp pellets, and concentrate; additionally, each cow was offered 8 kg of concentrate per day in the parlor at milking	Standard: 39% C	36

[1]AFBI (UK) = Agri-Food and Biosciences Institute, UK; AU (DK) = Aarhus University, Denmark; UCD (IE) = University College Dublin, Ireland.
[2]C = concentrate.

from 1 to 7, with 58 cows from Agri-Food and Biosciences Institute, UK (**AFBI**), 35 cows from Aarhus University, Denmark (**AU**), and 36 cows from University College Dublin, Ireland (**UCD**).

Diet Analysis

In the AFBI and AU herds, the cows were divided in 3 subgroups and offered different diets, which were designed to challenge the cows metabolically. The UCD cows were offered a diet reflecting local management practices. Diets offered are described in Table 1. Daily feed intake of individual cows was recorded by automated recording systems, Insentec (Markneesse, the Netherlands) in AU and UCD, and using Calan gates linked to an automatic cow identification system (American Calan, Northwood, NH) in AFBI, which allowed cows access to feed boxes mounted on a weigh scales (Griffith Elder, Bury St. Edmunds, UK). Individual diet components were sampled weekly at AU and UCD and daily at AFBI, dried at 85°C for DM content determination, and analyzed for chemical composition at Cumberland Valley Analytical Services' forage lab (Waynesboro, PA) using near-infrared spectroscopy. Daily DMI was calculated for each cow based on daily intake and DM content of diets. For all cows, a 3-d (± 1 DIM) moving average of DMI was calculated to smooth biases due to measurements. Individual N intake was calculated based on diet CP content divided by 6.25 (FAO, 2003) and DMI. Additionally, cows were weighed weekly at AFBI and UCD and daily at AU. Energy balance (**EB**) was calculated daily according to the National Research Council (NRC, 2001), taking into account body weight changes as described by De Koster et al. (2019). Additional details of the experiments, including diets offered, can be found in Little et al. (2019) and Krogh et al. (2019).

Milk Analysis for N Content and MIR Spectra

Milk yield was measured daily. Twice weekly, a.m. and p.m. milk samples were collected from each cow, following ICAR procedures (https://www.icar.org/Guidelines/02-Overview-Cattle-Milk-Recording.pdf). The samples were preserved at 4°C with bronopol 0.02%, and analyzed locally on FT2 and FT6000 spectrometers (Foss Analytics, Hillerød, Denmark) or at Walloon Agricultural Research Center (Gembloux, Belgium) on a Standard Lactoscope FT-MIR automatic (Delta Instruments, Drachten, the Netherlands). To enable future use of the models in the context of milk recording, which uses a blend of a.m. and p.m. milk samples collected over a 24-h period, models need to be developed based on daily spectra. Hence, a.m. and p.m. milk MIR spectra were combined into a unique daily spectrum via a weighted average using a.m. and p.m. milk yields. The spectra of the different instruments were standardized to allow them to be merged into a common data set, following the procedure described in Grelet et al. (2015). Fat, lactose, urea, and total protein content of milk samples were also generated locally from the MIR analysis, and a weighted average for each day was determined using a.m. and p.m. milk yields. Total protein output in milk was calculated daily, based on daily milk yields and biweekly milk total protein content, using the closest composition measure to the milk yield. Daily N output in milk was calculated as daily total protein output in milk divided by 6.38 (WHO and FAO, 2011).

Data Editing and Development of MIR Models

Nitrogen use efficiency was calculated as the ratio of grams of N in milk to grams of N intake, expressed as a percentage. Daily nitrogen losses through feces

and urine were estimated as grams of N intake minus grams of N in milk. Seven inconsistent records with daily DMI below 5 kg/d were discarded. The data set contained 4,824 daily records of NUE from 129 cows, with an average of 37.4 records per cow. In addition, the data set contained a total of 1,267 twice-weekly weighted spectra from the same cows, with an average of 9.8 records per cow. The spectra were merged with the NUE reference values from the same day, providing 1,119 records from 129 cows, with an average of 8.7 records per cow during the period from 4 to 50 d in milk. To prevent errors arising from analytical issues and incorrect associations between spectra and milk composition data, fat and protein models developed at Walloon Agricultural Research Center (unpublished data) were applied to the spectra, and the predictions generated were compared with predictions provided by the labs where the AFBI, AU, and UCD samples were originally analyzed. Records in which the difference between local predictions and lab predictions were greater than 0.2 g/100 mL were discarded, leading to a data set of 1,034 records. The MIR spectra were pretreated by a first derivative with a window of 5 wavenumbers. The spectral areas selected were constituted by 212 wavenumbers, from 968.1 to 1,577.5 cm^{-1}, 1,731.8 to 1,762.6 cm^{-1}, 1,781.9 to 1,808.9 cm^{-1}, and 2,831.0 to 2,966.0 cm^{-1} (Grelet et al., 2016). These areas were selected to exclude noisy parts of the spectrum induced by water and areas not repeatable among different instruments after analysis of common samples, to enable further transfer of the model to different instruments. Parity (1, 2, and 3 or more) and corresponding daily milk yield (**MY**; L/cow per d) were used as predictors as well. For that purpose, these additional variables and the spectral variables were concatenated and were all mean-centered to equally scale the distribution of spectral and additional data.

Models were developed using partial least squares (**PLS**) or support vector machine (**SVM**) regression methods. Number of PLS latent variables (**LV**) was selected visually through the point of break of root mean square error (**RMSE**) slope, where adding a new LV does not reduce substantially the RMSE, with a maximum of 16 LV. The SVM method selects a reduced number of samples, the support vectors, defining the best sparse deterministic regression relationship between the MIR data and the reference values. The SVM method was used after a PLS compression reducing the data set dimension to 14 latent variables, and the Library for Support Vector Machines (integrated software for support vector machines, https://www.csie.ntu.edu.tw/~cjlin/libsvm/) algorithm was used in this study with a radial basis function as kernel. Internal cross-validation was performed on the calibration data set, with 10 subsets randomly constituted. In this cross-validation scheme, records from the same cow with different DIM can be in both the calibration and the validation data sets. The models were also tested by performing a cow validation, randomly removing 25% of the cows to be used as a validation data set and calibrating with records of the remaining 75% of cows. Finally, the models were tested with a diet validation, removing each of the 7 diets one by one, as a validation set, and performing the calibration on the 6 remaining diets. The statistics of the models, in both calibration and validation steps, were expressed in terms of R^2, RMSE, relative error calculated as RMSE/average of the global data set, and ratio of standard deviation of global data set to RMSE (**RPD**).

Computations and models were carried out with programs developed in Matlab 2017b (Mathworks, Natick, MA) and the PLS toolbox version 8.5.1 (Eigenvector Research Inc., Wenatchee, WA).

RESULTS AND DISCUSSION

Descriptive Statistics

Individual daily NUE ranged from 9.7 to 81.7%, with an average of 36.9% and a standard deviation of 10.4%. Distribution of values is plotted in Figure 1. This range was very large compared with the literature. For example, previous studies reported ranges from 25 to 37% (Olmos Colmenero and Broderick, 2006), from 18 to 40% (Nadeau et al., 2007), from 15 to 40% (Calsamiglia et al., 2010), and from 8 to 42% (Castillo et al., 2000). However, the range in the present study was for individual cows, whereas the ranges in these other studies related to averages from groups of cows, or across time periods, which can explain the absence

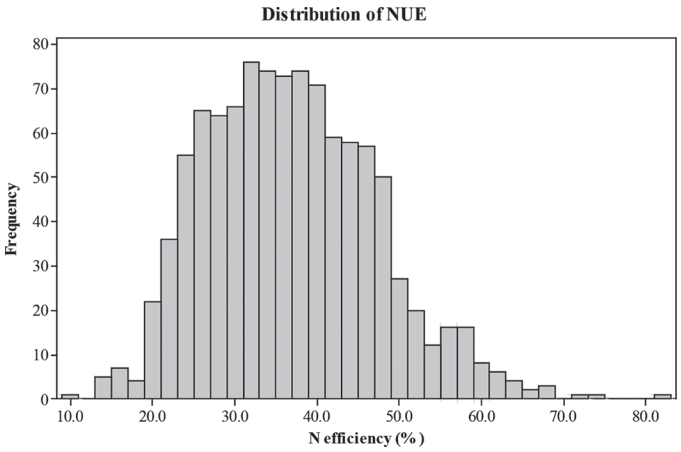

Figure 1. Distribution of daily individual nitrogen use efficiency (NUE) records (n = 1,034 records).

Table 2. Average production and nitrogen data associated with the 7 different diets

Diet[1]	MY[2] (kg/d)	N milk (%)	Weight (kg)	DMI (kg/d)	EB[3] (Mcal/d)	N intake (kg/d)	N diet (%)	N losses (kg/d)	NUE[4] (%)
AFBI (UK)									
High C	37.4	3.33	633	23.5	0.73	0.629	2.7	0.434	31
Low C	27.1	2.94	596	15.4	−5.72	0.408	2.6	0.282	32
Standard	32.6	3.09	604	19.8	−2.22	0.523	2.6	0.367	30
AU (DK)									
High starch	37.9	3.29	600	20.3	−1.21	0.448	2.2	0.256	44
High sugar	35.5	3.21	607	20.2	−3.00	0.462	2.3	0.284	39
Standard	39.5	3.27	594	20.6	−2.94	0.456	2.2	0.255	44
UCD (IE)									
Standard	33.8	2.99	655	18.5	−6.87	0.380	2.0	0.223	42

[1]AFBI (UK) = Agri-Food and Biosciences Institute, UK; AU (DK) = Aarhus University, Denmark; UCD (IE) = University College Dublin, Ireland; C = concentrate.

[2]MY = daily milk yield.

[3]EB = energy balance.

[4]NUE = N use efficiency.

of extreme values and the smaller variation. This large range was likely enhanced because of the different geographical areas, cow genotypes, and large variability of diets offered to the animals. Finally, the extremely high values may be explained by short-term declines in DMI (e.g., short-term illness or estrus) without a concomitant decline in milk N. The average NUE observed in the present study was in the same order of magnitude as that observed in other studies conducted in early lactation. For example, Cowan et al. (1981) observed an average NUE of 35% between DIM 1 to 112, and Law et al. (2009) observed average NUE between 35 and 42%, with diets differing in protein levels, during the period from DIM 1 to 151.

Measurements associated with the 7 different diets offered are reported in Table 2, with averaged NUE per diet ranging from 30 to 44%. The important differences observed between individual records and between groups of diets may suggest potential for improving NUE through nutrition and management practices, and possibly through genetics.

Pearson correlations between NUE, N losses, and other variables are reported in Table 3. As expected, NUE was highly correlated with both variables included in the NUE ratio, N intake (kg/d) and N production in milk (kg/d), and was also highly correlated with N losses. Nitrogen losses were more strongly influenced by N intake than by N production in milk. Regarding the 2 variables used to determine N intake, NUE was more closely linked with percentage of N in diet than with DMI. This suggests potential to improve NUE by modification of the N content of the diet, as reported by Castillo et al. (2001). A negative correlation of −0.67 was observed between NUE and EB, with more efficient animals having a greater negative EB. This could be explained by an indirect link, with cows having a low DMI in early lactation, and consequently low N intake, making the NUE ratio high, and low energy intake as well, leading to negative EB. From this correlation, it is not possible to know whether cows actually are efficient or whether they mobilize their N body reserves. Castillo et al. (2001) found that 98% of N intake was recovered in urine, feces, and milk, and mentioned that BW changes were not expected to affect N balance studies. However, Castillo and colleagues only considered animals after the peak of lactation, which was not the case in the present study. Other studies have found cows to be in negative N balance in early lactation (Cowan et al., 1981; Sutter and Beever, 2000), whereas Komaragiri and Erdman (1997) measured a mobilization of 21 kg of body protein between wk 2 and 5 after calving. The high mean NUE calculated in the present study suggests mobilization of body N reserves as well,

Table 3. Pearson correlation between individual nitrogen use efficiency (NUE), N losses, and production variables

Item	NUE (%)	*P*-value	N losses (kg/d)	*P*-value
DIM	−0.26	<0.001	0.31	<0.001
Parity	0.26	<0.001	0.08	<0.001
Weight (kg)	0.32	<0.001	0.12	<0.001
DMI (kg/d)	−0.20	<0.001	0.77	<0.001
N intake (kg/d)	−0.43	<0.001	0.93	<0.001
Diet N (%)	−0.67	<0.001	0.63	<0.001
EB[1] (Mcal/d)	−0.67	<0.001	0.63	<0.001
Milk yield (kg/d)	0.47	<0.001	0.17	<0.001
Milk fat (%)	−0.06	0.042	−0.01	0.759
Milk lactose (%)	−0.33	<0.001	0.32	<0.001
Milk N (%)	0.19	<0.001	0.03	0.417
Milk N production (kg/d)	0.51	<0.001	0.18	<0.001
Urea (mg/kg)	−0.30	<0.001	0.11	<0.001
N losses (kg/d)	−0.71	<0.001		

[1]EB = energy balance.

Table 4. Performance of PLS and SVM models[1] to predict individual N use efficiency (NUE) in cross-validation with 10 subsets (n = 1,034)

	PLS				SVM			
Predictor[2]	R^2cv[3]	Error (RMSEcv[4])	Relative error (%; RMSEcv/mean)	RPD[5]	R^2cv	Error (RMSEcv)	Relative error (%; RMSEcv/mean)	RPD
MIR	0.59	6.6	18	1.58	0.63	6.3	17	1.65
MIR+Parity	0.62	6.4	17	1.64	0.65	6.1	17	1.70
MIR+Parity+MY	0.72	5.5	15	1.89	0.74	5.3	14	1.97

[1]PLS = partial least square regression; SVM = support vector machines regression.
[2]MIR = mid-infrared spectra; MY = milk yield.
[3]R^2cv = coefficient of determination of cross-validation.
[4]RMSEcv = root mean square error of cross-validation.
[5]RPD = ratio of SD to RMSEcv.

leading to an artificially high short-term NUE that may decrease after peak lactation, when reconstitution of body reserves takes place. However, the present data set does not allow an estimation of true efficiency and losses, due to the absence of data on the N content in urine and feces. Such data would be necessary to avoid bias arising from potential negative N balance in early lactation. Indeed, if NUE or N losses are biased by N balance, improvement of those traits through management or genetic selection could lead to increased body tissue mobilization and health problems induced by severe negative EB. Additionally, it would be interesting to consider NUE and N balance after peak of lactation, to observe true efficiency over long periods and free from bias due to N balance.

MIR Models

The performance of PLS and SVM models in cross-validation with 10 subsets are reported in Table 4. The 3 PLS models were developed with 11, 9, and 14 LV, respectively. For both methods, use of parity and MY in addition to the MIR spectra improved the performance of the models in terms of R^2 of cross-validation, RMSE of cross-validation, relative error, and RPD. Addition of parity to the model may improve the predictions due to differences in growth between parities, which is hinted by the positive correlation between NUE and parity (Table 3). Addition of MY logically brought additional information to the NUE ratio. The SVM models provided better performance than did PLS. The best model is plotted in Figure 2 and was obtained by using SVM regression with MIR spectra, with parity and MY as predictors, resulting in relative error of 14% and RPD of 1.97. Based on those statistics, the model seems suitable to compare groups of cows and to discriminate among cows with low and high NUE values.

In the first cross-validation scheme, different records from the same cow can be used in the calibration and in the validation data set, potentially leading to overfitting of the results. Table 5 shows the results of both PLS and SVM models, randomly removing 25% of the cows to be used as a validation data set, and calibrating with records of the remaining 75% of cows. The PLS model was developed with 12 LV. In this step, the PLS and SVM methods provided equivalent performances. The R^2 decreased compared with 10-subset cross-validation, but this can be explained by the smaller range in the validation data set, with NUE values ranging from 17 to 68% instead of 9.7 to 81.7% in the initial data set. Indeed, as shown by Davies and Fearn (2006), R^2 is highly dependent on the distribution, especially the range of data; knowing this, it is more relevant to focus on RMSE and related variables (relative error and RPD) to evaluate performance of models. Relative error and RPD were in the same order of magnitude as that in the cross-validation step, resulting in 14% and 2.08, respectively, from the SVM model. This shows a good potential of the models to predict other cows offered similar diets to those in the calibration data set. From the NUE ratio, the information regarding daily N output in milk was already fully contained within the predictors, as the N content of milk was predicted from MIR, and milk yield was used as a predictor. However, it is of interest to estimate how effectively MIR can predict the second part of the ratio, the daily N intake. Table 6 reports the performance of predictions of DMI, N intake, and N losses in cow validation, using the same methodology as described, and using SVM regression. Intake of N and DMI are predicted with relative errors of 14 and 15%, respectively, which explains the error in the same order of magnitude obtained for NUE. Prediction of diet N percentage was not reported, as the percentage was identical for each cow having the same diet and cannot be considered a quantitative variable. The model predicted N losses with a relative error of 23%, which is larger than the relative error of NUE, even though it was calculated from the same variables.

When predicting NUE from milk urea, Jonker et al. (1998) obtained relative errors from 11 to 14%, and

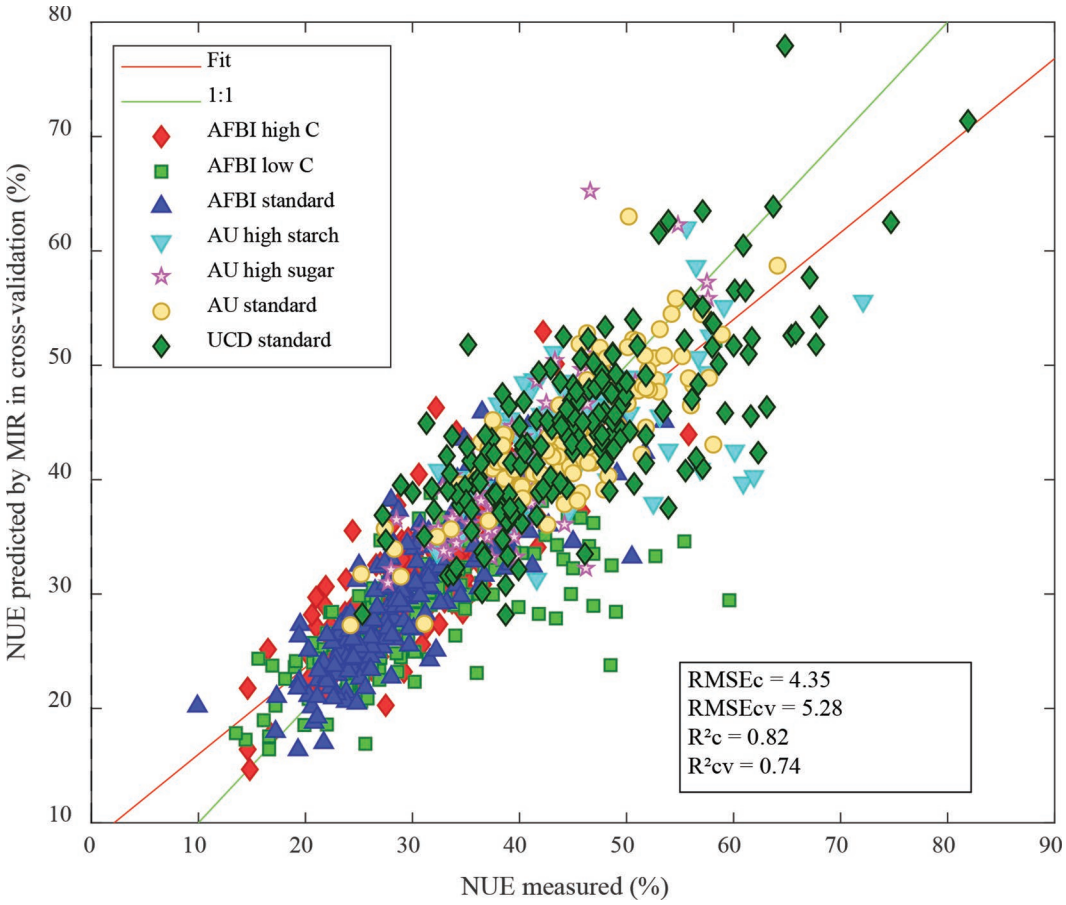

Figure 2. Plot of measured individual nitrogen use efficiency (NUE) of dairy cows compared with NUE values predicted by the support vector machines regression (SVM) model using mid-infrared (MIR) spectra, parity, and milk yield as predictors, in cross-validation with 10 subsets (n = 1,034). RMSEc = root mean square error of calibration; RMSEcv = root mean square error of cross-validation with 10 subsets; R^2c = coefficient of determination of calibration; R^2cv = coefficient of determination of cross-validation with 10 subsets.

Nousiainen et al. (2004) reported a relative error of approximately 8%. Thus, their models provide results similar to or better than the models developed in this study. However, as mentioned previously, milk urea levels have to be measured via chemical methods, which are both time-consuming and expensive. Alternatively, they can be predicted by MIR with a relative error of approximately 20%, which adds to the error of the model predicting NUE from MIR milk urea. Additionally, in the present data set, the correlation between predicted milk urea and NUE was only −0.3. Other works have predicted NUE from ^{15}N fractions in tissues (Cheng et al., 2011; Cantalapiedra-Hijar et al., 2016) but either resulted in models with low R^2 or concluded

Table 5. Cow validation: performance of PLS and SVM models[1] to predict individual nitrogen use efficiency (NUE) when 25% of cows are randomly removed to be used as a validation data set (n = 255), and calibrating with records of the 75% of cows remaining (n = 779)

	PLS				SVM			
Predictor[2]	R^2v[3]	Error (RMSEv[4])	Relative error (%; RMSEv/mean)	RPD[5]	R^2v	Error (RMSEv)	Relative error (%; RMSEv/mean)	RPD
MIR+Parity+MY	0.68	5.04	14	2.07	0.68	5.01	14	2.08

[1]PLS = partial least square regression; SVM = support vector machines regression.
[2]MIR = mid-infrared spectra; MY = milk yield.
[3]R^2v = coefficient of determination of cow validation.
[4]RMSEv = root mean square error of cow validation.
[5]RPD = ratio of SD to RMSEv.

that difficulties existed in predicting NUE using this method. As a consequence, the models obtained in the present study appear better suited to large-scale applications, especially due to the low cost and high speed of MIR analysis.

Finally, models were evaluated by performing a diet validation, by removing diets one by one, to be used as a validation data set, and calibrating using the records from the 6 remaining diets. We undertook this to simulate the application of the model to samples from a diet that differed from diets in the calibration data set. The 7 PLS models were developed based on 12 LV. Results are shown in Table 7. The resulting $R^2$of cow validation ranged from 0.06 to 0.68, but as mentioned earlier, due to reduced data set size and range, focusing on RMSE and relative error to evaluate the models was more reliable. Relative errors ranged from 12 to 34%, and SVM provided better results in most cases, except for the AU and UCD standard diets. For both AU and AFBI data, standard diets were predicted with a relative error in the same order of magnitude as in the 10-subset cross-validation and in the cow validation, with values from 12 to 16%. The low relative errors and the associated RPD, from 1.75 to 2.38, suggested that models can allow comparisons of groups of cows, discriminating among low and high NUE values when applied to those diets. Otherwise, the extreme diets in those 2 herds were predicted with relative errors ranging from 18 to 23%, with associated RPD from 1.25 to 1.56. Such results are expected, because extrapolation (predicting extreme diets not included in the calibration data set) is very dangerous in infrared analysis (Dardenne, 2010). It highlights the fact that samples from specific diets cannot be well predicted if those diets are not represented in the calibration data set. This element can be objectified by looking to the global H (**GH**), which is the standardized Mahalanobis distance between the validation records and the calibration data set. High GH means that the sample to be predicted is not covered by the calibration data set (Dardenne, 2010). In Table 7, the averaged GH per validation data set and the percentage of GH that are higher than 3 are reported. Both AFBI and AU standard diets show the lowest GH averages, 0.95 and 0.92, respectively, whereas AFBI and AU extreme diets show GH averages ranging from 1.04 to 1.35, indicating that those data sets were less well covered by the calibration data sets. Additionally, the UCD standard diet records were predicted with a relative error of 34% and an RPD below 1, even though the diet contained only "standard" feed components, and it might have been expected that this diet would be similar to, and largely covered by, the calibration data sets. However, the UCD records showed a GH average of 1.71 and 12% of records with a GH above 3, which highlights spectral differences between this data set and the other data sets and helps explain the poor quality of predictions. Those spectral differences could originate from factors affecting milk composition and MIR spectra such as genetics, diet, management, or season. Globally, in the present data set the correlation coefficient is 0.94 between relative error of models in diet validation and GH mean, as shown in Figure 3, displaying a strong link between the quality of predictions and the ability of a calibration data set to cover the variability of a new sample to be predicted. This is an argument in favor of quality assurance systems to avoid extrapolation when using MIR models, to ensure that any sample for prediction is sufficiently covered by the calibration data set to avoid inconsistent and incorrect predictions. It also implies the need to develop robust models to generate phenotypes for routine use. Robustness can be considered as the capacity of models to be useful in all scenarios and to provide good predictions in various conditions (Grelet et al., 2017). For this, more data must be integrated in the models to cover a maximum of variability regarding breeds, diets, DIM, management practices, seasons, MIR instruments, and geographic regions.

Table 6. Predictions of DMI, N intake, and N losses by SVM[1] when 25% of the cows are randomly removed to be used as a validation data set (n = 255), and calibrating with records of the 75% cows remaining (n = 779)

Item	Predictor[2]	Minimum	Maximum	Mean	SD	R^2v[3]	Error (RMSEv[4])	Relative error (%; RMSEv/mean)	RPD[5]
DMI (kg/d)	MIR+Parity+MY	8.8	36.2	19.9	4.53	0.66	2.71	14	1.67
N intake (kg/d)	MIR+Parity+MY	0.171	1.024	0.486	0.129	0.71	0.072	15	1.79
N losses (kg/d)	MIR+Parity+MY	0.044	0.809	0.311	0.11	0.59	0.070	23	1.57

[1]SVM = support vector machines regression.
[2]MIR = mid-infrared spectra; MY = milk yield.
[3]R^2v = coefficient of determination of cow validation.
[4]RMSEv = root mean square error of cow validation.
[5]RPD = ratio of SD to RMSEv.

Table 7. Diet validation: performance of PLS and SVM models[1] to predict individual nitrogen use efficiency (NUE) using mid-infrared spectra, parity, and milk yield as predictors when diets are removed one by one to be used as a validation data set, and calibrating with the records of the 6 remaining diets

			PLS				SVM			
Validation diet[2]	GH mean[3]	% GH >3[4]	R^2v[5]	Error (RMSEv[6])	Relative error (%; RMSEv/mean)	RPD[7]	R^2v	Error (RMSEv)	Relative error (%; RMSEv/mean)	RPD
AFBI high C	1.04	2	0.63	8.03	22	1.30	0.59	6.67	18	1.56
AFBI low C	1.33	7	0.54	8.31	23	1.25	0.43	8.13	22	1.28
AFBI standard	0.95	3	0.56	5.08	14	2.05	0.68	4.38	12	2.38
AU high starch	1.14	1	0.19	7.14	19	1.46	0.30	6.95	19	1.50
AU high sugar	1.35	6	0.50	7.93	22	1.31	0.57	7.51	20	1.39
AU standard	0.92	1	0.53	5.42	15	1.92	0.58	5.96	16	1.75
UCD standard	1.71	12	0.06	12.23	33	0.85	0.14	12.58	34	0.83

[1]PLS = partial least square regression; SVM = support vector machines regression.

[2]AFBI = Agri-Food and Biosciences Institute, UK; AU = Aarhus University, Denmark; UCD = University College Dublin, Ireland. C = concentrate.

[3]GH mean = mean of standardized Mahalanobis distances between the validation records and the calibration data set.

[4]% GH >3 = percentage of standardized Mahalanobis distances above 3.

[5]R^2v = coefficient of determination of diet validation.

[6]RMSEv = root mean square error of diet validation.

[7]RPD = ratio of SD to RMSEv.

Perspectives and Limitations

The results showed an interesting potential of MIR analysis of milk to predict individual NUE of dairy cows from 0 to 50 DIM. The methodology allowed prediction of NUE with a relative error of 14%, which seems reasonable to discriminate between low and high NUE, and might potentially be used to improve nutrition and herd management and to perform genetic studies. Indeed, in a further step in this work, the equation will be applied to the GplusE population of genotyped cows to check the genetic background of MIR-predicted NUE. This should allow an estimation of the heritability of NUE, its genetic correlations with other traits of interest and, finally, its use in genome-wide association studies. Regarding practical implementation, SVM provided better performance than PLS, but SVM is a complex method demanding high computing power, whereas PLS models are easy to transfer in routine application protocols. Consequently, the slight differences between the performance of the 2 methods justifies the implementation of PLS in the context of routine milk recording.

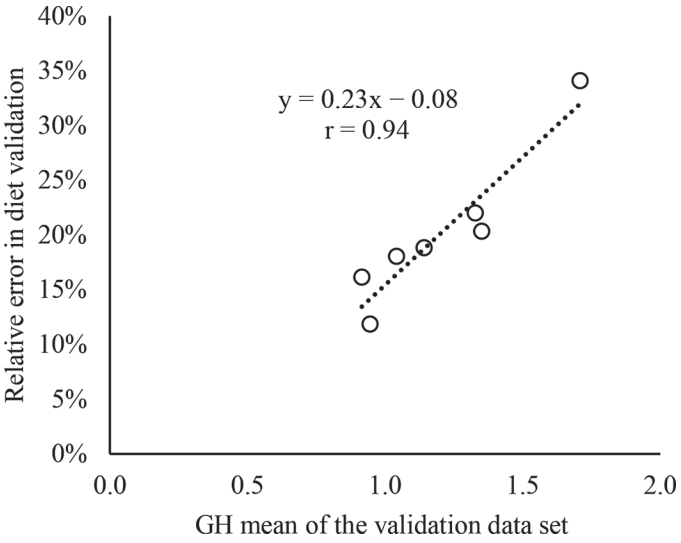

Figure 3. Plot of relative error (ratio of root mean square error of diet validation to mean) for each validation data set in diet validation, versus the averaged global H (standardized Mahalanobis distance, GH) between the validation records and the calibration data set.

However, the current models are not yet robust enough to be used at a large scale, especially in routine performance recording outside of the framework of the GplusE project. Models were developed with a limited number of cows and diets, and because MIR models can only be applied to spectra that are similar to information contained in the calibration data set, the current model is only valid for Holstein cows in early lactation with specific types of diets. This lack of robustness was highlighted by the heterogeneous results in the diet validation. The model developed is currently limited to a restricted set of conditions, as extrapolation to other circumstances may lead to biased predictions. However, to avoid the development of overly specific and multiple models (as for specific breeds, diets, etc.), MIR calibrations should be as general as possible. To reach this goal and enable use in routine milk recording and on a large scale, additional variability must be entered into the calibration data set by merging records from other breeds, diets, and countries to derive a universal model. This would be possible through international collabora-

tion, as NUE and spectral data are routinely recorded in numerous research farms. Additionally, NUE may be highly influenced by lactation stage, due to mobilization and storage of body reserves at different stages of lactation. Consequently, cows should be followed over the whole lactation to assess a global value of NUE. Further work is needed to validate the possibility of predicting NUE from MIR in late lactation as well. Finally, the absence of information on the N content in feces and urine in this study makes it impossible to calculate the N balance. Because data were recorded in early lactation, there is a risk of confusing negative N balance with artificially high NUE and increasing the difficulty induced by severe mobilization of body reserves by trying to improve NUE. Additional data on N content in urine and feces in the same stage of lactation are needed, to further study this potential bias.

CONCLUSIONS

The objective of this study was to evaluate the potential of MIR analysis of milk to predict NUE of individual dairy cows. The results obtained from the present data set indicate that the methodology can allow prediction of NUE with a relative error of 14%, which seems reasonable to discriminate between low and high NUE, to improve nutrition and management of herds and to perform genetic studies. However, the developed models are not robust enough at present to be routinely used, and collaborations are needed to increase data set variability to improve robustness and practical applicability.

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