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Comparison of the mid-infrared spectra and prediction equations developed from morning and evening milk samples from twice-a-day milked dairy cows

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ABSTRACT

Mid-infrared spectroscopy is a technology used globally for quantifying the concentration of fat, protein, lactose, and other constituents in the milk samples of both individual animals and bulk tank milk. Differences in the milk components and yield of cows are known to exist between morning and evening milk; nonetheless, differences in the spectra originating from the same cow from morning and evening milkings have never been investigated. Data were obtained from 2,602 dairy cows from 7 research farms in Ireland. A total of 199,288 morning milk spectra with associated evening milk spectra produced by the same cow within 24 h were available. Postediting, spectral data were available on the same 502 wavelengths in the mid-infrared region of the electromagnetic spectrum for all milk samples. Differences between morning and evening milk spectra produced by the same cow in a 24-h period were investigated using (1) the mean and SD of the difference between morning and evening spectra absorbance values, (2) the correlation between the morning and the respective evening wavelength absorbance values, and (3) the L_2 distance, all of which were quantified across stages of lactation, years, and farms. The average (SD) difference between the morning and the evening spectrum absorbance values produced by the same cow within 24 h was 0.00097 (0.008), and it was always larger than 0.055 for the wavelengths between 2,920 cm^{-1} and 2,947 cm^{-1}. The correlation between morning wavelength absorbance values and the respective evening wavelength absorbance values were all strong (i.e., >0.80) in the spectral region of 1,469 cm^{-1} to 1,473 cm^{-1}; weak correlations of <0.26 existed between morning and evening spectra wavelengths in the region of 1,593 cm^{-1} to 1,597 cm^{-1}. These trends in correlations generally persisted within different stages of lactation, years, and farms. Results from the L_2 distance indicated that early lactation morning and evening spectra were more different from each other than when compared in late lactation; no large differences in the L_2 distance across different farms and years were evident. The impact of a prediction equation developed from morning spectral data but applied to evening milk spectral data, and vice versa, was investigated. Nitrogen use efficiency (NUE) was the animal trait explored; the mean NUE (SD) in the validation dataset was 22.16 (4.86). The root mean square error for predictions developed and validated on morning spectra samples was 3.49; this increased to 3.85 for the same validation in the morning spectra when the prediction equation was developed using only evening spectra. Similarly, the root mean square error from predictions developed and validated on evening spectra samples was 3.46, which increased to 3.85 when the prediction equation was developed using only morning spectra. In conclusion, morning and evening milk spectra produced by the same cow within 24 h differ, particularly in some spectral regions; these differences affect the prediction performance of applied prediction equations.
Key words: milk chemistry, mid-infrared spectroscopy, chemometrics, milking

INTRODUCTION

Animals adapt to both spatial and temporal environmental variables, enabling them to regulate diverse functions based on the time of day and the season (Asher et al., 2015). Moreover, the metabolism rate in many organisms differ between day and night (Teng et al., 2021). Such variability can affect potential differences in milk composition across the day. Differences in the milk composition between morning and evening milk for mean fat and protein percentage have been reported (Everett and Wadell, 1970; Pavel and Gavan, 2011), as well as

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in the concentration of small molecules, metabolites, lipids, hormones, and cytokines in milk produced from the same dairy cow within a day (Teng et al., 2021). As differences in the morning and evening milk produced by the same cow within 24 h have been documented, differences could also exist in the mid-infrared (**MIR**) spectra generated from the milk produced by the same cow in consecutive morning and evening milkings.

Globally, milk samples collected from individual animals during routine milk testing are subjected to spectroscopy analysis in the MIR region of electromagnetic spectrum. The resulting spectral data are used to predict different milk quality measures (De Marchi et al., 2014) or animal-level features (McParland and Berry, 2016). In some studies (e.g., McParland et al., 2014, 2023; Frizzarin et al., 2024) prediction equations for animal-level features were separately developed for morning and evening milk samples. Other studies (McParland et al., 2024; Frizzarin et al., 2024) combined the morning and evening spectra from milk samples of dairy cows to develop a single prediction equation for animal-level features. McParland et al. (2024) averaged the absorbance of the morning and evening milk spectra from the same cow for predicting individual cow methane emissions, whereas Frizzarin et al. (2024) used a concatenation of the morning and evening milk spectra for predicting nitrogen use efficiency (**NUE**) in dairy cows. Both Frizzarin et al. (2024) and McParland et al. (2024) reported an improvement in the model prediction accuracy when the prediction equations were developed combining the morning and evening milk spectra compared with the equations developed using just the morning or just the evening milk spectra.

No previous study has explored the differences between the actual milk MIR spectra from the same cow collected in the morning and evening milking within a 24-h period. Therefore, the objectives of the present study were (1) to explore the differences, if any, between the MIR spectra from milk samples collected from the same dairy cow in the morning and evening milking; (2) identify the spectral regions that differ the most between morning and evening milk; and (3) quantify the effect on predictive performance, if any, when a prediction model for a cow-level feature developed using morning milk MIR spectra was applied to evening milk spectra to predict a phenotype, and vice versa.

MATERIALS AND METHODS

All data used in this study were from a pre-existing database. Therefore, because no human or animal subjects were used, this study did not require approval by an Institutional Animal Care and Use Committee or Institutional Review Board.

Table 1. Description of the research farms used in the study

Farm	Location within Ireland	Breeds[1]	Research trials	References	No. of records
1 Clonakilty	Southwest	Pure Ho-Fr and Ho-Fr crossbreed with Je and Norwegian Red	White clover plus perennial ryegrass effects on milk production	McClearn et al., 2019	25,657
2 Dairygold	Southwest	200 pure Ho-Fr and 100 pure Je	Effect of genetic improvement on performance	Buckley et al., 2019	29,863
3 Moorepark	Southwest	Pure Ho-Fr and crossbreed	White clover plus perennial ryegrass effects on performance; Restricted access to pasture in autumn	Enriquez-Hidalgo et al., 2018; Garry et al., 2021	39,689
4 Ballyhaise	North	Pure Ho-Fr crossbred Ho-Fr-Je	Different grazing stocking rates	Patton et al., 2016	3,589
5 Solohead	Southwest	Pure Ho-Fr and crossbreed	Extension of grazing season	Fenger et al., 2021	1,879
6 Johnstown	Southeast	Pure Ho-Fr and crossbreed	Nitrogen sources and transformation	Clagnan et al., 2019	1,100
7 Curtins	Southwest	Pure Ho-Fr and crossbreed Ho-Fr Je	Different grazing stocking rates	Coffey et al., 2018	17,511

[1]Ho-Fr = Holstein-Friesian; Je = Jersey.

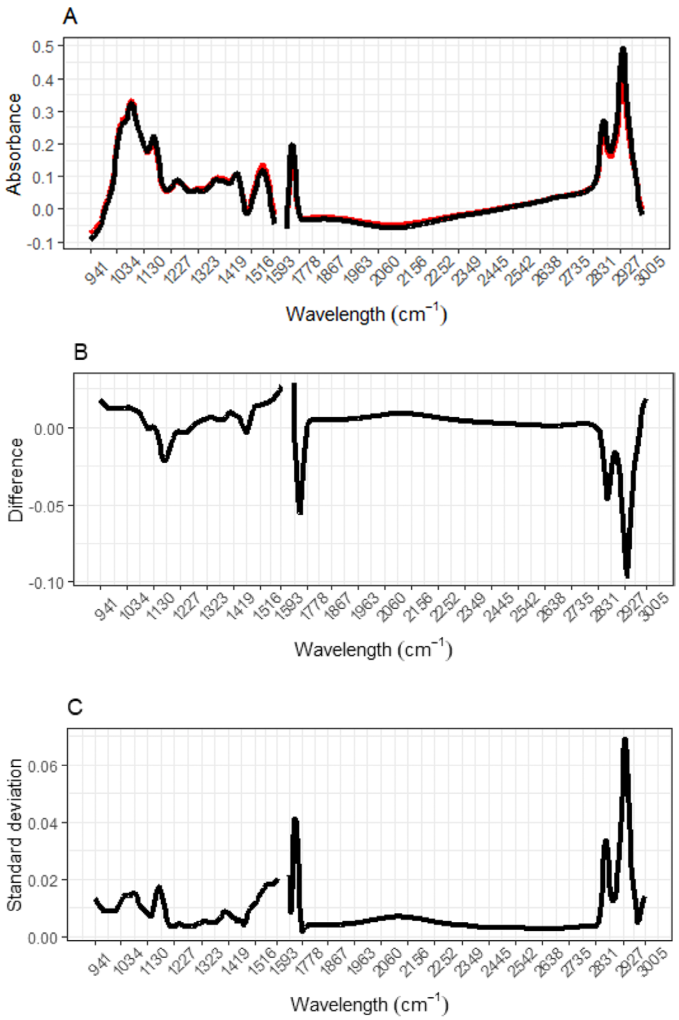

Figure 1. (A) Average morning (red) and evening (black) spectra, (B) difference between morning and evening spectra (i.e., morning spectrum minus the evening spectrum), and (C) SD of the difference between morning and evening spectra.

Data and Data Editing

All the data used in this study were collected between the years 2016 and 2020 inclusive, from 7 Teagasc research farms. Most of the data were from spring-calving cows fed a predominantly grazed grass diet. A summary of the herd characteristics and research trials undertaken across the study period considered in the analyses are in Table 1. The data represented different parities of cows (parities 1–10) as well as different stages of lactation (i.e., DIM from 5 to 305).

All cows were milked twice daily, generally at 0700 h and 1500 h. Individual cow milk samples for spectral analyses were collected on 2 consecutive days during the evening and the following morning milking. All milk samples were analyzed using the same MIR spectrometer (Foss MilkoScan FT6000; Foss Electric A/S) generating 1,060 transmittance spectral values. Transmittance values were transformed into absorbance values by taking the $\log_{10}$ of the reciprocal of the transmittance values. The wavelengths were standardized across time (Grelet et al., 2017) using the standardization coefficients provided as part of the European Milk Recording project ring testing program (European Milk Recording, 2022). Noise regions of the spectra related to water absorption were removed leaving a total of 502 wavelengths for each morning and evening milk sample; these represented the regions from 941 to 1,601 cm^{-1} and from 1,736 to 3,005 cm^{-1}. Spectral outliers were identified using the approach described in detail by Frizzarin et al. (2021). Briefly, the Mahalanobis distance was quantified for the morning and the evening spectra separately using the first 5 principal components of the spectra. Spectra with a Mahalanobis distance from the center greater than the 99th quantile of the quantified distances were subsequently removed. After outlier removal, only morning spectra with accompanying evening spectra were retained. The final dataset comprised 199,288 spectra from the morning milking with an accompanying 199,288 spectra from the evening milking from 2,602 cows.

Statistical Analyses

Comparison of Morning and Evening Spectrum. Mean differences between each morning wavelength absorbance value and the respective evening wavelength absorbance value for the same cow were compared using a 2-sided paired t-test. Because multiple t-tests were undertaken, one per wavelength, the resulting P-values were subjected to an adjustment to account for multiple testing using the method developed by Benjamini and Yekutieli (2001). Then, 2 separate correlation matrices were generated: (1) the pairwise correlations between all the morning wavelength absorbance values across the spectrum, and (2) the pairwise correlations between all the evening wavelength absorbance values across the spectrum. These 2 correlation matrices were then compared using the test implemented by Schott (2007). The test bases its comparison on the sum of squared differences between distinct entries of the tested sample correlation matrices. The test was implemented using the covTestR package (Barnard and Young, 2018) in the R software (R Core Team, 2023) with the null hypothesis being that no difference existed between the 2 correlation matrices.

Similarity between the morning and the respective evening milk spectra absorbance values produced by the same cow within 24 h was also determined using (1) the Pearson correlation between the absorbance value for a given wavelength from a milk sample collected in the morning versus that for the same wavelength collected in immediately previous evening milking from the same cow, and (2) the L_2 distance. The L_2 distance was computed as follows:

$$L_2\left(x_{AM}, x_{PM}\right) = \sqrt{\sum_{k=1}^{502}\left(x_{AMk} - x_{PMk}\right)^2},$$

where x_{AMk} and x_{PMk} are the observed absorbance values of the kth wavelength for the AMth and PMth spectra, respectively. The L_2 distance can be used as a measure of similarity between 2 curves with greater values indicating a more pronounced difference between the curves and a value of 0 means that 2 curves are equal to each other. The difference between the morning and the respective evening wavelength absorbance values, the correlations between the morning and the respective evening wavelengths absorbance values, and the L_2 distance were all investigated for the whole dataset but also separately within (1) stage of lactation, (2) year, and (3) farm. Differences in the mean L_2 distance across groups was tested using Wilcoxon rank-sum test, which is a nonparametric test. The 5 stages of lactation considered were early lactation between 5 to 60 DIM, early-mid lactation between 61 to 120 DIM, mid lactation between 121 to 180 DIM, mid-late lactation between 181 to 240 DIM, and late lactation between 241 to 305 DIM. The L_2 distance was also investigated after stratifying the data according to the difference in part-day milk yield within cow-day between the morning and evening milking.

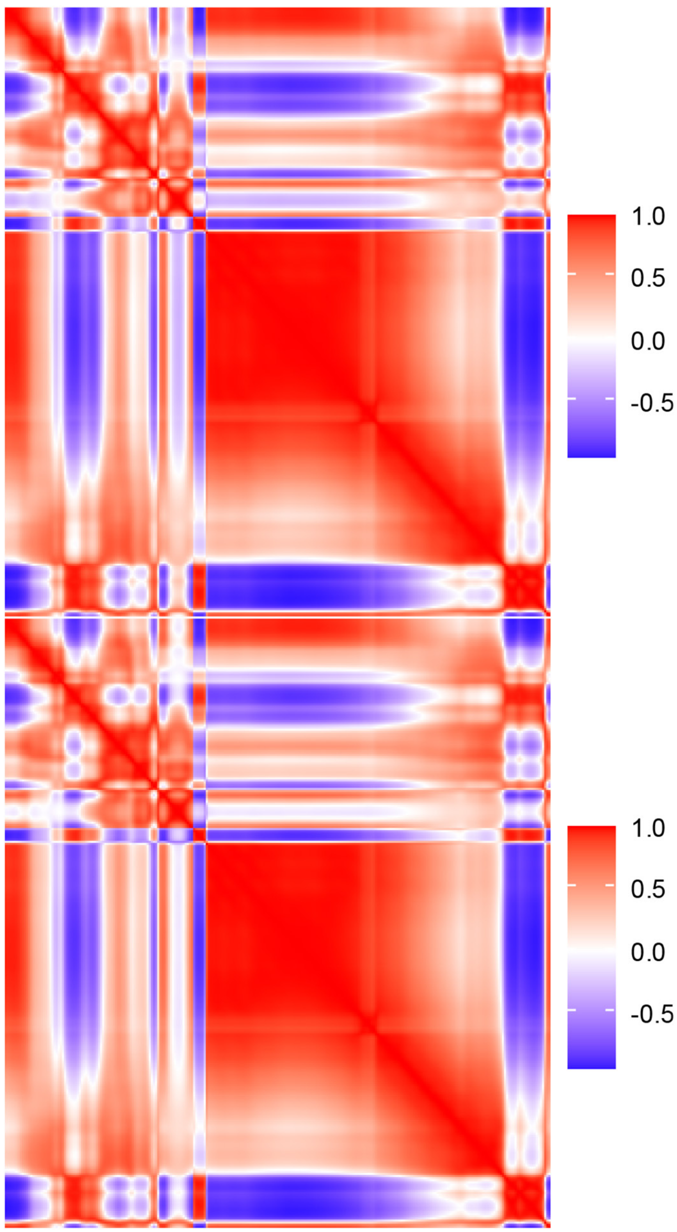

Figure 2. Correlation matrix for the morning (top) and evening (bottom) spectra.

Development of Prediction Equations. Using cow NUE as a case study, the effect of a prediction equation developed from morning spectral data being applied to evening spectral data, and vice versa, was assessed. The dataset used for this analysis was previously described in detail by Frizzarin et al. (2024). Briefly, 3,497 NUE records (with associated MIR data) from 1,195 cows were collected from 4 research farms across 11 yr. Nitrogen use efficiency was quantified as the sum of nitrogen in milk, nitrogen in the conceptus, nitrogen used for the growth, and nitrogen stored in the reserves divided by the sum of nitrogen intake and nitrogen mobilized from the reserves. The same NUE value was associated with an evening and a morning spectrum collected within 24 h. Four datasets were created: (1) a morning calibration dataset, (2) an evening calibration dataset, (3) a morning validation dataset, and (4) an evening validation dataset. An additional dataset was created, for comparative purposes, representing the calibration just described but where the weighted average of the MIR per day was calculated; the weighting factor used was the respective morning and evening milk yield. The partition of records into the calibration and validation datasets was random with the exception that records from the same cow could not be present in both the calibration and validation datasets. The morning calibration dataset contained 75% (i.e., 2,622 records) of the morning spectra along with the NUE value; the same 75% of NUE records associated with the evening spectra constituted the evening calibration dataset; the remaining 25% (i.e., 875 records) of the morning and evening spectra constituted the morning and evening validation dataset, respectively. The morning calibration dataset was used to develop a prediction equation for NUE, which was then applied to both the morning and evening validation datasets separately; in a separate analysis, the evening calibration dataset was used to develop an equation to predict

NUE, which was then validated in the morning and evening validation datasets separately. Lastly, the weighted average spectra calibration dataset was used to develop an equation to predict NUE, which was then validated in the morning and evening validation datasets separately. Partial least squares regression was used to develop the equations using the R package pls (Mevik et al., 2019). To develop the prediction equation, 531 wavelengths were used. The prediction accuracy was assessed using the correlation between the actual and the predicted values, the bias, as well as the root mean square error of prediction (**RMSE**). Differences in the prediction results obtained when the morning and the evening prediction equations were used to predict NUE on the same validation dataset was assessed using the F-test on the residuals (i.e., the residuals were quantified as actual NUE value minus the predicted NUE value).

RESULTS

The mean morning and the mean evening milk spectra are in Figure 1. Although the average morning spectra generally followed a similar pattern to the average evening spectra, the mean morning wavelength absorbance values were different (P < 0.05) from the respective evening wavelength absorbance values. The mean difference and the SD of the difference between the morning and the evening spectrum absorbance values (quantified as the morning spectrum absorbance values minus the evening spectrum absorbance values) produced by the same cow within 24 h are in Figure 1. The greatest difference, as well as the greatest SD of the difference, between the morning and evening milk spectral absorbance values was for the wavelengths spanning from 1,743 cm^{-1} to 1,763 cm^{-1} and from 2,854 cm^{-1} to 2,962 cm^{-1}. This was consistent when the mean difference and the greatest SD of the difference between the morning and evening milk spectral absorbance values were quantified within lactation stages.

The 2 correlation matrices among the wavelength absorbance values for the morning milking, and separately for the evening milking, are in Figure 2. Visually the pattern in the correlation matrices for the morning and evening milk samples looked similar but the test proposed by Schott (2007) suggested that the internal relationships among the absorbance values for the morning spectra differed (P < 0.05) from those among the absorbance values for the evening spectra.

The first versus the second principal components of the MIR spectra, which represented respectively 70% and 13% of the total spectral variance, for the morning and evening milk samples are separately presented in Figure 3; only 1 random record per milking day was retained leaving 878 data points (i.e., 439 morning and 439 evening spectra). Some overlap in the scatter plot of the 2 principal components existed between the morning and evening milk spectra.

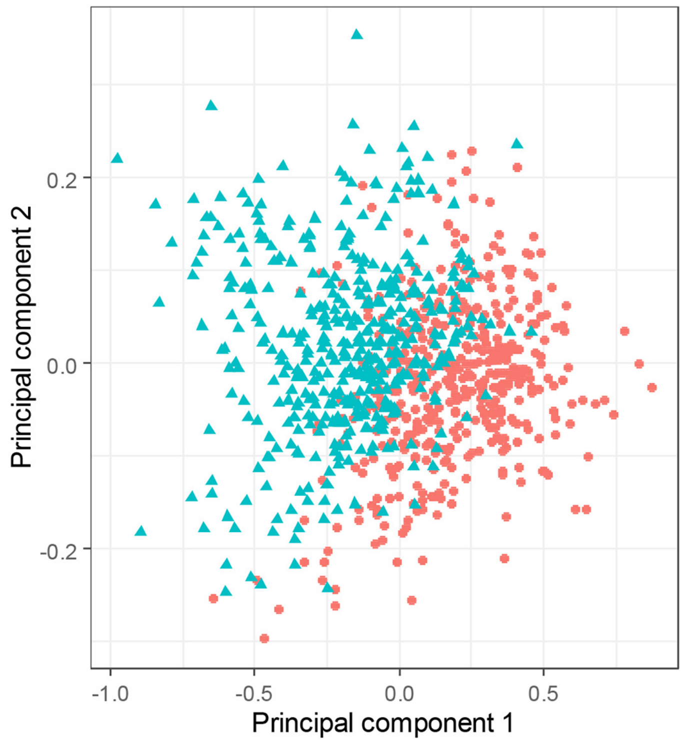

Figure 3. The first versus second principal components for the edited spectral data for the morning (red dots) and evening (blue triangles) milk samples. The first and the second principal components represented 70% and 13% of the total spectral variance, respectively.

Correlation Between Morning and Evening Spectra

The correlations between the wavelength absorbance values from the morning milk sample and the corresponding wavelength absorbance values from the evening milk sample from the same cow are in Figure 4. The correlations were strong (i.e., >0.80) in the spectral region between 1,469 cm^{-1} to 1,473 cm^{-1} but weak (i.e., <0.26) in the spectral region between 1,593 cm^{-1} to 1,597 cm^{-1}. Strong correlations (i.e., >0.70) were also observed between wavelength absorbance values in the regions between 1,227 cm^{-1} and 1,269 cm^{-1}, between 1,454 cm^{-1} and 1,477 cm^{-1}, between 1,543 cm^{-1} and 1,554 cm^{-1}, and between 2,978 cm^{-1} and 2,981 cm^{-1}. Weak correlations (i.e., <0.35) were evident between the wavelength absorbance values in the regions between 1,334 cm^{-1} and 1,346 cm^{-1}, between 1,400 cm^{-1} and 1,435 cm^{-1}, between 1,485 cm^{-1} and 1,508 cm^{-1}, between 1,585 cm^{-1} and 1,601 cm^{-1}, and between 2,993 cm^{-1} and 2,997 cm^{-1}.

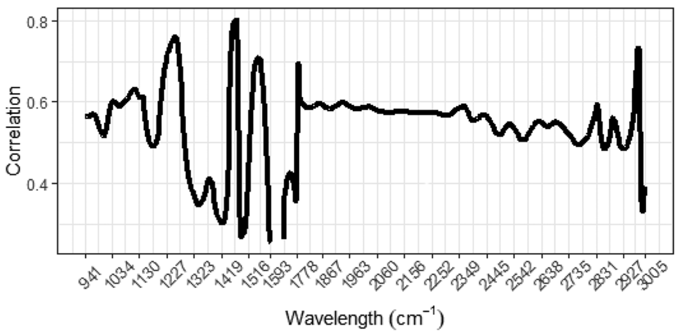

Figure 4. Correlation between each morning wavelength and the respective evening wavelength from spectra produced from the same cow.

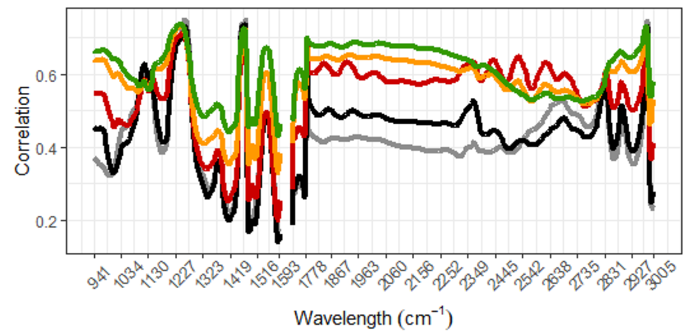

Figure 5. Correlation between each morning wavelength and the respective evening wavelength for spectra produced from the same cow using data between 5 and 60 DIM (gray line), between 61 and 120 DIM (black line), between 121 and 180 DIM (red line), between 181 and 240 DIM (orange line), and between 240 and 305 DIM (green line).

The correlations between the wavelength absorbance values from the morning and the respective evening spectra across stages of lactation are in Figure 5. Although the extent of the correlations between morning and evening spectra generally differed by stage of lactation, some spectral regions (i.e., 1,227–1,601 cm^{-1} and 1,736–2,156 cm^{-1}) had a similar correlation profile across stages of lactation, with similar peaks and troughs. The profile of the correlations between the morning and evening spectra across years (Figure 6) or across farms (Figure 7) varied widely in strength in the spectral regions between 941 cm^{-1} and 1,127 cm^{-1}, and between 2,250 cm^{-1} and 2,750 cm^{-1}, while having relatively constant values in the other spectral regions.

L_2 Distance

The mean (SD) L_2 distance between morning and evening spectra from milk samples of the same cow taken within 24 h across stages of lactation was 0.52 (0.25), 0.43 (0.23), 0.34 (0.19), 0.32 (0.17), and 0.30 (0.16) for early, early-mid, mid, mid-late, and late lactation, respectively. Therefore, the mean L_2 distance values decreased (i.e., became more similar) with stage of lactation and differed from each other ($P < 0.05$). The mean L_2 distance across years ranged from 0.37 to 0.40, and from 0.36 to 0.40 across farms. Lastly, the L_2 distance increased (i.e., became more different) as the difference in milk yield between the morning and evening milk yield increased ($P < 0.05$); the L_2 distance was equal to 0.35 (0.21), 0.36 (0.20), 0.38 (0.21), and 0.42 (0.23) when the difference between the morning and evening part-day milk yield was less than 5.1 kg, between 5.1 and 6.8 kg, between 6.8 and 8.7 kg, and greater than 8.7 kg, respectively.

Prediction Accuracy

The accuracy of predicting NUE using different combinations of the calibration and validation datasets based on morning or evening spectral data are in Table 2. When NUE was predicted from morning milk spectra using a prediction equation developed using evening milk spectra or the weighted average spectra instead of morning spectra, the RMSE increased (i.e., worsened) by 10.3% and 4.0%, respectively. Similarly, when NUE was predicted from evening milk spectra using a prediction equation developed using morning spectra or weighted average spectra instead of evening spectra, the RMSE increased by 11.3% and 9.5%, respectively. The bias when NUE was predicted from morning milk spectra using a prediction equation developed using evening spectra was −0.31, while it was −0.02 when the prediction equation was developed using morning spectra. Likewise, the bias when NUE was predicted from evening milk spectra using a prediction equation developed using morning spectra was 1.00, whereas it was just 0.02 when the prediction equation was developed using evening spectra.

DISCUSSION

In some countries, such as Ireland, cows are generally milked twice a day. Nonetheless, once-a-day milking is sometimes practiced (Berry et al., 2024), as well as milking 3 times a day. Differences in the milk composition are known to exist between morning and evening milk (Everett and Wadell, 1970; Pavel and Gavan, 2011). Specifically, fat in the morning milk is generally lower than that in the evening milk, with the opposite sometimes (Pavel and Gavan, 2011) but not always (Berry et al., 2006) being true for protein. These differences are associated with the time interval between milkings; total milk production is higher when the interval between milkings is longer, but hourly secretion rate is lower with longer intervals (Everett and Wadell, 1970). As milk spectral data are able to capture information on the milk

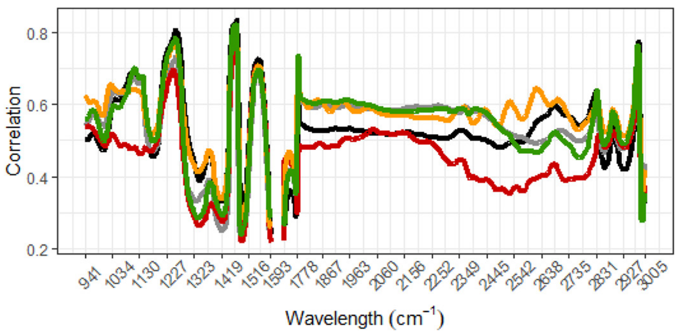

Figure 6. Correlation between each morning wavelength and the respective evening wavelength for spectra produced from the same cow using data collected in 2016 (gray line), 2017 (black line), 2018 (red line), 2019 (orange line), and 2020 (green line).

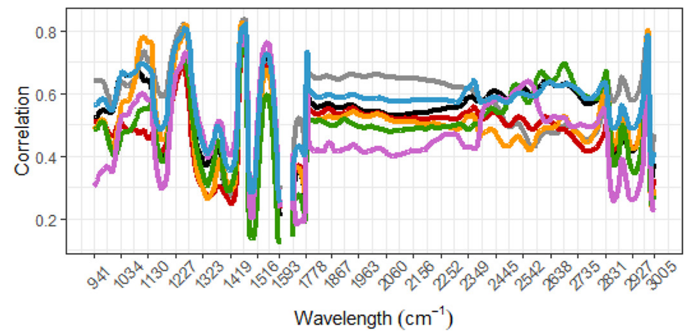

Figure 7. Correlation between each morning wavelength and the respective evening wavelength for spectra produced from the same cow using data collected in farm 1 (gray line), farm 2 (black line), farm 3 (red line), farm 4 (orange line), farm 5 (green line), farm 6 (purple line), and farm 7 (blue line).

components (e.g., fat, protein, and lactose), differences in the spectra generated from the same cow during the morning or evening milking undertaken within 24 h may exist. Identifying these differences can help recognize the chemical components that vary the most between the milk from different periods of the day. This knowledge can be exploited when prediction equations are developed from the milk MIR spectra.

Similarities and Differences Between Morning and Evening Spectra

Different metrics were used in the present study to investigate the similarities and differences between morning and evening milk spectra produced by the same cow in a 24-h period, such as (1) the mean and SD of the difference between morning and evening milk spectra absorbance values, (2) the correlation between the morning and the respective evening wavelength absorbance values, and (3) the L_2 distance. In a further series of analyses, the repeatability of the morning wavelengths absorbance values across stages of lactation and separately the repeatability of the evening wavelengths absorbance values across stages of lactation was quantified (Figure 8). The mean number of days between milk spectra records for an individual cow was 9 d.

The investigated metrics provided different insights on the differences between morning and evening milk spectra. Indeed, the mean difference and SD of the difference between the morning and the evening spectrum absorbance values can help identify the spectral regions that vary the most between morning and evening milking, whereas the correlation between the morning and the respective evening wavelength absorbance values is useful to identify regions in the spectra in which animals with high wavelength absorbance values for the morning milk have also high wavelength absorbance values for the evening milk. Furthermore, the L_2 distance is a measure of the overall similarity between a morning and the respective evening spectrum.

The spectral regions with the greatest difference and the greatest SD of the difference between the morning and the evening spectrum absorbance values also had weak to moderate correlations between the morning and the respective evening wavelength absorbance values (i.e., ranging from 0.40 to 0.42 for the spectral regions between 1,743 cm^{-1} and 1,763 cm^{-1} and from 0.48 to 0.57 for the spectral region between 2,854 cm^{-1} and 2,926 cm^{-1}). Results from the repeatability of both the morning and the evening wavelengths absorbance values within stages of lactation were often in agreement with results from the correlation analyses. Indeed, spectral regions with strong correlations between the morning and the respective evening wavelength absorbance values also had high repeatability across lactation for both the morning and the evening wavelength absorbance values. Nonetheless, the spectral regions with the weakest correlation between the morning and the respective evening wavelength absorbance values did not have the lowest repeatability of the morning and the evening wavelengths absorbance values.

The spectral regions with the greatest mean difference and the greatest SD of the difference between the morning and the evening spectrum absorbance values are associated with the fat content of the milk. In particular, the C–H stretching of $-CH_3$ and $-CH_2$ have been identified in the spectral region between 2,862 and 2,927 cm^{-1} of dairy cow milk with C = O stretching around 1,743 cm^{-1} (Grelet et al., 2015). Milk fat is the component whose concentration changes the most between morning and evening milking (McParland et al., 2019). Using the summary statistics provided by McParland et al. (2019) from part-day milk samples from Irish dairy cows, it was possible to calculate the variance of the difference in milk fat percent and in milk protein percent between morning and evening milk; the respective values were 0.47 percentage units^2 and 0.02 percentage units^2 signifying the greater variance in the difference between fat in the morning and evening milk samples of cows relative to protein. The spectral regions with the strongest and weakest correlations between morning and evening milk spectra absorbance values (i.e., between 1,469 cm^{-1} and 1,473 cm^{-1} and between 1,593 cm^{-1} and 1,597 cm^{-1}, respectively) have been associated with proteins (i.e., the amide I and amide II regions). The amide II vibration region is measured in the range between 1,450 cm^{-1} and 1,550 cm^{-1} (Tarapoulouzi et al., 2020), whereas the amide I vibration is measured in the range between 1,550 cm^{-1} and 1,650 cm^{-1} (Tarapoulouzi et al., 2020).

When investigated within lactation stages, the greatest mean and SD of the difference between the morning and the respective evening wavelength absorbance values was in early lactation. Similarly, the weakest correlations between morning and evening milk spectra absorbance values were observed in early lactation. Moreover, early lactation was also characterized by lower repeatability of both the morning and the evening wavelengths absorbance values compared with the repeatability quantified for milk collected in later stages of lactation. Finally, the L_2 distance quantified between morning and evening spectra wavelengths absorbance values was greater in early lactation compared with late lactation. These greater differences in morning and evening spectra in early lactation could be attributed to the greatest difference in morning and evening milk yield; the mean difference between morning and evening milking in early and late lactation was 8.17 and 4.88 kg, respectively.

Differences across farms and years in strength of the correlations between morning and evening milk spectra were mainly limited to the regions between 941 cm^{-1} and 1,227 cm^{-1} and between 2,349 cm^{-1} and 2,831 cm^{-1}. These regions have been associated with carbohydrates and minerals in milk corresponding to P–O stretching of phosphorus (i.e., between 950 cm^{-1} and 1,200 cm^{-1}), aldehydes (2,780 cm^{-1}; Bittante and Cecchinato, 2013), and multiple peaks of the ammonium bonds (2,400 cm^{-1} to 3,200 cm^{-1}; Bittante and Cecchinato, 2013). This is also consistent with results obtained from the L_2 distance. Indeed, a clear decrease in the L_2 distance was documented from early to late lactation, whereas no differences were documented in the L_2 distance across farms and years.

Practical Applications

Acknowledgment of the differences between morning and evening milk are particularly important when developing prediction equations for animal traits (Frizzarin et al., 2024; McParland et al., 2024). Indeed, although milk quality traits, such as milk fat, protein, or lactose content, are directly quantified from the milk sample (i.e., there is a direct relationship between the gold-standard measure and the MIR spectra), the same phenotypic value for animal traits like BCS change or NUE can be associated with 2 different spectra (i.e., 2 milk spectra generated from the milk milked from the same cow with 24 h). As morning and evening milk spectra provide different information, the inclusion of both the morning and the evening spectra in a prediction model could generate more accurate predictions; this was also the conclusion of both Frizzarin et al. (2024) and McParland et al. (2024), who used cow milk MIR data to predict NUE and methane emissions, respectively. Despite this, taking separate morning and evening milk samples and subjecting them both to spectral analysis in commercial farms is probably not feasible. Moreover, there is actually a transition away from collecting (and analyzing) individual morning and evening samples during herd testing (McParland et al., 2019). Nonetheless, International Committee for Animal Recording procedures (https://www.icar.org/Guidelines/02-Overview-Cattle-Milk-Recording.pdf) still suggest recording both the morning and the evening milk samples when undertaking milk herd testing.

Table 2. Correlation (r) between actual and predicted values as well as the root mean square error of prediction (RMSE) using partial least squares regression to estimate nitrogen use efficiency[1]

Calibration	Validation	r	RMSE
Morning	Morning	0.70	3.49[a]
Evening	Morning	0.62	3.85[b]
Average	Morning	0.67	3.63[c]
Evening	Evening	0.70	3.46[a]
Morning	Evening	0.66	3.85[b]
Average	Evening	0.67	3.79[b]

[a–c]Different superscript letters indicate that RMSE values are different ($P < 0.05$) from each other within validation dataset.

[1]Predictions were developed using morning, evening, or average morning and evening spectra; predictions were then applied to morning or evening spectra separately. The calibration dataset included 2,622 records, and the validation dataset included 875 records.

An alternative to average (McParland et al., 2024) or concatenate (Frizzarin et al., 2024) the wavelength absorbance values from the morning and the respective evening milk spectra, is to develop prediction equations separately for the morning and evening milk samples. Indeed, based on the results from the present study, the prediction accuracy of NUE was 10.3% lower when predictions were developed using the morning spectra but applied to the evening spectra relative to if it was applied to the morning spectra; a similar difference was observed for the opposite scenario. Developing the prediction of NUE using the weighted average spectra from the morning and evening milk samples and applying to

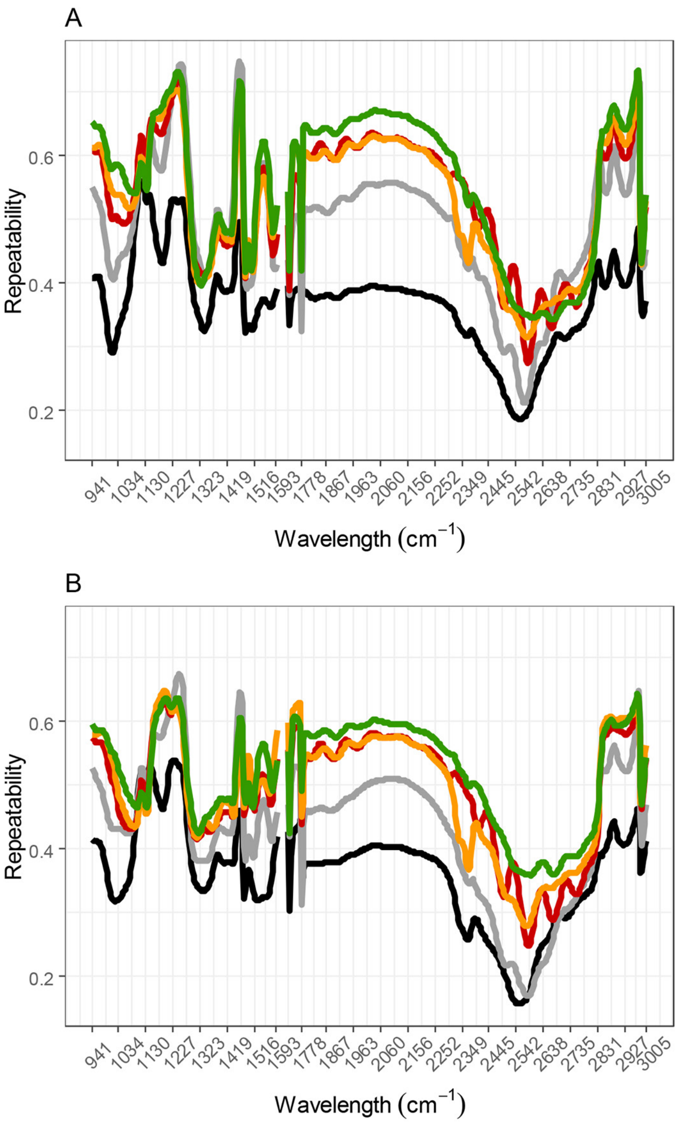

Figure 8. Repeatability for spectra collected during the morning milking (A) or during the evening milking (B) between 5 and 60 DIM (black), between 61 and 120 DIM (gray), between 121 and 180 DIM (red), between 181 and 240 DIM (orange), and between 240 and 305 DIM (green).

either the morning or evening milk spectra still resulted in poorer predictions compared with developing and applying the predictions using just the morning or just the evening milk separately. In fact, predictions developed using the average spectra and applied to morning milk were more similar to predictions developed and applied to just the morning milk samples (correlation and SD of the difference were 0.97 and 0.84 percentage units, respectively) compared with when the predictions were developed and applied to just the evening milk samples (correlation and SD of the difference were 0.94 and 1.63 percentage units, respectively); this is because morning milk yield was, on average, greater than evening milk yield with the former therefore contributing more to the weighted average spectra. Although the development of 2 different prediction equations generated more accurate predictions, the implementation and practical use of 2 prediction equations instead of one could be more complicated, especially where milking time and interval may differ between farms but also in farms operating once-a-day milk or robotic milking.

Based on the results from the present study, milk recording organizations should collaborate with researchers throughout the process of developing animal-level predictions from milk spectra. This is important to ensure the predictions developed are relevant to the type of milk samples (e.g., AM, PM, combined AM + PM, robot) being collected by the milk recording organizations. It should also be noted that the reduction in prediction performance when predictions equations developed for specific milking samples are applied to milk spectra from different sample types could pose challenges for international collaborations, in particular when milk recording practices vary between countries. This may also limit the ability to share equations across countries. For genetic evaluation bodies, genetic parameters should ideally be estimated for the separate predictions, as the (residual) variance for the trait under investigation may differ; or indeed, the genetic correlation between the same trait predicted values generated from different prediction approaches may not be one.

CONCLUSIONS

Results from the present study demonstrate differences in the MIR spectra of morning and evening milk. In particular, the study identified that (1) there are distinct internal relationships among the absorbance values for morning and evening milk spectra, (2) certain spectral regions exhibit substantial differences in absorbance values between morning and evening milk samples, and (3) other spectral regions had weak correlations between the absorbance values of morning and evening spectra. Notably, the spectral regions with large differences in absorbance values between morning and the respective evening are associated with milk fat content, whereas those with weak correlations are related to milk protein content; these differences are more pronounced in early lactation. Furthermore, the observed variability in absorbance values at different wavelengths between morning and evening samples can influence the accuracy of predicting animal-related traits from milk MIR.

NOTES

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Nonstandard abbreviations used: Ho-Fr = Holstein-Friesian; Je = Jersey; MIR = mid-infrared; NUE = nitrogen use efficiency; RMSE = root mean square error of prediction.

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