Differences in cow milk mid-infrared spectra collected during morning and evening milking and their implications

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Abstract

Individual cow milk samples are collected as part of routine milk recording; however differences across countries can exist on the recording scheme (e.g., only morning or evening milk samples, pooled morning and evening samples). Differences in the milk components are known to exist between morning and evening milk but possible differences in milk spectra generated from morning and evening milk have never been studied. To fill this gap in knowledge, a dataset of 199,288 morning milk spectra with an associated evening milk spectra produced by the same cow within 24 hrs was used. This dataset represented 2,602 dairy cows from 7 Irish research farms. Differences between morning and evening spectra were evaluated based on the mean and standard deviation of the difference between morning and evening spectral absorbance values, as well as the correlation between the morning and the respective evening wavelength absorbance values. Each of these were quantified across

1. Stages of lactation.
2. Years, and
3. Farms.

The largest mean difference between the morning wavelength absorbance values and the respective evening wavelength absorbance values was for wavelengths between 2,920 cm^{-1} and 2,947 cm^{-1}. Greater differences were found in milk produced in early lactation. Strong correlations (i.e., >0.80) between morning and evening spectra wavelengths were observed in the spectral region of 1,469 cm^{-1} to 1,473 cm^{-1} but weak correlations (i.e., <0.26) were evident between 1,593 cm^{-1} to 1,597 cm^{-1}. These trends in correlations were generally consistent within different stages of lactation, years, and farms. Furthermore, differences in morning and evening spectra materialized as a reduction in predictive ability of equations developed using morning spectra and applied to evening spectra, and vice versa. This degradation of performance was also observed when an equation developed using a daily spectrum (i.e., spectrum originating from weighted average of morning and evening spectrum, the weighting factor used was the respective morning and evening milk yield) was applied to predict information from a morning or an evening spectrum.

Keywords: mid-infrared spectroscopy, milking, predictions.

Introduction

Studies have reported differences in mean milk fat, protein, metabolites, and hormones between morning and evening milkings (Everett and Wadell, 1970; Pavel and Gavan, 2011; Teng *et al.,* 2021). Given these differences, variability may also exist in mid-infrared (MIR) spectra from milk collected at different day times.

Mid-infrared spectroscopy is widely used to predict milk quality and animal-level traits (De Marchi *et al.,* 2014; McParland and Berry, 2016). Some studies developed separate prediction models for morning and evening milk (McParland *et al.,* 2014, 2023; Frizzarin *et al.,* 2024), while others combined spectra from morning and evening milk to improve the prediction accuracy (McParland *et al.,* 2024; Frizzarin *et al.,* 2024). However, no study has directly compared MIR spectra from the same cow's morning and evening milkings within 24 hours. Therefore, this study aimed to

1. Identify differences in spectra generated from morning and evening milk collected from the same animal within 24 hours,
2. Identify spectral regions differing most between morning and evening milk spectra, and
3. Assess the impact on predictive performance of a model when applying to evening milk spectra prediction models developed using morning milk spectra, and vice versa.

Materials and methods

Data

This study used data collected between the years 2016 to 2020 from seven Teagasc research farms (Ireland). Data originated mainly from spring-calving cows on a grass-based diet. The dataset included cows in different parities (i.e., 1–10) and in different stages of lactation (i.e., DIM from 5 to 305). Cows were milked twice a day at 07:00 and 15:00, and milk samples were taken on consecutive days. Milk samples were then analyzed using a Foss MilkoScan FT6000 spectrometer. Absorbance values were transformed to transmittance and wavelengths were standardized across time using the European Milk Recording ring test program. The final dataset included 199,288 morning and 199,288 corresponding evening milk spectra from 2,602 cows.

Statistical analyses

Different analyses were undertaken to identify similarities and differences in morning and evening spectra produced by the same cow within 24 hours. These included

1. The mean and standard deviation of the difference per wavelength between morning and evening samples.
2. Comparison of the correlation matrices for morning and evening spectra, and
3. Pearson correlation between each individual wavelength. Analyses were performed on the whole dataset as well as within strata of lactation stage, year, or farm.

Using nitrogen use efficiency (NUE) as a case study, prediction models were developed separately for morning and evening milk spectra and validated using morning or evening spectra; only one NUE phenotype per day existed. An additional model was created using weighted average spectra per day. Partial least squares regression was used to develop the prediction models using 531 wavelengths. The accuracy of the prediction models was assessed using the Pearson correlation between the actual and the predicted NUE values, bias, and root mean square error. Differences in prediction accuracy were tested using the F-test on residuals.

Results

Morning vs. evening milk spectra

While both morning and evening spectra followed a similar pattern, mean differences ($P < 0.05$) were observed between morning and evening absorbance values. The largest mean difference and standard deviation of the difference occurred between 1,743–1,763 cm^{-1} and between 2,854–2,962 cm^{-1}, and this observation was consistent across lactation stages. Similarly, the correlation matrices among wavelength values within morning spectra and within evening spectra were similar, but the test introduced in Schott's (2007) indicated differences ($P < 0.05$) between morning and evening absorbance correlations. The correlations between morning and evening absorbance values for the same cow (Figure 1) were all strong (>0.80) in the 1,469–1,473 cm^{-1} region but weak (<0.26) in the 1,593–1,597 cm^{-1} region.

Across lactation stages (Figure 2), the correlations between morning and evening spectra wavelengths absorbance values had similar peaks and troughs in some spectral regions (1,227–1,601 cm^{-1}, 1,736–2,156 cm^{-1}), while differing in strength and shape in the other spectral regions. Across farms and years, the correlation between morning and evening spectra wavelength values showed variation in strength within the spectral regions of 941–1,127 cm^{-1} and 2,250–2,750 cm^{-1}, while remaining relatively stable in other regions.

NUE prediction accuracy

Table 1 details the NUE prediction accuracy using different calibration and validation datasets. When NUE was predicted from morning spectra using equations developed on evening spectra or weighted average spectra, the RMSE worsened by 10.3% and 4.0%, respectively, compared to use an equation developed on morning spectra. Similarly, when equations developed on morning or weighted average spectra were used to predict NUE from evening spectra, the RMSE increased (i.e., worsened) by 11.3% and 9.5%, respectively, compared to using an equation developed on evening spectra.

Discussion

Morning and evening milk of dairy cows differ in composition, with morning milk being generally lower in fat and sometimes (but not always) lower in protein (Berry *et al.*, 2006). These differences likely originate from differences in the preceding milking interval — longer intervals increase total yield but reduce hourly secretion rates (Everett

Table 1. 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 using morning, evening, or average morning and evening spectra. The prediction models were then applied to morning or evening spectra separately. The calibration dataset included 2,622 records, while the validation dataset included 875 records.

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 subscript letters indicate that RMSE values are different (P<0.05) from each other within validation dataset.

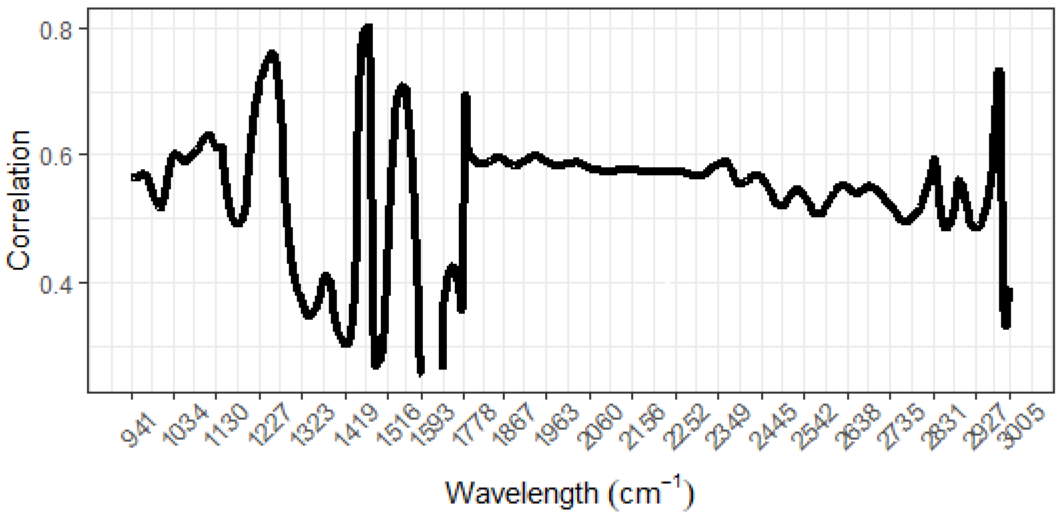

Figure 1. Correlation between each morning wavelength and the respective evening wavelength from spectra produced from the same cow

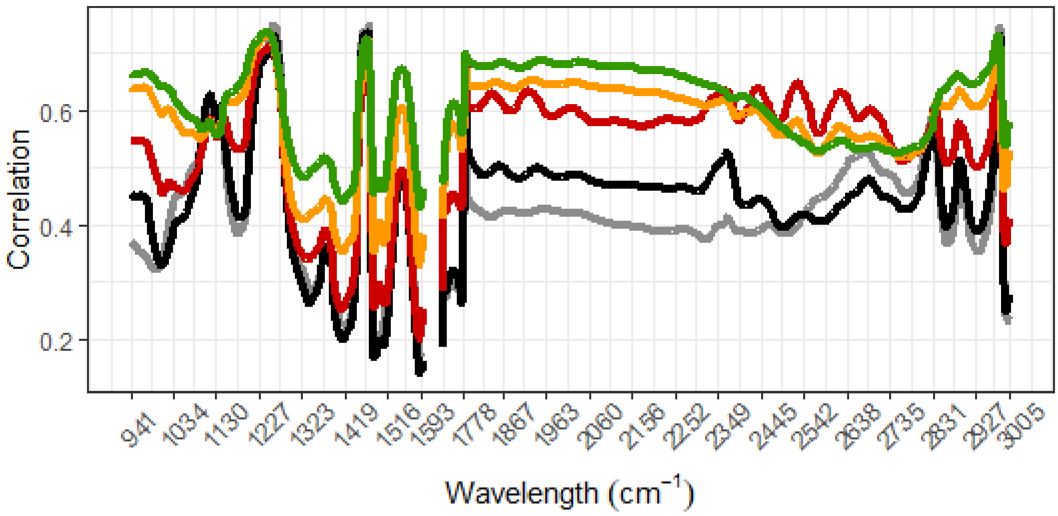

Figure 2. Correlations between each morning wavelength and the respective evening wavelength for spectra produced from the same cow using data between 5 and 60 DIM (grey line), between 61 and 120 DIM (black line), between 121 and 180 DIM (red line), between 181 and 240 DIM (orange line), and between 240 and 305 DIM (green line).

and Wadell, 1970). Since milk spectral data capture components like fat, protein, and lactose, differences in spectra may exist between morning and evening milk.

Each of the investigated similarity metrics provided unique insights. Mean difference and SD of the difference of the wavelengths absorbance values between morning and evening milk highlighted spectral regions with the greatest differences, while correlation analysis identified regions similar, or not, in the ranking of absorbance values between morning and evening milking of the same cow.

Spectral regions where the difference between morning and evening wavelength values were greatest were associated with regions linked with fat content. In contrast, Spectral regions with both the strongest and weakest morning-evening correlations are the ones typically associated with proteins.

The (mean and standard deviation of the) difference between morning and evening milk spectra was greatest in early lactation compared to other stages of lactation; similarly, the correlation between the spectral wavelengths between the morning and evening milk samples was weakest in early lactation compared to other stages of lactation. These greater differences between morning and evening spectra in early lactation are likely related to a greater difference in yield between the morning and evening milking in early lactation relative to others stage of lactation.

Differences in the pattern of the correlation between morning and evening spectra wavelength values across farms and years were mostly limited to 941–1,227 cm^{-1} and 2,349–2,831 cm^{-1}, which are spectral regions associated with carbohydrates and minerals.

Recognizing that differences in milk spectra exist between morning and evening milk is crucial when developing prediction models for animal-level phenotypes (Frizzarin *et al.,* 2024; McParland *et al.,* 2024). Unlike the concentration of milk components for individual milk samples, animal-level traits like NUE or methane emissions are measured on a daily basis and therefore the same gold standard value can be associated with the spectra for both milking times. Including both morning and evening spectra in prediction models improved the prediction accuracy in Frizzarin *et al* (2024) and McParland *et al* (2024) for NUE and methane emissions, respectively. Nonetheless, collecting separate morning and evening milk samples may be impractical for commercial farms, where herd testing increasingly relies on combined samples (McParland *et al.,* 2019). Alternatives to averaging or concatenating morning-evening spectra include developing separate prediction equations from the spectra of each milking.

Conclusions

This study reveals clear differences in MIR spectra originating from morning or evening milk. Key findings include:

1. Distinct relationships among milk spectra wavelength values within morning and evening spectra,
2. Certain spectral regions of the MIR were clearly different between morning and evening milk, and
3. Tthe spectral region between 1,593–1,597 cm^{-1} exhibited particularly weak correlations between the morning and evening spectral values.

Large absorbance differences between morning and evening spectra were linked to spectral regions associated with milk fat, while weak correlations between morning and evening spectra were associated with spectral regions linked with protein, with both effects more pronounced in early lactation. These spectral variations may impact the accuracy of animal trait predictions from milk MIR data.

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