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THE USE OF MID-INFRARED SPECTROMETRY TO PREDICT MILK FATTY ACID, ENERGY BALANCE AND METHANE EMISSIONS FOR AUSTRALIAN DAIRY COWS

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SUMMARY

The aim of the research was to develop prediction tools to help farmers select and manage their cows using mid-infra-red (MIR) spectroscopy. The prediction performance is evaluated on a range of complex phenotypes of energy balance, methane emissions and milk fatty acids using data from 120 Australian cows. Additionally, MIR prediction equations derived from the UK cows is also explored on the same Australia data. The accuracy (r2) of predicting energy balance using MIR from Australian cows was around 0.4, which is similar to the performance of applying the UK MIR prediction equations to Australian data of 0.39. The prediction accuracies of methane emissions and milk fatty acids were greater than 0.5. The next phase will focus on improving the accuracy and validating them against data from commercial populations.

INTRODUCTION

Mid-infrared spectral data are measures of the absorption of infrared rays at frequencies correlated to the vibration of specific chemical bonds within a molecule (Soyeurt et al., 2011). MIR prediction uses the absorbance of mid-infrared light through milk samples over a range of wavelengths to predict a given phenotype. To calculate a prediction equation that can be applied nationally, requires a reference population that has measurements of the phenotype of interest and MIR spectral data collected at the same time. The advantage of using MIR to predict these phenotypes is that the turnaround time back to farm can be fast and at little extra cost over standard milk tests, enabling reactive management decisions. To date, MIR prediction analysis has been applied to detailed milk fat and protein composition with promising prediction accuracies (Soyeurt et al., 2011). Furthermore, it has also been used to predict complex phenotypes, such as energy balance (McParland et al., 2011) and methane emissions (Dehareng et al., 2012).

As cows mobilise body fat in early lactation to sustain lactation (when feed requirements exceed intake), it is likely that special signatures of fatty acid composition are also observed in milk. There are several milk fatty acids in milk and having a greater understanding of how these are associated with energy balance may help to improve MIR predictions and could be worth breeding for in their own right.

The objective of this study was to predict a range of traits including several milk fatty acids, energy balance and methane emissions using MIR spectral data from a research herd in Victoria. In addition, a MIR prediction equation for energy balance developed using UK data and available commercially by National Milk Records (a UK milk recording organisation) was validated using this Australian data.

MATERIALS

Phenotypes and associated MIR spectral data were available from an experiment that ran from October to December 2015 consisting of 120 Australian Holstein lactating cows that calved in the spring at the research farm of the Department of Economic Development, Jobs, Transport, and Resources (DEDJTR) in Ellinbank, Victoria, Australia. The cows were divided into three batches of 40 cows to match the availability of automated feed intake equipment. Batches were balanced for days in milk (DIM), number of lactations and body weight. Cows had access to feed and water ad libitum with the diet consisting of cubes that were approximately 74% alfalfa hay, 25% crushed barley grain, and 1% minerals. The experimental duration was 32 days and the following measurements were performed: feed intake (for every meal), body weight after morning milking, body condition score 2x per week, milk yield (2x daily), milk fat percent, milk protein percent, and milk lactose percent 3 days per week at both morning and afternoon milking. MIR spectral data for each milking were obtained using a Bentley FTS instrument.

Traits. At 87-124 days in lactation, the records of 32 different types of fatty acids including saturated FA (C4:0 – C20:0), Saturated FA (10:1-18:1), Mono-unsaturated FA (18:2, 18:3) , and Trans FA (CLA) were collected for each sample twice across lactation. Across all the FA, Mahalanobis distance was calculated. Outlier samples and absorbance (with Mahalanobis distances >3) were removed.

Two equations obtained from Phuong et al (2016) were used to calculate energy balance (EB) from feed trial data. The first equation was energy intake minus energy output (EBinout), estimated using smoothed energy intake minus the energy required for maintenance, milk yield, and activity. The second equation was applied to calculate EB using milk composition only (Friggens et al., 2007).

Methane emissions were measured for each cow over a 5 day period that occurred within the 32d experiment using the SF6 tracer method of Deighton et al. (2014). Three phenotypes were calculated: 1) AvCH4, which was the mean of total methane emissions over 5 day period; 2) AvCH4yield, which was total methane yield divided by the actual feed intake over 5 day period; 3) AvCH4Intense, which was total methane yield divided by milk yield over the 5 day period.

Mid-infra-red spectral data. The MIR spectrum for each milk sample had 899 data points (absorbance) for wavelengths ranging from 649 to 3998 cm^{-1}. Using approaches developed by Grelet *et al.*, (2015), several pre-processing steps including removal of outliers, standardizing, smoothing, and noise removing were applied to the raw MIR data. As reported by Hewavitharana and van Brakel (1997), and De Marchi *et al.* (2012), two spectral regions (from 1603 to 1682 cm^{-1}; from 3006 to 3998 cm^{-1}) are either water absorbance or useless chemical information, these spectra were removed leaving 620 wavelengths for analysis.

METHODS

Partial least squares regression method. Partial least squares regression method is commonly used in the analysis of MIR spectral data (De Marchi *et al.*, 2012). An example is the SimPLS algorithm in R (R Development Core team, 2010) which was implemented for MIR prediction. To avoid over-fitting problems, 10 fold cross validation (10fold-CV) was used to assess the accuracy of the MIR prediction. The data sets were randomly separated into 10 subsets, and then each subset wass treated as a validation set while the other nine sets were combined as reference set. The accuracy was assessed as the coefficient of determination (**r^2**) calculated as the mean of 10 fold cross validation applied to prediction equations.

RESULTS AND DISCUSSION

We found a considerable number of significant correlations between energy balance and milk fatty acids, especially C:18 and C:20 (Figure 1). The high proportion of C:18 and C:20 during periods of negative energy balance is related to a high uptake of long chain fatty acids released from the mobilisation of body fat reserves (Bastin et al., 2011). Most of the correlations between energy balance and milk fatty acids were quite small. An explanation for these findings is the late lactation stage when the cows participated in the experiment. In the current experiment, days in milk of the cows varied from 87 to 124 which were >12 weeks where milk fatty acids would change very little or even remain constant.

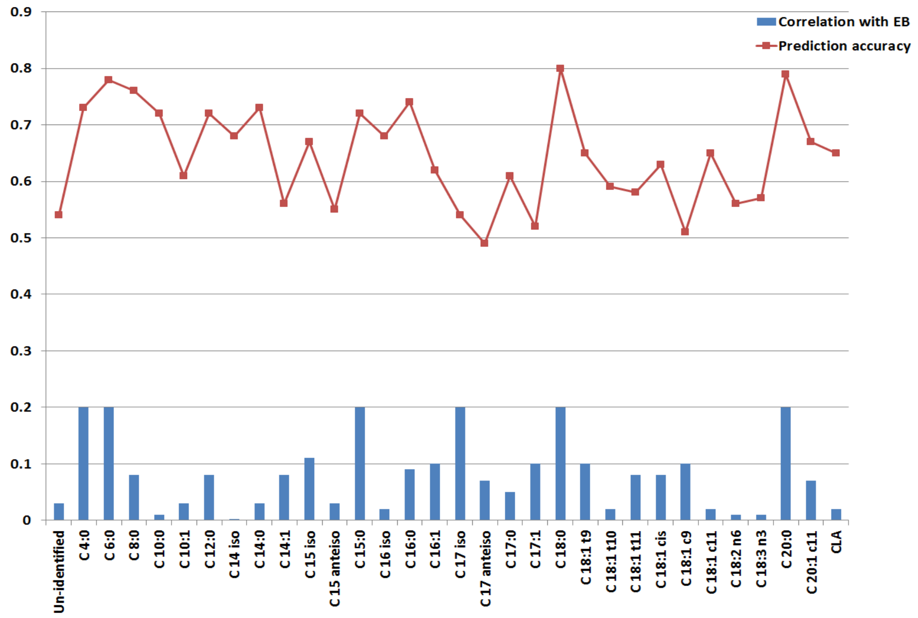

Figure 1. Pearson correlations between milk fatty acids and energy balance derived using individual cow data (blue bars) and the prediction accuracy using MIR data on the Fatty acids (red curves)

As shown in Figure 1, the accuracy of MIR prediction of milk fatty acid traits as determined by the coefficient of determination is higher than 0.50. For some fatty acids, for example, C4:0-C19:0, the prediction accuracy reached around 0.80.

Table 1. MIR prediction of two energy balance traits (predicted as the difference between energy intake and output; EBinout; energy balance using an equation applied to milk production data; EBalMilk) and three methane emission traits

Phenotypes		Country	r^{2*}	RMSE
Energy Balance	EBinout	AU	0.42	31.27
	EBinout	UK	0.39	-
	EBalMilk	AU	0.45	31.05
Methane Emission	AvCH4	AU	0.51	21.48
	AvCH4yield	AU	0.49	31.09
	AvCH4Intense	AU	0.52	20.45

*The accuracy was assessed as the coefficient of determination (r^2) and root mean square error of calibration (RMSE) calculated as the mean of 10 fold cross validation applied to prediction equations developed using Australian (AU) or UK reference datasets.

The prediction accuracy of energy balance and methane emissions using MIR is shown in Table 1. The prediction accuracy of EBinout is 0.42 with the root mean square error of calibration (RMSE) of 31.27. For the same trait, using the equation derived from the UK (estimated with a much larger reference dataset up to thousands of animals and a similar energy balance trait), we observed a similar accuracy (around 0.39). Similarly, the prediction accuracy of energy balance based on milk composition is around 0.45. The reasons that the accuracy in Australia was lower than observed in studies such as McParland et al (2011) include the fact that the reference population was not optimised for Australia; the phenotypes were subtly different to the energy balance used for the UK and it is possible that genotype by environment interactions exist.

Similarly, the accuracy of MIR prediction of the three methane emission traits ranges from 0.49 to 0.52 with the values of RMSE ranging between 20.45 and 31.09.

All the above results show that the accuracy of MIR prediction is currently lower than other comparable studies. The most likely explanation is that most previous studies have much larger reference size. For example, McParland et al (2011) had a large reference population of 6,665 test days from 465 lactations of 277 cows matched to MIR spectra. Our reference population in comparison is small. Therefore, the strategy of improving the prediction accuracy is to increase the reference population by expanding the number of phenotypes from research herds, or devising ways in which to measure energy balance on commercial farms.

CONCLUSION

Our analyses show MIR prediction is promising but needs further improvement. In the next phase, we are investigating ways in which more energy balance phenotypes can be collected from research and commercial dairy herds.

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