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## Mid-infrared spectrometry of milk as a predictor of energy intake and efficiency in lactating dairy cows

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### ABSTRACT

Interest is increasing in the feed intake complex of individual dairy cows, both for management and animal breeding. However, energy intake data on an individual-cow basis are not routinely available. The objective of the present study was to quantify the ability of routinely undertaken mid-infrared (MIR) spectroscopy analysis of individual cow milk samples to predict individual cow energy intake and efficiency. Feed efficiency in the present study was described by residual feed intake (RFI), which is the difference between actual energy intake and energy used (e.g., milk production, maintenance, and body tissue anabolism) or supplied from body tissue mobilization. A total of 1,535 records for energy intake, RFI, and milk MIR spectral data were available from an Irish research herd across 36 different test days from 535 lactations on 378 cows. Partial least squares regression analyses were used to relate the milk MIR spectral data to either energy intake or efficiency. The coefficient of correlation ( $R_{EX}$ ) of models to predict RFI across lactation ranged from 0.48 to 0.60 in an external validation data set; the predictive ability was, however, strongest ( $R_{EX} = 0.65$ ) in early lactation (<60 d in milk). The inclusion of milk yield as a predictor variable improved the accuracy of predicting energy intake across lactation ( $R_{EX} = 0.70$ ). The correlation between measured RFI and measured energy balance across lactation was 0.85, whereas the correlation between RFI and energy balance, both predicted from the MIR spectrum, was 0.65. Milk MIR spectral data are routinely generated for individual cows throughout lactation and, therefore, the prediction equations developed in the present study can be immediately (and retrospectively where MIR spectral data have been stored) applied to predict energy intake and efficiency to aid in management and breeding decisions.

**Key words:** feed efficiency, biomarker, mid-infrared spectrum, predictor

### INTRODUCTION

Animal feed efficiency is one component of the efficiency of the entire dairy sector. International initiatives using traditional- (Berry et al., 2014) and genomic-based (de Haas et al., 2012) approaches are underway to generate individual animal estimates of genetic merit for feed intake in lactating dairy cows. Incorporating estimates of genetic merit for feed intake in a breeding goal that also contains estimates of genetic merit for energy sinks is expected to improve feed efficiency (Berry and Crowley, 2013).

Residual feed intake (RFI) is commonly used in growing beef animals as a measure of feed efficiency (Berry and Crowley, 2013) and is increasing in popularity in lactating dairy cow populations (Coleman et al., 2010; Pryce et al., 2014). Residual feed intake in growing animals may be defined as the difference between actual energy intake and the energy intake predicted from the performance of the animal (Berry and Crowley, 2013). Because of lipid and protein body mass changes in lactating dairy cows, especially in early lactation (Berry et al., 2006), RFI in dairy cows may be defined as the difference between actual energy intake and both the energy demanded and supplied by the various energy sinks (e.g., milk production) and reservoirs (e.g., body lipids).

Irrespective of whether feed intake or RFI is incorporated in a national breeding objective, the lack of accurate estimates of genetic merit for either trait currently precludes inclusion in the breeding objective. Considerable interest exists in low-cost, easy-to-measure (biological) predictors of either feed/energy intake or RFI. Mid-infrared (MIR) spectroscopy is the study of the interaction between matter and electromagnetic waves in the 900 to 5,000  $\text{cm}^{-1}$  region and is the method used globally to routinely determine the fat, protein, and lactose concentration in milk. McParland et al. (2011, 2012) reported that the MIR spectrum of milk samples

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can predict both energy intake and energy balance. Because energy balance can be mathematically equivalent to RFI (Berry and Crowley, 2013; Savietto et al., 2014), we hypothesized that milk MIR spectral data could also predict individual cow RFI. The objective of the present study, therefore, was to quantify the ability of MIR spectra of individual cow milk samples to predict RFI. As milk MIR spectral data should be routinely available on all milk-tested animals at no marginal cost, if prediction of RFI from milk MIR were possible, the results from this study could be used in (1) day-to-day herd management, and (2) breeding programs to achieve genetic gain in feed efficiency in lactating dairy cows.

## MATERIALS AND METHODS

### Data

Data originated from an Irish dairy research herd, located at the Teagasc Animal and Grassland Research and Innovation Center (Moorepark, Fermoy, Co. Cork, Ireland) between the years 2008 and 2013, inclusive. The basal diet of the majority of cows was grazed grass and the data originated from a range of grazing studies (Ganche et al., 2013a,b; McCarthy et al., 2014). Individual cow DMI was periodically recorded at grass using the *n*-alkane technique and fecal grab samples (Dillon and Stakelum, 1989) up to 8 times across lactation. Details on the procedures used to collect and analyze the fecal grab samples have been provided elsewhere (Kennedy et al., 2008). The procedure provides a measure of DMI averaged across a week of sampling. In addition, a subset of cows ( $n = 25$ ) was housed in a freestall barn in early lactation (up to 39 DIM) and fed a TMR diet of maize silage, grass silage, soybean meal, and dairy concentrate (Moore et al., 2014). Individual DMI of housed cows was recorded daily using the Griffith Elder feeding system (Griffith Elder Ltd., Bury St. Edmunds, UK). The ME content of the grass was assumed to be  $0.0157 \times$  digestible OM of grass (AFRC, 1993). Grass ME intake was summed with the ME content of the concentrate fed (up to 5.3 kg daily, with an assumed energy content of 12.5 MJ; O'Mara, 1997). This information was used to compute effective energy intake (EEI) according to the formulas of Coffey et al. (2001).

Cows were milked twice daily at 0700 and 1500 h and individual cow milk yield was recorded at each milking. Individual cow milk samples were taken at consecutive p.m. and a.m. milkings once weekly. All milk samples were analyzed using the same MIR spectrometer (Foss MilkoScan FT6000; Foss Electric A/S, Hillerød, Denmark) and the resulting spectrum was stored. The Foss

MIR spectrum contains 1,060 data points that represent the absorption of infrared light through the milk sample at wavelengths in the 900 to 5,000  $\text{cm}^{-1}$  region. Spectral points in the region between 926 and 3,240  $\text{cm}^{-1}$  were retained for analysis. Informative wavelengths were identified through their associated X-loadings. Spectral data were transformed from transmittance to linear absorbance through a logarithmic transformation of the reciprocal of the wavelength values (Soyeurt et al., 2011).

Body weights of all cows were measured weekly following the a.m. milking using weighing scales. Body condition score was assessed by trained scorers every 2 to 3 wk on a scale of 1 to 5 (Edmonson et al., 1989). Cubic splines with 6 knot points at 20, 70, 120, 170, 220, and 270 DIM were fitted through individual test-day records of BW and BCS, with a covariance structure fitted among knot points. Body weight and BCS at each DIM were interpolated from the fitted splines. Forward differencing was used to estimate daily BW change ( $\Delta\text{BW}$ ) and daily BCS change ( $\Delta\text{BCS}$ ) for each DIM.

For comparative purposes, energy balance (EB) was calculated as the difference between effective energy intake and effective energy expended through milk production and maintenance according to the effective energy methodology of Banos and Coffey (2010).

### Computation of RFI

Milk yield, fat, protein, and lactose concentration as well as BW, BCS,  $\Delta\text{BW}$ , and  $\Delta\text{BCS}$  on test days that had a corresponding MIR spectral record and energy intake record were retained for subsequent analysis. Following edits, 1,535 a.m. and 1,335 p.m. spectral records from 535 lactations on 378 cows were available for analysis. Cows had between 1 and 4 spectral records per lactation.

Residual feed intake for test-day  $i$  ( $\text{RFI}_i$ ) was calculated as follows:

$$\text{RFI}_i = \text{EEI}_i - \left[ \sum_{n=1}^2 \text{DIM}^n + \text{Milk}_i + \text{Fat}_i + \text{Protein}_i + \text{Lactose}_i + \text{BW}_i^{0.75} + \text{BCS}_i + \text{BW}_i^{0.75} \times \text{BCS}_i + \Delta\text{BW}_{i-1,i} + \Delta\text{BCS}_{i-1,i} + \Delta\text{BW}_{i-1,i} \times \Delta\text{BCS}_{i-1,i} \right] \quad [1]$$

where  $\text{RFI}_i$  is residual feed intake on day  $i$ ,  $\text{EEI}_i$  is effective energy intake on day  $i$ ,  $\text{Milk}_i$  is milk yield on day  $i$ ,  $\text{Fat}_i$  is milk fat yield on day  $i$ ,  $\text{Protein}_i$  is milk protein yield on day  $i$ ,  $\text{Lactose}_i$  is milk lactose yield on day  $i$ ,  $\text{BW}_i^{0.75}$  is metabolic liveweight (i.e.,  $\text{BW}^{0.75}$ ) on day  $i$ ,

$BCS_i$  is BCS on day  $i$ ,  $\Delta BW_{i-1,i}$  is change in BW from day  $i - 1$  to day  $i$ , and  $\Delta BCS_{i-1,i}$  is change in BCS from day  $i - 1$  to day  $i$ . No multicollinearity existed in the model as quantified by the variance inflation factor.

Residual feed intake (calculated above) was also categorized into 3 different stages of lactation: 5 to 60 (RFI<sub>early</sub>;  $n = 301$ ), 60 to 180 (RFI<sub>mid</sub>;  $n = 666$ ), and 180 to 300 (RFI<sub>late</sub>;  $n = 368$ ) DIM. Records from cows housed during lactation contributed 12% to the early lactation records used in this study.

### Development of Prediction Equations

Partial least squares regression (PROC PLS; SAS Institute Inc., Cary, NC) was used to predict RFI, EB,  $\Delta BCS$ , and EEI from the MIR linear absorbance data. The data were sorted by the variable to be predicted and every eighth observation was removed from the calibration data set and retained for use in an external validation. This was done to optimize the robustness of the prediction equation, as samples in the calibration data set should represent the variation observed in the phenotype to be predicted (McParland et al., 2012). To facilitate an independent validation, all records from lactations originally selected for external validation were removed from the calibration data set and included in the external validation data set. Hence, the external validation data set was an independent group of lactations to the calibration data set.

The prediction model was developed using split-sample cross-validation in the calibration data set. In this approach, every 20th observation was removed from the calibration data set and predicted using a model developed from the data remaining in the calibration data set. This was iterated until every sample had been predicted once.

Prediction equations were calibrated and externally validated using 1 of 5 models: (1) using a.m. spectra only ( $n = 1,535$ ), (2) using p.m. spectra only ( $n = 1,335$ ), (3) using both a.m. and p.m. spectra jointly ( $n = 1,335$ ), (4) using the average of a.m. and p.m. spectra weighted by their corresponding milk yield ( $n = 1,335$ ), and (5) using both a.m. and p.m. spectra together with total test-day milk yield ( $n = 1,335$ ). An additional model was tested to predict RFI<sub>early</sub>, RFI<sub>mid</sub>, and RFI<sub>late</sub> using a.m. and p.m. spectra jointly as predictor variables (i.e., model 3 above).

In 2 separate analyses, RFI was recalculated using EEI predicted using a.m. and p.m. spectra and milk yield (i.e., predicted using model 5). First, RFI was defined according to Equation 1; however, measured EEI in the equation was substituted with predicted EEI. Second, RFI was defined as the difference between MIR-predicted EEI and predicted energy intake from

the regression coefficients derived in model 1 (i.e., using real EEI as the dependent variable).

Correlations between the measured variables (referred to as true RFI, true EB, true EEI, and true  $\Delta BCS$ ) were estimated, as were the correlations between the predicted respective variables. For the latter, daily predictions of each trait were the daily average of the predictions from the a.m. samples and p.m. samples (i.e., model 1 and 2, respectively). The significance of the difference between a correlation between 2 variables estimated using the true data and the corresponding correlation estimated using the predicted variables was determined following a Fisher  $r$ -to- $z$  transformation of the correlations (Fisher, 1915).

## RESULTS

The test days used in the present study were evenly distributed across lactation from 6 to 277 DIM. Mean values (SD in parentheses) of RFI, EB, EEI, and  $\Delta BCS$ , were 0 (30) MJ/d, 52 (35) MJ/d, 185 (38) MJ/d, and  $-0.0009$  (0.0026) units, respectively. As lactation progressed, although no decline in DMI was observed, EEI decreased, whereas simultaneously, milk yield decreased, resulting in improved (measured) EB in late lactation (Table 1). The modal number of model explanatory factors across all model scenarios was 14; however, they ranged between 8 (RFI predicted using p.m. spectra) and 19 ( $\Delta BCS$  predicted using either a.m. or p.m. samples).

### Prediction Accuracy Across Lactation

Cross-validation accuracy of prediction was stronger than the accuracy obtained from external validation ( $R_{EX}$ ) for all prediction equations, with the exception of RFI predicted using the weighted average of a.m. and p.m. spectra (Tables 2 and 3). Prediction equations calibrated using only p.m. milk samples were superior to those calibrated using only a.m. milk samples (Table 2). For the majority of the traits, predictions made using only p.m. milk samples had a lower root mean square error (RMSE), a slope between true and predicted values closer to unity, and a stronger correlation between true and predicted values in the external validation compared with predictions made using only a.m. milk samples. The difference in  $R_{EX}$  between equations using either only a.m. or only p.m. milk samples was sometimes large; differences in  $R_{EX}$  ranged from 0.03 (EB) to 0.18 ( $\Delta BCS$ ).

Prediction accuracy of EB improved when both a.m. and p.m. spectra were jointly included as predictor variables (Table 3). When the weighted average of a.m. and p.m. spectra was used for prediction, the bias of

**Table 1.** Mean (SD in parentheses) values of milk production, intake, measured energy balance, and change in BCS across stage of lactation

Trait <sup>1</sup>	Stage of lactation <sup>2</sup>		
	Early (n = 301)	Mid (n = 666)	Late (n = 368)
Milk (kg)	26 (6)	21 (5)	14 (3)
Fat (%)	4.2 (0.7)	4.0 (0.7)	4.7 (0.8)
Protein (%)	3.2 (0.3)	3.4 (0.3)	3.9 (0.4)
DMI (kg)	15 (4)	16 (3)	15 (3)
EEI (kg)	186 (41)	188 (35)	171 (33)
EB (MJ/d)	30 (39)	54 (30)	57 (25)
RFI (MJ/d)	2 (33)	-6 (28)	-1 (24)
$\Delta$ BCS (U)	-0.0048 (0.0019)	-0.0001 (0.0019)	0.0007 (0.0012)

<sup>1</sup>EEI = effective energy intake; EB = energy balance; RFI = residual feed intake;  $\Delta$ BCS = daily change in BCS.

<sup>2</sup>Early = <60 DIM; mid = 60 to 180 DIM; late = 180 to 305 DIM.

predicting EB was close to 0 and the slope between true and predicted values of EB was not different from 1. The addition of milk yield as a predictor variable failed to improve the accuracy of predicting EB. In contrast, the best model to predict EEI comprised a.m. and p.m. spectra and milk yield, all included as predictor variables (Table 3).

The weighted average of a.m. and p.m. spectra provided the most accurate prediction equation for  $\Delta$ BCS; the slope between true and predicted values of  $\Delta$ BCS was not different from 1 and the  $R_{EX}$  of  $\Delta$ BCS was 0.73. The accuracy of predicting  $\Delta$ BCS declined substantially when milk yield was included in the prediction model (Table 3).

Regardless of the prediction model used, RFI was more poorly predicted than EB. Moreover, accuracy statistics of the equations to predict RFI did not vary much across the alternative prediction model scenarios evaluated. Including a.m. and p.m. spectra jointly, or including milk yield as a predictor variable did not substantially improve the accuracy of prediction of RFI over prediction using p.m. milk samples alone.

However, using the weighted average of a.m. and p.m. spectra as predictors resulted in deterioration in prediction accuracy of RFI (Table 3).

#### Prediction Accuracy of RFI Within Lactation Stage

Accuracy of prediction of RFI changed throughout lactation (Table 4). In early lactation, despite a small calibration data set, the  $R_{EX}$  was 0.65, with a slope between true and predicted values of close to 1. In late lactation, however, the  $R_{EX}$  was 0.50, with a bias of 2.19 MJ/d. Although the slope of the regression of true on predicted RFI deteriorated from early to late lactation, the root mean square error was least in late lactation and greatest in early lactation.

#### Correlations Among Performance Variables

Correlations among RFI, EB, EEI, and  $\Delta$ BCS are in Table 5. Differences between correlations between true variables and corresponding correlations between predicted variables were statistically different ( $P <$

**Table 2.** Fitting statistics<sup>1</sup> of cross- and external-validation prediction equations using morning or evening milk samples

Trait <sup>2</sup>	Cross-validation			External validation					
	No.	RMSE	r	No.	Bias	RMSE	Slope (SE)	RPD	r
Morning milk samples									
EB	1,036	24.71	0.71	495	-0.39	26.66	0.69 (0.04)	1.27	0.62
EEI	1,026	28.57	0.64	505	3.26	29.75	0.84 (0.05)	1.25	0.60
$\Delta$ BCS	1,018	0.0017	0.76	513	0.0000	0.0022	0.58 (0.04)	1.22	0.57
RFI	1,032	25.27	0.50	499	-0.16	26.48	0.93 (0.08)	1.14	0.48
Evening milk samples									
EB	929	24.55	0.68	406	-2.3	23.73	0.79 (0.05)	1.31	0.65
EEI	930	27.76	0.65	405	-1.53	28.49	0.91 (0.05)	1.29	0.64
$\Delta$ BCS	927	0.0017	0.78	408	0.0000	0.0018	1.01 (0.04)	1.50	0.75
RFI	936	22.63	0.60	399	-1.9	23.88	0.88 (0.06)	1.22	0.58

<sup>1</sup>No. = number of samples; RMSE = root mean square error; RPD = ratio performance deviation; r = correlation between true and predicted values.

<sup>2</sup>EB = energy balance; EEI = effective energy intake;  $\Delta$ BCS = daily change in BCS; RFI = residual feed intake.



**Table 3.** Fitting statistics<sup>1</sup> of cross- and external-validation prediction equations

Trait <sup>2,3</sup>	Cross-validation			External validation					
	No.	RMSE	r	No.	Bias	RMSE	Slope (SE)	RPD	r
a.m. and p.m. spectra									
EB	929	23.38	0.71	406	-1.83	22.79	0.81 (0.04)	1.37	0.68
EEl	934	27.06	0.67	401	-0.08	31.01	0.58 (0.04)	1.19	0.55
ΔBCS	927	0.002	0.75	408	0.0000	0.002	0.62 (0.04)	1.22	0.58
RFI	936	22.14	0.62	399	-1.23	23.53	0.89 (0.06)	1.24	0.59
Weighted average a.m. and p.m. spectra									
EB	929	24.72	0.67	406	-0.03	23.41	0.92 (0.05)	1.33	0.66
EEl	934	24.03	0.75	401	-0.23	30.43	0.54 (0.04)	1.21	0.57
ΔBCS	927	0.002	0.77	408	-0.0001	0.002	0.99 (0.05)	1.46	0.73
RFI	936	23.89	0.53	399	-2.86	24.63	0.94 (0.07)	1.18	0.54
a.m. and p.m. spectra and milk									
EB	929	24.84	0.67	406	-1.47	24.02	0.85 (0.05)	1.30	0.64
EEl	934	23.77	0.76	401	-0.12	26.63	0.79 (0.04)	1.38	0.70
ΔBCS	927	0.002	0.77	408	0.0000	0.002	0.55 (0.04)	1.20	0.55
RFI	936	21.95	0.63	399	-1.23	23.39	0.88 (0.06)	1.25	0.60

<sup>1</sup>No. = number of samples; RMSE = root mean square error; RPD = ratio performance deviation; r = correlation between true and predicted values.

<sup>2</sup>a.m. and p.m. spectra = morning and evening milk spectra used jointly as predictors; weighted average a.m. and p.m. spectra = the average of morning and evening milk spectra weighted by their test-day milk yield used as predictors; a.m. and p.m. spectra and milk = morning and evening milk spectra plus total test-day milk yield used as predictors.

<sup>3</sup>EB = energy balance; EEl = effective energy intake; ΔBCS = daily change in BCS; RFI = residual feed intake.

0.001; with the exception of the difference between correlations between EEl and ΔBCS); however, differences tended to be biologically small. The correlation between true RFI and EB across the entire lactation was 0.85; the corresponding correlation between the MIR spectra-predicted equivalents was 0.65. The correlation, however, between MIR spectra-predicted RFI and true EB was 0.45 (results not shown).

The correlation between true RFI and EB changed throughout lactation, ranging from a minimum of 0.87 at DIM <60 to a maximum of 0.95 in mid lactation (120–180 DIM). The correlation between MIR spectra-predicted RFI and MIR spectra-predicted EB was also greatest between 120 and 180 DIM (r = 0.89). The correlation between RFI and EEl across lactation varied from 0.75 to 0.79, irrespective of whether the correlations were based on true or MIR spectra-predicted variables; hence,

56 to 62% of the variation in RFI was due to differences in EEl. The correlation between EB and ΔBCS was weaker ( $P < 0.001$ ) between the true traits (r = 0.35) compared with when the equivalent MIR spectra-predicted traits were correlated (r = 0.53; Table 5).

The correlation between true RFI and RFI calculated as the difference between MIR spectra-predicted EEl and required intake was 0.60 and 0.61 in the calibration and validation data sets, respectively and was similar ( $P > 0.05$ ) to the accuracy of predicting RFI directly from the MIR spectrum. Correlations between measured RFI and RFI calculated from MIR spectra-predicted EEl were 0.63 and 0.64 in the calibration and validation data sets, respectively, and thus were stronger ( $P > 0.05$  and  $P < 0.05$ , respectively) than the accuracy of predicting RFI directly from the MIR spectra.

**Table 4.** Fitting statistics<sup>1</sup> of cross- and external-validation prediction equations to predict residual feed intake across stage of lactation using morning and evening spectra

Stage <sup>2</sup>	Cross-validation			External validation				
	No.	RMSE	r	No.	Bias	RMSE	Slope (SE)	r
Early	253	25.92	0.65	48	1.46	24.13	0.92 (0.16)	0.65
Mid	524	22.12	0.63	142	0.99	21.81	0.91 (0.10)	0.59
Late	314	17.91	0.66	54	2.19	20.45	0.67 (0.16)	0.50

<sup>1</sup>No. = number of samples; RMSE = root mean square error; r = correlation between true and predicted values.

<sup>2</sup>Early = <60 DIM; mid = 60 to 180 DIM; late = 180 to 305 DIM.

**Table 5.** Correlations<sup>1</sup> between measured (above diagonal) and mid-infrared (MIR) spectra-predicted (below diagonal) energy balance (EB), energy intake (EEI), daily change in BCS ( $\Delta$ BCS), and residual feed intake (RFI)

Item	EB	EEI	$\Delta$ BCS	RFI
EB	—	0.69	0.35	0.85
EEI	0.57	—	0.00 <sup>2</sup>	0.79
$\Delta$ BCS	0.53	-0.06 <sup>2</sup>	—	0.01
RFI	0.65	0.75	-0.10	—

<sup>1</sup>Differences between correlations between measured variables and corresponding correlations between MIR spectra-predicted variables were statistically different ( $P < 0.001$ ).

<sup>2</sup>No difference between correlations between measured and MIR spectra-predicted variables.

## DISCUSSION

Interest in the feed intake complex and, in particular, energy intake and RFI is increasing because of the requirement to feed a growing and more affluent human population while simultaneously minimizing the environmental footprint of increased livestock production. In addition to the usefulness of energy intake and efficiency in breeding goals, monitoring individual animal or herd energy intake and efficiency has many other uses. Such uses include the evaluation of alternative management strategies (e.g., diet), monitoring animal or herd health, and potential benchmarking of herd performance (Berry and Crowley, 2013). Nonetheless, the main factor hindering the widespread use of energy intake and efficiency in management, but especially breeding strategies, is the limited availability of individual animal or group feed intake records. Although international initiatives are underway to implement international genetic (Berry et al., 2014) and genomic (De Haas et al., 2012) evaluations for feed intake in lactating dairy cows, the accuracy of the generated EBV is still low due to a paucity of individual cow feed intake data. Greater accuracy could be achieved with a larger population of animals with feed intake phenotypes. Using selection index theory applied to variance components from a meta-analysis of dairy cow genetic studies, Berry and Crowley (2013) reported that approximately 89% of the genetic variation in DMI could be explained by genetic differences in milk yield, liveweight, chest width, and stature. Variance components are, however, population specific, and represent the average of the population, but do not account for individual animal deviations. The scenario alluded to represents net feed efficiency (i.e., the genetic variation unexplained by the 4 index traits). Therefore, considerable interest still remains in low-cost tools to measure or predict feed intake or efficiency on a routine basis. Because information on most energy sinks in lactating dairy cows are routinely available (e.g., milk production) or can

easily be generated (e.g., liveweight), having accurate predictions of energy intake facilitates the derivation of all feed efficiency measures (Berry and Crowley, 2013). In the present study, we attempted to predict both energy intake and feed efficiency directly from the MIR spectrum of milk. For comparison purposes, we also attempted to predict EB measured as the differential between energy intake and output, but also approximated as  $\Delta$ BCS.

### *Milk MIR Spectroscopy as a Phenotyping Tool*

The potential of milk MIR spectroscopy as a phenotyping tool has already been comprehensively reviewed (Berry et al., 2013; De Marchi et al., 2014). A particular advantage of milk MIR spectroscopy is that all milk samples (i.e., all bulk tank samples and individual samples from milk-recorded animals) are subjected to MIR spectroscopy analysis to determine the fat, protein, and lactose concentrations (as well as other components). Therefore, the marginal cost of implementing a prediction equation based on the milk MIR spectra is negligible once an accurate prediction equation is developed. Due to the fact that milk samples are taken routinely, longitudinal data per cow or per herd are available, facilitating a greater ability to detect perturbations over time. McParland et al. (2011, 2012) previously documented the ability of milk MIR spectra to predict EB. Energy balance and RFI are, in principle, mathematically very similar (Savietto et al., 2014) and this was substantiated by the very strong correlation that existed in the present study between EB and RFI ( $r = 0.85$  across lactation). The correlation was particularly strong ( $r = 0.95$ ) in mid lactation when liveweight change is minimal, which is the main mathematical difference between the 2 traits.

### *Prediction of Feed Intake Complex with Milk MIR Spectroscopy*

A subset of the data set used in the present study was previously used to predict EB from milk MIR spectra (McParland et al., 2012) and the results observed in the present study are similar to those documented previously. The ability of the MIR spectrum to predict component traits of EB (i.e., milk fat, protein, and lactose concentrations) is well established (Biggs, 1978). The MIR spectrum, however, captures additional variation in EB, which is not simply an artifact of the mathematical part-whole relationship between the left-hand side and right-hand side of the prediction equations. This is because accuracy (i.e.,  $R_{EX}$ ) of predicting EB using test-day milk, fat, protein, and lactose yields alone as predictor variables was 0.38. This is considerably lower

than the accuracy of predicting EB from milk MIR on the same data set (0.68).

Body condition score change can also be used as a proxy for EB (Friggens et al., 2007). Prediction accuracy for  $\Delta$ BCS in the present study was greater than the prediction accuracy for EB calculated from energy intake and energy expenditure despite no part-whole mathematical relationship existing between  $\Delta$ BCS and the predictor variables. Loss of body condition is associated with mobilization of fat reserves. The MIR spectrum is an accurate predictor of individual milk fats (Soyeurt et al., 2011), including C18:0 and *cis*-9 C18:1, which are known to be stored in adipose tissue (Chilliard et al., 2000). These FA are released during body tissue mobilization or periods of negative EB, thus providing a biological rationale for how the milk MIR can predict  $\Delta$ BCS. Furthermore, Bastin et al. (2012) documented a genetic association between both C18:0 and *cis*-9 C18:1 predicted from the MIR spectrum and days open (i.e., fertility), and showed that in early lactation, when cows are typically in negative EB (Berry et al., 2006), the associations between milk FA derived from mobilization of body reserves and days open were positive (Bastin et al., 2012). In the current study, the correlation between true EB and  $\Delta$ BCS was weaker than the correlation between predicted EB and  $\Delta$ BCS (Table 5), indicating that predicted EB may be more strongly associated with lipolysis than true EB.

As defined in the present study, RFI is phenotypically independent of milk composition and  $\Delta$ BCS. Therefore, the predictive ability of RFI from milk MIR spectra suggests that the MIR spectroscopy also detects biological factors associated with RFI, which are the net of differences in milk yield (i.e., dilution), milk composition, and  $\Delta$ BCS.

Methane emissions account for approximately 6% of ingested energy of ruminants (Johnson and Johnson, 1995) and are, therefore, likely to contribute to differences in RFI among animals. Albeit from a relatively small data set, Dehareng et al. (2012) documented the ability of milk MIR spectra to predict methane emissions, which is one biological rationale to why the milk MIR can predict RFI. Based on the proportion of variation in RFI explained by the MIR spectra, however, it is very likely that other biological compounds related to RFI (either directly or indirectly) were also being detected in the milk. The contributors to differences in RFI, especially in dairy cows, have not been fully elucidated. Components detected in the milk MIR spectra, however, may include alternative protein structures as an artifact of, for example, differences in protein turnover, which is speculated to contribute to differences in RFI, in growing animals at least (Richardson and Herd, 2004).

Furthermore, data on animal-level factors not included in the prediction models in the current study, but that could be procured relatively cheaply to further improve the prediction of feed efficiency include animal activity measured using pedometers or accelerometers and body temperature measured using infrared tomography (Montanholi et al., 2009).

As EB and RFI are mathematically similar, the similarity in prediction accuracy for EB and RFI in the present study is not surprising. McParland et al. (2012) previously discussed why prediction accuracy of near unity is not expected for traits such as EB (and therefore RFI) because of the inherent errors within these gold-standard measures themselves. The ratio of the standard error of prediction to the standard deviation of the gold standard traits (**RPD**) is often used as an indicator of the usefulness of the prediction equation for practical purposes (Williams, 2007). Application of equations with an RPD less than 2.3 is not recommended, equations with an RPD between 2.4 and 3.0 can be considered for rough screening quality, and equations with an RPD greater than 3 can be considered for screening quality (Williams and Sobering, 1993). In the current study, the RPD of all prediction equations was less than 2 but must be interpreted in the context of the precision of the gold-standard variables. Contributing factors to the errors for all traits include measurement or recording errors (including gut fill), the smoothing of the liveweight and BCS data in the present study, and the applied regression coefficients to the energy sinks. McParland et al. (2012) showed differences in the ability of the MIR spectrum to predict energy intake across 2 research herds: the Langhill herd located at the Scottish Agricultural College (Edinburgh, UK) and the Teagasc Moorepark herd (Cork, Ireland). Although the research herds operated different management strategies and comprised different genetics, the main reason that intake prediction accuracy was greater in the Langhill herd compared with the Moorepark herd was likely due to differences in the recording of the energy intake data. Intake data in the Langhill herd were recorded automatically using Calan gates (American Calan Inc., Northwood, NH), whereas intake of the Teagasc Moorepark herd was recorded at grass using the *n*-alkane technique (averaged across a week).

Nonetheless, the stronger correlation between measured RFI and RFI calculated from MIR spectra-predicted EEI compared with the correlation between measured RFI and RFI predicted directly from the MIR spectrum suggests that, in practice, the RFI of animals should be calculated using MIR spectra-predicted EEI and not predicted directly from the MIR spectrum.

Of particular note, not directly related to the objective of this study, was the strong correlation between EB and RFI. Severity and duration of negative EB is well known to be associated with compromised reproductive performance (Beam and Butler, 1999) and more negative RFI is perceived to confer greater efficiency of production (Berry and Crowley, 2013). Breeding programs, therefore, that advocate selection for (lower) RFI in dairy and beef cows should do so with caution. Selection based on MIR spectra-predicted RFI would yield improved (true) efficiency at the expense of more negative (true) EB.

## CONCLUSIONS

The present study highlights the potential ability of routinely collected milk MIR spectra to predict energy intake and efficiency in lactating dairy cows either directly from the MIR spectrum, or derived using MIR spectra-predicted energy intake. Equations developed in this study are, however, specific to grass-fed cows. Populations with different production environments should develop their own equations or merge data with other production environments to develop robust equations. Moreover, generating more accurate gold-standard measures for the variables (e.g., large calorimetric studies) may aid in improving the prediction equations further, although such approaches have the disadvantage of removing some of the likely contributing factors to feed efficiency such as activity.

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