



Exploring the potential of ingestive behaviour, body measurements, thermal imaging, heart rate and blood pressure to predict dry matter intake in grazing dairy cows

B. Lahart^{1,2†}, E. Kennedy¹, M. Williams¹, M. Liddane¹, T.M. Boland², K. O'Sullivan³, F. Buckley¹

¹Teagasc, Animal and Grassland Research and Innovation Centre, Moorepark, Fermoy, Co. Cork, Ireland

²School of Agriculture and Food Science, University College Dublin, Belfield, Dublin 4, Ireland

³School of Mathematical Sciences, University College Cork, Cork, Ireland

Abstract

The objective of this study was to develop and validate models to predict dry matter intake (DMI) of grazing dairy cows using animal energy sinks and status traits in combination with traits related to grazing behaviour, body measurements, thermal imaging, heart rate and blood pressure. The dataset used to develop the models comprised 33 measurements from 113 Holstein-Friesian dairy cows. Multivariable regression models were constructed incorporating each independent variable into a benchmark model incorporating the energy sinks (milk yield [MY], fat %, protein % and body weight [BW]) and status traits (feeding treatment, parity and calving day of year). Of the 33 variables tested, 10 showed an association with DMI ($P < 0.25$). These variables were incorporated into a backward linear regression model. Variables were retained in this model if $P < 0.05$. Grazing bout duration and rumination mastication rate were retained in the final model. The inclusion of these variables in the model increased DMI prediction by 0.01 (coefficient of determination [R^2] = 0.85) compared to the benchmark model alone ($R^2 = 0.84$). The models were applied to data recorded on an independent herd of 51 dairy cows. The R^2 upon validation was 0.80 for the benchmark model and 0.79 for the model incorporating rumination mastication rate and grazing bout duration in combination with the benchmark variables. The separation of grazing bout duration and rumination mastication rate to predict DMI revealed rumination mastication rate slightly increases predictive accuracy upon external validation ($R^2 = 0.81$), whereas grazing bout duration did not ($R^2 = 0.78$). This suggests that grazing bout duration is not a robust trait for DMI prediction. Results from this study suggest that rumination mastication rate can slightly increase the accuracy of DMI prediction surpassing known energy sinks and status traits.

Keywords

Dairy cows • dry matter intake • grazing • modelling

Introduction

Feed efficiency is an important component of dairy systems (Connor, 2014) with genetic variation in feed efficiency of pasture-based dairy cows previously documented (Hurley *et al.*, 2017). A major obstacle to the direct inclusion of feed efficiency in dairy breeding programmes is routine access to large amounts of individual animal feed intake data from commercial dairy farms (Connor, 2014). The *n*-alkane technique (Mayes *et al.*, 1986) is the marker method commonly used to estimate dietary dry matter intake (DMI) in grazing dairy cows (McCarthy *et al.*, 2014; Coffey *et al.*, 2017). However, this method is expensive to employ and labour intensive; thus, it is unsuitable for the collection of DMI records at commercial farm level.

Known animal energy sinks such as body weight (BW) and milk production have previously been used as explanatory

variables in DMI prediction models for grazing dairy cows (Vazquez & Smith, 2000; Lahart *et al.*, 2019). The identification of alternative variables that explain additional variation on DMI may prove worthwhile, particularly if they have the potential to be recorded routinely on commercial farms. The advancement of precision agriculture technologies offers potential to develop novel methods of predicting the intake of grazing dairy cows. Nevertheless, the identification of traits that explain variation in DMI is required.

Grazing animals must autonomously harvest pasture to meet their energetic demands (Gregorini *et al.*, 2008). Therefore, differences in grazing behaviour amongst animals may explain some of the inter-animal variability in DMI. Prendiville *et al.* (2010) reported that grazing and rumination behaviour traits were associated with intake capacity in grazing dairy

[†]Corresponding author: B. Lahart

E-mail: ben.lahart@teagasc.ie

cows. Additionally, high intake capacity has been associated with larger gastrointestinal tracts per unit BW in dairy cows (Beecher *et al.*, 2014). Therefore, body measurements may offer potential to predict DMI.

An increase in the metabolic status of the animal such as heart rate is linked to increases in rates of feed digestion and the rate of heat production following eating (Brosh *et al.*, 1998). Heat produced due to maintenance requirements represents a large proportion of energy loss of animals (Montanholi *et al.*, 2009). Infrared thermography can identify variations in body temperature and has been reported as a predictor of DMI through detecting differences in temperature omitted through various body parts of growing Angus bulls (Montanholi *et al.*, 2009).

No study has explored the potential to predict DMI in grazing dairy cows using known animal energy sinks and status traits in combination with a comprehensive set of novel animal traits of this nature. The objective of this study was to (1) develop models to predict the individual animal DMI of grazing dairy cows within pasture-based systems using energy sinks and status traits in combination with novel animal traits relating to grazing behaviour, body measurements, thermal imaging, heart rate and blood pressure, and (2) validate the models on an independent group of animals.

Materials and methods

The animal procedures undertaken in this study were approved by the Teagasc Animal Ethics Committee and licenced by the Health Products Regulatory Authority in accordance with the protection of animals used for scientific purposes.

Initial measurements were conducted in 2015 on a herd of 135 Holstein-Friesian (HF) cows, at the Teagasc, Dairygold Research farm (Kilworth, Co. Cork, Ireland). These were part of the “Next Generation Herd” project described by O’Sullivan *et al.* (2019). This study comprised two genotypes of HF dairy cow divergent in Economic Breeding Index (EBI). The EBI, published by the Irish Cattle Breeding Federation (www.icbf.com), helps farmers identify the most profitable bulls and cows for breeding dairy herd replacements. The cows were all spring calving; the mean herd calving date was 21 February. There were 35 first, 32 second and 59 third lactation cows in the experiment. They were assigned to one of three pasture-based feed treatments – control (CTL), low grass allowance (LGA) and high concentrate (HC) treatments – which had a target post-grazing residual sward height of 4.5–5, 3.5–4.5 and 4.5–5 cm and a total concentrate allowance of 300, 300 and 1,200 kg/cow per lactation, respectively. The animals in all treatments were offered similar quality herbage throughout the grazing experimental period (O’Sullivan *et al.*, 2019). The experimental area was

a permanent grassland site containing a perennial ryegrass (*Lolium perenne* L.) dominated sward.

A second phase of measurements was conducted in 2017 to validate the findings from 2015. An independent herd comprising 51 HF dairy cows on the Curtin’s research farm at Teagasc Moorepark was used. The herd was a continuation of the study published by Coffey *et al.* (2017). The mean calving date of the herd was 22 February. The animals were managed under a rotational grazing system, similar to the CTL treatment of the initial experiment on a predominantly perennial ryegrass sward.

Animal measurements

DMI, milk production and BW

The DMI of each individual animal was estimated three times in 2015 at a herd average of 79, 107 and 205 d in milk (DIM). During the DMI estimation periods, the diet of the CTL and LGA feeding treatments comprised solely grazed grass, whereas the diet of the HC feeding treatment comprised grazed grass plus 3.6 kg of concentrate (DM). In 2017, DMI was estimated at a herd average 96 and 172 DIM. The diet of these cows comprised solely grazed grass. The DMI of each animal was estimated using the *n*-alkane technique as described by Mayes *et al.* (1986) and modified by Dillon (1993). This procedure involved dosing cows twice daily for a 12-d period using paper pellets each containing 500 mg C32-alkane (*n*-dotriacontane). On days 7–12, faeces were sampled prior to morning and evening milking. These samples were subsequently bulked (12 g/sample) and placed in a 40°C oven prior to being milled using a 1-mm sieve. Herbage representative of that grazed by the cows was sampled manually using a hand held electronic shears on days 6–11 of each DMI estimation period. The ratio of naturally occurring C33-alkane (tritriacontane) in the herbage to dosed C32-alkane was used to calculate DMI. The milk yield (MY) of each individual cow was recorded at each morning and evening milking during the DMI estimation periods using electronic milk meters (Dairymaster, Causeway, Co. Kerry, Ireland). The milk fat and protein constituents were determined by analysing milk sampled on successive evening and morning milkings once weekly, with a Milkoscan FT6000 (Foss Electric, Hilerød, Denmark). Body weight was recorded once during each DMI estimation period in both 2015 and 2017 using calibrated weighing scales (Dairymaster). The DMI, MY, milk constituents and BW records were subsequently averaged per year for 2015 and 2017, respectively.

Body measurements

Body measurements were recorded twice for all animals in 2015 at a herd average of 164 and 221 DIM. There were 12 measurements recorded: full chest girth, empty chest girth, full-body depth, heart girth, empty body depth, hip width, chest width, back length, head length, rump width, withers

height and muzzle circumference. The body measurements were carried out by two trained individuals using the methods outlined by Williams *et al.* (2019). The body measurement data were subsequently averaged per animal.

Grazing behaviour

The grazing behaviour of each cow was recorded once in 2015 at a herd average of 141 DIM using Institute of Grassland and Environmental Research (IGER) behaviour recorders that have been validated against visual observation (Rutter *et al.*, 1997) over a 24-h recording period. Recording commenced following morning milking once acclimatisation collars were detached and the IGER recorders attached. Acclimatisation collars had been attached 24 h previously to acclimatise the cows to the sensation of wearing recorders. Measurements were recorded on between one and five animals within each treatment per day. If a record was of a poor or unusable quality, an IGER recorder was attached to the animal for a further 24-h recording period. In total, one 24-h recording for each of the 126 cows was obtained in 2015. After the data were collected, files were downloaded and the jaw movements were analysed using the “Graze” analysis software (V.08, IGER, North Wyke, UK) (Rutter, 2000) from which outputs of the focal behaviour parameters such as grazing and rumination time, grazing and rumination bouts, grazing bites, rumination mastications and rumination boli were obtained. These data were subsequently used to extrapolate the parameters: grazing bout duration, rumination bout duration, bite rate, rumination mastication rate and rumination boli per rumination bout.

Grazing behaviour was recorded for each animal in 2017 during the DMI estimation periods using RumiwatchSystem (ITIN+HOCH, Laubibergstrasse, Liestal, Switzerland) over a 5-d period for each individual cow. The RumiwatchSystem was used in place of the IGER recording system used in 2015. The RumiwatchSystem has been validated against visual observation by Werner *et al.* (2018) to record grazing and rumination behaviour of dairy cows. The grazing behaviour data from the RumiWatch devices were downloaded to the RumiWatch Manager 2 (V.2.1.0.0, ITIN+HOCH, Laubibergstrasse, Liestal, Switzerland) and subsequently analysed using the RumiWatch Convertor software (V.0.7.3.36, ITIN+HOCH, Laubibergstrasse, Liestal, Switzerland), as described by Werner *et al.* (2018) to obtain similar focal behavioural parameters to the 2015 study. Data were subsequently categorised into similar behaviour parameters as the 2015 dataset.

Thermal imaging

Thermal images of the eyes, ribs, front and back hooves were captured twice in 2015 at a herd average of 181 and 212 DIM using an FLIR T430sc thermal camera (FLIR Systems Inc., Stockholm, Sweden) as described by Byrne *et al.* (2017). Images

were recorded following morning milking in a covered shed with no direct sunlight ensuring all cows were subjected to a similar ambient temperature at the time of measurement. Images of the left eye, ribs, fore and hind hooves were taken at 1.25, 2, 2 and 1 m from the cow, respectively. To ensure repeatability, three images of each body part were captured (Byrne *et al.*, 2017).

The thermal images were analysed and had temperatures extracted using Thermovision LabVIEW Toolkit 3.3 (FLIR Systems Inc.) as outlined by Byrne *et al.* (2017). The emissivity, ambient temperature, humidity, object distance and reflected temperature were adjusted in each image prior to analysis. Images of the eye and ribs were cropped to a pre-set area by one user. Eye images were cropped through drawing a rectangle around the outer edges of the cornea. The images of the ribs were cropped at the mid-point of the ribs. The average temperature of each body part was calculated from the images. Additionally, these values were subsequently averaged over the two measurement periods.

Heart rate

Heart rate was measured once in 2015 at a herd average of 137 DIM using a heart rate monitor (Polar V800, Polar Electro LTD, Warwick, UK). The heart rate monitor was fitted after morning milking for a 24-h period during which it continuously recorded the heart rate of each animal. The heart rate monitor was placed within a girth belt which was strapped around the chest of the animal. The heart rate monitor was placed on the chest of the animal at the location of the heart.

Blood pressure

Blood pressure was recorded twice in 2015 (111 and 138 DIM, respectively) using a blood pressure device (Suntech 247, SunTech, Morrisville, NC, USA). The blood pressure cuff was placed on the tail of each cow, 16 cm below the horizontal line of the hip bone. The blood pressure cuff was initially activated to acclimatise the animal to the device. Systolic and diastolic blood pressure was subsequently taken over four consecutive 10-min recording periods, with 2-min intervals between each period. All measurements were taken on all animals by the same operator.

Statistical analysis

Model development

All statistical analysis in this study was conducted using SAS v9.4 (SAS Institute Inc., Cary, NC, USA). Data collected in 2015 were used to develop prediction models for DMI (Table 1). Data were assessed for normality using SAS PROC UNIVARIATE. Outlying values were examined, and one animal was removed due to obvious errors in body measurement records. The relationship between DMI and each individual variable of interest was assessed to establish the strength and directions of the associations using PROC CORR and PROC GPLOT in SAS. After observing the correlations and scatter

Table 1: Phenotypic values of traits from the calibration dataset

Variable	Mean	±s.d.
Dry matter intake (kg)	17.5 ¹	1.96
Body weight (kg)	532	50.6
Milk yield (kg)	22.1	4.38
Fat %	4.2	0.51
Protein %	3.8	0.22
Parity	2.2	0.8
Calving day of year	52	16.4
Heart girth (cm)	192	7.0
Empty chest girth (cm)	237	14.5
Full chest girth (cm)	244	9.6
Empty body depth	125	15.7
Full-body depth	127	6.1
Back length (cm)	96	8.6
Hip width (cm)	42	3.0
Chest width (cm)	65	7.7
Rump width (cm)	26	2.9
Withers height (cm)	135	4.8
Head length (cm)	51	4.4
Muzzle circumference (cm)	45	2.1
Grazing mastications (<i>n</i>)	8,049	3,059.2
Grazing bites (<i>n</i>)	35,805	5,814.1
Grazing bite rate (<i>n</i> /min)	60	5.7
Grazing bouts (<i>n</i>)	9.4	2.35
Grazing time (min)	596	71.3
Grazing bout duration (min/bout)	67	18.1
Rumination bouts (<i>n</i>)	17.3	4.74
Rumination mastications (<i>n</i>)	30,324	7,016.1
Rumination boli (<i>n</i>)	499	115
Rumination time (min)	453	84.5
Rumination mastication rate (<i>n</i> /min)	66	5.3
Rumination bout duration (min/bout)	28	9.4
Rumination boli per bout (<i>n</i>)	30	9.5
Systolic blood pressure (mm Hg)	105	16.6
Diastolic blood pressure (mm Hg)	72	11.8
Heart rate (beats/min)	78	9.7
Thermal eye temperature (°C)	34	0.7
Thermal front left hoof temperature (°C)	26	1.3
Thermal front right hoof temperature (°C)	26	1.4
Thermal back left hoof temperature (°C)	28	1.3
Thermal back right hoof temperature (°C)	28	1.3
Thermal rib temperature (°C)	32	1.0

¹The average grass and concentrate dry matter intakes across the feeding treatments were 16.0 and 0 kg for the low grass allowance, 17.4 and 0 kg for the control plus 15.5 and 3.6 kg for the high-concentrate feeding treatment, respectively.

plots, heart girth was removed from the analysis as it had a correlation of >0.80 with the variable BW.

Initially, SAS PROC REG was used to develop a benchmark regression model for the prediction of DMI using known animal energy sinks (MY, fat %, protein % and BW) and status traits (parity [coded as dummy variables], feeding treatment [coded as dummy variables] and calving day of year). The effect of genotype (high EBI vs. average EBI) was investigated and in agreement with O'Sullivan *et al.* (2019) was non-significant ($P = 0.75$) and was omitted from the model. All other variables were retained in the regression model regardless of P value as they were deemed biologically plausible adjustment variables for DMI in grazing dairy cows (Kennedy *et al.*, 2003; McCarthy *et al.*, 2014; Lahart *et al.*, 2019). All seven variables were subsequently used as a benchmark model to which additional traits could be incorporated.

As an initial screening step, 33 separate multivariable linear regression models were constructed using SAS PROC REG. DMI was the dependent variable and the benchmark variables (energy sinks and status traits; forced into the model) along with one novel variable of interest were the independent variables. Only cows with values for all variables were included in the analysis ($n = 113$). Variables of interest $P < 0.25$ were retained for assimilation into a backward linear regression model. The backward linear regression model again included the adjustment variables (energy sinks and status traits) and the variables which passed the initial screening step. Variables remained in the final model where $P < 0.05$. The final models were repeated to incorporate the maximum number of cows with values for all novel traits significantly associated with DMI.

The coefficient of determination (R^2) was used to estimate the proportion of DMI explained by both the benchmark and the final models. Multi-collinearity was monitored in prediction models through the variance inflation factor (VIF) and the intercept-adjusted condition index. A VIF of >10 or an intercept-adjusted condition index >30 indicated multi-collinearity. Model residuals were standardised and normality checks were performed using SAS PROC UNIVARIATE.

Model validation

Data collected from the 2017 study were used to validate the benchmark and final models. Data were assessed for normality using SAS PROC MEANS and SAS PROC UNIVARIATE. Outlying values were examined, with no observations removed. A regression analysis was undertaken using SAS PROC REG to evaluate the prediction models. Criteria used to validate the predictive ability of the equation included the R^2 , the average bias, the slope between true and predicted DMI values, the root mean square error (RMSE) and relative prediction error (RPE) (Fuentes-Pila *et al.*, 1996; Derby, 2010; Zom *et al.*, 2012).

Results

The proportion of variation (R^2) in DMI explained by each individual trait in the benchmark model was as follows: MY = 0.66 ($P < 0.001$), milk fat % = 0.02 ($P = 0.18$), milk protein % = 0.04 ($P < 0.05$), BW = 0.38 ($P < 0.001$), feeding treatment = 0.35 ($P < 0.001$), parity = 0.33 ($P < 0.001$) and calving day of year = 0.01 ($P = 0.41$). When combined, MY, protein %, BW, parity and feeding treatment all remained significantly associated ($P < 0.05$) with DMI. The VIF and intercept-adjusted condition index indicated multi-collinearity was not present in the model.

There were 113 animals with records for all of the novel variables that were included in the analysis. The initial screening step revealed 10 of the 33 variables had a tendency towards association with DMI ($P < 0.25$): empty chest girth, rump width, hip width, grazing bout duration, number of rumination boli, rumination mastication rate, average front left hoof temperature, average front right hoof temperature, average back left hoof temperature and average back right hoof temperature. The subsequent step, backward linear regression, identified grazing bout duration, rumination mastication rate, rumination boli and right back hoof temperature, and remained in the model ($P < 0.05$) in addition to the benchmark variables. When the number of animals in the final model were maximised (animals with records for all the measurements retained in the final model; $n = 120$), rumination boli and right back hoof temperature became non-significant and were removed from the model. The R^2 of the final model was 0.85. The inclusion of the variables such as grazing bout duration and rumination mastication rate led to a 0.01 increase in the R^2 surpassing the benchmark model. Both the benchmark model (adjusted for animals in the final model; $n = 120$) and the final model are presented in Tables 2 and 3, respectively.

Validation

The models developed using the 2015 dataset were applied to the independent dataset generated in 2017 to predict DMI. The benchmark model predicted DMI of the independent dataset with an R^2 of 0.80 and an RMSE of 1.20 kg (Figure 1). The slope between true and predicted DMI was 1.68 (s.e. = 0.12; $b \neq 1$, $P < 0.05$). The prediction model resulted in an average bias of -0.20 and an RPE of 0.08. The residuals from this model were normally distributed. The final model incorporating grazing bout duration, rumination mastication rate and the benchmark variables predicted DMI of the independent dataset with an R^2 of 0.79 and an RMSE of 1.23 kg (Figure 2). The slope between true and predicted DMI was 1.65 (s.e. = 0.12; $b \neq 1$, $P < 0.05$). The prediction model

Table 2: Partial regression coefficients, s.e. and P values associated with the benchmark model to predict DMI (number of records = 120; coefficient of determination = 0.84)

Variables	Partial regression coefficient (95% CI)	s.e.	P value
Intercept	-0.38 (-4.78, 4.02)	2.22	0.865
Body weight (kg)	0.007 (0.003, 0.012)	0.002	0.002
Milk yield (kg)	0.29 (0.22, 0.36)	0.03	<0.001
Fat %	0.36 (-0.06, 0.79)	0.21	0.089
Protein %	1.60 (0.62, 2.57)	0.49	0.002
Feeding treatment	–	–	<0.001
LGA vs. CTL	-0.81 (-1.20, -0.42)	0.20	<0.001
HC vs. CTL	0.32 (-0.14, 0.78)	0.23	0.167
Parity	–	–	0.003
Parity 2 vs. 1	0.77 (0.27, 1.28)	0.25	0.003
Parity 3 vs. 1	0.32 (-0.32, 0.96)	0.32	0.320
Calving day of year	-0.003 (-0.013, 0.007)	0.005	0.542

CI = confidence interval; CTL = control; DMI = dry matter intake; HC = high concentrate; LGA = low grass allowance.

Table 3: Partial regression coefficients, s.e. and P values associated with the final model to predict DMI (number of records = 120; coefficient of determination = 0.85)

Variables	Partial regression coefficient (95% CI)	s.e.	P value
Intercept	-1.87 (-6.35, 2.62)	2.16	0.396
Body weight (kg)	0.007 (0.002, 0.011)	0.002	0.003
Milk yield (kg)	0.27 (0.20, 0.34)	0.03	<0.001
Fat %	0.38 (-0.03, 0.79)	0.21	0.068
Protein %	1.26 (0.30, 2.22)	0.49	0.011
Feeding treatment	–	–	<0.001
LGA vs. CTL	-0.75 (-1.13, -0.37)	0.19	<0.001
HC vs. CTL	0.64 (0.17, 1.12)	0.24	0.008
Parity	–	–	<0.001
Parity 2 vs. 1	1.00 (0.48, 1.51)	0.26	<0.001
Parity 3 vs. 1	0.65 (-0.01, 1.31)	0.33	0.052
Calving day of year	-0.006 (-0.016, 0.032)	0.005	0.193
Graze bout duration	0.012 (0.003, 0.021)	0.004	0.007
Rumination mastication rate	0.038 (0.006, 0.070)	0.016	0.021

CI = confidence interval; CTL = control; DMI = dry matter intake; HC = high concentrate; LGA = low grass allowance.

resulted in an average bias of -0.35 and an RPE of 0.09. The residuals from this model were normally distributed.

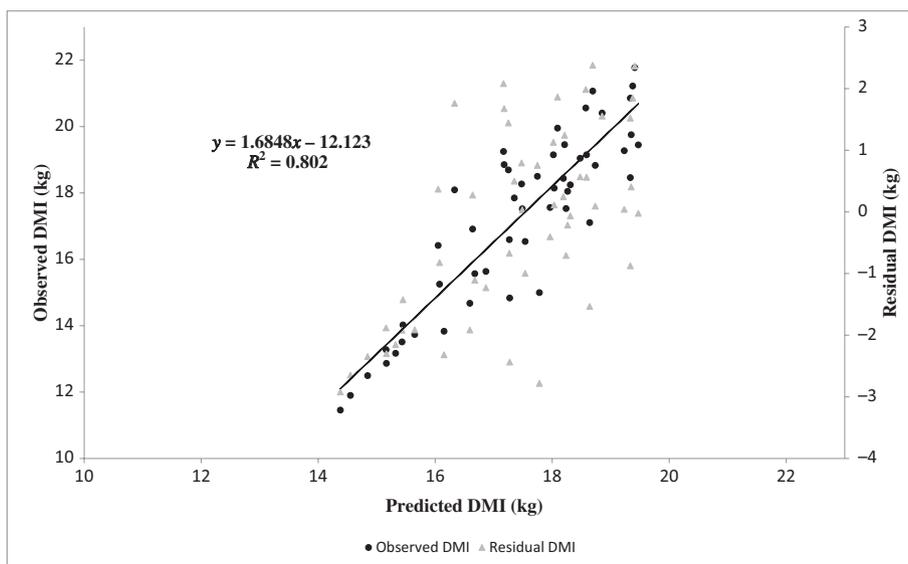


Figure 1. The relationship between the observed and predicted dry matter intake (DMI) for validation of the benchmark model.

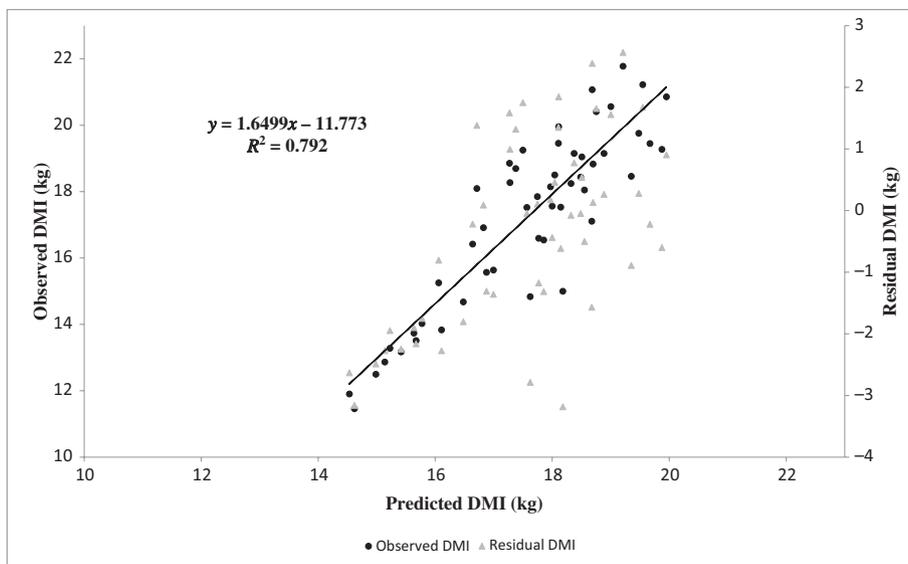


Figure 2. The relationship between observed and predicted dry matter intake (DMI) for validation of the final model.

Removing grazing bout duration and incorporating rumination mastication rate alone with the benchmark variables slightly increased the predictive accuracy ($R^2 = 0.81$; $RMSE = 1.16$) when compared to the benchmark model alone (Figure 3), while the slope between true and predicted DMI was 1.70 ($s.e. = 0.12$; $b \neq 1$, $P < 0.05$). The prediction model resulted in an average bias of -0.20 and an RPE of 0.08. In contrast,

grazing bout duration alone in combination with benchmark variables resulted in a lower predictive accuracy compared to the benchmark model with an R^2 of 0.78 and an RMSE of 1.26 (data not shown), while the slope between true and predicted DMI values was 1.64 ($s.e. = 0.12$; $b \neq 1$, $P < 0.05$). The prediction model resulted in an average bias of -0.34 and an RPE of 0.09.

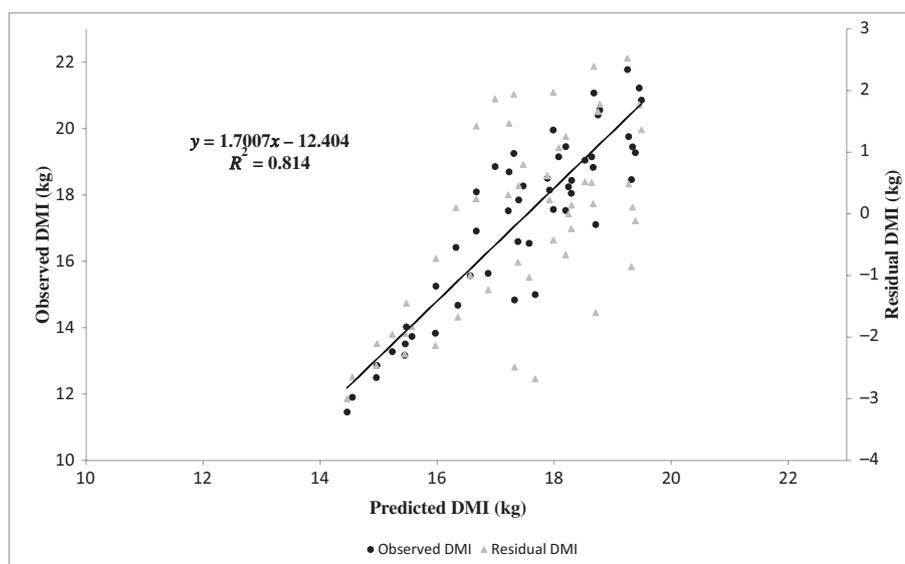


Figure 3. The relationship between observed and predicted dry matter intake (DMI) for validation of the model solely comprising rumination mastication rate in combination with the benchmark variables.

Discussion

There is considerable genetic variation in feed efficiency of dairy cows (Hurley *et al.*, 2017). However, with the exception of Australia (Pryce *et al.*, 2015) and the Netherlands (Manzanilla-Pech *et al.*, 2017), the inclusion of the trait in breeding indexes has been limited. At present, partial selection for gross feed efficiency exists within the EBI through simultaneous negative selection of BW and positive selection of milk production (Berry & Pryce, 2014). Nevertheless, O'Sullivan *et al.* (2019) did not observe superior feed efficiency (milk output/DMI) with high EBI dairy cows under grazing conditions. Thus, direct selection for feed efficiency may be required to improve the trait. The energy sinks have previously been reported as good predictors of DMI (Kennedy *et al.*, 2003; McCarthy *et al.*, 2014). Such data are readily available on commercial dairy farms. For instance, half the Irish dairy herd is routinely milk recorded (Roche *et al.*, 2017), while weighing scales are widely available for the purpose of weighing growing replacement animals. Nonetheless, identifying additional traits correlated with DMI may prove useful in explaining further variation in DMI and possibly unexplained variation in feed efficiency. The aim of this study was to assess if various novel animal traits could explain variation in DMI surpassing known animal energy sinks and status traits in grazing dairy cows.

Evaluation of prediction models

The R^2 of the models is similar to that of previous studies that developed DMI regression models for grazing dairy cows (Vazquez & Smith, 2000; Coleman *et al.*, 2010; Rombach *et al.*, 2019). Body weight was a significant predictor of DMI. Animals with a larger BW have a greater energetic requirement for the maintenance of metabolic functions (McDonald, 2002). Each 100 kg increase in BW was associated with a 0.70 kg increase in DMI. In agreement with Vazquez and Smith (2000) and Rombach *et al.* (2019), MY was a significant contributor to the prediction of DMI. This is not surprising, as dairy cows eat primarily to meet their energetic requirements for milk production (Holmes *et al.*, 2002). As a result, there is a strong genetic relationship between milk production and DMI in dairy cows (Manzanilla-Pech *et al.*, 2014), meaning high-yielding cows have a greater DMI than lower-yielding cows (Buckley *et al.*, 2000). In agreement with previous studies (Kennedy *et al.*, 2003; McCarthy *et al.*, 2014), increased parity was associated with increased DMI. Unsurprisingly, the feeding treatments were also strong predictors of DMI as O'Sullivan *et al.* (2019) reported significant differences across the feeding treatments for DMI.

Grazing bout duration was significantly associated with DMI in the final model. Rombach *et al.* (2019) reported that total eating time per day was positively associated with herbage DMI in a prediction model for grazing dairy cows. Within this study, each 1-min increase in grazing bout duration was associated with a 0.12 kg increase in DMI. Rumination

mastication rate was also positively associated with DMI. Animals ruminate to break down ingested feed into smaller particles for further digestion (Van Soest, 1994). In grazing animals, a greater number of rumination mastications could indicate a higher level of feed to be digested, hence a greater DMI. Interestingly, recent research has also reported positive associations between DMI and rumination mastication rate in grazing steers and heifers (Lahart *et al.*, 2020) as well as between DMI and the number of daily rumination mastications in lactating beef cows at pasture (Williams *et al.*, 2019). Collectively, this suggests that ruminating activity is a useful predictor of DMI in grazing cattle regardless of sex, breed and physiological state.

There was a marginal (+0.01) improvement in the R^2 of the model when grazing bout duration and rumination mastication rate were combined with the benchmark model. Clement *et al.* (2014) reported no improvement to DMI prediction of lactating dairy cows when rumination time was used in combination with a model comprising milk production, BW and DIM (Clement *et al.* 2014). Williams *et al.* (2019) observed a 0.24 increase in the R^2 for DMI prediction when traits pertaining to linear body scores and daily rumination mastications were combined with known energy sinks and status traits in a study with lactating beef cows. However, the energy sinks and status traits in their study explained significantly less variation in DMI ($R^2 = 0.45$) compared to the current study ($R^2 = 0.84$). This may be partly related to the fact that Williams *et al.* (2019) estimated MY, whereas MY was directly recorded in the current study.

None of the other novel traits were significantly associated with DMI. It should be acknowledged that traits such as heart rate, blood pressure, body measurements and thermal imaging were not recorded concurrent with DMI estimation. The labour-intensive nature of the measurements prevented the simultaneous recording of DMI with these measurements. It is unclear if this affected associations with DMI. Although traits such as thermography are relatively repeatable (Byrne *et al.*, 2017).

Validation of prediction models

The RPE of the models upon validation (0.08–0.09) indicates satisfactory DMI prediction (Fuentes-Pila *et al.*, 1996). It is difficult to achieve fitting statistics close to unity in prediction models for grazing animals due to the use of marker techniques which estimate DMI, not directly measuring the trait. The alkane technique as used in this study can be poor at estimating within animal variation in DMI due to differences in selective grazing, digestion and recovery rates between animals (Dove *et al.*, 2000). However, the fitting statistics in the current study did not seem to be impaired by the technique and were greater than fitting statistics reported by O'Neill *et al.* (2013), who developed herd average grass DMI prediction models for grazing dairy cows using animal and

sward variables. It should be highlighted that the DMI data in the present study were the mean of multiple estimates which likely led to the improved fitting statistics. Nevertheless, there was a large slope when actual DMI was regressed on predicted DMI, signifying that the model overestimated low DMI values and underestimated high DMI values. The incorporation of more records into the prediction model may alleviate this.

Grazing bout duration and rumination mastication rate in combination were not useful at increasing the predictability of DMI surpassing the energy sinks upon external validation. Grazing behaviour was recorded using IGER recording devices in the 2015 calibration study, whereas it was recorded using RumiWatch recording devices in the 2017 validation study. Nonetheless, previous research by both Rutter *et al.* (1997) and Werner *et al.* (2018) has shown the two devices to be as accurate as visual observation. When the traits were separated, rumination mastication rate did prove to improve the predictability of DMI, whereas grazing bout duration did not. There was a considerable difference in grazing bout duration in the 2015 calibration study (67 min/bout) compared to the 2017 validation study (86 min/bout; data not shown), whereas there was little difference between rumination mastication rate in 2015 (66 chews/min) compared with 2017 (65 chews/min; data not shown). The discrepancies in grazing bout duration between the calibration and validation studies may be due to differences in concentrate supplementation (one-third of the animals in the calibration study received concentrate supplementation), environmental conditions and photoperiod, all of which can influence the trait (Gregorini *et al.*, 2006; O'Sullivan *et al.*, 2019).

Application of prediction models to improve feed efficiency

Alternative methods of selecting for feed efficiency have been proposed; both De Haas *et al.* (2015) and Pryce *et al.* (2015) have demonstrated that genomic selection can be used as a method of selecting for feed intake and feed efficiency. These genomic breeding values have been derived predominantly from feed intake records of animals in indoor environments. However, genotype by environmental interactions need to be considered when predicting breeding values for grazing dairy cows, as genetic correlations between the two feeding systems tend to be low (Berry *et al.*, 2014). Thus, actual phenotypes of grazing dairy cows may be required to make substantial genetic gain for feed efficiency. The energy sinks have been proposed as suitable predictors of DMI (Manzanilla-Pech *et al.*, 2017). However, further detail is warranted, specifically to explain true variation in net feed efficiency (residual feed intake) between animals (De Haas *et al.*, 2015). Rumination mastication rate in the current study explained additional variation in the feed intake complex surpassing the energy sinks upon external validation. Albeit the improvement was

small, however, the trait possibly represents true variation in net feed efficiency between animals. Data on feeding and rumination time are routinely recorded on commercial dairy farms using accelerometer devices for heat detection and health monitoring. Given the rapid pace at which these technologies are developing, it may be possible to record rumination intensity in the future. The decision to invest in collecting such information relies on the marginal response in genetic gain in the overall breeding index from measuring such data (Berry & Crowley, 2013). Ideally, these traits should be genetically correlated with DMI but should also explain genetic variation in DMI surpassing the energy sinks (De Haas *et al.*, 2015). It is unclear if the marginal phenotypic variation in the feed intake complex explained by rumination mastication rate within the current study would translate through to genetic gain in feed efficiency. Further work assessing the genetic associations amongst feeding behaviour traits such as rumination mastication rate and feed intake and efficiency in grazing cattle may be beneficial.

Conclusion

The current study aimed to evaluate a range of animal traits with regard to their ability to explain additional variation in DMI in grazing dairy cows over and above known animal energy sinks and status traits. Despite the comprehensive measurements undertaken, rumination mastication rate was the only trait identified that could increase the accuracy of DMI prediction upon external validation. If routinely available, the trait may be a useful contributor to breeding for improvements in feed efficiency of grazing dairy cows in the future.

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