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Evaluation of nitrogen excretion equations for ryegrass pasture-fed dairy cows

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ABSTRACT

Accurate and precise estimates of nitrogen (**N**) excretion in faeces and urine of dairy cattle may provide direct tools to improve N management and thus, to mitigate environmental pollution from dairy production. Empirical equations of N excretion have been evaluated for indoor dairy cattle but there is no evaluation for cows fed high proportions of fresh forage. Therefore, the objective of the current study was to evaluate N excretion equations with a unique data set of zero-grazing experiments. Through literature searches, 89 predictive equations were identified from 13 studies. An independent data set was developed from seven zero-grazing experiments with, in total, 55 dairy Holstein-Friesian cows. Models' performance was evaluated with statistics derived from a mixed-effect model and a simple regression analysis model. Squared sample correlation coefficients were used as indicators of precision and based on either the best linear unbiased predictions ($\boldsymbol{R^2_{BLUP}}$) or model-predicted estimates ($\boldsymbol{R^2_{MDP}}$) derived from the mixed model and simple regression analysis, respectively. The slope ($\boldsymbol{\beta_0}$), the intercept ($\boldsymbol{\beta_1}$) and the root mean square prediction error (**RMSPE**$_{\mathbf{m\%}}$) were calculated with the mixed-effect model and used to assess accuracy. The root mean square prediction error (**RMSPE**$_{\mathbf{sr\%}}$) and the decomposition of the mean square prediction error were calculated with the simple regression analysis and were used to estimate the error due to central tendency (mean bias), regression (systematic bias), and random variation. Concordance correlation coefficient (**CCC**) were also calculated with the simple regression analysis model and were used to simultaneously assess accuracy and precision. Considering both analysis models, results suggested that urinary N excretion (**UN**; R^2_{MDP} = 0.76, R^2_{BLUP} = 0.89, RMSPE$_{m\%}$ = 17.2, CCC = 0.82), total manure N excretion (**ManN**; R^2_{MDP} = 0.83, R^2_{BLUP} = 0.90, RMSPE$_{m\%}$ = 11.0, CCC = 0.84) and N apparently digested (**NAD**; R^2_{MDP} = 0.97, R^2_{BLUP} = 0.97, RMSPE$_{m\%}$ = 5.3, CCC = 0.95) were closely related to N intake. Milk N secretion was better predicted using milk yield as a single independent variable (MilkN; R^2_{MDP} = 0.77, R^2_{BLUP} = 0.97, RMSPE$_{m\%}$ = 6.0, CCC = 0.74). Additionally, DM intake was a good predictor of UN and ManN and dietary CP concentration of UN and ManN. Consequently, results suggest that several evaluated empirical equations can be used to make accurate and precise predictions concerning N excretion from dairy cows being fed on fresh forage.

Implications

The extensive use of nitrogen in dairy cattle operations results in considerable nitrogen pollution of the environment. Good nutritional management can have a positive impact on nitrogen utilization and help reduce nitrogen pollution. However, direct tools to improve nitrogen management are needed. Accurate and precise predictions of nitrogen excretion from different dietary conditions may provide an on-farm tool to improve nitrogen utilization at farm or animal levels. In this study we evaluated 89 empirical equations and we detected those that are more precise and accurate for cattle fed high amounts of fresh forage.

Introduction

Environmental pollution from livestock production raised concerns worldwide and became a challenge for the scientific community to study further and provide practical solutions towards its mitigation. Nitrogen (**N**) is an essential element for life and is a key factor for the proper functioning of the ecosystems, controlling species composition and generally securing nature's diversity

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(Steinfeld et al., 2006). However, its extensive use to achieve high productivity of land and animals ranks agriculture as the main contributor to the circulation of anthropogenic N in ecosystems (Sutton et al., 2011). This circulation of surplus N leads to multiple negative effects on both atmospheric and aquatic environments and is characterised as the N-cascade phenomenon (Galloway et al., 2003; 2004). Dairy farming systems are major contributors to the surplus N in the environment because they tend to have a low N use efficiency (Foskolos and Moorby, 2018) and pasture-based systems are heavily based on livestock grazing (Gonzalez-Mejia et al., 2018) that limits the implementation of modern technologies for improved manure management (Soteriades et al., 2018).

An increase in N use efficiency provides an effective strategy to improve N management and mitigate environmental pollution caused by dairy cattle farming (Calsamiglia et al., 2010; Foskolos and Moorby, 2018). Improved N use efficiency may be achieved either at the farm or animal level. From a biological point of view, improved efficiency of N use at the animal level can be achieved through better nutritional management (Huhtanen and Hristov, 2009; Broderick, 2018), and mathematical models can provide a powerful tool to achieve it (Van Amburgh et al., 2019). For this purpose, accurate and precise prediction models for N excretion and other important parameters involved in N use efficiency are necessary. Several recent studies evaluated empirical equations to predict N excretion in cattle (Johnson et al., 2016; Angelidis et al., 2019) but such analysis has not been carried out for cattle fed high dietary proportions of fresh forage. Moreover, the data sets that developed these equations included either a low proportion of forage (46–68 and 42–57% on a DM basis for Huhtanen et al. (2008) and Nousiainen et al. (2004), respectively) or some diets that were 100% forage (Castillo et al., 2000). In high-forage diets, cows consume large amounts of rapidly degradable N and depending on the availability of rapidly degradable energy and rumen balance of protein and energy, these might lead to poor feed N utilisation (Hoekstra et al., 2007). However, it is unknown whether empirical equations are capable of predicting feed N utilisation accurately in high-forage diets. Considering that pasture-based systems are the principal method of dairy farming in many areas of the world, the objective of the current study was to evaluate the accuracy and precision of empirical equations used to predict N excretion of dairy cows fed diets containing high proportions of fresh forage.

Material and methods

Predictive equations of nitrogen excretion and utilisation

A literature search was conducted using Google Scholar and Scopus for the selection of the predictive equations evaluated in the current study with the following keywords: N excretion or output or balance, dairy cows or cattle and equations. This search led to 2 080 and 248 studies on Google Scholar and Scopus, respectively. However, we selected 13 studies that developed 267 predictive equations, which not only included parameters that can regularly be measured at a farm level such as BW and milk yield but also included feed intake data and milk chemical composition parameters. The selection criteria for the chosen studies included (a) studies that developed empirical equations mostly regarding the prediction of N excretion, (b) publication in peer-reviewed journals (except for a single conference paper), (c) equations from these studies were applicable to lactating dairy cows. Although we found 267 empirical equations from the published articles, we selected 89 that could be evaluated using our own data set of input variables. For example, we did not measure milk urea nitrogen, and, therefore, all equations required milk urea nitrogen as an input were excluded. A full list of these studies is given in Supplementary Material S1, along with the description and abbreviation of the equations in the Supplementary Tables. Particularly: (1) total DM solid excretion (**DME**; kg/d), total manure excretion (**TME**; kg/d) and urine volume (l/d) in Supplementary Table S1; (2) urinary N excretion (**UN**) (g/d) in Supplementary Table S2; (3) faecal N excretion (**FN;** g/d) in Supplementary Table S3, (4) total manure N excretion (**ManN**; g/d) and ratio of UN to ManN (**UN/ManN**; g/kg) in Supplementary Table S4; and (5) milk N secretion (**MilkN**; g/d)**;** milk N use efficiency (**MNE**; g/kg), N apparently digested (g/d) and N balance (g/d) in Supplementary Table S5.

Evaluation data set

Data for this evaluation were derived from seven zero-grazing experiments that included individual records of 55 Holstein-Friesian lactating dairy cows. Two of these experiments have been published (Miller et al., 2001; Moorby et al., 2006), while remainder have not (J.M. Moorby, unpublished data). This data set was developed for two reasons: (i) data from these experiments have not been used to derive the above-mentioned predictive equations, resulting in an independent evaluation data set, and (ii) in N balance measurements, total collections of faeces and urine were performed. All work with animals used in the seven experiments referred to above was conducted in accordance with the requirements of the UK Animals (Scientific Procedures) Act 1 986 and with approval of the Institute of Grassland and Environmental Research's Local Ethical Review Committee or Aberystwyth University's Animal Welfare and Ethical Review Body.

Animal trials and sample collection

The duration of each experiment was three weeks. The first two weeks were used for animal adaptation to the diets, and the last one for sampling. These experiments were part of an examination of the use of novel grasses for animal production and efficiency. At each experiment, eight Holstein-Friesian lactating dairy cows were randomly allocated to one of two dietary treatments with the exception of the fifth experiment in which seven cows were used. In four of the experiments, diets were inclusive of a standard dairy compound concentrate fed at a rate of 4 kg/day (210 g CP/kg, 13.5 MJ metabolisable energy/kg), which was offered in two equal proportions at each milking (approximately at 0800 h and 1400 h). Forages were offered fresh on an *ad libitum* basis. However, in the fifth (unpublished) experiment, dairy cows in early lactation were used as part of zero-grazing work carried out during the early grazing season. Four cows were allocated on the first dietary treatment and were fed perennial ryegrass, cv. AberDart, and four were allocated in the second treatment and were fed perennial ryegrass cv. Fennema. Grass was fed *ad libitum* with an inclusion of a dairy concentrate at a rate of 3 kg/day. In the first of the following two unpublished experiments, four early-to-mid-lactation multiparous Holstein-Friesian dairy cows were fed a control ryegrass variety (cv. Premium) and four were fed the perennial ryegrass variety Aberwolf. In the second unpublished experiment, four early-to-mid-lactation multiparous Holstein-Friesian dairy cows were fed the same control ryegrass variety (cv. Premium), while four were fed fresh white clover (on average 4.2 kg DM daily). Diets were offered at a constant 80:20 forage:concentrate ratio (offered on a DM basis). The amount of concentrate offered to each animal was calculated using a three-day rolling average of forage intake. A detailed description of the cattle housing, forage management, measurements and sample analysis was provided in Milleret al. (2001) and is described in Supplementary Material S2.

Measurements were carried out the same way at each experiment. Body weight was recorded before morning feeding on the first day of the sampling week. Individual animal milk yields and feed intake were recorded daily. Feed intake was measured as DM offered minus DM refused. During each experiment, forage samples were collected for further analysis to obtain information concerning their nutritional composition. During the measurement week, forage and concentrate samples were collected from each of the feed containers before being offered to the cows (morning and afternoon). Samples were composited to generate one sample per treatment per day and were stored frozen before freeze-drying and grinding for analysis. Individual milk samples from four consecutive milkings were taken during the N balance measurement period and five millilitres of milk was collected and preserved with a LacTab milk preservative tablet (Thompson and Capper, Runcorn, Cheshire, UK) for further analysis.

Nitrogen balance

At the final week of each experiment, and for a period of six days, an individual total collection of faeces, urine and milk was carried out for each cow for the determination of N partitioning and whole tract diet digestibility. Urine and faeces were separated using externally applied separators (Moorby et al., 2000). Furthermore, N balance, MNE and ManN were estimated as follows:

$$\text{N balance} = \text{N intake} - (\text{UN} + \text{FN} + \text{MilkN})$$

$$\text{MNE} = \text{MilkN}/\text{N intake}$$

$$\text{ManN} = \text{UN} + \text{FN}$$

Statistical analysis

Statistical analysis was conducted using JMPv13 (SAS Institute Inc.). The current study implemented two different statistical models to provide a more robust evaluation as suggested by Van Amburghet al. (2015) and Higgset al. (2012). For the data sets, a mixed-effect model using the restricted maximum likelihood procedure was used to analyse the data as proposed by St-Pierre (2001):

$$Y_{ij} = \beta_0 + \beta_1 X_{ij} + s_i + b_{1i} X_{ij} + \varepsilon_{ij}$$

where Y_{ij} = the outcome for the dependent variable Y at observation j of the continuous variable X in experiment i, β_0 = the overall intercept across all experiments, s_i = the random effect of experiment i, β_1 = the overall slope of Y on X across all experiments, b_{1i} = the random effect of experiment i on the slope of Y on X, X_{ij} = the model-predicted data associated with observation j of the variable X in experiment i, and ε_{ij} = random variation.

From the mixed-effect model, the reported slope and intercept ($\boldsymbol{\beta_0}$ and $\boldsymbol{\beta_1}$, respectively) were used to indicate accuracy, and the squared sample correlation coefficient based on the best linear unbiased predictions ($\boldsymbol{R^2_{BLUP}}$) was used as an indicator of precision. Root mean square prediction error expressed as a percentage of the observed mean (**$\text{RMSPE}_{\text{m\%}}$**) and variance component (%) were calculated using the mixed-effect model and used to assess accuracy. The $\text{RMSPE}_{\text{m\%}}$ values were calculated by dividing the RMSPE values by the observed mean (Patra, 2015).

In addition, simple regression analysis was performed using a model of the form:

$$Y_i = \beta'_0 + \beta'_1 X_i + e_i$$

where Y_i = the expected outcome for the dependent variable Y at observation i of the continuous variable X, β'_0 = the intercept, β'_1 = the slope, X_i = the model-predicted value of the continuous variable X, and e_i = error.

Following the methodology of Tedeschi (2006), we calculated several statistics from the simple linear regression model. The squared sample correlation coefficient based on model-predicted estimates ($\boldsymbol{R^2_{MDP}}$) was used as indicator of precision. The root mean square prediction error was expressed as a percentage of the observed mean (**$\text{RMSPE}_{\text{sr\%}}$**). The decomposition of the mean square prediction error (**MSPE**) was used to give an estimation of the error due to central tendency (mean bias), regression (systematic bias), and random variation (Tedeschi, 2006). Concordance correlation coefficient (**CCC**) were used to simultaneously assess accuracy and precision. The concordance correlation coefficient (Lin and I-Kuei, 1989) can vary from zero to one, with a value of one indicating that no deviation from the $Y = X$ line has occurred. In the current study, accuracy was treated as the ability of the model to predict known values well, and precision was treated as the ability of the model to predict similar values consistently (Tedeschi, 2006).

Results

Data set description

The evaluation data set was developed with the objectives to collect input variables necessary to run the equations, and then evaluate the output variables of interest (Table 1). The CP concentration of forages used in the experiments ranged from 113 to 202 g/kg DM, and that of supplemented concentrates from 193 to 220 g/kg DM. Similarly, water soluble carbohydrates ranged from 126 to 247 g/kg DM in forages and that of supplemented concentrates from 57 to 171 g/kg DM. Taking into account that DM intake was on average 17.6 ± 1.8 kg/d, the CP intake ranged from 1.6 to 3.7 kg/d. This experimental set-up resulted in a wide range of output variables of interest. Indeed, UN, FN and MNE were on average 137.7 g/d ± 67.6, 141.5 ± 24.0, and 286.2 ± 72.5 g/kg, respectively.

Evaluation of excreta predictive equations

For the prediction of DME, we found four equations that used DM intake or milk yield as inputs and one that used milk yield, BW and milk true protein (Supplementary Table S1). Regarding the mixed model, two equations that used DM intake as an input (DME 1 and DME 3) resulted in higher R^2_{BLUP} and $\text{RMSPE}_{\text{m\%}}$ values compared with the equations that used milk yield as an input variable. In the same direction, considering the simple regression analysis, those that used milk yield as an input variable (DME 2, 4 and 5 equations) predicted DME poorly with R^2_{MDP} close to zero, while those that used DM intake as an input resulted in better predictions (Table 2). Indeed, the third equation for the prediction of DME demonstrated the best performance but the low CCC value suggests poor accuracy and precision. A similar situation was observed for TME, since the four evaluated equations had the same inputs as DME equations. The overall performance was poor whether mixed model or simple linear regression analysis was applied, with even negative R^2_{BLUP} and low CCC values. Based on mixed model, the best performance was observed for the TME 1 and TME 4 equations, demonstrating the highest R^2_{BLUP} and the lower $\text{RMSPE}_{\text{m\%}}$. Similarly, with the simple linear regression analysis, TME 1 and TME 4 demonstrated the highest R^2_{MDP}, and the lower $\text{RMSPE}_{\text{sr\%}}$, but even in this case, the accuracy and precision were poor (CCC = 0.12 and 0.09, respectively). Furthermore, we detected two equations to predict urine volume excretion using either BW or a combination of milk composition (e.g., milk fat concentration), feed (dietary CP concentration) and animal (BW) characteristics. Using the mixed model, both equations were performed with high precision (R^2_{BLUP} = 0.82

Table 1
Descriptive statistics of ryegrass pasture-fed dairy cows and production characteristics.

Item	n	Mean	SD	Minimum	Maximum
Diet composition					
Forages					
OM, g/kg DM		923	9.3	905	943
CP, g/kg DM		152	26.2	113	202
NDF, g/kg DM		496	43.0	449	587
ADF, g/kg DM		269	38.3	239	331
WSC, g/kg DM		184	38.0	126	247
ME, MJ/kg DM		11.6	0.6	10.2	12.6
Concentrate					
OM, g/kg DM		907	12.9	894	923
CP, g/kg DM		203	10.7	193	220
NDF, g/kg DM		299	98.0	148	380
ADF, g/kg DM		164	46.0	64	195
WSC, g/kg		87	39.0	57	171
Animal Data					
BW, kg	55	616	50	522	773
MBW, kg	55	124	7.6	109	146
Intakes					
DMI, kg/d	55	17.6	1.8	12.2	20.5
OMI, kg/d	55	16.2	1.6	11.2	19.1
NI, g/d	55	442.8	108.1	256.2	667.0
MEI, MJ/MBW	55	1.6	0.2	1.0	2.0
Production Inputs					
MY, kg/d	54	23.9	8.2	9.6	40.0
MTP, g/g MY/d	55	0.031	0.004	0.02	0.004
MCP, g/kg MY/d	46	33.7	5.6	23.2	43.5
MF, g/g MY/d	55	0.1	0.5	0.02	3.7
N excretion					
UN, g/d	55	137.7	67.6	53.6	300.2
FN, g/d	55	141.5	24.0	87.4	232.4
MilkN, g/d	54	123.8	37.4	63.1	199.9
ManN, g/d	55	246.2	99.8	81.3	483.1
UN/ManN, g/kg	55	519.4	131.7	310.6	813.6
Other Parameters					
Proportion of dietary forage, g/kg DM	55	806.3	31.5	712.2	868.2
MNE, g/kg NI	54	286	72.5	122	430
DM solid excretion, kg/d	55	5.9	0.5	4.4	6.6
Total manure excretion, kg/d	55	75.7	10.6	55.5	99.6
Urine volume excretion, l/d	55	28.3	6.6	17.2	42.9
N apparently digested, g/d	55	301.3	92.2	152.1	505.1
Nitrogen balance, g/d	54	39.9	41.7	120.4	−48.6

Abbreviations: OM = organic matter; ME = metabolisable energy; WSC = water soluble carbohydrates; MBW = metabolic BW; DMI = DM intake; OMI = organic matter intake; MEI = metabolisable energy intake; MY = milk yield; MTP = milk true protein; MCP = milk CP; MF = milk fat; NI = nitrogen intake; UN = urinary nitrogen excretion; FN = faecal nitrogen excretion, MilkN = milk nitrogen secretion; ManN = total manure nitrogen excretion; UN/ManN = proportion of UN to ManN; MNE = milk nitrogen use efficiency (MilkN/NI).

Table 2
Equation adequacy statistics for the prediction of ryegrass pasture-fed dairy cows' DM (solids) excretion (DME, kg/d), total manure excretion (TME, kg/d) and Urine volume excretion, (UVE, l/d).

	Mixed model							Simple regression					
					Variance component (%)						MSPE partitioned (%)		
Equation	β_0	β_1	R^2_{BLUP}	$RMSPE_{m\%}$	Exp	Sl	Res	R^2_{MDP}	CCC	$RMSPE_{sr\%}$	U^M	U^S	U^R
DME 1	1.8	0.6	0.53	5.1	27.7	0.0	72.3	0.36	0.16	24.0	88.3	4.2	7.5
DME 2	5.8	0.0	0.24	6.8	0.0	54.6	45.4	0.00	0.01	29.4	71.3	21.3	7.4
DME 3	2.0	0.6	0.53	5.1	27.7	0.0	72.3	0.36	0.18	16.9	86.2	5.2	8.6
DME 4	5.8	0.0	0.24	6.8	0.0	59.2	40.8	0.00	0.00	29.4	80.9	13.4	5.7
DME 5	5.0	0.1	0.17	6.8	14.5	0.0	85.5	0.03	0.03	29.4	81.7	12.7	5.6
TME 1	−9.3	1.5	0.51	10.7	26.4	0.2	73.4	0.28	0.12	26.4	78.6	5.8	24.6
TME 2	27.8	0.9	−0.08	12.9	43.8	0.0	56.2	0.00	0.01	27.3	72.3	0.1	27.6
TME 3	23.3	0.9	−0.08	12.9	43.9	0.0	56.1	0.00	0.01	24.8	66.3	0.1	33.6
TME 4	−9.0	1.5	0.48	10.7	26.5	0.2	73.3	0.24	0.09	28.9	81.2	1.2	17.6
UVE 1	20.5	0.4	0.82	10.3	80.2	0.0	19.8	0.45	0.22	31.6	66.7	3.5	29.8
UVE 2	9.9	0.8	0.84	9.9	82.5	0.0	17.5	0.13	0.05	30.8	49.3	0.0	50.7

Abbreviations: β_0 = the overall intercept across all experiments, β_1 = the overall slope of Y on X across all experiments, R^2_{BLUP} = correlation coefficient based on BLUP, $RMSPE_{m\%}$ = root mean square prediction error expressed as % of the observed mean, percentage of variance related to the effect of study (Exp), differences in slope (Sl) between study (study × prediction) and random variation (Res); R^2_{MDP} = correlation coefficient based on model predictions, CCC = concordance correlation coefficient, $RMSPE_{sr\%}$ = root mean square prediction error expressed as % of the observed mean, MSPE = mean square prediction error, U^M = percentage of error due to mean bias, U^S = percentage of error due to systematic bias, and U^R = percentage of error due to random variation ($U^M + U^S + U^R = 100$).

and 0.84 for UVE 1 and UVE 2, respectively) and moderate accuracy ($RMSPE_{m\%}$ = 10.3 ad 9.9 for UVE 1 and UVE 2, respectively). Furthermore, for both equations, the variance component (%) of the mixed model suggested that variation was attributed mostly to the random effect of the study (=80.2 and 82.5%, respectively). However, the linear regression analysis suggested that UVE 1 was better performed than UVE 2 even though the accuracy and precision were low (CCC = 0.22 and 0.05 for UVE 1 and UVE 2, respectively).

Evaluation of nitrogen excretion predictive equations

A total of 58 equations that predict UN, FN and ManN were identified. Specifically, our research identified 20, 13 and 25 equations to predict UN (Supplementary Table S2), FN (Supplementary Table S3) and ManN (Supplementary Table S4), respectively. We identified several equations that performed well and others that severely underperformed. Equations UN 2, UN 5, UN 6, and UN 20 were performed with the highest precision and accuracy as suggested by the high R^2_{BLUP} and the low $RMSPE_{m\%}$ of the mixed model analysis. Among them, UN 20 had the lowest $RMSPE_{m\%}$ (=15.3). Similar results were observed for these equations when evaluated with the simple regression analysis, resulting in higher R^2_{MPD}, CCC and lower $RMSPE_{sr\%}$ values compared with the rest UN equations (Table 3). In this direction, UN 2 had the lowest $RMSPE_{sr\%}$ and the decomposition of the MSPE suggested that 52 % was attributed to the error due to random variation, while mean bias (U^M = 26.4%) was the main element to explain bias. A visual interpretation of the UN 2 equation is presented in Supplementary Fig. S1. Furthermore, several equations that underperformed were identified, such as equations UN 8, UN 9, and UN 11 that resulted in the lowest CCC values (Table 3).

The equations that predict FN included DM intake, organic matter intake, N intake, UN and MilkN as independent variables. However, few equations were performed accurately and precisely. Mixed model analysis indicated that the R^2_{BLUP} ranged from 0.58 to 0.60, and FN equations had very similar $RMSPE_{m\%}$ values (ranged from 11.2 to 11.5%). Furthermore, the variance component analysis of the mixed model indicated that variation was attributed mostly to the residuals, except for FN 6 and FN 13. Regarding the simple regression, the R^2_{MDP} ranged from 0.19 to 0.53 (Table 3), while FN 2 had the highest CCC (=0.72), and equations FN 2, FN 8, and FN 9 had the lowest $RMSPE_{sr\%}$. However, some equations underperformed with both statistical analyses, such as FN 6 (a visual interpretation of FN 6 is depicted in Supplementary Fig. S2).

An alternative to UN and FN individual predictions is the prediction of ManN. In this case, we identified 25 equations in the literature, and several of them performed with high accuracy and precision for pasture-based cows. Several independent variables were used in these equations, such as BW, dietary CP concentration, DM intake, N intake, metabolisable energy, milk yield, proportion of dietary forage. However, the equations that use N intake as an independent variable showed a remarkable prediction ability

Table 3
Equation adequacy statistics for the prediction of ryegrass pasture-fed dairy cows' urinary nitrogen excretion (UN, g/d) and faecal nitrogen excretion (FN, g/d).

	Mixed model							Simple regression					
					Variance component (%)						MSPE partitioned (%)		
Equation	β_0	β_1	R^2_{BLUP}	$RMSPE_{m\%}$	Exp	Sl	Res	R^2_{MDP}	CCC	$RMSPE_{sr\%}$	U^M	U^S	U^R
UN 1	−26.5	0.8	0.86	18.5	44.3	0.0	55.7	0.73	0.54	53.8	76.8	2.1	21.1
UN 2	30.6	0.7	0.89	17.2	67.9	0.0	32.1	0.76	0.82	29.3	26.4	20.6	52.0
UN 3	7.3	0.8	0.86	17.9	44.7	0.0	55.3	0.76	0.79	32.9	39.4	19.2	41.0
UN 4	25.1	0.7	0.88	17.6	61.7	0.0	38.3	0.71	0.75	38.6	39.4	19.2	41.0
UN 5	16.7	0.7	0.89	16.8	44.6	0.0	55.4	0.81	0.80	35.1	42.6	23.4	34.0
UN 6	16.4	0.7	0.89	16.9	44.9	0.0	55.1	0.81	0.81	34.2	42.8	21.7	35.5
UN 7	21.5	0.7	0.88	17.1	35.1	0.0	64.9	0.84	0.80	38.2	47.2	25.9	26.9
UN 8	150.4	−0.1	0.85	20.2	83.9	0.0	16.1	0.01	0.06	92.2	11.6	61.7	26.7
UN 9	24.2	0.6	0.87	18.1	49.5	0.0	50.5	0.75	0.48	30.0	56.0	9.6	34.4
UN 10	−59.4	1.8	0.88	17.6	61.7	0.0	38.3	0.71	0.65	31.8	1.1	15.3	83.6
UN 11	−59.2	1.5	0.88	17.6	61.7	0.0	38.3	0.71	0.51	39.0	28.2	18.0	53.8
UN 12	17.7	0.7	0.87	18.0	50.4	0.0	49.6	0.74	0.78	34.6	38.8	16.0	45.2
UN 13	−81.6	1.2	0.88	17.6	61.7	0.0	38.3	0.71	0.53	46.5	65.0	1.2	33.8
UN 14	−41.6	1.0	0.90	16.6	52.0	0.0	48.0	0.80	0.71	36.5	62.9	0.0	37.1
UN 15	44.6	0.5	0.92	20.0	92.7	0.0	7.3	0.77	0.70	44.0	32.5	53.2	14.3
UN 16	48.5	0.5	0.92	20.0	92.7	0.0	7.3	0.77	0.71	43.6	30.2	55.7	14.1
UN 17	50.2	0.5	0.91	15.8	88.3	0.0	11.7	0.66	0.66	47.2	43.5	25.7	30.8
UN 18	68.2	0.4	0.90	16.4	87.8	0.0	12.2	0.55	0.66	46.5	23.0	37.4	39.6
UN 19	−74.2	1.3	0.88	17.6	61.7	0.0	38.3	0.71	0.65	34.1	24.2	7.7	68.1
UN 20	47.2	0.6	0.92	15.3	87.6	0.0	12.4	0.76	0.81	30.7	27.2	28.6	44.2
FN 1	33.2	0.7	0.58	11.4	8.1	0.0	91.9	0.53	0.71	12.5	4.5	10.8	84.7
FN 2	31.3	0.8	0.56	11.5	11.5	0.0	88.5	0.53	0.72	12.5	5.4	10.4	84.2
FN 3	26.7	1.0	0.58	11.3	26.9	0.0	73.1	0.47	0.48	21.7	54.3	14.7	31.0
FN 4	41.0	0.6	0.58	11.4	22.3	0.0	77.7	0.49	0.45	23.0	62.8	10.4	26.8
FN 5	49.8	0.5	0.58	11.3	36.0	0.0	64.0	0.41	0.41	23.0	58.1	11.3	30.7
FN 6	71.5	0.5	0.60	11.2	53.0	0.0	47.0	0.19	0.41	16.5	4.9	15.8	79.3
FN 7	49.8	0.6	0.58	11.3	36.0	0.0	64.0	0.41	0.63	13.5	0.3	14.7	85.0
FN 8	−2.8	1.0	0.58	11.4	8.1	0.0	91.9	0.53	0.66	12.3	11.9	0.1	88.0
FN 9	−9.2	1.0	0.58	11.4	8.1	0.0	91.9	0.53	0.67	11.8	3.1	0.2	96.7
FN 10	56.6	0.5	0.58	11.4	8.1	0.0	91.9	0.53	0.48	24.8	56.2	22.2	21.6
FN 11	54.3	0.6	0.58	11.4	8.1	0.0	91.9	0.53	0.62	17.9	31.4	27.7	40.9
FN 12	66.6	0.6	0.58	11.4	8.1	0.0	91.9	0.53	0.68	15.6	10.8	36.0	53.2
FN 13	66.7	0.5	0.60	11.2	53.0	0.0	47.0	0.19	0.31	20.8	37.4	11.1	51.5

Abbreviations: β_0 = the overall intercept across all experiments, β_1 = the overall slope of Y on X across all experiments, R^2_{BLUP} = correlation coefficient based on BLUP, $RMSPE_{m\%}$ = root mean square prediction error expressed as % of the observed mean, percentage of variance related to the effect of study (Exp), differences in slope (Sl) between study (study × prediction) and random variation (Res); R^2_{MDP} = correlation coefficient based on model predictions, CCC = concordance correlation coefficient, $RMSPE_{sr\%}$ = root mean square prediction error expressed as % of the observed mean, MSPE = mean square prediction error, U^M = percentage of error due to mean bias, U^S = percentage of error due to systematic bias, and U^R = percentage of error due to random variation ($U^M + U^S + U^R = 100$).

(Table 4). For example, using mixed model methodology, ManN 3, ManN 9 and ManN 11 resulted in high R^2_{BLUP} (0.90, 0.88 and 0.88, respectively). Moreover, ManN 9 had the closest to 0 β_0 (=2.2) and the closest to 1 β_1 (=0.9). In this direction, simple regression analysis for the same equations resulted in high R^2_{MDP} values (0.83, 0.79 and 0.79, respectively), while Man 11 had the lowest $RMSPE_{sr\%}$ (=16.4% of the mean). The decomposition of MSPE for Man 11 suggested that 78% was attributed to the random variation, and 11.6% to mean bias. Considering the CCC values, these equations predicted accurately and precisely ManN (CCC = 0.84, 0.81 and 0.87 for ManN 3, ManN 9 and ManN 11, respectively; Table 4).

The UN/ManN equations were performed in a similar way, probably because all of them used dietary CP concentration as the independent variable. The mixed model analysis resulted in high precision (R^2_{BLUP} ranged from 0.82 to 0.84), and the linear regression analysis in moderate precision (R^2_{MDP} ranged from 0.60 to 0.61). Regarding the results from simple linear regression, these three equations demonstrated similar precision and accuracy as assessed by the CCC values that ranged from 0.56 to 0.62. Among them, UN/ManN 3 equation had the lowest $RMSPE_{sr\%}$, and the MSPE partitioned (%) suggested that mean bias and random variation were the main elements of error.

Evaluation of animal-related and efficiency predictive equations

Equations to predict MilkN included DM intake, N intake, metabolisable energy intake and milk yield as independent variables and the results are presented in Table 5. All equations had high R^2_{BLUP} values (0.84–0.97). A single equation (MilkN 1) by Banninket al. (1999) is the only one that used milk yield as a single independent variable and had the best predictive power compared with the rest listed, resulting in high precision and accuracy when evaluated with the mixed model (R^2_{BLUP} = 0.97, β_0 = −20.4, β_1 = 1.1, $RMSPE_{m\%}$ = 6.0% of the mean). This performance was also supported by the results of the simple regression that resulted in high R^2_{MDP} (=0.77) and the highest CCC value (=0.74). Moreover, the decomposition of the MSPE suggested that 62.7% was attributed to the percentage of error due to random variation, and 36.5% to mean bias.

For MNE prediction equations, dietary CP concentration, CP intake, metabolisable energy, N intake and MilkN were the predictor inputs. Based on mixed model, MNE 3 equation resulted in the highest R^2_{BLUP} value (=0.82). However, simple regression suggested that it did not predict MNE with accuracy and precision as assessed by the low CCC value (=0.30). The other equations evaluated also did not fit our data well, thus resulting in low prediction ability for all. The prediction of N apparently digested was very well estimated when N intake was the independent variable in both mixed model (R^2_{BLUP} = 0.97) and simple linear regression analyses (R^2_{MDP} = 0.97 and CCC = 0.95), while the equation that predicts nitrogen balance resulted in low R^2_{BLUP} (=0.42), and in high β_0 (=469.1) using mixed model analysis. Similarly, considering simple linear regression, it resulted in very low R^2_{MDP} (=0.12) and CCC value (=0.07; Table 5), suggesting that it is inaccurate.

Discussion

In modern dairy farming, it is necessary to incorporate effective tools to improve nutritional management, with a focus on reducing both the economic cost and environmental impact of production. To help with this, fast, reliable and accurate predictions of N excretion and N use efficiency are required. Several studies focused on

Table 4
Equation adequacy statistics for the prediction of ryegrass pasture-fed dairy cows' total manure nitrogen excretion (ManN, g/d) and proportion of Urinary nitrogen to ManN (UN/ManN, g/kg).

	Mixed model							Simple regression					
					Variance component (%)						MSPE partitioned (%)		
Equation	β_0	β_1	R^2_{BLUP}	$RMSPE_{m\%}$	Exp	Sl	Res	R^2_{MDP}	CCC	$RMSPE_{sr\%}$	U^M	U^S	U^R
ManN 1	373.8	−0.2	0.86	13.6	85.8	0.0	14.2	0.00	0.01	56.1	55.4	9.5	35.1
ManN 2	65.1	0.7	0.88	11.3	50.1	0.0	49.9	0.79	0.82	22.4	36.0	24.4	39.6
ManN 3	61.0	0.7	0.90	11.0	46.2	0.0	53.8	0.83	0.84	21.1	35.9	27.3	36.8
ManN 4	47.2	0.7	0.90	10.9	44.3	0.0	55.7	0.83	0.79	24.9	54.5	18.4	27.1
ManN 5	−8.00	0.9	0.88	11.3	50.1	0.0	49.9	0.79	0.81	18.6	30.3	0.5	69.2
ManN 6	48.4	0.7	0.89	11.5	43.3	0.0	56.7	0.81	0.83	20.5	39.5	18.2	42.3
ManN 7	431.6	−0.4	0.86	13.6	85.8	0.0	14.2	0.00	0.01	65.2	69.4	3.9	26.7
ManN 8	−107.0	1.1	0.85	12.1	51.4	0.0	48.6	0.68	0.50	34.6	67.8	0.5	31.7
ManN 9	2.2	0.9	0.88	11.3	50.1	0.0	49.9	0.79	0.81	19.7	38.8	2.0	59.2
ManN 10	−23.9	0.9	0.90	10.9	42.2	0.0	57.8	0.84	0.69	29.7	78.2	1.8	20.0
ManN 11	37.9	0.8	0.88	11.3	50.1	0.0	49.9	0.79	0.87	16.4	11.6	10.4	78.0
ManN 12	28.0	0.8	0.87	12.1	41.3	0.0	58.7	0.78	0.80	20.0	48.8	8.2	43.0
ManN 13	27.0	0.8	0.87	12.3	38.1	0.0	61.9	0.78	0.73	25.9	59.3	5.6	35.1
ManN 14	22.4	0.8	0.87	12.1	41.3	0.0	58.7	0.78	0.77	23.2	51.1	6.4	42.5
ManN 15	26.5	0.8	0.87	11.9	37.8	0.0	62.2	0.81	0.82	20.0	45.2	8.7	46.1
ManN 16	217.1	0.2	0.85	13.6	85.3	0.0	14.7	0.07	0.08	55.5	61.8	4.8	33.4
ManN 17	325.3	−0.1	0.86	13.6	85.8	0.0	14.2	0.00	0.02	57.4	40.4	27.3	32.3
ManN 18	228.3	0.1	0.83	13.9	86.5	0.0	13.5	0.02	0.05	73.0	68.5	10.4	21.1
ManN 19	227.2	0.2	0.83	13.9	86.4	0.0	13.6	0.02	0.06	55.3	51.3	11.8	36.9
ManN 20	121.5	0.5	0.88	12.3	77.9	0.0	22.1	0.59	0.70	26.6	21.9	26.6	51.5
ManN 21	16.4	0.8	0.88	11.3	50.1	0.0	49.9	0.79	0.78	23.1	51.4	5.7	42.9
ManN 22	20.5	0.8	0.88	11.3	50.1	0.0	49.9	0.79	0.79	22.8	49.7	6.7	43.6
ManN 23	7.5	0.8	0.89	11.3	48.1	0.0	51.9	0.80	0.76	24.7	58.8	4.0	37.2
ManN 24	31.6	0.8	0.90	10.8	51.0	0.0	49.0	0.83	0.84	19.9	41.6	10.3	48.1
ManN 25	28.0	0.8	0.81	10.8	48.4	0.0	51.6	0.83	0.82	21.3	50.1	7.2	42.7
UN/ManN 1	−97.5	1.1	0.84	8.3	67.1	0.0	32.9	0.61	0.56	17.1	50.6	0.4	49.0
UN/ManN 2	−28.4	0.9	0.82	8.6	55.3	0.0	44.7	0.60	0.62	16.8	49.5	0.3	50.2
UN/ManN 3	−48.5	1.0	0.84	8.3	67.1	0.0	32.9	0.61	0.59	16.8	49.6	0.0	50.4

Abbreviations: β_0 = the overall intercept across all experiments, β_1 = the overall slope of Y on X across all experiments, R^2_{BLUP} = correlation coefficient based on BLUP, $RMSPE_{m\%}$ = root mean square prediction error expressed as % of the observed mean, percentage of variance related to the effect of study (Exp), differences in slope (Sl) between study (study × prediction) and random variation (Res); R^2_{MDP} = correlation coefficient based on model predictions, CCC = concordance correlation coefficient, $RMSPE_{sr\%}$ = root mean square prediction error expressed as % of the observed mean, MSPE = mean square prediction error, U^M = percentage of error due to mean bias, U^S = percentage of error due to systematic bias, and U^R = percentage of error due to random variation ($U^M + U^S + U^R = 100$).

Table 5
Equation adequacy statistics for the prediction of ryegrass pasture-fed dairy cows' milk nitrogen secretion (MilkN, g/d); milk nitrogen use efficiency (MNE, g/kg); nitrogen apparently digested (NAD, g/d) and NB = nitrogen balance (NB, g/d).

	Mixed model							Simple regression					
					Variance component (%)						MSPE partitioned (%)		
Equation	β_0	β_1	R^2_{BLUP}	$RMSPE_{m\%}$	Exp	Sl	Res	R^2_{MDP}	CCC	$RMSPE_{sr\%}$	U^M	U^S	U^R
MilkN 1	−20.4	1.1	0.97	6.0	86.5	0.0	13.4	0.77	0.74	27.7	36.5	0.8	62.7
MilkN 2	88.1	0.4	0.84	13.1	71.6	0.0	28.3	0.05	0.46	27.7	12.9	18.3	68.8
MilkN 3	15.6	0.9	0.85	12.1	75.1	0.0	24.8	0.29	0.41	26.1	6.0	0.1	93.9
MilkN 4	22.0	0.8	0.85	12.8	75.1	0.0	24.8	0.29	0.45	25.3	0.2	1.2	98.6
MilkN 5	21.4	0.9	0.85	12.8	75.1	0.0	24.8	0.29	0.38	27.8	15.9	0.1	84.0
MilkN 6	45.2	0.6	0.84	13.0	78.0	0.0	22.0	0.31	0.47	25.7	3.1	5.6	91.3
MilkN 7	30.1	0.8	0.85	12.8	75.1	0.0	24.8	0.29	0.37	26.7	0.1	1.0	98.9
MilkN 8	24.8	0.8	0.86	12.1	81.6	0.0	18.4	0.21	0.43	16.9	9.7	3.2	87.1
MNE 1	−158.0	1.6	0.74	12.6	80.3	0.0	19.7	0.10	0.19	23.9	0.1	4.3	95.6
MNE 2	−88.0	0.8	0.80	11.8	79.7	0.0	20.3	0.14	0.27	23.4	1.2	0.9	97.9
MNE 3	−53.5	1.1	0.82	11.5	79.7	0.0	20.3	0.14	0.30	23.9	3.6	0.6	95.8
MNE 4	−53.5	1.2	0.80	12.0	76.5	0.0	23.5	0.15	0.24	23.3	1.5	0.4	98.1
MNE 5	−16.4	1.0	0.78	12.1	75.0	0.0	25.0	0.16	0.27	23.5	3.1	0.0	96.9
MNE 6	−75.3	1.2	0.76	12.1	80.6	0.0	19.4	0.16	0.27	23.1	0.7	1.1	98.2
MNE 7	397.0	−0.6	0.80	11.8	79.7	0.0	20.3	0.14	0.20	53.3	35.3	47.4	17.3
NAD 1	−23.6	1.1	0.97	5.3	8.1	0.0	91.9	0.97	0.95	8.6	41.8	17.9	40.3
NB 1	469.1	2.6	0.42	71.8	76.7	0.0	23.3	0.12	0.07	204.1	69.8	9.4	20.8

Abbreviations: β_0 = the overall intercept across all experiments, β_1 = the overall slope of *Y* on *X* across all experiments, R^2_{BLUP} = correlation coefficient based on BLUP, $RMSPE_{m\%}$ = root mean square prediction error expressed as % of the observed mean, percentage of variance related to the effect of study (Exp), differences in slope (Sl) between study (study × prediction) and random variation (Res); R^2_{MDP} = correlation coefficient based on model predictions, CCC = concordance correlation coefficient, $RMSPE_{sr\%}$ = root mean square prediction error expressed as % of the observed mean, MSPE = mean square prediction error, U^M = percentage of error due to mean bias, U^S = percentage of error due to systematic bias, and U^R = percentage of error due to random variation ($U^M + U^S + U^R = 100$).

predicting N excretion from dairy cows fed silage-based diets and/or total mixed ratios (Huhtanen et al., 2008; Higgs et al., 2012; Johnson et al., 2016) but, to our knowledge, this is the first study that has evaluated empirical equations with a unique data set of cows fed diets containing high proportions of fresh forages. Moreover, our data set included seven experiments in which total collection of faeces and urine took place. In these seven experiments, forages of different chemical composition were fed creating a wide range of input and output values. Forage concentrations of CP and water soluble carbohydrates in particular varied widely because the forages used in some work were developed to express higher water soluble carbohydrate concentrations, the so-called high sugar grasses, and this occurs at the expense of either CP or NDF concentrations (Soteriades et al., 2018). In the current experiments, cows were supplemented with a flat-rate of concentrate feeds, which is a standard procedure in some pasture-based systems (Hills et al., 2015; Moorby et al., 2016) when animals receive concentrates during milking. The mean N intake of 442.8 ± 108.1 g/d in our data set is within the range found in other evaluation studies with housed dairy cattle (Higgs et al., 2012; Johnson et al., 2016) even though it falls within the lower limits mainly due to the restricted concentrate intake. Taking into account that MNE improvement is the required goal of improved N management (Foskolos and Moorby, 2018) with dairy cattle, the current data set included a wide range of observed MNE, including situations of severe N use inefficiency (min MNE = 122 g/kg N intake) and high N use efficiency (max MNE = 430 g/kg N intake).

Model evaluation is an important step not only to assess model adequacy but also to highlight important gaps and limitations of scientific knowledge (Tedeschi et al., 2014). On the one hand, linear regression analysis has been used to investigate the relationship between parameters and to assess the degree of precision and accuracy of models or equations (Tedeschi, 2006). However, simple linear regression may lead to misinterpretation of biological relationships (St-Pierre, 2001), assuming the absence of error in the independent variable, which is not true in this case. On the other hand, mixed-effect models have been suggested and implemented in several model evaluations (Higgs et al., 2012; Van Amburgh et al., 2015). However, the use of mixed model methodology also has limitations because it reduces the applicability of a model or an equation to different environments (experiments or farms). Therefore, in the current work, both statistical models were used to provide a holistic evaluation of the selected equations. Certainly, this resulted in differences in evaluation statistics of empirical equations depending on the model used.

Prediction of dairy cattle excreta is important because this estimation is required to improve on-farm N management not only for housed cattle but also for pasture-based systems. In several pasture-based systems, cows have restricted grazing time (Kennedy et al., 2009) meaning that indoor facilities have to be designed accordingly. Moreover, in assessing the environmental impact of pasture-based systems, calculations for manure distribution on pasture are needed (Styles et al., 2018). The current study included several equations to predict dairy cattle excreta outputs, but our results suggested that none of them are accurate and precise in DME, TME and urine volume excretion for dairy cows fed large amounts of fresh forage. Available empirical equations rely on simple inputs, mainly milk yield and DM intake (ASAE, 2005; Nennich et al., 2005). It has been reported that DME and TME are closely related to DM intake (Nennich et al., 2005). However, the evaluated equations performed poorly with our data set. Similarly, two equations evaluated to predict urine volume excretion performed poorly. It was evident that for cows fed high amounts of fresh forage, milk yield is not a reliable input variable. Although the current data set included milk yield values ranging from 9.6 to 40.0 kg/d, this did not result in a wide range of actual DME and TME. For example, DME predicted with the second listed equation ranged from 6.0 to 8.9 kg/d, and at the same time, the observed DME ranged from 4.4 to 6.6 kg/d, thus predictions overestimated outputs of excreta significantly. This probably occurred because of the high intercept of the equation (5.073) that limited the sensitivity of responses in different milk yield values, suggesting the need to refine prediction equations to include cows fed high amounts of forage. However, at present, if an estimation of

DME and TME is necessary, equations that use DM intake as an input would be most suitable.

Prediction of the excretion of N in faeces and urine is a key factor for whole-farm N management, which is why many previous studies have attempted to do this. The empirical equations used to predict UN utilise BW, CP, DM intake, N intake, metabolisable energy, metabolisable energy intake, milk yield and dietary CP concentration as independent variables (Castillo et al., 2000; Huhtanen et al., 2008; Kebreab et al., 2010; Spek et al., 2013). It has been reported that there is a curvilinear relationship between UN and N intake (Castillo et al., 2000; Kebreab et al., 2001) and that dietary CP concentration is considered a good predictor of UN (Spek et al., 2013). Generally, an increase in dietary N intake results in an increase in excreted N and in a reduction in MNE (Dijkstra et al., 2011). Indeed, in our study, several equations that use N intake as the independent variable predicted UN accurately and precisely (e.g., UN 2, UN 5, and UN 6) and similar results were also observed on equations that include dietary CP concentration as a predictor input, as indicated by the UN 20 model that performed with the lowest $RMSPE_{m\%}$ value (=15.3% of the mean). Furthermore, Kebreabet al. (2010) reported that there is a clear interaction between N in feeds and energy that affects the relative partitioning of feed N between faeces, urine, and milk. Thus, to allow for possible reductions in feed N concentrations, feed energy density and availability also need to be taken into consideration. This interaction between N and energy was observed in equation UN 6, which was one of the three best predictive equations evaluated in this study.

It was previously reported that FN is closely related to DM intake and there is a strong linear relationship between FN and N intakes (Castillo et al., 2000; Kebreab et al., 2001). Moreover, when N intake and DM intake were both included in the equations, the prediction ability improved (Huhtanen et al., 2008). However, our results suggested that even though FN is more accurately predicted when N intake is the single predictor variable (FN 2; $RMSPE_{sr\%}$ = 12.5), the mixed model analysis suggested that FN equations performed much alike as indicated by the $RMSPE_{m\%}$ values, while the overall precision was relatively low. Van Amburghet al. (2015) demonstrated the insensitivity of FN to increasing levels of N intake when energy was the first limiting factor of the diet in lactating dairy cows. In this case, protein digestibility was proposed to be the driving force in N excreted in faeces, because N losses may result from microbial degradation of dietary protein in the rumen (Tamminga et al., 1994). However, besides the low practical value of such equation due to the difficulty to collect this measurement on a farm level, we found no equation that uses N digestibility as a predictor variable. Interestingly, we identified several equations that were good at predicting ManN (UN + FN). Even though several predictor variables, such as BW, milk yield, dietary CP concentration, DM intake and N intake (Yan et al., 2006), were used as predictor inputs in empirical equations to predict ManN, those that performed best for pasture-fed lactating cows used N intake as a single predictor. Yanet al. (2006) reported that equations that use BW and milk yield, as single independent variables or combined, had low prediction ability and large standard error, but when N intake was included, the prediction ability improved. In our evaluation, such equations also resulted in considerable error but by including N intake, prediction accuracy was greatly improved, even leading to the lowest $RMSPE_{m\%}$ values. Moreover, Huhtanenet al. (2008) determined ManN as N intake minus MilkN, assuming zero N balance (i.e. accretion or loss of body N), which is valid for mature high yielding dairy cows at certain times of lactation, i.e. mid-lactation. As for the prediction of UN/ManN, Huhtanenet al. (2008) reported a close interaction between UN/ManN and dietary CP concentration, which can also be observed from our results.

In the literature, MilkN and N intakes appear to have a linear relationship (Kebreab et al., 2001), while Castilloet al. (2000) reported this relationship to be strong at levels below 400 g of N intake. Regardless of this, in our study, MilkN was better predicted by the equation (MilkN 1) of Banninket al. (1999) using milk yield as a single predictor. Moreover, all equations either using milk yield or N intake, DM intake, and metabolisable energy intake resulted in high accuracy and precision with the mixed model statistical analysis suggesting that the biological relationship between Milk N and milk yield or nutrient intake is strong. Indeed, Danielet al. (2016) demonstrated that Milk N is a function of both milk protein and net energy supply. However, when linear regression analysis was used, only milk yield equations were accurate and precise, but this does not mean that nutrient intake does not affect Milk N. As Bachet al. (2008) demonstrated, there are several non-dietary management factors that affect milk yield and Milk N. Concerning the prediction of MNE, Huhtanenet al. (2008) reported that dietary CP concentration and rumen protein balance were the best predictors. Also, N intakes exceeding 400 g/d have been found to reduce N use efficiency for milk N production (Castillo et al., 2000). However, in our study, dietary CP concentration indeed resulted in high R^2_{BLUP} values, but the evaluated equations do not result in any useful prediction ability.

We also evaluated a single equation for the estimation of N apparently digested, and considering both mixed model and simple regression analyses, it resulted in high R^2_{BLUP}, low $RMSPE_{m\%}$ and high CCC values which indicate both prediction accuracy and precision. Finally, nitrogen balance is estimated as N intake minus total N excreted/secreted and, in our study, this was predicted by N intake in a single equation. This equation resulted in low R^2_{BLUP}, and $RMSPE_{m\%}$ in the mixed model analysis, also to low R^2_{MDP}, and with a poor CCC, in the simple regression model.

A list of the best performing empirical equations and their reference studies can be found in Table 6, and a visual interpretation in Fig. 1. Based on our results, easily measured inputs such as BW and milk yield, when used alone or combined, led to a significant

Table 6
List of the best performing evaluated equations of ryegrass pasture-fed dairy cows' predictions.

Item	Predicting	Equation	Study
1	UN 2	UN = 30.4 × $e^{0.0036 \times NI}$	Castillo et al. (2000)
2	UN 5	UN = 27 + 0.844 × NI − 13 × DMI	Huhtanen et al. (2008)
3	UN 6	UN = 104 + 0.855 × NI − 13.2 × DMI − 6.8 × ME	Huhtanen et al. (2008)
4	FN 2	If NI = 200 − 400, FN = 0.38 × NI, or NI > 400, FN = 0.23 × NI + 41	Castillo et al. (2000)
5	ManN 3	ManN = −1 + 0.937 × NI − 5.7 × DMI	Huhtanen et al. (2008)
6	ManN 11	(Forage Type: Fresh Grass) ManN = 0.711 × NI − 21	Yan et al. (2006)
7	MilkN 1	MilkN = 46.62 + 3.681 × MY	Bannink et al. (1999)
8	N apparently digested 1	NAD = −42.5 + 0.738 × NI	Bannink et al. (1999)

Abbreviations: UN = urinary nitrogen excretion (g/d); NI = nitrogen intake (g/d); DMI = DM intake (kg/d); ME = metabolisable energy (Mj/kg DM); FN = faecal nitrogen excretion (g/d); ManN = manure nitrogen excretion (g/d); MilkN = milk nitrogen secretion (g/d); MY = milk yield (kg/d); NAD = nitrogen apparently digested (g/d).

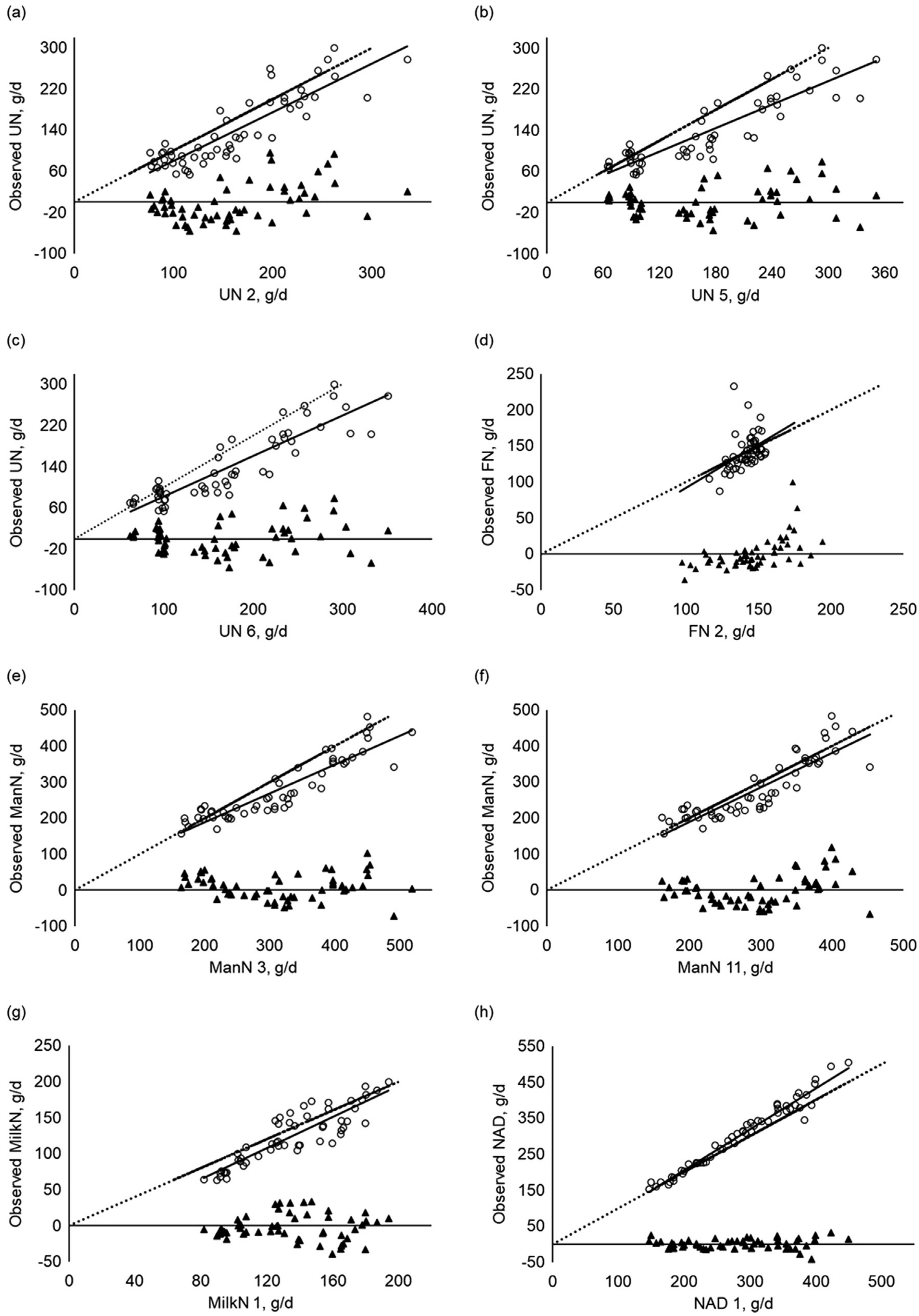

Fig. 1. Demonstration of the best performing predictive equations for ryegrass pasture-fed dairy cows. Observed versus prediction (○) for (a) urinary nitrogen excretion (UN) 2 (Castilloet al., 2000). (b) UN 5 (Huhtanenet al., 2008). (c) UN 6 (Huhtanenet al., 2008). (d) Faecal nitrogen excretion (FN) 2 (Huhtanenet al., 2008). (e) Manure nitrogen excretion (ManN) 3 (Huhtanenet al., 2008). (f) ManN 11 (Yanet al., 2006). (g) Milk nitrogen secretion (MilkN) 1 (Banninket al., 1999) and (h) nitrogen apparently digested (NAD) 1 (Banninket al., 1999). Mixed model residuals are also shown on the graphs (▲). The dotted line from the origin is meant to indicate unity.

inaccuracy in the prediction of UN and ManN. Therefore, these equations are impractical to make accurate and precise estimations in pasture-based systems and for reliable conclusions, nutrient intake and more complex records should be considered.

Conclusion

Our results suggest that UN is closely associated with N intake, DM intake and metabolisable energy, MilkN with milk yield, and N apparently digested with N intake. Furthermore, the prediction accuracy and precision of ManN equations that use BW and milk yield as inputs greatly improve when N intake or dietary CP concentration are included. Consequently, easily measured inputs (BW and milk yield), alone or combined, cannot be considered for use in an effective N management strategy in pasture-based systems, since they cannot be used to accurately and precisely estimate N excretion variables. However, several empirical equations, evaluated in this study, can be used as direct tools to better manage N excretion from dairy cattle fed with high amounts of fresh forage daily.

Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.animal.2021.100311.

Ethics approval

All work with animals used in the seven experiments referred to above was conducted in accordance with the requirements of the UK Animals (Scientific Procedures) Act 1 986 and with approval of the Institute of Grassland and Environmental Research's Local Ethical Review Committee or Aberystwyth University's Animal Welfare and Ethical Review Body. No specific reference numbers were issued following approval.

Data and model availability statement

None of the data were deposited in an official repository.

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Declaration of interest

None.

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