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Prediction of nitrogen excretion from data on dairy cows fed a wide range of diets compiled in an intercontinental database: A meta-analysis

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ABSTRACT

Manure nitrogen (N) from cattle contributes to nitrous oxide and ammonia emissions and nitrate leaching. Measurement of manure N outputs on dairy farms is laborious, expensive, and impractical at large scales; therefore, models are needed to predict N excreted in urine and feces. Building robust prediction models requires extensive data from animals under different management systems worldwide. Thus, the study objectives were (1) to collate an international database of N excretion in feces and urine based on individual lactating dairy cow data from different continents; (2) to determine the suitability of key variables for predicting fecal, urinary, and total manure N excretion; and (3) to develop robust and reliable N excretion prediction models based on individual data from lactating dairy cows consuming various diets. A raw data set was created based on 5,483 individual cow observations, with 5,420 fecal N excretion and 3,621 urine N excretion measurements collected from 162 in vivo experiments conducted by 22 research institutes mostly located in Europe (n = 14) and North America (n = 5). A sequential approach was taken in developing models with increasing complexity by incrementally adding variables that had a significant individual effect on fecal, urinary, or total

manure N excretion. Nitrogen excretion was predicted by fitting linear mixed models including experiment as a random effect. Simple models requiring dry matter intake (DMI) or N intake performed better for predicting fecal N excretion than simple models using diet nutrient composition or milk performance parameters. Simple models based on N intake performed better for urinary and total manure N excretion than those based on DMI, but simple models using milk urea N (MUN) and N intake performed even better for urinary N excretion. The full model predicting fecal N excretion had similar performance to simple models based on DMI but included several independent variables (DMI, diet crude protein content, diet neutral detergent fiber content, milk protein), depending on the location, and had root mean square prediction errors as a fraction of the observed mean values of 19.1% for intercontinental, 19.8% for European, and 17.7% for North American data sets. Complex total manure N excretion models based on N intake and MUN led to prediction errors of about 13.0% to 14.0%, which were comparable to models based on N intake alone. Intercepts and slopes of variables in optimal prediction equations developed on intercontinental, European, and North American bases differed from each other, and therefore region-specific models are preferred to predict N excretion. In conclusion, region-specific models that include information on DMI or N intake and MUN are required for good prediction of fecal, urinary, and total manure N excretion. In absence of intake data, region-specific complex equations using easily and routinely measured variables to predict fecal, urinary, or total manure N excretion may be used, but these equations have lower performance than equations based on intake.

Key words: manure nitrogen excretion, prediction model, dairy cow

INTRODUCTION

Ruminants are the most important source of milk for humans, providing 852 million tons of milk in 2019, of which 80% was produced by dairy cattle (OECD-FAO, 2020). Increasing demand for dairy products has led to expansion of dairy herds globally. However, the environmental impact of livestock-based food production is of concern because livestock manure generates nitrous oxide (N_2O) and ammonia (NH_3) emissions, and nitrate leaching into soil and ground water, contributing to air, water, and soil pollution (FAO, 2002). Nitrous oxide is an important greenhouse gas, with 265 times greater global warming potential than CO_2 over a 100-yr period (IPCC, 2007). Emissions of N_2O can occur from hydrolysis, nitrification, and denitrification of N constituents mainly in urine, in particular urea,

purine derivatives, creatine, and creatinine (Dijkstra et al. 2013a). Although urea is quantitatively the most important N compound in urine, other N compounds are important as well in N_2O emissions related to urine (Dijkstra et al. 2013a). Farm animals are considered the main contributors to NH_3 emissions, contributing, for instance, 50% of NH_3 emissions in the United States (Hristov et al., 2011). When urine and feces are mixed, urease present in feces rapidly converts urea excreted in urine into NH_3 and NH_4^+, resulting in NH_3 volatilization (Bougouin et al., 2016). Therefore, urine N is the main contributor to NH_3 emission from manure (Hristov et al., 2011).

Overall, ruminants are relatively inefficient at utilizing feed N, with 60 to 80% of dietary N being excreted mainly through urine outputs (Kebreab et al., 2001). The nondigested N, from endogenous or feed sources, is excreted in feces (Moore et al., 2014), whereas urinary N may only arise from digested and absorbed N, or mobilized body N. Although 50 to over 90% of total urine N is in the form of urea N (Bougouin et al., 2016), diet composition affects levels of various urinary N compounds and consequently susceptibility to losses after excretion (Dijkstra et al., 2013a). It has also been shown that reduced dietary N supply is accompanied by a decline in the proportion of the easily volatile urea in urine N (Dijkstra et al., 2013a). Hence, large variation in urinary N excretion compared with N excretion in feces presents an opportunity to manipulate diets to reduce urinary N excretion.

Predictive tools such as empirical or mechanistic models for estimating N excretion in manure are useful, especially because measurement techniques are laborious and costly and difficult to apply in the field (Hristov et al., 2019). Several models have been developed to predict total N excretion from lactating dairy cows to assess the efficiency of dairy production and to calculate national inventories of N_2O emissions. For example, IPCC (2006), revised by IPCC (2019), methodologies for predicting N_2O emissions from livestock manure using either default (tier 1 or 2) or country-specific estimates (tier 2 or 3) for total manure N excretion are recommended. However, proportions of N excreted in either urine or feces determine the fate of N as source of pollution (Tamminga, 1996). Therefore, models predicting both urine and feces N excretion separately are of interest and could improve N emissions estimation, resulting in a more accurate environmental impact assessment.

Therefore, the objectives of the present study were (1) to collate an intercontinental database with data on N intake and excretion in feces and urine for individual lactating dairy cows in different locations of the world and fed on a wide variety of diets; (2) to determine the

suitability of key variables for predicting N excretion in feces and urine, as well as total manure N excretion; and (3) to develop simplified but robust and reliable models that may facilitate prediction of N excretion in urine and feces.

MATERIALS AND METHODS

Databases and Variable Selection

This study is an element of the Global Network project and the Feed and Nutrition Network, which is an activity of the Livestock Research Group of the Global Research Alliance for Agricultural Greenhouse Gases (https://globalresearchalliance.org). A database for analysis was created using measurements made on individual animals at research locations from different parts of the world. The following information, all on an individual animal basis, was included: (1) fecal and urinary N excretion measured by either total collection or by marker methods for fecal N, (2) DMI, (3) dietary nutrient contents [CP, NDF, ADF, ether extract (**EE**), and starch] and their apparent total-tract digestibility, and (4) lactation performance [milk yield (**MY**) and concentrations of fat, protein (**MProt**), lactose, and MUN in milk]. Daily intakes of diet nutrients (CP, NDF, ADF, EE, starch) were calculated based on individual DMI and dietary chemical composition within experiments. Intake of N was calculated as CP intake/6.25 when not reported by the collaborators. A total of 5,483 observations were compiled, with 5,420 fecal N excretion and 3,621 urine N excretion measurements, from individual lactating dairy cows of different breeds (e.g., Holstein, Ayrshire, Holstein × Jersey). The raw data set included DMI and diet chemical composition, animal characteristics, and lactation performance from the 162 in vivo experiments (see details in Supplemental Table S1, https://data.mendeley.com/datasets/2d3ff88t5g/1; Kebreab, 2022) that were carried out in Europe (Belgium, Denmark, Finland, France, Germany, Ireland, Norway, the Netherlands, Sweden, Switzerland, and the United Kingdom), in North America (the United States and Canada), in Central and South America (Costa Rica and Chile), and in Oceania (New Zealand). The intercontinental data set includes all data from the locations mentioned.

The data set included diets that tested effects of dietary carbohydrate source (26% of all experiments), CP content (23% of all experiments), and supplemental lipids (6% of all experiments), or were categorized as control diets (28% of all experiments). In several experiments, additives were tested (e.g., tannins, monensin, enzymes, yeasts, essential oils, or other plant extracts;

17% of all experiments). Measurements of N excretion in feces and in urine were conducted using total feces and urine collection or using digestibility markers [acid-insoluble ash, chromium oxide (Cr_2O_3), indigestible NDF, titanium dioxide (TiO_2), ytterbium (Yb), and ytterbium (III) chloride ($YbCl_3$)] and creatinine as urine volume marker with spot sampling approaches, or using total manure collection and digestibility markers for fecal N excretion and calculating urinary N excretion by difference. Total manure N excretion was obtained by total collection or calculated as the sum of N excreted in feces and urine.

Data Preselection for Model Development

An exploratory data screening was performed to evaluate the data for completeness, consistency in nomenclature of each variable, and presence of outliers (Pyle, 1999). Data sets were screened for outliers by visually checking boxplots in R (boxplot function; version 0.98.1102, R Foundation for Statistical Computing) and using the interquartile range (**IQR**) method (Zwillinger and Kokoska, 2000). The IQR method was applied on subsets that included intercontinental data, European data, or North America data separately. A factor of 1.5 for extremes was used in constructing markers to identify outliers, as shown in Equations [i–iii]:

$$IQR = \text{Third Quartile (Q3)} - \text{First Quartile (Q1)};$$
[i]

$$\text{Lower Fence} = Q1 - IQR \times 1.5; \qquad [ii]$$

$$\text{Upper Fence} = Q3 + IQR \times 1.5. \qquad [iii]$$

As a result, the revised data set, used for model development, contained 5,409 observations on fecal N excretion (<1% removed from initial data set) and 3,584 observations on urine N excretion (1% removed) from 162 experiments. Measured variables and their summary statistics are given in Table 1 for the intercontinental data set, such information for North American and European data sets are given in Supplemental Tables S2 and S3 (https://data.mendeley.com/datasets/2d3ff88t5g/1; Kebreab, 2022). To compare model performances on the same basis, evaluation data sets were created for fecal, urinary, and total manure N excretion and per region, to have complete information on N excretion, DMI, BW, dietary nutrient content (CP and NDF), nutrient intake (N and NDF), and lactation performance (MY, MProt, and MUN). As a result, the evaluation data sets containing intercontinental data for fecal, urinary, and total manure N excretion had

Table 1. Summary descriptive statistics of variables used for model development[1]

Input variable	n	Mean	SD	Min	Max
Animal characteristics					
DIM (d)	5,416	111	68.0	1	304
BW (kg)	4,892	641	76.6	432	850
Dietary nutrient content (% of DM)					
CP	5,142	16.4	1.55	12.4	20.5
NDF	4,924	35.4	5.62	20.4	49.9
ADF	3,373	20.3	2.93	13.3	27.7
EE	5,482	4.2	1.35	1.7	8.5
Starch	5,482	20.7	5.48	7.8	33.8
Nutrient digestibilities (%)					
dCP	4,285	67.3	5.93	51.0	83.5
dNDF	3,083	54.4	11.61	21.3	86.3
dADF	1,593	49.9	13.57	17.9	86.8
dEE	844	63.6	11.99	28.9	94.7
dStarch	1,378	97.6	1.51	92.8	100.0
Performance variables					
DMI (kg/d)	5,452	21.4	4.19	9.9	33.0
N intake (g/d)	5,219	569.1	130.41	207.4	944.0
MY (kg/d)	5,385	32.1	9.30	7.3	56.9
MFat (%)	5,191	3.92	0.626	2.25	5.51
MProt (%)	4,813	3.26	0.310	2.42	4.07
MLact (%)	5,117	4.70	0.221	4.09	5.31
MUN (mg/dL)	4,350	11.2	4.541	1.5	24.2
Nitrogen excretion (g/d)					
Fecal nitrogen	5,409	184.0	50.38	46.8	322.1
Urinary nitrogen	3,621	175.5	66.22	7.0	365.2
Total manure nitrogen	3,629	358.4	96.14	97.0	633.0

[1]Summary statistics of the intercontinental data set (after outlier removal) used for model development. EE = ether extract; dCP, dNDF, etc. = digestibility of respective nutrients; MY = milk yield; MFat = milk fat; MProt = milk protein; MLact = milk lactose; Min = minimum; Max = maximum.

3,445, 2,489, and 2,490 individual observations, respectively. Summary statistics are described in Table 2.

Statistical Analyses

Random-Effects Model Analysis and Model Development. Linear mixed-effects models were constructed to predict fecal, urinary, and total manure N excretion (g/d) using the final data sets with intercontinental data, European data, or North America data separately. Mixed-effect models were also developed using the international database, excluding data from New Zealand, Costa Rica, and Chile, to test the models' accuracy, as farming systems in these countries might be different from the others. No differences in accuracy were observed; thus, data from New Zealand, Costa Rica, and Chile were kept in the international database (data not shown). Random-effect meta-analysis approaches (St-Pierre, 2001) were applied, and N excretion was predicted by fitting a mixed-effect model using the lmer (Bates et al., 2015) procedure of the R statistical language (R Core Team, 2017; version 3.3.0). Several models, as explained hereafter, were developed with different categories of independent variables used as fixed effects, and with experiments included as random effects. The potential predictors among the different categories [DMI; dietary nutrient contents (CP, NDF, ADF, EE, and starch), intakes of diet nutrient contents (N, NDF), lactation performance (MY, milk fat concentration, MProt, milk lactose concentration, and MUN)] were individually tested for their effects on fecal, urinary, and total manure N excretion and selected when $P < 0.10$ (Supplemental Tables S5, S6, and S7; https://data.mendeley.com/datasets/2d3ff88t5g/1; Kebreab, 2022). To evaluate the individual effect, mixed-effect models were applied, including each individual variable as a fixed effect and experiment as random effect.

The first set of N excretion prediction models began with simple models based on variables that had a significant individual effect on N excretion ($P < 0.10$). Variables where pairwise Pearson correlations for predictors had an absolute value of $|r| \geq 0.5$ were not included simultaneously in model development to avoid multicollinearity, which decreased statistical power and could lead to the exclusion of significant predictor variables during model construction (Graham, 2003). For instance, DMI and milk production had a Pearson correlation coefficient of 0.73 (Supplemental Table S4; https://data.mendeley.com/datasets/2d3ff88t5g/1; Kebreab, 2022) and consequently were not used together in the same model. Overall, if DMI was in the equa-

Table 2. Summary descriptive statistics of variables from the international data set used for model evaluation[1]

Variable	Feces N evaluation data set					Urine N evaluation data set					Total manure N evaluation data set				
	n	Mean	SD	Min	Max	n	Mean	SD	Min	Max	n	Mean	SD	Min	Max
Dietary nutrient concentration															
CP (%)	3,445	16.5	1.48	12.5	20.5	2,489	16.4	1.48	12.5	20.5	2,490	16.4	1.48	12.5	20.5
NDF (%)	3,445	35.1	5.51	20.4	49.8	2,489	35.2	5.82	20.4	49.8	2,490	34.5	5.84	20.43	49.8
Performance variables															
DMI (kg/d)	3,445	22.2	3.77	9.9	33.0	2,489	22.0	3.83	9.9	32.8	2,490	21.9	3.81	9.9	32.8
N intake (g/d)	3,445	584.8	116.78	222.4	927.2	2,489	576.2	117.29	222.4	895.0	2,490	575.8	116.92	222.4	927.2
Milk yield (kg/d)	3,445	33.5	8.63	7.3	56.9	2,489	33.4	8.25	7.3	56.9	2,490	33.3	8.23	7.3	56.9
MProt (%)	3,445	3.23	0.299	2.43	4.06	2,489	3.21	0.299	2.45	4.06	2,490	3.21	0.298	2.45	4.06
MUN (mg/dL)	3,445	11.4	4.46	1.5	24.2	2,489	10.4	4.11	1.5	24.0	2,490	10.5	4.12	1.5	24.0
Nitrogen excretion[2] (g/d)	3,445	191.6	48.33	46.8	322.1	2,489	184.4	61.60	7.0	365.2	2,490	191.6	87.82	115.1	632.6

[1]MProt = milk protein; Min = minimum; Max = maximum.
[2]Nitrogen excretion for fecal, urinary, and total manure N according to the data set.

tion, then the other variables included did not include intake of specific nutrients (in units of mass per time) but uncorrelated nutrient concentrations (in units of mass per unit of diet DM). The simple N excretion prediction models were developed based on separate variable categories, namely DMI only (**DMI_M**), N intake only (**N intake_M**), nutrient intakes (**Diet_intake_M**), dietary nutrient contents (**Diet_M**), and lactation performance variables (**Milk_M**). For the more complex models, all significant independent variables among different variable categories were used (**Full_M**). Another complex model was developed using variables that could be easily and routinely measured on field (**Full_Field_M**) such as dietary composition, MY, and milk composition variables. Simple models based on MUN (**MUN_M**), or on MUN and N intake (**MUN_Nintake_M**) were only developed with urinary and total manure N excretion, because MUN reflects surplus of N to energy in the body, which is probably not related to fecal N. For each category, intercontinental and location-specific models were developed: Europe (Belgium, Denmark, Finland, France, Germany, Ireland, Norway, the Netherland, Sweden, Switzerland, the United Kingdom) and North America (the United States and Canada). No models were developed for the other locations (New Zealand, Costa Rica, and Chile) as there were too few observations recorded, compared with the other regions. However, observations from these 3 countries were included in the intercontinental database.

The mixed-effect models development approach used in this study enabled analysis of fixed effects of independent preselected variables, as well as experiment-specific deviation of the N excretion response, which was taken into account as a random effect. The general mixed-effect model for single and multiple regressions is represented as follows:

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \ldots + \beta_n X_n + Rj + e, \quad [iv]$$

where β_0 denotes the fixed effect of intercept; X_1 to X_n denote the fixed effects of predictor variables, and β_1 to β_n are the corresponding slopes; Rj denotes the random effect of the jth experiment (to capture variations such as different regional weather conditions, measurement methods used, research protocols, and more); and e is the within-experiment error. All variables that had a P-value < 0.10 for their individual relationship with N excretions were further used for model development. The Bayesian information criterion (**BIC**) was computed for each model. Models with the lowest BIC were selected as the best models to predict each N excretion

response at each level of complexity (see below). The BIC was calculated as follows: n log (SSE_p/n) + (log n) p, where p is the number of regression coefficients, n is the sample size, and SSE_p is error sum of squares. A model with a smaller BIC is preferred because it reaches a balance between goodness of fit and model complexity. Variance inflation factor analysis was carried out to test independence of predictors in complex models, because influential cases can affect the validity and robustness of meta-analysis conclusions (Sutton et al., 2000; Viechtbauer and Cheung, 2010). Thus, residuals were visually inspected for any pattern. Studentized residuals offer the possibility to identify an observation with a high leverage, which arises when an observation influences the regression model to such an extent that the estimated regression function is pulled toward that potential observation (St-Pierre, 2007). To account for this, any studentized residuals $|\geq 3.0|$ were removed from the data set.

Model Evaluation. Prediction accuracy of each N excretion mixed-effect model was evaluated using the revised k-fold cross-validation method (James et al., 2014), based on the evaluation region-specific data set (total of 3,445, 2,489, and 2,490 observations for fecal, urinary, and total manure N excretion in the intercontinental evaluation data set, for instance), with folds composed of individual experiments (k = 127, 105, and 105 experiments for fecal, urinary, and total manure N excretion, respectively). Each individual fold was treated as an evaluation set, where the prediction of N excretion of each fold was calculated using the model that was fitted from the remaining folds. In this cross-evaluation method, the predictions of all folds were used to conduct model evaluation metrics as will be described.

A combination of model evaluation metrics was used to assess model performance. Root mean square of prediction error (**RMSPE**), expressed as a fraction of the observed mean was calculated, where a smaller RMSPE indicates better model predictive ability. The mean square prediction error was decomposed into mean bias (**MB**) and slope bias (**SB**) deviations to identify systematic biases. The MB and SB were calculated as shown in Equations [v] and [vi]:

$$MB = \left(\bar{P} - \bar{O}\right)^2; \qquad [v]$$

$$SB = \left(S_p - r \times S_o\right)^2, \qquad [vi]$$

where $\bar{P}$ and $\bar{O}$ denote the predicted and observed means, S_p denotes the standard deviation (SD) of predicted values, S_o denotes the SD of observations, and r denotes the Pearson correlation coefficient.

The concordance correlation coefficient (**CCC**; Lin, 1989) was calculated as follows:

$$CCC = r \times C_b, \qquad [vii],$$

where

$$C_b = \left[\frac{\left(v + \dfrac{1}{v} + u^2\right)}{2}\right]^{-1}; \qquad [viii]$$

$$v = S_o/S_p; \qquad [ix]$$

$$u = \left(\bar{P} - \bar{O}\right)\big/\left(S_o \times S_p\right)^2, \qquad [x]$$

where $\bar{P}$, $\bar{O}$, S_o, and S_p are as previously defined, v provides a measure of scale shift, and u provides a measure of location shift. The CCC evaluates the degree of deviation between the best-fit line and the identity line (y = x). Therefore, a CCC of a model closer to 1 is an indication of better model performance. When using different data sets to compare the performance of models, the ratio of RMSPE to SD of the data (observed values) can be used, namely RMSPE-observations SD ratio (**RSR**), as it takes standardized model performance relative to the variability in observations in different data sets (Moriasi et al., 2007). The RSR was calculated as shown in Equation [xi]:

$$RSR = \frac{RMSPE}{S_o}. \qquad [xi]$$

Smaller RSR (<1) indicates better performance given the variability of observations.

RESULTS

Database

Summary statistics of the final data set, which included data from 162 experiments used for model development, are presented in Table 1. Dry matter intake per cow ranged from 9.9 to 33.0 kg/d, and milk production ranged from 7.3 to 56.9 kg/d. On average, N excretion per cow in feces was 184.0 g/d (SD ± 50.38) and in urine was 175.5 g/d (SD ± 66.22), and total manure N excretion was 358.4 g/d (SD ± 96.14). Most of the experimental diets were fed as TMR or forage and concentrate separately, and included grass silage (35.9%, n = 1,969) or corn silage (32.7%, n = 1,792) as the main forage source, or a forage mix (12.4%, n =

680). Only 4.6% (n = 251) included grass hay as the main forage source, and only 3.9% (n = 215) included pasture grass. Some other forages were used as main source, such as cereal, clover, or alfalfa silage, as well as corn stover (5.0%; n = 275). Nutrient contents varied greatly among diets: in terms of dietary DM, CP contents varied between 12.4 and 20.5% with SD ± 1.55%, NDF between 20.4 and 49.9% with SD ± 5.62%, EE between 1.7 and 8.5% with SD ± 1.35%, and starch between 7.8 and 33.8% with SD ± 5.48%.

Mixed-Effect Models for Fecal N Excretion

Models to predict fecal N excretion are presented in Table 3. Daily fecal N excretion was positively correlated with individual variables such as DMI, N intake, dietary contents of CP and NDF, MY, and MProt. Negative relationships with fecal N excretion were observed with MUN with North American data (Equation [4]; Table 3).

Positive linear relationships between N excretion in feces and DMI or N intake were observed with data from the different continents and had similar accuracy of prediction (Equations [1] and [2]; Table 3). Nonetheless, differences in accuracy were observed between continents. Both DMI_M and N intake_M models led to prediction errors of 17.7 and 17.4%, respectively, when models were developed from North America data. The DMI_M and N intake_M models led to greater prediction errors when based on European data, with 19.9 and 19.6% error, respectively. Similar results were observed for models based on DMI and N intake with intercontinental data (19.0 and 18.8% prediction error, respectively). The greatest RSR and the lowest CCC were observed with DMI_M models based on European data (Table 3) compared with the other locations, whereas the lowest RSR and the greatest CCC were observed with N intake_M models based on intercontinental data (Table 3) compared with the other locations. Negligible MB and SB were observed with these models regardless of location. The DMI_M tended to slightly underpredict excretion at the lower end of N excretion and overpredict excretion at the higher end of fecal N excretion with North American and European data (Figure 1). When models were based on nutrient intake data, N intake was selected as the best predictor for all the different locations. The simple models Diet_M included dietary CP content in the best model with intercontinental and European data (Equation [3]; Table 3), and led to greater prediction error than models using DMI or N intake (RMSPE increased for these 2 locations, with RMSPE values of 25.5 and 25.3%, respectively). With the North American data, dietary NDF content was chosen as the best predictor and led

to a RMSPE of 23.3%. The RSR was greater than 1.0 compared with previously cited models, regardless of the location. In addition, MB was observed with intercontinental and European data (5.52 and 8.35%), and SB was observed in the North American model (11.13%). The Milk_M included MY and MProt to predict fecal N excretion for all locations (Equation [4]; Table 3), and MUN was included with North American data. Models with intercontinental and European data were of comparable accuracy, with the prediction error ranging from 23.4% and 24.3%, respectively. The RMSPE was slightly lower with the model based on North American data (20.3%; Equation [4], Table 3). The RSR was <1.0 for all locations, along with negligible MB or SB (<3%) except for MB of 4.35% with European data.

Variables used in the complex Full_M models (Equation [5]; Table 3) were not the same among continents. Models based on intercontinental and European data both included DMI, dietary CP and NDF, and MProt (only with intercontinental data), whereas only DMI was used in models based on North American data. The model Full_M for North America led to similar prediction performance (RMSPE of 17.7%) compared with the simple model N intake_M for this location (17.4%). The Full_M models using intercontinental and European data led to prediction error (RMSPE of 19.1 and 19.8%, respectively) similar to the DMI_M (19.0 and 19.9% prediction error for intercontinental and European data) but greater than N intake models for intercontinental data (18.8% prediction error). Negligible bias was observed with the Full_M models (<3%), but the Full_M models tended to underpredict excretion at the lower end of fecal N excretion and overpredict excretion at the higher end of N excretion with North American and European data (Figure 2).

The complex Full_Field_M model led to better prediction error and RSR than models based on dietary nutrient content variables, prediction error and RSR similar to models based on lactation performance, but greater prediction error than all the other models for all locations (RMSPE >20%). Variables selected in the Full_Field_M model were not the same among locations. Only MY was used for all locations, whereas dietary NDF was used in models based on intercontinental and European data, and MProt was used for intercontinental and North American models (Equation [6]; Table 3).

Mixed-Effect Models for Urinary N Excretion

Models to predict urinary N excretion are presented in Table 4. In the simple models, N excretion in urine had positive relationships with DMI, intakes of N and

Table 3. Prediction equations of fecal N excretion (g/d per cow) according to different categories and model performance evaluation

Equation number	Category[1]	Location	Prediction equations[2]	n	RMSPE, %	RSR	MB	SB	CCC
						Model performance[3]			
1	DMI_M	Intercontinental	$2.7 (\pm2.99) + 8.4 (\pm0.11) \times DMI$	3,445	19.0	0.75	0.21	0.04	0.60
		Europe	$2.3 (\pm3.31) + 8.4 (\pm0.13) \times DMI$	2,306	19.9	0.80	0.31	0.16	0.54
		North America	$11.1 (\pm7.19) + 8.4 (\pm0.29) \times DMI$	1,089	17.7	0.79	0.00	0.09	0.56
2	N intake_M	Intercontinental	$32.0 (\pm2.90) + 0.27 (\pm0.004) \times N \text{ intake}$	3,445	18.8	0.75	0.20	0.02	0.61
		Europe	$35.4 (\pm3.15) + 0.25 (\pm0.004) \times N \text{ intake}$	2,306	19.6	0.79	0.32	0.28	0.57
		North America	$24.5 (\pm7.15) + 0.29 (\pm0.009) \times N \text{ intake}$	1,089	17.4	0.78	0.00	0.00	0.56
3	Diet_M	Intercontinental	$136.7 (\pm11.60) + 0.25 (\pm0.07) \times CP$	3,445	25.5	1.01	5.52	1.13	0.04
		Europe	$105.0 (\pm11.10) + 0.34 (\pm0.06) \times CP$	2,306	25.3	1.02	8.35	1.76	0.06
		North America	$95.2 (\pm27.15) + 0.37 (\pm0.085) \times NDF$	1,089	23.3	1.04	0.38	11.13	−0.06
4	Milk_M	Intercontinental	$28.1 (\pm8.67) + 2.3 (\pm0.08) \times MY + 24.2 (\pm2.11) \times MProt$	3,445	23.4	0.93	2.98	0.42	0.25
		Europe	$44.3 (\pm10.20) + 2.2 (\pm0.10) \times MY + 17.5 (\pm2.51) \times MProt$	2,306	24.3	0.98	4.35	0.23	0.18
		North America	$-14.0 (\pm18.29) + 2.8 (\pm0.16) \times MY + 43.8 (\pm4.33) \times MProt - 1.3 (\pm0.39) \times MUN$	1,089	20.3	0.91	0.16	0.05	0.29
5	Full_M	Intercontinental	$-188.5 (\pm21.58) + 8.3 (\pm0.21) \times DMI + 0.28 (\pm0.07) \times CP + 0.36 (\pm0.031) \times NDF + 7.2 (\pm2.36) \times MProt$	3,445	19.1	0.76	0.29	0.09	0.59
		Europe	$-154.1 (\pm22.61) + 8.0 (\pm0.29) \times DMI + 0.28 (\pm0.08) \times CP + 0.32 (\pm0.04) \times NDF$	2,306	19.8	0.79	0.15	0.33	0.56
		North America	$5.3 (\pm9.46) + 8.5 (\pm0.32) \times DMI$	1,089	17.7	0.79	0.00	0.09	0.56
6	Full_Field_M	Intercontinental	$-85.6 (\pm21.37) + 1.9 (\pm0.12) \times MY + 22.6 (\pm0.04) \times MProt + 0.39 (\pm0.039) \times NDF$	3,445	24.2	0.96	3.25	0.01	0.20
		Europe	$25.6 (\pm19.09) + 1.4 (\pm0.14) \times MY + 0.27 (\pm0.044) \times NDF$	2,306	24.7	0.99	4.51	0.57	0.15
		North America	$-17.7 (\pm22.77) + 2.3 (\pm0.20) \times MY + 43.7 (\pm5.49) \times MProt$	1,089	20.4	0.91	0.22	0.01	0.28

[1]Model categories are as follows. Simple models: DMI only (DMI_M), N intake only (N intake_M), dietary nutrient contents variables (Diet_M). Complex models: all significant independent variables among variable categories were used for the full model selection (Full_M); all significant independent variables easily and routinely measured on field (Full_Field_M). Diet_Intake_M (dietary nutrient intake variables) were identical to N_Intake_M equations and are therefore not shown. Locations are as follows. Intercontinental: Belgium, Canada, Chile, Costa Rica, Denmark, Finland, France, Germany, Ireland, the Netherlands, New Zealand, Norway, Sweden, Switzerland, the UK, and the US. Europe: Belgium, Denmark, Finland, France, Germany, Ireland, Norway, the Netherlands, Sweden, Switzerland, and the UK. North America: the US and Canada.

[2]In parentheses: ±SE. DMI (kg/d); CP = dietary CP content (g/kg DM); NDF = dietary NDF content (g/kg DM); N intake (g/d); MY = milk yield (kg/d); MProt = milk protein (%); MUN (mg/dL).

[3]n = number of observations used for model evaluation; RMSPE = root mean square prediction error; RSR = RMSPE-observations SD ratio; MB = mean bias as a percentage of mean square prediction error; SB = slope bias as a percentage of mean square prediction error; expressed as a percentage of observed mean daily N excretion in feces; RSR = RMSPE-observations SD ratio; MB = mean bias as a percentage of mean square prediction error; SB = slope bias as a percentage of mean square prediction error; CCC = concordance correlation coefficient.
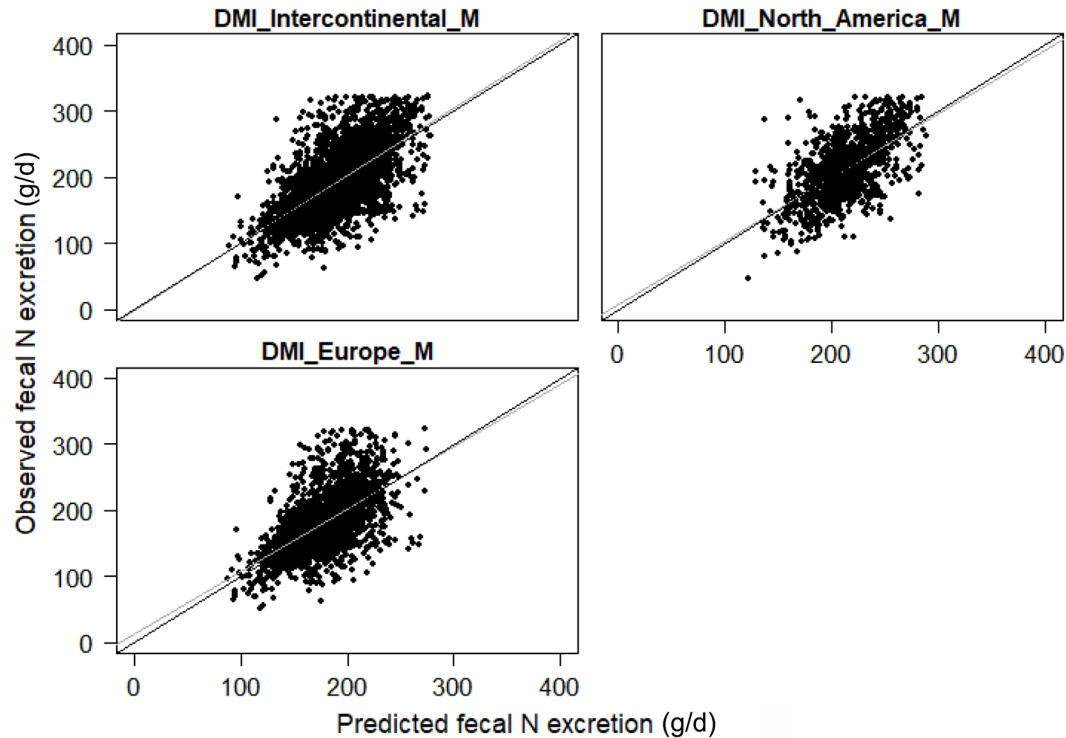
Figure 1. Predicted vs. observed values based on fecal N excretion (g/d per cow) prediction equations with DMI_M (DMI only) for all locations. Gray lines represent the fitted regression line for the relationship between predicted and observed values, and black lines represent the identity line (y = x).

NDF, and dietary CP content, as well as with MY, MProt, and MUN. Only with North American data, a negative relationship was observed between urinary N excretion and dietary NDF (Equation [10]; Table 4).

The simple N intake_M models for urine N excretion for the different locations, based on N intake, led to RMSPE of 26.1% with European data, 27.2% with intercontinental data, and 27.1% with North American data (Equation [8]; Table 4). The RSR was <1.0 for these models, and negligible MB and SB were observed (<3%). The N intake_M models tended to overpredict at the lower end of urinary N excretion and underpredict at the higher end of urinary N excretion, in particular with North American data (Figure 3).

The other simple models, such as DMI_M, Diet_M, Milk_M, and MUN_M, led to lower prediction performances than N intake_M models regardless of the location, with RMSPE varying between 27.7 and 33.9%. Models based on dietary CP or both dietary CP and NDF led to MB (>3%; Equation [10]). With North American data, Equation [10] led to 17.2% SB. Models based on milk performance all included MY, MProt, and MUN (Equation [11]; Table 4), and negligible MB or SB were observed (<3%). Models based on MUN only (Equation [12]) had the worst performance of all simple models considered.

The simple MUN_Nintake_M models based on MUN and N intake led to lower RMSPE, compared with previously cited simple models, which varied between 25.0% (with North American data) and 26.5% (with intercontinental data). These models led to SB with North American data (>3%, Equation [13]; Table 4).

The complex Full_M models were based on N intake and MUN for intercontinental and European data, but were different from the North American data, with dietary NDF being included along with N intake and MUN as predictors. This latter model led to RMSPE of 28.9% and RSR of 0.99, along with SB (>3%; Equation [14]; Table 4), whereas intercontinental and European Full_M models led to 28.0 and 26.8% prediction error, respectively (Equation [14]; Table 4). These 3 models tended to overpredict at the lower end of urinary N excretion and underpredict at the higher end of urinary N excretion for North America data in particular (Figure 4).

The complex Full_Field_M model (Equation [15]; Table 4) had a lower prediction error than the Full_M model, regardless of the location. The same variables were used for intercontinental and European models (MY, MProt, MUN, and dietary CP), and dietary NDF content was included in the North American model. All these models led to negligible MB and SB (<3%).

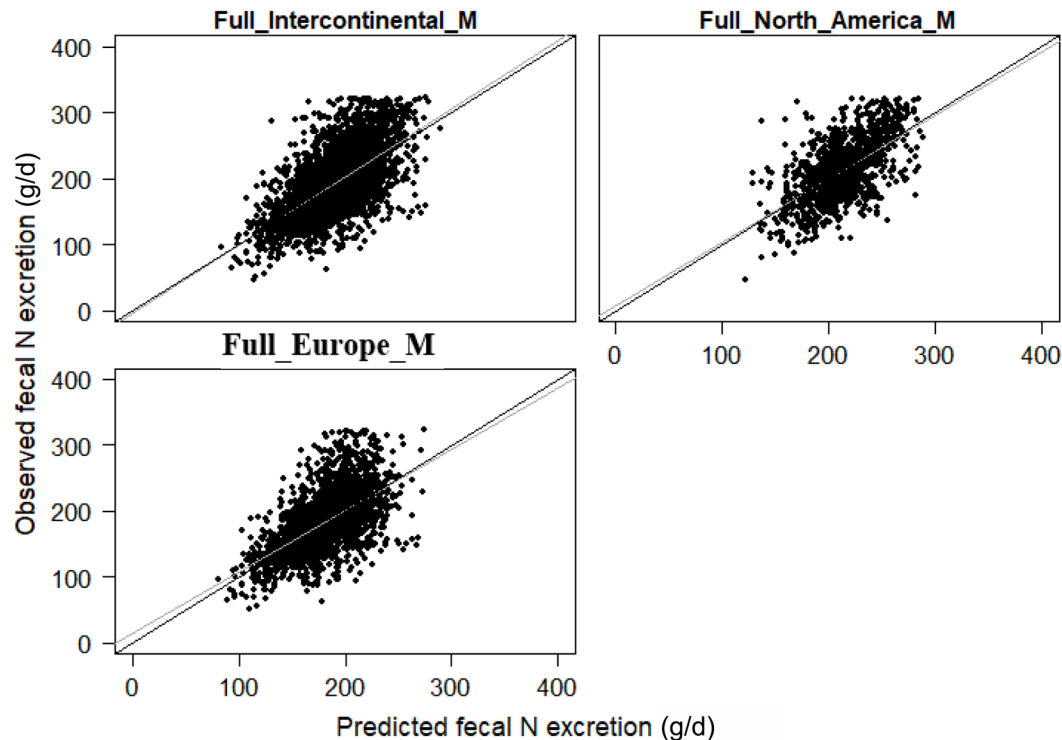**Figure 2.** Predicted vs. observed values based on fecal N excretion (g/d per cow) prediction equations at the highest level of complexity (Full_M) for all locations. Gray lines represent the fitted regression line for the relationship between predicted and observed values, and black lines represent the identity line (y = x).

Mixed-Effect Models for Total Manure N Excretion

Models for predicting total manure N excretion are presented in Table 5. Positive relationships were observed with DMI, N intake, diet CP content, MY, MProt, and MUN. Negative relationships were observed with dietary NDF content only for the simple model. In Full_M, dietary NDF was included with a positive sign.

The N intake_M models based on N intake led to the lowest prediction errors among simple models (≤14.0%; Table 5) for intercontinental, European, and North American data, along with the lowest RSR among simple models, and negligible MB and SB (<3%). Overall, the N intake_M model tended to slightly overpredict at the lower end of total manure N excretion and underpredict at the higher end of total manure N excretion (Figure 5). The DMI_M had greater RMSPE (ranging from 14.5 to 17.7%) compared with N intake models for all locations.

The other simple models (i.e., Diet_M, Milk_M, and MUN_M) had lower prediction ability than N intake models with larger RMSPE and RSR, along with some models having MB or SB (>3%; except for Milk_M). For models based on dietary parameters, milk performance, or MUN only, better results were observed with North American data compared with other locations. For instance, Equation [20], based on MUN, led to an RMSPE of 19.4% with North American data, along with negligible MB and SB, whereas intercontinental and European models led to RMSPE of 23.3 and 24.2%, with MB for intercontinental data only. The Diet_Intake_M models all included N intake as a predicting variable.

Once N intake is included along with MUN, the prediction performance is similar to models based on N intake only. In addition, the N intake and MUN model and the Full_M model (Equations [21] and [22]; Table 5) led to prediction error (RMSPE <14%) similar to intercontinental, European, and North American data compared with N intake_M. In the complex Full_M models, N intake, MProt, and MUN were used as predictors with intercontinental and North American data, whereas N intake and dietary NDF content were selected with European data. The RSR was <0.70 for these models, and negligible MB was detected (<3%). Overall, the Full_M model tended to overpredict at the lower end of total manure N excretion and underpredict at the higher end of total manure N excretion (Figure 6).

The complex Full_Field_M model had RMSPE greater (>17.0%) than the Full_M model and the N

Table 4. Prediction equations of urinary N excretion (g/d per cow) according to different categories and model performance evaluation

Equation number	Model development[1]		Prediction equations[2]	Model performance[3]					
	Category	Location		n	RMSPE,%	RSR	MB, %	SB, %	CCC
7	DMI_M	Intercontinental	$57.1\ (\pm6.20) + 5.4\ (\pm0.22) \times$ DMI	2,489	30.1	0.90	1.83	0.43	0.30
		Europe	$38.3\ (\pm7.46) + 6.2\ (\pm0.29) \times$ DMI	1,412	30.4	0.90	0.85	0.03	0.33
		North America	$87.9\ (\pm11.66) + 4.3\ (\pm0.37) \times$ DMI	944	27.9	0.96	1.98	0.20	0.17
8	N intake_M	Intercontinental	$32.9\ (\pm4.99) + 0.25\ (\pm0.007) \times$ N intake	2,489	27.2	0.81	1.42	0.64	0.48
		Europe	$20.2\ (\pm5.65) + 0.28\ (\pm0.008) \times$ N intake	1,225	26.1	0.78	0.97	0.07	0.56
		North America	$63.5\ (\pm10.79) + 0.20\ (\pm0.013) \times$ N intake	944	27.1	0.93	1.82	0.72	0.22
9	Diet_Intake_M	Intercontinental	$86.5\ (\pm8.16) + 10.3\ (\pm0.69) \times$ NDF intake	2,489	32.5	0.97	3.61	0.01	0.15
		Europe	$26.0\ (\pm12.30) + 14.3\ (\pm1.32) \times$ NDF intake	1,225	31.8	0.94	1.39	0.25	0.24
		North America	$132.2\ (\pm10.22) + 7.7\ (\pm0.79) \times$ NDF intake	944	28.4	0.98	1.77	0.48	0.10
10	Diet_M	Intercontinental	$-110.1\ (\pm13.07) + 1.7\ (\pm0.08) \times$ CP	2,489	30.4	0.91	4.42	0.38	0.31
		Europe	$-97.7\ (\pm11.86) + 1.6\ (\pm0.07) \times$ CP	1,225	28.9	0.86	4.15	0.66	0.41
		North America	$-149.2\ (\pm60.53) + 3.0\ (\pm0.30) \times$ CP $- 0.50\ (\pm0.101) \times$ NDF	944	32.1	1.10	0.90	17.19	0.06
11	Milk_M	Intercontinental	$-17.4\ (\pm11.48) + 1.8\ (\pm0.12) \times$ MY $+ 20.9\ (\pm2.52) \times$ MProt $+ 6.2\ (\pm0.26) \times$ MUN	2,489	31.0	0.93	2.13	0.03	0.26
		Europe	$-31.9\ (\pm13.36) + 2.4\ (\pm0.15) \times$ MY $+ 20.1\ (\pm2.86) \times$ MProt $+ 6.5\ (\pm0.30) \times$ MUN	1,225	31.5	0.94	0.95	0.40	0.26
		North America	$6.02\ (\pm23.39) + 1.1\ (\pm0.19) \times$ MY $+ 24.7\ (\pm5.43) \times$ MProt $+ 5.4\ (\pm0.52) \times$ MUN	944	27.7	0.95	2.70	0.92	0.18
12	MUN_M	Intercontinental	$106.9\ (\pm5.91) + 6.1\ (\pm0.26) \times$ MUN	2,489	32.6	0.98	3.29	0.05	0.15
		Europe	$99.4\ (\pm7.35) + 6.4\ (\pm0.30) \times$ MUN	1,225	33.9	1.01	1.89	2.73	0.10
		North America	$126.6\ (\pm9.67) + 5.3\ (\pm0.51) \times$ MUN	944	28.1	0.96	2.47	1.23	0.14
13	MUN_Nintake_M	Intercontinental	$-8.2\ (\pm6.09) + 0.25\ (\pm0.007) \times$ N intake $+ 4.1\ (\pm0.25) \times$ MUN	2,489	26.5	0.79	0.64	0.01	0.55
		Europe	$-0.32\ (\pm5.82) + 0.23\ (\pm0.007) \times$ N intake $+ 4.2\ (\pm0.23) \times$ MUN	1,225	25.9	0.77	0.34	0.18	0.59
		North America	$0.59\ (\pm11.46) + 0.20\ (\pm0.012) \times$ N intake $+ 5.2\ (\pm0.46) \times$ MUN	944	25.0	0.86	2.60	3.72	0.37
14	Full_M	Intercontinental	$-4.4\ (\pm9.63) + 0.22\ (\pm0.012) \times$ N intake $+ 3.9\ (\pm0.37) \times$ MUN	2,489	28.0	0.84	2.58	1.01	0.44
		Europe	$-15.8\ (\pm17.53) + 0.27\ (\pm0.032) \times$ N intake $+ 2.0\ (\pm0.62) \times$ MUN	1,225	26.8	0.80	2.17	1.50	0.51
		North America	$151.6\ (\pm35.44) + 0.20\ (\pm0.013) \times$ N intake $+ 4.3\ (\pm0.50) \times$ MUN $- 0.45\ (\pm0.101) \times$ NDF	944	28.9	0.99	1.50	3.67	0.19
15	Full_Field_M	Intercontinental	$-192.8\ (\pm16.23) + 1.4\ (\pm0.07) \times$ CP $+ 1.4\ (\pm0.12) \times$ MY $+ 20.1\ (\pm2.79) \times$ MProt $+ 3.5\ (\pm0.29) \times$ MUN	2,489	26.5	0.79	0.64	0.01	0.55
		Europe	$-191.8\ (\pm17.97) + 1.3\ (\pm0.08) \times$ CP $+ 1.9\ (\pm0.17) \times$ MY $+ 19.4\ (\pm3.40) \times$ MProt $+ 3.0\ (\pm0.34) \times$ MUN	1,225	25.9	0.77	0.34	0.18	0.59
		North America	$-295.6\ (\pm67.22) + 1.1\ (\pm0.20) \times$ MY $+ 25.3\ (\pm5.47) \times$ MProt $+ 3.9\ (\pm0.57) \times$ MUN $+ 2.5\ (\pm0.32) \times$ CP $- 0.33\ (\pm0.121) \times$ NDF	944	25.7	0.88	2.42	0.93	0.35

[1] Model categories are as follows. Simple models: DMI only (DMI_M), N intake only (N intake_M), nutrient intakes variables (Diet_intake_M), dietary nutrient contents variables (Diet_M), lactation performance variables (Milk_M), MUN only (MUN_M); MUN and N intake (MUN_Nintake_M). Complex models: all significant independent variables among variable categories were used for the full model selection (Full_M); all significant independent variables easily and routinely measured on field (Full_Field_M). Locations are as follows. Intercontinental: Belgium, Canada, Chile, Costa Rica, Denmark, Finland, France, Germany, Ireland, the Netherlands, New Zealand, Norway, Sweden, Switzerland, the UK, and the US. Europe: Belgium, Denmark, Finland, France, Germany, Ireland, Norway, the Netherlands, Sweden, Switzerland, and the UK. North America: the US and Canada.

[2] In parentheses: ±SE. DMI (kg/d); CP = dietary CP content (g/kg DM); NDF (g/kg DM); N intake (g/d); NDF intake (g/d); MY = milk yield (kg/d); MProt = milk protein (%); MUN (mg/dL).

[3] n = number of observations used to evaluate equations; RMSPE = root mean square prediction error, expressed as a percentage of observed mean daily N excretion in urine; RSR = RMSPE-observations SD ratio; MB = mean bias as a percentage of mean square prediction error; SB = slope bias as a percentage of mean square prediction error; CCC = concordance correlation coefficient.
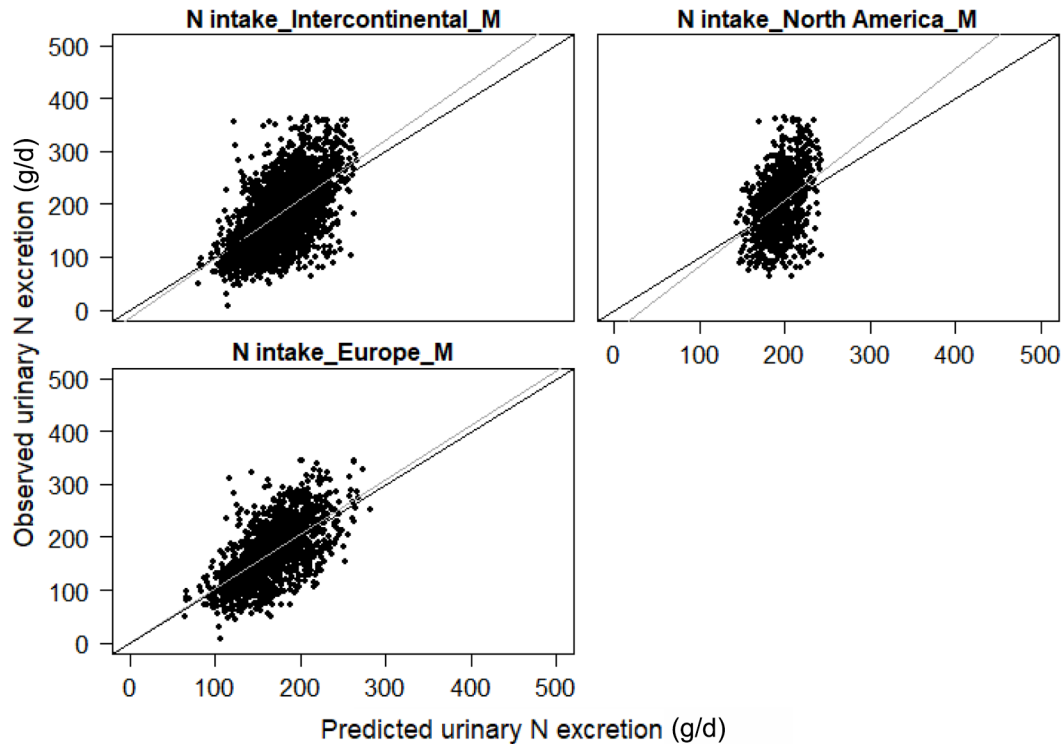
Figure 3. Predicted vs. observed values based on urinary N excretion (g/d per cow) prediction equations with N intake_M (N intake only) for all locations. Gray lines represent the fitted regression line for the relationship between predicted and observed values, and black lines represent the identity line (y = x).

intake_M model and similar to the models based on DMI, except with North American data. Milk yield, MProt, and dietary CP were used as predictors in Full_Field_M models for all locations, whereas MUN was used in the intercontinental and North American models (Equation [23]; Table 5). Slope bias was present for European models (3.72%), whereas negligible bias was observed for the other locations (<3.0%).

DISCUSSION

In this study, we identified key predictor variables for fecal, urinary, and total manure N excretion in lactating dairy cows, and we evaluated the prediction accuracy of the developed models. Many different prediction models have already been published for beef and dairy cattle (Nennich et al., 2005; Spek et al., 2013a; Reed et al., 2015). However, in the current evaluation we used a larger database, encompassing many areas of the world, with individual measurements. In addition, the compilation of 162 experiments contained a large variety of diets from experiments conducted primarily with Holstein cows (91.0% of all studies) and across North America, Europe, and these continents combined with Central and South America (Costa Rica, and Chile)

and Oceania (New Zealand). The diets encompassed a large variety of feeds, but the major ingredients were corn silage and grass silage. Therefore, the results from this meta-analysis will be most applicable to Holstein cows fed corn silage- or grass silage-based diets.

Key Predictors of N Excretion

Excretion of N in feces, urine, and total manure was positively related to both DMI and N intake. The DMI is related to endogenous N and undigested microbial N, which end up in feces, leading to logical prediction ability of DMI for fecal N excretion. Increasing DMI increases N excretion simply because greater feed consumption means greater N consumption. However, N intake does a better job than DMI in explaining urinary N excretion and therefore total manure N excretion. It makes sense that urinary N is much more affected by N intake, as any surplus of N absorbed that cannot be used for milk N or body N accretion, will have to leave via urine. Reed et al. (2015) developed prediction equations of N excretion in feces and urine in dairy cows from data collected over 30 years during USDA energy metabolism studies (United States). They also noticed a better ability of N intake to predict urinary and total
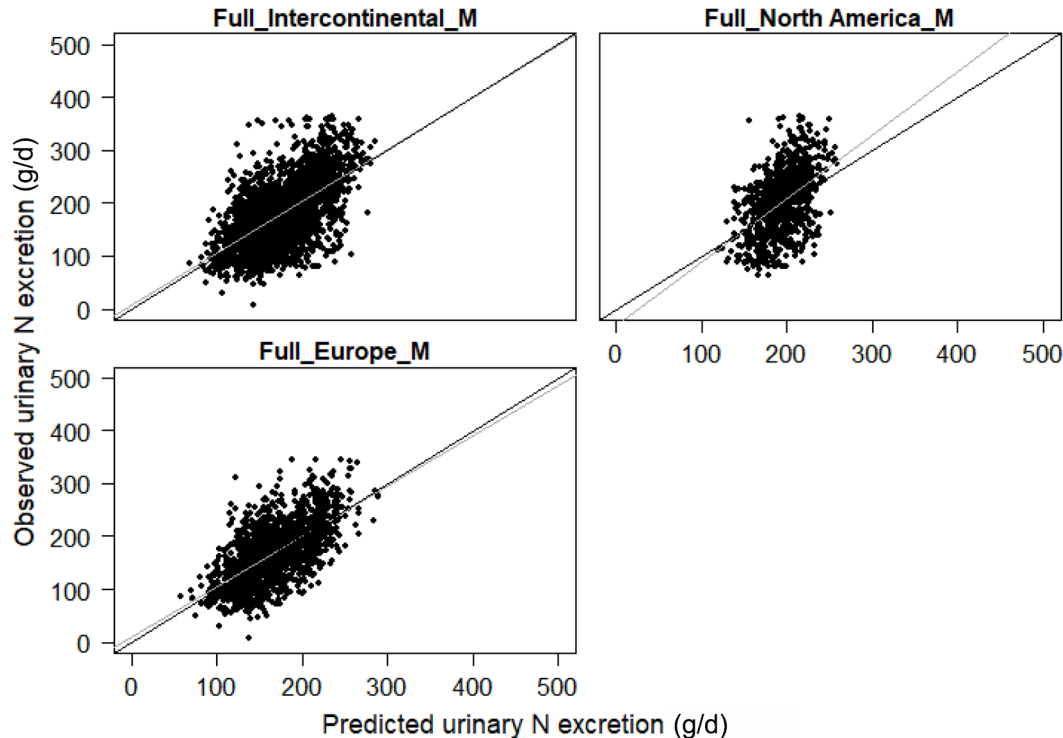
Figure 4. Predicted vs. observed values based on urinary N excretion (g/d per cow) prediction equations at the highest level of complexity (Full_M) for all locations. Gray lines represent the fitted regression line for the relationship between predicted and observed values, and black lines represent the identity line (y = x).

manure N excretion. In addition, they showed that, for fecal N excretion, DMI was better suited than N intake. In the current study, using DMI or N intake led to very similar results in fecal N prediction. Huhtanen et al. (2008) also found DMI to be a better predictor of fecal N excretion than N intake, but prediction accuracy improved when both were included together.

The data set used by Reed et al. (2015) was not as broad as the one used in the present study, but predictor coefficients were similar between studies, even with the intercontinental models. Nonetheless, prediction performance of fecal N excretion based on DMI was better in Reed et al. (2015), with prediction error of 12.6%, than in the current study (17.7% with North American data). The complex fecal N excretion model developed in Reed et al. (2015) used different variables (DMI, dietary ME, NDF and CP contents, and DIM) and led to lower RMSPE (11.4%) than in the current study (Full_M led to 17.7% with North American data). The prediction abilities were lower in the current study for total manure N excretion models than in the study by Reed et al. (2015). When N intake was used to predict urinary excretion, it led to similar prediction error, with RMSPE of 23.7% in Reed et al. (2015) and 27.1% in the current study with North American data.

Endogenous N and protein synthesis from ruminal microorganisms and subsequent undigested microbial N are important sources of fecal N, as explained before. In addition, undigested feed protein from forage or concentrates could also contribute to N excretion in feces. Greater DMI and N intake increase rumen microbial growth and N absorption across the digestive tract, but greater DMI also increases the digesta passage rate, resulting in increased fecal DM and N excretion in feces and decreased CP digestibility (Dijkstra et al., 2013b). However, CP digestibility might have a minor contribution compared with DMI, which is directly linked to metabolic and endogenous N and subsequent N losses in feces (Huhtanen et al., 2008). Nevertheless, variation in dietary N supply particularly affects urinary N output (Huhtanen et al., 2008), and nitrogen intake has been shown to be the main driver of N losses in dairy cows (Kebreab et al., 2010), milk N efficiency (Huhtanen and Hristov, 2009), and NH_3 emissions from manure (Hristov et al., 2011). Increasing N intake increases the amount of N excreted in urine as urea N in particular, which may lead to higher NH_3 emissions from manure (Weiss et al., 2009). Colmenero and Broderick (2006) showed that any increase in N intake with diets containing more than 16.5% CP was

Table 5. Prediction equations of total N excretion (g/d per cow) according to different categories and model performance evaluation

	Model development[1]			Model performance[3]						Prediction equations[2]
Equation number	Category	Location	n	RMSPE, %	RSR	MB, %	SB, %	CCC		
16	DMI_M	Intercontinental	2,490	16.4	0.71	1.04	1.08	0.65		$54.5\ (\pm7.06) + 14.4\ (\pm0.26) \times DMI$
		Europe	1,422	17.7	0.75	0.62	0.70	0.59		$40.1\ (\pm8.56) + 14.9\ (\pm0.33) \times DMI$
		North America	940	14.5	0.76	0.52	0.36	0.59		$84.2\ (\pm12.94) + 13.5\ (\pm0.44) \times DMI$
17	N intake_M	Intercontinental	2,490	13.4	0.58	1.16	1.64	0.79		$49.0\ (\pm5.08) + 0.56\ (\pm0.007) \times N\ intake$
		Europe	1,422	13.6	0.57	1.54	2.14	0.79		$43.4\ (\pm5.81) + 0.57\ (\pm0.008) \times N\ intake$
		North America	940	13.3	0.69	0.34	2.05	0.66		$68.8\ (\pm11.23) + 0.53\ (\pm0.015) \times N\ intake$
18	Diet_M	Intercontinental	2,490	20.8	0.90	5.31	1.30	0.33		$40.1\ (\pm24.61) + 2.2\ (\pm0.11) \times CP - 0.13\ (\pm0.038) \times NDF$
		Europe	1,422	20.3	0.86	4.92	1.65	0.40		$-29.0\ (\pm17.24) + 2.2\ (\pm0.10) \times CP$
		North America	940	19.6	1.02	0.19	4.49	0.04		$-80.5\ (\pm74.75) + 3.0\ (\pm0.44) \times CP$
19	Milk_M	Intercontinental	2,490	19.7	0.85	1.90	1.09	0.41		$-41.0\ (\pm15.92) + 5.1\ (\pm0.16) \times MY + 6.0\ (\pm0.35) \times MUN + 54.5\ (\pm3.60) \times MProt$
		Europe	1,422	20.6	0.87	0.70	0.00	0.40		$-42.1\ (\pm17.92) + 5.9\ (\pm0.20) \times MY + 6.7\ (\pm0.39) \times MUN + 45.1\ (\pm3.91) \times MProt$
		North America	940	17.9	0.93	0.77	0.00	0.24		$-71.8\ (\pm33.68) + 4.2\ (\pm0.29) \times MY + 4.1\ (\pm0.74) \times MUN + 85.5\ (\pm7.98) \times MProt$
20	MUN_M	Intercontinental	2,490	23.3	1.00	4.35	0.03	0.07		$290.7\ (\pm8.46) + 6.1\ (\pm0.38) \times MUN$
		Europe	1,422	24.2	1.02	2.57	2.42	0.03		$267.3\ (\pm10.51) + 7.1\ (\pm0.47) \times MUN$
		North America	940	19.4	1.01	0.56	1.22	0.00		$362.1\ (\pm13.42) + 3.7\ (\pm0.79) \times MUN$
21	MUN_Nintake_M	Intercontinental	2,490	13.4	0.58	0.74	0.63	0.79		$30.0\ (\pm6.02) + 0.55\ (\pm0.008) \times N\ intake + 2.5\ (\pm0.25) \times MUN$
		Europe	1,422	13.7	0.58	1.04	1.16	0.79		$31.2\ (\pm6.84) + 0.55\ (\pm0.009) \times N\ intake + 2.1\ (\pm0.27) \times MUN$
		North America	940	13.0	0.68	0.50	2.44	0.67		$33.1\ (\pm12.94) + 0.53\ (\pm0.15) \times N\ intake + 3.2\ (\pm0.55) \times MUN$
22	Full_M	Intercontinental	2,490	13.4	0.58	0.79	0.69	0.79		$-24.5\ (\pm20.13) + 0.54\ (\pm0.017) \times N\ intake + 2.5\ (\pm0.48) \times MUN + 14.8\ (\pm5.00) \times MProt$
		Europe	1,422	13.5	0.57	1.50	2.34	0.79		$-53.1\ (\pm41.52) + 0.45\ (\pm0.034) \times N\ intake + 0.4\ (\pm0.11) \times NDF$
		North America	940	12.9	0.67	0.49	2.53	0.68		$-56.1\ (\pm25.38) + 0.54\ (\pm0.021) \times N\ intake + 2.6\ (\pm0.68) \times MUN + 26.4\ (\pm6.42) \times MProt$
23	Full_Field_M	Intercontinental	2,490	17.6	0.76	2.47	2.60	0.56		$-269.4\ (\pm29.98) + 4.5\ (\pm0.22) \times MY + 1.9\ (\pm0.51) \times MUN + 63.0\ (\pm5.09) \times MProt + 1.6\ (\pm0.13) \times CP$
		Europe	1,422	17.2	0.73	2.53	3.72	0.60		$-192.9\ (\pm35.12) + 4.5\ (\pm0.32) \times MY + 45.1\ (\pm6.96) \times MProt + 1.5\ (\pm0.13) \times CP$
		North America	940	17.9	0.93	0.37	0.88	0.28		$-497.6\ (\pm79.61) + 4.1\ (\pm0.32) \times MY + 3.2\ (\pm0.85) \times MUN + 88.0\ (\pm8.73) \times MProt + 2.6\ (\pm0.43) \times CP$

[1]Model categories are as follows. Simple models: DMI only (DMI_M), N intake only (N intake_M), dietary nutrient contents variables (Diet_M), lactation performance variables (Milk_M), MUN only (MUN_M), MUN and N intake (MUN_Nintake_M). Complex models: all significant independent variables among variable categories were used for the full model selection (Full_M); all significant independent variables easily and routinely measured on field (Full_Field_M). Models based on intake variables led to models using N intake, as for Equation [17]. Locations are as follows. Intercontinental: Belgium, Canada, Chile, Costa Rica, Denmark, Finland, France, Germany, Ireland, the Netherlands, New Zealand, Norway, Sweden, Switzerland, the UK, and the US. Europe: Belgium, Denmark, Finland, France, Germany, Ireland, Norway, the Netherlands, Sweden, Switzerland, and the UK. North America: the US and Canada.

[2]In parentheses: ±SE. DMI (kg/d); CP (g/kg DM); N intake (g/d); NDF intake (g/d); MY = milk yield (kg/d); MProt = milk protein (%); MUN (mg/dL).

[3]n = number of observations used to evaluate equations; RMSPE = root mean square prediction error, expressed as a percentage of observed mean daily N excretion; RSR = RMSPE-observations SD ratio; MB = mean bias as a percentage of root mean square prediction error; SB = slope bias as a percentage of root mean square prediction error; CCC = concordance correlation coefficient.
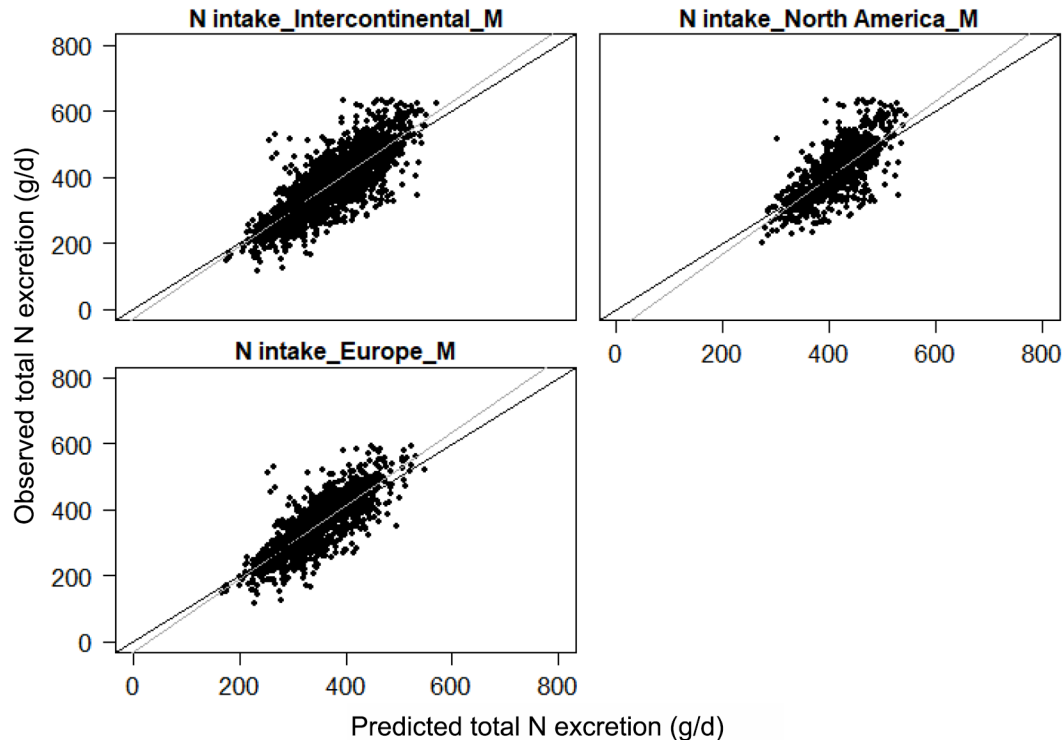
Figure 5. Predicted vs. observed values based on total manure N excretion (g/d per cow) prediction equations with N intake_M models (N intake only) developed for all locations. Gray lines represent the fitted regression line for the relationship between predicted and observed values, and black lines represent the identity line (y = x).

lost mainly as urinary urea N. There are 2 main sources of urinary urea N: surplus of rumen degradable N (relative to rumen available energy for microbes), leading to NH_3-N absorption, and surplus absorbed amino acid-N (relative to what the body can use for milk protein and body accretion N), which is deaminated and gives rise to NH_3 production (Spek et al., 2013a). Results of the present study hence agree with previous findings that N intake is the main driver of total manure N excretion.

In complex models, along with N intake, Reed et al. (2015) used both dietary contents of NDF and CP as predictors of fecal, urinary, and total N excretion. In our study, dietary CP was used in simple models as well as in fecal Full_M models and urinary and total N excretion Full_field_M models, whereas dietary NDF was not included in fecal Full_M models for North American data. However, urinary Full_Field_M included dietary NDF content with North American data. Dietary NDF was included in simple fecal and urinary Diet_M models developed with North American data as well as in simple total manure N Diet_M models with intercontinental data. In the simple models, the coefficient sign for NDF was positive when predicting fecal N excretion and negative for urinary N excretion in our study but was positive in Reed et al. (2015),

whereas the coefficient sign was positive for dietary CP in both studies.

The effect of dietary CP content on N excretion has been well studied (Wattiaux and Karg, 2004; Colmenero and Broderick, 2006; Aguerre et al., 2010), and results generally compare well with the present study. Decreasing dietary CP contents from 17.5 to 12.5% reduced urine N excretion by 59% (and by 69% when additional rumen-protected methionine was supplied), total N emissions from slurry by 72%, and N_2O emissions from farmyard manure with urine-rich slurry by 78% (Kröber et al., 2000; Külling et al., 2001). The present results are also in line with several studies which indicated that increasing dietary CP content linearly increased N excreted in feces and urine (Colmenero and Broderick, 2006; Weiss et al., 2009; Spek et al., 2013a). It has been shown that any increase in dietary protein or N intake would lead to substantial increases in urinary loss (Van Soest, 1994), with any surplus of N ingested in excess of requirements being excreted in urine (Castillo et al., 2000). Moreover, the true urea N part of urine N decreases more with decrease in CP content than the nonurea N part in urine N (Spek et al., 2013b).

It is well known that dietary factors, such as the content of dietary CP (Marini et al., 2004; Huhtanen
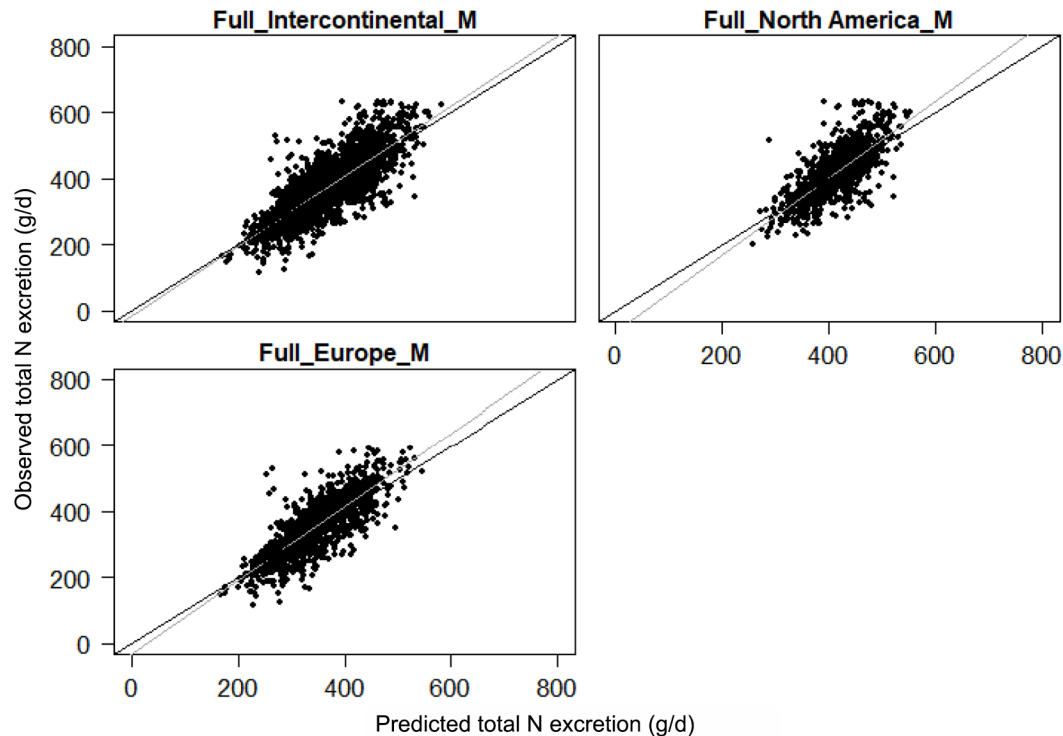
Figure 6. Predicted vs. observed values based on total manure N excretion (g/d per cow) prediction equations at the highest level of complexity (Full_M) for all locations. Gray lines represent the fitted regression line for the relationship between predicted and observed values, and black lines represent the identity line (y = x).

and Hristov, 2009) or diet digestibility (Theurer et al., 2002), affect apparent N utilization in ruminants. Apparent N utilization is reported to increase and urinary N excretion to decrease with highly digestible diets containing cereal grains such as barley (Cohen et al., 2006). As discussed by Dijkstra et al. (2018), apparent N utilization and excretion are not exclusively driven by dietary protein concentration but also depend on dietary carbohydrate composition and digestible energy supply, with decreased milk N output with high-fiber diets compared with high-starch isoenergetic diets. In their complex models, Reed et al. (2015) observed a positive relationship between dietary NDF and urinary and total N excretion but a negative relationship between dietary NDF and fecal N excretion. However, as mentioned before, the results of our study are different. Indeed, dietary NDF content had a positive effect on fecal N excretion in the Diet_M models (North American data only) and Full_M models (intercontinental and European data), and a negative effect on urinary N excretion in simple or Full_M models (North American data only) and on total manure N excretion in simple models (intercontinental data only). These differences may be due to the variability in NDF contents or nature within studies. Indeed, the nature of the NDF used in this study is more diverse than in that of Reed et

al. (2015), due to the larger and more diverse source of data collected from all around the world, and not only in a specific region in the United States. In addition, we can speculate that N excretion is indirectly linked to dietary NDF, because both are related to DMI, and that presumably greater NDF content will coincide with lower DMI, leading to lower N excretion in urine and total manure. Indeed, it has been shown that greater dietary NDF content is usually linked to lower DMI (Arelovich et al., 2008), which in turn is linked to lower N excretion. However, high dietary intakes and high amounts of fiber in the diet are also associated with greater endogenous N losses from the gut (Ouellet et al., 2002), which has a major contribution to fecal N excretion (Huhtanen et al., 2008), and thus might explain the positive relationship between dietary NDF and fecal N excretion. In addition, Castillo et al. (2000) also reported that fiber-based supplements tended to increase fecal N excretion compared with starch-based concentrates.

In several models, MProt was positively related to fecal, urinary, and total manure N excretion. Increasing dietary CP content has been reported to increase milk protein yield (Colmenero and Broderick, 2006), and greater level of dietary CP will most likely have greater N excretion, as mentioned before. Milk urea N was also

positively related to both urinary and total manure N excretion, and MUN was selected in Full_M models for every region, except for the Full_M model to predict total N excretion with European data. A positive linear relationship between N excreted in urine and MUN was found also by Kauffman and St-Pierre (2001). Increasing N intake leads to a more pronounced increase in urinary N excretion than in fecal N excretion (Kebreab et al., 2001) and generally results in an elevated concentration of MUN. In addition, MUN, urine volume, and urinary excretion of total N and urea N all increased in response to dietary CP content. Milk urea N concentration has already been suggested as a predictor of N excretion in urine by Jonker et al. (1998), who proposed a simple model of N balance from 4 in vivo studies, gathering 70 observations in dairy cows from North America. Spek et al. (2013b) compared 5 equations to predict urinary N excretion based on MUN, and observed large differences between these equations in predicted urinary N excretion. Spek et al. (2013a) performed a meta-analysis using MUN as a predictor of urinary N excretion with data from Europe and North America demonstrating different relationships for both data sources. In our study, we also noticed different prediction performances between Europe and North America, which could be partly explained by location-specific diets.

Simple models including only MUN led to higher predictive errors for urinary N excretion compared with total manure N excretion. However, models based on MUN and predicting urinary N excretion led to 28.1 to 33.9% error. From a theoretical viewpoint, one would expect MUN to correspond better to urinary N excretion than to total manure N excretion, as both urine urea N excretion and MUN are a function of blood urea nitrogen concentration. However, not all urine N is urea N, and it has been shown that variation exists in the fraction of urea N in urine N (62 to 86% of urinary N excreted with urea), as discussed via a modeling approach by Dijkstra et al. (2018). The accuracy of MUN as a predictor of urinary N excretion may improve when various factors that affect this relationship can be taken into account (reviewed by Spek et al., 2013b). These factors include BW, water intake, dietary CP content, and frequency of milking and feeding. Not surprisingly, MUN and N intake used simultaneously in urinary and total manure N excretion models led to better prediction performance than MUN alone. For urinary N excretion, the MUN and N intake used simultaneously led to better prediction performance than N intake only. For total manure N excretion models, simple equations based on N intake and complex equations based on N intake and MUN led to similar prediction performance. Thus, MUN does not help in explaining more variation.

This is in contrast to urinary N excretion, where MUN added to N intake explained some more variation in urinary N excretion.

Application of N Excretion Models

The simplest models for N excretion in feces and urine based on N intake led to about 18 to 27% prediction errors, depending on the location. These models led to better prediction ability for total manure N excretion by reducing the error to about 13 to 14%. Simple models based on dietary composition or milk parameters seemed more adapted for total manure N excretion than for fecal or urinary N excretion. This may be partly related to difficulties in measurement techniques to determine fecal and urinary N excretion (Hristov et al., 2019), where errors in measurement in fecal N excretion may be counterbalanced by errors in urinary N excretion and vice versa.

We observed that the accuracy of prediction of fecal, urinary, or total manure N excretion was similar or best with models that included dietary intakes, either DMI or N intake, along with a few other covariates, such as dietary CP, NDF, MProt, or MUN. These other covariates may reflect factors that modify the partitioning of N excreted in urine and feces. Using all available variable information did not consistently improve prediction performance with the full models compared with simpler models and between locations. Models that include DMI or N intake showed better performance than models without DMI or N intake, indicating that these variables are among the main drivers of N losses in either feces or urine. One important reason that N excretion is related to N intake is simply that the variation in N intake is large (200–900 g of N/d). But DMI and N intake also account for variation in energy and protein supply (i.e., DMI, N intake), or ruminal and postruminal protein supply, protein retained by the animal, and protein secreted in milk, which determines the amount of digested and undigested N as well as the fraction of digested N that is not retained by the animal and excreted in feces and urine.

Models generally differed between locations, with different coefficients, prediction performance, and, in some cases, variables included in models; thus, models are location-specific. For instance, all simple models to predict fecal N excretion performed better with North American data than with intercontinental or European data. For urinary N excretion, N intake models performed better with European data than with intercontinental or North American data. Intercontinental models were developed based on a data set containing a greater proportion of European data compared with North American or other location data (73.3%

vs. 23.0% and 3.6%, respectively). Thus, logically the greater proportion of European data influenced the results toward better prediction for intercontinental models compared with North American. For different models (Diet_M, Full_M, or Full_Field_M) derived from intercontinental, European, or North American data, different predicting variables were sometime used. Overall, a need exists to use location-specific models when predicting N excretion in feces, urine, or total manure, and the discrepancies are probably due to the different types of diets used among regions of the world. This is not an unexpected result, as previous analyses of enteric methane prediction models in dairy cattle or beef cattle following a similar approach to ours came to similar conclusions (Niu et al., 2018; van Lingen et al., 2019). Dufrasne et al. (2013) also pointed out the importance of environmental factors that would directly affect animal productivity and hence N retained by the animal, such as heat stress, disease, BW, and feeding behavior, which are indirectly linked to location.

Estimation of DMI and, along with this, N intake is still challenging on farm in practice because it is not routinely monitored. Voluntary DMI prediction equations require individual animal information that is in part routinely available (milk production and composition, DIM), or in part not routinely available (BW; e.g., NRC, 2001; Lahart et al., 2019), or are based on dietary energy content and energy requirements for maintenance, milk production, body growth, and pregnancy. Nonetheless, variables not yet routinely available could be obtained with calculations based on feeding systems or from milking robot records, specifically for BW, which is recorded at each milking visit. But even then, it remains to be shown whether intake estimations are accurate enough to provide an advantage over milk parameters based on intake estimations, as milk parameters are obtained as accurate data. In addition, current research is oriented toward the use of Fourier-transform mid-infrared spectroscopy of milk components, which shows promise for predicting N use efficiency, as shown by Grelet et al. (2020). Milk N, which is used in models developed in this study, is already routinely estimated using mid-infrared spectroscopy with a high degree of accuracy. Thus, the use of full models to predict total manure N excretion using MY, milk protein content, and diet CP content, which could be easily obtained on farm with confinement systems, is preferred only if N intake data are not available. Otherwise, simple models based on MUN and N intake or full models based on N intake, dietary NDF, MUN, and milk protein content would be better suited. For urinary N excretion, in absence of intake data, full models based on diet and milk parameters would be preferred. Simple models based on milk parameters would be chosen for fecal N

excretion, but, if intake data are available, then simple models based on N intake would be preferred. Thus, information on intake is required for good prediction of N excretion, in particular of total manure N excretion. In absence of intake data, milk and diet composition parameters may be used, but prediction accuracy of N excretion will decrease.

CONCLUSIONS

Models based on DMI or N intake can be used to predict fecal and total manure N excretion with good accuracy, and urinary N excretion with satisfactory accuracy. Prediction accuracy may be somewhat further improved by adding diet composition or milk parameters to intake parameters in complex models. In absence of intake data, models using diet composition and milk performance parameters could be used to predict fecal, urinary, and total manure N excretion, but with greater prediction error and occurrence of MB or SB, or both. Intercepts and slopes of variables in optimal prediction equations developed on intercontinental, European, and North American bases differed from each other, and region-specific models are preferred to predict N excretion. Complex, region-specific models to predict total manure N excretion should be used when inputs are available, whereas simple location-specific models based on DMI or N intake should be used for fecal and urine N excretion prediction.

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