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Prediction of nitrogen use in dairy cattle: a multivariate Bayesian approach

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Abstract. Quantification of dairy cattle nitrogen (N) excretion and secretion is necessary to improve the efficiency with which feed N is converted to milk N (ENU). Faecal and urinary N excretion and milk N secretion are correlated with each other and thus are more accurately described by a multivariate model that can accommodate the covariance between the three observations than by three separate univariate models. Further, by simultaneously predicting the three routes of excretion and taking advantage of the mass balance relationships between them, covariate effects on N partitioning from feed to faeces and absorbed N and from absorbed N to milk and urine N and animal ENU can be estimated. A database containing 1094 lactating dairy cow observations collated from indirect calorimetry experiments was used for model development. Dietary metabolisable energy content (ME, MJ/kg DM) increased ENU at a decreasing rate, increased the efficiency with which feed N was converted to absorbed N and decreased the efficiency with which absorbed N was converted to milk N. However, the parameter estimate of the effect of ME on post-absorption efficiency was not different from zero when the model was fitted to a data subset in which net energy and metabolisable protein were at or above requirement. This suggests the effect of ME on post-absorption N use is dependent on the energy status of the animal.

Additional keywords: protein nutrition, modelling.

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Introduction

Efforts to improve nitrogen (N) utilisation by dairy cattle are motivated by the environmental and economic impacts of N lost from dairy production systems. Nitrate leaching from manure to surface and ground waters contributes to eutrophication, and has led to a 30-fold increase in hypoxic zones in the US in the past 50 years (McCubbin *et al.* 2002; Diaz and Rosenberg 2008). Manure ammonia volatilisation can cause respiratory damage to humans and animals in proximity to accumulation sites, resulting in economic losses of up to US\$12 billion/year (McCubbin *et al.* 2002). Transformations of ammonia and nitrate via nitrification and denitrification processes make urinary N, in particular, an important source of nitrous oxide (Dijkstra *et al.* 2013a), which is a greenhouse gas with a global warming potential 298 times that of carbon dioxide (IPCC 2006). In addition, N excretion has a direct impact on the producer's economy because N in feed not captured in milk or meat reduces utilisation efficiency, with a resultant economic loss. Finally, N release to the environment is regulated through legislations such as the *US Environmental Protection Agency's Clean Water Act* (US Senate 2002). Due to these economic, environmental and health impacts, considerable resources are directed at finding strategies to mitigate N losses

from dairy production. Diet manipulation to improve the efficiency of N use (ENU) is one such strategy that has the potential to influence animal, farm and ecosystem efficiency.

At the individual animal level in lactating cows, ENU can be defined as the proportion of ingested N that is secreted in milk. The estimated theoretical maximum ENU, based on known physiological and biochemical relationships, is 0.43 (Dijkstra *et al.* 2013b; N secreted in milk true protein only); however, the observed efficiencies in both production and experimental settings are usually much lower (Calsamiglia *et al.* 2010). In their review of N utilisation in dairy cattle, Castillo *et al.* (2000) found variable results with respect to improving ENU. The physiological mechanisms that drive N utilisation are numerous and, although some may be discretely predictable, their integration to predict whole animal N efficiency is not dependable. Of the diet-based strategies for influencing ENU reviewed, reducing diet crude protein (CP) was the only strategy that conclusively improved ENU (Castillo *et al.* 2000). Other methods to manipulate the amount, rate, and site of digestion of dietary energy have had variable effects on N utilisation despite popular theories about the effects of energy–protein synchrony and post-ruminal starch digestion (Reynolds *et al.* 1996; Rius

et al. 2010). Mathematical models of N use and digestion become an important tool to investigate the discrepancy between theory and practice through identification and quantification of dietary and animal factors that influence ENU.

Several studies have reported empirical, univariate models to predict N excretion from dairy cattle (Jonker *et al.* 1998; Kebreab *et al.* 2000, 2002, 2010; Nennich *et al.* 2006; Yan *et al.* 2006; Huhtanen *et al.* 2008; Marini *et al.* 2008; Higgs *et al.* 2012). These studies range in model complexity, development methods and selected covariates. All models included either N intake (NI) or dry matter intake (DMI) as a significant predictor of each form of N excretion – faecal (FN), urinary (UN), milk (MN) and total manure (TN) N. In agreement with biological and mass-balance principles, the model-predicted relationships between intake and excretion are always positive. As N intake increases, however, the relative increase in MN is smaller than the increase in TN, resulting in a decrease in ENU (Castillo *et al.* 2000; NRC 2001). There is less consensus, however, about which additional covariates (e.g. digestible energy content (MJ/kg DM, DE), metabolisable energy content (MJ/kg DM, ME), neutral detergent fibre (% DM, NDF), and ether extract (%DM, EE)) improve prediction of N excretion.

Metabolisable energy content and ME intake have been proposed as covariates in recent studies to predict N excretion (e.g. Huhtanen *et al.* 2008; Kebreab *et al.* 2010) on the basis of biological principles. Increasing rumen-available energy is expected to increase microbial fermentation and thus microbial N production, increasing the supply of digestible N (Dijkstra *et al.* 1998; NRC 2001). However, a proportion of this microbial N is nucleic acid N, and various products from purine metabolism (allantoin, uric acid, xanthine and hypoxanthine) are excreted in urine (Dijkstra *et al.* 2013a). Evidence also suggests that diet energy content influences post-absorption N metabolism by increasing milk protein synthesis independent of its effects on digestible protein supply (Reynolds *et al.* 1996; Raggio *et al.* 2006; Rius *et al.* 2010). Assuming the previous statements are accurate, diet energy content, as represented by ME, is expected to have a positive effect on ENU at the following two independent stages of N use within the dairy cow: first, increasing the proportion of NI that is digested and absorbed (although some absorbed N is unavoidably excreted in urine); and second, increasing the proportion of absorbed N that is secreted in milk.

Extant N excretion models are predominantly univariate and are incapable of distinguishing between the pre- and post-absorptive effects of ME on ENU. Multivariate models, however, improve prediction because the covariance among correlated dependent variables FN, UN and MN is accommodated and inference is gained. To our knowledge, the only existing multivariate model of N excretion was proposed by Kebreab *et al.* (2010). Although their results indicated ENU increases with metabolisability (MJ ME/MJ GE), the structure for the mean vector in which each mean is described by a linear combination of NI and ME does not facilitate estimation of the separate effect of ME on the two phases of N partitioning. Incorporation of known biological relationships in the mean structure of a multivariate model to describe and predict FN, UN, and MN simultaneously will enable distinction between the two phases of N partitioning and the variables that influence them. The objectives of the present study were to (1) develop a

multivariate model of FN, UN and MN incorporating the biological relationships inherent in N partitioning, and (2) investigate the effect of dietary energy on N partitioning.

Materials and methods

Data sources

The dataset used for model development consists of 1094 observations collected from 40 indirect calorimetry studies conducted at USDA's former Energy and Metabolism laboratory in Beltsville, MD, over 30 years. The data contain detailed chemical analyses of diet and total nutrient excretions. The data have a wide range of dietary parameters that encompass most diets used in current production settings (Table 1). In total, 298 individual animals were used across experiments, including primiparous and multiparous lactating cows from Holstein and Jersey breeds. Animals appear in multiple studies, and thus are not fully nested within study. For details of data collection and consolidation methods, see Wilkerson *et al.* (1997).

Multivariate model development

A Bayesian framework was adopted for model development as it naturally accommodates the model hierarchy required for the proposed mixed-model structure. Further, advances in computing power and Markov Chain Monte Carlo (MCMC) simulation facilitate parameter estimation and interpretation when analytical solutions are complex or intractable. A multivariate normal distribution was assumed in which FN, UN, and MN residuals are assumed to be correlated to accommodate the covariance among the three forms of excretion. The model for the data conditional on model parameters is described by

$$\mathbf{Y}_{ijk} \sim N_3(\boldsymbol{\mu}_{ijk}, \boldsymbol{\Sigma}), \quad (1)$$

where \mathbf{Y}_{ijk} is the 3×1 vector of the k th record ($k = 1, \dots, n_{ij}$) on the j th animal ($j = 1, \dots, J$) in the i th study ($i = 1, \dots, I$); the

Table 1. Units, means, standard deviations and ranges of dietary and animal parameters in the dataset used for model development

Covariate	Unit	Mean	s.d.	Min.	Max.
Nitrogen intake	g/day	432	145.8	79.9	932
Dry matter intake	kg/day	16.5	4.27	3.94	29.4
Gross energy	MJ/kg DM	18.9	0.52	17.8	20.7
Digestible energy	MJ/kg DM	12.5	0.95	8.53	15.8
Metabolisable energy	MJ/kg DM	10.8	0.90	6.85	14.6
Organic matter	%DM	93.6	1.11	87.9	96.3
Dry matter content	%	65.3	19.8	30.2	100
Ether extract	%DM	2.78	0.97	0.99	7.02
Neutral detergent fibre	%DM	34.3	7.44	14.9	76.1
Acid detergent fibre	%DM	20.0	4.20	7.67	47.1
Lignin	%DM	4.43	1.44	0.52	9.42
Crude protein	%DM	16.2	2.51	5.17	23.5
Milk	kg/day	23.4	10.1	0.29	56.6
Milk crude protein	%	3.25	0.39	2.30	5.75
Milk fat	%	3.67	0.79	1.43	7.60
Day in milk	day	162	79	11	398
Age	month	64.2	24.5	25	180
Bodyweight	kg	594	88.4	302	854

mean vector μ_{ijk} is modelled as a function of a matrix of covariates \mathbf{X}_{ijk} and parameters; Σ and is the 3×3 residual variance covariance matrix. Some covariates were standardised ($\mathbf{X}^S = \frac{\mathbf{X} - \bar{\mathbf{X}}}{S_x}$ where S_x is the standard deviation of \mathbf{X}) so as to facilitate computation and are designated by a superscript 'S'. The components of the mean vector were modelled as

$$\begin{bmatrix} \mu_{ijk}^{(\text{FN})} \\ \mu_{ijk}^{(\text{UN})} \\ \mu_{ijk}^{(\text{MN})} \end{bmatrix} = \begin{bmatrix} \beta_0 + \beta_1 \times \text{NI}_{ijk}^S + \beta_2 \times \text{ME}_{ijk}^S + S_{1i} + A_{1i} \\ (\text{NI}_{ijk} - \text{FN}_{ijk} - \text{RN}_{ijk}) \times (1 - P_{ijk}) + S_{2i} + A_{2j} \\ (\text{NI}_{ijk} - \text{FN}_{ijk} - \text{RN}_{ijk}) \times P_{ijk} + S_{3i} + A_{3i} \end{bmatrix}, \quad (2)$$

where $\mu_{ijk}^{(\text{FN})}$, $\mu_{ijk}^{(\text{UN})}$, $\mu_{ijk}^{(\text{MN})}$ are the expectations of the three responses; β_0 is the expected value of $\mu_{ijk}^{(\text{FN})}$ at mean values of NI and ME; β_{1-2} are the slopes describing the linear relationships between the dependent variable FN and NI (NI_{ijk}^S), and ME content of the diet (ME_{ijk}^S) respectively. RN_{ijk} is the observed retained N, which is defined as the ingested N (g/day) that is not accounted for in milk, faeces or urine. It includes both positive and negative values and represents the N that is either retained within the animal or lost from the animal on the day of the record. In the present data, RN has a mean of 12.9 (g/day) and a standard deviation of 27.2 (g/day). P_{ijk} is the partition coefficient (ranges between 0 and 1) describing the partition of the absorbed N into urine or milk, modelled through the following functional form:

$$P_{ijk} \sim N(\mu_{ijk}^{(P)}, \sigma_P^2),$$

$$\mu_{ijk}^{(P)} = \frac{1}{1 + e^{(\beta_3 + \beta_4 \times \text{DIM}_{ijk}^S + \beta_5 \times \text{ME}_{ijk}^S)}}, \quad (3)$$

where $\frac{1}{1+e^{-\beta_3}}$ is the expected value of P at mean values of DIM and ME; and β_4 is the estimate of the effect of DIM on P_{ijk} ; and β_5 is the estimate of the effect of ME on P_{ijk} . The functional form specified for $\mu_{ijk}^{(P)}$ behaves as a link function that maps values of $\mu_{ijk}^{(P)}$ into the (0, 1) interval. Further, placing a negative value on the exponent causes the response of $\mu_{ijk}^{(P)}$ to DIM and ME to be in the same direction as their coefficients. Specifically, a positive estimate for β_5 corresponds to increases in the estimate for $\mu_{ijk}^{(P)}$ as ME increases, while a negative estimate for β_5 corresponds to decreases in the estimate for $\mu_{ijk}^{(P)}$ as ME increases. The random effects $S_{.i}$ and $A_{.j}$ were incorporated into $\mu_{ijk}^{(\text{FN})}$, $\mu_{ijk}^{(\text{UN})}$, and $\mu_{ijk}^{(\text{MN})}$ to account for study and animal variability in the model structure where the $S_{.i}$ is the random effect associated with the i th study and $A_{.j}$ is the random effect associated with the j th animal. Each vector of random effects was assumed to be distributed as $N_3(\mathbf{0}, \Omega_{(S, A)})$, where $\Omega_{(S, A)}$ is a 3×3 positive definite matrix that specifies the covariance structure of the random effects.

By designating $\mu_{ijk}^{(\text{UN})}$ and $\mu_{ijk}^{(\text{MN})}$ to be complementary proportions of the difference between NI and the sum of FN_{ijk} and RN_{ijk} , the mean structure preserves the mass-balance relationships among the three levels of excretion. Biologically, UN and MN are modelled as a partition of the remaining ingested NI after FN has been removed and net N retention or mobilisation (RN) has been accounted for. In addition, the model allows for separation of the impact of ME on N

partitioning into two phases. The first phase estimates the effect of ME on the partitioning of NI into absorbed N and FN through estimation of β_2 . In the second phase, the impact of ME on the partitioning of absorbed N to UN and MN is estimated through estimation of β_5 . The model assumes that all NI is accounted for in FN, UN, MN, and RN. Although retained N measured through N balance trials can include significant mean and slope bias (Spanghero and Kowalski 1997), the methods used to generate the current data resulted in minimal mean and no evident slope bias; supporting the mass-balance assumption required for the model.

Most parameters were given minimally informative priors with large variances such that $\beta_{0-2; 6-8} \sim N(0, 10^6)$. However, the parameters acting on the partition coefficient, P_{ijk} , were given slightly more informative priors due to the link function used to estimate this parameter. The range of the proportion of absorbed N excreted in milk (0.01, 0.75) can be used as an estimate of the true biological range of P . Assuming P falls within this range and using the endpoints as inputs to the inverse of the link function (3) to solve for $-(\beta_3 + \beta_4 \times \text{DIM}_{ijk}^S + \beta_5 \times \text{ME}_{ijk}^S)$, we find that this sum is constrained to a range smaller than $(-5, 5)$. Thus, β_{3-5} were also assigned Gaussian priors with a zero mean but with variances set to 1000 because within the expected range of the true parameters, a prior distribution of $N(0, 1000)$ is still a minimally informative prior. Minimally informative Gaussian priors were specified for the study (S_i) and animal (A_j) random effects with means of zero and large variances. Random effect means were monitored within each simulation to check that their values remained close to zero. The prior for the variance covariance matrix of the data (Σ) and of the random effects ($\Omega_{(S, A)}$) were modelled as an inverse Wishart distributions ($IW_3(\mathbf{I}_3, 3)$), where \mathbf{I}_3 is the identity matrix of order three.

Model performance and evaluation

Parameters were estimated by MCMC methods for which all simulations were conducted in OpenBugs software (Lunn *et al.* 2009). In short, the MCMC method simulates the posterior densities by constructing Markov Chains, for which limiting distributions approximate the posterior density, and using Monte Carlo integration to compute integrals and expectations. As a general rule, the error in the Monte Carlo approximation is reduced by increasing the number of samples, up to a point at which no further gain in accuracy is achieved in the approximation of the posterior summaries. A minimum of 100 000 iterations after the burn-in period were saved to obtain posterior distribution estimates. Initial values were chosen from within designated parameter spaces using a random-number generator. Convergence was assessed through visual inspection of multiple-chain history, Gelman–Rubin diagnostic plots, and a comparison of the Monte-Carlo error and standard deviation (Ntzoufras 2009). Auto-correlation was visually assessed and chains were thinned by a factor of 10. A burn-in period of 10 000 samples was sufficient to reach convergence.

Several techniques were used to assess model fit, accuracy and precision. Model assumptions were checked with univariate residual normal probability plots, plots of standardised residuals against centred predicted values, and residual density plots. Model fit was assessed using the deviance information criteria

(DIC) which is a combined measure of model fit and complexity with goodness of fit indicated by smaller values (Spiegelhalter *et al.* 2002). Observed values were plotted against the predicted values to visually assess deviations from the line of unity as an indication of model bias and accuracy. The root mean-square prediction error (RMSPE)

$$\text{RMSPE} = \sqrt{\frac{(\mathbf{Y} - \mathbf{E}[\mathbf{Y}])'(\mathbf{Y} - \mathbf{E}[\mathbf{Y}])}{n}} \quad (4)$$

was tracked within simulations to quantitatively assess model prediction accuracy. Here, \mathbf{Y} is the vector of observations (UN, FN or MN), $\mathbf{E}[\mathbf{Y}]$ is the vector of the posterior means of the expected values (UN, FN or MN) and n is the number of observations.

Univariate models in which the covariance between FN, UN and MN was assumed to be zero and a multivariate model as described by Kebreab *et al.* (2010) were fitted to the data for comparison of model fit and accuracy. In both cases, the model for the data follows that outlined in Eqn 1; however, in the univariate setting, the off-diagonal elements of Σ are set to zero. The mean structures for the univariate models are

$$\begin{aligned} \mu_{ijk}^{(\text{FN})} &= \beta_0 + \beta_1 \times \text{NI}_{ijk}^S + \beta_2 \times \text{ME}_{ijk}^S + S_{1i} + A_{1j} \\ \mu_{ijk}^{(\text{UN})} &= \beta_3 + \beta_4 \times \text{NI}_{ijk}^S + \beta_5 \times \text{ME}_{ijk}^S + S_{2i} + A_{2j} \\ \mu_{ijk}^{(\text{MN})} &= \beta_6 + \beta_7 \times \text{NI}_{ijk}^S + \beta_8 \times \text{ME}_{ijk}^S + S_{3i} + A_{3j} \end{aligned} \quad (5)$$

where $\beta_{0,3,6}$ are the expected values of FN, UN, and MN at mean NI and ME; $\beta_{1,4,7}$ are the parameter estimates of the effect of NI on FN, UN, and MN; $\beta_{2,5,8}$ are the parameter estimates of the effect of ME on FN, UN, and MN. The random effects of study (S_i^*) and animal (A_j^*) were assumed to be mutually independent for each form of excretion and assumed to be distributed as $N(0, \sigma_{(S, A)}^2)$.

In the case of the multivariate model described by Kebreab *et al.* (2010), the data, as described by Eqn 1, have the following mean structure:

$$\begin{bmatrix} \mu_{ijk}^{(\text{FN})} \\ \mu_{ijk}^{(\text{UN})} \\ \mu_{ijk}^{(\text{MN})} \end{bmatrix} = \begin{bmatrix} \beta_0 + \beta_1 \times \text{NI}_{ijk}^S + \beta_2 \times \text{ME}_{ijk}^S + S_{1i} + A_{1j} \\ \beta_3 + \beta_4 \times \text{NI}_{ijk}^S + \beta_5 \times \text{ME}_{ijk}^S + S_{2i} + A_{2j} \\ \beta_6 + \beta_7 \times \text{NI}_{ijk}^S + \beta_8 \times \text{ME}_{ijk}^S + S_{3i} + A_{3j} \end{bmatrix}, \quad (6)$$

where the $S_{.i}$ and $A_{.j}$ parameters have the same interpretation as the univariate model but the random effects for each study and animal are modelled as a 3×1 vector distributed as $N_3(\mathbf{0}, \Omega_{(S, A)})$ as with the proposed model. The RMSPE was used for comparison of model prediction accuracy and the DIC was used for comparison of model fit.

Finally, so as to investigate whether the effect of ME on ENU changes with the overall energy and N balance of the cow, the proposed model was fitted to a subset of the data in which the dietary net energy for lactation (NE_L) and metabolisable protein (MP) were supplied at or above the animal's calculated requirement level. The requirements of NE_L were calculated

by dividing the NE of the observed milk production by the efficiency of using ME for lactation, and the MP requirements for lactation (MP_L) were calculated by dividing the observed milk protein (kg) by the efficiency of using MP for lactation (0.67) (NRC 2001). The supplied NE_L for lactation was calculated by the difference in NE consumed and the sum of NE requirements for pregnancy and maintenance (NE requirements for growth were assumed to be negligible for multiparous cows), on the basis of the recommendations of NRC (2001). Supplied MP_L was calculated as the difference between NI and the sum of FN and MP requirements for maintenance (in units of g MP N) where the MP requirements for maintenance were also estimated on the basis of NRC (2001) recommendations. The resulting dataset included 260 observations from 31 studies and 115 animals. In addition, NI ranged from 88 to 813 g with a mean of 396 g; ME ranged from 8.2 to 13.5 MJ/kg DM with a mean of 10.9 MJ/kg DM; and DIM ranged from 45 to 393 days with a mean of 179 days.

Results

The posterior distribution means and standard deviations for β and Σ parameters are listed in Table 2. The parameters β_{1-2} estimate the effects of NI and ME on FN excretion respectively. The estimates were similar for all three models fitted to the full dataset (Models 1–3). Furthermore, the standard deviations were relatively small compared with posterior means, which is an indication of a small variance in the sampling distributions of these parameters and stability in the parameter estimation. As expected, FN increased with increasing NI and decreased with increasing ME. The results for $\beta_{4,5,7,8}$ (Eqns 5, 6), estimating the effect of NI and ME on UN and MN, were also similar for the univariate and the multivariate model proposed by Kebreab *et al.* (2010). Consequently, incorporation of the covariance did not affect the mean structure estimates and only influenced the residual variance. The effect of ME on UN and MN, represented by β_5 and β_8 in Models 1 and 2 (Eqns 5, 6), were positive, predicting an increase in milk and urine N as ME content of diet increases. However, β_8 is much smaller than β_5 . Thus, for a unit increase in ME, in the univariate models and the multivariate model proposed by Kebreab *et al.* (2010), UN was expected to increase by ~2.5 g more than MN. In addition, the coefficient of variation ($\text{CV} = \sigma/\mu$) for β_8 was relatively large (0.513) and the 95% credible interval encompassed zero (−0.00032, 3.36), which suggests that the relationship of ME with MN was more variable than its relationship with UN and its effect may be negligible.

In the proposed model (Eqn 2, Table 2), β_4 and β_5 estimate the effect of DIM and ME on the proportion of absorbed N secreted in milk (P) instead of their effect on UN. The negative values for both parameter estimates predicted a decrease in P for every unit increase in DIM or ME. The CVs for β_4 and β_5 were 0.052 and 0.19 respectively. Although the CV for β_5 is large compared with other parameters, the 95% credible interval remains negative as (−0.0852, −0.0399). The variance of P as estimated by σ_x^2 was 0.0198. Plugging the estimates of β_{3-5} into the link function used to predict $\mu^{(P)}$ or $\text{E}[P]$ (Eqn 3, Table 2), yielded the expected change in P as DIM and ME change. When DIM and ME were at their respective means,

$E[P|DIM^S = 0, ME^S = 0] = 0.422$. Holding ME constant and increasing DIM by one standard deviation yielded $E[P|DIM^S = 1, ME^S = 0] = 0.372$, a decrease of 0.049. Similarly, holding DIM constant, and increasing ME by one standard deviation yielded $E[P|ME^S = 1, DIM^S = 0] = 0.407$, a decrease of 0.015. Fig. 1 shows the expected value of P when the DIM is held constant at its mean ($E[P|DIM^S = 0]$) over the range of ME within the data. So as to assess the range of predicted values of $E[P|DIM^S = 0]$, 1000 samples of $E[P|DIM^S = 0]$ were generated within the MCMC simulation for each observed value of ME and plotted against their ME values. The grey points represent a single sample and the black

line is the mean of each sample. For comparison with the post-absorptive N partitioning, the effect of ME on N partitioning to absorbed N and FN ($P_{Dig} = 1 - (E[FN]/NI)$, $E[P_{Dig}|NI^S = 0]$) was also sampled and plotted against ME in Fig. 2. As expected, the proportion of ingested N absorbed increased with increasing ME; however, the change in P_{Dig} per unit of ME was much larger than the change in P . Consequently, the range of $E[P_{Dig}|NI^S = 0]$ over the observed ME contents was roughly (0.50, 0.80), double the length of the range of $E[P|DIM^S = 0]$, which was approximately (0.35, 0.50).

The estimates from the proposed model fit to the data subset are also given in Table 2. The estimates for β_{4-6} have large

Table 2. Model parameter estimates (s.d. in parentheses) to predict faecal (FN), urinary (UN) and milk (MN) nitrogen

Model 1 is three univariate models with the mean structure defined in Eqn 5, Model 2 is the multivariate model proposed by Kebreab *et al.* (2010) and has the mean structure of Eqn 6. Models 3 and 4 are the proposed mean structure defined in Eqn 2 that incorporates mass-balance relationships among the three forms of excretion, fit to the full and subsetted (observations that meet NRC (2001) requirements for net energy and metabolisable protein for lactation) data, respectively. Note that not all β parameters are comparable across models due to differences in model mean structure. β_0 is the expected value of FN at mean nitrogen intake (NI, g/day) and metabolisable energy (ME, MJ/kg DM) for all models. β_1 and β_2 are the estimates of the effect of NI and ME on FN for all models. β_3 is the expected value of UN at mean NI and ME for Models 1 and 2. For Models 3 and 4, the expectation of the proportion of absorbed N secreted in milk (P) at mean NI and ME is given by $\frac{1}{1+e^{-\beta_3}}$. β_4 and β_5 are the effects of NI and ME on UN for Models 1 and 2. For Models 3 and 4, β_4 and β_5 are the effects of days in milk (DIM) and ME on P through the link function $P = \frac{1}{1+e^{-(\beta_3 + \beta_4 DIM + \beta_5 ME)}}$. For Models 1 and 2, β_6 is the expected value of MN at mean NI and ME and β_7 and β_8 are the effects of NI and ME on MN. σ_{FN}^2 , σ_{UN}^2 , and σ_{MN}^2 are residual variance of FN, UN and MN respectively $\sigma_{FN}\sigma_{UN}$, $\sigma_{FN}\sigma_{MN}$, $\sigma_{UN}\sigma_{MN}$ are the covariance between FN and UN, FN and MN and UN and MN respectively

Model	β_0	β_1	β_2	β_3	β_4	β_5	β_6	β_7	β_8	σ_{FN}^2	σ_{UN}^2	σ_{MN}^2	$\sigma_{FN}\sigma_{UN}$	$\sigma_{FN}\sigma_{MN}$	$\sigma_{UN}\sigma_{MN}$
1	141 (2.36)	42.1 (0.72)	-13.4 (0.55)	152 (3.68)	45.5 (1.53)	4.22 (1.12)	112 (2.85)	34.7 (1.13)	1.68 ^A (0.855)	145 (7.58)	603 (31.7)	360 (18.7)			
2	141 (2.29)	42.6 (0.72)	-13.2 (0.55)	152 (3.59)	46.2 (1.53)	4.34 (1.13)	111 (2.75)	34.7 (1.15)	1.67 ^A (0.861)	146 (7.59)	614 (32.5)	363 (19.0)	-120 (11.9)	46.8 (8.70)	-256 (20.0)
3	141 (1.92)	43.4 (0.66)	-13.8 (0.54)	-0.316 (0.0121)	-0.207 (0.0109)	-0.0619 (0.0115)				177 (9.9)	342 (31.2)	342 (31.2)	-152 (13.0)	152 (13.0)	-342 (31.2)
4	142 (3.03)	124 (5.26)	-79.0 (5.53)	0.576 ^A (0.557)	-1.62 (0.599)	-0.607 ^A (0.426)				200 (20.4)	1087 (108)	749 (80.3)	-167 (41.8)	14.7 (30.8)	289 (65.4)

^AThe 95% credible interval of the parameter contains zero.

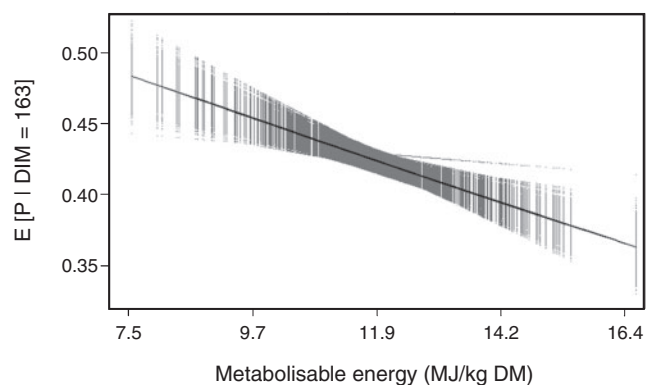


Fig. 1. Plot of 1000 Markov Chain Monte Carlo samples from the proposed model (Eqn 2)-expected values of the proportion of absorbed nitrogen excreted in milk (P) at 163 days in milk (DIM) ($E[P|DIM = 163]$) for each observed value of metabolisable energy. Grey points are individual samples and the black line is the mean of that sample.

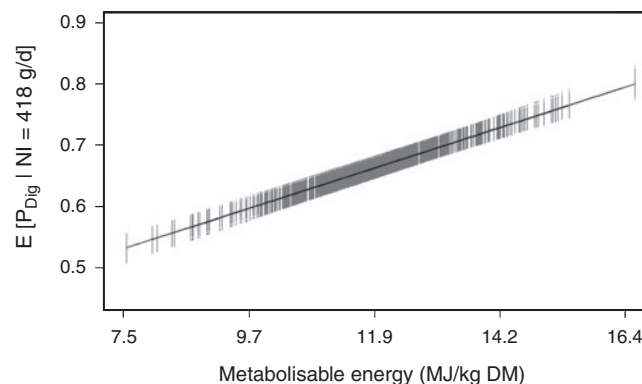


Fig. 2. Plot of 1000 Markov Chain Monte Carlo samples from the proposed model (Eqn 2)-expected values of the proportion of ingested nitrogen (NI) that is not excreted in faeces (P_{Dig}) at the mean NI of 418 g/day. The expectation, $E[P_{Dig}|NI = 418 \text{ g/day}]$, is calculated as $1 - \frac{E[\text{FecalN}|NI=418\text{g/d}]}{NI=418\text{g/d}}$. Grey points are individual samples and the black line is the mean of that sample.

standard deviations and all contain zero within their 95% credible interval. In addition, the variance of P was estimated as $\sigma_X^2 = 0.0433$, more than twice as large as the estimate with the full dataset. These results show that the post-absorptive efficiency of N use is dependent on energetic state of the animal and that there are other factors, not accounted for in the present model, that influence P .

The estimates for the individual residual variances from the univariate model and for Σ for the multivariate models are also given in Table 2. The estimates for $\Sigma_{11,22,33}$ were similar between the univariate model and the multivariate model by Kebreab *et al.* (2010), indicating that no reduction in residual error occurred in moving from the univariate to the multivariate model. In the multivariate model of Kebreab *et al.* (2010), the estimated covariances between FN and UN (Σ_{12}) and UN and MN (Σ_{23}) were negative, while the covariance between FN and MN (Σ_{13}) was positive. Using the covariance estimates from Model 2, the conditional expectations of MN given FN ($E[MN|FN = \mu_{ijk}^{(FN)} + 1]$) or MN given UN ($E[MN|UN = \mu_{ijk}^{(UN)} + 1]$) would increase by 0.32 g and decrease by 0.42 g respectively. Similarly, the decrease in the conditional expectation of UN given FN ($E[UN|FN = \mu_{ijk}^{(FN)} + 1]$) was 0.82 g. The residual variances for UN and MN ($\Sigma_{22, 33}$) for the proposed model (Model 3)

Table 3. Nitrogen excretion model deviance information criterion (DIC) and root mean-square prediction error (RMSPE)

Model 1 is three univariate models with the mean structure of Eqn 7, Model 2 is the multivariate model proposed by Kebreab *et al.* (2010) and has the mean structure of Eqn 8. Model 3 is the proposed mean structure defined in Eqn 2 that incorporates mass-balance relationships among the three forms of excretion. Dhat is a measure of how well the model fits the data. pD is the penalty for the effective number of parameters

Model	DIC	Dhat	pD	RMSPE _{FN} (%)	RMSPE _{UN} (%)	RMSPE _{MN} (%)
1	27 080	26 020	529	12.8 (0.30)	23.6 (0.41)	22.2 (0.26)
2	26 450	25 650	399	12.8 (0.30)	23.4 (0.39)	22.2 (0.26)
3	12 770	11 710	533	12.8 (0.24)	14.7 (0.05)	19.4 (0.07)

were smaller than those of the other two models. The covariances between UN and MN are larger and are not suitable for interpretation because the model forces UN and MN to be strongly negatively correlated as a function of the N partitioning structure.

Model evaluation results are presented in Table 3. The change in DIC between the univariate model and the multivariate model proposed by Kebreab *et al.* (2010) was 630, which indicated an improvement in model fit from inclusion of the covariance parameters. The model developed in the current study further reduced the DIC by 13 680, indicating a great improvement in model fit due to the change in the mean structure of the model. As expected from the results for the fixed effect parameter estimates, prediction accuracy estimated by the RMSPE was not improved in the multivariate model by Kebreab *et al.* (2010). However, the prediction errors for the proposed model decreased slightly. The observed FN, UN, and MN values were plotted against their predicted values in Fig. 3 and did not reveal any significant biases. Plots of the residuals versus the centred predicted values (not shown) did not reveal any departures from normality.

Discussion

The Bayesian framework applied in the present study accommodates description of the cross-classified covariance matrix of the data and the nested model structure required for the proposed model. Further, application of MCMC simulation in a Bayesian setting makes estimation of the complete posterior distribution of model parameters and any desired predictions easily accessible, even when analytical solutions are highly complex. By tracking predictions that are of particular interest within a simulation, the distribution of that value can be used for inference instead of relying solely on the mean.

The proposed multivariate model in which a partition coefficient was used to represent the proportion of absorbed N secreted in milk, described the trivariate observation of N excretion in urine and faeces and secretion in milk using biological principles. In addition to improvements in model fit,

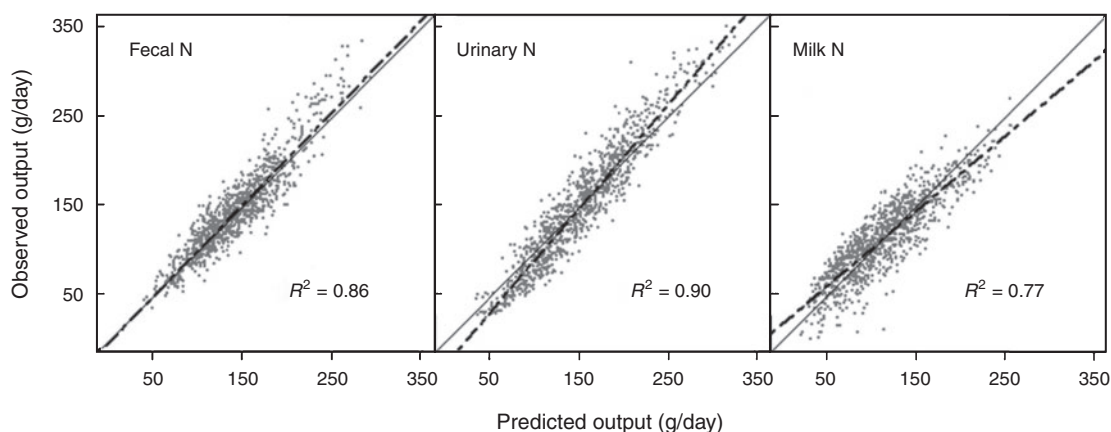


Fig. 3. Plot of faecal, milk and urinary nitrogen observations against their predicted values given by the proposed model of nitrogen excretion (Eqn 2). The solid lines are the line of unity and the dashed lines are the predictions of observed values regressed on predicted values.

as indicated by a large decrease in DIC, prediction accuracy was also improved as indicated by reductions in the RMSPE for UN and MN. The greatest advantage of this model structure, however, is the inference that can be drawn about N partitioning and the variables that influence it. Specifically, the model facilitates simultaneous estimation of apparent N digestibility and the efficiency of converting absorbed N into MN. Heuristically, N use can be thought of as being divided into two main components – digestion and metabolism – and the proposed model facilitates distinct and simultaneous estimation of covariate effects on these two components of N use. The present study focused on the effect of dietary energy content, as represented by ME, on N partitioning but more variables can and should be investigated in the future. Metabolisable energy, whether calculated from measured energy intakes and losses or from tabular feed values, does not represent a specific nutrient but is rather the aggregate energy from all nutrients that is available for productive purposes. There are many diet formulations that deliver the same ME, including diets that vary in CP, carbohydrate and fat concentration, which undoubtedly affect N partitioning differently. Future work should focus on specific nutrients that contribute to ME, so as to strengthen the biological inference on the nutritive factors that drive N use in dairy cattle. In addition, a systematic variable-selection process under the proposed model has potential to improve fit and prediction accuracy while revealing other covariates that influence N partitioning.

The first stage of N partitioning in the dairy cow is from ingested N into faeces and N absorbed through the digestive tract. The results of the present study were in broad agreement with other works that suggest that FN decreases with increasing energy content of the diet as described by ME or metabolisability (a measure highly correlated with ME) (Tamminga 1992; Castillo *et al.* 2000; Kebreab *et al.* 2010). On the basis of the current dataset, the proportion of NI excreted in faeces varies widely with diet ME content. Assuming the mean NI of 418 g/day, the model predicted that FN could vary from 63 g/day for diets with high ME contents to 207 g/day for diets with low ME content. Conversely, the model predicted that the amount of absorbed N increases with increasing ME content and is predicted to range from 211 g/day to 355 g/day over the range of observed ME for a fixed NI of 418 g/day. This is a desirable relationship as the N in feed must first be absorbed before it can be deposited in tissue or secreted in milk. There are many explanations for the positive relationship between absorbed N and ME, the simplest of which is that ME is a constituent of DE and they are highly correlated with each other. Changes in ME could also reflect changes in the structural and non-structural carbohydrate fractions in the diet which can affect both protein degradation and synthesis rates in the rumen (Bach *et al.* 2005), leading to variation in feed N digestibility, and thus changes in FN.

The model estimated the effect of ME on the partitioning of absorbed N to milk and urine was not as large as the estimated effect on the partitioning between absorbed N and FN. However, because $E[P]$, the expected value of the proportion of absorbed N secreted as milk, was predicted to decrease with increasing ME, the results contradict other findings in the literature. For example, Kebreab *et al.* (2010) found a negative relationship between metabolisability and UN in both their

multivariate and univariate models. However, fitting their models to the current dataset consistently found the opposite. A reason for the discrepant estimates between datasets could be that the current data contain a mixture of hay- and corn silage-based diets while the diets in the data used by Kebreab *et al.* (2010) were mostly based on grass silage. Rius *et al.* (2010) found a significant treatment effect when comparing the effect of high-energy and low-energy diets on post-absorptive N efficiency and concluded that dietary net energy content improved post-absorptive efficiency. However, there are some noteworthy differences between their analysis and ours that undermine any direct comparison to the present study, including their use of estimated MP as their measure of absorbed N, the more empirical and flexible structure of the ANOVA model, and their use of rumen bypass protein supplements to directly manipulate rumen-undegradable protein (Rius *et al.* 2010).

Despite the predicted negative relationship between the post-absorptive efficiency and ME, overall ENU is still predicted to increase with increasing ME (Fig. 4). The reason for this apparent inconsistency is the relative size of the effect of ME on pre- and post-absorption N utilisation. At the mean NI of 418 g/day, total absorbed N is expected to increase by 13.8 g for every 0.9 MJ/kg DM increase in ME. In contrast, MN is expected to increase only by 5.7 g for an increase in 0.9 MJ/kg DM of ME at the mean NI. The reason the efficiency declines with increasing ME is because the denominator (absorbed N) of post-absorptive N efficiency is increasing at a faster rate than the numerator (MN) as a function of ME. The predicted decrease in post-absorptive efficiency is likely a result of the large increases in the digestibility of N (ranging from 0.40 to 0.80) seen within the dataset and the estimated relationship of apparent N digestibility to ME content.

The significant change in the β_{3-5} estimates when the model was fit to the data subset restricted to observations for which the requirements for NE_L for lactation and MP_L were at least met, demonstrated that increasing energy and N balance eliminates the negative relationship between ME and post-absorptive N efficiency. This result is a reminder that data used for model

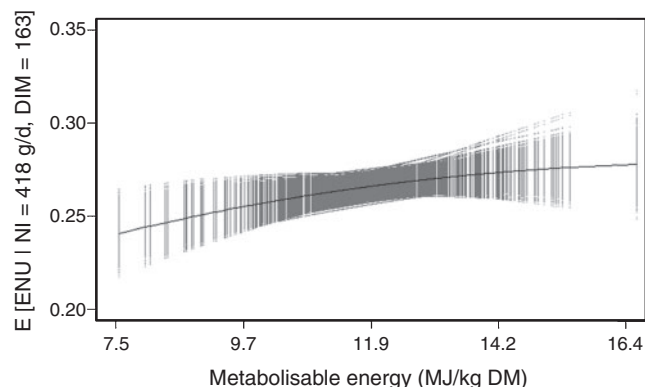


Fig. 4. Plot of 1000 Markov Chain Monte Carlo samples from the proposed model (Eqn 2)-expected values of the efficiency of nitrogen use (ENU) when a nitrogen intake is 418 g/day and days in milk (DIM) is 163 for each observed value of metabolisable energy. Where the expectation $E[\text{ENU} | \text{NI} = 418 \text{ g/day}, \text{DIM} = 163 \text{ days}]$ is calculated as
$$\frac{E[\text{Milk N} | \text{NI} = 418 \text{ g/d}, \text{DIM} = 163 \text{ d}]}{\text{NI} = 418 \text{ g/d}}$$

development are a representative sample of the population on which inference can be made. Over the wide range of diets and intake levels encompassed in the current complete dataset, post-absorptive N efficiency declines with ME. Within the smaller range of diets in the data subset or with diets that alter the rate of absorbed N as a function of ME, such as those that alter the profile of absorbed AA, different relationships most likely hold. The proposed model structure can be used to assess the effect of dietary covariates on pre- and post-absorptive N efficiency in different experimental settings and direct future investigations into ENU. In particular, nutrients that contribute to ME such as fibre, carbohydrate and fat fractions and feeds that change the post-rumen profile of AA, could be assessed for their potential impact on post-absorptive N efficiency by including them as covariates in the proposed model.

Conclusions

Improvement in dairy-cattle ENU is an important strategy to mitigate the environmental impacts of N losses and improve dairy production economy. The proposed model structure (Eqn 2) represents an improvement in model fit and prediction accuracy and can be used for further investigation into dietary and animal factors that affect ENU in dairy cattle. On the basis of the current investigation, increasing diet ME content increases ENU with diminishing returns (Fig. 4) as a result of a decreasing post-absorptive N efficiency as a function of ME. However, the relationship of ME with post-absorption N partitioning should be investigated further because it is dependent on the energy status of the animal and does not distinguish between the nutrient sources of the energy.

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