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Nitrogen and truly digestible protein efficiencies of dairy cows fed fresh herbage- or maize forage-based diets: a meta-analysis

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ABSTRACT

Understanding how dietary forage influences dairy cows' use of nitrogen (**N**) and protein resources may help decrease N losses to the environment, which is a major issue in the livestock sector. The present study evaluated the effects of cow and diet characteristics on N use efficiency and the efficiency of truly digestible protein in the intestine (**PDI**) (i.e., protein use efficiency at the metabolic level), depending on whether the base forage is fresh herbage or maize forage. To this end, a meta-analysis was performed of a dataset composed of 22 experiments that included 266 observations of individual *in vivo* whole-tract digestibility and N balance for lactating dairy cows fed either fresh herbage diets indoors (Fresh herbage, n = 113) or maize forage diets (Maize, n = 153). The dataset compiled dietary and zootechnical variables such as feed intake, chemical composition of the diet, milk yield (**MY**), milk composition and cow characteristics. The nutritional value of diets, cow protein requirements and PDI use efficiency were calculated for all diets according to the INRA 2018 feeding system for ruminants. The dataset was then analysed using mixed-effect models, considering the experiment and the cow as random effects. Feed intake, MY and dietary PDI concentration were greater for Maize diets than Fresh herbage diets, while dietary N and energy concentrations were greater for Fresh herbage diets. Dietary N concentration influenced N use efficiency the most, while the ratio of dietary protein concentration to energy concentration influenced PDI efficiency the most. N and PDI use efficiency averaged 23.9 and 72.0%, respectively, for the Fresh herbage diet type, and 30.6 and 74.7%, respectively, for the Maize diet type. This study highlighted that cows use N and PDI in similar ways regardless of whether they are fed fresh herbage or maize diets. In the range of variation studied, each increase in N use efficiency increased PDI use efficiency by the same degree, regardless of the type of diet. This suggests that PDI use efficiency can be predicted from N use efficiency, which is easier to calculate.

Implications

Dairy cow diets can be based on different types of forage, such as fresh herbage or maize forage, which have a wide range of characteristics. The present meta-analysis studied the effects of diet type (fresh herbage- or maize forage-based diets) on cow nitrogen and protein use. It highlighted that cows used nitrogen and protein in similar ways regardless of whether they were fed fresh herbage or maize diets. It thus increased understanding of the effects of dietary characteristics on nitrogen use and thus can help to decrease nitrogen losses to the environment.

Introduction

To ensure their sustainability, dairy cattle farms must decrease their nitrogen (**N**) losses to the environment, which, in excess, lead to soil acidification, climate change and eutrophication (Tamminga, 1992; Lesschen et al., 2011; Peyraud et al., 2014). To decrease these impacts on the environment, one solution is to increase dietary N use by cows for milk yield (**MY**), which decreases N loss in manure. To quantify N use by cows, N use efficiency is frequently calculated as N exported in milk divided by N intake (Castillo et al., 2000; Calsamiglia et al., 2010). The N use efficiency of dairy cows is usually ca. 25% but can vary widely (from 10-45%) depending on both diet and cow characteristics (Huhtanen and Hristov, 2009; Aizimu et al., 2021). The INRA feeding system for ruminants developed another efficiency indicator based on truly digestible protein (**PDI**, equivalent to metabolisable protein; INRA, 2018) that quantifies the use of protein by the cows' metabolism – PDI use efficiency – which equals the sum of protein exported by cows for lactation, gestation, growth and maintenance divided by the PDI supply. N use efficiency is always smaller than PDI use efficiency (Omphalius et al., 2019; Laroche et al., 2022) because it considers only N exported in milk (i.e., in both protein

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and non-protein) as a N requirement of the cow, while considering the other uses of N as inefficiency. Conversely, PDI use efficiency is a marginal efficiency which focuses on protein catabolism (protein wasted by cow metabolism) and does not consider N degradation and use by ruminal microbiota or N digestion. Thus, the characteristics of the diet could influence these two efficiencies in different ways.

To date, few studies have examined the effects of diet and cow characteristics on PDI use efficiency (Cantalapiedra-Hijar et al., 2018; Lapierre et al., 2018; Omphalius et al., 2019; Laroche et al., 2022). We hypothesised that N and PDI use efficiencies respond differently to changes in diet and cow characteristics, as their respective equations include different variables related to cows' N metabolism (Cantalapiedra-Hijar et al., 2018; INRA, 2018). Thus, the first objective of this study was to identify the variables that explain variations in N and PDI use efficiencies the most.

Dairy farms have a wide diversity of feeding practices that can influence N and PDI use efficiencies. Cow diets are based on a variety of forage types, such as fresh herbage grazed or offered indoors, conserved forage (e.g., maize silage, herbage silage, hay) with concentrates, or both. Maize forage-based diets usually contain more concentrates than fresh herbage-based diets. Fresh herbage can have a high N concentration, especially of non-protein N, while some conserved forages, such as maize forage, have relatively low N concentrations, whose N needs to be balanced with high-protein concentrates (French Livestock Institute, 2010; INRA, 2018). Fresh herbage-based diets could lead to lower voluntary DM intake and greater diet DM digestibility than maize forage-based diets (Bargo et al., 2002a; INRA, 2018; Brito et al., 2022). These differences are likely to influence N and PDI use efficiencies (Phuong et al., 2013; Brito et al., 2022). Thus, the second objective of this study was to determine whether the relationships between N or PDI use efficiency and diet and cow characteristics are the same for fresh herbage- and maize forage-based diets. To meet the study's objectives, a meta-analysis was performed using experimental data from cows fed fresh herbage- or maize forage-based diets.

Material and methods

Experimental dataset

The data used for the meta-analysis came from the CowNflow database (Ferreira et al., 2021a), which includes 414 individual observations of *in vivo* whole-tract digestibility and N balance from 28 experiments with Holstein cows. The experiments were conducted at the INRAE experimental dairy farm of Méjusseaume (Le Rheu, France; https://doi.org/10.15454/yk9q-pf68) under controlled conditions from 1983 to 2019. The meta-data and the criteria that the experiments needed to meet to be included in the CowNflow database were described by Ferreira et al. (2021b). Briefly, the data included weekly observations per cow (one row per cow × measurement week) of DM intake (**DMI**), MY, along with the proportions of feeds in the diet and their chemical composition. Most experiments were conducted according to Latin square or switchback designs, with each cow receiving one treatment per period and treatments changing each period. The main treatments included the amount or composition of concentrates, the proportion and composition of forage (e.g., herbage species, forage processing), dietary N concentration, the frequency of feed distribution and the feeding level (*ad libitum* vs restricted feeding).

For the meta-analysis, data were selected for lactating cows only (from 7 weeks of lactation at the start of the experiment to a maximum of 60 weeks at the end of the experiment) fed either fresh herbage-based diets or maize forage-based diets (Fresh herbage and Maize diet types, respectively), as these two diet types contained enough data for robust statistical analysis. Six data were removed because milk data were missing, the cow was drying up or the net energy (**UFL**) concentration of the fresh herbage was inconsistent (INRA, 2018). The meta-analysis thus focused on a subset of 266 observations for 97 dairy cows (dataset available: https://doi.org/10.57745/7MEYNT). Specifically, the Fresh herbage and Maize diet types included 11 experiments each, with 113 and 153 observations, respectively. The majority of the Fresh herbage diets were concentrate-free (80 of the 113 observations). The dataset contained mainly data on multiparous cows (239 of the 266 observations). Cows were in mid- or late lactation (29 ± 10.0 lactation weeks) and weighed 619 ± 70.6 kg (Table 1).

Some variables came directly from the database, such as DMI (kg/day), dietary N concentration (g/kg DM), organic matter (**OM**) digestibility (g/g), non-digestible OM (g/kg DM), MY (kg/day), milk true protein concentration (**MPC**, g/kg) and N use efficiency (%). Other variables were estimated using the INRA (2018) feeding system: dietary PDI and UFL (1 UFL = 7.37 MJ of net energy for lactation; INRA, 2018) concentrations (g/kg DM and UFL/kg DM, respectively). Finally, other variables were calculated using both the database and estimated data from the INRA (2018) feeding system, such as PDI use efficiency (%) (Eq. (1) below).

Calculation of nutritional value of feeds and diets

The nutritional values of all feeds, including PDI and UFL concentrations, were estimated using the INRA (2018) feeding system and the PrevAlim module of INRAtion® V5 software (INRA, 2020) based on their components and chemical composition provided by the database (Ferreira et al., 2021a). Then, the nutritional value of the entire diet was calculated considering the three main sources of digestive interactions between these feeds: the feeding level, percentage of concentrates in the diet and rumen protein balance (CP intake minus the non-ammonia CP flowing from the duodenum) (INRA, 2018). Dietary UFL and PDI concentrations were calculated using INRAtion® V5 software based on the nutritional values of the feeds calculated previously and the DMI of each feed from the database.

Efficiency calculations

Nitrogen use efficiency (%), calculated as N exported in milk (g/day) divided by N intake (g/day), was provided by the CowNflow database (Ferreira et al., 2021a). PDI use efficiency (%) was calculated as the sum of protein exported for MY (**MPY**, g/day), gestation (g/day), growth (g/day) and maintenance (including scurf protein and endogenous faecal protein, **EFP**, g/day, equivalent to metabolic faecal protein, g/day), divided by the available PDI (PDI intake minus endogenous urinary protein, **EUP**, g/day). The gestation, growth, scurf proteins, EFP, and EUP were calculated using INRAtion® V5 software (INRA, 2020) based on BW, age, gestation stage and feed intake, which were also provided by the database. The PDI use efficiency equation also considers the body protein balance (g/day), depending on the net energy balance (UFL/day) (INRA, 2018). When the net energy balance was positive, the cow fixed protein in its tissues, and body protein balance was considered a protein expenditure (INRA, 2018):

$$\text{PDI use efficiency} = \frac{\text{MPY} + \text{Gestation} + \text{Growth} + \text{Scurf} + \text{EFP} + \text{Body protein balance}}{\text{PDI intake - EUP}} \times 100 \tag{1}$$

When the net energy balance was negative, the cow mobilised protein from its tissues, and body protein balance was considered a protein source (INRA, 2018):

Table 1
Descriptive statistics of dairy cow characteristics, nutritional value of the diets, performance, nitrogen use efficiency and truly digestible protein use efficiency per diet type.

Variable	Fresh herbage (n = 113)					Maize (n = 153)				
	Mean	SD	Median	Min	Max	Mean	SD	Median	Min	Max
Experiment year	1 996	12.3	1 991	1 983	2 014	2 007	10.6	2 008	1 990	2 019
Lactation number	3.08	1.833	3.00	1.00	8.00	3.16	1.487	3.00	1.00	7.00
Age (month)	63.1	26.18	55.6	31.9	132	62.4	22.74	55.1	30.9	128
BW (kg)	579	67.4	578	430	740	649	57.1	645	545	803
Lactation stage (week)	31.4	6.68	30.3	16.3	50.3	26.7	11.50	24.3	7.9	59.7
DMI (kg/day)	14.4	2.39	14.9	8.0	19.4	20.6	2.50	20.7	12.0	29.6
Percentage of concentrates in the diet (%)	7.4	12.64	0	0	42.3	26.9	7.33	25.0	17.2	51.8
N intake (g/day)	405	108.6	380	218	689	494	95.1	491	273	732
MY (kg/day)	18.0	4.99	17.8	7.0	29.8	28.8	6.94	28.6	10.7	47.0
MPC (g/kg)	31.2	2.53	31.3	25.2	38.8	31.3	3.44	30.6	25.1	43.3
N in milk (g/day)	92.1	23.36	93.6	37.3	145.3	148.3	28.43	149	65.8	217.5
Dietary N (g/kg DM)	28.4	7.29	28.9	16.6	42.6	24.0	3.62	23.6	17.2	38.4
Dietary OM digestibility (g/g)	0.794	0.0358	0.799	0.698	0.884	0.718	0.0260	0.720	0.666	0.801
Dietary non-digestible OM (g/kg DMI)	184	32.2	183	103	272	265	23.7	265	189	314
Dietary PDI (g/kg DM)	85.8	7.35	86.3	73.5	104.9	88.4	6.95	89.1	68.4	111.8
Dietary UFL (UFL/kg DM)	0.99	0.068	1.00	0.81	1.14	0.91	0.025	0.91	0.85	0.99
Dietary PDI:UFL ratio (g/UFL)	86.6	6.62	93.4	71.3	101.1	97.7	6.92	97.7	81.0	115.3
Dietary rumen protein balance[1] (g/kg DM)	32.0	37.62	35.4	−33.5	110.4	5.7	14.88	3.2	−20.7	38.9
PDI intake (g/day)	1 239	238.2	1 235	728	1 878	1 818	267.6	1 858	987	2 574
UFL intake (UFL/day)	14.3	2.53	14.7	8.2	18.8	18.6	2.15	18.7	11.2	25.4
N use efficiency (%)	23.9	7.56	22.4	8.8	44.2	30.6	5.65	30.5	15.4	44.1
PDI use efficiency[2] (%)	72.0	8.14	70.8	53.0	93.0	74.7	7.52	74.7	51.2	94.6

Abbreviations: DMI = DM intake; Max = maximum; Min = minimum; MPC = milk true protein concentration; MY = milk yield; OM = organic matter; PDI = protein truly digestible in the small intestine (INRA, 2018); UFL = net energy (1 UFL = 7.37 MJ of net energy for lactation; INRA, 2018).
[1] Rumen protein balance = CP intake minus non-ammonia CP flowing at the duodenum (INRA, 2018).
[2] PDI use efficiency from the INRA (2018) feeding system for ruminants (INRA, 2018).

$$\text{PDI use efficiency} = \frac{\text{MPY} + \text{Gestation} + \text{Growth} + \text{Scurf} + \text{EFP}}{\text{PDI intake - EUP} + |\text{Body protein balance}|} \times 100 \tag{2}$$

The body protein balance was calculated from the net energy balance assuming that PDI increased by 33 g per unit increase in UFL (INRA, 2018). The net energy balance was calculated by INRAtion® V5 software as net energy supply minus the net energy expenditure for lactation, gestation, growth and maintenance.

Statistical analysis

The meta-analysis used covariance analyses to describe the effects of the two diet types and other diet and cow characteristics on N and PDI use efficiencies. Variables that described performance (live weight, lactation stage, parity, MY and milk composition) and diet (chemical composition, nutritional values, percentage of concentrates and feeding level) were investigated as candidate explanatory variables for N and PDI efficiencies. To capture the variability due to the diversity of experiments and cows, these two types of variables were considered random intercepts in the models. Mixed-effect models were built using the lme4 package (Bates et al., 2015) of RStudio software (v. 1.3) according to the following model:

$$Y_{ijk} = \mu + Diet_k + B_{1k}X_{1ijk} + B_{2k}X_{2ijk} + \cdots + B_{lk}X_{lijk} + Experiment_i + Cow_j + e_{ijk} \tag{3}$$

with Y_{ijk} the dependent variable (N use efficiency or PDI use efficiency); μ the intercept across diets, experiments and cows; $Diet_k$ the fixed intercept of diet type k (k = Fresh herbage or Maize); X_{lijk} the l explanatory variables (1… l predictors); B_{lk} the regression coefficients of the corresponding l^{th} explanatory variable (X_{lijk}) for diet type k; $Experiment_i$ and Cow_j the random intercepts of the experiment and cow nested in the experiment, respectively, with i = 1…22 experiments and j = 1… 97 cows; and e_{ijk} the residual error. The random effects of $Experiment_i$ and Cow_j were assumed to be normally distributed, with a mean of 0 and a SD of 1.

An initial exploratory study tested many combinations of explanatory variables. Its results were used to select the candidate explanatory variables that were robust in explaining the variance and were biologically relevant for predicting efficiency. The independence of each candidate variable was examined using graphical analysis and by calculating Pearson's correlation coefficients (r). Ultimately, seven explanatory variables that were not strongly correlated with each other (r < 0.8) (Akoglu, 2018) were selected in the model: DMI, dietary N concentration, PDI and UFL concentrations, the dietary PDI:UFL ratio, MY and MPC.

Linear and quadratic effects of each variable were considered. Interactions between the diet type and each explanatory variable were also tested. After calculating models that contained all seven variables, non-significant variables or interactions were removed individually from the most to least explanatory, and the model was recalculated until only significant variables and interactions remained. The diet type was included in all models. The quality of each model was evaluated using three indicators: RSD (calculated with the sigma function), as a measure of model precision; marginal R2, as the proportion of variance explained by the model's fixed effects (Nakagawa and Schielzeth, 2013; calculated with the r.squaredGLMM function) and Akaike's information criterion, as a measure of model parsimony. The most parsimonious models (i.e., with the lowest Akaike's information criterion, assuming a difference of at least two Akaike's information criterion points between two models; Arnold, 2010) obtained for N use efficiency and PDI use efficiency were called Neff_n and PDIeff_n, respectively, with n the number of significant variables in the model, not counting the diet type factor. Model residuals were assessed graphically for all explanatory variables. The type III sums of squares of Neff_n and PDIeff_n were calculated using the Satterthwaite method (Kuznetsova et al., 2017). To identify the variables that had the greatest weight in each model, the variable that explained efficiency the least (i.e., smallest sum of squares) in Neff_n and PDIeff_n was then removed stepwise until the model contained only one variable and the diet type factor (i.e., until Neff_1 and PDIeff_1, a method also used by Phuong et al., 2013).

The relationships between Neff_n or PDIeff_n and its main explanatory variables were represented graphically as follows: fitted efficiency values were calculated for each data using all model coefficients except for the explanatory variable on the x-axis of the graph and the diet type effect. Then, to represent both diet types on the same graph more clearly, the fitted values were centred with the means per diet type for the same explanatory variables used above (Table 1; Supplementary Material S1).

The relationship between PDI use efficiency (dependent variable PDIeff) and N use efficiency (explanatory variable Neff) was studied using a mixed-effect model, considering the effect of diet type ($Diet_k$, k = Fresh herbage or Maize) on the intercept and the interaction between N use efficiency and the diet type (Eq. (4). $Experiment_i$ and Cow_j variables were considered random effects on the intercept, and all non-significant variables and interactions were removed individually from the most to least explanatory.

$$PDIeff_{ijk} = \mu + Diet_k + Neff_k X_{ijk} + Experiment_i + Cow_j + e_{ijk} \quad (4)$$

Results

Dataset description

The mean DMI and MY were 6.2 and 10.8 kg/day greater for the Maize diet than for the Fresh herbage diet, while the MPC was similar for both diets (31.3 ± 3.08 g/kg) (Table 1). The mean lactation stage was 5 weeks shorter for the Maize diet than for the Fresh herbage diet, while the mean BW was 70 kg greater for the Maize diet. The Maize diet experiments contained fewer primiparous observations than did those for the Fresh herbage diet (5.2 and 16.8% of cows, respectively).

Nitrogen use efficiency and PDI use efficiency were 6.7 and 2.7% points greater for the Maize diet than for the Fresh herbage diet. Nitrogen intake and N in milk were 89.0 and 56.2 g/day greater for the Maize diet than for the Fresh herbage diet. The percentage of concentrates in the diet was greater for the Maize diet than for the Fresh herbage diet (+19.5% points), while the OM digestibility was 0.076 g/g lower for the Maize diet. The dietary N and UFL concentrations were 4.4 g/kg DM and 0.08 UFL/kg DM lower for the Maize diet than for the Fresh herbage diet, while the dietary PDI concentration and PDI:UFL ratio were 2.6 g/kg DM and 11.1 g/UFL greater for the Maize diet.

Nitrogen use efficiency

The most parsimonious (lowest Akaike's information criterion) N use efficiency model (Neff_9) included seven significant explanatory variables and two significant interactions ($P < 0.05$), not counting the diet type factor, with linear effects of DMI; quadratic effects of the dietary N concentration, MY and MPC; and two interactions between diet type and DMI or MY (Table 2). The intercept differed significantly between diet types for all N use efficiency models except for Neff_6, 5 and 4. The DMI and dietary N concentration were negatively correlated with N use efficiency, while MY and MPC were positively correlated with it. The decrease in N use efficiency as the DMI increased was significantly lower ($P < 0.05$) for the Maize diet than for the Fresh herbage diet (−1.32 and −1.73% points for each 1 kg increase in DMI, respectively). The increase in N use efficiency as the MY increased was significantly lower ($P < 0.05$) for the Maize diet than for the Fresh herbage diet (1.72 and 1.88% points for each 1 kg increase in MY increase, respectively).

The Neff_9 model also had the greatest precision among N use efficiency models (marginal R^2 = 0.97 and RSD = 1.01). The gradual removal of the least explanatory variable had little effect on RSD from Neff_9 to Neff_5 (Fig. 1). Conversely, from Neff_5 to Neff_1, the marginal R^2 decreased by 0.33 and the RSD increased by 1.76. The variables that influenced N use efficiency the most were dietary N concentration, MY, DMI and MPC, as indicated by models Neff_1 to Neff_4. For a given MY, N use efficiency was lower for the Maize diet than for the Fresh herbage diet (Fig. 2d). Conversely, for a given dietary N concentration, DMI or MPC (Fig. 2b, f and h, respectively), N use efficiency was greater for the Maize diet than for the Fresh herbage diet.

Truly digestible protein use efficiency

The most parsimonious PDI use efficiency model (PDIeff_9) included eight significant explanatory variables and one significant interaction ($P < 0.05$), not counting the diet type factor, with linear effects of the DMI and dietary UFL concentration; quadratic effects of the dietary PDI:UFL ratio, MY and MPC; and the interaction between diet type and the dietary PDI:UFL ratio (Table 3). The intercept differed significantly between diet types for all PDI use efficiency models except for PDIeff_7. The DMI, dietary UFL concentration and PDI:UFL ratio were negatively correlated with PDI use efficiency, while the MY and MPC were positively correlated with it. The decrease in PDI use efficiency as the dietary PDI:UFL ratio increased was significantly greater ($P < 0.05$) for the Maize diet than for the Fresh herbage diet (−3.69 and −3.55% points for each 1 g increase in the PDI:UFL ratio, respectively). The PDIeff_9 model also had the greatest precision among PDI use efficiency models (marginal R^2 = 0.94 and RSD = 0.97). The gradual removal of the least explanatory variable had little effect on model precision from PDIeff_9 to PDIeff_7 (Fig. 1). Conversely, from PDIeff_6 to PDIeff_1, the marginal R^2 decreased by 0.44 and the RSD increased by 2.51. The variables that influenced PDI use efficiency the most were the dietary PDI:UFL ratio, MY, dietary UFL concentration and DMI, as indicated by the models PDIeff_1 to PDIeff_4.

For a given dietary PDI:UFL ratio or DMI (Fig. 3b and h, respectively), PDI use efficiency was greater for the Maize diet than for the Fresh herbage diet, while for a given MY (Fig. 3d), PDI use efficiency was lower for the Maize diet.

The PDI use efficiency and N use efficiency were positively correlated, with a slope of 0.99 ± 0.047 for both diet types (Fig. 4). Intercepts differed significantly between diet types: for a given N use efficiency, PDI use efficiency was 4.2% points lower for the Maize diet than for the Fresh herbage diet (44.5 and 48.7%, respectively).

Discussion

Explaining variations in nitrogen use efficiency and truly digestible protein use efficiency

The meta-analysis enabled us to study N and PDI use efficiencies from a large dataset of individual lactating dairy cows fed either a Fresh herbage or Maize diet. This study considered between-cow variability due to differences in cow characteristics known to influence N use efficiency (Huhtanen et al., 2015; Cantalapiedra-Hijar et al., 2018). As in previous studies, dietary N concentration had one of the greatest influences on N use efficiency (Huhtanen et al., 2008; Huhtanen and Hristov, 2009). Conversely, the ratio of dietary protein concentration to energy concentration had the greatest influence on PDI efficiency, which is consistent with the results of Vérité and Delaby (2000) and Lapierre et al. (2023). These two variables partly reflect the quality

Table 2
Mixed-effect regression models of nitrogen use efficiency (Neff) in dairy cows including the diet type (Fresh herbage or Maize) and explanatory variables.

Model	Model coefficients[1,2]								Model metrics		
	Intercept (%)	DMI (kg/day)	Diet N (g/kg DM)	Diet N² (g^2/kg^2 DM)	MY (kg/day)	MY² (kg^2/day^2)	MPC (g/kg)	MPC² (g^2/kg^2)	marginal R^2	RSD	AIC
Neff_9[3]	FH: 21.4 MA: 18.5	FH: −1.73 MA: −1.32	−2.49	0.0286	FH: 1.88 MA: 1.72	−0.0132	2.087	−0.0213	0.970	1.01	886
Neff_8	FH: 24.5 MA: 19.7	FH: −1.70 MA: −1.39	−2.46	0.0280	1.95	−0.0163	1.807	−0.0166	0.969	1.02	889
Neff_7	FH: 42.0 MA: 36.6	FH: −1.70 MA: −1.36	−2.43	0.0274	1.97	−0.0170	0.705	–	0.970	1.01	888
Neff_6	41.5	−1.47	−2.46	0.0280	1.90	−0.0155	0.687	–	0.967	1.03	902
Neff_5	49.7	−1.46	−2.30	0.0251	1.08	–	0.636	–	0.936	1.16	1 012
Neff_4	32.9	−1.47	−0.94	–	1.03	–	0.651	–	0.914	1.55	1 111
Neff_3	FH: 52.6 MA: 54.4	−1.24	−0.90	–	0.81	–	–	–	0.881	1.72	1 205
Neff_2	FH: 40.0 MA: 37.9	–	−0.87	–	0.48	–	–	–	0.799	2.52	1 357
Neff_1	FH: 48.0 MA: 51.0 t	–	−0.87	–	–	–	–	–	0.602	2.90	1 443

Abbreviations: AIC = Akaike information criterion; DMI = DM intake; FH = fresh herbage; MA = maize; MPC = milk true protein concentration; MY = milk yield.
[1] All explanatory variables in the model are significant at $P < 0.05$.
[2] The intercept column shows the intercept for each diet when the effect of the diet type factor is significant ($P < 0.05$) or a trend ("t", $P < 0.10$). When only one intercept is given, it applies for both FH and MA diets (diet type factor non-significant). This notation also applies to the slope coefficients of variables that have significant (or non-significant if only one coefficient) interactions with diet type.
[3] Number of variables and interactions in the model not counting the diet type factor.

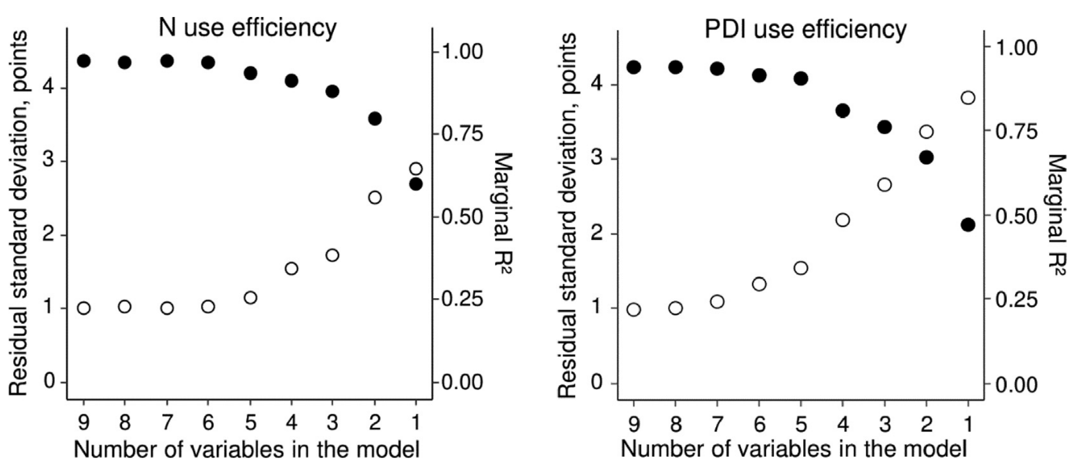

Fig. 1. Relation between residual SD (○) or marginal R^2 (●) and the number of variables and interactions, not counting diet type, in the regression model that explains variations in nitrogen use efficiency or truly digestible protein use efficiency of dairy cows. Abbreviations: PDI = protein truly digestible in the small intestine (INRA, 2018).

of the diet, which appeared to be a major determinant of efficiency. For both N and PDI use efficiency models, the most significant explanatory variables were moreover logically those used in their respective equations. Once DMI, MY, MPC and diet composition are included in the models, the other cow and diet characteristics known to affect the efficiency (e.g., the lactation stage; Lapierre et al., 2023) are no longer significant, as their effects on efficiency are largely due to their impacts on these four components.

Comparing the efficiencies of Fresh herbage and Maize diets

The relations between N or PDI use efficiency and explanatory variables were similar for the two diet types. Few significant interactions were observed between explanatory variables and the diet type (two for N use efficiency and one for PDI use efficiency), which indicates that the efficiency responses to diet and cow characteristics generally did not depend on the diet type. Cows used N and protein in similar ways regardless of the diet type. These results agreed with those of Brito et al. (2022), who compared the effects of grazing and indoor systems on N use efficiency, and found that cows used nutrients similarly among systems. In the present study, the differences in efficiency between the Fresh herbage diet and Maize diet could be explained mainly by the mean DM intake, dietary N and PDI concentrations and MY for each diet type. For a given dietary N concentration or DMI, the greater N use efficiency for the Maize diet was likely due to the diet's higher MY and N in milk than those for the Fresh herbage diet (Fig. 2). Moreover, for a given DMI, the Maize diet had a lower dietary N concentration, which decreased N intake. Conversely, for a given MY, the lower N use efficiency for the Maize diet could be explained by its greater DMI, which increased N intake. When all of these variables were included in the model (Neff_9), N use efficiency was greater for the Fresh herbage diet, perhaps due to the diet's greater dietary OM digestibility and UFL concentration than those of the Maize diet. Some studies observed that increasing dietary energy concentration without changing N and protein concentrations increased N use efficiency (Aizimu et al., 2021; Beltran et al., 2022; Laroche et al., 2022), which could be due to an increase in N use by the ruminal microbiota or in the milk protein yield (Laroche et al., 2022). The higher N use efficiency for the Fresh herbage diet may

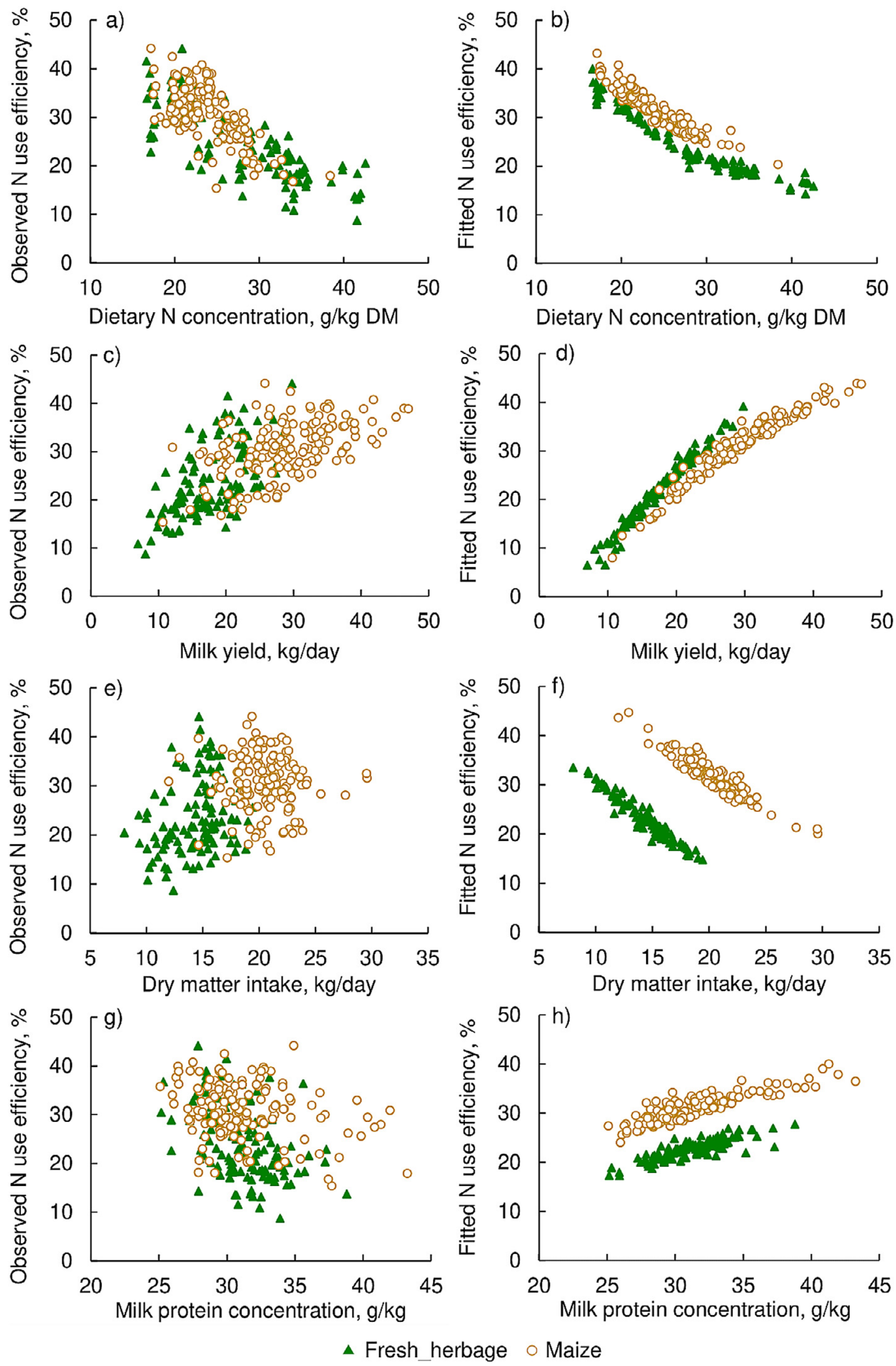

Fig. 2. Relation between observed and fitted nitrogen use efficiency of dairy cows and (a and b) dietary nitrogen concentrations, (c and d) milk yield, (e and f) DM intake and (g and h) milk true protein concentrations. N use efficiency fitted with the Neff_9 model. For each observation, the efficiency was calculated using all model coefficients except those related to the diet type and the explanatory variable on the x-axis. The efficiency was then centred on the mean value per diet type.

also have been due to more non-protein N in milk, which increased total N in milk (Bargo et al., 2002a; Bargo et al., 2002b; Couvreur et al., 2007; Ferreira et al., 2021b).

For a given MY, the greater PDI use efficiency for the Fresh herbage diet was due to the diet's lower DMI than that for the Maize diet, like for the N use efficiency (Fig. 3). The dietary PDI concentration was similar between diet types. Thus, for a given DMI, the difference in PDI use efficiency between diet types was due mainly to variations in protein exported in milk (g/day). Therefore, the greater MY for the Maize diet could largely explain its greater PDI use efficiency for a given PDI:UFL ratio or DMI. When all of these variables were included in the PDI use efficiency model (PDIeff_9), the Maize diet was more efficient than the Fresh herbage diet, perhaps due in part to the Maize diet's greater EFP. Milk protein yield and EFP were the largest protein expenditures (56.0 ± 5.64% and 25.5 ± 4.29% of the total protein expenditure, respectively), while the other protein expenditures (scurf, gestation, growth and endogenous urinary protein) were relatively small (INRA, 2018). The EFP was calculated from the DMI and dietary non-digestible OM concentration as follows (INRA, 2018):

Table 3
Mixed-effect regression models of truly digestible protein use efficiency (PDIeff) in dairy cows, including the diet type (Fresh herbage or Maize) and explanatory variables.

Model	Model coefficients[1,2]									Accuracy metrics		
	Intercept (%)	DMI (kg/day)	Diet UFL (UFL/kg DM)	Diet PDI:UFL (g/UFL)	Diet (PDI:UFL)^2 (g^2/UFL^2)	MY (kg/day)	MY^2 (kg^2/day^2)	MPC (g/kg)	MPC^2 (g^2/kg^2)	marginal R^2	RSD	AIC
PDIeff_9[3]	FH: 238 MA: 253	−1.71	−69.5	FH: −3.551 MA: −3.692	0.0147	2.24	−0.0161	4.37	−0.0478	0.941	0.97	1 002
PDIeff_8	FH: 218 MA: 220	−1.72	−71.6	−2.960	0.0111	2.23	−0.0160	4.27	−0.0462	0.941	0.99	1 006
PDIeff_7	267	−1.63	−72.1	−2.928	0.0109	2.28	−0.0175	1.21	–	0.936	1.09	1 039
PDIeff_6	FH: 263 MA: 265	−1.58	−68.2	−2.701	0.0097	1.36	–	1.16	–	0.916	1.31	1 118
PDIeff_5	FH: 175 MA: 177	−1.58	−62.3	−0.900	–	1.33	–	1.15	–	0.908	1.54	1 158
PDIeff_4	FH: 203 MA: 208	−1.12	−56.5	−0.877	–	0.95	–	–	–	0.811	2.17	1 351
PDIeff_3	FH: 187 MA: 189 t	–	−47.9	−0.916	–	0.64	–	–	–	0.764	2.66	1 426
PDIeff_2	FH: 132 MA: 138	–	–	−0.814	–	0.58	–	–	–	0.673	3.37	1 515
PDIeff_1	FH: 139 MA: 150	–	–	−0.778	–	–	–	–	–	0.473	3.82	1 588

Abbreviations: AIC = Akaike information criterion; DMI = DM intake; FH = fresh herbage; MA = maize; MPC = milk true protein concentrations; MY = milk yield; PDI = protein truly digestible in the small intestine; UFL = net energy (1 UFL = 7.37 MJ of net energy for lactation; INRA, 2018).
[1] All explanatory variables in the model are significant at $P < 0.05$.
[2] The intercept column shows the intercept for each diet when the effect of the diet type factor is significant ($P < 0.05$) or a trend ("t", $P < 0.10$). When only one intercept is given, it applies for both FH and MA diets (diet type factor non-significant). This notation also applies to the slope coefficients of variables that have significant (or non-significant if only one coefficient) interactions with diet type.
[3] Number of variables and interactions in the model not counting the diet type factor.

$$\text{EFP (g/day)} = (5.7 + 0.074 \times \text{non-digestible OM concentration (g/kg DMI)}) \times \text{DMI (kg/day)}$$

The EFP was 278 and 522 g/day for the Fresh herbage diet and the Maize diet, respectively. This large difference in EFP between diet types was due to the lower DMI and greater dietary OM digestibility of the Fresh herbage diet.

Comparing nitrogen use efficiency and truly digestible protein use efficiency

For a given PDI use efficiency, the lower N use efficiency for the Fresh herbage diet than for the Maize diet may have been related to the high degradable N and non-protein N concentrations of the Fresh herbage diet. Indeed, cows fed fresh herbage diets consume more N but less PDI per kg of DMI than cows fed maize diets (Fig. 4; Demarquilly, 1975; INRA, 2018). This meta-analysis highlighted that N use efficiency and PDI use efficiency were strongly related, with a slope of 0.99, which indicates that each increase in N use efficiency increased PDI use efficiency to the same degree regardless of the diet type, despite the different variables in their respective equations. These results agreed with those of Cantalapiedra-Hijar et al. (2018), who observed a strong correlation (r = 0.80) between PDI use efficiency and N use efficiency. That study also found that the variables related to N metabolism correlated more with N use efficiency than those related to N ruminal fermentation and digestion. In the present meta-analysis, the strong relation between N use efficiency and PDI use efficiency indicates that variations in N use efficiency depend mainly on cow N metabolism. Therefore, PDI use efficiency can be predicted from N use efficiency, which is easier to calculate based on cow performance and diet characteristics.

Conclusion

This study showed that the most significant explanatory variables for variations in both N and PDI use efficiency were logically those used in their respective equations. Among these variables, those that reflect diet quality (i.e., dietary N concentration for N use efficiency and the ratio of dietary protein concentration to energy concentration for PDI efficiency) were the most influential. Moreover, cows used N and PDI in similar ways regardless of whether they were fed the Fresh herbage diet or Maize diet. The main differences in efficiency between these two diet types were due to differences in intake, digestibility and diet composition. Finally, regardless of the diet type, each increase in N use efficiency increased PDI use efficiency to the same degree, despite large differences in their respective equations and absolute values. Thus, N use and PDI use efficiencies seem to reflect the same variations in cow N use.

Supplementary material

Supplementary Material for this article (https://doi.org/10.1016/j.animal.2025.101511) can be found at the foot of the online page, in the Appendix section.

Ethics approval

This meta-analysis study has not been submitted for ethics approval. However, all procedures in the experiments used in this study complied with the ethical standards of the relevant national and institutional committees on animal experimentation at the time they were performed.

Data and model availability statement

The data that support the study findings are deposited in an official repository and are publicly available (Etalab open licence 2.0) in Ferreira et al. (2021a) [DataINRAE https://doi.org/10.15454/FKDGTG] and Delagarde R., 2025 [Extraction of the CowN-flow dataset for the present paper by Ferreira et al., DataINRAE https://doi.org/10.57745/7MEYNT]. Information can be made available from the authors upon request.

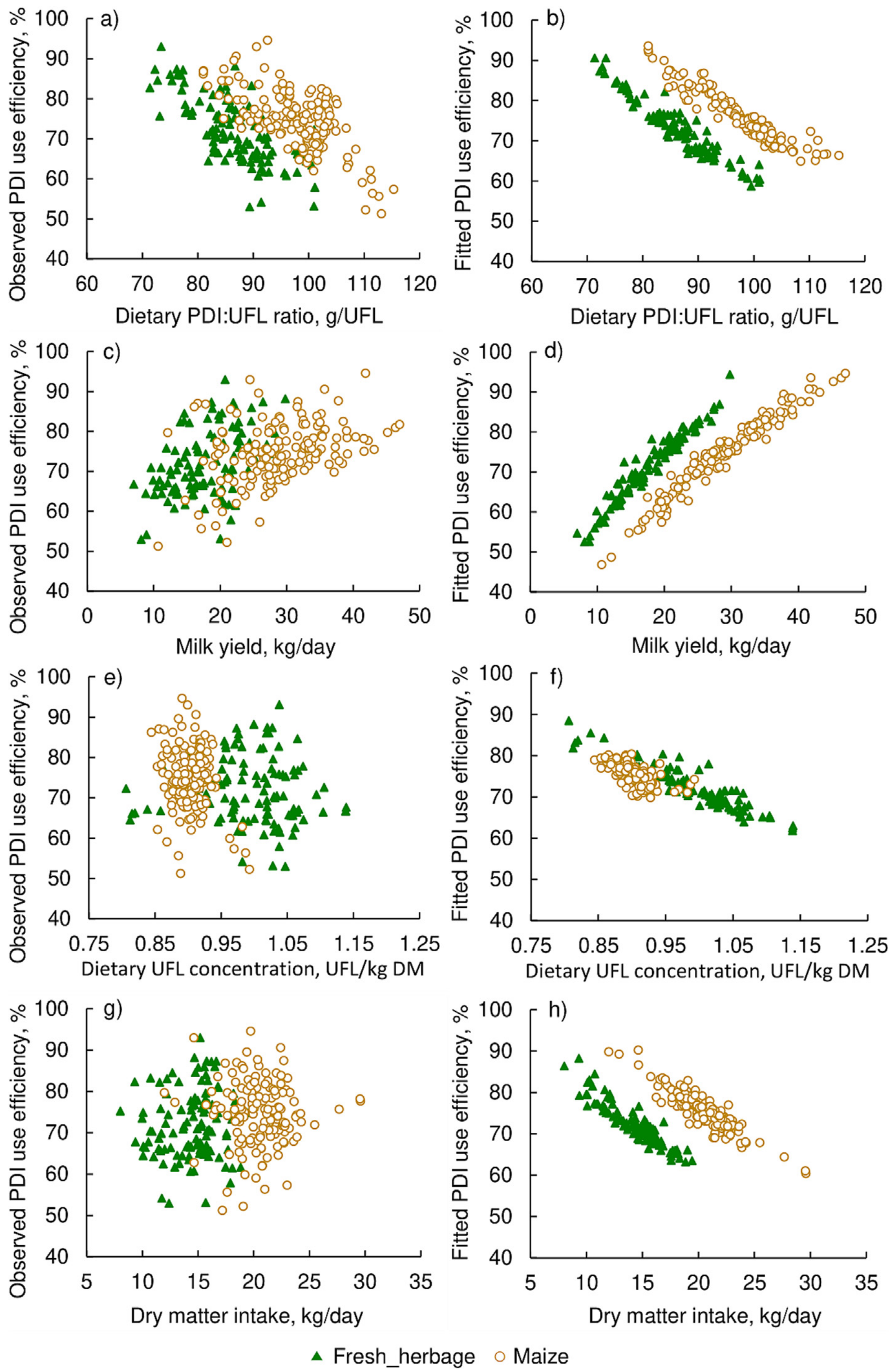

Fig. 3. Relation between observed and fitted truly digestible protein use efficiency of dairy cow and (a and b) the dietary truly digestible protein:net energy ratio, (c and d) milk yield, (e and f) dietary net energy concentration and (g and h) DM intake. Abbreviations: PDI = protein truly digestible in the small intestine; UFL = net energy (1 UFL = 7.37 MJ of net energy for lactation; INRA, 2018). PDI use efficiency fitted with the PDIeff_9 model. For each observation, the efficiency was calculated using all model coefficients except for the diet type and the explanatory variable on the x-axis. The efficiency was then centred on the mean value per diet type.

Declaration of Generative AI and AI-assisted technologies in the writing process

During the preparation of this work the author(s) did not use any AI and AI-assisted technologies.

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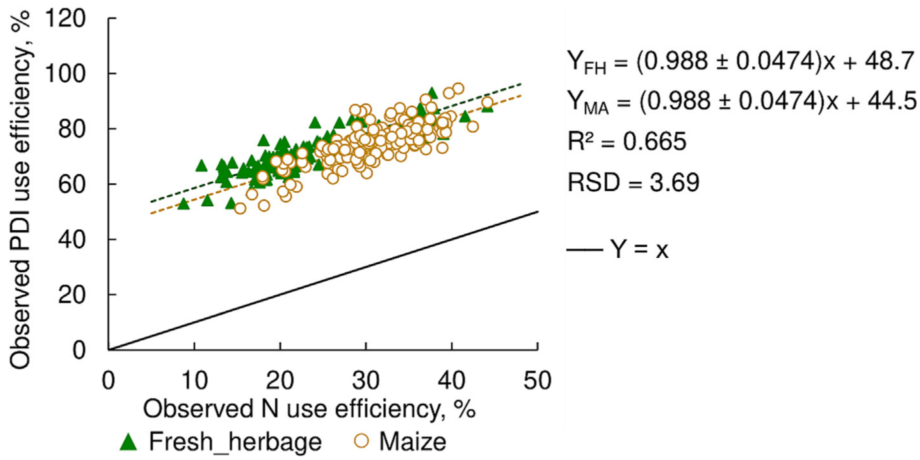

Fig. 4. Relation between observed truly digestible protein use efficiency and nitrogen use efficiency of dairy cows per diet type (Fresh herbage or Maize). Abbreviations: FH = fresh herbage; MA = maize; PDI = protein truly digestible in the small intestine.

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Declaration of interest

None.

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References

Aizimu, W., Al-Marashdeh, O., Hodge, S., Dewhurst, R.J., Chen, A., Zhao, G., Talukder, S., Edwards, G.R., Cheng, L., 2021. Estimation of nitrogen use efficiency for ryegrass-fed dairy cows: model development using diet- and animal-based proxy measures. Dairy 2, 435–451.
Akoglu, H., 2018. User's guide to correlation coefficients. Turkish Journal of Emergency Medicine 18, 91–93.
Arnold, T.W., 2010. Uninformative parameters and model selection using Akaike's Information Criterion. Journal of Wildlife Management 74, 1175–1178.
Bargo, F., Muller, L.D., Delahoy, J.E., Cassidy, T.W., 2002a. Performance of high producing dairy cows with three different feeding systems combining pasture and total mixed rations. Journal of Dairy Science 85, 2948–2963.
Bargo, F., Muller, L.D., Varga, G.A., Delahoy, J.E., Cassidy, T.W., 2002b. Ruminal digestion and fermentation of high-producing dairy cows with three different feeding systems combining pasture and total mixed rations. Journal of Dairy Science 85, 2964–2973.
Bates, D., Mächler, M., Bolker, B., Walker, S., 2015. Fitting linear mixed-effects models using lme4. Journal of Statistical Software 67, 1–51.
Beltran, I.E., Calvache, I., Cofre, R., Salazar, F., Keim, J.P., Morales, A., Pulido, R.G., Alfaro, M., 2022. Nitrogen intake and its partition on urine, dung and products of dairy and beef cattle in Chile. Agronomy-Basel 12, 15.
Brito, A.F., Almeida, K., Oliveira, A.S., 2022. Production performance, nutrient use efficiency, and predicted enteric methane emissions in dairy cows under confinement or grazing management system. Translational Animal Science 6, txac028.
Calsamiglia, S., Ferret, A., Reynolds, C.K., Kristensen, N.B., van Vuuren, A.M., 2010. Strategies for optimizing nitrogen use by ruminants. Animal 4, 1184–1196.
Cantalapiedra-Hijar, G., Dewhurst, R.J., Cheng, L., Cabrita, A.R.J., Fonseca, A.J.M., Noziere, P., Makowski, D., Fouillet, H., Ortigues-Marty, I., 2018. Nitrogen isotopic fractionation as a biomarker for nitrogen use efficiency in ruminants: a meta-analysis. Animal 12, 1827–1837.
Castillo, A.R., Kebreab, E., Beever, D.E., France, J., 2000. A review of efficiency of nitrogen utilisation in lactating dairy cows and its relationship with environmental pollution. Journal of Animal and Feed Sciences 9, 1–32.
Couvreur, S., Hurtaud, C., Marnet, P.G., Faverdin, P., Peyraud, J.L., 2007. Composition of milk fat from cows selected for milk fat globule size and offered either fresh pasture or corn silage-based diet. Journal of Dairy Science 90, 392–403.
Demarquilly, C., 1975. Éléments nouveaux concernant l'utilisation de l'énergie et de l'azote par les ruminants. Fourrages 62, 35–46.
Ferreira, M., 2023. Nitrogen use efficiency and excretion in dairy cows fed combination of fresh herbage and maize silage. PhD thesis, L'Institut Agro Rennes-Angers, France.
Ferreira M., Delagarde R., Edouard N., 2021a. CowNflow: A dataset on nitrogen flows and balances in dairy cows fed maize forage or herbage-based diets. https://doi.org/10.15454/FKDGTG. Accessed 16 February 2021.
Ferreira, M., Delagarde, R., Edouard, N., 2021b. CowNflow: a dataset on nitrogen flows and balances in dairy cows fed maize forage or herbage-based diets. Data in Brief 38, 107393.
French Livestock Institute (Institut de l'élevage), 2010. Guide pratique de l'alimentation du troupeau bovin laitier, 1st edition. Editions Quae, Institut de l'élevage, Versailles, France.
Huhtanen, P., Hristov, A.N., 2009. A meta-analysis of the effects of dietary protein concentration and degradability on milk protein yield and milk N efficiency in dairy cows. Journal of Dairy Science 92, 3222–3232.
Huhtanen, P., Nousiainen, J.I., Rinne, M., Kytola, K., Khalili, H., 2008. Utilization and partition of dietary nitrogen in dairy cows fed grass silage-based diets. Journal of Dairy Science 91, 3589–3599.
Huhtanen, P., Cabezas-Garcia, E.H., Krizsan, S.J., Shingfield, K.J., 2015. Evaluation of between-cow variation in milk urea and rumen ammonia nitrogen concentrations and the association with nitrogen utilization and diet digestibility in lactating cows. Journal of Dairy Science 98, 3182–3196.
INRA, 2018. INRA Feeding System for Ruminants. Wageningen Academic, Wageningen, Netherlands.
INRA, 2020. INRAtion® V5 software. Retrieved on 13th December 2021 from https://www.inration-ruminal.fr/en/.
Kuznetsova, A., Brockhoff, P.B., Christensen, R.H.B., 2017. lmertest package: tests in linear mixed effects models. Journal of Statistical Software 82, 1–26.
Lapierre, H., Larsen, M., Sauvant, D., Van Amburgh, M.E., Van Duinkerken, G., 2018. Review: converting nutritional knowledge into feeding practices: a case study comparing different protein feeding systems for dairy cows. Animal 12, 457–466.
Lapierre, H., Martineau, R., Hanigen, M.D., Ouellet, D.R., 2023. Review: how the efficiency of utilization of essential amino acids can be applied in dairy cow nutrition. Animal 17, 1008833.
Laroche, J.-P., Gervais, R., Lapierre, H., Ouellet, D.R., Tremblay, G.F., Halde, C., Boucher, M.-S., Charbonneau, E., 2022. Milk production and efficiency of utilization of nitrogen, metabolizable protein, and amino acids are affected by

protein and energy supplies in dairy cows fed alfalfa-based diets. Journal of Dairy Science 105, 329–346.
Lesschen, J.P., Van den Berg, M., Westhoek, H.J., Witzke, H.P., Oenema, O., 2011. Greenhouse gas emission profiles of European livestock sectors. Animal Feed Science and Technology 166–167, 16–28.
Nakagawa, S., Schielzeth, H., 2013. A general and simple method for obtaining R2 from generalized linear mixed-effects models. Methods in Ecology and Evolution 4, 133–142.
Omphalius, C., Lapierre, H., Guinard-Flament, J., Lamberton, P., Bahloul, L., Lemosquet, S., 2019. Amino acid efficiencies of utilization vary by different mechanisms in response to energy and protein supplies in dairy cows: study at mammary-gland and whole-body levels. Journal of Dairy Science 102, 9883–9901.
Peyraud, J.L., Cellier, P., Donnars, C., Vertès, F., 2014. Réduire les pertes d'azote dans l'élevage : expertise scientifique collective. Editions Quae, Versailles, France.
Phuong, H.N., Friggens, N.C., de Boer, I.J.M., Schmidely, P., 2013. Factors affecting energy and nitrogen efficiency of dairy cows: A meta-analysis. Journal of Dairy Science 96, 7245–7259.
Tamminga, S., 1992. Nutrition management of dairy cows as a contribution to pollution control. Journal of Dairy Science 75, 345–357.
Vérité, R., Delaby, L., 2000. Relation between nutrition, performances and nitrogen excretion in dairy cows. Annales De Zootechnie 49, 217–230.