Prediction of urinary nitrogen and urinary urea nitrogen excretion by lactating dairy cattle in northwestern Europe and North America: A meta-analysis

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ABSTRACT

A meta-analysis was conducted on the effect of dietary and animal factors on the excretion of total urinary nitrogen (UN) and urinary urea nitrogen (UUN) in lactating dairy cattle in North America (NA) and northwestern Europe (EU). Mean treatment data were used from 47 trials carried out in NA and EU. Mixed model analysis was used with experiment included as a random effect and all other factors, consisting of dietary and animal characteristics, included as fixed effects. Fixed factors were nested within continent (EU or NA). A distinction was made between urinary excretions based on either urine spot samples or calculated assuming a zero N balance, and excretions that were determined by total collection of urine only. Moreover, with the subset of data based on total collection of urine, a new data set was created by calculating urinary N excretion assuming a zero N balance. Comparison with the original subset of data allowed for examining the effect of such an assumption on the relationship established between milk urea N (MUN) concentration and UN. Of all single dietary and animal factors evaluated to predict N excretion in urine, MUN and dietary crude protein (CP) concentration were by far the best predictors. Urinary N excretion was best predicted by the combination of MUN, CP, and dry matter intake, whereas UUN was best predicted by the combination of MUN and CP. All other factors did not improve or only marginally improved the prediction of UN or UUN. The relationship between UN and MUN differed between NA and EU, with higher estimated regression coefficients for MUN for the NA data set. Precision of UN and UUN prediction improved substantially when only UN or UUN data based on total collection of urine were used. The relationship between UN and MUN for the NA data set, but not for the EU data set, was substantially altered when UN was calculated assuming a zero N balance instead of being based on the total collection of urine. According to results of the present meta-analysis, UN and UUN are best predicted by the combination of MUN and CP and that, in regard to precision and accuracy, prediction equations for UN and UUN should be derived from the total collection of urine.

Key words: milk urea nitrogen, urinary nitrogen, dairy cattle, meta-analysis

INTRODUCTION

Nitrogen (N) losses via excreted feces and urine in dairy cattle are associated with losses of N from the farming system through ammonia volatilization, nitrate leaching, and dissipation of N as N_2O, NO, and NO_2 (de Vries et al., 2001). With regard to such environmental concerns, great interest has been noted in investigating the potential of specific on-farm measures to reduce N losses, preferably without reducing milk production. Nitrogen digested and not excreted as milk protein is, in large part, excreted as urea N in urine. On-farm indicators including MUN concentration (mg of N/dL) may be attractive to monitor the excretion of urinary urea N (**UUN**; g of N/d) or total urinary N (**UN**; g of N/d). Several studies focused on the relationship between MUN and UN (Jonker et al., 1998; Kauffman and St-Pierre, 2001; Nousiainen et al., 2004; Zhai et al., 2005; Zhai et al., 2007). Jonker et al. (1998) and Nousiainen et al. (2004) performed meta-analyses analyzing the relationship between MUN and UN on data sets containing data from multiple trials. Published meta-analyses have either been based solely on data from North America (**NA**) or data from northwestern European countries (**EU**). Jonker et al. (1998) based their analysis on 3 NA trials, whereas Nousiainen et al. (2004) based their analysis on a large data set of 50 EU trials with grass-silage-based diets. In all trials used by Nousiainen et al. (2004), concentrates were offered at a flat rate irrespective of milk yield and UN was not based on total collection of urine but calculated from the difference between N intake and excretion of N in feces and milk, assuming a zero N balance. However, it is known from various studies in lactating dairy cows (Spanghero and Kowalski, 1997; Eriksson et al., 2004; Colmenero and Broderick, 2006), mice (Costa et al.,

1968), and humans (Young et al., 1981) that the N balance in general is positive due to losses of N from the organism not measured in urine, milk, and feces. Furthermore, differences in the MUN-UN relationships established in these studies might be related to differences in herd management, climatic conditions, type of diet, concentrate to roughage ratio of the diet, genetic makeup of the cows, or differences in techniques used to measure UN and UUN. Recently, the effect of such factors has been reviewed by Spek et al. (2013). During the last decade, more attention has been paid to the relationship between MUN and UUN instead of UN, because UUN is most strongly related to ammonia emission (Burgos et al., 2007). At present, only a few studies (Burgos et al., 2007; Powell et al., 2011) have focused on prediction of UUN by MUN. No studies have been published on the prediction of UUN from multiple animal- and dietary-related factors. For the present study, we hypothesized that the prediction accuracy of UN and UUN may be improved by selection of only those trials where UN and UUN are analyzed based on total collection of urine, instead of estimating UN and UUN based on the difference between N intake and excretion of N in milk and feces, or based on analysis of UN and UUN in urine spot samples with daily volume of urine estimated from creatinine levels in the same urine spot samples. For practical and animal welfare reasons, it might be argued to determine UN based on the difference between N intake and N excreted in feces and milk instead of using indwelling urine catheters. However, no studies have been carried out that have tested whether the relationship between MUN and UN is actually similar for UN derived from total collection of urine, or for UN calculated as the difference between N intake and N excreted in feces and milk.

The first objective of this study was to quantify the relationship between various dietary and animal factors and UN or UUN for either EU or NA data sets and to compare their respective prediction equations. The second objective of this study was to test whether the accuracy and precision of UN and UUN prediction equations are affected by the method of measuring UN and UUN, namely, estimation of UN and UUN from urine spot samples or by calculations assuming a zero N balance, versus UN and UUN determined by total collection of urine only.

MATERIALS AND METHODS

Data Set Selection

Studies were selected that contained at least information on (1) the partitioning of N excretion in urine, feces, and milk, (2) MUN, (3) DMI (kg/d) and com-

position of the ration, and (4) milk production and fat and protein content in milk. Mean treatment data (n = 200) from 47 trials carried out in NA (n = 118) and EU (n = 82) were used. A description of this data set (referred to as complete data set) is presented in Table 1 and contained 193 observations on UN (n = 111 for NA and n = 82 for EU) and 98 observations on UUN (n = 57 for NA and n = 41 for NA). The appendix provides a reference list of 41 studies describing these 47 trials. Some studies described multiple trials, which explains the presence of more trials than studies. In several studies, excretion of UN and UUN was determined based on spot samples taken from the urine, or based on the calculation of UN as the difference between N intake and excretion of N in milk and feces (i.e., zero N balance). A reduced data set (referred to as reduced data set) was developed including only observations on UN or UUN from studies where urine was collected quantitatively. This reduced data set contained 123 observations on UN (n = 55 for NA and n = 68 for EU) and 63 observations on UUN (n = 22 for NA and n = 41 for EU). The number of observations in the reduced data set where both UN and UUN were measured was 56 (n = 15 for NA and n = 41 for EU). To evaluate the effect of the assumption of a zero N balance on the results obtained, a new data set (number of data hence identical to that of the reduced data set) was created from the reduced data set in which data on urine N excretion were replaced by values calculated under assumption of a zero N balance.

Independent and Dependent Factors

The list of independent factors that were tested for their capacity to explain observed variation in UN and UUN included animal factors and dietary factors. These independent factors are presented in Table 1 under the headings Animal factors and Dietary factors. The dependent factors in the data set were UUN and UN. Some dietary values were missing with respect to ash, starch, and NDF. These missing values were predicted based on typical composition using the Dutch feeding tables (CVB, 2007). For all diets, values were predicted for RDP, RUP, rumen-undegradable starch, digestible protein available in the small intestine (**DVE**), rumen-degradable protein balance (**OEB**), and NE_L using the Dutch feeding tables (CVB, 2007).

Statistical Procedure

Multiple regression analyses were carried out with the MIXED procedure in SAS (SAS Institute Inc., Cary, NC) with trial included as random effect and all other factors as fixed effects. Fixed effects were nested

Table 1. Characterization of data sets used for the statistical evaluation

Item	Northwestern Europe			North America		
	n	Mean	SD	n	Mean	SD
Animal factors						
BW (kg)	76	607	28.1	114	649	56.4
DIM	76	133	54.1	118	129	58.9
DMI (kg/d)	82	18.9	2.45	118	23.4	2.29
Milk (kg/d)	82	25.5	4.51	118	34.7	6.79
MUN (mg of N/dL)	82	12.5	5.07	118	13.1	3.59
Milk fat (%)	82	4.36	0.392	118	3.52	0.432
Milk protein (%)	82	3.33	0.210	118	3.05	0.262
Dietary factors[1]						
Corn silage (% in DM)	82	15	23.5	118	22	15.8
Grass silage or herbage (% in DM)	82	47	30.3	118	5	11.6
Legume (% in DM)	82	7	14.1	118	25	16.8
Roughage (% in DM)	82	68	15.3	118	52	9.4
CP (% in DM)	82	16.1	2.55	118	17.1	1.82
RDP (% in DM)	76	10.0	2.08	118	10.2	1.65
RUP (% in DM)	76	5.9	1.17	118	7.0	1.37
OEB (% in DM)	76	2.1	2.02	118	2.4	1.73
DVE (% in DM)	76	8.2	1.37	118	8.9	1.48
NE_L (MJ/kg of DM)	76	6.6	0.36	118	6.6	0.47
NDF (% in DM)	76	39.6	5.41	118	30.8	5.39
NDF forage (% in DM)	76	31.2	7.72	118	20.5	6.49
NDF forage to NDF total diet ratio	76	0.78	0.104	118	0.68	0.199
Starch (% in DM)	76	13.2	8.35	118	25.4	6.88
RUS (% in DM)	76	2.5	2.20	118	7.7	3.08
Ash (% in DM)	76	8.5	1.63	118	7.4	1.39
Nitrogen flows[2] (g of N/d)						
N intake	82	485	85.0	118	637	89.6
N milk	82	133	21.0	118	166	32.1
N feces	82	159	31.9	111	223	62.9
UN	82	185	69.1	111	212	56.0
UUN	41	152	85.7	57	168	47.9
NUUN	35	45	12.0	50	44	19.5
N balance	68	10	30.9	55	54	38.8

[1]OEB = rumen-degraded protein balance (Tamminga et al., 1994); DVE = intestinal digestible protein (Tamminga et al., 1994); RUS = rumen undegradable starch.

[2]UN = N excreted in urine; UUN = urea N excreted in urine; NUUN = non-urea-N excreted in urine. N balance = calculated as N-intake minus N excreted in urine, feces, and milk where UN is based on total collection of urine.

within continent (EU or NA). Univariate regression analysis of fixed factors on UN was carried out to determine those factors that explained most variation in UN from the complete data set (Table 2). Those factors that explained more than 50% of the variation in UN were combined with each of the other individual animal and dietary related factors by means of multivariate regression analysis. Based on the criteria of R^2, root mean square prediction error (**RMSPE**), and Akaike's information criterion (**AIC**) it was decided whether the other factors improved the model fit. It was assumed that observations within trial were equally correlated to each other and, therefore, the covariance structure was modeled as compound symmetry. The R^2 values presented apply to the regression of predicted values of fixed effects to observed values, hence excluding the contribution of variation associated with random effect. Likewise, the RMSPE was calculated from the residuals of the fixed effects. The RMSPE was calculated accord-

ing to Jonker et al. (1998). In practice, the trial effect is unknown and was not included in the calculation of RMSPE and R^2, and therefore better reflects the prediction accuracy to be expected when applying the models. A COVTEST statement was included in the PROC MIXED statement to test for the effects of fixed factors on changes in between-trial variation. Data from one of the trials of Tas et al. (2006) were discarded because of very low MUN values that resulted in UN to MUN ratios more than 3 standard deviations higher than the average.

RESULTS AND DISCUSSION

Data Set Evaluation

The complete data set showed a large range in CP (9.4–24.1% DM), MUN (3.8–30.2 mg of N/dL), DMI (13.8–30.2 kg/d), and milk production (15.5–45.5

Table 2. Effect of single factors on N excretion in urine in the complete data set as judged from the *P*-value of these single factors and the goodness of fit parameters R^2 and AIC^1

Item	*P*-value	R^2	AIC
Animal factors			
MUN (mg of N/dL)	<0.001	0.72	1,683
BW (kg)	0.050	0.11	1,932
DIM	0.480	0.07	1,979
Milk (kg/d)	0.010	0.01	2,025
Milk protein (%)	0.449	0.07	2,019
Milk fat (%)	0.350	0.05	2,021
DMI (kg/d)	0.008	0.02	2,021
Dietary factors[2]			
CP (% in DM)	<0.001	0.79	1,700
RDP (% in DM)	<0.001	0.36	1,750
RUP (% in DM)	<0.001	0.38	1,903
OEB (% in DM)	<0.001	0.37	1,779
DVE (% in DM)	<0.001	0.29	1,925
NE_L (MJ/kg of DM)	0.075	0.12	1,955
NDF (% in DM)	0.518	0.01	1,970
NDF forage (% in DM)	0.974	0.06	1,972
Starch (% in DM)	<0.001	0.28	1,943
RUS (% in DM)	<0.001	0.21	1,950
Ash (% in DM)	0.004	0.14	1,955
Roughage (% in DM)	0.814	0.10	2,032

[1]Coefficient of determination (R^2) is calculated from the total sums of squares of the model and the sums of squares explained by the fixed effect part of the model only; AIC = Akaike information criterion, a measure of the relative goodness of fit of the model; a smaller value means a better model fit.

[2]OEB = rumen-degraded protein balance (Tamminga et al., 1994); DVE = intestinal digestible protein (Tamminga et al., 1994); RUS = rumen undegradable starch.

kg/d). The NA diets were largely based on corn silage and alfalfa and contained on average 52% roughage, whereas the EU diets were largely based on grass silage and clover silage and contained on average 68% roughage. As a consequence, the NA formulated rations contained on average 25.4% starch (DM basis) and EU rations contained 13.2% starch (DM basis). On average, NA cows produced 9.2 kg/d more milk (34.7 vs. 25.5 kg/d), consumed 4.5 kg of DM/d more feed (23.4 vs. 18.9 kg of DM/d), and were 42 kg heavier (649 vs. 607 kg) than the EU cows. Higher standard deviations were found in the EU studies compared with NA studies for MUN (SD of 5.07 vs. 3.59), UN (SD of 69.1 vs. 56.0), and UUN (SD of 85.7 vs. 47.9). Daily N intake was on average 152 g higher in NA studies and daily excretion of N in milk, urine, and feces were 33, 27, and 64 g higher, respectively. Average MUN was 0.6 mg/dL higher in NA compared with EU, in line with the higher UN and UUN, but lower than would be expected considering the 27 (UN) and 16 (UUN) g of N/d difference between EU and NA. Compared with EU data, fecal N excretion was 40% higher for NA cows, whereas DMI and CP content for NA were only 24 and 6% higher, respectively. The positive relation between fecal N excretion and DMI might explain the higher fecal N excretion for NA. Huhtanen et al. (2008) observed a 9.9-g increase in fecal N per kilogram increase of DMI. Regression of fecal N excretion on DMI in this study resulted in 11.3 ± 1.07 and 12.1 ± 2.03 g of fecal N/kg of DMI for EU and NA, respectively. The intercept was 17.4 ± 48.77 g of fecal N/d higher for NA and not statistically different from EU. Hristov et al. (2005) found a 76% higher fecal N excretion for diets supplemented with starch compared with diets supplemented with fiber and ascribed this difference in fecal N to differences in the amounts of microbial N synthesized in the large intestine and excreted in the feces. The 17.4-g higher fecal N excretion for NA, not being explained by DMI, might be explained by the larger amounts of rumen-undegradable starch reaching the large intestine for NA diets, resulting in an increased synthesis and fecal excretion of microbial N.

Explanatory Factors

From all independent factors tested, the animal factor MUN and the dietary factor CP are the single explanatory factors in the model that explained most of the observed variation of UN (R^2 of 0.72 and 0.79, respectively; Table 3) and of UUN (R^2 of 0.87 and 0.81, respectively; Table 4). Combining MUN and CP further improved the explanation of observed variation of UN and UUN (Tables 3 and 4). Although a substantial portion of variation in UN in the complete data set was explained by the single factors RUP (38%), RDP (36%), OEB (37%), and DVE (29%; Table 2), these fac-

tors did not increase the portion of explained variation when added to the model that already included MUN and CP. For UUN, a substantial part of the variation in UUN could be explained by the single factors RDP (50%), OEB (49%), DVE (20%), and RUP (18%), but, similar to UN, these factors did not increase the portion of explained variation when added to the model that already included MUN and CP. The prediction of UN was only slightly further improved when DMI was added to the model that already included MUN and CP. Milk production and BW were significant factors in explaining variation in UN when added to the model that already included MUN and CP. However, the model fit, as judged by the RMSPE and R^2, did not improve and therefore these factors were excluded from the models presented in Tables 3 and 4.

Effect of Dietary and Animal Factors on UN

More variation of UN in the complete data set was explained by CP ($R^2 = 0.79$) than by MUN ($R^2 = 0.72$), whereas, in the reduced data set, MUN explained slightly more variation than CP ($R^2 = 0.85$ vs. 0.82). However, both the complete and reduced data sets had lower AIC values when MUN was included in the model, thus favoring MUN above CP as a predictor of UN. Results from a meta-analysis by Nousiainen et al. (2004) suggest that, based on R^2 and root mean square errors values, CP is a better predictor of UN than MUN (R^2 of 0.85 vs. 0.74). However, Nousiainen et al. (2004) did not report AIC values and, therefore, based on the combined results of the present study and the study of Nousiainen et al. (2004), it is not possible to conclude which predictor, CP or MUN, is preferred to predict UN. The relationship established between MUN and UN was almost identical for the complete and reduced data set, for EU as well as for NA (Figure 1; Table 3). Results of the univariate regression of UN on MUN for NA and EU were similar to values reported by Kauffman and St-Pierre (2001), Kohn et al. (2002), Nousiainen et al. (2004), and Zhai et al. (2005) at low to moderate MUN values (MUN < 10 mg of N/dL). Predicted values of UN from the studies of Kauffman and St-Pierre (2001), Kohn et al. (2002), Nousiainen et al. (2004), and predicted values of NA in the present study diverged (became larger) at higher MUN values from predicted values in the study of Zhai et al. (2005) and predicted values of EU in the present study (Figure 1).

Multiple regression of MUN and CP on UN improved the explanation of UN substantially as judged by the AIC, R^2, and RMSPE for both the complete (model 3; Table 3), reduced (model 7; Table 3), NA, and EU data sets. Furthermore, this model lowered the differences

in regression coefficients for MUN, but increased the differences in regression coefficients for CP between EU and NA. Besides the increase in differences between regression coefficients for CP for EU and NA, the differences in intercepts between NA and EU increased as well, from 10.2 to 65.8 g of N/d for the complete data set and from 15.7 to 58.0 g of N/d for the reduced data set, respectively, indicating an interaction between CP and MUN. Indeed, a positive significant interaction between MUN and CP was observed for the complete data set ($P = 0.006$) and reduced data set ($P = 0.006$). Apparently, CP and MUN are not completely confounded in explaining variation in UN, as can be observed from the increased explanation of variation in UN when both factors are included in the model compared with univariate models containing either MUN or CP. The interaction between CP and MUN is another indication that CP and MUN are not completely confounded with respect to explaining variation in UN. Nousiainen et al. (2004) observed a positive linear relationship between MUN and CP; MUN (mg of N/dL) = $1.7 \times$ CP (% in DM) $- 14.2$ with an R^2 of 0.78. In the present study, a similar relationship was established between CP and MUN for EU ($R^2 = 0.75$) but a weaker one for NA ($R^2 = 0.47$; Figure 2). The fact that a substantial part of the variation in MUN is not explained by CP indicates that, at a given level of CP, the level of MUN varies due to factors other than CP. Factors that might explain this unexplained variation in MUN are numerous and related to differences in nutrition and management and differences between cows. For instance, nutritional factors that affect MUN, but are unrelated to CP, include the partition of CP in RDP and RUP (Broderick et al., 1993), the dietary intake of salt and water (Burgos et al., 2001; Spek et al., 2012), or dietary energy content (Broderick and Clayton, 1997). Examples of management factors that affect MUN, but not CP, are the time and frequency of feeding and milking, which can both affect the diurnal pattern of MUN (Gustafsson and Palmquist, 1993; Friggens and Rasmussen, 2001). Differences between cows with respect to milk protein production might affect the partition of degraded and absorbed protein into milk protein and urea formed with protein catabolism, thereby affecting the relationship between MUN and CP (Huhtanen and Hristov, 2010). Furthermore, the need of the animal to retain N at low levels of N intake affects the renal regulation of urea excretion. Several studies in sheep (Schmidt-Nielsen et al., 1958), goats (Eriksson and Valtonen, 1982), and cattle (Thornton, 1970) show that a reduction in CP can result in a reduced renal urea clearance rate and increased renal urea reabsorption. These kidney-related factors affect the pool of urea and the distribution of urea in the cow and, as a result, affect

Table 3. Prediction of urinary nitrogen excretion (UN; g of N/d) by means of MUN (mg of N/dL) concentration, dietary CP concentration (% in DM), and DMI (kg/d)[1]

Model	Data set	μ[2] SE P-value	EU[3] SE P-value	MUN SE P-value EU	MUN SE P-value NA[8]	CP SE P-value EU	CP SE P-value NA	DMI SE P-value EU	DMI SE P-value NA	n[4]	R[2][5]	RMSPE[6]	AIC[7]
1	Complete	29.3	7.1	11.92**	14.08					193	0.72	33.1	1,683
		10.38	14.41	0.470	0.657								
		0.007	0.621	<0.001	<0.001								
2	Complete	−223.9	10.2			24.81	25.47			193	0.79	28.6	1,700
		22.61	29.53			1.089	1.294						
		<0.001	0.730			<0.001	<0.001						
3	Complete	−132.2	65.8	7.73	7.52	9.63	14.42			193	0.85	24.7	1,609
		21.46	33.39	1.086	0.962	2.294	1.745						
		<0.001	0.051	<0.001	<0.001	<0.001	<0.001						
4	Complete	−168.9	19.1	7.55	7.84	9.64	13.37	4.55	2.17	193	0.86	24.2	1,578
		29.43	41.10	1.015	0.9100	2.143	1.700	0.895	1.182				
		<0.001	0.646	<0.001	<0.001	<0.001	<0.001	<0.001	0.069				
5	Reduced	16.7	13.0	12.03*	14.31					123	0.85	26.4	1,050
		12.94	15.82	0.466	0.824								
		0.206	0.413	<0.001	<0.001								
6	Reduced	−230.5	15.7			24.65	25.72			123	0.82	28.6	1,094
		32.98	39.16			1.219	1.880						
		<0.001	0.690			<0.001	<0.001						
7	Reduced	−123.8	58.0	8.27	8.95	8.87	12.43			123	0.93	18.8	1,012
		27.23	36.91	1.077	1.235	2.256	2.237						
		<0.001	0.120	<0.001	<0.001	<0.001	<0.001						
8	Reduced	−148.8	8.9	8.06	8.79	8.91	11.97	4.06	1.58	123	0.93	18.3	988
		39.79	49.42	1.019	1.213	2.132	2.178	0.915	1.583				
		<0.001	0.858	<0.001	<0.001	<0.001	<0.001	<0.001	0.322				

[1]Models 1 to 4 are based on studies where UN was determined based on total collection of urine, urine spot samples, or zero N balance calculations (data set Complete), whereas models 5 to 8 are based on studies where UN was determined based on total collection of urine only (data set Reduced).

[2]μ = intercept value.

[3]EU = data are valid for northwestern Europe only, and this factor is included in the model to be able to create separate intercepts and regression coefficients for North America and Europe.

[4]n = number of observations.

[5]Coefficient of determination is calculated from the total sums of squares of the model and the sums of squares explained by the fixed effect part of the model.

[6]RMSPE = root mean square prediction error based on the residuals from the fixed effect part of the model only.

[7]AIC = Akaike information criterion, a measure of the relative goodness of fit of the model; a smaller value means a better model fit.

[8]NA = data are valid for North America only, and this factor is included in the model to be able to create separate regression coefficient for North America and northwestern Europe.

*$P \leq 0.05$, **$P \leq 0.01$; asterisks denote a significance of difference in regression coefficients for MUN, CP, or DMI between EU and NA.

Table 4. Prediction of urinary urea nitrogen excretion (UUN; g of N/d) by means of MUN (mg of N/dL) concentration and dietary CP concentration (% in DM)[1]

Model	Data set	μ^2 SE P-value	EU[3] SE P-value	MUN SE P-value		CP SE P-value		n[4]	R[25]	RMSPE[6]	AIC[7]
				EU	NA[8]	EU	NA				
9	Complete	−20.8	−26.6	14.09	14.40			98	0.87	23.7	825
		11.05	15.74	0.535	0.701						
		0.075	0.095	<0.001	<0.001						
10	Complete	−380.7	56.2			28.70	32.00	98	0.81	29.0	874
		38.03	47.03			1.523	2.182				
		<0.001	0.236			<0.001	<0.001				
11	Complete	−197.6	74.3	10.97	8.77	7.19	14.62	98	0.92	18.9	788
		35.78	46.23	1.248	1.231	2.645	2.829				
		<0.001	0.112	<0.001	<0.001	0.008	<.001				
12	Reduced	−31.4	−17.2	14.15	14.57			63	0.93	19.9	523
		16.21	19.14	0.541	0.93						
		0.077	0.374	<0.001	<0.001						
13	Reduced	−470.4	144.6			28.77*	36.82	63	0.81	33.6	564
		61.95	69.07			1.657	3.355				
		<0.001	0.042			<0.001	<0.001				
14	Reduced	−146.9	2.7	10.38	11.32	8.92	9.19	63	0.96	15.5	500
		62.82	67.87	1.119	1.839	2.315	4.771				
		0.038	0.968	<0.001	<0.001	0.004	0.060				

[1]Models 9 to 11 are based on studies where UUN was determined based on total collection of urine, urine spot samples, and zero N balance calculations (data set Complete), whereas models 12 to 14 are based on studies where UUN was determined based on total collection of urine only (data set Reduced)

[2]μ = intercept value.

[3]EU = data are valid for northwestern Europe only, and this factor is included in the model to be able to create separate intercepts and regression coefficients for North America and Europe.

[4]n = number of observations.

[5]Coefficient of determination is calculated from the total sums of squares of the model and the sums of squares explained by the fixed effect part of the model only.

[6]RMSPE = root mean square prediction error based on the residuals from the fixed effect part of the model only.

[7]AIC = Akaike information criterion, a measure of the relative goodness of fit of the model; a smaller value means a better model fit.

[8]NA = data are valid for North America only, and this factor is included in the model to be able to create separate regression coefficient for North America and northwestern Europe.

*$P \leq 0.05$; an asterisk denotes a significance of difference in regression coefficients for MUN, CP, or DMI between EU and NA.

concentrations of urea nitrogen in blood plasma and MUN. All sources of variation mentioned above might underlie the moderate relationship observed between MUN and CP. The same sources of variation may be responsible for the added effect of MUN and CP in predicting UN. In the present study, indications were also noted for an increase in renal recycling of urea at low dietary CP, as observed from differences in quantities of urinary urea N excreted per unit increase in MUN at low and high protein diets. For example, regression of UUN on MUN for the reduced data set resulted in lower regression coefficients for MUN for EU (8.01 ± 2.683) for a subdata set with only CP values lower than average CP, compared with regression coefficients for EU (13.70 ± 0.759) for a subdata set with CP values higher than average CP. These differences in regression coefficients of MUN between high- and low-protein subdata sets in predicting UUN are also reflected by the positive interaction between CP and MUN in the complete ($P = 0.006$) and reduced ($P = 0.006$) data

sets in predicting UN, even though inclusion of the CP × MUN interaction did not improve model fit. These differences in regression coefficients for MUN found in the subdata set with low CP values and those observed in the subdata set with high CP values might explain differences in UN-MUN relationships found between various studies, and might also explain differences in UN-MUN relationships observed in the present data set between NA and EU data.

Addition of DMI to the model containing MUN and CP (models 4 and 8; Table 3) slightly improved the model fit of UN without a pronounced effect on regression coefficients of MUN and CP (Table 3). However, the difference in intercept values between EU and NA was reduced. The effect of DMI on UN was larger for EU than NA and is likely related to the positive and significant relation between CP and DMI for NA in the complete ($P = 0.044$) and reduced ($P = 0.040$) data set, whereas no such relationship was established for EU in the complete ($P = 0.530$) and reduced (P
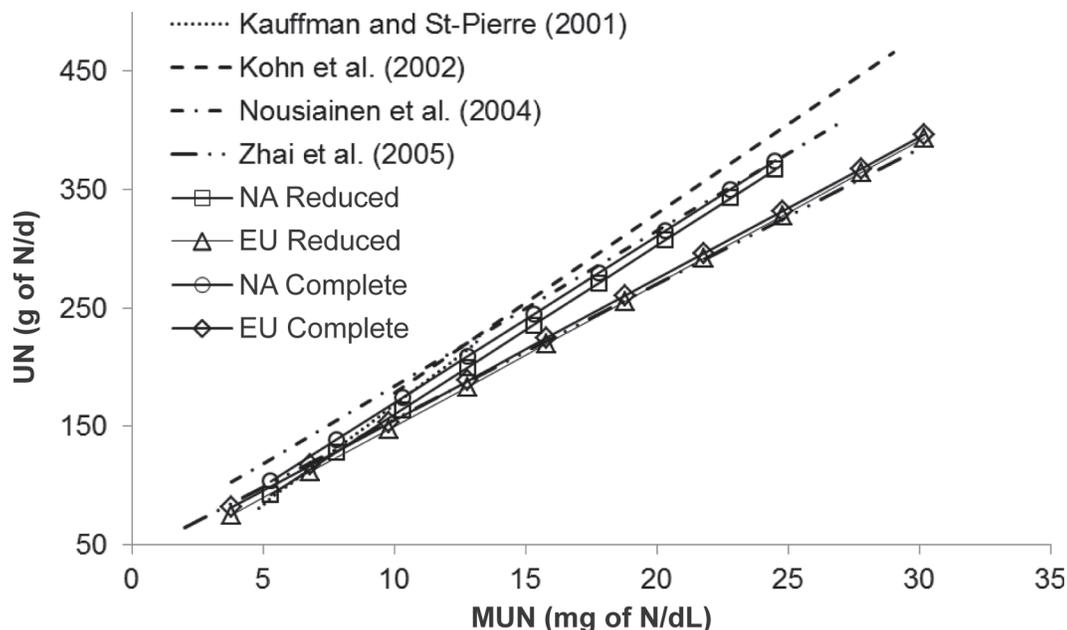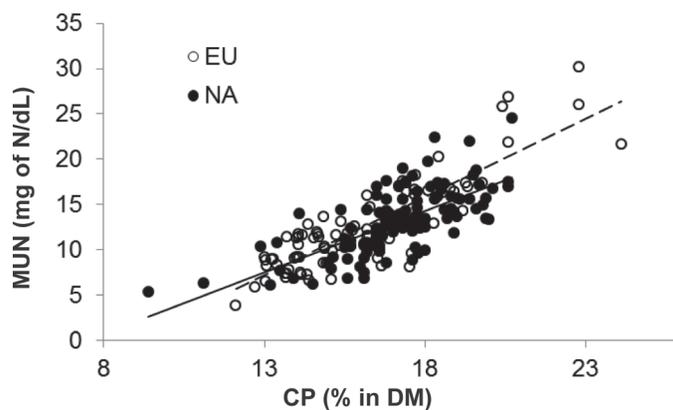
Figure 1. Relationship between MUN (mg of N/dL) and urinary nitrogen excretion (UN; g of N/d) for northwestern Europe (EU) and North America (NA). Urinary nitrogen excretion is determined based on the complete data set (Complete; n = 193) or based on the reduced data set (Reduced; n = 123) in this study and for other studies (Kauffman and St-Pierre, 2001; Kohn et al., 2002; Nousiainen et al., 2004; Zhai et al., 2005).

= 0.590) data set. The authors of the present study have no explanation for these differences in CP-DMI relationships between NA and EU.

Effect of Dietary and Animal Factors on UUN

Variation in UUN was largely explained by MUN (models 9 and 12; Table 4) and CP (models 10 and 13; Table 4) with the complete as well as the reduced data set. Milk urea nitrogen explained a larger fraction (R^2 = 0.87–0.93) of observed variation in UUN than CP (R^2 = 0.81), with 93% of variation in UUN explained for the in EU data and 75% in the NA data. The fact that more variation of UUN was explained in EU compared with NA data may be related to the larger variation in the EU data versus the NA data for UUN (152 ± 85.7 vs. 168 ± 47.9 g of N/d) and MUN (12.5 ± 5.07 vs. 13.1 ± 3.59 mg of N/dL). The slope of MUN on UUN was similar for EU (14.09 and 14.15 for the complete and reduced data set, respectively) and NA (14.40 and 14.57 for the complete and reduced data set, respectively); whereas the intercept value of UUN tended to be lower for the EU than the NA data set for both the complete and reduced data set. Burgos et al. (2007) and Powell et al. (2011) observed regression coefficients for MUN of 14.4 and 16.2 g of UUN/d per unit of MUN, respectively. The model prediction of Burgos et al. (2007) was based on a single study, whereas the model prediction of Powell et al. (2011)

was based on a simple regression of UUN on MUN data from 9 studies without correction for trial effect. The reason that a higher regression coefficient for MUN was found by Powell et al. (2011) compared with Burgos et al. (2007) and results from the present study might be the consequence of not accounting for trial effect, as shown to be necessary by St-Pierre (2001). Extrapolation of the model prediction of UUN by CP (model 13;

Figure 2. Relationship between dietary CP content (% in DM) and MUN (mg of N/dL) for northwestern Europe (EU; n = 82) and North America (NA; n = 118) based on the complete data set. For NA (solid regression line), MUN = −10.1 ± 2.29 + 1.36 ± 0.133 × CP, R^2 = 0.47. For EU (dashed regression line), MUN = −15.3 ± 1.80 + 1.73 ± 0.111 × CP, R^2 = 0.75.
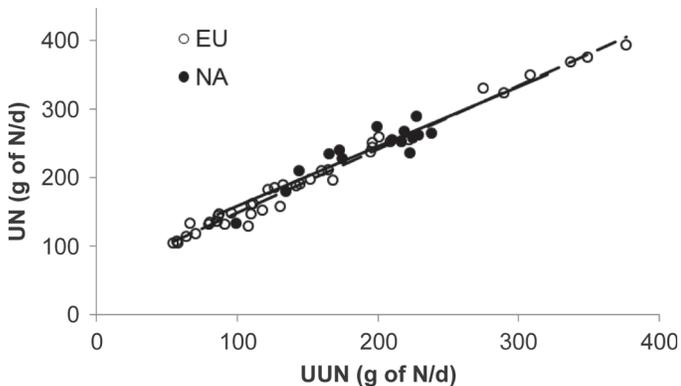
Figure 3. Relationship between urinary urea nitrogen excretion (UUN; g of N/d) and urinary total nitrogen excretion (UN; g of N/d) for northwestern Europe (EU; n = 41) and North America (NA; n = 15) based on the reduced data set. For NA (solid regression line), UN = 72.3 ± 22.49 + 0.866 ± 0.1147 × UUN, R^2 = 0.81. For EU (dashed regression line), UN = 55.4 ± 3.14 + 0.932 ± 0.0181 × UUN, R^2 = 0.99.

Table 4) suggests that UUN would become zero at a CP of 12.8 and 11.3% with the NA and the EU data, respectively, suggesting that these CP values indicate the highest possible N utilization with no loss of urea excreted in urine. Few studies have determined minimal UN or UUN excretion in dairy cows. In one study, Wohlt et al. (1978) measured a UN of 35 to 38 g of N/d at CP of 9.4 to 10.3%, a UN of 57 to 63 g of N/d at CP of 11.6 to 12.0%, and a UN of 115 to 137 g of N/d at CP of 14.2 to 14.4%. In another study, Ørskov and MacLeod (1982) measured minimal UN and UUN in pregnant, nonlactating Friesian cows receiving no dietary N at all and observed UN ranging from 34.2 to 42.0 g of N/d and UUN ranging from 17.8 to 26.0 g of N/d. The similarity in UN values observed in the study of Ørskov and MacLeod (1982) and those observed in the study of Wohlt et al. (1978), at CP ranging from 9.4 to 12.0%, indicate that the cow is able to reduce the excretion of UN to 34 to 42 g of N/d. A third study, a review on urea excretion and recycling to the gastrointestinal tract (Reynolds and Kristensen, 2008), demonstrated that ruminants are able to reduce urea excretion at low CP, and observed that at a CP of 7.5% the amount of urea excreted as a proportion of urea produced became close to zero. The database used in the present study indicates an average nonurea urinary nitrogen excretion (g of N/d) of 45 ± 12.0 and 44 ± 19.5 g of N/d for the EU and NA data set, respectively (Table 1). It was expected that regression coefficients for MUN and CP versus UUN would be similar to those obtained in the UN equation, as UUN and UN are strongly related (Burgos et al., 2005; Figure 3). This was indeed the case for the NA data set, where MUN regression coefficients of UN models for both the complete (14.08)

and reduced (14.31) data sets (Table 3) were similar to the MUN regression coefficients (14.40 and 14.57, respectively) of the UUN models (Table 4). However, for the EU data set, MUN regression coefficients of the UN models for the complete (11.92) and reduced (12.03) data sets (Table 3) were lower than the MUN regression coefficients (14.09 and 14.15, respectively) of the UUN models (Table 4). Removal of extreme MUN observations (>25 mg of N/d) from the EU data set reduced the MUN regression coefficients for UUN from 14.15 to 12.71 in the reduced data set and from 14.09 to 12.62 in the complete data set, and became similar to those obtained for UN. The observations with MUN values higher than 25 mg of N/dL in the EU data set were, therefore, probably responsible for this effect. Regression coefficients of CP were higher in the UUN models for the complete and reduced data set (models 10 and 13; Table 4) compared with those for the UN models, in particular for the NA data set.

Similar to the prediction of UN, the combination of MUN and CP in both the complete and reduced data set (models 11 and 14, respectively; Table 4) resulted in a better prediction of UUN than with MUN or CP as the single explanatory factor, lowering the AIC and RMSPE substantially. The same physiological explanation as given for the added effect of MUN and CP in explaining UN holds for UUN. Addition of other dietary or animal factors did not improve the explanation of UUN.

Between-Trial Variation

Between-trial variation for UN in a model containing only the class variable continent (NA or EU) was substantially reduced when MUN (reduction of 63 and 80% for the complete and reduced data set, respectively), CP (reduction of 77 and 80% for the complete and reduced data set, respectively), or the combination of MUN and CP (reduction of 82 and 93% for the complete and reduced data set, respectively) was included in the model. Between-trial variation for UUN in a model containing only the class variable continent (NA or EU) was substantially reduced when MUN (reduction of 78 and 91% for the complete and reduced data set, respectively), CP (reduction of 73 and 70% for the complete and reduced data set, respectively), or the combination of MUN and CP (reduction of 87 and 97% for the complete and reduced data set, respectively) was included in the model. Trial effect was significant in all cases except for the multivariate model containing MUN and CP that predicted UUN for the reduced data set (P = 0.066). The reduction in between-trial variation upon including MUN, CP, or the combination of MUN and CP in the model is not surprising
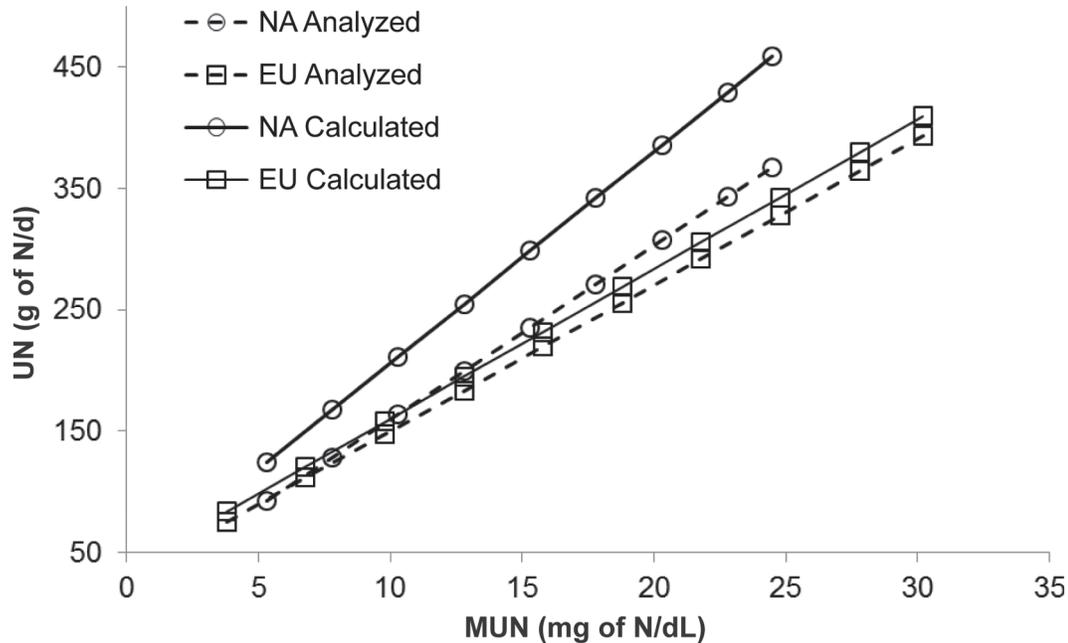
Figure 4. Relationship between MUN (mg of N/dL) and urinary nitrogen excretion (UN; g of N/d) based on data from the reduced data set for northwestern Europe (EU; n = 68) and North America (NA; n = 55). Urinary nitrogen excretion is determined based on total collection of urine (Analyzed) or based on calculation of UN as the difference between N intake and N excreted in feces and milk (Calculated). The UN Analyzed prediction model is presented in model 5 (Table 3). The UN Calculated prediction model is $\mu = 31.4 \pm 21.22$; EU = 5.6 ± 25.94; EU regression coefficient for MUN = 12.33 ± 0.764; NA regression coefficient for MUN = 17.46 ± 1.352; Akaike's information criterion = 1,168; root mean square prediction error = 43.8; $R^2 = 0.79$.

and can be explained by the variation in UN and UUN explained by MUN and CP. The significance of trial effect in almost all cases indicates additional explainable variation is present.

Precision and Accuracy

A more precise explanation of UN and UUN was obtained with the reduced data set, containing only observations on UN and UUN based on the total collection of urine, compared with the complete data set, as judged by the R^2 and RMSPE. The RMSPE obtained for UN using both MUN and CP as independent variables in the model was reduced by 24% (from 24.7 to 18.8; models 3 and 7 in Table 3). The RMSPE obtained for UN using MUN, CP, and DMI as independent variables in the model was reduced by 24% as well (from 24.2 to 18.3; models 4 and 8 in Table 3). The RMSPE for the UUN data set using both MUN and CP as independent variables in the model was reduced by 18% (from 18.9 to 15.5; models 11 and 14 in Table 4). These substantial reductions in RMSPE for the reduced data set (vs. complete data set) demonstrates the importance of analyzing UN and UUN based on the total collection of urine instead of determining UN and UUN from urine spot samples or

by assuming zero N balance. Although for the reason of precision it is best to estimate UN based on total collection of urine (UN-analyzed), from a practical and animal welfare point of view it might be preferred to derive UN by assuming a zero N balance and calculate UN as the difference between N intake and N excreted in feces and milk (UN calculated). Although precision of UN calculated will be lower, little is known about the effect of calculating UN based on zero N balance on accuracy. The MUN-UN calculated relationship and the MUN-UN analyzed relationship for the same reduced data set were compared and it appeared that these relationships were similar for EU but substantially different for NA (Figure 4). For the EU data set, the combined intercept and the slope were 29.7 and 12.03 $\pm$ 0.466 for the analyzed data, and 37.0 and 12.33 $\pm$ 0.764 for the calculated data. The relationship between UN and MUN for the NA data set had a higher regression coefficient for MUN when UN was calculated (17.46 $\pm$ 1.352; Figure 4) as compared with UN measured (14.31 $\pm$ 0.824; model 5 in Table 3). These results indicate that the accuracy of MUN in predicting UN is sensitive to the method of UN determination. The higher UN calculated, compared with UN analyzed, can only be explained by a high positive N balance for NA. The reduced data set average N balance was 54 $\pm$ 38.8 g

of N/d for NA and substantially larger than the average N balance for EU of 10.1 ± 30.9 g of N/d (Table 1). It is unclear why the N balance is higher for the NA than for the EU data set. Spanghero and Kowalski (1997) observed from a meta-analysis on N balance trials (35 studies, 27 from NA and 8 from EU) an average positive N balance of 39 g of N/d, which seems in line with the findings in the present study for the NA data set. Spanghero and Kowalski (1997) also observed a positive relationship between digestible N intake and N balance. This positive relationship might also explain the difference in N balance between EU and NA, as N intake (and probably also digestible N intake) was on average 31% higher for NA (637 g of N/d) compared with EU (485 g of N/d).

CONCLUSIONS

Variation in UN and UUN for EU and NA was best explained by the combination of MUN and CP. Addition of DMI to the model further improved the explanation of UN for EU, but not for NA. Other animal and dietary factors tested in this study did not improve or only marginally improved the model fit of UN and UUN. The relationship between UN and MUN differed between NA and EU, with higher estimated regression coefficients for MUN for the NA data set. Prediction precision of UN and UUN was improved substantially by including only those studies in which UN and UUN were based on total collection of urine instead of being derived from spot samples or by assumption of zero N balance. In regard to precision and accuracy, relations between MUN and UN should be based on UN derived from total collection of urine, whereas establishing relationships between MUN and UN, where UN is calculated as N intake minus N excreted in milk and feces, is likely to yield inaccurate and imprecise relationships. We concluded that on-farm prediction of UN or UUN can be substantially improved by using MUN and CP, compared with either MUN or CP alone, and might help in monitoring and in taking policy measures to reduce environmental N losses.

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APPENDIX

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