Short communication: Milk urea nitrogen as a predictor of urinary nitrogen and urea nitrogen excretions of late-lactation dairy cows fed nitrogen-limiting diets

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ABSTRACT

Our objectives were to assess the relationships between milk urea N (MUN), serum urea N (SUN), urine N (UN), and urinary urea N (UUN) in late-lactation cows fed N-limiting diets and compare these relationships with those previously established. Data were from a pen-based study in which 128 Holstein cows had been assigned to 1 of 16 pens in a randomized complete block design to assess the effects of diets containing 16.2, 14.4, 13.1, and 11.8% crude protein (CP, dry matter basis) during a 12-wk period. At least half of the cows in each pen were randomly selected to collect pen-level samples of serum and urine in wk 3, 7, and 11, when wk in lactation averaged 35, 39, and 43, respectively. A mixed model was developed to study the relationship of MUN with SUN, UN, and UUN. Week of lactation did not affect the relation between MUN and SUN across dietary treatments. However, we found a week $\times$ MUN interaction, suggesting that between wk 35 and 43 of lactation, UN excretion decreased from 89 to 73 g/d (-17 g/d) when MUN was 6.0 mg/dL (11.8% dietary CP) but increased from 142 to 149 g/d ($+7$ g/d) when MUN was 13.3 mg/dL (16.2% dietary CP). These effects were essentially due to changes in UUN excretion, which declined from 54 to 37 g/d (-17 g/d) and increased from 112 to 117 g/d ($+5$ g/d) when MUN was 6.0 and 13.3 mg/dL, respectively. When MUN was 11.2 mg/dL (15% dietary CP), UN and UUN excretions remained constant over time. Based on root mean squared prediction error and the concordance correlation coefficient, these data did not conform to most previously published prediction equations because of both mean and slope biases. The discrepancy could have resulted from difference in study design (cow vs.

pen as experimental unit), dietary treatments (energy vs. N-limiting diets), frequency of measurement and duration of adaptation period (single measurement after 1 to 3 wk of adaptation vs. repeated measurements over a 12-wk period), method for determining urine volume (total collection vs. spot sampling), and the assay used to measure MUN. However, our data captured changes in kidney physiology that warrant further studies of long-term renal adaptation to N-limiting diets.

Key words: milk urea nitrogen, urinary nitrogen, kidney, dietary crude protein

Short Communication

Manure N from livestock operations is a global concern, in part because of undesirable emission of ammonia and nitrous oxide (Dijkstra et al., 2013; Montes et al., 2013). Of the total N consumed by a dairy cow in North America, approximately 26% is secreted in milk, whereas 33 and 35% are excreted in the urine and feces, respectively (Spek et al., 2013a). Fecal N is bound to complex and stable organic structures and thus is relatively resilient to environmental losses. In contrast, urinary N (UN), which is made up primarily of urea, is the main source of undesirable emissions. Starting on the barn floors (Aguerre et al., 2010), the mixing of feces with urine promotes the conversion of urinary urea N (UUN) to ammonia N, with ensuing emission that continues throughout the entire manure management chain (Hou et al., 2015). When excess N is removed from the diet of lactating cows, daily excretions of UN and UUN can be reduced without penalizing milk protein yield (Colmenero and Broderick, 2006). As a highly water-soluble molecule, urea released into the general circulation by the liver equilibrates rapidly with body fluids, including milk (Gustafsson and Palmquist, 1993), leading to a strong correlation between urea N concentration in blood and milk (Broderick and Clayton, 1997). Subsequent research has proven MUN to

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be a reliable predictor of UN (Kauffman and St-Pierre, 2001; Kohn et al., 2002; Spek et al., 2013a), UUN (Burgos et al., 2007; Powell et al., 2011; Spek et al., 2013a), and ammonia emission (Burgos et al., 2010). Using a whole-farm model, Powell et al. (2014) suggested that each 1 mg/dL decrease in MUN (in the range of 16 to 10 mg/dL) was associated with a 16.6 g/d reduction in UUN and 7 to 12% reduction in emissions of both ammonia and nitrous oxide, depending on manure management chain. These predictions, however, should not be generalized until the relationship between UUN and MUN has been assessed under a variety of conditions. Except for Kauffman and St-Pierre (2001), who used 2 diets, one lower (13%, DM basis) and the other higher (17%) than considered optimal for dietary CP content, most published models that predict UN and UUN from MUN have been created with data collected from cows fed diets with sufficient or excessive levels of CP (Wattiaux and Karg, 2004; Burgos et al., 2007) or from meta-analyses revealing above-optimal levels of dietary CP (Jonker et al., 1998; Spek et al., 2013a). Although stage of lactation has not been identified as a major influencing factor (Burgos et al., 2007; Spek et al., 2013a), research in rodents and humans has shown a substantial effect of pregnancy on kidney function, renal urea filtration, and sodium and water balances (i.e., urine volume; Cheung and Lafayette, 2013). As highlighted in Kohn et al. (2005), kidney function is central to the relationship between BUN (mg/dL) and UN excretion (g/d) across animal species. In addition, the release of urea in the general circulation by the liver may be altered in the last weeks of lactation as the efficiency of utilization of MP decreases as pregnancy progressively takes priority over milk protein synthesis (NRC, 2001). Our recent study (Barros et al., 2017) suggested that cows responded better than expected when fed MP-deficient diets for 12 wk, prompting us to explore in a preliminary study the changes in kidney function as a mechanism to enhance N recycling when N-limiting diets are fed (Barros and Wattiaux, 2015). Thus, in this study, we hypothesized that the relationships of MUN to serum urea N (**SUN**), UN, and UUN in late-lactation cows fed N-limiting diets for 12 wk may be unique and differ from previously published reports. Our objectives were to investigate these relationships with a data set collected during the experiment reported in Barros et al. (2017) and to compare the prediction of UN and UUN from MUN from this particular data set with previously established prediction equations.

As described in Barros et al. (2017), 128 Holstein cows (mean $\pm$ SD) that were 32 ± 7 wk of lactation at the start of the trial were stratified by parity ($2.5 \pm$

1.3 lactations) and days pregnant (86 ± 25 d) and then randomly assigned to 1 of 16 pens (experimental units), each including 2 primiparous and 6 multiparous cows. After a 3-wk covariate period, pens were randomly allocated to 1 of 4 diets containing 16.2, 14.4, 13.1, or 11.8% CP (DM basis) for 12 wk. Diets were offered once a day as TMR (at 0700 h) and contained 65% forage (with almost equal proportion of corn silage and alfalfa silage), 13.5% high-moisture corn, and a 21.5% concentrate mix (DM basis). The CP reduction in the diet was achieved by replacing soybean meal with soy hulls in the concentrate mix. Chemical composition of feed and TMR as well as NRC (2001) predictions of nutrient supplies were presented in Barros et al. (2017). The MP balance averaged -109 , -187 , -336 , and -398 g/d for the 16.2, 14.4, 13.1, or 11.8% CP, respectively. For all diets, the supply of MP was predicted to be more limiting than the supply of NE_L . Cows were milked twice a day at 0600 and 1700 h. Details on milk sampling protocol and BW measurements can be found in Barros et al. (2017). In this study, MUN was determined by AgSource Laboratory (Menomonie, WI) using a CombiFoss 6600 FT +/-FC (Foss Electric, Hillerød, Denmark). Pen values for MUN were calculated from milk samples from all cows in the pen. Blood and urine spot samples were collected in wk 35, 39, and 43 of lactation (wk 3, 7, and 11 of the experimental period) from at least half of the cows in each pen (a subset of 4 cows: 1 primiparous and 3 multiparous). Using evacuated serum tubes, blood samples were collected approximately 4 h after feeding from the coccygeal vein or artery of cows that had been randomly selected once at the beginning of the trial. Serum was isolated after coagulated blood was centrifuged at $1,000 \times g$ for 15 min at room temperature. Pen-level composite samples were prepared and stored at -20°C until determination of urea N with a colorimetric assay (Chaney and Marbach, 1962). Mid-stream urine samples were collected after manual stimulation of the region around the vulva 6 h before and 6 h after feeding on a subset of 4 cows (1 primiparous and 3 multiparous) randomly selected at each sampling. Samples were acidified immediately by diluting 15 mL of urine in 60 mL of 0.072 N H_2SO_4 and stored at -20°C until analysis. Pen-level composite samples were analyzed for total N with a combustion assay (Leco FP-2000 Nitrogen Analyzer, Leco Instruments Inc., St. Joseph, MI), for urea N with an automated colorimetric assay (Broderick and Clayton, 1997), and for creatinine (Lachat Quik-Chem 8000 FIA, Lachat Instruments, Milwaukee, WI). Excretions of UN and UUN were computed using pen-level concentrations, and estimates of urine volume based on a creatinine excretion of 29 mg/kg of BW per day

(Valadares et al., 1999; Tebbe and Weiss, 2018), which has been shown to be independent of DIM (Chizzotti et al., 2008).

Univariate regressions were performed using Microsoft Excel (Microsoft Corp., Redmond, WA) to determine coefficients of determination among MUN, SUN, UN, and UUN excretions. In addition, mixed models were developed to investigate the relationship of MUN with SUN and UUN as lactation progressed: $y_{ij} = \mathbf{X}_{ij}^T \boldsymbol{\beta} + \rho_i + \varepsilon_{ij}$, where y_{ij} is the observation for the j th sampling week ($j = 1$ to 3) from the i th pen ($i = 1$ to 16); $\mathbf{X}_{ij}^T$ is the transpose of the covariate vector; $\boldsymbol{\beta}$ is the vector of parameter estimates; ρ_i is the random effect of pen i ; and ε_{ij} is the residual error. In the full model the fixed-effect covariate vector included the intercept, MUN, week, and MUN $\times$ week interaction. The full model was progressively built by adding covariates one at a time in the order listed. Models were fit using R statistical software with the lme4 package using REML estimation (Bates et al., 2013).

Our review of extant UN and UUN prediction equations yielded 6 equations to predict UN and 5 equations to predict UUN. Two methods were used to evaluate the equations: the root mean squared prediction error (RMSPE) and the concordance correlation coefficient (CCC; Lin, 1989, 2000). The RMSPE provides a mea-

sure of error in the units of the predicted value and as a percentage of the mean observed value. The RMSPE was calculated as follows:

$$\text{RMSPE} = \left[\left(\mathbf{y} - \mathbf{X}\hat{\boldsymbol{\beta}} \right)^T \left(\mathbf{y} - \mathbf{X}\hat{\boldsymbol{\beta}} \right) n^{-1} \right]^{\frac{1}{2}},$$

where $\mathbf{y}$ is the vector of observations; $\mathbf{X}$ is the matrix of explanatory variables; $\hat{\boldsymbol{\beta}}$ is the vector of fixed-effect parameter estimates; n is the number of observations in the study ($n = 48$); and the superscript T denotes the transpose function. The RMSPE was decomposed into the error of central tendency, the error due to regression, and the error of dispersion as described by Bibby and Toutenburg (1977). The CCC combines measures of both precision and accuracy to determine how far the observed data deviate from the line of perfect concordance (a line at 45° on a square scatter plot), with values close to 1 indicating agreement between observed and predicted (Lin, 1989, 2000). The CCC was calculated using the *epi.ccc* function in the epiR package (Stevenson et al., 2014) in R statistical software (R Core Group, Vienna, Austria).

For our data set MUN explained 65% of the variation in SUN, and the regression slope (0.746; Table 1) was intermediate between the values of 0.62 and 0.89

Table 1. Parameter estimates (SE in parentheses) of multiple linear mixed model regression of serum urea N (SUN, mg/dL), urinary N (UN, g/d), and urinary urea N (UUN, g/d) from MUN (mg/dL), week of lactation, and MUN $\times$ week of lactation interaction

Dependent variable	Item	Intercept	MUN (mg/dL)	Week	MUN $\times$ week	$\hat{\sigma}_P^1$	$\hat{\sigma}_R^2$
SUN	Mean (SE)	2.12 (1.055)	0.746 (0.1003)	—	—	1.14	1.21
	P-value ³	NA ⁴	<0.001				
SUN	Mean (SE)	2.26 (0.752)	0.752 (0.1005)	0.00551 (0.052184)		1.13	1.23
	P-value	NA	<0.001	0.878			
SUN	Mean (SE)	4.00 (8.562)	0.589 (0.7932)	−0.0550 (0.23159)	0.00470 (0.02166)	1.11	1.25
	P-value	NA	NA	NA	0.820		
UN	Mean (SE)	35.6 (6.94)	8.05 (0.659)	—	—	6.89	8.79
	P-value	NA	<0.001				
UN	Mean (SE)	47.2 (7.96)	7.96 (0.679)	−0.278 (0.3659)		7.30	8.71
	P-value	NA	<0.001	0.498			
UN	Mean (SE)	203 (52.6)	−6.76 (4.877)	−4.48 (1.426)	0.400 (0.1335)	8.02	7.64
	P-value	NA	NA	NA	0.004		
UUN	Mean (SE)	−3.32 (6.84)	8.64 (0.649)	—	—	7.34	7.86
	P-value	NA	<0.001				
UUN	Mean (SE)	15.3 (14.59)	8.43 (0.675)	−0.433 (0.3256)		7.49	7.55
	P-value	NA	<0.001	0.230			
UUN	Mean (SE)	163 (45.1)	−5.58 (4.183)	−4.43 (1.226)	0.382 (0.1149)	8.49	6.51
	P-value	NA	NA	NA	0.001		

¹Estimates of the random error due to the pen.

²Estimate of the residual random error.

³P-value between the null (simpler) and alternate model (null model with stepwise addition of MUN, week, and MUN $\times$ week interaction) estimated from chi-squared distribution of likelihood ratio test.

⁴Not applicable.

mg/dL reported by Broderick and Clayton (1997) and Kauffman and St-Pierre (2001), respectively. Also, in our data set, dietary CP level and MUN explained 77 and 80% of the variation in UN, respectively. These values agreed closely with the corresponding 72 and 79% reported in Spek et al. (2013a). However, the regression slope of 8.05 g of UN/d per unit of MUN found in this study (Table 1) was 68 and 49% of the values reported in Jonker et al. (1998) and Kauffman and St-Pierre (2001), respectively. These latter authors have shown that UN prediction from MUN can be improved by accounting for variation in BW (Jersey vs. Holstein). Our results indicated only a marginal improvement in a model with MUN adjusted for BW, most likely due to small differences in BW among the Holsteins in the study. In agreement with Colmenero and Broderick (2006), UUN in our study comprised a decreasing percentage of UN with decreasing dietary CP: 81, 74, 68, and 54% for dietary CP of 16.2, 14.4, 13.1, and 11.8%, respectively. Also, dietary CP level and MUN explained 80 and 88% of the variation in UUN, respectively. However, the regression slope between MUN and UUN (8.64 g of UUN/d per unit of MUN; Table 1) was 43 and 35% smaller than the slopes reported in Powell et al. (2011) and Spek et al. (2013a), respectively.

Results of the mixed model analysis are shown in Table 1. In predicting SUN, neither the week nor the MUN × week interaction were significant, indicating that the relationship between MUN and SUN was independent of week of lactation covered in our data set. In contrast, for the models that predicted UN and UUN from MUN, the week effect alone was not significant, but the MUN × week interaction was significant ($P < 0.01$). This finding highlighted the necessity of including the interaction term to predict both UN and UUN in our data set. Using our data to derive dietary CP from MUN ($y = 8.25 + 0.601 x$; $R^2 = 0.999$) and the models presented in Table 1, MUN of 11.2 mg/dL corresponded to a diet of 15% CP, which resulted in excretions of 127 g/d for UN and 95 g/d of UUN that remained independent of week of lactation. However, for MUN >11.2 mg/dL (dietary CP >15%), UN and UUN excretions were predicted to increase as lactation progressed. For example, when MUN was 13.3 mg/dL (16.2% of dietary CP), UN was predicted to increase from 142 to 149 g/d (+7 g/d) and UUN was predicted to increase from 112 to 117 g/d (+5 g/d) between wk 35 and 43 of lactation. Conversely, for MUN <11.2 mg/dL (dietary CP <15%), UN and UUN excretions were predicted to decrease between wk 35 and 43 of lactation. For example, when MUN was 6.0 mg/dL (11.8% dietary CP), UN was predicted to decrease from 89 to 73 g/d (−17 g/d) and UUN was predicted to decrease from 54 to 37 g/d (−17 g/d) between wk 35 and 43

Table 2. Analysis of errors associated with predicting urinary N (UN, g/d) and urinary urea N (UUN, g/d) observed in this study using previously established prediction equations¹

Equation no.	Dependent variable	Equation	Reference	RMSPE (g/d)	RMSPE (% of mean)	ECT	ER	ED	CCC
1	UN	$12.54 \times \text{MUN}$	Jonker et al. (1998)	80.1	68.9	96.2	1.38	2.71	0.109
2	UN	$0.0259 \times \text{MUN} \times \text{BW}$	Kauffman and St-Pierre (2001)	88.2	75.9	81.2	15.7	1.24	0.296
3	UN	$0.0283 \times \text{MUN} \times \text{BW}$	Wattiaux and Karg (2004)	107.0	92.1	83.1	14.4	0.84	0.237
4	UN	$16.7 + 14.3 \times \text{MUN}$	Spek et al. (2013a)	48.4	41.6	80.1	12.5	4.42	0.495
5	UN	$230.5 + 25.72 \times \text{CP}$	Spek et al. (2013a)	22.3	19.2	31.3	39.0	22.4	0.809
6	UN	$148.8 + 8.79 \times \text{MUN} + 11.97 \times \text{CP} + 1.58 \times \text{DMI}$	Spek et al. (2013a)	35.2	30.3	62.4	27.5	5.59	0.667
7	UUN	$-23.1 + 14.4 \times \text{MUN}$	Burgos et al. (2007)	42.2	50.7	78.6	12.6	5.09	0.586
8	UUN	$-34.2 + 16.23 \times \text{MUN}$	Powell et al. (2011)	50.9	61.1	76.9	16.3	3.50	0.524
9	UUN	$-31.4 + 14.57 \times \text{MUN}$	Spek et al. (2013a)	36.6	44.0	70.9	17.9	6.76	0.655
10	UUN	$-470.4 + 36.82 \times \text{CP}$	Spek et al. (2013a)	50.4	60.5	61.0	32.7	3.78	0.566
11	UUN	$-146.9 + 11.32 \times \text{MUN} + 9.19 \times \text{CP}$	Spek et al. (2013a)	23.9	28.7	22.2	59.1	9.95	0.831

¹Equations were evaluated with the root mean squared prediction error (RMSPE); the concordance correlation coefficient (CCC); and the decomposition of RMSPE into mean bias (error of central tendency; ECT), slope bias (error due to regression; ER), and error due to dispersion (ED).

of lactation. In contrast to these results, DIM (129 ± 58.9) was not a significant explanatory factor for UN or UUN in the meta-analysis of Spek et al. (2013a), and stage of lactation (early, mid, and late) had no effect on MUN, plasma urea N, or UUN in the study of Burgos et al. (2007). The former and latter studies predicted 114 and 121 g/d of UUN at any point of the lactation for MUN of 10 mg/dL, but for our data set, the same MUN value predicted 86, 83, and 81 g/d of UUN for wk 35, 39, and 43 of lactation, respectively.

The analysis of errors (RMSPE and CCC) associated with predicting our observed UN and UUN values using previously established prediction equations is presented in Table 2 and Figure 1. Models that predicted UN excretion showed an RMSPE ranging from 22.3 to 107.0 g/d (19.2 to 92.1% of the mean). All models exhibited high mean bias ($>31\%$ of the total error)

and slope bias [except for the model of Jonker et al. (1998), equation 1 in Table 2]. Similarly, models that predict UUN excretion showed RMSPE ranging from 22.9 to 50.9 g/d (28.7 to 61.1% of the mean). All the UUN prediction models showed large mean and slope bias beyond acceptable according to the guidelines by Reed et al. (2015). We believe that our data set did not conform with current models for several possible reasons associated with experimental design and protocol as well as cow biology. First, our excretion data were estimated from spot sampling rather than measured by total urine collection. Working with a group of 18 cows, Tebbe and Weiss (2018) concluded that average urine output did not differ between the 2 methods despite marked difference for some individual cows. Because the data analyzed in our study were pen-based averages, the residual random error of our prediction models

Figure 1. (A) Observed urine N (UN) versus predicted UN excretion using 6 published equations: (a) Jonker et al. (1998), $UN = 12.54 \times MUN$; (b) Kauffman and St-Pierre (2001), $UN = 0.0259 \times MUN \times BW$; (c) Wattiaux and Karg (2004), $UN = 0.0283 \times MUN \times BW$; (d) Spek et al. (2013a), $UN = 16.7 + 14.3 \times MUN$; (e) Spek et al. (2013a), $UN = 230.5 + 25.72 \times CP$; (f) Spek et al. (2013a), $148.8 + 8.79 \times MUN + 11.97 \times CP + 1.58 \times DMI$. (B) Observed urinary urea N (UUN) versus predicted UUN excretion using 5 published equations: (g) Burgos et al. (2007), $UUN = -23.1 + 14.4 \times MUN$; (h) Powell et al. (2011), $UUN = -34.2 + 16.23 \times MUN$; (i) Spek et al. (2013a), $UUN = -31.4 + 14.57 \times MUN$; (j) Spek et al. (2013a), $UUN = -470.4 + 36.82 \times CP$; (k) Spek et al. (2013a), $UUN = -146.9 + 11.32 \times MUN + 9.19 \times CP$.

was reduced when compared with data from individual animals; thus, the total prediction error will be smaller than individual animal data but the systematic biases will represent a larger portion of the remaining error. Care should be taken when interpreting the error decomposition as the systematic biases are expected to appear larger than a similar analysis with individual animal data. Second, cows in our study were at a much later stage of lactation than in previous studies. Week of lactation in our study averaged 39 but was 22.3, 12.0, and 18.4 in Kauffman and St-Pierre (2001), Wattiaux and Karg (2004), and Spek et al. (2013a), respectively. In the work of Burgos et al. (2007), average week of lactation for early-, mid-, and late-lactation cows was 18, 25, and 32, respectively. Third, in our study diets were N limiting. Except for the study of Kauffman and St-Pierre (2001), which included a dietary treatment with 13% CP, most other studies from which prediction equations have been created used levels of dietary CP that supplied an adequate to excessive amount of N. For example, dietary CP was 16.4, 16.6, 17.8, and 18.0% in Wattiaux and Karg (2004) and 15, 17, 19, and 21% in Burgos et al. (2007). Similarly, the North American data set of the meta-analysis of Spek et al. (2013a) included 118 treatment means with $17.1 \pm 1.8\%$ CP. Fourth, in our study, measurements were repeated on cows that remained on the same dietary treatment for up to 12 wk compared with a single measurement performed after 1 to 3 wk of adaptation in most other studies cited here. Fifth, the variability in MUN obtained from different assays (Broderick, 2003) or equipment (Peterson et al., 2004) may also be a source of between-studies variation. Nevertheless, under our experimental conditions, we may have captured changes in renal functions due to long-term exposure to distinct levels of dietary CP, with changes in UUN excretion explaining the changes in UN excretion. In their review of factors affecting the MUN:UN ratio, Spek et al. (2013b) concluded that both dietary CP and physiological state of the animal influence the amount of urea reabsorption that occurs in the kidney (see also Burgos et al., 2007). Alteration of urea reuptake by the kidneys might provide a physiological basis of the significant effect of MUN by week of lactation in predicting UN and UUN from MUN observed in this study. Because UUN is easily lost to the environment, inaccuracies in UUN predictions may lead to inaccuracies in estimates of N loss from the farm and related environmental impacts. Thus, we hope that our findings will stimulate further research in methods for measuring or predicting urine volume, UN, and UUN under a wide range of experimental conditions and in the mechanisms that govern changes in kidney reabsorption of urea throughout the lactation and reproduction cycle of dairy cows.

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