A Statistical Evaluation of Animal and Nutritional Factors Influencing Concentrations of Milk Urea Nitrogen[1]

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ABSTRACT

Data from 35 trials with 482 lactating cows fed 106 diets were used to study the effects of animal and dietary factors on the relationship between milk and blood urea N and the value of milk urea N in the assessment of protein status. In two trials, urea N in whole blood and in blood plasma were closely related ($r^2 = 0.952$); the slope was not significantly different from 1.0, and the intercept was not significantly different from 0. Regression of milk urea N on blood urea N with a mixed effects model using all 2231 observations yielded the equation: milk urea N (milligrams of N per deciliter) = 0.620 × blood urea N (milligrams of N per deciliter) + 4.75 ($r^2 = 0.842$); this model accounted for a significant interaction of cow and blood urea N. Single factors that yielded significant regressions on milk urea N with the mixed effects models were dietary crude protein (CP) (percentage of dry matter; $r^2 = 0.839$), dietary CP per megacalorie of net energy for lactation (NE_L) ($r^2 = 0.833$), excess N intake ($r^2 = 0.772$), N efficiency ($r^2 = 0.626$), and ruminal NH_3 ($r^2 = 0.574$). When all factors were analyzed at once, 12 were significant in a mixed effects model. Blood urea N, body weight, yield of fat-corrected milk, dietary CP content, excess N intake, dry matter intake, and days in milk were positively related to milk urea N, and parity, milk and fat yield, dietary CP per unit of NE_L content, and NE_L intake were negatively related to milk urea N. In one trial, the mean urea concentration was 35 times greater in urine than in milk; lower proportions of total urea excretion in milk were observed in the a.m. sampling (1.8%) than in the p.m. sampling (3.3%). Measuring urea N in a composite milk sample from the whole day substantially improved reliability of data. The number of cows fed a specific diet that must be sampled to determine mean milk urea N within 95% confidence intervals with half widths of 1.0 and 2.0 mg of N/dl was estimated to be 16 and 4, respectively.

(**Key words**: blood urea N, milk urea N, milk yield, dietary protein)

Abbreviation key: **BUN** = blood urea N, **dNE_L** = NE_L estimated from apparent DM digestibility, **MUN** = milk urea N, **nNE_L** = NE_L computed from NRC (25) tables, **PUN** = plasma urea N.

INTRODUCTION

Urea is the primary form of excretory N in mammals, and concentrations of blood urea N (**BUN**) have long been known to reflect inefficient utilization of dietary CP by ruminants (22). Urea equilibrates rapidly throughout body fluids, including milk, and the concentration of milk urea N (**MUN**) is thought to reflect the concentration of BUN (28). Therefore, MUN may serve as an index of inefficient N utilization in the lactating dairy cow (1, 27, 29). Milk NPN constitutes 5 to 6% of total milk N of which MUN typically contributes about half (14). Concentrations of MUN might be used to monitor dietary CP intake more closely relative to requirements because 1) excess N intake may impair reproductive performance, possibly through elevated urea concentrations in fluids in the urogenital tract (12, 17, 21); 2) consumption of excess CP increases energy requirements by 13.3 kcal of digestible energy/g of excess N (25); 3) protein supplements are the most costly feed ingredients; and 4) excess N excretion has a negative environmental impact. Nelson (26) speculated that the use of MUN data to adjust ration protein and energy contents could, by reducing feed costs or improving performance, repay the costs for MUN analyses by 10-fold or more.

Concentrations of MUN and BUN were determined in a number of lactation studies in which milk yield and various other animal and dietary characteristics

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also were measured. Our objectives were to conduct a statistical evaluation on this data file 1) to quantify the effect of various animal and dietary factors on the relationship between MUN and BUN and 2) to quantify the value of MUN in the assessment of protein status of the lactating cow.

MATERIALS AND METHODS

The data analyzed in this study were collected in 35 conventional lactation trials conducted with 482 Holstein cows of known parity, BW, and DIM that were fed 106 different diets (Table 1). The following measurements were used in the statistical analyses (Table 2): MUN concentration; concentration of either BUN or blood plasma urea N (**PUN**); mean daily yields of milk, 3.5% FCM, protein, fat, and SNF; DM efficiency (milk yield/DMI); dietary content (percentage of DM) of CP, NDF, and **nNE_L** [NE_L computed from NRC (25) tables]; dietary CP per unit of nNE_L; intake of DM, CP, and nNE_L; N efficiency (milk N yield/N intake, assuming milk protein contained 6.38% N); and excess N intake (total N intake – milk N yield). In 19 trials (trials 3 to 7, 11, 12, 14, 17, 21 to 25, 29 to 32, and 35; Table 1), 50 ruminally cannulated cows were used in switchback arrangements of dietary treatments to collect 254 measurements of ruminal NH_3 of cows fed 69 of the diets. In 12 trials (trials 3, 5, 12 to 14, 18, 20 to 24, and 29; Table 1), 697 fecal grab samples also were collected from 189 of the cows fed 41 of the diets; an internal marker, either acid-insoluble ash or indigestible ADF, was used to estimate apparent digestibility of dietary DM. The content of NE_L estimated from apparent DM digestibility (**dNE_L**) was computed by substituting apparent DM digestibility for TDN in the equation dNE_L (megacalories per kilogram of DM) = 0.0245 × TDN (percentage of DM) – 0.12 (25). Dietary CP per dNE_L also was computed.

TABLE 1. Trial summary.

Trial no.	Diets	Cows	Observations	BUN or PUN[1]	Reference
	(no.)				
1	2	8	32	PUN	(7)
2	2	8	16	PUN	(7)
3	3	21	63	BUN	(2)
4	4	16	64	PUN	(6)
5	4	16	64	PUN	(2)
6	2	12	24	BUN	(3)
7	4	20	80	BUN	(3)
8	4	20	80	BUN	(8)
9	4	20	80	BUN	(8)
10	4	24	96	PUN	(8)
11	2	22	88	PUN	(4)
12	3	22	22	PUN	(24)
13	6	22	44	PUN	(24)
14	2	14	56	PUN	(11)
15	4	18	72	PUN	(4)
16	4	18	72	PUN	(4)
17	4	24	96	PUN	(9)
18	4	16	64	PUN	(11)
19	4	8	32	PUN	G. Broderick, 1990, unpublished data
20	3	23	65	PUN	G. Broderick, 1991, unpublished data
21	3	6	18	PUN	(20)
22	4	20	80	PUN	(5)
23	4	20	59	PUN	G. Broderick, 1992, unpublished data
24	4	20	60	PUN	(5)
25	6	6	30	BUN and PUN	(23)
26	6	16	96	BUN and PUN	(23)
27	6	16	96	BUN and PUN	(23)
28	4	24	94	PUN	G. Broderick, 1993, unpublished data
29	4	52	104	PUN	(15)
30	4	12	48	BUN	(35)
31	4	24	96	BUN	(35)
32	4	28	112	BUN	G. Broderick, 1995, unpublished data
33	4	24	48	PUN	G. Broderick, 1996, unpublished data
34	4	24	48	PUN	G. Broderick, 1996, unpublished data
35	4	8	32	PUN	G. Broderick, 1996, unpublished data

[1]BUN = Blood urea N; PUN = plasma urea N.

Concentrations of MUN and BUN or PUN were determined in all trials using the same diacetyl monoxime colorimetric assay adapted to a continuous flow analyzer (34). In all trials, daily composites were prepared from milk collected at a.m. and p.m. samplings; composites were deproteinized with trichloroacetic acid (32). Deproteinized milk was analyzed for MUN. In 2 trials (trials 21 and 30; Table 1), milk collections from a.m. and p.m. samplings also were individually deproteinized and analyzed for MUN. Concentrations of BUN or PUN were measured at 4 h after feeding in all trials; blood was collected into heparin from the jugular vein in 2 trials (trials 1 and 2; Table 1) and from the coccygeal artery or vein in 33 trials (trials 3 to 35; Table 1). Urea was determined on 584 samples of whole blood (BUN) in 8 trials (trials 3, 6 to 9, and 30 to 32; Table 1) and on 1426 samples of blood plasma (PUN) that had been deproteinized with 3% (wt/vol) sulfosalicylic acid (10) in 24 trials (trials 1, 2, 4, 5, 10 to 24, 28, 29, and 33 to 35; Table 1). Both BUN and PUN were determined on 222 samples in 3 trials (trials 25 to 27; Table 1); only the BUN data from these 3 trials were used in the overall statistical analyses. In 1 trial (trial 30; Table 1), total urine collections were made over 72 h as six separate 12-h samples; urine from the last two 12-h samples plus the corresponding a.m. and p.m. milk samples also were analyzed for urea N.

A linear regression model (30) was used to assess the relationship between BUN and PUN data from the 3 trials (trials 25 to 27; Table 1) in which both were determined. The Mixed procedure of SAS (31) was the primary tool used to construct mixed effects regression models. A series of single factor regressions were fit to describe the relationship between MUN and BUN and to establish the extent to which MUN could be used to predict various quantities. For these single factor regressions [i.e., MUN on BUN; CP, CP/nNE_L, CP/dNE_L, N efficiency (milk N yield/N intake), excess N intake (total N intake – milk N yield), and ruminal NH_3 on MUN], a mixed effects model was fit with both fixed effects (slope and intercept) and random effects (slope and intercept) for the independent variables. The random effects allowed for the possibility that, in the relationship between dependent and independent variables, each cow had her own slope and intercept that varied randomly from cow to cow. In this context, the slope and intercept from the fixed effects model represent a mean slope and a mean intercept averaged over all cows. In fitting these models, the random effect of slope was not significant for the dependent variables (N efficiency and ruminal NH_3) and, thus, was removed from the model in those cases. Whether significant or not, the random effect for intercept was left in the model to account for the design of the experiments, wherein each cow appeared several times in a given trial.

In a mixed effects model, there is no standard definition of R^2 as in standard (fixed effects) regressions. To assess overall model fit, the general linear models procedure of SAS (30) was used to fit a fixed effects regression of the dependent variable on the independent variable. To reflect the presence of random effects in the mixed effects model, a term representing cow was added to the model. In addition, in those cases in which a random slope effect was present in the mixed effects model (all cases except N efficiency and ruminal NH_3), a term for the interaction of the cow and dependent variable was included in the model. The resultant value of R^2 is reported here.

To explore more fully those factors that might affect MUN, a multiple regression with random effects was fit, again using the Mixed procedure of SAS (31). This model was constructed using a combination of three steps of forward selection and backward elimi-

TABLE 2. Single factors regressed on milk urea N (MUN) using mixed effects models.[1]

Independent variable	Dependent variable
BUN (mg of N/dl)	MUN (mg of N/dl)
MUN (mg of N/dl)	BUN (mg of N/dl)
	Parity
	DIM
	BW (kg)
	DMI (kg/d)
	Milk yield (kg/d)
	FCM Yield (kg/d)
	Protein yield (kg/d)
	Fat yield (kg/d)
	SNF Yield (kg/d)
	DM Efficiency (milk yield/DMI)
	N Efficiency (milk N/N intake)
	Dietary CP (% of DM)
	Dietary NDF (% of DM)
	Dietary dNE_L (Mcal/kg of DM)
	Dietary CP per unit of nNE_L (g/Mcal)
	CP Intake (kg/d)
	Excess N intake (g/d)
	nNE_L Intake (Mcal/d)
	NPN Intake (g/d)
	Dietary dNE_L (Mcal/kg of DM)
	CP per Unit of dNE_L (g/Mcal)
	dNE_L Intake (Mcal/d)
	Ruminal NH_3 (mg of N/dl)

[1]BUN = Blood urea N, dNE_L = dietary NE_L computed from apparent DM digestibility estimated during the respective trials using internal markers, nNE_L = dietary NE_L computed from NRC (25) tables, and excess N intake = total N intake – milk N secretion.

nation. First, MUN was regressed on each independent variable (Table 2) in models that included random and fixed effects (intercept and slope) for the independent variables. We made note of each case in which the random slope effect was significant. Second, a multiple regression, mixed effects model was fit that included all of the previously mentioned independent variables in addition to a random intercept effect and a random slope effect for each independent variable for which there was a significant random slope effect in the first series of models. Third, in a stepwise manner, nonsignificant terms were deleted from the model, except that the random effect for intercept was retained for the reasons described previously. The R^2 for this model was constructed using fixed effects multiple regression including all independent variables from the final step plus terms for cow and the interaction of cow and BUN (to reflect the significant random effect for BUN in the random effects model in the final step). In all cases, significance was determined at $P < 0.10$.

To estimate the sample sizes necessary to determine mean MUN concentrations with 95% confidence intervals of 1.0 and 2.0 mg of N/dl, the general linear models procedure of SAS (30) was used to fit a one-way ANOVA for MUN values in which diet number was the treatment and only the initial observation was used for each cow to avoid the complication of random effects. The root mean square error from this model was used as the estimate of σ, the standard deviation of a MUN value for a cow within a given diet. The half width (HW) of the 95% confidence interval (CI) for mean MUN is given by the following equations (33):

$$HW = 1.96 \times \sigma/\sqrt{n},$$

and

$$CI = \text{mean} \pm HW$$

where 1.96 is the two-tailed t value for $P = 0.05$ for a very large number of degrees of freedom for σ [Table A.3 in (33)]. Solving for n (the number of observations necessary to determine the mean MUN within the 95% CI) yields

$$n = (1.96 \times \sigma/HW)^2.$$

RESULTS AND DISCUSSION

Figure 1 shows the simple regression of PUN on BUN in the 3 trials (trials 25 to 27; n = 226; Table 1) in which both PUN and BUN were determined. The correlation coefficient ($r^2 = 0.918$) indicated that the relationship was strong. Data from 1 trial (trial 27; Table 1) appeared to deviate to a greater extent (Figure 1). Regression using only data from the other 2 trials (trials 25 and 26; n = 118; Table 1) yielded a stronger relationship ($r^2 = 0.952$); the slope was not significantly different from 1.0, and the intercept was not significantly different from 0 (intercept = –0.136). These results were taken to mean that BUN and PUN yielded virtually the same value, and the term BUN will be used to describe urea N concentration in both total blood and deproteinized blood plasma.

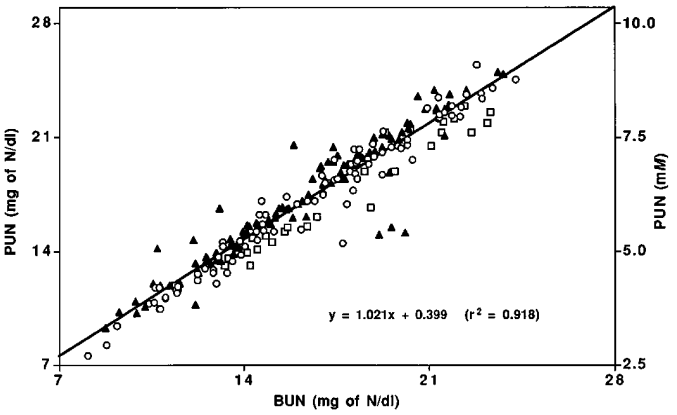

Figure 1. Linear regression of blood plasma urea N (PUN) on blood urea N (BUN) in the three trials in which both were analyzed (Table 1): trial 25 (□), trial 26 (○), trial 27 (▲).

The overall mixed effects model for regression of MUN on BUN using all data (Figure 2) indicated a strong association ($r^2 = 0.842$). Although the magnitudes of the slopes (0.62 vs. 0.60) and intercepts (4.8 vs. 5.1) were similar for the mixed effects model and a simple linear regression model, linear regression on MUN on BUN was not as well correlated ($r^2 = 0.588$) because the mixed effects model accounted for the significant interaction of cow and BUN, whereby each cow had her own slope for MUN on BUN. The high correlation of MUN and BUN was expected (28). Through the course of the day, the same concentration patterns were observed for MUN and BUN (blood serum) in individual cows; MUN concentrations tended to lag about 1 h behind BUN concentrations (19). In our trials, only a single blood sample was taken from each cow at 4 h after feeding; blood samples taken to determine BUN concentrations were obtained as early as the same day to as long as 3 d after milk samples were collected to determine MUN concentrations. Gustafsson and Palmquist (19) observed that urea in blood serum peaked about 3 h after feeding. Therefore, BUN concentrations likely

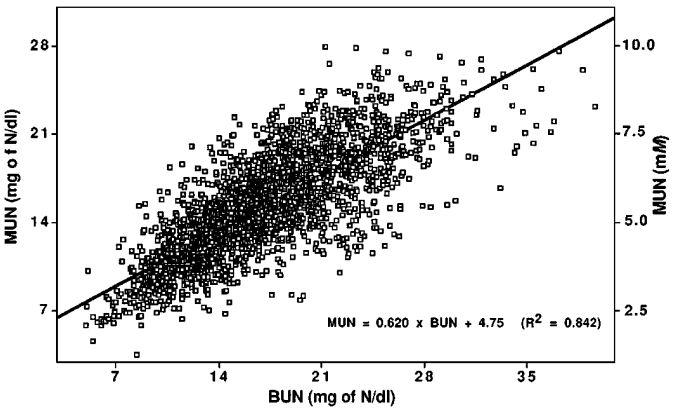

Figure 2. Regression of milk urea N (MUN) on blood urea N (BUN) using all data in the mixed effects model.

were near maximal at the time of blood sampling in our trials, which may explain the slope of 0.62 from our regression of MUN on BUN (Figure 2).

The six single factors that yielded, in single regressions, significant regressions on MUN concentration using mixed effects models are presented in Table 3. Dietary CP concentration, expressed on both a DM or energy basis (using either nNE_L or dNE_L), had the strongest relationship with MUN concentrations. Associations were not as strong for the regressions on MUN concentration of two factors that were clearly related to the utilization of dietary CP, excess N intake ($r^2 = 0.772$) and N efficiency ($r^2 = 0.626$). Of the six factors, ruminal NH_3 was most poorly associated ($r^2 = 0.574$) with MUN. Urea in body fluids, including urea in milk, results not only from excess protein degradation in the rumen but also from N inefficiency caused by an excess supply of protein to the tissues. Absorbable protein that is not converted to milk protein is catabolized for energy, and this N contributes to the urea pool, some of which appears as BUN and MUN.

The relationships in Table 3 may be useful in a number of applications. For example, reliable estimates of MUN could be used in the field to identify diets that were relatively low, or high, in CP concentration, either on a DM or energy basis. Dietary CP content (percentage of DM) could be estimated from MUN concentration using the first equation in Table 3.

The variables that contributed ($P < 0.10$) to the regression of MUN on multiple factors using the mixed effects model are listed in Table 4. Large *t* values indicated that milk yield (actual milk and FCM), parity, dietary CP (percentage of DM), and especially BUN concentration made the most significant contributions to the model. However, a number of other factors also were significant ($P < 0.10$) in the model, which indicated that MUN concentrations observed in lactating cows in the field are influenced by multiple animal and dietary characteristics. Three factors, fat yield, DMI, and DIM, were less significant than the others; *P* values for these factors ranged from 0.05 to 0.10. A number of factors (parity, milk and fat yield, CP per unit of nNE_L, and nNE_L intake) were negatively related to MUN concentration (Table 4). Oltner and Wiktorsson (27) used the negative relationship between MUN and dietary energy density to identify diets with concentrations of CP that were too low. Those researchers reported that MUN concentrations below 14 mg of N/dl indicated insufficient CP per unit of dietary energy. The negative relationship with parity indicated that MUN declined as cows progressed through succeeding lactations. A decline in MUN concentration with increased milk volume may be anticipated. In their reviews, De-

TABLE 3. Single factor regressions on milk urea N (MUN) using mixed effects models.

Factor[1]	Equation	ddf[2]	r^{2}[3]
CP (% of DM)	Y = 0.269 MUN (mg of N/dl) + 13.7	479	0.839
CP/nNE_L (g/Mcal)	Y = 1.79 MUN (mg of N/dl) + 84.4	479	0.833
CP/dNE_L (g/Mcal)	Y = 2.59 MUN (mg of N/dl) + 85.3	201	0.878
N Efficiency	Y = –0.004 MUN (mg of N/dl) + 0.309	1744	0.626
Excess N intake (g of N/d)	Y = 11.0 MUN (mg of N/dl) + 313	477	0.772
Ruminal NH_3 (mg of N/dl)	Y = 0.686 MUN (mg of N/dl) + 6.43	205	0.574

[1]CP/nNE_L = Dietary CP per unit of NE_L, where NE_L was computed from NRC (25) tables; CP/dNE_L = dietary CP per unit of NE_L, where NE_L was computed from apparent DM digestibility estimated during the respective trials using internal markers; N efficiency = milk N secretion/N intake; and excess N intake = total N intake – milk N secretion.

[2]Denominator degrees of freedom.

[3]Coefficient of determination constructed for the mixed effects model.

Peters and Cant (13) and Emery (16) reported that dietary protein was positively correlated with milk protein content, and milk protein and fat concentrations generally were negatively correlated. Because dietary CP concentration was highly (t = 9.51; P < 0.001) positively related to MUN, one may surmise that a negative relationship existed between milk fat and MUN. However, FCM yield was positively correlated with MUN, despite the negative relationships between both milk and fat yield and MUN. Interestingly, yield of protein and SNF and N efficiency did not make significant contributions to the overall mixed effects model.

On the farm, milk often is sampled for analysis at only one of the daily milkings. We assessed the importance of differences in MUN concentrations between a.m. or p.m. milkings using data from 2 trials (trials 21 and 30; Table 1) in which both were determined by comparing MUN in milk collected in the a.m. and p.m. with mean MUN and BUN. Over both trials, the association of BUN to MUN in milk collected in the a.m. was slightly stronger (r^2 = 0.686; Figure 3) than was the association of BUN to MUN in milk collected in the p.m. (r^2 = 0.526; Figure 3). These two regression lines had different slopes (P < 0.02) and intercepts (P < 0.0001). As expected, the regression of mean MUN concentration on BUN lay between the other two regressions lines (Figure 3) and explained more of the variation (r^2 = 0.737) in BUN than did MUN in milk collected at either the a.m. or p.m. milking. In both trials, cows were milked from 0400 to 0500 h and from 1600 to 1700 h. Cows were fed at about 1000 h, and blood was sampled at 4 h after feeding. If BUN concentrations followed a pattern similar to that reported earlier (19), then the greatest changes in BUN, and presumably MUN, would occur during the 12-h period represented by the p.m. sampling, which might account for the greater variation and lower correlation coefficient for the p.m. samples.

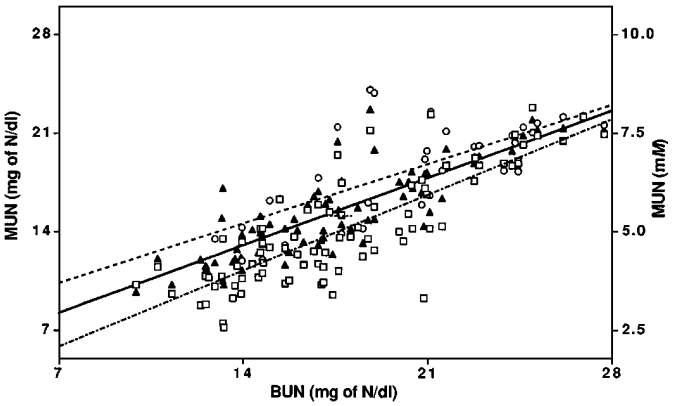

Figure 3. Linear regression of milk urea N (MUN) determined in milk from a.m. samplings (□; ·-·; Y = 0.768X + 0.443 (r^2 = 0.686)] or p.m. samplings [o; - - -; Y = 0.608X + 6.001 (r^2 = 0.526)] or determined from the mean MUN from a.m. and p.m. samplings [▲; —; Y = 0.688X + 3.328 (r^2 = 0.737)] on blood urea N (BUN) using data from 2 trials (trials 21 and 30; Table 1).

TABLE 4. Parameters that made significant contributions to the regression of milk urea N (MUN) on multiple factors using the mixed effects model.[1,2]

Parameter or factor[3]	Estimated coefficient	SE	t	P[4]
MUN (mg of N/dl) =				
Intercept	–4.713	1.897	–2.48	0.013
BUN (mg of N/dl)	0.484	0.013	37.05	<0.001
Parity	–0.175	0.045	–3.90	<0.001
BW (kg)	0.003	0.001	2.55	0.011
Milk yield (kg/d)	–0.101	0.028	–3.63	<0.001
3.5% FCM Yield (kg/d)	0.187	0.053	3.52	<0.001
Fat yield (kg/d)	–1.802	0.940	–1.92	0.056
CP (% of DM)	0.843	0.089	9.51	<0.001
CP/nNE$_L$ (g/Mcal)	–0.059	0.019	–3.18	<0.001
Excess N intake (g of N/d)	0.007	0.003	2.59	0.010
DMI (kg/d)	0.103	0.055	1.88	0.061
nNE$_L$ Intake (kg/d)	–0.133	0.053	–2.48	0.013
DIM	0.003	0.001	1.93	0.054

[1]BUN = Blood urea N; CP/nNE$_L$ = dietary CP per unit of NE$_L$, where NE$_L$ was computed from NRC (25) tables; and excess N intake = total N intake – milk N secretion.

[2]Coefficient of determination constructed for the mixed effects model. Denominator df = 1249; R^2 = 0.875.

[3]There were 2226 observations for each factor used in this model. Crude protein per unit of dNE$_L$ and dNE$_L$ intake, where dietary NE$_L$ was computed from apparent DM digestibility (n = 697 observations), and ruminal NH_3 (n = 254 observations) were omitted from this model because of too few observations.

[4]Student's t and its associated P value.

In one of the 2 trials (trial 30; Table 1), total urine collection and urinary urea N analysis were completed for the 12-h periods corresponding to MUN analyses in a.m. and p.m. milk samples (Table 5). Urine volume excreted during the 12 h preceding the a.m. milking was greater than that for the 12 h preceding the p.m. milking; the reverse was true for milk yield. Urinary urea N and MUN followed similar patterns in that concentrations of both were higher in p.m. samples than in a.m. samples. As expected, urinary urea concentration greatly exceeded MUN concentration (18). Urea N was, respectively, 38 and 32 times more concentrated in urine collected during the a.m. and p.m. sampling times than in milk collected during the a.m. and p.m. sampling times. Gonda and Lindberg (18) found that the mean concentration of urinary urea was 39 times greater than the concentration of MUN. Lower MUN concentrations in a.m. milk samples than in p.m. milk samples resulted in

TABLE 5. Concentration and excretion of urea N in urine and milk over 12-h periods ending at 0400 and 1600 h (trial 30; Table 1).[1]

Item	Period ending		SEM[2]
	0400 h	1600 h	
Urine volume, L/12 h	20.4	14.7	0.4
UUN, mg of N/dl	460.1	510.5	15.0
Urinary urea, g of N/12 h	92.5	73.4	2.6
Milk volume, L/12 h	13.5	15.1	0.3
MUN, mg of N/dl	11.99	16.04	0.37
Milk urea, g of N/12 h	1.60	2.41	0.07
Total urea, g of N/12 h	94.1	75.8	2.6
Milk urea/total urea, %	1.78	3.29	0.17

[1]UUN = Urinary urea N; MUN = milk urea N.

[2]Each a.m. (0400 h) versus p.m. (1600 h) comparison was different ($P < 0.001$).

lower amounts and proportions of total urea excretion in a.m. milk samples (1.8%) than in p.m. milk samples (3.3%) (Table 5). These data clearly indicated that MUN concentration patterns were not symmetrical over the two halves of the day and imply that switching milk sampling back and forth between a.m. and p.m. may confound interpretation of MUN data. Also, mean MUN values, or MUN in daily milk composites, were more reliable than MUN in samples from individual milkings as indicators of BUN concentration.

The number of cows fed a specific diet that must be sampled to determine the mean MUN concentration for cows fed that diet within 95% confidence intervals with half widths of 1.0 or 2.0 mg of N/dl was estimated using data from regressing MUN on diet number as described previously. Overall mean MUN was 14.8 mg of N/dl; root mean square error, an estimate of σ (the standard deviation of a MUN value for a cows fed a given diet), was 2.07. Using confidence intervals of 1.0 or 2.0 mg of N/dl and solving for n yielded:

$$n_{1.0} = (1.96 \times 2.07/1)^2 = 16.5, \text{ and}$$
$$n_{2.0} = (1.96 \times 2.07/2)^2 = 4.1.$$

This information may be used to develop recommendations for sampling milk for MUN analysis. Based on our within-diet variation in MUN, sampling milk from at least 4 cows would be needed to estimate MUN for a given diet. Although the added precision gained by sampling 16 cows may not be necessary, sampling milk from 4 cows fed a specific diet should be considered as a minimum. Milk samples representing the 24-h d substantially improve reliability of MUN data. Switching sampling between a.m. and p.m. milkings, and presumably among more frequent milkings (three or four times daily), confounds interpretation of MUN data. Generally, sampling bulk tank milk would probably have little value unless used in conjunction with a dietary change that affected all cows contributing milk to the tank. For example, it may be speculated that a sudden change in the forage that contributes a large proportion to the diet, such as replacing an alfalfa silage containing 18% CP and making up one-third of dietary DM with another silage containing 21% CP (i.e., increasing dietary CP by one percentage unit) would be reflected in an increase in MUN of 3.7 mg of N/dl in bulk tank milk using the equation in Table 3 that relates dietary CP percentage with MUN.

All but 2 (trials 1 and 2; Table 1) of the 35 trials were conducted using the dairy herd at the US Dairy Forage Center research farm (Prairie du Sac, WI), and variation might not have been as great as would be encountered among commercial herds in the field. However, data from the Dairy Forage Center were collected over 15 yr, reflecting substantial animal turnover within the herd and different cropping protocols and seasons. All MUN and BUN analyses were made using the diacetyl monoxime colorimetric assay (34). Determinations of MUN in the field are made by DHI Cooperatives using either automated infrared or urease analyses (G. L. Hustad, 1997, personal communication). We have not compared MUN results between our method and these other procedures.

Concentrations of MUN may serve as a guide for identifying diets that provide too little or too much protein. Mean MUN concentration was 14.8 mg of N/dl, but MUN concentration ranged from 3 to 28 mg of N/dl; BUN concentration ranged from 5 to 40 mg of N/dl. Carroll et al. (12) found that conception rates declined in dairy cows that had excessive urea concentrations in vaginal mucus; presumably BUN and MUN would reflect urea concentrations in the vaginal mucus. Jordan et al. (21) and Ferguson and Chalupa (17) both reported that excessive amounts of CP in the diet depressed reproductive efficiency.

CONCLUSIONS

Statistical analyses using both linear regression and mixed effects models were conducted on a large set of MUN data obtained from feeding studies with lactating dairy cows. Concentrations of BUN and MUN were found to be highly correlated. Concentrations of MUN were more closely related to dietary CP concentrations, expressed either on a DM or energy basis, than to N efficiency or ruminal NH_3. When all factors were analyzed at once with a mixed effects

model, BUN, BW, FCM yield, dietary CP content, excess N intake, DMI, and DIM were positively related to MUN in the model, and parity, milk and fat yield, dietary CP per unit of NE_L content, and NE_L intake were negatively related to MUN in the model. Protein and SNF yield, dietary NDF and NE_L content, DM and N efficiency, and CP intake were not significant in the model. In 2 trials (trials 21 and 30; Table 1), different relationships were found between BUN and MUN when assessed from MUN in milk collected at either the a.m. or p.m. milking; BUN was more highly correlated with mean daily MUN concentration. Under the conditions of these trials, daily composite milk samples from 16 or 4 cows would have to be analyzed to estimate mean MUN concentration within a 95% confidence interval with half widths of 1 or 2 mg of N/dl.

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