

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

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Introduction

Urea is the primary form in which N is excreted in mammals, and elevated concentrations of blood urea N (BUN) are known to reflect inefficient utilization of dietary CP. Urea equilibrates rapidly throughout body fluids, including milk, and concentrations of milk urea N (MUN) are closely related to BUN (Gustafsson and Palmquist, 1993). Therefore, MUN can serve as an easily sampled indicator of BUN. Last year, we reported on relationships, obtained by analyzing mean data from 22 feeding trials, of a number of dietary and milk yield factors to MUN concentration. Since that report, we have performed a more complete analysis using observations for individual cows rather than mean data. Also, data were added from 28 more diets fed in 13 additional trials. Our objective was to conduct a statistical evaluation on this data set to quantify: 1) the effect of various animal and dietary factors on the relationship between MUN and BUN, and 2) the value of MUN for assessing protein status of the lactating cow.

Materials and Methods

Data were collected in 35 conventional lactation trials conducted with 482 Holstein cows of known parity, BW and DIM and fed 106 different diets; intakes of DM, CP and estimated NE_L , change of BW, BUN concentrations, and production of milk, fat, protein and lactose was determined. A total of 2231 measurements of MUN and BUN was made during these studies. In 20 trials, ruminal NH_3 was measured in 50 cannulated cows fed 69 diets. In one trial, total urinary urea N concentration and excretion were determined and compared to MUN in AM and PM milk. Concentrations of MUN and BUN were determined in these trials using a diacetyl monoxime colorimetric assay adapted to the Technicon AutoAnalyzer. Linear and mixed

effects regression models in SAS were used to study the relationship of: 1) BUN to plasma urea N (PUN); 2) MUN to BUN; and 3) MUN to various quantities. The number of samples necessary to determine mean MUN concentration within 95% confidence intervals of 1.0 and 2.0 mg N/dL also was estimated. Data from 27 trials already are published in nine papers, two manuscripts in press, one abstract, and one thesis; data from eight trials are unpublished.

Results and Discussion

Regression of data from two trials yielded a strong relationship of BUN to PUN ($r^2 = 0.952$) with slope not different from 1.0 and intercept not different from 0. Thus, BUN and PUN are virtually the same, and the term BUN can be used to describe urea concentration in both total blood and deproteinized blood plasma. The overall, mixed effects model for regression of MUN on BUN using all the data (Fig.1) indicated a strong correlation ($R^2 = 0.842$). Although the magnitude of slopes (0.62 versus 0.60) and intercepts (4.8 versus 5.1) was similar for the mixed effects model and a simple linear regression model, linear regression of MUN on BUN was not as well correlated ($R^2 = 0.588$). This is because the mixed effects model accounted for a significant cow-by-BUN interaction, whereby each cow had its own slope for MUN on BUN. It was expected that MUN and BUN would be highly correlated (Rook and Thomas, 1985). In our trials, only a single blood sample was taken from each cow 4 h after feeding. Gustafsson and Palmquist (1993) observed that urea in blood serum peaked about 3 h after feeding; therefore, BUN concentrations likely were near maximum at blood sampling time in our trials. This may explain the slope of 0.62 from our regression of MUN on BUN (Fig.1).

Single factors that yielded significant regressions on MUN concentrations using the mixed effects models were: dietary CP concentration [expressed either per unit DM ($R^2 = 0.839$) or per unit NE_L ($R^2 = 0.833$)], excess N intake ($R^2 = 0.772$), N-efficiency ($R^2 = 0.626$), and ruminal NH_3 ($R^2 = 0.574$). Urea in body fluids including milk probably reflects N inefficiency due to both excess protein degradation in the rumen and excess amino acid supply to the tissues. This may explain why MUN was better correlated to dietary CP content than to ruminal NH_3 . When all factors were analyzed at once with the mixed effects model, 12 made significant ($P < 0.10$) contributions (Table 1): BUN, BW, FCM yield, dietary CP content, excess N intake, DMI, and DIM were positively related to MUN; parity, milk and fat yield, dietary CP/ NE_L content, and NE_L intake were negatively related to MUN. Protein and SNF yield, dietary NDF and NE_L content, DM- and N-efficiency, and CP intake were not significant in the model. Thus, MUN concentrations will be influenced by multiple animal and dietary characteristics.

On the farm, milk often is sampled for DHI analysis at only one of the daily milkings. In two trials, we determined MUN in both AM and PM milk samples. Over both trials, BUN was

associated more strongly to MUN in AM milk ($R^2 = 0.686$) than to MUN in PM milk ($R^2 = 0.526$); the two regressions had different slopes ($P < 0.02$) and intercepts ($P < 0.0001$). As expected, regression of mean MUN concentration on BUN explained more of the variation ($R^2 = 0.737$) in BUN than did MUN in either AM or PM milk. In one of these two trials, total urine collection and urinary urea N analyses were made for the 12-h periods corresponding to MUN in AM and PM milk (Table 2). Urine volume excreted during the 12-h preceding the AM milking was greater than that for the 12-h preceding the PM milking; the reverse was true for milk yield. Urinary urea N and MUN followed similar patterns in that concentrations of both were higher in PM than in AM secretions. As expected, urinary urea concentration greatly exceeded MUN: urea N was 38 and 32 times more concentrated in AM and PM urine than in AM and PM milk. Gonda and Lindberg (1994) found that urinary urea concentration averaged 39 times greater than MUN. Lower MUN concentrations in AM than PM milk resulted in lower amounts and proportions of total urea excretion in AM (1.8%) than in PM (3.3%) milk (Table 2). These data clearly indicated that MUN concentration patterns were not symmetrical over the two

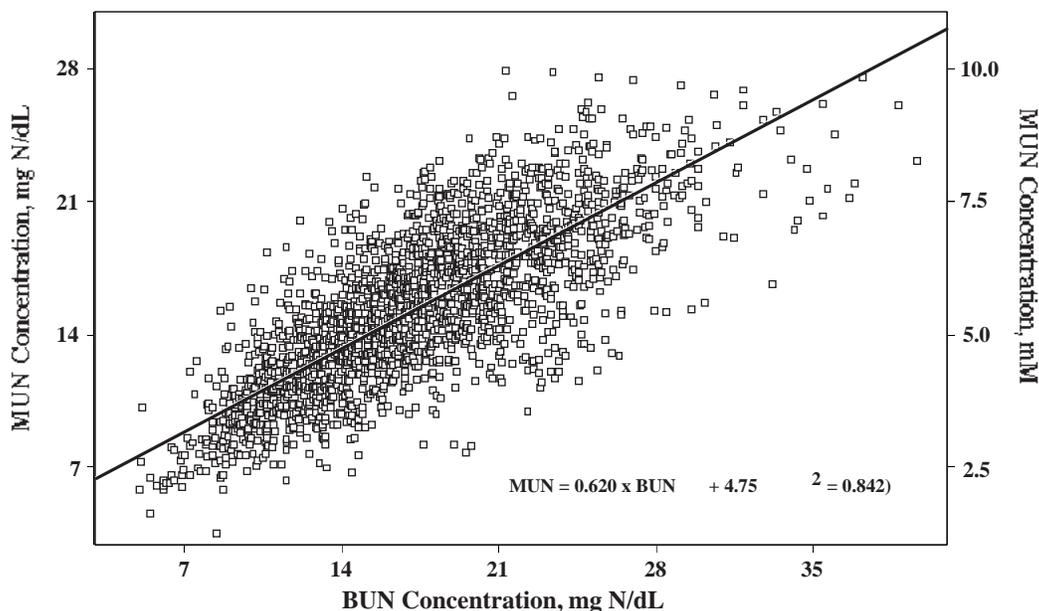


Figure 1. Regression of milk urea N (MUN) concentration on blood urea N (BUN) concentrations using all 2231 observations in the mixed effects model.

halves of the day and imply that switching milk sampling back and forth between AM to PM milkings is not appropriate. Composite milk samples representing the 24-h day will improve MUN reliability.

The numbers of cows fed a specific diet that must be sampled to determine the mean MUN concentration on that diet, within 95% confidence intervals of 1.0 or 2.0 mg N/dL, were estimated to be 16.5 and 4.1, respectively. This information may be used to develop recommendations for sampling milk for MUN analysis. Although the added precision gained by sampling 16 cows may not be necessary, our within-diet variation in MUN indicated that sampling milk from at least four cows would be the minimum needed to estimate MUN on a given diet. Milk samples representing the 24-h day will substantially improve reliability of MUN data. Switching sampling among AM to PM milkings, and presumably among more frequent 3X or 4X milkings, will confound interpretation of MUN data. Generally, sampling bulk tank milk probably would have little value unless used in conjunction with a dietary change that affected all the cows contributing milk to the tank. For example, if a lower protein alfalfa silage were replaced with one higher in protein such that dietary CP increased from 17 to 18% CP, the increase in MUN should be predictable. Rearranging the equation relating dietary CP% to MUN yields: $MUN = (\%CP - 13.7) / 0.269$, increasing dietary CP by 1 percentage unit, will increase MUN by 3.7 mg N/dL in bulk tank milk.

Summary

Statistical analyses using both linear regression and mixed effects models were conducted on a large set of MUN data obtained in feeding studies with lactating dairy cows. Concentrations of BUN and MUN were found to be highly correlated. Level of MUN was more closely related to dietary CP concentration, 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; parity, milk and fat yield, dietary CP/ 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 two trials, different relationships were found between BUN and MUN when assessed from MUN in either AM or PM milk collected in our twice daily milking scheme; BUN was more highly correlated to mean daily MUN concentration. Daily composite milk samples from 4 cows should be analyzed to estimate mean MUN concentration within a 95% confidence interval of +/- 2 mg N/dL.

References

- Gonda, H. L. and J.-E. Lindberg. 1994. Evaluation of dietary nitrogen utilization in dairy cows based on urea concentrations in blood, urine and milk, and on urinary concentration of purine derivatives. *Acta Agric. Scand., Sect. A* 44:236-245.
- Gustafsson, A. H. and D. L. Palmquist. 1993. Diurnal variation of rumen ammonia, serum urea, and milk urea in dairy cows at high and low yields. *J. Dairy Sci.* 76:475-484.
- National Research Council. 1989. *Nutrient Requirements of Dairy Cattle*. 6th rev. ed. Update. Natl. Acad. Press, Washington, DC.
- Rook, J.A.F. and P. C. Thomas. 1985. Milk secretion and its nutritional regulation. Ch. 8 in *Nutritional Physiology of Farm Animals* (J.A.F. Rook and P. C. Thomas, eds.). Longman Group, Limited, London.

Table 1. Parameters making significant contributions to the regression of milk urea N (MUN) on using the multiple factor, mixed effects model.¹ Denominator df = 1249; R² = 0.875.⁴

Parameter or factor ²	Estimated coefficient	SE	<i>t</i>	<i>P</i> ³
MUN (mg N/dL) =				
Intercept	- 4.713	1.897	- 2.48	0.013
BUN (mg N/dL)	0.484	0.013	37.05	< 0.001
Parity	- 0.175	0.045	- 3.90	< 0.001
Body weight (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/NE _L (g/Mcal)	- 0.059	0.019	- 3.18	< 0.001
Excess N intake (g N/d)	0.007	0.003	2.59	0.010
DMI (kg/d)	0.103	0.055	1.88	0.061
NE _L intake (kg/d)	- 0.133	0.053	- 2.48	0.013
DIM	0.003	0.001	1.93	0.054

¹BUN = Blood urea N; CP/NE_L = dietary CP/NE_L, where NE_L was computed from NRC (1989) tables; NE_L = NE_L intake computed from NRC (1989) tables; excess N intake = total N intake - milk N secretion.

²There were 2226 observations for each factor used in this model. Ruminal NH₃ was omitted from this model because of too few observations.

³Student's *t* and its associated *P*-value.

⁴Coefficient of determination determined for the mixed effects model.

Table 2. Concentration and excretion of urea N in urine and milk over 12-h periods ending at 4:00 AM and 4:00 PM.¹

Item	12-h Period ending at		SEM ²
	4:00 AM	4:00 PM	
Urine volume, L/12 h	20.4	14.7	0.4
UUN, mg N/dL	460.1	510.5	15.0
Urinary urea, g N/12 h	92.5	73.4	2.6
Milk volume, L/12 h	13.5	15.1	0.3
MUN, mg N/dL	12.0	16.0	0.37
Milk urea, g N/12 h	1.60	2.41	0.07
Total urea, g N/12 h	94.1	75.8	2.6
Milk urea/total urea, %	1.78	3.29	0.17

¹UUN = Urinary urea N; MUN = milk urea N; SEM = standard error of the mean.

²Each AM versus PM comparison was significantly different (*P* < 0.001).