Prediction of Ammonia Emission from Dairy Cattle Manure Based on Milk Urea Nitrogen: Relation of Milk Urea Nitrogen to Urine Urea Nitrogen Excretion

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ABSTRACT

The objectives of this study were to assess the relationship between urinary urea N (UUN) excretion (g/d) and milk urea N (MUN; mg/dL) and to test whether the relationship was affected by stage of lactation and the dietary crude protein (CP) content. Twelve lactating multiparous Holstein cows were randomly selected and blocked into 3 groups of 4 cows intended to represent early [123 ± 26 d in milk (DIM); mean $\pm$ standard deviation], mid (175 ± 3 DIM), and late (221 ± 12 DIM) lactation stages. Cows within each stage of lactation were randomly assigned to a treatment sequence within a split-plot Latin square balanced for carryover effects. Stage of lactation formed the main plots (squares) and dietary CP levels (15, 17, 19, and 21% of diet dry matter) formed the subplots. Graded amounts of urea were added to the basal total mixed ration to linearly increase dietary CP content while maintaining similar concentrations of all other nutrients among treatments. The experimental periods lasted 7 d, with d 1 to 6 used for adjustment to diets and d 7 used for total collection of urine as well as milk and blood sample collection. Dry matter intake and yields of milk, fat, protein, and lactose declined progressively with lactation stage and were unaffected by dietary CP content. Milk and plasma urea-N as well as UUN concentration and excretion increased in response to dietary CP content. Milk and urine urea-N concentration rose at increasing and decreasing rates, respectively, as a function of plasma urea-N. The renal urea-N clearance rate differed among lactation stages and dietary CP contents. The relationship between UUN excretion and MUN differed among lactation stages and diverged from linearity for cows in early and late lactation. However, these differences were restricted to very high MUN concentrations. Milk

urea N may be a useful tool to predict the UUN excretion and ultimately NH_3 emission from dairy cattle manure.

Key words: milk urea nitrogen, urine urea N excretion, ammonia emission

INTRODUCTION

There are growing concerns with regards to NH_3 emission into the atmosphere and its impact on the environment and human health. Ammonia emission is a process in which the N in animal manure (mainly urea-N in urine) is hydrolyzed to NH_3 by urease enzymes, dissociates to ionized and un-ionized forms in solution, and volatilizes by convective mass transfer to the air above the slurry, barn floor, or soil surface (Monteny and Erisman, 1998). Once emitted, NH_3 can be rapidly converted to NH_4^+ aerosol by reactions with acidic species found in ambient aerosols. As an aerosol, NH_4^+ contributes directly to the formation of particulate matter with a diameter of $2.5 \mu\text{m}$ or less ($\text{PM}_{2.5}$) and ecosystem fertilization, acidification, and eutrophication (NRC, 2003). Particulate matter is also associated with increased incidence of cardiorespiratory morbidity and mortality (Samet and Krewski, 2007).

Urea represents the majority of N-containing compounds in cattle urine, accounting for 50 to 90% of urinary N, and has the greatest potential for NH_3 volatilization (Bussink and Oenema, 1998). Accordingly, urinary urea N (UUN) excretion is associated with NH_3 emissions from dairy cattle manure (James et al., 1999) and can be used to establish reasonable estimates for potential NH_3 emission (Cassel et al., 2005).

Milk urea N concentration has emerged as a potentially useful tool to predict N excretion in urine and utilization efficiency in lactating dairy cows. The linear relationship between MUN and urinary N excretion was derived from the observations that the amount of N excreted by a cow in the urine was proportional to the concentration of urea in blood, which, in turn, was proportional to the concentration of urea in milk (Jonker et al., 1998). However, the proportionality be-

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tween urea concentration in blood and urea excreted in urine (i.e., the assumption that fractional rate of urea clearance from the blood by the kidney is constant) over a wide range of BUN concentrations was contested (Kauffman and St-Pierre, 2001). There is still debate as to how robust the relationships are over a wide range of data; therefore, there is a need for additional research to elucidate the reasons for the different relationships.

The prospective use of MUN for the estimation of UUN excretion and potential NH_3 emission is evident. However, the extent and type of relationship between UUN excretion and MUN over a wide range of N intake and the effect of physiological factors, such as stage of lactation, must be addressed before predictive models can be developed. The overall objective of the current study was to evaluate the potential of MUN as a predictor of NH_3 emissions from dairy cattle manure. The specific objectives of the experiment described herein were to assess the relationship between MUN and UUN, and to test whether the relationship was affected by stage of lactation and dietary CP content. The association of NH_3 emission with UUN excretion and MUN concentration will be reported in a future article.

MATERIALS AND METHODS

Cows, Experimental Design, and Diets

Twelve lactating multiparous Holstein cows were randomly selected from the University of California Dairy Teaching and Research Facility and blocked into 3 groups of 4 cows intended to represent early (123 ± 26 DIM; mean $\pm$ SD), mid (175 ± 3 DIM), and late (221 ± 12 DIM) lactation stages. Cows within each stage of lactation were randomly assigned to a treatment sequence within a split-plot Latin square balanced for carryover effects. Stage of lactation formed the main plots (squares) and dietary CP levels (15, 17, 19, and 21% of diet DM) formed the subplots. A basal TMR (15% CP, diet DM) was fed for 7 d before the start of the experiment. Thereafter, graded amounts of urea were added to the basal TMR to obtain a linear increase in dietary CP content. The experimental periods lasted 7 d, with d 1 to 6 used for adjustment to diets and d 7 used for total collection of urine and feces as well as milk and blood sample collection.

Cows were fed twice daily at 0700 and 1900 h throughout the experiment. Cows were housed in individual pens measuring 6.1×4.6 m during d 1 to 6 of the experimental period. On d 7, cows were moved to individual pens fitted with rubber mats modified to create a tie-stall arrangement. Water and feed were available for ad libitum consumption at all times. All experimental procedures were approved by the Institution

Animal Care and Use Committee at the University of California, Davis.

Sample Collection

The amounts of feed offered and orts were measured daily for each cow throughout the experiment. Daily samples of TMR and orts were pooled within each period for DM and chemical analyses. A portion of each sample was oven-dried at 100°C for 15 h to determine DM content. The remainder was air-dried at room temperature, ground through a 1-mm screen (Wiley mill, Arthur H. Thomas, Philadelphia, PA), and stored at room temperature until analyzed.

Milk yield was measured and recorded twice daily at approximately 0630 and 1830 h using Westfalia Systemat milk meters (Westfalia, Elk Grove Village, IL). Proportional milk samples from consecutive evening and morning milkings were taken on d 7 of each period and preserved with 2-bromo-2-nitro-propane-1,3-diol (Dairy and Food Labs, Inc., Modesto, CA). Evening samples were kept refrigerated (4°C) until after the morning sampling when all samples were warmed to 40°C in a water bath, composited by cow, and analyzed.

Urine was collected using an indwelling Foley catheter (24 French, 75-cc balloon; C. R. Bard, Covington, GA) that was inserted immediately after the afternoon milking on d 6 of the experimental period. Urine volume was measured for 4 consecutive intervals of approximately 6 h (0700 to 1300 h, 1300 to 1830 h, 1900 to 0100 h, and 0100 to 0630 h) on d 7 of each experimental period. Before each milking, cows were stimulated to urinate and urine was collected. Catheters were then clamped shut, cows were led to the milking parlor, milked (1830 to 1900 h and 0630 to 0700 h), and immediately returned to their stalls; the tubing was reconnected to the catheters. The adaptation period was considered sufficient time for the animal to adapt to a new diet because the only diet ingredient that was changing was the amount of urea that was soluble in the rumen. Also, plasma urea N (**PUN**) values were stable over the 24-h period after d 7, which is another indication that the animal reached a steady state between treatments. Urine containers (20 L, cleaned and rinsed thoroughly but not sterilized) were attached to catheters at 0700, 1300, 1900, and 0100 h. Urine was preserved by embedding the collection jugs in ice-cold water tubs. This procedure was planned to avoid urea-N loss by inhibiting enzyme-catalyzed urea hydrolysis while allowing its eventual conversion to NH_3 under controlled laboratory conditions using flux chambers. The efficacy of the urine preservation procedure was determined in preliminary experiments in which nearly 100% of urea was recovered compared with recovery

achieved using acid-treated urine. From each collection interval, a 100-mL aliquot of urine was immediately stored (-20°C), whereas a second portion (500 mL) was kept at 4°C until urine collection was completed and used to create a weighted composite sample to represent the 24-h period. An aliquot of the urine composite sample was stored at -20°C , whereas the remaining fraction was used for subsequent NH_3 emission measurements and analyzed immediately for urea. Urine volume and weight were recorded for each collection interval. The rate of renal urea-N clearance (L/kg of BW per d) was calculated as urine volume $\times$ urinary creatinine concentration/(plasma creatinine concentration $\times$ BW).

Four blood samples (10 mL each) were collected from a coccygeal vein at 0600, 1200, 1800, and 2400 h (i.e., 0000 h, midnight) starting on d 7 of the experimental period. Samples were collected by venipuncture (20-gauge needle) into sterile evacuated tubes containing 0.117 mL of 15% EDTA solution (Becton Dickinson and Co., Franklin Lakes, NJ), placed on ice, and centrifuged at $10,000 \times g$ for 20 min to obtain plasma. Plasma was stored at -20°C until analyzed for urea-N concentration.

Analytical Procedures

All chemical analyses of feeds were performed by a commercial laboratory (Cumberland Valley Analytical Services, Inc., Maugansville, MD). Dry matter of forages was determined by heating in a forced-air oven to 105°C for 3 h. Ash, ADF, selected minerals, and CP in the feed were determined according to AOAC (2000; methods 942.05, 973.18, 985.01, and 990.03, respectively) with modifications. Glass microfiber filters with $1.5\text{-}\mu\text{m}$ particle retention (Whatman International, Maidstone, UK) instead of a fritted glass crucible were used for ADF. Samples (0.5 g) were ashed for 2 h at 535°C and then digested in an open crucible for 20 min in 15% nitric acid on a hotplate. Selected samples for mineral analyses were then diluted to 50 mL and analyzed using inductively coupled plasma spectroscopy. Fiber residue from the ADF sample was recovered on the $1.5\text{-}\mu\text{m}$ particle retention filter. Neutral detergent fiber was determined using Whatman 934-AH glass microfiber filters with $1.5\text{-}\mu\text{m}$ particle retention (Whatman) and according to the methods described by Goering and Van Soest (1970). Soluble protein was measured using a borate-phosphate method (Krishnamoorthy et al., 1982).

Milk was analyzed for fat, protein, lactose, and SNF (AOAC, 1990). Urine, plasma, and milk samples were assayed for urea N concentration by a diacetyl-monoxime method (Marsh et al., 1957) using a Technicon au-

toanalyzer (Technicon Instruments Corp., Tarrytown, NY).

Statistical Analysis

Production and milk, plasma, and urine urea N concentrations, excretion, and clearance measurements were analyzed using Proc MIXED of SAS (SAS Institute, 1999) according to the following model:

$$Y_{ijkl} = \mu + S_i + c_{j(i)} + P_k + L_l + SL_{il} + E_{ijkl}, \quad [1]$$

where Y_{ijkl} = dependent variable measured for the i th stage of lactation, the j th cow within the i th stage of lactation, during the k th period, and the l th level of dietary CP content; μ = overall mean; S_i = fixed effect of the i th stage of lactation, $i = 1, 2, 3$; $c_{j(i)}$ = random effect of the j th cow within the i th stage of lactation, $j = 1, 2, 3, 4$; P_k = fixed effect of the k th period, $k = 1, 2, 3, 4$; L_l = fixed effect of the l th level of dietary CP content, $l = 1, 2, 3, 4$; SL_{il} = interaction term of the i th stage of lactation with the l th level of dietary CP content; and E_{ijkl} = error term $\sim N(0, \sigma_e^2)$.

Linear and quadratic effects of CP level were estimated by orthogonal contrast (Littell et al., 1996).

Plasma and urine urea N measurements over time were analyzed using Proc MIXED of SAS (SAS Institute, 1999) according to the following model:

$$Y_{ijklm} = \mu + S_i + c_{j(i)} + P_k + L_l + SL_{il} + T_m + ST_{im} + LT_{lm} + SLT_{ilm} + E_{ijklm}, \quad [2]$$

where symbols are as above with the following additions: Y_{ijklm} = dependent variable measured for the i th stage of lactation, the j th cow within the i th stage of lactation, during the k th period, for the l th level of dietary CP content, and in the m th time of sampling; T_m = fixed effect of m th time of sampling, $m = 1, 2, 3, 4$; ST_{im} = interaction term of the i th stage of lactation with the m th time of sampling; LT_{lm} = interaction term of the l th level of dietary CP content with the m th time of sampling; and SLT_{ilm} = interaction term of the i th stage of lactation with the l th level of dietary CP content, and m th time of sampling; and E_{ijklm} = error term $\sim N(0, \sigma_e^2)$.

Errors within cows and periods (repeated measures due to 4 equally spaced sampling intervals) were modeled using a first-order autoregressive covariance structure (Littell et al., 1996).

To determine the effect of stage of lactation on the relationships between MUN and PUN, UUN and PUN, and UUN excretion and MUN, a sequence of mixed models was fitted with Proc MIXED (SAS Institute,

Table 1. Sequence of equations used to determine final models¹

Hypotheses and models	Variable tested	MUN vs. PUN	MUN vs. FD ²	RD	UUN vs. PUN	UUN vs. FD	RD	UUNg vs. MUN	UUNg vs. FD	RD	Equation
Ho: All stage of lactation slopes are equal to zero $Y_{ijk} = S_i + c_{j(i)} + P_k + K_i X_{ijk} + E_{ijk}$	K_i	<0.001	<0.001		<0.001	<0.001		<0.001	<0.001		[3]
Ho: Homogeneity of slopes across stages of lactation $Y_{ijk} = \mu + S_i + c_{j(i)} + P_k + \beta_0 X_{ijk} + \beta_i X_{ijk} + E_{ijk}$	β_i	0.049 ³	0.585		0.311	0.420		0.006	0.201		[4]
Ho: SOL intercept are zero; individual slopes model $Y_{ijk} = \mu + S_i + c_{j(i)} + P_k + \beta_i X_{ijk} + E_{ijk}$	S_i	0.420						0.342			[5]
Ho: SOL intercept are zero; common slope model $Y_{ijk} = \mu + S_i + c_{j(i)} + P_k + \beta_0 X_{ijk} + E_{ijk}$	S_i		0.567		0.721	0.828			0.343		[6]
Ho: Overall means are zero; reduced models $Y_{ijk} = \mu + c_{j(i)} + P_k + \beta_i X_{ijk} + E_{ijk}$	μ	0.517									[7]
$Y_{ijk} = \mu + c_j + P_k + \beta_0 X_{ijk} + E_{ijk}$	μ		0.285		0.008	0.037		0.015 ⁴	0.016		[8]
Ho: Quadratic trend and overall mean not different from zero $Y_{ijk} = \mu + c_j + P_k + \beta_0 X_{ijk} + \delta_0 X_{ijk} X_{ijk} + E_{ijk}$	δ_0	<0.001	0.020		0.025	0.742			0.129		[9]
	μ	<0.001			0.760			0.027 ⁵			
Ho: Quadratic coefficient not different from zero $Y_{ijk} = \mu + c_{j(i)} + P_k + \beta_0 X_{ijk} + \delta_i X_{ijk} X_{ijk} + E_{ijk}$	δ_1 Early δ_2 Mid δ_3 Late							0.020 0.479 0.044			[10]
Final models $Y_{ijk} = c_j + P_k + \beta_0 X_{ijk} + E_{ijk}$				X							[11]
$Y_{ijk} = \mu + c_j + P_k + \beta_0 X_{ijk} + E_{ijk}$						X		X^4	X		[8]
$Y_{ijk} = \mu + c_j + P_k + \beta_0 X_{ijk} + \delta_0 X_{ijk} X_{ijk} + E_{ijk}$		X						X^5			[9]
$Y_{ijk} = c_j + P_k + \beta_0 X_{ijk} + \delta_0 X_{ijk} X_{ijk} + E_{ijk}$					X						[12]

¹The Y and X variable combinations are MUN vs. plasma urea N (PUN), urine urea N (UUN) vs. PUN, and UUNg (UUN excretion) vs. MUN. Units are mg/dL for all variables except UUNg (g/d). Y_{ijk} = dependent variable measured for the ith stage of lactation (SOL), the jth cow within the ith SOL, and during the kth period; where $i = 1, 2, 3$; $j = 1, 2, 3, 4$; $k = 1, 2, 3, 4$; μ = overall mean; S_i = fixed effect of the ith SOL; $c_{j(i)}$ = random effect of the jth cow within the ith SOL; P_k = fixed effect of the kth period; K_i = regression coefficient of X for the ith SOL; X_{ijk} = Predictor for the jth cow, within the ith SOL, during the kth period; β_0 = overall regression coefficient of Y on X and β_i = deviation of regression coefficient of Y on X of ith SOL from the overall regression coefficient, or linear slope coefficient; δ_0 = quadratic regression coefficient; δ_i = quadratic coefficient for the ith SOL; and E_{ijk} = error term $\sim N(0, \sigma_e^2)$.

²FD = full data set; RD = reduced data set, MUN values not considered >25 mg/dL.

³This value was not significant when tested with the quadratic equation.

⁴Mid lactation cows only.

⁵Early and late lactation cows considered as one group.

1999). All relationships between variables were evaluated with a full data set and a reduced data set, which did not consider data with MUN values >25 mg/dL. Table 1 contains equations [3] to [12] and the general format for the sequence of models developed. To determine the effect of stage of lactation on the slope of the regressions, equation [3] was fitted to test the hypothesis that all stage of lactation slopes were equal to zero (Littell et al., 1996). A significant effect of K_i indicated that the linear regression was different for at least 1 stage of lactation. To determine whether the stages shared a common slope, equation [4] was fitted to test the hypothesis that slopes are equal across stages of lactation. A significant β_i effect indicated that the slope of MUN on PUN and UUN excretion on MUN were different for at least 1 stage of lactation for the full data set; therefore, individual slopes were fitted for each stage of lactation according to equation [5]. There was insufficient evidence to reject the hypothesis that slopes are equal across stages of lactation for all other relation-

ships (Table 1); therefore, a common slope model was used according to equation [6]. The stage of lactation intercept (S_i) was evaluated for both equations [5] and [6] to determine whether it differed from zero. The stage of lactation intercepts were not significant in all cases. Equations [7] and [8] tested whether the overall means were different from zero.

All relationships were quadratic (equations [9] and [10]) with the full data set and linear with the reduced data set. Although both linear and quadratic models were reduced similarly to reach a final model for a given relationship, only the relevant steps are presented. The restriction that the sum of period effects equals zero was used when testing if the overall mean was not different from zero and in the prediction equations of the final models.

Stage of lactation only had an influence in the relationships between UUN excretion and MUN for the full data set in which early and late lactation were quadratic (equation [10]) yet the quadratic coefficients

Table 2. Ingredient and nutrient composition of experimental diets

Component	Dietary CP, % of DM			
	15	17	19	21
Ingredient, as-fed basis				
Alfalfa hay, chopped	32	31.8	31.5	31.3
Oat hay, chopped	12	11.9	11.8	11.7
Corn, steam-flaked	19	18.9	18.7	18.6
Almond hulls	17	16.9	16.8	16.6
Barley, steam-rolled	7.5	7.4	7.4	7.3
Whole cottonseed	6	6	5.9	5.9
Molasses, cane	3	3	3	2.9
Fat, yellow grease	2	2	2	2
Urea, prilled	0	0.7	1.5	2.2
Salt, trace mineral	1.5	1.5	1.5	1.5
Nutrient, % of DM				
CP	15.1	16.6	18.6	20.7
Soluble protein	4.6	5.4	8.3	9.6
ADF	22.0	22.3	22.5	22.6
NDF	31.9	32.1	32.3	32.3
Ash	7.13	7.11	6.99	7.34
NE _L , ¹ Mcal/kg	1.56	1.56	1.55	1.55
Ca	0.71	0.67	0.64	0.67
P	0.34	0.32	0.30	0.31
Mg	0.42	0.42	0.40	0.40
K	1.67	1.72	1.77	1.69
Na	0.32	0.30	0.31	0.30

¹Calculations based on NRC (1989).

for early and late were not different from each other, thus reducing to equation [9]. The mid lactation reduced to a linear model (equation [8]). The quadratic model for the UUN vs. PUN relationship showed that the overall intercept was not significant (equation [9], $P = 0.76$) and was reduced to equation [12]. All the relationships for the reduced datasets resulted in the same general equation [8] except for MUN vs. PUN, which reduced to equation [11].

RESULTS AND DISCUSSION

Cows, Experimental Design, and Composition of Diets

Ingredient and nutrient composition of the diets is given in Table 2. Addition of graded amounts of urea resulted in a linear increase in dietary CP content, whereas the remainder of all other nutrients concentrations were largely unchanged. All experimental diets were within 2.5% of the target CP concentration. Soluble protein accounted for about one-third of the basal TMR dietary CP content and increased to almost one-half at the greatest level of urea inclusion.

Intake and Milk Production

The interaction between stage of lactation and level of dietary CP content was not significant for all intake and milk production measurements ($P = 0.14$ to 0.79);

therefore, only the tests on main effects are presented (Table 3). Dry matter and N intakes were greatest in the early lactation group and decreased progressively as groups advanced in lactation. Yields of milk and milk constituents followed a similar trend, whereas milk composition remained unchanged.

Supplementation of a low-protein diet with urea had no effect on DMI (Table 3), in agreement with results from previous studies (Sannes et al., 2002). As intended, addition of graded amounts of urea to a basal TMR resulted in a linear increase in N intake by lactating dairy cows. Dietary CP content had no effect on milk yield and milk composition. In agreement with our findings, Olmos Colmenero and Broderick (2006) and Leonardi et al. (2003) observed no effect of dietary CP content on milk yield of dairy cows when dietary CP was increased from 13.5 to 19.4% and 16.1 to 18.9%, respectively.

Urea Nitrogen Metabolism and Excretion

The effect of level of dietary CP content on PUN and MUN concentration as well as UUN excretion was different among lactation stages (Table 4); but they were restricted to the highest CP level for MUN, PUN, and UUN excretion vs. dietary CP content ($P = 0.007$, 0.015 , and 0.003 , respectively; Figure 1). The concentration of urea in milk, plasma, and urine was not different among lactation stages but was influenced by dietary CP content, in agreement with previous studies (Kaufman and St-Pierre, 2001; Sannes et al., 2002; Olmos Colmenero and Broderick, 2006).

The concentration of urea-N in plasma and urine was different ($P < 0.001$) among sampling times (Figure 2). The concentration of urea-N in urine paralleled that of plasma for all sampling times with the exception of the 2400 h sample. Diurnal patterns in the concentration of urea in blood (Lefcourt et al., 1999) and urine (Gonda and Lindberg, 1994) of lactating dairy cows were previously reported. The time of sampling at which the UUN concentration was greatest (2400 h) coincided with the collection interval in which urine excretion was lowest (data not shown), possibly a consequence of inactivity and reduced urination frequency.

There was an interaction ($P < 0.001$) between dietary CP content and time of sampling for PUN (Figure 3). Plasma urea N concentration was greatest at the earliest sampling time for the 19 and 21% CP levels. The time of sampling had no effect on PUN for the 15 and 17% CP diets. It is unlikely that the high PUN at 0600 h was feed induced because cows were fed at 12-h intervals and blood samples were taken before feeding. Interestingly, the dietary CP by time of sampling interaction and the declining trend in PUN with time of sam-

Table 3. Means for intake, milk production and composition by lactating dairy cows different lactation stages fed diets varying in protein concentration

Item	Stage of lactation (SOL)			SEM	Dietary CP, % of DM				SEM	SOL	Contrast ¹	
	Early	Mid	Late		15	17	19	21			L	Q
											<i>P</i> -value ²	
DMI, kg/d	25.5	24.3	20.0	1.29	22.5	23.7	23.3	23.5	0.81	0.034	0.090	0.143
N intake, g/d	698.9	666.3	545.2	37.49	500.6	600.0	688.6	758.0	24.32	0.041	<0.001	0.253
Milk, kg/d	38.7	28.8	23.1	3.37	29.9	31.3	29.7	30.0	2.03	0.028	0.693	0.418
Yield, kg/d												
Fat	1.86	1.07	0.92	0.196	1.27	1.37	1.26	1.24	0.127	0.017	0.503	0.370
Protein	1.10	0.83	0.69	0.103	0.86	0.90	0.86	0.87	0.062	0.055	0.873	0.475
Lactose	2.03	1.33	1.13	0.207	1.48	1.57	1.47	1.48	0.125	0.031	0.607	0.368
SNF	3.49	2.53	2.06	0.315	2.66	2.79	2.65	2.68	0.189	0.029	0.704	0.443
Composition, %												
Fat	4.80	3.57	3.92	0.361	4.06	4.20	4.10	4.01	0.257	0.096	0.763	0.513
Protein	2.84	2.86	2.97	0.104	2.87	2.88	2.91	2.89	0.064	0.651	0.390	0.554
Lactose	5.24	4.45	4.77	0.245	4.82	4.85	4.82	4.79	0.152	0.124	0.690	0.630
SNF	9.01	8.72	8.87	0.113	8.86	8.86	8.88	8.86	0.073	0.238	0.911	0.856

¹Effect of CP content; L = linear and Q = quadratic trends.

²Probability of a significant effect of stage of lactation or a linear or quadratic effect of dietary CP.

pling for the highest CP treatment were also observed by Kauffman and St-Pierre (2001).

Plasma urea N concentration responded quadratically to supplementation of a basal ration with graded amounts of urea that resulted in a linear increase in dietary CP content (Table 4). This finding differs from the observations of Olmos Colmenero and Broderick (2006), who reported a linear response in PUN for a similar range (13.5 to 19.4% of DM) of dietary CP treatments. The difference in the type of response might be due to the source of dietary N; the latter study added solvent-extracted soybean meal, mainly RUP, to replace rolled high-moisture shelled corn in a basal ration, whereas the current study used urea, a highly rumen-soluble source of N, to supplement a basal ration. The quadratic response of PUN, increasing at an increasing rate as a function of CP levels in the diet, suggests that

the amount of NH₃ released in the rumen may have exceeded the capacity of microbes to utilize it, leading to a greater proportion of NH₃ absorbed through the rumen wall for urea synthesis in the liver and its subsequent increased concentration in systemic circulation.

The MUN concentration response was quadratic (similar to PUN) and ranged from 7.9 to 24.5 mg/dL when the CP content of the diet increased from 15.1 to 20.7% (Table 4). This is in agreement with previous findings by Broderick (2003), who reported a slight improvement in the fit of regression equation for the relationship between MUN and dietary CP content when a quadratic term was included in the model for lactating dairy cows fed 15.1 to 18.4% dietary CP. At the greatest CP level, average MUN values were 2 times greater than the mean value (12.5 mg/dL) observed at the greatest CP level in Kauffman and St-Pierre (2001). The

Table 4. Means for MUN, plasma urea N (PUN), and urine urea N (UUN) concentration, excretion, and clearance by lactating dairy cows on different lactation stages fed diets varying in protein concentration

Item	Stage of lactation (SOL)			SEM	Dietary CP, % of DM				SEM	SOL	Contrast ¹	
	Early	Mid	Late		15	17	19	21			L	Q
											<i>P</i> -value ²	
MUN, ³ mg/dL	16.39	15.30	14.56	0.904	7.90	11.96	17.28	24.53	0.687	0.393	<0.001	0.005
PUN, ⁴ mg/dL	17.08	16.51	15.70	0.796	8.23	12.96	18.68	25.85	0.602	0.495	<0.001	0.012
UUN, mg/dL	613	662	588	54.0	355	526	700	903	38.0	0.629	<0.001	0.418
UUN, ⁵ g/d	197.3	207.6	182.6	10.26	85.9	149.4	227.5	320.6	8.06	0.274	<0.001	0.028
Clearance, ⁶ L/kg of BW d	1.57	1.68	1.77	0.042	1.49	1.67	1.77	1.77	0.046	0.026	<0.001	0.061

¹Effect of dietary CP. L = linear and Q = quadratic trends.

²Probability of a significant effect of stage of lactation or a linear or quadratic effect of dietary CP.

³Stage of lactation × dietary CP interaction was significant (*P* = 0.027).

⁴Stage of lactation × dietary CP interaction was significant (*P* = 0.029).

⁵Stage of lactation × dietary CP interaction was significant (*P* = 0.01).

⁶Renal urea-N clearance rate.

Figure 1. Stage of lactation (○ = early, × = mid, and ▲ = late) by dietary CP interaction: a) MUN, b) plasma urea N (PUN); and c) urinary urea N (UUN) excretion.

Figure 2. Plasma urea N (PUN, ○) and urine urea N (UUN, ●) vs. time of sampling.

Figure 3. Plasma urea N (PUN) vs. time of sampling at 4 levels dietary of CP (○ = 15, × = 17, ▲ = 19, and ■ = 21%).

MUN concentration for individual observations ranged between 5.3 and 31.8 mg/dL, thus providing a wide range of MUN values to adequately evaluate the relationship between MUN and UUN excretion.

There was a close association between PUN and MUN (Figure 4), reflecting the rapid equilibration of blood urea into milk (Gustafsson and Palmquist, 1993). However, unlike previous studies (Roseler et al., 1993; Broderick and Clayton, 1997; Kauffman and St-Pierre, 2001), a slight quadratic term (0.01 ; $P < 0.001$) in the relationship between MUN and PUN was obtained when all MUN values were used (Figure 4). The positive quadratic coefficient is in accordance with the response pattern of MUN and PUN to changes in dietary CP content. The difference in the type of relationship among studies may relate to the range of urea-N concentrations used to develop the equation, because when MUN values >25 mg/dL (reduced data set) were not included, the relationship was linear [$\text{MUN} = 0.93 (\pm 0.007) \times \text{PUN}$]. Several investigators reported regression equations for the MUN-PUN relationship often

Figure 4. Milk urea N vs. plasma urea N (PUN) for early (○), mid (×), and late (▲) lactation cows. The solid line represents the regression equation [$\text{MUN} = 2.29 (\pm 0.55) + 0.61 (\pm 0.07) \times \text{PUN} + 0.01 (\pm 0.002) \times \text{PUN}^2$; $R^2 = 0.99$].

Figure 5. Urine urea N (UUN) vs. plasma urea N (PUN) for early (○), mid (×), and late (▲) lactation cows. The solid line represents the regression equation $[UUN = 47.67 (\pm 3.94) \times PUN - 0.49 (\pm 0.13) \times PUN^2; R^2 = 0.94]$.

differing in both intercept and slope. Kauffman and St-Pierre (2001) attributed the general disagreement among reports to differences in sampling time. At least 2 possible sources of variation may account for the differences in regression equations; namely, time of sampling relative to time of feeding or time of day. In the current trial, PUN values represented average urea-N concentration of 4 blood samples taken at 6-h intervals, whereas MUN concentrations were measured on a composite of 2 consecutive milk samples collected at 12-h intervals within 30 min of blood collection before feeding, thus minimizing the influence of time of sampling relative to time of day and time of feeding. If urea diffused passively from blood to milk then, for composited or otherwise averaged measurements within the linear range of urea-N concentrations, the regression equation of MUN on PUN should intercept at the origin and have a slope equal (or close) to unity.

The concentration of UUN was also affected by dietary CP content, but the response was linear. This finding is significant because it provides initial evidence to examine the assumption of a constant urea filtration rate by the kidney. It follows that if the relationship between MUN concentration and UUN excretion is to be linear; that is, if UUN is to be proportional to MUN, then the pattern of urea concentration in milk, plasma, and urine should be similar. The difference in the response of urea-N concentration between milk and urine is more clearly seen when the relationship between UUN and PUN (Figure 5) is compared with that of MUN and PUN (Figure 4). First, the magnitude of the difference in slopes, between 30- and 40-fold, reflects the capacity of the kidney to concentrate urea in the urine. Second, the scatter in the relationship between UUN and PUN reveals a greater variability in the transfer process between urea in plasma to urine than to milk. Third, the opposing response of MUN and UUN

to increasing PUN concentration implies that at very high PUN concentrations, more urea goes into the milk rather than into urine. Collectively, these observations point to a divergence in the pattern of MUN and UUN over a wide range of PUN.

Urine urea N excretion increased quadratically in response to a linear increase in dietary CP content (Table 4). Our findings are in contrast to those of Olmos Colmenero and Broderick (2006), who reported a linear effect of dietary CP content on UUN excretion. The amount of urea-N excreted in urine was almost equivalent (91 vs. 85.9 g/d) at 15% CP, but at the greatest CP level (~21% CP), UUN excretion in our study (321 g/d) was 54% greater than that of Olmos Colmenero and Broderick (2006; 208 g/d) possibly reflecting the differences in RUP between N sources, as previously discussed. An extensive analysis of N utilization in lactating dairy cows by Castillo et al. (2000) revealed that the excretion of N in urine increased linearly with N intake up to 400 g/d and increased exponentially thereafter. In the current study, N intake was greater than 400 g of N/d for all treatments so that a 51% increase in N intake resulted in a 273% increase in UUN excretion.

Renal urea-N clearance rates were different among lactation stages and dietary CP content (Table 4). The renal clearance of urea-N increased as lactation progressed; cows in late lactation cleared about 12 and 5% greater plasma volume than early and mid lactation cows, respectively, to remove an equivalent amount of urea-N. The renal urea-N clearance rate in the present study averaged 1,170 L/d for a 700-kg animal, which compared favorably with the average renal urea-N clearance rate estimated from published data (Sannes et al., 2002) at 1,163 L/d for lactating dairy cows weighing 690 kg. The quadratic response of renal urea-N clearance rate to dietary CP content indicates that the kidneys may have a limited ability to filter urea at high urea loads as previously proposed by Sannes et al. (2002). Taken together, our results provide evidence that the assumption of a constant renal urea filtration rate does not hold over a wide range of PUN and MUN concentrations.

Relationship Between UUN Excretion and MUN

The relationship between UUN excretion and MUN is presented in Figure 6. Slopes of the UUN excretion–MUN relationship were different among lactation stages ($P < 0.001$). The slopes from equation [10] indicated that the quadratic coefficients were different from zero in early and late ($P = 0.02$ and 0.04 , respectively), but not mid ($P = 0.48$), lactation cows. Furthermore, multiple comparison of the slopes revealed that early- and late-lactation quadratic coefficients were not differ-

Figure 6. Urine urea N (UUN) excretion vs. MUN. The solid line represents the regression equation for mid (x) lactation cows [$UUN = -37.33 (\pm 11.62) + 16.01 (\pm 0.48) \times MUN$; $R^2 = 0.99$]. The dashed line represents the regression equation for early (o) and late (▲) lactation [$UUN = -49.95 (\pm 21.18) + 18.67 (\pm 2.58) \times MUN - 0.17 (\pm 0.07) \times MUN^2$; $R^2 = 0.97$].

ent from each other ($P = 0.65$). Therefore, the stages of lactation were grouped into an early-late lactation stage group for a quadratic equation [9] and a mid lactation group for a linear equation [8].

The following regression equation was developed from equation [8] for mid lactation cows ($R^2 = 0.99$):

$$UUN \text{ (g/d)} = -37.33 (\pm 11.62) + 16.01 (\pm 0.48) \times MUN \text{ (mg/dL)} \quad [13]$$

and from equation [9] for early and late lactation cows ($R^2 = 0.97$):

$$UUN \text{ (g/d)} = -49.95 (\pm 21.18) + 18.67 (\pm 2.58) \times MUN \text{ (mg/dL)} - 0.17 (\pm 0.07) \times MUN^2 \quad [14]$$

The quadratic trend in the UUN excretion–MUN relationship for cows in early and late lactation confirms our contention, based on pattern of urea-N concentration in bodily fluids as well as stage of lactation and dietary CP-induced variation in renal urea-N clearance rates, that the relationship between UUN excretion and MUN concentration is not linear over a wide range of MUN values.

For practical application, it was desirable to further reduce the model to eliminate the need for separate equations for each stage of lactation. It appeared that the difference in slopes in the UUN excretion–MUN relationship among lactation stages, and the divergence from linearity for the early-late lactation group, may have been due to the very high MUN concentration (>25 mg/dL) observations from the high-CP diet. Therefore, the effect of stage of lactation on the relationship between UUN excretion and MUN concentration was re-

evaluated with a reduced data set in which MUN values >25 mg/dL were omitted to represent a linear range of UUN excretion relative to MUN. The following equation was developed to predict UUN excretion based on MUN (where $MUN \leq 25$ mg/dL; $R^2 = 0.96$):

$$UUN \text{ (g/d)} = -23.1 (\pm 9.13) + 14.4 (\pm 0.55) \times MUN \text{ (mg/dL)} \quad [15]$$

The range of MUN values used by Jonker et al. (1998) was 1.3 to 24.7 mg/dL with an average of 15.67 mg/dL. A similar range was apparently used by Broderick and Clayton (1997), based on Figure 2 of their paper, although the range was not reported. In a field trial at 2 commercial dairy herds in California, the average MUN concentration was around 15% with a range of about 8 to 22 mg/dL (Burgos et al., 2005). Therefore, equation [15] appears to be applicable to diets under commercial conditions.

As expected, the lactation stages slope were no longer different ($P = 0.34$) in the UUN excretion–MUN relationship with the reduced data set. The intercept was common among lactation stages and differed from zero ($P = 0.34$ and 0.02 , respectively). A negative intercept was not anticipated and its physiological interpretation is not immediately evident. The slope in UUN excretion–MUN relationship represents 80% of the regression coefficient for Holstein cows proposed by Kauffman and St-Pierre [2001; Urinary N (g/d) = $17.6 (\pm 0.56) MUN \text{ (mg/dL)}$], which is within the range of proportion of total urinary N excreted as urea by cattle (Bussink and Oenema, 1998). As comparable data become available, it will be interesting to compare regression coefficients obtained under different conditions (i.e., dietary treatments and physiological factors) and integrate the data to develop robust predictive models.

CONCLUSIONS

The concentration of urea-N in milk, plasma, and urine was affected by dietary CP content, but the response was different among the pools. The relationship of MUN and UUN to PUN diverged over a wide range of PUN. The renal urea-N clearance rate was different among stages of lactation and dietary CP contents. The relationship between UUN excretion and MUN concentration was different among lactation stage and diverged from linearity for early and late lactation over a wide range of MUN values. However, these differences were restricted to very high MUN concentrations. Thus, the prediction of UUN excretion based on MUN concentration depends on the range of MUN concentration. Milk urea N can be used to predict UUN excretion and may be extended to estimate NH_3 emissions from dairy

cattle manure because there is a strong relationship between UUN excretion and NH_3 emissions. Given the space restrictions, this evidence will be reported in a future article.

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