J. Dairy Sci. 84:2284–2294

The Relationship of Milk Urea Nitrogen to Urine Nitrogen Excretion in Holstein and Jersey Cows[1]

A. J. Kauffman and N. R. St-Pierre
Department of Animal Sciences,
The Ohio State University, Columbus 43210

ABSTRACT

The objectives of this study were to assess the relationship between urinary nitrogen excretion (UN, g/d) and milk urea nitrogen concentration (MUN, mg/dl) and whether the types of carbohydrates fed interacts with the dietary CP and the breed (size) of cows to affect this relationship. Eight multiparous cows (four Holstein and four Jersey) were fed four different diets in a 2 × 2 factorial arrangement of levels of crude protein (13 and 17%) and levels of neutral detergent fiber (30 and 40%). The experimental design was a split plot Latin square with breeds forming the main plots and diets forming the subplots. Experimental periods were 3 wk in length, with d 1 to 14 used for adjustment and d 15 to 19 used for a total collection of urine and feces. Crude protein concentrations had a significant effect on milk, milk fat and protein production, plasma urea N, MUN, and on N balance measurements (N intake, fecal and urinary N excretion, milk N production, N retention, apparent N digestibility, and N efficiency). Neutral detergent fiber levels had no effect on any production parameters or N balance measurements. The relationship between urinary N and MUN was linear over the range of MUN values observed and different for the two breeds. The breed effect on the UN-MUN relationship was no longer significant ($P = 0.63$) when body weight (BW) was included in the model. The optimal allometric coefficient for BW was 0.96 and was not different from 1.0. Therefore, the following equation is proposed to predict UN excretion based on MUN and BW: UN (g/d) = 0.0259 (± 0.0006) BW (kg) × MUN (mg/dl).

(**Key words:** milk urea nitrogen, urinary N excretion, crude protein, neutral detergent fiber)

Abbreviation key: ADICP = acid detergent insoluble CP, **BUN** = blood urea nitrogen, **MUN** = milk urea nitrogen, **MFN** = metabolic fecal nitrogen, **NDICP** = neutral detergent insoluble CP, **NI** = nitrogen intake, **PUN** = plasma urea nitrogen, **UN** = urinary nitrogen.

INTRODUCTION

The environmental impact of nutrient waste from agriculture has become an area of concern as ways are sought to produce more food with less land. One of the nutrients targeted is nitrogen (Kohn et al., 1997). Nitrogen is released into the environment through the urine and feces of livestock as undigested N fractions and as the end products of N metabolism. Dairy cows contribute an estimated minimum of 750,000 metric tons of N per year into the environment of the United States (St-Pierre and Thraen, 1999).

Urea is the major end product of N metabolism in most mammals, including ruminants (Merchen, 1993). Although the majority of urea is excreted in the urine, some diffuses into the milk. Due to advances in technology and laboratory automation of milk urea N (**MUN**) determination, many DHIA have adopted the technology as an additional service to their members. In practice, MUN has been proposed as an indicator of the protein nutrition status and efficiency of N utilization for dairy cows (Hof et al., 1997; Roseler et al., 1993; Shepers and Meijer, 1998), and MUN is now being investigated as a predictor of N output to the environment (Jonker et al., 1998).

Blood urea nitrogen (**BUN**) concentration directly affects the concentration of MUN (Oltner and Wiktorsson, 1983; Roseler et al., 1993) because urea freely diffuses from blood to milk (Gustafsson and Palmquist, 1993). In turn, BUN is affected by several factors, including level of CP in the diet (Baker et al., 1995; Oltner, et al., 1985; Roseler et al., 1993) and carbohydrate composition (Lykos et al., 1997). Jonker et al. (1998) proposed a simple, but integrated, model of N balance in dairy cows that was based on the following empirical relationship between urinary N (**UN**) and MUN:

$$\text{UN (g/d)} = 12.54 * \text{MUN (mg/dl)}, \quad [1]$$

Received January 22, 2001.
Accepted May 7, 2001.
Corresponding author: N. R. St-Pierre; e-mail: st-pierre.8@osu.edu.
[1]Salaries and research support were provided by state and federal funds appropriated by The Ohio Agricultural Research and Development Center, The Ohio State University, Manuscript no. 5-01AS.

therefore linking MUN with the partitioning of N intake (**NI**). If the assumption is that the kidney is a constant urea filter (i.e., that the fractional rate of urea clearance from the blood by the kidney is constant) and that blood urea diffuses rapidly into milk, then the relationship should be linear. However, we hypothesized that these assumptions would not hold over a wide range of BUN. Not all urea filtered by the kidney is excreted into urine and a varying proportion dependent on urine concentration is reabsorbed as the tubular fluid passes through the proximal convoluted tubules (Schmidt-Nielsen and Osaki, 1958). Additionally, we hypothesized that breed would affect the UN-MUN relationship; however, breeds of cattle could differ in this relationship because of a scale factor (body size). Therefore, the objectives of this study were to determine: 1) whether the empirical UN-MUN relationship is linear over a wide range of MUN, 2) whether the type of carbohydrate fed interacts with dietary CP levels to affect MUN and its relationship with UN, and 3) whether the breed of cattle has an effect on the UN-MUN relationship, and if present, whether it is a consequence of body size differences.

MATERIALS AND METHODS

Experimental Design and Treatments

Four Holstein and four Jersey cows were used. Animals were selected to ensure that the assumption of zero N balance (no net accumulation or net loss of N) was reasonably met. For this reason, experimental cows were all multiparous and past peak lactation, averaging 156 DIM (range = 96 to 209). The experimental design was a split-plot Latin square with breed forming the main plots (squares) and dietary treatments forming the subplots. Diets were arranged in a 2 × 2 factorial with CP levels (13 and 17%) and NDF (30 and 40%) as the main factors. The experimental periods were 3 wk in length, with d 1 to 14 used for adjustment and d 15 to 19 used for a 5-d total collection of urine and feces. Day 20 was used for blood collection, and no data collection took place on d 21. Hereafter, d 1 to 14 will be referred to as the adaptation period, and d 15 to 20, the collection period.

Diets and Cow Management

Diets were fed in TMR with a forage-to-concentrate ratio maintained at 50:50 across all diets (Table 1). Crude protein and NDF levels in the diets were adjusted by changing the relative proportions of corn, soybean meal, and soybean hulls. Diets were fed twice per day ad libitum (5 to 10% refusal). Diets were adjusted weekly based on DM analysis from the prior week. For the collection period, cows were tied in metabolism stalls inlaid with rubber mats, and no additional bedding was used in these stalls.

During the adaptation periods, the cows were milked in the parlor with the rest of the research herd at approximately 0600 and 1600 h. During the collection periods, the cows were milked in the metabolic tie stalls with portable inline milking units, equipped with DHIA sampling devices to measure milk volume and collect samples. Cows were milked at approximately 0630 and 1700 h during the collection periods.

Sample Collection

Feed intake and orts were measured and sampled daily for each cow during the collection period. These samples were kept refrigerated until the end of the collection period, at which time they were composited by cow and the resulting samples were frozen and stored at –20°C for later analysis.

Milk weights were recorded daily, and three samples were taken for each cow at each milking during the collection period. One samples was preserved with 2-bromo-2-nitropropane-1,3-diol, and the other two were kept without preservative. The unpreserved samples were frozen immediately and stored at –20°C for later analysis. The preserved samples were kept refrigerated until delivered to DHI Cooperative, Inc. (Powell, OH) for analyses.

Fecal weights were recorded and sampled daily. Daily samples were kept refrigerated for the duration of the collection period. At the end of each collection period, the samples were composited by cow and kept frozen at –20°C until analyzed.

Urine was collected with an external harness. Two rubber mesh patches were glued lateral to the tailhead on the cow's back, and a third patch was glued distal to the vulva opening to anchor the urine collection tube. A rubber tube, 7 cm in diameter, was tied to the patches to cover the vulva opening. The other end of the tube was attached to a 20-L carboy for total urine collection. Sufficient amounts of HCl (9 *N*) were used in each carboy to keep the pH ≤ 5. Urine weight was recorded daily, and a 500-ml sample was taken and kept refrigerated. Aliquots were composited by volume, frozen, and stored at –20°C until analyzed.

Blood samples were collected on d 20 of each period, following the total fecal and urine collection. Blood samples were collected from each cow at 0, 2, 4, and 8 h postprandial. Samples were taken from the tail vein during the first period and from the jugular vein for the remaining three periods. The blood was collected in sodium heparin collection tubes and kept on ice until

Table 1. Ingredients and nutrient composition of experimental diets.

	Treatments			
	13% CP		17% CP	
Item	30% NDF	40% NDF	30% NDF	40% NDF
	(% of DM)			
Dietary components				
Alfalfa hay, chopped	17	17	17	17
Corn silage	33	33	33	33
Ground shelled corn	35	19.3	25.9	10.0
Soybean hulls	4.9	21.5	4.9	21.8
Soybean meal (48% CP)	2.1	1.2	11.2	10.3
Distillers dried grains	3.8	3.8	3.8	3.8
Urea, prilled	0.28	0.28	0.28	0.28
Pellet binder	1.0	1.0	1.0	1.0
Molasses, dried	1.0	1.0	1.0	1.0
Limestone	0.58	0.58	0.58	0.58
Dicalcium phosphate	0.58	0.58	0.58	0.58
Salt, trace mineralized[1]	0.42	0.42	0.42	0.42
Potassium-magnesium sulfate	0.11	0.11	0.11	0.11
Magnesium oxide	0.08	0.08	0.08	0.08
Mineral and vitamin mix[2]	0.15	0.15	0.15	0.15
Nutrient composition				
CP	13.2	13.9	16.9	16.5
RDP[3]	8.5	9.2	11.1	11.1
RUP[3]	4.7	4.7	5.8	5.4
NDF	33.6	40.9	32.4	41.6
ADF	20.8	27.2	20.4	28.4
NFC[4]	46.4	38.8	43.7	35.2
NDICP[5]	2.62	3.24	2.71	3.27
ADICP[6]	1.19	1.58	1.21	1.56
Lignin	2.93	3.40	2.93	3.64
Ether extracts	3.41	3.23	3.26	3.09
Ash	6.01	6.46	6.40	7.09
NE_L (Mcal/kg)[3]	1.66	1.63	1.66	1.63

[1]Contained 0.01% Co, 0.025% Cu, 0.14% I, 0.1% Fe, 0.2% Mn, and 0.26% Zn.

[2]Contained 0.7% Cu, 0.017% Se, 1.4% Zn, 3430 IU of vitamin A/g, 860 IU of vitamin D/g, and 14 IU of vitamin E/g.

[3]Calculated according to NRC (1989) for diet components.

[4]Nonfiber carbohydrates = 100 − (CP + ash + ether extract + NDF − NDICP).

[5]NDICP = Neutral detergent insoluble CP.

[6]ADICP = Acid detergent insoluble CP.

centrifuged. The plasma was removed and frozen at –20°C until analyzed.

Sample Analysis

The composite feed and orts samples were dried at 55°C, and ground through a Wiley mill (2-mm screen; Arthur H. Thomas, Philadelphia, PA). The ground samples were then analyzed for N by the Kjeldahl method (AOAC, 1990). Fiber analyses included NDF, ADF, lignin, neutral detergent insoluble CP (**NDICP**), and acid detergent insoluble CP (**ADICP**; Robertson and Van Soest, 1981; Van Soest et al., 1991). The preserved milk samples were analyzed for CP, fat, SCC, and MUN. The MUN was determined by a diacetyl monoxime assay on a Skalar SAN Plus segmented flow analyzer (Skalar, Inc., Norcross, GA). The accuracy of the method has been investigated previously (De Jong et al., 1992). In a preliminary trial, we verified its accuracy using 53 milk samples with a range of 4 to 35 mg/dl of MUN compared with a standard diacetyl monoxime colorimetric assay (Sigma Kit #535; Sigma Diagnostics, 1990). Results showed that the SKALAR results were repeatable (r = 0.99) and accurate (σ_e^2 = 0.5) for MUN of less than 25 mg/dl.

Plasma samples were assayed in duplicate for plasma urea N (***PUN***) using Sigma Kit #535 (Sigma Diagnostics, St. Louis, MO).

Both fecal and urine samples were analyzed for N using the Kjeldahl method (AOAC, 1990). Fecal samples were analyzed for N on a wet basis to avoid possible losses of NH_3. A sample was also dried at 55°C and analyzed for NDF (Van Soest et al., 1991).

Statistical Analysis

Production and total collection data were analyzed using Proc Mixed of SAS (1999) according to the following model:

$$Y_{ijklm} = \mu + B_i + c_{j:i} + P_k + N_l + F_m + NF_{lm} + BN_{il} + BF_{im} + BNF_{ilm} + E_{ijklm}, \quad [2]$$

where,

μ = overall mean;
B_i = fixed effect of the i^{th} breed, i = 1, 2;
$c_{j:i}$ = random effect of the j^{th} cow within the i^{th} breed, j = 1, 2, 3, 4, ~ N (0, σ_c^2);
P_k = fixed effect of the k^{th} period, k = 1, 2, 3, 4;
N_l = fixed effect of the l^{th} nitrogen level (CP), l = 1, 2;
F_m = fixed effect of the m^{th} NDF level, m = 1, 2;
NF_{lm} = interaction term of the l^{th} nitrogen level with the m^{th} NDF level;
BN_{il} = interaction term of the i^{th} breed with the l^{th} nitrogen level;
BF_{im} = interaction term of the i^{th} breed with the m^{th} NDF level;
BNF_{ilm} = interaction term of the i^{th} breed, l^{th} nitrogen level, and m^{th} NDF level; and
E_{ijklm} = error term ~ N(0, σ_e^2).

Blood urea N measurements were analyzed using Proc Mixed of SAS (1999) according to the following model:

$$Y_{ijklmn} = \mu + B_i + c_{j:i} + P_k + N_l + F_m + NF_{lm} + BN_{il} + BF_{im} + BNF_{ilm} + T_n + Bt_{in} + NT_{lm} + FT_{mn} + BNFT_{ilmn} + E_{ijklmn}, \quad [3]$$

where symbols are defined in [2] with the following additions:

T_n = fixed effect of the n^{th} time of sampling, n = 1, 2, 3, 4;
BT_{in} = interaction term of the i^{th} breed with the n^{th} time of sampling;
NT_{ln} = interaction term of the l^{th} nitrogen level with the n^{th} time of sampling;
FT_{mn} = interaction term of the m^{th} NDF level with the n^{th} time of sampling; and
$BNFT_{ilmn}$ = interaction term of the i^{th} breed, l^{th} nitrogen level, m^{th} NDF level, and n^{th} time of sampling.

Errors within cows and period (repeated measures due to the four times of sampling) were modeled using a first-order autoregressive covariance structure.

A series of sequential mixed models were fitted with Proc Mixed of SAS (1999) to determine the effect of breed on the relationship between UN excretion and MUN. The first model was:

$$UN_{ijkl} = B_i + c_{j:i} + P_k + K_i M_{ijk} + E_{ijkl}, \quad [4]$$

where symbols are as defined in [2] with the following additions:

UN_{ijkl} = urinary N excretion (g/d);
K_i = regression coefficient of milk urea N for breed i;
M_{ijk} = milk urea N for the j^{th} cow, within the i^{th} breed, during the k^{th} period; and
E_{ijkl} = error term ~ N(0, σ_e^2).

The absence of a μ term in the model indicates that individual intercepts were fitted for each cow within breed and period. This model focuses on the effect of breed on the slope of the regression and the type III F-statistic corresponding to K_i tests the hypothesis that all slopes are equal to zero (Littell, et al., 1996). A significant effect of K_i indicated that the slope of the linear regression of UN on MUN was different from zero for at least one breed. Therefore, a second model was fitted as follows:

$$UN_{ijkl} = \mu + B_i + c_{j:i} + P_k + \beta_0 M_{ijk} + \beta_i M_{ijk} + E_{ijkl}, \quad [5]$$

where symbols are as previously defined with the following changes:

β_0 = overall regression coefficient of UN on MUN and
β_i = deviation of regression coefficient of UN on MUN of $breed_i$ from the overall regression coefficient.

A significant β_i effect indicated that the regression coefficient (slope) of UN on MUN was different for the two breeds. The following model was fitted to determine whether the two breeds shared a common intercept:

$$UN_{ijkl} = \mu + B_i + c_{j:i} + P_k + \beta_i M_{ijk} + E_{ijkl}. \quad [6]$$

The effect of B_i was nonsignificant ($P = 0.59$), indicating that the linear regressions of UN on MUN have a common intercept for both breeds. An F-test on μ indicated that the common intercept was not different from zero

($P = 0.72$). Therefore, a fourth model was fitted to obtain separate estimates of slopes for each breed:

$$UN_{ijkl} = c_{j:i} + P_k + \beta_i M_{ijk} + E_{ijkl}. \quad [7]$$

To test whether the effect of breed on the regression coefficient of UN on MUN was due to BW difference between breeds, we fitted the following model:

$$UN_{ijkl} = c_{j:i} + P_k + \beta_i M_{ijk} + \beta_0 W_{ijk} M_{ijk} + E_{ijkl}, \quad [8]$$

where,

W_{ijk} = BW of the j^{th} cow within the i^{th} breed, during the k^{th} period.

The β_i effect was not significant ($P = 0.65$), indicating that the effect of the two breeds on the UN-MUN relationship appeared to be solely due to their BW difference. Therefore, the following reduced model was fitted:

$$UN_{ijkl} = c_{j:i} + P_k + \beta_0 W_{ijk} M_{ijk} + E_{ijkl}. \quad [9]$$

To test whether the BW effect in the above model would be better expressed as an allometric expression of BW, we fitted a series of nonlinear mixed models of the following form:

$$UN_{ijkl} = c_{j:i} + P_k + \beta_0 (W_{ijk})^b M_{ijk} + E_{ijkl}. \quad [10]$$

Using the residual log likelihood as the objective function, a line search was done on the parameter b within the bracket $0.5 < b < 1.2$.

RESULTS AND DISCUSSION

Plasma and MUN and Milk Production

The results for milk production measurements, MUN, and PUN are reported in Table 2. None of the interactions between main effects was significant; hence, only the tests on the main effects are reported. The NDF concentration of the diets had no effect on milk composition. This indicates that the amount of effective NDF and physically effective NDF (Mertens, 1997) must have been sufficient to maintain a proper rumen environment, even when cows were fed the 30% NDF diets. No difference was found in MUN concentrations between Holstein and Jersey cows. Breed had a significant effect on all milk components with the exception of MUN and PUN. Rodriguez et al. (1997) reported a significant effect of breed on MUN, with milk from Holstein cows 40% higher in MUN than milk from Jersey cows. However, MUN was expressed as grams

Table 2. Least squares means for milk production and composition by Holstein and Jersey cows fed diets differing in CP and NDF.

	Treatments					Breed			P value[1]		
	13% CP		17% CP								
	30% NDF	40% NDF	30% NDF	40% NDF	SEM[3]	Holstein	Jersey	SEM[4]	CP	NDF	Breed
Milk, kg/d	27.8	26.5	28.6	28.6	1.70	35.5	20.2	2.20	0.036	NS	0.002
Milk protein, %	3.55	3.57	3.65	3.57	0.13	3.26	3.91	0.16	NS	NS	0.033
Milk fat, %	4.16	4.13	4.23	4.20	0.20	3.58	4.77	0.28	NS	NS	0.017
Milk protein, kg/d	0.96	0.91	1.02	0.99	0.05	1.14	0.80	0.06	0.033	NS	0.006
Milk fat, kg/d	1.11	1.03	1.14	1.16	0.07	1.25	0.96	0.08	0.017	NS	0.049
MUN, mg/dl	6.09	6.83	12.55	12.36	0.33	9.44	9.47	0.28	<0.001	NS	NS
PUN, mg/dl	9.55	10.04	15.89	15.56	0.48	12.8	12.7	0.48	<0.001	NS	NS

[1]NS = Nonsignificant ($P > 0.10$).
[2]The interaction effect of CP × sampling time ($P = 0.01$) was significant. No other interactions were significant.
[3]The standard errors for the 17% CP and 30% NDF diet were slightly higher because one cow on this diet was removed from the experiment in period 4.
[4]The reported SEM is for Holstein cows. The SEM for Jersey cows was slightly higher because one cow was removed from the experiment in period 4.
[5]MUN = Milk urea N.
[6]PUN = Plasma urea N.

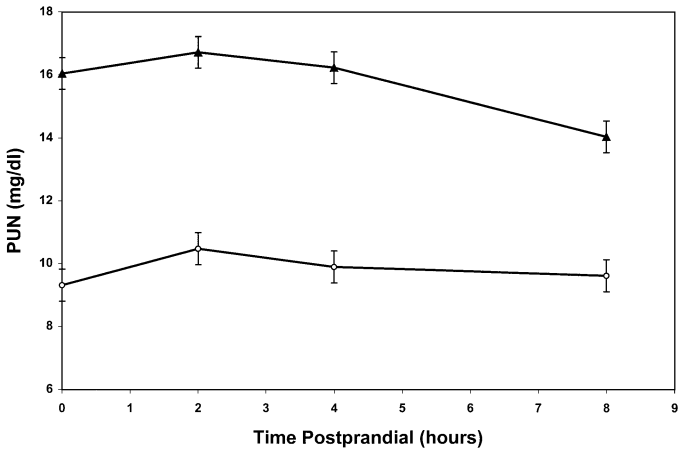

Figure 1. Plasma urea N (PUN) versus time postprandial at two levels of dietary CP (○ = 13%, ▲ = 17%). The interaction of CP × time was significant ($P = 0.01$).

per 100 g of total milk N. Milk from Jersey cows has a much higher N concentration than milk from Holstein cows. If MUN is the result of a simple diffusion of urea from blood to milk, then the difference observed by Rodriguez et al. (1997) for the two breeds may be entirely due to the basis of expression as opposed to true breed differences. In the experiment of Rodriguez et al. (1997), MUN for Holstein cows was only 10% higher than MUN for Jersey cows when we recalculated MUN as milligrams per deciliter. It is unlikely that this difference was significant, but this cannot be established from the data reported in the original paper.

Concentrations of MUN and PUN were affected by dietary CP concentration in the present study, in agreement with results from other studies (Erbersdobler et al., 1980; Baker et al., 1995; Broderick and Clayton, 1997). The RDP concentration increased from an average of 8.8% of DM in the low CP diets to an average of 11.1% of CP in the high CP diets. The increased RDP would result in a higher production of ruminal NH_3 without necessarily a corresponding increase in ruminal NH_3 utilization.

The pattern of response of PUN over time is shown in Figure 1. The cows fed the 17% CP diets had higher PUN concentrations throughout the sampling period, even before the morning feeding. The time of sampling had no effect on PUN for the 13% CP diets. This is in contrast to the 17% CP diets in which a significant downward trend of PUN as observed. Thus, the PUN difference between the 13 and 17% CP diets decreased linearly ($P = 0.006$) throughout the sampling period.

The response in milk production for cows on the 17% CP diets may be explained as either a response to increased CP concentration above requirements or as a consequence of feeding an insufficient amount of CP on the 13% CP diets. The 13% CP-30% NDF diet and the 13% CP-40% NDF diets provided 89 and 99% of NRC (1989) requirements, respectively. The milk production response seen when CP was increased with 40% NDF diets supports the view that cows respond in milk production to the supply of dietary CP provided above requirements (St-Pierre and Thraen, 1999; Vérité and Peyraud, 1988), whereas the response to increased CP concentration with 30% NDF diets could be explained in the traditional nutrient requirement framework (NRC, 1989).

The concentration of MUN was closely associated with the concentration of PUN (Figure 2). This is in agreement with other reports (Baker et al., 1995; Broderick and Clayton, 1997; Roseler et al., 1993). The regression equation in Figure 2 is in agreement with that reported by Roseler et al. (1993; MUN = 0.88 (± 0.05) PUN – 1.32 (± 0.90)) but is in disagreement with that of Broderick and Clayton (1997; MUN = 0.62 BUN + 4.75, SE not given). The sampling of blood on a separate day from milk may have caused the relatively large positive intercept for the regression of Broderick and Clayton (1997). Other studies have also reported a positive intercept (Oltner et al., 1985; Refsdal, 1983), whereas a negative intercept was found in others (Oltner and Wiktorsson, 1983; Roseler et al., 1993), including the current study. The differences in the reported intercepts may be the result of time of sampling. The concentration of BUN would be higher than MUN during periods when BUN is increasing and lower than MUN during periods when BUN is decreasing (Gustafsson and Palmquist, 1993). The overall difference between MUN and PUN concentrations is difficult to in-

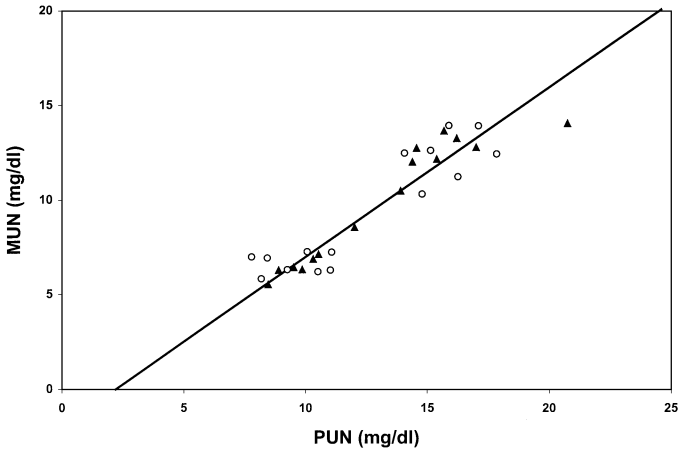

Figure 2. Milk urea N (MUN) versus plasma urea N (PUN) for Holstein (▲) and Jersey cows (○). The solid line represents the regression equation (MUN = 0.89 (± 0.07) × PUN – 1.96 (± 0.96); $r^2 = 0.84$).

terpret. Urea diffuses passively from blood to milk (Gustafsson and Palmquist, 1993). On the 13% CP diets, cows showed little variation in PUN across time (9.79 ± 0.34 mg/dl). The PUN exceeded the mean MUN (6.46 ± 0.23 mg/dl) at all sampling time points. Under a diffusion process, this could only be achieved if the rate of diffusion of urea from blood to the mammary alveoli was considerably slower than the rate of water secretion into the alveoli by mammary epithelial cells. Urea is a larger molecule and diffuses about 20% more slowly than water (Guyton, 1982).

Milk urea N concentrations (Table 2) were considerably lower and in a more narrow range than anticipated before the experiment. We propose two reasons to why this occurred. First, the expected MUN values were based on the model set forth by Jonker et al. (1998). Under this model, a cow excretes 12.54 g of UN per unit of MUN. In the current study, considerably more UN were excreted per unit of MUN. Thus the MUN concentrations were lower. Second, it was hypothesized that dietary NDF concentration would affect MUN. This is because of reduced ruminal carbohydrate degradability (Herrera-Saldana et al., 1990). In the current trial, the carbohydrates in soyhulls were apparently sufficiently digested in the rumen to provide an adequate level of energy for microbial protein synthesis.

Intake and Digestibility

The intake, excretion, and apparent digestion of DM were not affected by either CP or NDF concentrations in the diet (Table 3). However, as expected, the Holstein cows ate, excreted, and digested more DM than the Jersey cows. The level of NDF in the diet did not affect DMI in the present study. Apparently the additional NDF from soyhulls did not cause the diets to exceed the physical fill capacity of the digestive tract. The DM apparently digested tended to be higher ($P = 0.08$) with the 17% CP diets, indicating that the extra CP supplied was highly digestible and/or that the additional CP intake improved the digestibility of other dietary components. Dry matter and OM digestibilities have been reported to increase as dietary CP concentration increases (Van Soest, 1994). The apparent DM digestibility was the same for both breeds, which was expected (Ferrell, 1993). Breed significantly affected the intake and excretion of NDF. This would be expected because of the difference in DMI between the breeds. However, NDF digestibility was the same for both breeds. The apparent NDF digestibility was affected by the NDF concentration in the diet; cows fed the higher NDF diets had a digestibility that was 10.8 percentage units higher. The NDF levels of the high NDF diets were raised with soyhulls, which contain an average of 67%

Table 3. Least squares means for intake, fecal, and digestibility of DM and NDF by Holstein and Jersey cows fed diets differing in CP and NDF.

	Treatment					Breed			P value[1]		
	13% CP		17% CP								
	30% NDF	40% NDF	30% NDF	40% NDF	SEM[2]	Holstein	Jersey	SEM[3]	CP	NDF	Breed
DMI, kg/d	20.1	20.5	20.8	21.2	1.0	24.4	16.9	1.2	NS	NS	0.005
Fecal DM, kg/d	7.28	7.38	7.16	7.41	0.44	8.55	6.07	0.52	NS	NS	0.015
DM Apparently digested, kg/d	12.8	13.1	13.7	13.8	0.7	15.8	10.9	0.7	0.085	NS	0.003
Apparent DM digestibility, %	63.9	63.7	65.8	65.1	1.1	65.0	64.0	0.8	NS	NS	NS
NDF Intake, kg/d	6.61	8.38	6.75	8.76	0.38	8.99	6.27	0.43	NS	<0.001	0.004
Fecal NDF, kg/d	4.24	4.35	4.11	4.50	0.29	5.01	3.59	0.30	NS	NS	0.017
NDF Apparently digested, kg/d	2.38	4.03	2.63	4.27	0.21	3.95	2.69	0.19	NS	<0.001[4]	0.003[4]
Apparent NDF digestibility, %	36.0	48.1	39.0	48.7	2.2	44.3	42.9	1.8	NS	0.001	NS

[1]NS = Nonsignificant ($P > 0.10$).
[2]The standard errors for the 17% CP and 30% CP diet were slightly higher because one cow on this diet was removed from the experiment in period 4.
[3]The reported SEM is for Holstein cows. The SEM for Jersey cows was slightly higher because one Jersey was removed from the experiment in period 4.
[4]Breed × NDF interaction significant ($P = 0.007$). No other interactions were significant.

NDF (NRC, 1989). The extent of ruminal digestion of NDF in soyhulls is still debated. Van Soest and Fox (1992) argued that because of a high rate of passage at high intakes, there should be a severe depression in digestibility. At a feeding level of three times maintenance, the total tract digestibility of soyhulls would be only 56% (Van Soest and Fox, 1992). However, total tract NDF digestibility was found to increase from 54.5 (SE = 3.4) to 63.0% (SE = 3.4) when soyhulls increased from 0 to 50% of the concentrate (Nakamura and Owen, 1989). This response is similar to the increase in total tract NDF digestibility (37.5% at 30% NDF; 48.4% at 40% NDF) seen in the current trial when soyhulls were increased from 9.8 to 43.6% of the concentrate (4.9 to 21.8% of dietary DM). These NDF digestibilities are also in agreement with the results obtained by Cunningham et al. (1993).

Nitrogen Balance

Results of the N balance data are reported in Table 4. Breed had a significant effect on N intake because the Holstein cows had a higher DMI than did the Jersey cows. The amount of N apparently absorbed and apparent N digestibility were significantly higher for the 17% CP diets. The increase in apparent N digestibility can be the result of two things: 1) supplemental N with a higher true digestibility than true digestibility of N in the 13% CP diets, or 2) a dilution of metabolic fecal N (**MFN**). In Figure 3, the linear relationships between N absorbed and NI (also known as a Lucas test; Van Soest, 1994) are given for each breed. The intercepts for the two breeds represent an estimate of MFN losses and were –117 (± 25) g/d for the Holstein cows and –93 (± 19) g/d for the Jersey cows, or 4.8 to 5.5 g/kg of DMI. The two intercepts are not statistically different (P = 0.15) and were similar to the intercept (–97.0) reported by Jonker et al. (1998) for Holstein cows. The slopes were the same for the two breeds (0.83 ± 0.037). They represent an estimate of the true digestibility of the average protein in the diet. This slope is in agreement with the slope (0.83 ± 0.02) reported by Jonker et al. (1998). Therefore, the increased apparent digestibility of the 17% CP diets appears to be a result of a dilution of MFN.

As the CP level in the diet increased, the efficiency of converting absorbed N to milk N decreased substantially (Table 4), dropping from 60.3 and 52.3% in the 13% CP diets to 42.5 and 42.7% in the 17% CP diets. Consequently, the amount of urine N increased because most of the additional absorbed N was not used for additional milk N production.

Across NDF levels, the N intake on the 17% CP diets was 122 g/d higher than on the 13% CP diets (Table 4). Likewise, the absorbed N on the 17% CP diets was 106 g/d higher than on the 13% CP diets. Therefore, the additional protein had a calculated digestibility of 86.1% (105/122) across NDF concentration and breed. Furthermore, of this additional 105 g/d of absorbed protein, only 10 g/d were partitioned into additional milk N (milk protein ÷ 6.38, Table 4). From this, the marginal efficiency with which the absorbed N was incorporated into milk N was calculated as 9.5% (10/105).

Relationship Between MUN and UN Excretion

The relationship between UN excretion and MUN is presented in Figure 4. The regression lines are those obtained form [7]. The difference in the slopes between the two breeds was significant ($P < 0.001$). The intercept was not different between the two breeds ($P = 0.59$) and also was not different from zero (P = 0.72), which is similar to the results of Jonker et al. (1998).

The regression equation for Holstein cows is:

$$\text{UN (g/d)} = 17.6\ (\pm\ 0.56)\ \text{MUN (mg/dl)} \qquad [11]$$

and the equation for Jersey cows is:

$$\text{UN (g/d)} = 11.8\ (\pm\ 0.62)\ \text{MUN (mg/dl)}. \qquad [12]$$

The combined model has an R^2 = 0.98 and a residual mean square (estimate of σ_e^2), = 81.5. The regression coefficient for the Holstein cows was 40% higher than in the equation proposed by Jonker et al. (1998; UN (g/d) = 12.54 MUN (mg/dl)). Two-thirds of the data used to generate the latter equation were from studies that used infrared spectroscopy to measure MUN (R.A. Kohn, personal communication), which has been shown to be biased upward (overpredict true MUN; Wilson et al., 1998).

Estimation of the parameters of model [8] showed that the breed effect on the UN-MUN relationship lost its significance ($P = 0.63$) when the model included BW as a continuous variable. Furthermore, the optimum exponent on the BW parameter [10] was found to be 0.96 and was not different from 1.0 (P = 0.80). These findings imply that the breed does not have to be considered in the UN-MUN relationship when a linear adjustment based on BW is done, eliminating the need for separate equations. The equation determined to predict UN excretion based on MUN and BW is the following:

$$\text{UN (g/d)} = 0.0259\ (\pm\ 0.0006)\ \text{BW (kg)} \times \text{MUN (mg/dl)}. \qquad [13]$$

(R^2 = 0.98; residual mean square = 74.1).

Without loss of generality, both sides of equation [13] can be divided by BW, thus canceling out the BW term

Table 4. Least squares means for N utilization by Holstein and Jersey cows fed diets differing in CP and NDF.

	Treatment					Breed			P value[1]		
	13% CP		17% CP								
	30% NDF	40% NDF	30% NDF	40% NDF	SEM[2]	Holstein	Jersey	SEM[3]	CP	NDF	Breed
N Intake, g/d	429	460	572	560	27	599	411	31	<0.001	NS	0.004
Fecal N, g/d	178	184	198	197	12	218	161	14	0.028	NS	0.028
N Apparently absorbed, g/d	251	276	374	363	20	382	251	20	<0.001	NS	0.003
Urine N, g/d	93	101	190	185	6	166	118	6	<0.001[4]	NS	0.002[4]
Milk N[5], g/d	151	143	159	155	8	179	125	9	0.033	NS	0.006
Apparent N Retention[6], g/d	7	32	25	23	16	36	7	12	NS	NS	0.072
N, % of intake											
Feces	41.5	40.3	34.6	35.4	1.4	36.4	39.2	1.1	<0.001	NS	0.056
Urine	22.3	22.4	33.7	32.8	1.3	27.1	28.5	1.3	<0.001	NS	NS
Absorbed (Digestibility)	58.6	59.6	64.6	64.6	1.4	63.6	60.1	1.1	<0.001	NS	0.056
Milk	35.2	30.9	28.2	27.7	1.3	30.1	30.9	0.9	<0.001	0.092	NS
Retained	1.0	6.4	3.5	4.1	3.0	6.4	1.4	2.2	NS	NS	0.082
Productive[7]	36.2	37.3	31.7	31.8	2.2	36.5	31.7	1.8	0.028	NS	0.092
N, % of absorbed											
Urine	37.1	36.6	50.8	51.0	2.6	43.6	47.2	2.2	<0.001	NS	NS
Milk	60.3	52.3	42.5	42.7	2.6	47.0	50.0	1.8	<0.001	0.10	NS
Retained	2.6	11.1	6.7	6.3	4.7	9.4	2.8	3.3	NS	NS	0.089
Productive[7]	62.9	63.4	49.2	49.0	2.7	56.4	52.8	2.2	<0.001	NS	NS

[1]NS = Nonsignificant ($P > 0.10$).

[2]The standard errors for the 17% CP and 30% NDF diet were slightly higher because one cow was removed from the experiment in period 4.

[3]The reported SEM is for Holstein cows. The SEM for Jersey cows was slightly higher because one cow was removed from the experiment in period 4.

[4]Breed × CP interaction was significant ($P < 0.001$).

[5]Calculated as CP/6.38.

[6]Calculated by difference (Retention = N intake − fecal N − urine N − milk N).

[7]Productive N = milk N + retained N.

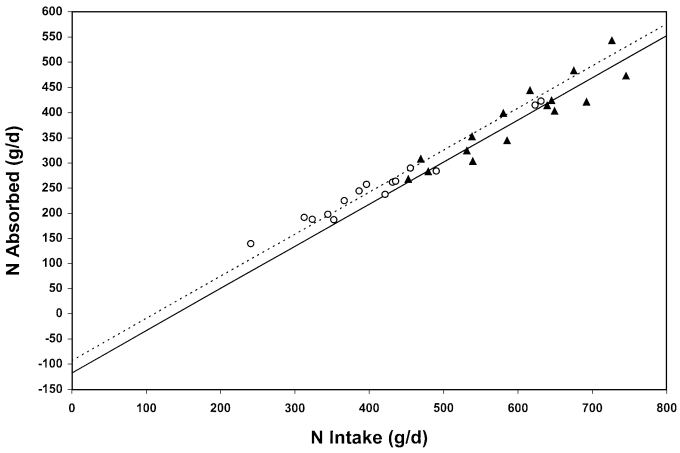

Figure 3. Nitrogen absorbed (NA) versus N intake (NI) for Holstein (▲) and Jersey (○) cows. The solid line represents the regression equation for Holstein cows (NA = 0.83 (± 0.04) NI – 117 (± 25)). The dashed line represents the regression equation for Jersey cows (NA = 0.83 (± 0.04) NI – 93 (± 19)).

on the right side of the equation. The left side of the equation becomes UN/BW and represents grams of UN excreted per kilogram of BW. This relationship is presented in Figure 5 and shows graphically the absence of a breed effect on the UN-MUN relationship when observations were corrected for BW.

Assuming that the kidney filters a constant fraction of MUN from blood per unit time, then

$$\text{UN (mg/d)} = \text{V (dl/d)} \times k_f\text{(MG/MG)} \times \text{BUN (mg/dl)}, \quad [14]$$

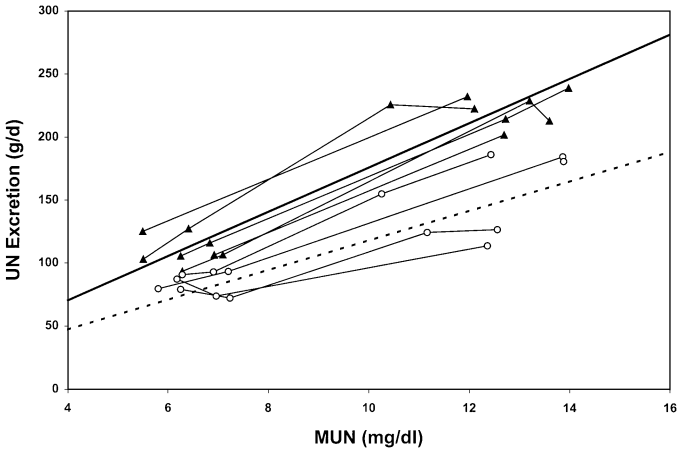

Figure 4. Relationship of urinary N excretion (UN) to milk urea N (MUN) concentration for Holstein (▲) and Jersey (○) cows. The solid line represents the regression equation for Holstein cows (UN = 17.6 (± 0.56) MUN); the dashed line represents the regression equation for Jersey cows (UN = 11.8 (± 0.62) MUN)). Each solid, thin line connects observations common to a cow.

where V is the volume of blood flow through the kidney and k_f is the MUN fraction cleared. Under the additional assumption that urea diffuses rapidly from blood to milk (i.e., MUN is equal to BUN), [14] becomes:

$$\text{UN (mg/d)} = \text{V (dl/d)} \times k_f\text{(mg/mg)} \times \text{MUN (mg/dl)}. \quad [15]$$

Equation [15] has the same form as [11] and [12] and provides a physiological interpretation to the parameter estimates in [11], [12], and [13]. Thus, [13] implies an estimated renal clearance rate of 2.59 (± 0.06) L/kg of BW per day. Renal clearance rate is estimated at 1756 L/d and 1217 L/d for a 678- and 470-kg animal, respectively (the mean liveweight for Holstein and Jersey cows in this experiment). Jonker et al. (1998) estimated a renal clearance rate of 1254 L/d for cows averaging 598 kg. This equates to a renal clearance rate of 2.10 L/kg of BW per day, a value which is lower ($P <$ 0.0001, calculated from a simple t test) than the estimate in the present study. A renal clearance study to estimate the glomerular filtration rate of urea over a wide range of BUN would resolve the discrepancies between these two estimates.

CONCLUSIONS

Milk urea N was related linearly to UN excretion over the range of MUN concentrations of 5 to 14 mg/dl, but this range of MUN was insufficient to adequately challenge the assumption of a linear relationship between UN and MUN.

The NDF concentration of the diet had no significant effect on any of the production variables measured and

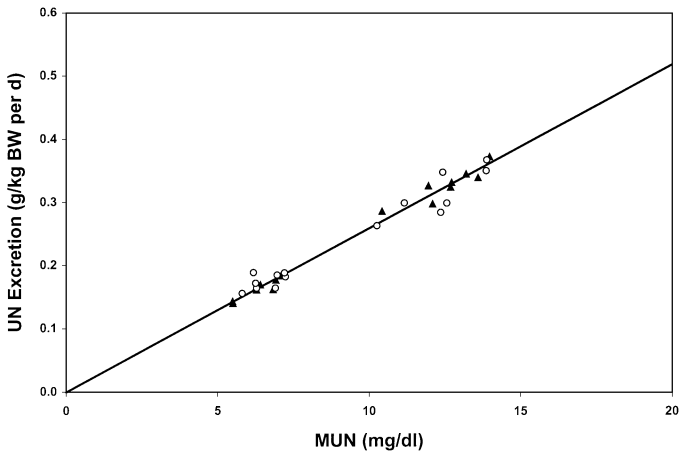

Figure 5. Urinary N (UN) excretion per unit of BW as a function of milk urea N (MUN) for Holstein (▲) and Jersey (○) cows. The solid line represents the regression equation UN/BW = 0.0259 (± 0.0006) MUN.

did not affect the relationship between UN and MUN. Thus, the traditional thinking that NFC concentration has a direct effect on MUN must be revised. Our results are in line with one of the implications of the Jonker et al. (1998) model that ruminal carbohydrate digestibility and efficiency of ruminal N fermentation can only affect MUN indirectly through either an increase in milk N excretion, a decrease in N intake, or an increase in fecal N with a net result of reducing urinary N excretion.

The effect of breed on the UN-MUN relationship was considerable but fully explained by BW differences. Allometric regressions showed that UN is directly proportional to BW at a constant MUN. Thus, BW and not other allometric transforms (e.g., metabolic weight) should be used to predict UN excretion from MUN in mature dairy cattle.

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