Sources of Variation in Milk Urea Nitrogen in Ohio Dairy Herds

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ABSTRACT
The purpose of this study was to estimate the amount of variation in milk urea nitrogen (MUN) concentrations attributable to test-day, individual cow, and herd effects and to describe factors associated with MUN measurements in Ohio dairy herds. The data came from 24 Holstein herds, half of which were classified as low producing (LP) [rolling herd average (RHA) milk production < 7,258 kg] and half as high producing (HP) herds (RHA production > 10,433 kg). MUN concentration was measured from cow’s monthly test-day milk samples. The data were analyzed using multilevel modeling technique in MLwiN, separately for LP and HP herds.

The unadjusted mean MUN was 13.9 mg/dl for the HP herds and 11.3 mg/dl for the LP herds. The variance structure was different between the two groups. Most of the variability was found at test-day level in the LP herds, but at herd level in HP herds. MUN was lowest during the first month of lactation, and also season was associated with MUN in both groups. Test-day milk yield, milk fat percentage, and SCC were associated with MUN in the HP herds. With significant explanatory variables in the model, proportionally more of the variation was explained at herd level and less at test day level in both groups.

Lower variability in MUN between test days in the HP herds may indicate more consistent day-to-day feeding and management within a herd. The great variability between test days should be considered when interpreting MUN and samples should be collected at the same time of the day to minimize day-to-day variability. (Key words: milk urea nitrogen, variation, multilevel modeling)

Abbreviation key: MUN = milk urea nitrogen, RHA = rolling herd average.

INTRODUCTION
Several studies have shown that milk urea nitrogen (MUN) concentration is related to dietary CP intake, the percentage of rumen degradable and undegradable protein as well as protein-energy ratio in the diet (Oltnner and Wiktorsson, 1983; Roseler et al., 1993; Baker et al., 1995). The normal/target values for MUN are considered to be within the range from 10 to 15 mg/dl (Carlsson and Pehrson, 1994; Moore and Varga, 1996). Thus, MUN concentrations can be used as a practical tool to monitor dietary CP and energy intake relative to requirements. This type of monitoring can play an important role in dairy herd management, because 1) excess protein (N) intake may impair reproductive performance; 2) consumption of excess CP increases energy requirements; 3) protein supplements are costly feed ingredients; and 4) excess N excretion has a negative environmental impact (Broderick and Clayton, 1997).

Based on experimental studies, a number of other factors in addition to feed intake and dietary composition, are also known to be related to MUN concentrations. Such factors are sampling time, method of analysis, DIM, BW, parity, and milk yield of a cow. Only a few studies have been published that have used field data from several herds and have evaluated non-nutritional factors affecting MUN (Eicher et al., 1999; Godden et al., 2001b). However, when interpreting MUN values in field conditions, these other factors affecting MUN concentrations should also be considered.

A common scenario encountered in epidemiologic studies of animal populations is the clustered structure of the data (McDermott and Schukken, 1994). Until recently, lack of affordable and user-friendly software as well as inadequate computing power have been obstacles to the use and application of multilevel models (also known as hierarchical models, mixed models, or random-effects models) (Greenland, 2000). In epidemiologic studies, the use of this methodology has been motivated by the purpose of accounting for the clustered data structure (Chriel et al., 1999; Gröhn et al., 1999; Rajala-Schultz et al., 1999) or by the goal of estimating...
the contribution of the various levels of organization to the total variance of the parameter of interest (Dohoo et al., 2001).

The main purpose of this research was to estimate the amount of variation in MUN concentrations attributable to test-day, individual cow, and herd effects and to describe nondietary sources of variation in and factors associated with MUN measurements.

MATERIALS AND METHODS

Data

The data for this study came from 24 Holstein herds that were enrolled in the Ohio Dairy Herd Improvement (DHI) Cooperative, Inc. (Columbus, OH). The herd selection process as well as the association between MUN concentrations and fertility of cows in these herds has been described earlier in detail (Rajala-Schultz et al., 2001). Briefly, the study population was selected to represent Ohio Holstein dairy herds with either high or low milk production and was randomly selected from the client herds of Ohio DHI, fulfilling the following criteria: high producing herds (n = 12) were defined as having a rolling herd average (RHA) milk production greater than 10,433 kg (23,000 lb) and low producing herds (n = 12) were defined as having a RHA milk production less than 7258 kg (16,000 lb). Initially, we had information from all lactations that started anytime during 1998 in these herds. The follow-up period lasted through May 1999. Approximately 2% of the cows in the study population calved twice during 1998, but information from the latter lactation was excluded. In the final dataset, 12,939 test-day observations from 1681 cows from 24 herds were available.

The milk samples for determination of urea nitrogen were collected by the DHI supervisors during their regular monthly farm visits. All samples in this study were taken during normal milking time, in some herds twice but in some herds only once a day. If tested twice during a day, the samples were mixed together. Urea measurements were performed spectrophotometrically at the Ohio DHI laboratory, which is accredited by National DHIA, using an automated procedure with a Skalar Segmented Flow Analyzer. Information on test-day dates, herd milk production level, cow’s test-day milk yield, milk fat, and protein percentage, SCC, DIM on each test day, calving date and parity of a cow was also available. Days in milk were categorized into 9 mo of lactation, in 30-d increments—DIM greater or equal to 240 d formed the last category and was used as the reference level in the modeling process. Parity had two levels: primiparous (lactation 1) and multiparous cows (lactation 2 or higher). Season of the test days was divided into four categories: winter (Dec–Feb), spring (March–May), summer (June–Aug), and autumn (Sept–Nov).

Statistical Analysis

The outcome of interest was the individual cow test-day milk urea nitrogen concentration. The data were analyzed using multilevel modeling technique with MLwiN (Rasbash et al., 2000). Different levels of organization were herd, cow, and test day. The potential explanatory variables that were considered were herd milk production, cow’s test-day milk production and milk fat and total protein percentage, parity, month of lactation, season of the test day, and SCC (expressed as linear score in the modeling process). Initially, all data were analyzed together, but because of problems with heteroskedasticity (inequality of variances based on visual examination of plots of residuals versus predicted values from the final model), data were divided into two datasets, separating the data from the high producing and the low producing herds.

Initial screening was performed using PROC MIXED in SAS (version 8.1) (Littell et al., 1996) to find explanatory variables that were unconditionally (no other variables in the model) associated with MUN concentrations. After that, a simple random intercept model containing only a constant was run using restricted iterative generalized least-squares algorithm in MLwiN, obtaining restricted maximum likelihood estimates of parameters (Dohoo et al., 2001). All independent variables which were associated with the outcome in the initial screening at P < 0.25 were included in the multivariate models. Variables treated as continuous (SCC and milk fat and protein percentage) were centered at the mean value of the variable in the respective datasets for a meaningful interpretation (Dohoo et al., 2001). Then, the least significant variable was dropped, one at a time, until all variables that remained in the model were significant (Wald test, P < 0.05). However, all levels of categorical variables were kept in the model even if only some of them were statistically significant. Then, all first-order interaction terms between the significant main effects were included and kept in the model, if significant at P < 0.05 level.

The adequacy of the final models and the assumptions of normality and homogeneity of variance were evaluated by examining normal probability plots and plots of residuals versus predicted values in all levels of the data structure. Other diagnostics (to find outlying or highly influential data points) were performed by plotting raw, standardized, and deletion residuals, and leverage and influence values at each organizational level (Rasbash et al., 2000). The total residual variance
and the proportion of variance on each level of data hierarchy were computed. The reduction in the variance estimates between the intercept only and the final models with significant fixed effects was evaluated.

RESULTS

Descriptive Statistics

The structure of the data is presented in Table 1. Data from 12 high producing and 12 low producing Holstein herds and 1681 cows in Ohio were available. Descriptive data from these herds are presented in Table 2. The average herd size among the low-producers was 50 cows, ranging from 26 to 97 cows, and among the high producers, it was 90 cows, ranging from 49 to 165 cows. The overall, unadjusted mean MUN concentration in high producing herds was 13.9 mg/dl (SD 3.7) and 11.3 mg/dl (SD 5.4) in the low producing herds. In general, the MUN values tended to be higher and their standard deviations smaller in the high producing herds than in the low producing herds. The herd average MUN concentrations and RHA milk production are presented in Figure 1. The lowest average MUN concentration in a high producing herd was 10.1 mg/dl (RHA milk production 11,226 kg) and the highest was 19.2 mg/dl (RHA milk production 10,908 kg). The range for MUN concentrations in low producing herds was from 5.0 to 15.1 mg/dl. Average daily milk yield was 35.4 kg in the high and 23.6 kg in the low producing herds. Milk fat and protein percentages were 3.6 and 3.2% in the high and 3.8 and 3.3% in the low producing herds, respectively.

Variance Structure of the Data

The total variance of MUN concentrations was greater in the low producing herds than in the high producing herds (31.6 vs. 16.6 in the random intercept only model and 30.3 vs. 16.7 in the final model with significant fixed effects, respectively; Tables 3 and 4). From the simple random intercept-only model without any fixed effects, for the low producing herds, the proportions of variance that were explained at herd, cow, and test-day levels were 32.2, 0.2, and 67.6%, respectively. The variance structure of the data from the 12 high producing herds differed from that in the low producing herds; the proportions of variance that were explained at herd, cow, and test-day levels in the high producing herds in the random intercept model were 49.0, 12.1, and 38.9%, respectively. Thus, in the low producing herds, the majority of the variance (67.6%, i.e., approximately two-thirds) was explained at test-day level, whereas in the high producing herds, the greatest proportion of variance (49.0%) was observed at herd level (between-herd variability). One-third of the variance in the low producing herds was at herd level, and 38.9% of variance in high producing herds originated from test day variability. While almost none of the variability (0.2%) in the low producing herds was explained at cow level, 12.1% of the variance in the high producing herds was.

These variance estimates on herd, cow, and test-day levels changed slightly when the significant fixed effects were added (the final model): in the low producing herds, 49.0, 1.4, and 38.9% and in the high producing herds 55.8, 10.6, and 33.6% of the variance was explained at herd level, cow and test-day levels, respectively. When all the significant fixed effects were in the model,
Table 2. Descriptive statistics (means with standard deviations in parenthesis) for the 24 study herds enrolled in Ohio DHI.

<table>
<thead>
<tr>
<th></th>
<th>Low producing herds</th>
<th>High producing herds</th>
</tr>
</thead>
<tbody>
<tr>
<td>RHA milk production, kg</td>
<td>6850 (204)</td>
<td>10,916 (409)</td>
</tr>
<tr>
<td>Milk protein %</td>
<td>3.3 (0.4)</td>
<td>3.2 (0.3)</td>
</tr>
<tr>
<td>Milk fat %</td>
<td>3.8 (0.9)</td>
<td>3.6 (0.8)</td>
</tr>
<tr>
<td>MUN, mg/dl</td>
<td>11.3 (5.4)</td>
<td>13.9 (3.7)</td>
</tr>
<tr>
<td>SCC, cells/ml</td>
<td>423,000 (1.1 × 10^6)</td>
<td>304,000 (899,000)</td>
</tr>
</tbody>
</table>

the proportion of variance explained at herd level increased, and the proportion explained at test day level decreased, for both groups. When the fit of the final models was checked, one herd among the high producing group appeared to be an outlier. Omitting that herd from the analysis did not change the coefficients of the parameters in the final model, but the proportion of variance on the different levels of data, as expected, changed. Without this herd, 42.4, 13.5, and 44.1% of the variation in the random intercept only model were at herd, cow, and test day levels, respectively. In the final model, the respective proportions were 46.4, 12.8, and 40.8%. Thus, without this herd, less of the variation remained on herd level and more was contributed to test-day level (and also slightly more to cow level). The change in the proportions of variance from the intercept only model to the final model was similar with and without this outlying herd: more of the variance was explained at herd level and less at test-day level, when the significant fixed effects were included than without them.

In the low producing herds, the diagnostics based on the final model indicated that there was a minor violation of the assumption of normality of error terms. Distribution of MUN concentrations was skewed to the right in these herds and a logarithmic transformation of the outcome variable was done and models run again. There was a slight improvement in the fit of the model based on visual inspection of the residual plots, but because of a more complicated interpretation of the results with the log-transformed outcome, only the results from the models using MUN on the original scale are presented.

Factors Associated with MUN

The results from the final models for the low and high producing herds are presented in Table 5. For the high producing herds, the results are from the model with all the 12 herds included in the analysis, but the parameter estimates for (i.e., the coefficients of) the significant fixed effects did not change regardless of whether all the herds were included or if the one outlying herd was excluded from the analysis. The intercept of 13.4 mg/dl in the low producing herds indicates the mean MUN concentration for a cow in summer in late lactation (month of lactation ≥ 9) and the value of 14.2 mg/dl in the high producing herds indicates the mean MUN level also for a cow in summer in late lactation, in the lowest milk production quartile (milk yield < 29.4 kg/d) with average milk fat percentage of 3.6% and linear score of 4.4.

Concentrations of MUN were significantly associated with month of lactation (Table 5). They were lowest during the first month of lactation in both production groups, 3.2 mg/dl lower in the high production group during the first month, and 2.6 mg/dl lower in the low production group than at the end of lactation (i.e., mo 9 and onward). MUN concentration increased for several months after parturition, then decreased and leveled off during mid-lactation. In the high producing herds, MUN concentrations were significantly lower until the seventh month of lactation than at the end of lactation. In the low producing herds, MUN concentrations were not significantly different after the fourth month of lactation when compared to the concentrations at the end of lactation. Also, season of the year when the milk

### Table 3. Proportion of variance in milk urea nitrogen (MUN) explained at each level of the data hierarchy in the intercept only model for 12 low and 12 high producing Holstein herds belonging to Ohio DHI.

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>Low production herds</th>
<th>High production herds</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>% of variance</td>
<td>Variance</td>
</tr>
<tr>
<td>Herd</td>
<td>32.2</td>
<td>10.17</td>
</tr>
<tr>
<td>Cow</td>
<td>0.2</td>
<td>0.06</td>
</tr>
<tr>
<td>Test day</td>
<td>67.6</td>
<td>21.32</td>
</tr>
<tr>
<td>Total variance</td>
<td>31.55</td>
<td>16.55</td>
</tr>
</tbody>
</table>

\(^1\)Standard error of the variance estimate of the parameter.
Table 4. Proportion of variance in MUN explained at each level of the data hierarchy in the final model with significant fixed effects for 12 low and 12 high producing Holstein herds belonging to Ohio DHI.

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>% of variance</th>
<th>Variance</th>
<th>SE</th>
<th>% of variance</th>
<th>Variance</th>
<th>SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Herd</td>
<td>34.0</td>
<td>10.27</td>
<td>4.22</td>
<td>55.8</td>
<td>9.32</td>
<td>3.82</td>
</tr>
<tr>
<td>Cow</td>
<td>1.4</td>
<td>0.43</td>
<td>0.17</td>
<td>10.6</td>
<td>1.77</td>
<td>0.11</td>
</tr>
<tr>
<td>Test-day</td>
<td>64.6</td>
<td>19.56</td>
<td>0.43</td>
<td>33.6</td>
<td>5.61</td>
<td>0.09</td>
</tr>
<tr>
<td>Total variance</td>
<td></td>
<td>30.26</td>
<td></td>
<td></td>
<td>16.70</td>
<td></td>
</tr>
</tbody>
</table>

1Standard error of the variance estimate of the parameter.

e samples were collected was significantly associated with MUN. In the low producing herds, MUN concentrations were highest during summer and significantly lower in winter, spring and fall (2.5, 1.8, and 2.8 mg/dl lower than in summer, respectively). In the high producing herds, the differences among seasons were much smaller, but significant for winter and spring and in fact, MUN concentrations were lowest during summer. Parity of the cows was not significantly associated with MUN (P > 0.05); however, in both groups, first-lactation cows tended to have lower MUN than older cows (approximately 0.2 to 0.4 mg/dl lower for lactation 1 cows).

Test-day milk yield, milk fat, and protein percentages and SCC were not significantly associated with MUN in the low producing herds. In the high production group, on the other hand, test-day milk yield was positively associated with MUN. Cows in the highest production quartile (milk yield > 41.2 kg/d; cut-off points based on the data from cows in these high production

Table 5. Fixed effects from multilevel linear models of milk urea nitrogen concentrations in 12 low and 12 high producing Holstein dairy herds in Ohio.

<table>
<thead>
<tr>
<th>Effect</th>
<th>Low herds</th>
<th>High herds</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>13.35 (0.95)</td>
<td>14.22 (0.88)***</td>
</tr>
<tr>
<td>Month of lactation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>−2.63 (0.29)***</td>
<td>−3.20 (0.13)***</td>
</tr>
<tr>
<td>2</td>
<td>−1.14 (0.27)*</td>
<td>−1.59 (0.12)***</td>
</tr>
<tr>
<td>3</td>
<td>−0.78 (0.26)</td>
<td>−0.93 (0.12)***</td>
</tr>
<tr>
<td>4</td>
<td>−1.14 (0.25)*</td>
<td>−0.50 (0.11)*</td>
</tr>
<tr>
<td>5</td>
<td>−0.44 (0.24)</td>
<td>−0.50 (0.11)*</td>
</tr>
<tr>
<td>6</td>
<td>−0.26 (0.24)</td>
<td>−0.43 (0.10)*</td>
</tr>
<tr>
<td>7</td>
<td>0.59 (0.25)</td>
<td>−0.53 (0.10)*</td>
</tr>
<tr>
<td>8</td>
<td>0.15 (0.26)</td>
<td>−0.17 (0.11)</td>
</tr>
<tr>
<td>9+</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Test day season</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Winter</td>
<td>−2.53 (0.19)***</td>
<td>0.36 (0.08)***</td>
</tr>
<tr>
<td>Spring</td>
<td>−1.79 (0.21)***</td>
<td>0.47 (0.09)***</td>
</tr>
<tr>
<td>Summer</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Fall</td>
<td>−2.80 (0.20)***</td>
<td>0.002 (0.08)</td>
</tr>
<tr>
<td>Test day milk yield</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;29.4 kg</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>29.4–34.9 kg</td>
<td>ns</td>
<td>0.24 (0.08)</td>
</tr>
<tr>
<td>34.9–41.2 kg</td>
<td>ns</td>
<td>0.51 (0.09)***</td>
</tr>
<tr>
<td>&gt;41.2 kg</td>
<td>ns</td>
<td>0.81 (0.11)***</td>
</tr>
<tr>
<td>Milk fat percentage</td>
<td>ns</td>
<td>0.33 (0.05)***</td>
</tr>
<tr>
<td>Linear score</td>
<td>ns</td>
<td>−0.21 (0.02)***</td>
</tr>
</tbody>
</table>

1Coefficients presented only for variables which were significant (P < 0.05) or at least one level of categorical variable was significant.

2Parameter estimates and their standard errors.

3Categories are based on the cut-off values of quartiles for the high producing herds.

4Fat percentage centered at the mean value of 3.6% for the high producing herds.

5Linear score centered at the mean value of 4.4 for the high producing herds.

ns = Not significant.

*P < 0.05.

**P < 0.01.

***P < 0.001.
herds) had on average 0.8 mg/dl higher MUN than cows in the lowest production quartile. In the initial bivariate screening, both protein and fat percentage were associated with MUN. When fat and protein were simultaneously in the model, protein content of milk, however, was no longer significant. Milk fat percentage was positively associated with MUN in the high producing group: with increasing milk fat percentage concentration of MUN increased. Milk SCC (expressed as linear score) was negatively associated with MUN in the high production herds (P < 0.01). With a unit increase in linear score, MUN concentration decreased by 0.2 mg/dl. Rolling herd average milk production was not associated with MUN in either the low or the high producing herds.

**DISCUSSION**

Variance Structure of the Data

The use of the multilevel modeling technique allowed us to evaluate the proportion of variance in MUN attributed to each level of organization in the data. In their recent paper, Dohoo et al. (2001) discussed different estimation methods and computation of variance components in multilevel models when evaluating sources of variation in reproductive performance of dairy cows. The overall variability in MUN concentrations was greater in the low than in the high producing herds. It has been shown that the concentration of urea in milk varies with changes in the amount and proportion of energy and protein in the diet of cows (Oltner and Wiktorsson, 1983; Carlsson and Pehrson, 1994; Roseler et al., 1993). Given that diets, forage, and feed quality, and feeding practices vary considerably between herds, it was expected that the herd-level variability would account for most of the variance in MUN (Carlsson et al., 1995). In the present study, this was true, however, only for the high producing group, whereas in the low producing group, most of the variability was explained at test-day level. This finding may be attributed to the general observation that in high producing herds the day-to-day management and feeding practices as well as forage and/or feed quality can be expected to be more consistent than in the low producing herds. The high producing herds fed their cows TMR, whereas 10 of the low producing herds were component fed (information was missing from the other herds and therefore, was not used in the modeling process). This difference could also possibly explain some of the higher variability in MUN among the low producing herds.

Most of the variation in MUN in the present study was observed at test day level, approximately 65 to 68% in the low producing herds and 34 to 39% in the high producing herds. Several researchers have reported a distinct diurnal pattern in MUN (Gustafsson and Palmqvist, 1993; Carlsson and Bergstrom, 1994; Rodriguez et al., 1997), with the peak in MUN related to and following the time of feeding by 3 to 5 h. All the samples in this study were collected at normal milking times, in some herds twice, but in some herds only once a day. If tested twice, the samples were mixed together. We did not, however, have detailed information about the time of the day (i.e., a.m. or p.m. milking) of collection of individual samples for each herd and the results could not be adjusted for that. Thus, a portion of the observed day-to-day variability in our data can probably be explained by different sampling times. Broderick and Clayton (1997) reported that MUN concentration patterns were not symmetrical over the two halves of the day, implying that switching back and forth between a.m. and p.m. sampling may confound interpretation of MUN data. Thus, from a practical standpoint, if the sampling time within a herd can be kept constant, that source of variability will be reduced, making the interpretation of MUN easier and more reliable and more useful for assisting in management decisions.

When the independent variables were added to the model, the overall variance in MUN remained approximately the same within the high production herds, but decreased slightly within the low production group. Also, the proportion of variability explained at test-day level decreased and the proportion explained at herd level increased in both groups. All the significant explanatory variables, in fact, were measured at test day level (test-day milk yield, season of the test day, month of lactation, and SCC) and therefore, it seems logical that the residual variability at test-day level decreased with these variables in the model. Also, since most of the variability in the low producing herds was observed at test-day level, it appears reasonable that the overall variance decreased in this group when the explanatory variables were in the model.

It is generally recommended that MUN data be interpreted at a group level, partly because cows are managed in groups, but also because of large variation observed in MUN measurements between cows (Broderick and Clayton, 1997; Schepers and Meijer, 1998; Godden et al., 2001a). The present study found very little of the variability in MUN to be explained at cow level and thus our results would not support the above reason (i.e., variation between cows) for the need to interpret MUN at group level. Regardless, it would not be recommended to intervene (such as changing ration formulation for the entire herd) based on a MUN measurement from an individual cow or a small group of cows. The earlier recommendation to use group level data is largely based on studies where samples were analyzed using the infrared technique. Ohio DHI ana-
lyzes MUN spectrophotometrically by an automated procedure with a Skalar Segmented Flow Analyzer, i.e., using a so-called “wet chemistry” method. This indirect chemical test is considered a reference method for MUN analysis (Godden et al., 2000). Many North American DHI laboratories have adopted the infrared method for MUN analysis, as this same technique has long been used to determine the levels of milk fat, protein, and SCC. Godden et al. (2000) found a good overall agreement between these two tests at group level, but a relative lack of agreement between the two tests for individual cows. They concluded that milk samples routinely collected and analyzed by IR at DHI are suitable for measurement of milk urea concentration if the data are interpreted at group, not individual animal level.

**Factors Associated with MUN**

MUN concentrations in Ohio dairy herds were lowest during the first month of lactation. This observation agrees with several other reports (Carlsson et al., 1995; Eicher et al., 1999; Godden et al., 2001b). Schepers and Meijer (1998), however, found no significant association between MUN and stage of lactation in feeding trials, which controlled for nutritional factors. In the present study, after the first month of lactation, MUN concentration increased for 2–3 months, reaching a peak roughly at the same time when cows typically reach peak milk yield. Carlsson et al. (1995) observed the same with cows that were housed inside (i.e., were not grazing). Especially high producing cows have difficulty in meeting their requirements for energy, and they are in a negative energy balance at the beginning of lactation (Carlsson et al., 1995; Butler, 2000; de Vries and Veerkamp, 2000). Lower MUN concentrations at the beginning of lactation could be related to and explained by the inability of cows to ingest sufficient amount of feed, which could lead to, or be result of, suboptimal function of the ruminal flora (Carlsson et al., 1995).

MUN concentrations were significantly higher during summer than other times of the year in the low producing herds. All except one of the low producing herds had their cows on pasture during summer, but we did not have information on what portion of their diet the cows received from pasture. Higher MUN values in summer in these herds could, however, be explained by cows having access to fresh pasture, which typically contains highly degradable protein and has high protein/energy ratio (Westwood et al., 1998; Soriano et al., 2001). This could also partially explain the high proportion of variability in MUN explained at test-day level among these low producing herds. Several other researchers have reported high MUN concentrations during the summer (Carlsson et al., 1995; Westwood et al., 1998; Godden et al., 2001b). Wittwer et al. (1999) found bulk milk urea concentrations to be highest during spring and lowest during summer in grazing herds in Southern Chile, but they also reported that CP content of grass is much higher in spring than in summer in Southern Chile. The effect of season was much less evident in the high than in the low producing herds. Only one of the high producing herds had their cows on pasture and thus changes in nutrient levels and protein content in their day-to-day feeding were probably much smaller than in the low producing herds and therefore the MUN levels showed less variability from season to season. Lower MUN concentrations in summer than in winter and spring for high producing herds could be related to lower DMI due to the heat, and thus lower protein intake in summer.

At herd level, the high producing herds (RHA > 10,433 kg), on average, had higher MUN concentrations than the low producing herds (RHA < 7258 kg). Within these production groups, however, there was no association between RHA milk production and the average MUN concentration in the herd. The lowest average MUN concentration at herd level among the high producing herds was 10.1 mg/dl, and this was observed in the second highest producing herd (with RHA milk production of 11,226 kg). Cows in high producing herds with low MUN are likely utilizing protein in their diets very efficiently. Our observation from herds with over 11,000 kg of RHA production with MUN concentrations between 10 and 11 mg/dl would suggest that it is possible to achieve high production levels and have relatively low MUN concentrations. This, in fact, suggests that in many herds monitoring MUN concentrations and making appropriate changes in rations could provide an opportunity for a better protein feeding efficiency, which could potentially be an opportunity to reduce feed costs and improve profitability.

At individual cow level, a significant positive association was observed between test-day milk yield and MUN concentration in the high producing herds, but not in the low producing herds. Broderick and Clayton (1997) reported that fat-corrected milk yield was positively correlated with MUN and Oltner et al. (1985) also observed a positive correlation between milk yield and milk urea concentration. Gustafsson and Carlson (1993), on the other hand, found no significant correlations between these parameters and Carlson et al. (1995) found only a weak positive correlation between the daily milk yield and concentration of MUN.

Milk fat and protein percentages were not associated with MUN concentrations in the low producing herds. In the high producing herds, however, milk fat percentage was positively related to MUN. Godden et al. (2001b), on the other hand, reported a negative nonlin-
ear association between milk urea and both milk fat and total protein percentages. Although their results were statistically significant, the authors questioned their biological and economic significance. In the present study, MUN and milk protein percentage was negatively associated, but not statistically significant when fat percentage was included in the model. Baker et al. (1995) and Roseler et al. (1993) reported that the true protein in milk is influenced by the level of CP and protein type (proportion of rumen degradable and undegradable protein) in the diet. Milk fat, however, was not affected by the treatment (isocaloric diets, varying in RDP, RUP, and CP levels) in their studies (Roseler et al., 1993; Baker et al., 1995). The higher MUN concentrations in the high producing herds, in general, might be attributed to likely higher protein levels in the diets in these herds compared with what might be expected in the low producing herds. The interest of the present study was not to study associations between MUN and diet, and we did not have any information of the actual diets and their nutritional components in the study herds and, therefore, are not able to draw direct conclusions about the relationship of MUN with dietary factors.

We found a significant negative association between SCC (linear score) and MUN in the high producing herds but no association in the low producing herds. Little research has been published exploring the association between MUN and SCC. Godden et al. (2001b) reported a negative association between cow-level MUN and linear score in 60 commercial Ontario Holstein herds over a 13-mo period, but no association between herd average linear score and herd average MUN. Faust et al. (1997) reported that MUN values were lowest for samples with largest SCC. These observations would agree with our finding that increasing SCC was associated with decreasing MUN. However, a positive association between SCC and milk NPN levels (which includes urea) has also been reported (Ng-Kwai-Hang et al., 1985). Also, DePeters and Ferguson (1992) reviewed earlier studies and reported that milk from mastitic cows was lower in casein and higher in noncasein protein (which would include urea).

Parity was not significantly associated with MUN in the present study in either production group. First-lactation cows tended to have lower concentrations of MUN than older cows, but the difference was not statistically significant. This finding agrees with previous studies that have reported lower MUN concentrations in first-parity cows; the differences have either been statistically significant, but numerically minor (Oltner et al., 1985; Godden et al., 2001b) or not significant at all (Canfield et al., 1990; Carlsson et al., 1995).

Even though we had an equal number of herds in both groups, the high producing herds were larger and thus contributed more cows and test-day observations into the analyses; almost two-thirds of all of the observations were from high producing herds. Thus, larger sample size might have enabled us to observe statistically significant results more easily among the high producing herds. Also the higher level of variability in the low producing herds (in addition to a smaller sample size) likely affected our ability to identify statistically significant results in this group.

**CONCLUSIONS**

The use of a multilevel modeling technique enabled us to estimate the proportion of variance in different levels of organization in the MUN data. Most of the variation in MUN was explained at test-day level in the low producing herds, but at herd level among the high producing herds. Lower variability at test-day level in high producing herds possibly indicates more consistent day-to-day management, feeding, and forage quality in these herds than in low producing herds. The results suggest that the high variability from test day to test day needs to be considered when interpreting MUN data in the field. It is important to take same type of samples (e.g., composite samples) at the same time of the day to minimize day-to-day variability in MUN measurements. Mean MUN concentrations in some of the high producing herds were between 10 to 11 mg/dl. Using MUN to monitor and adjust ration energy-protein balance might provide an opportunity to reduce feed costs and to improve profitability of the herd.

**REFERENCES**


