Effect of herd, parity, stage of lactation and milk yield on urea concentration in milk

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ABSTRACT: The objective of this study was to determine how non-nutritional factors such as herd, parity, days in milk, milk production, and milk components affect the concentration of milk urea. A retrospective, observational study comprising analyses of individual cow records from monthly dairy herd improvement milk tests including milk urea tests was conducted. Six commercial Holstein dairy herds were involved in the investigation from October 2000 to September 2003. The data were evaluated using the mixed linear model with repeated measures, and parameters were estimated by the REML method. Significant differences in milk urea concentrations were observed between herds ($P < 0.0001$), with a significant variability between test dates within herds. Significant effects of parity ($P = 0.0003$), days in milk ($P < 0.0001$), and their interaction were also found. Higher concentrations of milk urea were determined in the cows on the first and second parity (5.63 and 5.62 mmol/l, respectively) compared to the groups of cows on the third and fourth parity (5.47 mmol/l). It was found that milk urea concentration increased with the square of milk yield ($P < 0.0001$), while a negative quadratic relationship was found between milk urea concentration and milk fat % ($P < 0.0001$). It was concluded that milk urea concentration should be evaluated in association with days in milk, milk yield, milk fat %, and milk protein %.

Keywords: dairy cow; milk urea; non-nutritional factors

The concentration of milk urea (MU) is a useful measurement for assessing whether the balance between the cow's intake of protein and energy is correct. Concentrations are variable from herd to herd and between cows in the same herd. To interpret the milk urea concentration correctly, it is important to take into account other factors besides the cow's diet (Carlsson et al., 1995), because, for example, non-nutritional factors explained 13.3% of the variation in MUN (Arunvipas et al., 2003), and production and environmental factors explained 37% of the MU variation in individual cows (Hojman et al., 2004).

One of the most important non-nutritional factors is herd. Rajala-Schultz and Saville (2003) tested the variability of MUN in 12 low-producing herds (rolling herd average milk production < 7.258 kg) and 12 high-producing herds (rolling herd average milk production > 10.433 kg). They found lower variability in milk urea nitrogen (MUN) between test days in the high-producing herds. This may indicate more consistent day-to-day feeding and management within a herd. However, housing factors (tie stall vs. free stall), TMR versus component feeding, feeding frequency, and synchrony of offering forages and concentrates were not associated with herd mean MU (Godden et al., 2001a). Eicher et al. (1999a) stated that herd was a global concept and most likely, a herd effect on MUN would be related to feeding and management procedures. Further studies are needed to more precisely define herd and feeding characteristics with explanatory

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variables, in order to obtain better defined diagnostic models. These findings emphasize the need to use an approach at the herd level and should make researchers aware that herds are not homogeneous in their relationships between MUN and explanatory variables.

The stage of lactation also influences the level of MUN, although the results of different authors are not consistent. Emanuelson et al. (1993) found in a feeding experiment the highest level of MU between the 60th and 90th day of lactation. Carlsson et al. (1995) noticed a low level of MU at the start of lactation independently of parity and season. Richardt et al. (2001) found that fixed effects for the first and second lactation periods versus the third lactation period were – 31.6 mg/l MU and – 2.1 mg/l MU, respectively.

In the study of Arunvipas et al. (2003) MUN was lowest in the first month of lactation but increased rapidly during the first two months followed by a slower increase over the next two months. Also, Hojman et al. (2004) and Giger et al. (1997) found that MU levels increased as lactation progressed. This result does not agree with Ng-Kwai-Hang et al. (1985), who reported that milk non-protein nitrogen rapidly declined after calving, and then gradually rose through to the end of lactation. However, Hoffmann and Steinhofel (1990), Faust et al. (1997), and Schepers and Meijer (1998) found no variation by stage of lactation.

MU has been reported to be lower in first-lactation cows than second- or later-lactation animals in several studies (Peterson and Waldern, 1981; Ng-Kwai-Hang et al., 1985; Oltner et al., 1985; Carroll et al., 1988; Canfield et al., 1990; Barton et al., 1996; Arunvipas et al., 2003; Hojman et al., 2004). Other studies reported only minor differences, with no significant association between parity and MU (Canfield et al., 1990). Gooden et al. (2001a) and Richardt et al. (2001) found statistically significant but numerically small difference in MU due to parity, while other studies found no age effect (Kaufmann, 1982; Kaim et al., 1983; Gustafsson et al., 1987; Ropstad et al., 1989; Hoffmann and Steinhofel, 1990; Carlsson et al., 1995; Eicher et al., 1999a).

MU was higher in high-producing herds than in low-producing herds (Kaufmann, 1982; Macleod et al., 1984; Oltner et al., 1985; Carlsson et al., 1995; Hojman et al., 2004). Arunvipas et al. (2003) found correlation between MUN and milk production (0.173) similar to that reported by Ferguson et al. (1997) (0.178). According to the study of Jonker et al. (1999), a 2 000-kg increase in milk production per lactation was associated with a 2.6 mg/100 ml increase in mean MUN. In the study of Arunvipas et al. (2003) an increase of 1 kg of milk production per day increased the MUN concentration by 0.05 mg/100 ml. In this situation, an increase in milk production of 2 000 kg over a 305-day lactation period would increase MUN by 0.33 mg/100 ml, which is a much smaller increase than that reported by Jonker et al. (1999). In contrast, other studies (Carlsson, 1989; Ropstad et al., 1989; Gustafsson and Palmquist, 1993; Eicher et al., 1999b) found no correlation between MUN and milk yield.

Some researchers found the relationship between MU and milk total protein percentage negative and between MU concentration and fat percentage positive (Hojman et al., 2004). In the study of Arunvipas et al. (2003) the correlations of MUN with protein% and fat% were –0.212 and –0.117, respectively. Ferguson et al. (1997) reported a correlation of –0.138 for protein% and 0.0135 for fat%. The relationship between MUN and milk fat was quadratic (Arunvipas et al., 2003). This form of the relationship was also reported by Godden et al. (2000). Faust et al. (1997) and Carlsson (1989) found no relationship between MUN and percentages of protein and fat, but these authors did not evaluate the possibility of a quadratic association.

Our objective was to determine how non-nutritional factors such as breed, parity, days in milk, milk production, and milk components affect the concentration of MU.

MATERIAL AND METHODS

A retrospective, observational study was conducted by analyzing individual cow records from monthly dairy herd improvement (DHI) milk tests including an MU test. Six commercial Holstein dairy herds participated in the investigation from October 2000 to September 2003. All cows were milked twice daily, housed in free stalls, and fed a TMR twice a day, but no distinction was made for management style and feeding scheme. Test day observations were combined into a dataset that included herd code, date of test, milk yield, milk protein content, milk fat content, MU concentration (mmol/l), breeding date, days in milk (DIM), and parity. Laboratory measurements were performed by the Czech-Moravian Breeders' Corporation, Inc. (No. 1312.2-CSN EN ISO/IEC 17025). The

MU concentration (mmol/l) was measured using a standardised procedure based on the rate of electrical conductivity change during urea hydrolysis with Ureakvant 2 (Agro-servisis, Olomouc, the Czech Republic).

Each production variable was screened for normality and the presence of the outliers by visual assessment of the distribution and by calculation of kurtosis and skewness. Records with milk protein content and milk fat content exceeding intervals < 2%, 5.5% > and < 2%, 6.5% >, respectively, were excluded from the analysis to remove the outliers. Lactation length was fixed at 300 days and was divided into 10 DIM intervals of 30 days each, starting from parturition. Later lactation records were omitted from the analysis because of the rapidly decreasing presence of these data, the same as records of cows after the 5th and later calving. Lactations with fewer than 5 test day records were discarded. The complete data set consisted of 12 991 records from 1 221 cows on the first to fourth parity. Descriptive statistics for MU, DIM, parity, milk yield, and fat and protein content were calculated. Test day records were averaged for each individual lactation and individual herd to obtain means at lactation and herd levels. The data were evaluated using a mixed linear model with repeated measures. Parameters were estimated by the REML method using a Mixed procedure in SAS (SAS, 2004).

The model was structured to determine the effect of herd, test date, DIM, and parity on MU concentration (the dependent variable) and to observe the effect of parity on lactation curves. The variable test date was entered as random within the herd to control for random test day effect and for the fact that observations were repeated within the herd on different test days (because of the high co-linearity the seasonal effect could not be included). To investigate the relationship between test day MU concentrations and production variables test day milk production, fat and protein percentages were added as covariates to the model. Quadratic terms and two-way interactions were also included. Effects that were not significant ($P < 0.05$) in the final multivariate model were subsequently removed by backwards elimination. The following statistical model was used:

$$Y_{ijklm} = \mu + H_i + d(H)_{ji} + P_k + D_l + PD_{kl} + \alpha_1 m_{ijklm} + \alpha_2 m_{ijklm}^2 + ß_1 p_{ijklm} + ß_2 p_{ijklm}^2 + \gamma_1 f_{ijklm} + \gamma_2 f_{ijklm}^2 + e_{ijklm}$$

where:

Y_{ijklm} = test day MU concentration in mmol/l
μ = overall mean
H_i = herd (i = 1 to 6)
$d(H)_{ji}$ = random effect of test date j within herd i, $d(H)_{ji} \sim N(0, \sigma_d^2)$
P_k = parity (k = 1 to 4)
D_l = days in milk (l = 1 to 10), intervals of 30 days each, starting from parturition
PD_{kl} = the interaction between parity and DIM interval
m_{ijklm} = test day kilograms of milk
p_{ijklm} = test day protein percentage
f_{ijklm} = test day fat percentage
$\alpha_1, \alpha_2, ß_1, ß_2, \gamma_1, \gamma_2$ = appropriate regression coefficients
e_{ijklm} = random residual, $e_{ijklm} \sim N(0, \sigma_e^2)$

In the model, random (co)variances between days in milk intervals within lactation were summarized by residual R matrix which was assumed to be a block diagonal with identical 10 × 10 submatrices, each corresponding to an individual lactation:

$$R = \begin{bmatrix} R_1 & & & \\ & R_2 & & \\ & & \ldots & \\ & & & R_4 \end{bmatrix} = \text{where } R_1 = R_2 = \ldots = R_n$$

As alternatives, the compound symmetry, unstructured, autoregressive of order 1 and the Toeplitz covariance structures were compared. The Toeplitz covariance structure was found to be the most appropriate in accordance with Akaike's Information Criterion (AIC) and the Schwartz Bayesian Criterion (SBC) (Littell et al., 2000). The least squares means were calculated, and multiple comparisons were made, with P-values adjusted using Tukey's procedure.

RESULTS AND DISCUSSION

Descriptive statistics

In this data set, the average number of cows and their lactations per herd was 196.83 ± 73 and 269 ± 103.18 with the range from 50 to 240 cows and 76 to 352 lactations, respectively (Table 1). The MU content and production variables ranged at the herd level from 4.13 to 7.79 mmol/l, 22.57 to 31.40 kg, 3.97 to 4.28% and 3.31 to 3.47% for MU, milk, fat and protein content, respectively. Average parity was 1.91, but there were included 753, 459, 293 and 156 lactations of cows in parity 1, 2, 3 and 4. The frequency of testing cows in individual lactations ranged from 5 to 10, and the DIM was

Table 1. Descriptive statistics

Variable	Mean	Std	Min	Max
Unique herd records (*n* = 6)				
Cow count	196.83	73.05	50.00	240.00
Lactation count	269.00	103.18	76.00	352.00
MU (mmol/l)/test day	5.55	1.39	4.13	7.79
Milk (kg)/test day	27.15	3.11	22.57	31.40
Fat (%)/test day	4.12	0.11	3.97	4.28
Protein (%)/test day	3.38	0.05	3.31	3.47
Unique lactation records (*n* = 1 661)				
Parity	1.91	1.00	1.00	4.00
DIM	155.16	25.81	73.40	242..40
Testing frequency [test/lactation]	7.94	1.49	5.00	10.00
Test day records (*n* = 12 991)				
MU (mmol/l)	5.75	2.00	1.17	12.99
Milk (kg)	27.10	8.28	2.10	69.20
Fat (%)	4.11	0.76	2.00	6.50
Protein (%)	3.38	0.35	2.02	5.25
DIM	155.24	80.10	10.00	300.00

normally distributed with the mean 155.16 ± 25.81. The MU was skewed to the right with the mean 5.75 mmol/l and range from 1.77 to 12.99 mmol/l. Production data were normally distributed with the means of 27.10 ± 8.29 kg, 4.12 ± 0.77%, 3.38 ± 0.36% for milk, fat and protein content. The range of DIM on the test day was from 10 to 300 days.

Factors associated with milk urea

A significant variability between test dates within the herd was confirmed by means of the likelihood ratio statistic (Self and Liang, 1987). It can be assumed that factors relating to management decisions are more significant for the variability than the effect of the season, which is included in the random effect of the test date within herds. With regard to the findings of other authors (Rajala-Schultz and Saville, 2003), who reported that most variability can be explained at the herd and the test date level, a significant effect of the herd ($P < 0.0001$) on MU content has also been demonstrated. The highest content of MU, 7.64 mmol/l, was found in herd 4 (Table 2). It significantly differed ($P < 0.05$) from LSM in all other herds, similar to the second highest estimation of MU content in herd 1 (6.57 mmol/l). On the contrary, the lowest MU content was estimated in herd 1 (4.30 mmol/l). The effect of herd and test date on MU content is related to the different ratio of energy and protein in the feeding dose, as was reported, e.g., by Carlsson and Pehrson (1994). Moreover, the significant effect of the test day in individual herds may be attributed to less consistent day-to-day management and feeding practice, as well as to forage and feed quality (Rajala-Schultz and Saville, 2003) in the period under study. With regard to the findings of other authors

Table 2. Least square means of MU concentration by herd categories

	Herd					
	1	2	3	4	5	6
n	2 127	2 516	1 839	2 883	2 957	649
MU concentration (mmol/l)	6.57^{a}	4.30^{b}	4.99^{bc}	7.64^{d}	5.26^{c}	4.51^{bc}
SE	0.272	0.215	0.218	0.179	0.190	0.215

Different letters of superscript mean significant difference at the level of $P < 0.05$

Table 3. Least square means of MU concentration by parity categories

	Parity			
	1	2	3	4
n	5 874	3 633	2 262	1 222
MU concentration (mmol/l)	5.63[a]	5.62[a]	5.47[b]	5.47[b]
SE	0.090	0.092	0.095	0.101

Different letters of superscript mean significant difference at the level of $P < 0.05$

(Rajala-Schultz and Saville, 2003; Wattiaux et al., 2005), the effects of herd and test day are among the most important causes of MU variability in milk. This also correspond to the recommended interpretation of MUN data at the level of whole cow groups with uniform management (Godden et al., 2001a). Although no significant decrease in residual error was recorded when the random effect of the cow was included in the model, in agreement with Rajala-Schultz and Saville (2003), changes in management and feeding based on individual results or just a small group of cows cannot be recommended.

In the hypothesis tests, significant effects of parity ($P = 0.0003$), DIM ($P < 0.0001$) and their interaction ($P < 0.05$) were found. Two significantly different groups of cows can be distinguished in LSM concentration of MU according to the parity (Table 3). Higher MU concentrations (5.63 and 5.62 mmol/l) were estimated in cows in their first and second lactations, in contrast to groups in their third and fourth lactations (5.47 mmol/l). Johnson and Young (2003) also recorded the highest MUN concentration in cows in the first lactation, with significant results in overall means with relatively small differences between lactations. Godden et al. (2001b) also reported small but significant differences between lactations, but with the lowest MUN concentrations in cows in the first lactation, like many other authors (Carlsson et al., 1995 and others). However, these authors compared only cows in the first lactation with older ones (two and more calvings).

The effect of the lactation phase on MU content was found to be one of the most important factors ($P < 0.0001$). The lowest level of MU concentration in the first month of lactation was estimated to be (5.19 ± 0.1 mmol/l). It was significantly lower ($P < 0.05$) than MU content in all other periods of lactation (see Figure 1). In the following two months, the content of MU relatively rapidly increased, and maximum concentration was achieved in the 5[th] month of lactation. These results fully correspond to those of Arunvipas et al. (2003). These authors observed an increase of MU concentration during the first two months of lactation with the peak in the fourth month of lactation. Carlsson et al. (1995) and Hojman et al. (2004) also recorded maximum

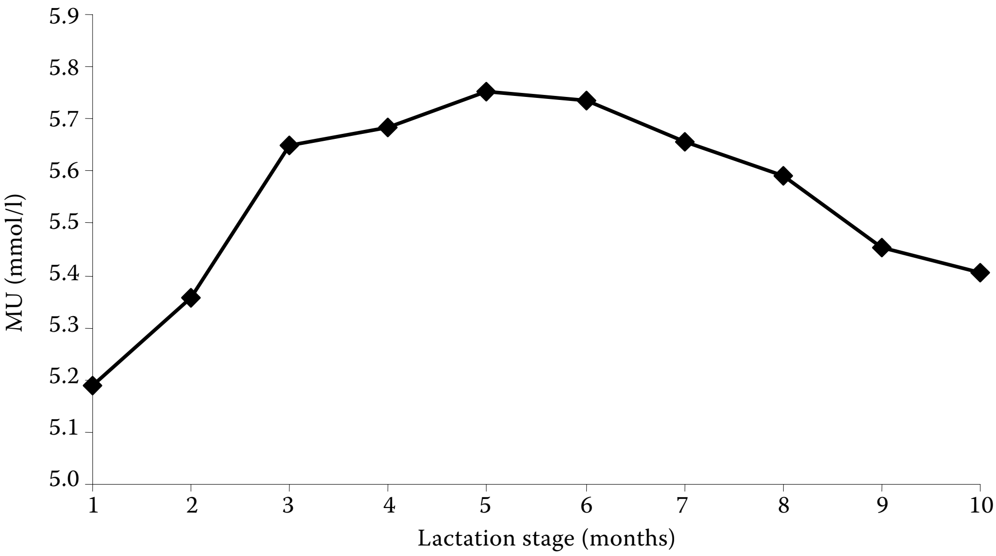

Figure 1. Influence of lactation stage (in months) on MU concentration (mmol/l)

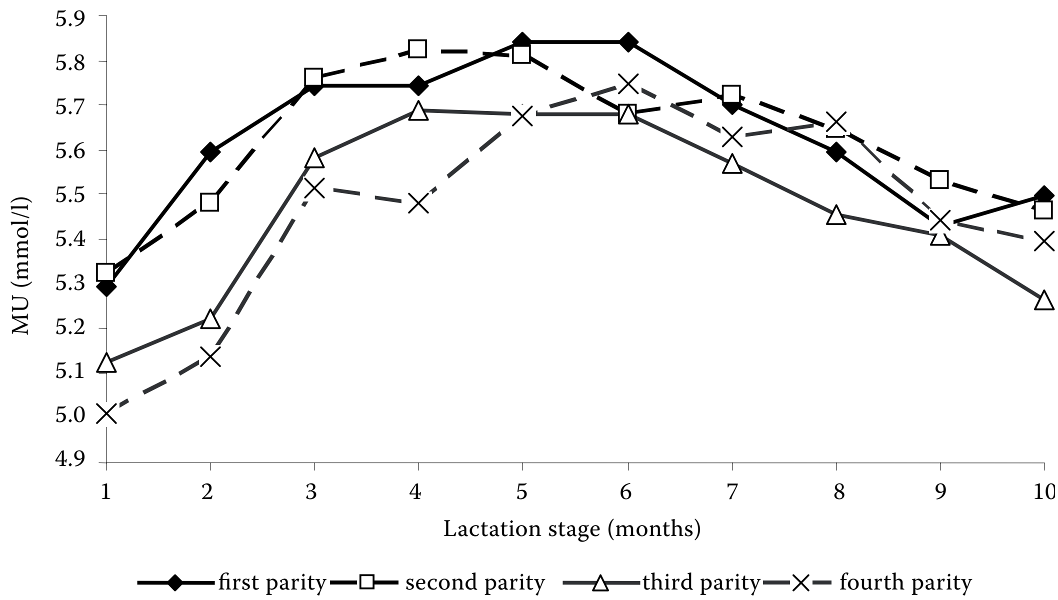

Figure 2. Fixed effect of lactation stage (in months) on MU concentration (mmol/l) in different parities

MUN content in the 3rd – 6th months of lactation. On the contrary Ng-Kwai-Hang et al., (1985) reported that milk non-protein nitrogen rapidly decreased and gradually increased up to the end of lactation. Carlsson et al. (1995) explained the lower MUN content in the first month of lactation as the inability of cows to digest a sufficient amount of feed at the beginning of lactation, which resulted in a relatively lower intake of proteins.

The interactions of the parity and the phase of lactation in relation to the above results confirm a higher MU concentration during the first and second lactations than in later lactations. It is evident from Figure 2 that there are higher MU concentrations in the first two lactations, with more marked differences in the first phases of lactation. In the second month of lactations, significant differences ($P < 0.05$) in MU content were recorded between the first and third and fourth lactations (5.59 and 5.22, resp. 5.13 mmol/l). In subsequent months, no significant differences in MU concentrations were recorded. The general trend of changes in MU content in the course of lactation is identical in all lactations. However, it is evident that MU concentration increases more slowly in the 3rd and 4th lactations and at its peak does not reach the values estimated in the 1st and 2nd lactations.

It was recorded that there is a significant relationship between the amounts of milk produced and the content of milk components together with MU concentration. The results in Table 4 show that MU content significantly increases ($P < 0.0001$) with the square milk yield ($\alpha^2 = 0.000705$). Carlsson et al. (1995) and Godden et al. (2001b) reported a relation between milk yield capacity and MUN. The same results were obtained by (Johnson and Young, 2003). Significant positive association was observed between test day milk yield and MUN concentration in the high-producing herds, but not in the low-producing ones (Rajala-Schultz and Saville (2003).

Similarly, the concentration of MU is significantly ($P < 0.0001$) but negatively influenced by the content of milk fat ($\gamma_2 = -0.01437$). A significant effect on protein content was found in both linear ($P < 0.05$) and quadratic ($P < 0.001$) terms of re-

Table 4. Parameters with significant contributions to the regression of MU concentration on multiple factors using the mixed effects model

Factor	Estimated coefficient	SE	*P*
Square of milk (kg)	0.000705	0.000035	< 0.0001
Square of fat (%)	–0.01437	0.001761	< 0.0001
Protein (%)	–0.7448	0.3615	0.0394
Square of protein (%)	0.1464	0.05181	0.00047

gression. The slope of the linear term expresses a negative relation of MU content ($\beta_1 = -0.7448$), whereas the slope of the quadratic term is positive ($\beta_2 = 0.1464$). In the study of Arunvipas et al. (2003) a positive relationship between MUN concentration and milk yield was found, while a negative relationship was found with regard to milk protein percentage and quadratic relationship with milk fat percentage (lower MUN at low and high fat percentage). A positive nonlinear association between MU and milk yield may be attributed to increased milk production which resulted from increased levels of dietary protein fed (Chalupa, 1984; Oldham, 1984). The question arises of whether the changes in concentration of MUN in the course of lactation are due to DIM, or whether they are directly related to milk production (Johnson and Young, 2003).

CONCLUSIONS

MU concentration in these data were positively associated with the square of milk yield, while a negative quadratic relationship was found between MU concentration and milk fat. It was concluded that MU concentration should be evaluated in association with parity, DIM, milk yield, milk fat percentage, and milk protein percentage. These variables should be considered as potential sources of misinterpretation in exploring the relationship between MU and nutritional management or measures of performance. This study was a necessary step in validating the use of MU measurements from DHI samples as a tool to assist dairy producers.

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