Genetic Parameters for Milk Urea Nitrogen in Relation to Milk Production Traits

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ABSTRACT

The aim of this study was to estimate genetic parameters for test-day milk urea nitrogen (MUN) and its relationships with milk production traits. Three test-day morning milk samples were collected from 1,953 Holstein-Friesian heifers located on 398 commercial herds in the Netherlands. Each sample was analyzed for somatic cell count, net energy concentration, MUN, and the percentage of fat, protein, and lactose. Genetic parameters were estimated using an animal model with covariates for days in milk and age at first calving, fixed effects for season of calving and effect of test or proven bull, and random effects for herd-test day, animal, permanent environment, and error. Coefficient of variation for MUN was 33%. Estimated heritability for MUN was 0.14. Phenotypic correlation of MUN with each of the milk production traits was low. The genetic correlation was close to zero for MUN and lactose percentage (−0.09); was moderately positive for MUN and net energy concentration of milk (0.19), fat yield (0.41), protein yield (0.38), lactose yield (0.22), and milk yield (0.24), and percentage of fat (0.18), and percentage of protein (0.27); and was high for MUN and somatic cell score (0.85). Herd-test day explained 58% of the variation in MUN, which suggests that management adjustments at herd-level can reduce MUN. This study shows that it is possible to influence MUN by herd practice and by genetic selection.

Key words: dairy cattle, genetic parameter, milk yield, urea

INTRODUCTION

Milk urea nitrogen has become an important trait now that, due to new European legislation, the Netherlands will start using MUN to monitor mineral efficiencies of herds (LNV, 2006). Milk urea is synthesized as a consequence of an imbalance between dietary nitrogen

and energy in the rumen, and protein synthesis inefficiency (DePeters and Ferguson, 1992). As the main non-protein source of nitrogen in milk, MUN reflects the efficiency of nitrogen utilization and the nitrogen output toward the environment.

The amount of urea in blood, plasma, urine, and milk is related to the CP and energy percentage of the feed (Roseler et al., 1993), which suggests a relationship between urea and energy balance (DePeters and Ferguson, 1992) and possibly with the energy concentration of the milk. Cows in early lactation have a ruminal flora that is not adapted to the shift to high protein diets after parturition. The consequential mismatch in energy and protein may lead to an increased MUN in the first months of lactation (Jorritsma et al., 2003).

The apparent relationships of MUN with nitrogen excretion in milk and urine suggest that decreased MUN will decrease environmental pollution with nitrogen. Milk urea nitrogen might be used as a selection tool, and therefore, information on genetic parameters is needed. Estimates of genetic parameters for MUN have resulted in heritabilities between 0.06 and 0.44, and in low phenotypic correlations with production traits such as fat and milk yield (Wenninger and Distl, 1993; Wood et al., 2003; Mitchell et al., 2005). The range in estimates is broad, as are the numbers of animals and the types of models used, and gives no clear indication of the heritability or genetic correlations of MUN for the Dutch Holstein-Friesian population.

Our study aims to estimate heritability for MUN, phenotypic and genetic correlations of MUN with SCS, percentages of fat, protein, and lactose, yields of fat, protein, lactose, and milk, and net energy concentration of the milk (**NEm**), and quantify the effects of herd-test day on MUN.

MATERIALS AND METHODS

Animals

This study is part of the Milk Genomics Initiative, which focuses on the genetic background of detailed milk composition. As part of this study, milk samples of 1,953 first-lactation cows on 398 commercial herds in

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the Netherlands were collected. The cows were selected such that at least 5 selected cows per herd were present at the start of the experiment. Each cow was between 5 and 220 DIM of first lactation at the start of the experiment, implying a restricted range in date of calving. Cows were over 87.5% Holstein-Friesian.

The cows were sired by 1 of 50 young bulls (857 cows), 1 of 5 proven bulls (909 cows), or other proven bulls (187 cows). The pedigree of each of the 1,953 selected cows was supplied by NRS (Arnhem, the Netherlands).

Milk Samples

Cows were sampled at 3 test-day mornings during February to June 2005. Cows were milked twice daily, but only the morning milk samples were analyzed for milk composition. Sodium azide (0.03% wt/wt) was added to the milk samples. Time between subsequent samples in the same herd ranged from 4 to 8 wk. A total of 5,737 samples were collected. A total of 156 records were discarded, mainly because there were fewer than 2 samples per cow, fewer than 3 animals per herd-test day class, or cows were more than 335 DIM. In total, 5,581 test-day records were analyzed for MUN, SCC, and percentage traits. For yield traits, 5,292 records were analyzed because milk yield was missing for 289 records.

Analysis

For each sample, MUN and percentage of fat, protein, and lactose were determined by infrared spectroscopy using a Fourier transform interferogram (MilkoScan FT 6000, Foss Electric, Hillerød, Denmark) at the laboratory of the Milk Control Station (Zutphen, the Netherlands). A calibrated regression curve was used to calculate parameter values from the peak pattern resulting from infrared spectroscopy. For MUN, about 95% of the control samples had a difference between a pair of duplicated samples less than 5 mg/100 g. Yields of MUN, fat, protein, and lactose were calculated by multiplying percentages with milk yield.

Somatic cell count was determined using a Fossomatic 5000 (Foss Electric) and analyzed as log-transformed SCS. Net energy concentration of each milk sample (NEm in MJ/kg) was calculated as $NEm = 0.384 (\%fat) + 0.223 (\%protein) + 0.199 (\%lactose) - 0.108$ (Tyrrell and Reid, 1965).

Variance components and genetic parameters were estimated using a repeatability animal model in AS-Reml (Gilmour et al., 2002):

$$y_{ijklmno} = \mu + b_1 \times \text{dim}_i + b_2 \times e^{-0.05 \times \text{dim}} + b_3 \times \text{afc}_j + b_4 \times \text{afc}_j^2 + \text{season}_k + \text{scode}_l + \text{htd}_m + A_n + pe_o + e_{ijklmno}, \quad [1]$$

where $y_{ijklmno}$ = dependent variable corresponding to the o th test-day observation of cow n with a sire code of l , calving age of first calving j during season k and at DIM i on herd test day m ; μ = general mean; dim_i = DIM (time between calving and date of sample), modeled with a Wilmink curve (Wilmink, 1987); afc_j = covariate describing the effect of age at first calving; season_k = 3 classes for season of calving: summer (June-August 2004), autumn (September-November 2004), and winter (December 2004-February 2005); scode_l = fixed effect accounting for differences between groups of proven bull daughters and young bull daughters; htd_m = random effect defining groups of animals sampled in the same herd on the same day; A_n = random additive genetic effect of animal n ; pe_o = random permanent environmental effect cow n ; and $e_{ijklmno}$ = random residual effect.

Heritabilities and repeatabilities were estimated using univariate analyses. Correlations were estimated using model [1] and bivariate analyses. Starting values for variance structures in bivariate analyses were based on results of univariate analysis.

Heritability (h^2) was calculated as:

$$h^2 = \frac{\sigma_A^2}{\sigma_A^2 + \sigma_{pe}^2 + \sigma_e^2}, \quad [2]$$

where σ_A^2 = additive genetic variation, σ_{pe}^2 = permanent environment variation, and σ_e^2 = residual variation.

The repeatability (r) estimates the correlation between consecutive samples of the same cow in time. Repeatability was calculated as

$$r = \frac{\sigma_A^2 + \sigma_{pe}^2}{\sigma_A^2 + \sigma_{pe}^2 + \sigma_e^2}. \quad [3]$$

The proportion of variance due to htd (h_{htd}) was calculated as

$$h_{htd} = \frac{\sigma_{htd}^2}{\sigma_{htd}^2 + \sigma_A^2 + \sigma_{pe}^2 + \sigma_e^2}, \quad [4]$$

where σ_{htd}^2 = herd-test day variation.

RESULTS

Mean and Coefficient of Variation

Means and coefficients of variation for MUN and milk production traits, based on 3 test-day morning milk

Table 1. Means and coefficients of variation for MUN and milk production traits measured on 3 morning milk samples of 1,953 primiparous HF^a cows

Trait	n	Mean	CV
Urea (mg/100 g)	5,576	20.39	33
Urea yield (g)	5,587	2.65	40
Fat (%)	5,581	4.30	17
Protein (%)	5,581	3.52	8
Lactose (%)	5,581	4.63	3
Fat yield (kg)	5,292	0.55	20
Protein yield (kg)	5,292	0.46	20
Lactose yield (kg)	5,292	0.60	22
Milk yield (kg)	5,292	13.00	21
SCS ¹	5,579	3.90	28
NEm ² (MJ/kg)	5,581	3.25	10

¹SCS = log-transformed SCC.²NEm = net energy concentration of the milk sample (Tyrrell and Reid, 1965).

samples, are in Table 1. Coefficient of variation for MUN was high (33%) compared with other milk production traits; moderate for yield traits (around 20%) and fat percentage (17%); and low for percentage of protein (8%) and lactose (3%). Coefficient of variation for NEm (10%) was half of the variation of the yield traits and one-third of the variation of MUN.

Heritability and Repeatability

Heritabilities for MUN and the milk production traits are in Table 2. Heritability for MUN was 0.14, which was higher than that for SCS (0.08), but lower than those for milk production traits. The heritability estimate was moderate for yield of MUN (0.28), protein (0.34), fat (0.37), milk (0.44), and lactose (0.47); and

high for percentage of protein (0.60), fat (0.52), and lactose (0.64), as well as for NEm (0.56).

Repeatabilities are also in Table 2. For MUN, the repeatability was relatively low (0.43), which suggests that the correlation of MUN between test-days was lower than for the other traits. For SCS, the difference between repeatability and heritability was large (0.66). Much variation in SCS can therefore be explained by permanent environmental effects.

Effect of Herd-Test Day and DIM

Table 2 shows the proportion of total variance explained by herd-test day. For MUN, herd-test day explained 58% of the variation, whereas for SCS this was only 1%. Variation due to herd-test day for percentage traits was 8% for fat, 6% for protein, and 5% for lactose. The ratio of genetic variance to herd-test day variance showed that for all traits, except MUN and MUN yield, genetic effects were much larger than herd-test day effects.

Days in milk had significant effects ($P < 0.05$) on all traits, except for NEm, although NEm increased slightly with advancing lactation (result not shown). Mean MUN (Figure 1) peaked around the third month of lactation and decreased thereafter.

Correlations

The phenotypic and genetic correlations among MUN and percentage traits are in Table 3. Phenotypic correlations among MUN and percentage traits were low, ranging from -0.06 through 0.11. Genetic correlation was low to moderate among MUN and percentage of

Table 2. Phenotypic variance (σ_p^2), ratio ($\sigma_A^2/\sigma_{htd}^2$), heritability (h^2), repeatability (r), and proportion of variation due to herd-test day (h_{htd}) for MUN and milk production traits estimated from 3 morning milk samples of 1,953 primiparous HF cows¹

Trait	σ_p^2	Ratio ²	h^2	SE	r	SE	h_{htd}	SE
Urea (mg/100 g)	18.64	0.11	0.14	0.05	0.43	0.02	0.58	0.01
Urea yield (g)	0.51	0.30	0.28	0.07	0.58	0.02	0.49	0.02
Fat (%)	0.51	6.81	0.52	0.09	0.68	0.01	0.07	0.01
Protein (%)	0.08	9.04	0.60	0.10	0.85	0.01	0.06	0.01
Lactose (%)	0.02	14.69	0.64	0.10	0.72	0.01	0.04	0.01
Fat yield (kg)	0.01	4.60	0.37	0.08	0.69	0.01	0.08	0.01
Protein yield (kg)	0.01	2.96	0.34	0.08	0.79	0.01	0.10	0.01
Lactose yield (kg)	0.01	8.57	0.47	0.09	0.83	0.01	0.05	0.01
Milk yield (kg)	6.47	8.35	0.44	0.09	0.84	0.01	0.05	0.01
SCS ³	1.13	5.63	0.08	0.04	0.74	0.01	0.01	0.00
NEm ⁴ (MJ/kg)	0.10	9.46	0.56	0.10	0.75	0.01	0.06	0.01

¹ $\sigma_p^2 = \sigma_A^2 + \sigma_{pe}^2 + \sigma_e^2$; $h^2 = \sigma_A^2/(\sigma_A^2 + \sigma_{pe}^2 + \sigma_e^2)$; $r = (\sigma_A^2 + \sigma_{pe}^2)/(\sigma_A^2 + \sigma_{pe}^2 + \sigma_e^2)$; and $h_{htd} = \sigma_{htd}^2/(\sigma_{htd}^2 + \sigma_A^2 + \sigma_{pe}^2 + \sigma_e^2)$.

²Ratio of $\sigma_A^2/\sigma_{htd}^2$ = ratio between animal variance and variance due to herd-test day.

³SCS = log-transformed SCC.

⁴NEm = net energy concentration of the milk sample (Tyrrell and Reid, 1965).

Figure 1. Mean MUN during advancing DIM. ■ = mean MUN from data (means are estimated for classes of DIM). Curve is modeled after the following formula, based on model [1]: $MUN = 22.389 - 0.009 \times DIM - 76.7 \times e^{-0.05 \times DIM}$.

lactose (−0.09), fat (0.19), and protein (0.27). Genetic correlation of MUN with SCS was high (0.85). Standard errors of the estimates were high.

The phenotypic and genetic correlations among MUN and yield traits are in Table 4. Phenotypic correlations were low, ranging from −0.05 to 0.06, with an exception for the correlation of MUN with MUN yield, which was 0.84. Genetic correlations were moderate among MUN and yield traits, ranging from 0.22 to 0.41, except for the correlation of MUN with MUN yield, which was 0.77. Genetic correlations among yield traits were consistently high, ranging from 0.58 for fat with lactose yield, to 0.89 for milk with protein yield.

DISCUSSION

In our study, average MUN was 20.39 mg/100 g of milk. Butler et al. (1996) reported a MUN of 22.8 mg/dL for nonpregnant cows, 21.3 mg/dL for cows later identified pregnant, and overall mean values of 22.3

mg/dL. Other studies reported lower means of around 12 to 13 mg/dL (Wood et al., 2003; Mitchell et al., 2005), although the range was similar to our results (1 to 50 mg/dL). In our study, MUN was measured per 100 g, which is slightly less than 1 dL, though it is considered to be approximately the same unit.

The international use of AI bulls makes it unlikely that the large difference in mean is due to different genetic level of Holstein populations. More likely, the difference is due to feed, because Dutch diets are generally high in protein.

Heritability of milk production traits described in this study were in range of those reported in literature (e.g., Hayes et al., 1984; Ikonen et al., 1999; Wood et al., 2003). For test-day MUN, Mitchell et al. (2005) estimated a heritability for first-parity cows of 0.22 when using infrared spectroscopy, and 0.14 when using wet chemistry techniques to determine MUN, which was equal to the estimate found in our study (0.14). Wenninger and Distl (1993) estimated heritabilities for MUN in 2 German breeds: 0.06 for German Simmental and 0.25 for German Brown. Wood et al. (2003) estimated a higher heritability for infrared-determined MUN of 0.44, using random regression analysis of at least 4 test-day samples per cow with heterogeneous variance structures based on DIM.

Results are sometimes presented as lactation yields based on 305-d production. This 305-d production increases the heritability because residual variance is decreased when taking an overall value of 10 test-days. Recalculating heritabilities based on 305-d production led to heritabilities of 0.17 for MUN and 0.32 for MUN yield in this study.

Phenotypic correlations of MUN with the other traits were low, ranging from −0.06 to 0.11. Broderick and Clayton (1997) found negative phenotypic correlations of MUN with milk yield, fat yield, and NEm. Godden et al. (2001) also found a negative correlation of MUN with milk yield but found a correlation near zero for

Table 3. Phenotypic¹ (below diagonal) and genetic (above diagonal, SE in parentheses) correlations among MUN and percentage of fat, protein, and lactose, SCS, and NEm estimated from 3 morning milk samples of 1,953 primiparous HF cows

Trait	Percentage (%)					
	MUN	Fat	Protein	Lactose	SCS ²	NEm ³
MUN (mg/100 g)		0.19 (0.20)	0.27 (0.19)	−0.09 (0.19)	0.85 (0.19)	0.21 (0.20)
Fat (%)	0.07		0.72 (0.08)	−0.18 (0.14)	0.39 (0.25)	0.99 (0.01)
Protein (%)	0.11	0.51		−0.35 (0.04)	0.27 (0.26)	0.79 (0.06)
Lactose (%)	−0.06	−0.09	−0.07		−0.44 (0.21)	−0.14 (0.14)
SCS ²	0.00	0.06	0.06	−0.24		0.36 (0.25)
NEm ³	0.07	0.98	0.66	0.00	0.04	

¹Phenotypic correlations had SE around 0.02.

²SCS = log-transformed SCC.

³NEm = net energy concentration of the milk (Tyrrell and Reid, 1965).

Table 4. Phenotypic¹ (below diagonal) and genetic correlations (above diagonal, SE in parentheses) among MUN and yield of MUN, fat, protein, lactose and milk, estimated from 3 morning milk samples of 1,953 primiparous HF cows

Trait	MUN (mg/100 g)	MUN (g)	Yield (kg)			
			Fat	Protein	Lactose	Milk
MUN (mg/100 g)		0.77 (0.09)	0.41 (0.19)	0.38 (0.20)	0.22 (0.21)	0.24 (0.22)
MUN yield (g)	0.84		0.66 (0.11)	0.85 (0.06)	0.82 (0.07)	0.87 (0.05)
Fat yield (kg)	0.06	0.46		0.75 (0.08)	0.58 (0.11)	0.59 (0.11)
Protein yield (kg)	0.05	0.64	0.76		0.86 (0.04)	0.89 (0.03)
Lactose yield (kg)	0.00	0.60	0.66	0.91		NE ²
Milk yield (kg)	-0.05	0.68	0.67	0.92	NE ²	

¹Phenotypic correlations had SE around 0.02.²NE = nonestimable.

MUN with fat yield. A weak positive phenotypic correlation of MUN with fat and protein percentages was observed in our study as well as in a few others (Weninger and Distl, 1993; Godden et al., 2001).

We found a strong genetic correlation of milk urea with SCS (0.85). Our genetic correlation was surprising because the phenotypic correlation was weak (0.00). The phenotypic correlation was in line with other studies that demonstrated only a slight increase in nonprotein nitrogen with increasing SCC (Ng-Kwai-Hang et al., 1985), no significant effect of SCC on urea (Eicher et al., 1999), and a negative correlation of -0.01 (Godden et al., 2000). The genetic correlation in our study, however, suggests that the same genetic mechanism is associated with SCS and MUN, e.g., possibly due to changes in protein metabolism during episodes of mastitis.

The very high genetic correlation of fat percentage with NEm (0.99) suggests that these are genetically similar traits. The NEm reflects the energy concentration of the milk and might therefore be related to the energy status of the cow, as is MUN (Jorritsma et al., 2003). The genetic correlation between MUN and NEm, however, seemed low to moderate (0.21) with a high standard error. Results indicate that NEm is almost completely dependent on fat percentage and that protein metabolism and MUN are not strongly related to NEm.

Wood et al. (2003) found a weak genetic correlation of MUN with milk yield (0.11). In our study this correlation was in the same range, although slightly higher (0.24). Wood et al. (2003) found correlations close to zero for MUN with both fat yield (0.01) and protein yield (0.04), whereas in our study the correlations were moderate for MUN with both fat yield (0.41) and protein yield (0.38). The genetic correlation of MUN yield with both fat yield (0.66) and protein yield (0.85) was high, suggesting that yield traits are related.

A number of studies reported significant effects of DIM on MUN, but the direction of the effect was inconsistent. There was an increase of MUN with advancing

DIM from around the second month onward (e.g., Wood et al., 2003), leading to a curve for MUN similar to those for fat and protein percentage. Jonker et al. (1998), however, found a decrease of MUN with advancing DIM from around the second month onward, leading to a curve for MUN similar to that for milk yield. Jorritsma et al. (2003) hypothesized that MUN might be increased under a negative energy balance, suggesting a peak in MUN during early lactation like Jonker et al. (1998). Our data suggest a peak between the second and third month of lactation and a slight decrease in MUN with advancing DIM.

As in our study, Wood et al. (2003) found the effect of herd to be the most significant effect on MUN. This effect might be due mainly to feed differences among herds. It has been suggested that energy and total CP intake (Roseler et al., 1993) and feeding time (Gustafsson and Palmquist, 1993) affect MUN. In our study, herd-test day had small effects on fat percentage (7%) and fat yield (8%), suggesting small effects of feed on fat, whereas other studies have shown an effect of diet on fat (e.g., Keady et al., 2001). Herd-test day includes not only effects of management and feed but also possible effects of sampler, measurement technique, and season of sampling. Season of sampling has been suggested to affect MUN, with MUN being higher in the summer than in winter (Wattiaux et al., 2005).

In most countries protein yield takes an important place in the national selection index (Miglior et al., 2005) and has been associated with an increase in MUN. The present study shows moderate positive genetic correlations of MUN with yield traits, and strong positive genetic correlations of MUN yield with yield traits, suggesting that selection on protein yield indeed leads to an increase in MUN, but standard errors were high. An increase in MUN might have several causes: inefficient ruminal degradation of protein, less efficient protein synthesis in the mammary gland, or changes in conversion processes elsewhere. The use of MUN in new legislation and the possible relationship between

increased MUN and decreased fertility (e.g., Melendez et al., 2000) require a decrease of MUN.

The large coefficient of variation (33%) for MUN in combination with a heritability of 0.14 suggests that there are possibilities to change or control MUN by means of selection. Further research, however, is needed to identify the biological pathways that are affected when selecting for a decreased MUN. The large fraction of the variance due to herd-test day (0.58) indicates that breeding is not the only way to change MUN. Management strategies can play an important role in controlling MUN.

CONCLUSIONS

Our aim was to estimate genetic parameters for MUN and to evaluate its relationships with milk production traits. Heritability of MUN was low. Phenotypic correlations of MUN with milk production traits were close to zero, and genetic correlations were low to moderate and positive. This suggests that selection for milk production traits tends to increase yield of MUN. Results from this study show that it is possible to influence MUN by herd practice (as reflected by the high amount of variation explained by herd-test day) and by genetic selection.

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