

Genetic Analysis of MUN and Lactose and their Relationships with Economically Important Traits in Canadian Holstein Cattle

F. Miglior^{1,2}, A. Sewalem^{1,2}, J. Jamrozik³, G. Kistemaker², D. M. Lefebvre⁴, and R. K. Moore⁴

¹Agriculture and Agri-Food Canada, Dairy and Swine Research and Development Centre, Lennoxville, QC, Canada

²Canadian Dairy Network, Guelph, ON, Canada

³Centre for the Genetic Improvement of Livestock, University of Guelph, Guelph, ON, Canada

⁴Valacta, Ste-Anne-de-Bellevue, QC, Canada

Introduction

Traditional milk recording by DHI organizations collects milk weights and samples for each cow.

Milk samples are sent to the lab for analysis of fat and protein content, and for the count of somatic cells. More recently, DHI labs are analyzing the milk samples also for milk urea nitrogen (MUN) and for the percentage of lactose. The Programme d'Analyse des Troupeaux Laitiers du Québec (PATLQ) has been collecting data in Quebec dairy herds on lactose since 2001 and MUN since 1997. While data on MUN is also being collected in other Canadian provinces, testing for lactose percentage in Canada is currently done exclusively in Quebec by PATLQ. Concentrations of MUN are measured at Canadian DHI labs by infrared technology. Infrared MUN values are calculated from prediction equations that use spectrum analyses and are an indirect measure of MUN. MUN can also be measured by wet chemistry methods, which directly measure concentration of urea nitrogen in milk samples. Because of higher costs of wet chemistry analysis, infrared methodology is commonly used by DHI in Canada.

MUN is a normal nonprotein nitrogen component in milk. Urea is a major end-product of nitrogen metabolism in dairy cows. It is synthesized primarily in the liver and transported in blood to the kidney to be excreted in urine. From the blood, its concentration equilibrates rapidly with other body fluids, including milk (Gustafsson and Palmquist, 1993). Urea originates mainly from excess ammonia released from dietary protein degradation in the rumen or from deamination of amino acids in excess of requirements. Small amounts can also be derived from arginine catabolism in the mammary gland (Nousiainen *et al.*, 2004). MUN has been used

as a non-invasive measurement to monitor the animal's protein status and the efficiency of nitrogen utilization (Broderick and Clayton, 1997; Moore and Varga, 1986; Jonker *et al.*, 1998; Eicher *et al.*, 1999). Concentration of MUN in Ontario herds has been shown to be heritable with low genetic correlations with production traits (Wood *et al.*, 2003). Lower heritability values for MUN have been found in two US studies (Vallimont *et al.*, 2003; Mitchell *et al.*, 2005).

The level of water secretion into milk largely determines the fat and protein content of milk. The rate of water secretion is mostly determined by the rate of lactose synthesis, because lactose is the major factor responsible for the osmolality of milk. Lactose percent has been found highly heritable (0.53) in Holstein cows from Michigan (Welper and Freeman, 1992).

The objectives of this research were to estimate a) heritabilities of MUN and lactose in the first three parities, b) their genetic relationships with milk, fat, protein and SCS, and c) breeding values for MUN and lactose percentage in Canadian Holsteins.

Materials and Methods

Test day records collected from 2001 to 2004 were provided by PATLQ for Holstein cows from Quebec farms. The data included test date, milk yield, fat, protein and lactose percentages, SCC, MUN concentration, times milking, and a flag indicating supervised/unsupervised control. All samples were analysed by mid-infrared spectroscopy using Fossomatic 4000 milk analysers (Foss Electric, Hillerød Denmark) calibrated (wavelength = 9.6 μ m) weekly. Calibration samples were analysed for anhydrous lactose by HPLC (IDF Standard 198 / ISO

22662) and for MUN by pH difference (IDF Standard 195 / ISO 14637). Records from DIM lower than 5 and greater than 305 days were eliminated. Only records from the first 3 parities which contain data for all production traits on a given test day, including lactose and MUN, were kept. If parity 3 was present, parities 1 and 2 were also present, and if parity 2 was present, parity 1 was also present. Data for breeding value estimation included an additional year of calving (2005) and had lower editing restrictions in comparison with the analysis of variance component estimation, as missing traits and/or parities were allowed. Only records from 5 to 305 DIM from the first three parities were included. The final edited data set included 1,317,798 test day records from 245,283 cows from 4691 herds.

In order to estimate variance components, a random sample of test day records was extracted from the original data set. Herds were required to have a minimum of 20 cows in the data set to be randomly selected (approx 5 cows per year). A total of 5,022 cows from 91 herds (average 55 cows / herd) with 60,645 test day records were randomly selected. The total number of animals (cows with own records + pedigree) included 15,532 animals.

Genetic parameters were estimated by Bayesian methods with Gibbs sampling using six separate four-trait random regression test-day models. The six four-trait analyses were performed for the following group of traits:

1. Milk, Lactose percentage, MUN, SCS
2. Milk, Lactose kg, MUN, SCS
3. Fat percentage, Lactose percentage, MUN, SCS
4. Protein percentage, Lactose percentage, MUN, SCS
5. Milk, Fat kg, Protein kg, SCS
6. Milk, Fat percentage, Protein percentage, SCS

All six models required that the four traits used for each analysis were recorded on a given test day. The model for a single trait (milk, fat, protein, SCS, MUN or lactose) can be expressed in matrix notation by the equation:

$$\mathbf{Y}_m = \mathbf{H}_m \mathbf{h}_m + \mathbf{X}_m \mathbf{b}_m + \mathbf{W}_m \mathbf{p}_m + \mathbf{Z}_m \mathbf{u}_m + \mathbf{e}_m$$

where: $\mathbf{Y}_m$ = is a vector of observations from lactations 1, 2 and 3, for trait m ($m=1, \dots, 4$), $\mathbf{h}_m$ = is a vector of fixed herd - test-day effects, $\mathbf{b}_m$ = is a vector of fixed regression coefficients for age/parity /season of calving effects, $\mathbf{p}_m$ = is a vector of random regression coefficients for permanent environmental effects, $\mathbf{u}_m$ = is a vector of random regression coefficients for animal genetic effect, $\mathbf{e}_m$ = is a vector of residuals, and $\mathbf{H}_m$, $\mathbf{X}_m$, $\mathbf{W}_m$, and $\mathbf{Z}_m$ are incidence matrices assigning observations to effects.

Regression curves were modeled using Legendre polynomials of order four, defined as:

$$\begin{aligned} Z_{t0} &= 1.0, Z_{t1} = 3.0^{0.5}x, Z_{t2} = 5.0^{0.5}(1.5x^2 - 0.5), \\ Z_{t3} &= 7.0^{0.5}(2.5x^3 - 1.5), Z_{t4} = 9.0^{0.5}(35x^4 - 30x^2 \\ &+ 3)/8, \text{ where } x = 2(t-5)/300-1 \text{ is standardized time and } t \text{ is days in milk (5 to 305 days).} \end{aligned}$$

The multiple trait model (4 traits at a time) was:

$$\mathbf{Y} = \mathbf{Hh} + \mathbf{Xb} + \mathbf{Wp} + \mathbf{Zu} + \mathbf{e}$$

where

$$\mathbf{y} = [y_1', \dots, y_4']', \mathbf{h} = [h_1', \dots, h_4']', \mathbf{b} = [b_1', \dots, b_4']', \mathbf{p} = [p_1', \dots, p_4']', \mathbf{a} = [a_1', \dots, a_4']' \text{ and } \mathbf{e} = [e_1', \dots, e_4']';$$

$$\mathbf{H} = \sum \mathbf{H}_i, \mathbf{X} = \sum \mathbf{X}_i, \mathbf{W} = \sum \mathbf{W}_i \text{ and } \mathbf{Z} = \sum \mathbf{Z}_i \text{ (direct sum of matrices).}$$

Expectations and covariance structure for random effects can be described in matrix notation as:

$$\mathbf{E}(\mathbf{y}) = \mathbf{Hh} + \mathbf{Xb}, \mathbf{E}(\mathbf{p}) = \mathbf{0}, \mathbf{E}(\mathbf{u}) = \mathbf{0}, \mathbf{E}(\mathbf{e}) = \mathbf{0}$$

and $\mathbf{V}(\mathbf{p}) = \mathbf{P}, \mathbf{V}(\mathbf{a}) = \mathbf{G}, \mathbf{V}(\mathbf{e}) = \mathbf{E},$

where

$\mathbf{P} = \mathbf{I} \otimes \mathbf{P}_0$, $\mathbf{G} = \mathbf{A} \otimes \mathbf{G}_0$ (direct product of matrices), $\mathbf{A}$ is the additive relationship matrix, and $\mathbf{P}_0$ and $\mathbf{G}_0$ are 60 x 60 covariance matrices for environmental and genetic regression coefficients, respectively. Finally, $\mathbf{E}$ is a block-diagonal residual covariance matrix consisting of different co-variances for each combination of parity and interval of day in milk. These intervals were defined as 5 to 45, 46 to 115, 116 to 205, and 206 to 305 days in milk. Random effects of the model were assumed to be normally distributed, and the conditional

distribution of the data was also normal. In addition, residual effects were assumed to be independent between cows and different DIM. Flat prior distributions were assumed for all fixed effects and inverted Wishart distributions were used as priors for covariance matrices. Prior values for covariances were as estimated previously from Holstein data (Muir and Kistemaker, 2004) and they were used with minimal number of degrees of freedom. Posterior means of (co)variance components were estimated using 90,000 samples after a burn-in of 10,000 samples for each four-trait model. Daily heritability was defined as a ratio of genetic variance to the sum of genetic, permanent environmental (PE) and residual variances for each day in milk from 5 to 305 days, and averaged across the entire lactation for each of the first three lactations. Genetic correlations were calculated using (co)variances of the first regression coefficients as described by Wood *et al.* (2003). Breeding values for milk yield, lactose percentage, MUN and SCS were estimated using a 4-trait multiple-lactation random regression test-day animal model on the full edited data set. Variance components estimated with model 1 were used. The model for breeding value estimation was equivalent to the model used for parameter estimation with the addition of genetic groups, defined by country, sex and year of birth. Pearson correlations between EBV of all traits evaluated in Canada with lactose and MUN EBV were then estimated for bulls born after 1990 with at least 20 daughters with lactose and MUN records.

Results and Discussion

Descriptive statistics of the sample (N=5022) for all production traits by parity are shown in Table 1. Table 2 shows raw phenotypic correlations among all traits in the original edited data set. Phenotypically, MUN was low correlated with all traits, while lactose percentage was positively correlated with yield traits and negatively correlated with percentages, as well as with SCS. Lactose yield followed the same pattern of lactose percentage, but with much higher phenotypic correlations with yield traits. Phenotypic correlation between lactose yield and percentage was moderate and positive (.498).

Heritabilities and correlations for some trait-pair combinations were averaged across separate four-trait analyses. Original heritabilities and correlations (genetic and permanent environment) resulted from the 6 separate four-trait analyses are in Miglior *et al.* (Unpublished data, 2006). Average heritabilities for all traits are presented in Table 4 for each parity. Heritabilities for lactose yield and percentage were high, ranging from 0.466 to 0.539. There are very few genetic studies on lactose. Welper and Freeman (1992) using a lactation model estimated heritability of .26 for lactose yield and .53 for lactose percent in Michigan Holstein cows. Heritabilities for MUN (0.384 to 0.414) and SCS (0.189 to 0.338) were lower. Wood *et al.* (2003) estimated higher heritabilities for MUN in Ontario herds, ranging from .44 to .59. They used the same model and software used in this study. However, because of short period of data collections (less than 2 years), lactations 1 to 3 were analyzed separately. Heritabilities for Lactose percentage and SCS tended to increase over parities. There was no clear pattern across lactations for MUN and lactose percentage. As expected, heritabilities for fat (0.533 to 0.555) and protein (0.561 to 0.586) percentages were markedly higher than heritabilities for fat (0.350 to 0.369) and protein (0.392 to 0.423) yield. Overall heritabilities for protein tended to be higher than correspondent heritabilities for fat. Heritabilities for milk yield were very close to heritabilities of lactose kg.

Table 4 shows genetic correlations among production traits. Correlations were averaged across lactations and separate four-trait analyses. Correlations for some trait-pairs are missing as they were not covered by the 6 separate four-trait analyses. MUN had a moderate and positive genetic correlation with fat percentage (0.425) and lower with protein percentage (0.200). MUN had a desirable negative genetic correlation with SCS (-0.190). Wood *et al.* (2003) found low genetic correlations of MUN with production traits (-.05 to .32). All genetic correlations between lactose percentage and other traits were close to zero, with one exception: a genetic correlation of -.202 with SCS. Lactose yield seemed to be genetically the same trait as milk yield, as their genetic correlation was very close to 1 (0.979). In a

study by Welper and Freeman (1992) lactose percentage was negatively correlated with volumes of milk, fat and protein (-.16 to -.30) and SCS (-.11), and positively correlated with fat and protein percentages (.16 to .29). The same investigation found a small genetic correlation between lactose yield and percent (.10) and high correlations between lactose yield with milk (.92), fat (.68) and protein yield (.89), and negatively correlated with fat (-.35) and protein percent (-.37). Similar pattern in genetic correlations were found by Roman and Wilcox (2000) in Jerseys from a Florida experimental farm. As expected, negative genetic correlations were found in the current study between milk yield with fat and protein percentages, -0.494 and -0.538, respectively. There are no prior estimates in literature from a test day model.

Average genetic correlations among parities within trait are shown in Table 5. Correlations among parities for MUN, lactose percentage and yield were quite high like for milk yield. Genetic correlations among parities for SCS were again quite low, confirming previous studies carried out in Canada (Jamrozik *et al.*, 1998; Muir and Kistemaker, 2003). Genetic correlations among parities for fat and protein percentages were much higher than correspondent values for fat and protein yield.

Table 6 shows bull EBV correlations higher than .10 or lower than -.10 between the trait of interest and MUN and lactose percentage. Highest correlation for MUN was with fat percentage (.287), while for lactose percentage was with lactation persistency (.329).

Conclusion

Average daily heritabilities were moderately high for MUN (0.384 - 0.414), lactose yield (0.466 to 0.539), and lactose percentage (0.478 to 0.508). Lactose yield was highly correlated with milk yield (0.979). Lactose percentage and MUN were not genetically correlated with milk yield. However, lactose percentage was significantly correlated to SCS (-0.202). MUN was correlated with fat (0.425) and protein percentages (0.20). Genetic correlations among parities were high for MUN, lactose percentage and yield. Bull EBV of MUN were correlated with fat percentage EBV (.287), while lactose

percentage EBV were correlated with persistency EBV (.329). Research is underway to assess the relationships between MUN and lactose percentage with fertility traits.

References

- Broderick, G.A. & Clayton, M.K. 1997. A statistical evaluation of animal and nutritional factors influencing concentrations of milk urea nitrogen. *J. Dairy Sci.* 80, 2964–2970.
- Eicher, R., Bouchard, E. & Bigras-Poulin, M. 1999. Factors affecting milk urea nitrogen and protein concentrations in Quebec dairy cows. *Prev. Vet. Med.* 39, 53–63.
- Gustafsson, A.H. & Palmquist, D.L. 1993. Diurnal variation of rumen ammonia, serum urea and milk urea in dairy cows at high and low yields. *J. Dairy Sci.* 76, 475–484.
- Jamrozik, J., Schaeffer, L.R. & Grignola, F. 1998. Genetic parameters for production traits and somatic cell score of Canadian Holsteins with multiple trait random regression model. *6WCGALP.* 23, 303–306.
- Jonker, J.S., Kohn, R.A. & Erdman, R.A. 1998. Using Milk Urea Nitrogen to Predict Nitrogen Excretion and Utilization Efficiency in Lactating Dairy Cows. *J. Dairy Sci.* 81, 2681–2692.
- Mitchell, R.G., Rogers, G.W., Dechow, C.D., Vallimont, J.E., Cooper, J.B., Sander-Nielsen, U. & Clay, J.S. 2005. Milk urea nitrogen concentration: heritability and genetic correlations with reproductive performance and disease. *J. Dairy Sci.* 88, 4434–4440.
- Moore, D.A. & Varga, G. 1986. BUN and MUN: Urea nitrogen testing in dairy cattle. *Compend. Food Anim.* 18, 712–720.
- Muir, B. & Kistemaker, G.J. 2003. Estimation of genetic parameters for the Canadian test-day model with Legendre polynomials for coloured breeds based on more recent data. <http://www.cdn.ca/committe.htm>. Accessed November 11, 2004.
- Nousiainen, J., Shingfield, K.J. & Huhtanen, P. 2004. Evaluation of milk urea nitrogen as a diagnostic of protein feeding. *J. Dairy Sci.* 87, 386–398.
- Vallimont, J.E., Rogers, G.W., Holden, L.A., O'Connor, M.L., Cooper, J.B., Dechow, C.D. & Clay, J.S. 2003. Milk urea nitrogen and conception rate: a population study using test

day records. *J. Dairy Sci.* 81(Suppl. 1), 239. (Abstr.)

Welper, R.D. & Freeman, A.E. 1992. Genetic parameters for yield traits of Holsteins, including lactose and somatic cell score. *J. Dairy Sci.* 75, 1342-1348.

Wood, G.M., Boettcher, P.J., Jamrozik, J., Jansen, G.B. & Kelton, D.F. 2003. Estimation of genetic parameters for concentration of milk urea nitrogen. *J. Dairy Sci.* 86, 2462-2469.

Table 1. Descriptive statistics of test day records in random sample used for variance component estimation (N=5,022 cows).

Parity	Test day		Milk kg		Fat %		Protein %		SCC '000/ml		MUN mg/dL		Lactose %	
	records	Cows	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
1	30,432	5,022	27.2	6.3	3.81	0.64	3.31	0.31	169	496	10.63	3.39	4.71	0.17
2	20,599	4,658	31.9	8.9	3.78	0.67	3.31	0.34	229	593	11.19	3.41	4.58	0.20
3	9,614	3,479	33.9	9.6	3.79	0.67	3.27	0.35	286	724	11.26	3.40	4.53	0.23
Total	60,645	5,022	29.9	8.3	3.80	0.66	3.31	0.33	170	499	10.92	3.41	4.63	0.21

Table 2. Phenotypic correlations among production traits.

	Fat (kg)	Protein (kg)	Fat (%)	Protein (%)	SCS	MUN	Lactose (kg)	Lactose (%)
Milk	0.787	0.936	-0.362	-0.520	-0.083	-0.031	0.989	0.252
Fat (kg)		0.805	0.260	-0.250	-0.058	0.083	0.771	0.161
Protein (kg)			-0.238	-0.205	-0.062	-0.011	0.921	0.208
Fat (%)				0.470	0.045	0.182	-0.370	-0.168
Protein (%)					0.093	0.082	-0.529	-0.248
SCS						-0.100	-0.110	-0.227
MUN							-0.042	-0.072
Lactose (kg)								0.382

Table 3. Average heritabilities by parity.

	Parity		
	First	Second	Third
Milk	0.518	0.431	0.468
Fat (kg)	0.369	0.350	0.362
Protein (kg)	0.423	0.392	0.410
Fat (%)	0.555	0.533	0.543
Protein (%)	0.576	0.561	0.586
SCS	0.189	0.272	0.338
MUN	0.394	0.384	0.414
Lactose (kg)	0.539	0.466	0.490
Lactose (%)	0.478	0.506	0.508

Table 4. Average genetic correlations among production traits (averaged across analyses and lactations)

	Fat (kg)	Protein (kg)	Fat (%)	Protein (%)	SCS	MUN	Lactose (kg)	Lactose (%)
Milk	0.566	0.894	-0.494	-0.538	0.001	-0.094	0.979	0.096
Fat (kg)		0.684	---	---	-0.045	---	---	---
Protein (kg)			---	---	0.014	---	---	---
Fat (%)				0.659	-0.109	0.425	---	0.058
Protein (%)					0.009	0.200	---	0.017
SCS						-0.190	-0.024	-0.202
MUN							-0.092	-0.041
Lactose (kg)								---

Table 5. Genetic correlations among parities

	Parity		
	1 vs. 2	1 vs. 3	2 vs. 3
Milk	0.786	0.737	0.897
Fat (kg)	0.741	0.603	0.791
Protein (kg)	0.739	0.585	0.793
Fat (%)	0.961	0.887	0.951
Protein (%)	0.887	0.863	0.838
SCS	0.543	0.395	0.562
MUN	0.846	0.744	0.870
Lactose (kg)	0.807	0.714	0.892
Lactose (%)	0.831	0.760	0.845

Table 6. Bull EBV correlations between MUN and lactose percentage with other traits ($r \geq \text{absolute}(.10)$)

	Lactose (%)		MUN
SCS	-0.164	Milk	-0.106
Mammary System	0.100	Protein (%)	0.144
Milk	0.101	Fat (kg)	0.208
Median Suspensory	0.112	Fat (%)	0.287
Udder Depth	0.128		
LPI	0.139		
Persistency	0.329		

