Genetic Relationships Between Milk Fatty Acids and Fertility of Dairy Cows

C. Bastin[1], H. Soyeurt[1,2], S. Vanderick[1], and N. Gengler[1,2]

[1] *Animal Science Unit, Gembloux Agro-Bio Tech, University of Liège, B-5030 Gembloux, Belgium*
[2] *National Fund for Scientific Research, B-1000 Brussels, Belgium*

Introduction

Fertility traits are potentially difficult to measure, they are often not readily available and they have low heritabilities. Consequently, indicators traits are of interest to increase accuracy of estimated breeding values for fertility as long as these traits are easier to measure, have higher heritabilities, and are well correlated with fertility. Previous studies presented the opportunity to use correlated traits in genetic evaluation of fertility such as milk, fat, protein yields, body condition score, or type traits (Wall *et al.,* 2003; de Jong, 2005). Furthermore milk fatty acid (**FA**) profile has been suggested to be related to energy balance status of dairy cows in early lactation (Stoop *et al.,* 2009) and could therefore be considered as an indicator trait for fertility.

The objective of this study was to investigate the opportunity to use FA traits as indicators for fertility in genetic evaluations. First genetic correlations between fertility and production traits and contents in milk of 17 groups and individual FA were estimated. Second, effects of including FA EBVs available in the Walloon Region of Belgium (dUNSAT and dMONO) within the combined female fertility index (**CFF**) were studied.

Materials and Methods

Estimation of genetic correlations

Data. Daily milk yield (kg), fat yield (kg), protein yield (kg), fat content (g/dl of milk), protein content (g/dl of milk), and days open (**DO**) records of first-parity Holstein cattle were from the routine Walloon genetic evaluation. Predicted contents (g/dl of milk) of individual or groups of FA used in this study are listed in Table 1. They were predicted by applying the calibration equations developed by Soyeurt *et al.* (2011) on spectra generated during routine milk recording. The 7 FA groups are saturated FA

(**SFA**), unsaturated (**UFA**), monounsaturated (**MUFA**), polyunsaturated (**PUFA**), short chain fatty acids (**SCFA**) including FA with 4 to 10 carbons, medium chain fatty acids (**MCFA**) including FA with 12 to 16 carbons, and long chain fatty acids (**LCFA**) including FA with 17 to 22 carbons. To eliminate potentially abnormal records, FA values below the first percentile and above the 99th percentile were deleted. Finally cows were required to have both days open record and all production and FA records (for at least 2 test-days). Final dataset included 143,332 FA and production records and 29,792 DO records from 29,792 cows in 1,170 herds. Pedigree data were limited to animals born after 1985, retaining 91,032 animals.

Model. A total of 22 two-trait models were run:

$$\mathbf{y} = \mathbf{X\beta} + \mathbf{Hh} + \mathbf{Ww} + \mathbf{Zp} + \mathbf{Za} + \mathbf{e}$$

where **y** was the vector of observations (DO and one of the production or FA traits)**; β** was the vector of the following fixed effects: for production and FA traits, 1) herd × test-day, 2) gestation stage, 3) minor lactation stage (classes of 5 DIM), and 4) major lactation stage (classes of 73 DIM) × age at calving × season of calving class of 14 DIM; for DO, 1) herd, 2) year × month of calving, 3) age at calving × season of calving; **h** was the vector of the herd × year of calving random effect for DO, **w** was the vector of herd × period of calving random regression coefficients for FA and production traits, **p** was the vector of permanent environmental random effect for DO and the vector of permanent environmental random regression coefficients for production traits and FA, **a** was the vector of additive genetic random regression coefficients for FA and production traits and the vector of additive genetic random effect for DO, **e** was the vector of residuals and **H, X, W, Z** were incidence matrices. Regression curves were modeled using Legendre polynomials of second-order. Covariance matrices for environmental and genetic effects combined the variance for

DO, the (co)variance for random regression components for FA or production traits, and the covariance between DO and random regression components for FA or production traits. Random effects were assumed to be normally distributed and residual variances were assumed to be independent and constant over the lactation. Variance components estimation was performed using Gibbs sampling.

Integration of FA breeding values in CFF

Currently in the Walloon Region of Belgium, development of genetic evaluations for FA focuses on SFA and MUFA (Gengler *et al.,* 2010). EBVs for these traits are estimated as dUNSAT and dMONO, two indices that represent the relative part of milk fat that is unsaturated or mono-unsaturated.

Furthermore, the female fertility index published in the Walloon Region of Belgium (Vanderick *et al.,* 2009) is composed of 2 sub-indexes: 1) the direct female fertility index (**DFF**) which is a linear combination of the Interbull international female fertility proofs available on the Walloon scale, and 2) the indirect female fertility index (**IFF**) which is a linear combination of breeding values of fertility-correlated traits. Traits used for the definition of IFF are milk yield, protein yield, somatic cell score (**SCS**), stature, body depth, overall udder score, overall feet and legs score, final conformation score and body condition score (**BCS**) or angularity if BCS is not available. Two IFFs are therefore defined: IFF_{BCS} and IFF_{ANG}. All indexes were updated in November 2010 to integrate changes related to the new definition of EBVs for BCS (Bastin et al., 2010) using the methodology described by Vanderick *et al.* (2009) on a dataset including 604 bulls that had female fertility indexes in June 2010 in 6 foreign countries (Canada, France, Germany, Italy, the United States and the Netherlands). The same data set was used to investigate the opportunity to include dUNSAT and dMONO as additional indicator traits within IFF_{BCS} and IFF_{ANG} defining two new indices that included dUNSAT and dMONO: IFF_{BCS-FA} and IFF_{ANG-FA}. Then IFF_{BCS-FA} and IFF_{ANG-FA} were combined with DFF into CFF_{FA} using the same coefficient as for CFF. Finally, changes in reliability were assessed and sub-indexes were compared for a total of 779 bulls that had values for all indexes.

Results and Discussion

Genetic parameters

Descriptive statistics and lactation heritabilities of studied traits are presented in Table 1. Heritability for DO presented in Table 1 was the average heritabilities through the 22 analyses. Heritability for DO was similar to estimation by Mayeres *et al.* (2006) for pregnancy rate. However heritabilities for milk, fat, and protein yields were slightly lower than those used in routine genetic evaluations. Lactation heritabilities for FA ranged between 0.52 for C18:1 *cis-9* and 0.70 for C17:0. Results showed that *de novo* synthetized FA (C4:0 to C14:0 and half of C16:0) had greater heritabilities than FA originating from the diet and from body fat mobilization (LCFA and PUFA). This is in line with previous studies (Bastin *et al.,* 2011; Stoop *et al.,* 2007).

Table 1. Mean and standard deviation of DO (n=29,792) and production and FA (expressed in g/dl of milk) traits (n=143,332); lactation heritabilities (h²) and lactation correlations with DO (r_{DO}) are also presented.

Traits	Mean	SD	h²	r_{DO}
DO	147	83	0.05	-
Milk (kg)	23.08	5.99	0.31	0.51
Fat (kg)	0.904	0.226	0.29	0.42
Protein (kg)	0.765	0.187	0.29	0.38
Fat (%)	3.964	0.544	0.68	-0.15
Protein (%)	3.343	0.324	0.67	-0.34
SFA	2.793	0.461	0.68	-0.12
MUFA	1.129	0.206	0.58	-0.15
PUFA	0.167	0.032	0.69	-0.16
UFA	1.310	0.226	0.60	-0.16
SCFA	0.348	0.063	0.68	-0.10
MCFA	2.134	0.412	0.68	-0.13
LCFA	1.625	0.307	0.56	-0.13
C4:0	0.106	0.018	0.63	-0.03
C6:0	0.074	0.013	0.67	-0.07
C8:0	0.046	0.009	0.68	-0.11
C10:0	0.109	0.027	0.68	-0.15
C12:0	0.132	0.035	0.69	-0.18
C14:0	0.467	0.087	0.68	-0.13
C16:0	1.236	0.269	0.67	-0.11
C17:0	0.030	0.004	0.70	-0.20
C18:0	0.407	0.093	0.60	-0.06
C18:1 *cis-9*	0.803	0.167	0.52	-0.13

Lactation genetic correlations between DO and production traits and FA contents in milk are presented in Table 1. Daily genetic correlations
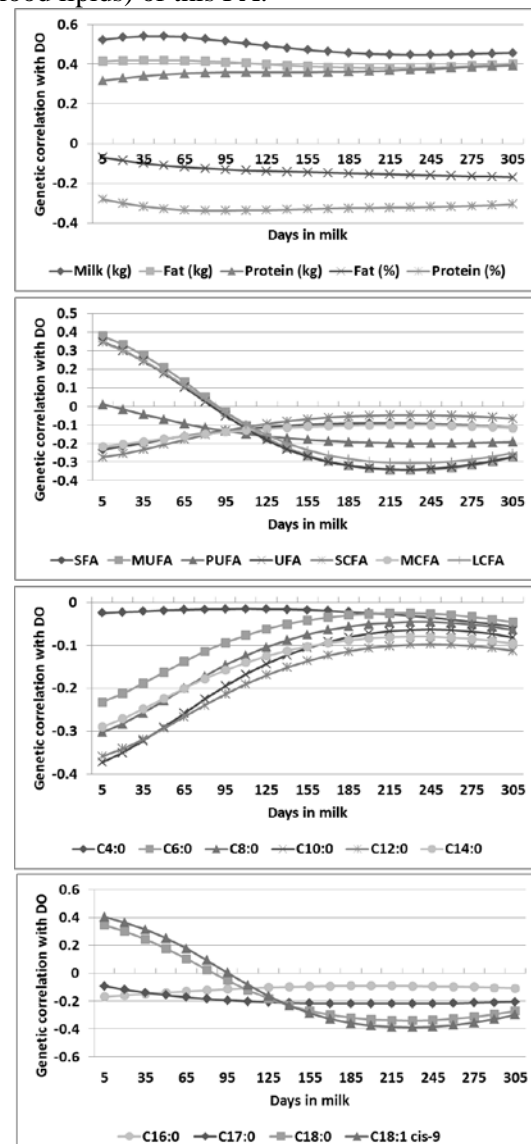
between DO and production traits and FA contents in milk are presented in Figure 1. Daily genetic correlations between DO and yields were positive and did not change greatly over DIM. They ranged between 0.45 and 0.54 for milk yield, between 0.36 and 0.42 for fat yield, and between 0.32 and 0.39 for protein yield. Lactation correlations with DO were 0.51 for milk yield, 0.42 for fat yield, and 0.38 for protein yield. This is in agreement with previous studies indicating that selection for higher yields would decrease fertility performances (e.g. Veerkamp *et al.,* 2001).

Although genetic correlations between fat content in milk and DO was low, negative, and stable along the lactation (they ranged from -0.17 to -0.07), genetic correlations between DO and FA contents in milk varied over the lactation. For UFA, MUFA, LCFA, C18:0, and C18:1 *cis-9*, genetic correlations with DO were positive in early lactation and became negative after 100 DIM. For the other groups and individual FA, genetic correlations with DO were negative along the whole lactation. The great variation of daily correlations along the lactation explained why lactation heritabilities between DO and FA contents in milk were low and ranged between -0.20 and -0.03. Patterns of genetic correlations between fertility and FA contents in milk could be related to cows' physiology in early lactation.

First, at initiation of lactation, cows are in negative energy balance, causing mobilization of adipose FA and incorporation of C18 FA in milk (Palmquist *et al.,* 1993; Barber *et al.,* 1997). Therefore the FA composition of milk has a much higher proportion of C18:0 and C18:1 *cis-9* when lipolysis is high (i.e. when the cow is in negative energy balance). Consequently higher contents of C18:0 and C18:1 cis-9 in milk could be associated to negative energy balance and therefore to poor fertility performances. Genetic correlation at 5 DIM was 0.40 between DO and C18:1 *cis-9* and 0.35 between DO and C18:0 indicating that higher contents of C18 FA in milk is related to higher number of days when the cow is not pregnant.

Second, concomitant with the release of adipose FA in milk in early lactation, the high uptake of LCFA inhibits *de novo* synthesis of FA by mammary gland tissue through the inhibition of acetyl-coenzyme A carboxylase. This inhibition increases with increasing chain lengths

(Palmquist *et al.,* 1993). Lower contents of C6:0 to C14:0 FA in milk could therefore be associated with high body fat mobilization and thus with poor fertility performances. Genetic correlations at 5 DIM between DO and C6:0 to C14:0 ranged between -0.37 for C10:0 and -0.23 for C6:0. Furthermore, the synthesis of C4:0 is not inhibited in early lactation because it originates in pathways independent of the acetyl coenzyme A carboxylase pathway. Therefore the genetic correlation between DO and content of C4:0 in milk was close to 0. Finally, genetic correlation between C16:0 content in milk and DO was slightly negative and did not vary a lot along the lactation. This is probably due to the double origin (*de novo* synthesis and circulating blood lipids) of this FA.

Figure 1. Daily genetic correlations between days open (DO) and production traits and contents of fatty acids (FA) in milk (g/dl of milk).

Table 2. No. of bulls in 6 classes of reliabilities for IFF_{BCS}, IFF_{BCS-FA}, IFF_{ANG}, IFF_{ANG-FA}, CFF, and CFF_{FA}.

Classes of reliability	IFF_{BCS}	IFF_{BCS-FA}	IFF_{ANG}	IFF_{ANG-FA}	CFF	CFF_{FA}
rel < 0.75	1	1	1	1	15	13
0.75 <= rel < 0.80	16	13	5	1	11	11
0.80 <= rel < 0.85	54	42	20	13	23	20
0.85 <= rel < 0.90	162	118	84	55	34	34
0.90 <= rel < 0.95	455	501	509	535	214	195
rel => 0.95	91	104	160	174	482	506

After 150 DIM, genetic correlations between DO and contents of FA in milk were all negative and ranged between -0.38 at 230 DIM for C18:1 *cis-9* at 230 DIM to -0.02 for C4:0 at 150 DIM. These correlations indicated that selection for higher contents in milk of MUFA and LCFA in mid to late lactation would improve fertility. Finally PUFA content in milk was not highly associated to fertility along the lactation.

Integration of dUNSAT and dMONO in CFF

Table 2 presents the number of bulls in 6 classes of reliabilities for the current fertility indexes (IFF_{BCS}, IFF_{ANG}, and CFF), and the new indexes that included FA traits (IFF_{BCS-FA}, IFF_{ANG-FA}, and CFF_{FA}). Results indicated that on average, reliabilities of indexes increases when FA traits were included in the index. Results in Table 2 indicate that the number of bulls with reliabilities greater than 0.90 increased from 545 to 605 when FA were included in IFF_{BCS} and from 669 to 709 in IFF_{ANG}.

Conclusion

Results of this study indicated that FA contents in milk could be used as indicators traits in genetic evaluation for fertility. Contents of FA in milk had high lactation heritabilities: from 0.52 to 0.70. Genetic correlations between FA contents in milk and DO varied over the lactation and could be related to the energy balance status of cows in early lactation. The highest correlations with DO were observed for C18:1 *cis-9* (which is an indicator of body fat mobilization): 0.42 at 5 DIM and -0.40 at 230 DIM. New fertility indicators traits such as C18:1 *cis-9* content in milk at 5 DIM or at 230 DIM could be defined in order to take into account changes of genetic correlations along the lactation. However selection for improved C18:1 *cis-9* content at 230 DIM should be preferred towards the objective to select for cows that are more fertile and that produce milk with higher contents of MUFA. Finally, the opportunity of including FA traits in the current Walloon fertility index was also investigated and indicated that including dUNSAT and dMONO within the IFF index would increase its reliability.

Acknowledgments

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