Genetic Parameter Estimation for Milk Fatty Acids in three French dairy Cattle Breeds

A. Gion[1], H. Larroque[2], M. Brochard[3], F. Lahalle[3 4], D. Boichard[1]
[1] INRA, UMR1313 GABI, 78350, Jouy-en-Josas, France
[2] INRA, UR631 SAGA, 31326,Castanet, France
[3] Institut de l'Elevage, Paris, France
[4] CNIEL, Paris, France
armelle.gion@jouy.inra.fr

Abstract

Genetic parameters of milk composition in fatty acids (FA) were estimated in the three French Montbeliarde (MO), Normande (NO) and Holstein (HO) cattle breeds. Data originated from the PhénoFinlait project and from the national genetic database. They contained 101,858 test-day records from 21,947 cows in first lactation. Each test-day was characterized by its profile in FA in milk, as estimated from Mid-Infrared (MIR) spectrometry. Three different models were used to analyze either individual test-day data, or lactation means. Test-day saturated FA (SAT) had higher heritability estimates (from 0.16 to 0.44) than unsaturated FA (UNSAT). FA measurements were highly genetically correlated across different stages of lactation except in the beginning of the lactation. Genetic correlations between FA in milk were positive across SAT, also positive across UNSAT, and negative between SAT and UNSAT. SAT were positively correlated to fat content in milk. The definition of the traits, expressed in percentage of milk or fat, was discussed.

Introduction

For a long time, milk of cows, goats and ewes has been strongly used in human food. Although the image of milk and dairy products is still very good, nutritional concern increases about the nature of milk fat. Cow milk fat contains a low proportion of UNSAT and a high proportion of short and medium-chain SAT, mainly C14:0 and C16:0. Both have been associated with increased levels of cholesterol and increased risk of cardiovascular disease (Stoop et al. 2008).

In France, a number of organizations formed a consortium to carry out a large scale project called PhénoFinlait (PFL). Its main objective is to study the fine composition of milk in FA and proteins in bovine, ovine and caprine species. In the framework of this project, equations were developed to predict about 80 FA (or ratio and group of FA) from MIR spectra (Ferrand et al., 2010). These equations were then used to build a large data base for fine composition of milk predicted from MIR spectra collected on a large scale in routine milk analysis laboratories. Additional surveys were carried out by milk recording technicians to record management and feeding systems. And technicians from AI center especially collected blood sample of about 18,000 females. For bovine, 8,000 cows were selected to be genotyped, to detect Quantitative Trait Locus (QTL) and pave the way for a genomic evaluation on milk FA composition.

Before including molecular information, the first compulsory step is a good understanding of the genetic determinism of the traits through conventional genetic parameters.

Material and Methods

Animal and Milk samples

More than 425,000 milk samples were collected from 86,458 cows in 1,023 herds, between November 2009 and December 2010. Cows were distributed into the three main French dairy breeds, *i.e.* MO, NO and HO. Herds were selected according to their geographical locations (16 regions), their history in milk recording, their size and their number of potential PFL cows. A PFL cow was defined as a cow with first or second calving in end 2009 or beginning 2010 and born from one of a list of preselected AI young bulls. For each herd enrolled in the PFL project, the data base contains information of 5 to 6 PFL test-day (*i.e.* in addition to milk recording, milk MIR spectra and animal feeding information were collected) for all the milking cows (PFL or not). In addition, the other test-day of the same lactations were extracted from the national genetic database. Data were then edited according to the following criteria: days in milk (DIM) between 8 and 350 days (370 in HO), at least 3 test-day in total and 2 PFL test-day in the lactation, first lactation and known sire.

Statistical Models

Variance components and genetic parameters were estimated by using several animal models with the REML software WOMBAT (Meyer, 2006), . Model 1 was a single-trait mixed model with a permanent environment effect:

$$\mathbf{y} = \mathbf{X\ß} + \mathbf{Za} + \mathbf{Zp} + \mathbf{e}$$

where **y** is the vector of test-day observations, **ß** is the vector of fixed effects: herd * test-day, stage of lactation, month of calving * year of calving, **a** is the vector of random genetic effect normally distributed $N(\mathbf{0}, \mathbf{G}\sigma_g^2)$, **p** is the vector of random permanent environment effect $N(\mathbf{0}, \mathbf{I}\sigma_p^2)$ and **e** is the vector of random residual $(\mathbf{0}, \mathbf{I}\sigma_e^2)$. **X** and **Z** are incidence matrices.

Model 2 was used to assess if the different measures over the lactation are repeated expressions of the same trait, or expressions of different traits. The lactation was divided into 4 periods (T1 to T4): 8-50 days, 51-120 days, 121-200 days and 201-350 days. Information after 350 days of lactation was deleted for the three breeds. Each period was considered as a trait and the four traits were analyzed together in a multivariate analysis for each FA. The multi-trait mixed model included the same fixed and random effects as model 1.

Model 3 addressed the genetic relationship between FA. This analysis was carried out with average measures of FA along the lactation. Individual FA at each test-day were first adjusted for lactation stage (with the GLM procedure of SAS) and then averaged over the lactation. The average was obtained by using milk yield or fat content as weights. Then the model used included the fixed effect of the herd and year*month of calving, and a random genetic effect.

Expression of FA content

Calibration equations provide FA concentration in milk (expressed in g/dL of milk). Using the density of milk (1.030 g/cm^3), these FA contents were expressed in grams per 100g of milk. Using the fat content, these FA contents in milk were converted into contents in milk fat (g/100g of fat). FA predictions were adjusted for absorbance drift of the instrument, thanks to reference samples with known FA content analyzed each month on each instrument. To avoid outliers, data deviating from more than 3 standard deviations were discarded.

Results and Discussions

Impact of units

FA could be expressed in proportion of fat or milk. Whereas the first one just illustrates the FA proportion in fat, the second one accounts for the total amount of fat in milk. FA in fat of the diet is often considered by the milk industry and nutritionists as the trait of interest for human health. However, a milk sample produced by one cow is not representative of the diet. A more important parameter is the balance of FA in the resulting bulk milk of a farm or even a region, and this balance not only depends on FA balance in individual milk samples but also on the contribution of each milking to the overall pool, which also depends of fat concentration in milk (and also on milk quantities). Therefore we consider that FA in milk is more relevant for genetic improvement because it is closer to the final parameter of interest, the FA balance in the total fat of the human diet.

Heritability

Tables 1 and 2 summarize heritability estimates with model 1 (single trait mixed-model with repeated data) for the three breeds. Data in g/100g of milk and in g/100g of fat were analyzed; standard errors were always under 0.05 and therefore were not reported in the table.

For each breed and for the majority of SAT, contents in milk were more heritable than contents in fat, except for C4:0 and C6:0. Similar observations were made in previous studies on milk composition.

Table 1: heritability of test-day FA - in g/100g milk

	MO	NO	HO
SAT	0.32	0.34	0.28
MONO	0.13	0.18	0.15
POLY	0.21	0.24	0.22
UNSAT	0.11	0.14	0.14
C16:0	0.32	0.34	0.28
C14:1c9	0.40	0.38	0.32
C18:1c9	0.13	0.16	0.11
C18:2c9t11	0.17	0.14	0.11

Table 2: heritability of test-day FA - in g/100g fat

	MO	NO	HO
SAT	0.22	0.19	0.16
MONO	0.22	0.20	0.15
POLY	0.24	0.23	0.20
UNSAT	0.23	0.14	0.13
C4:0	0.44	0.41	0.31
C16:0	0.27	0.18	0.21
C14:1c9	0.35	0.22	0.29
C18:1c9	0.21	0.19	0.17
C18:2c9t11	0.18	0.15	0.14

In this study, results show higher heritability for SAT than UNSAT. This result could be partially explained by the dual origin of the FA: short (C4:0 to C10:0) and a fraction of medium chain SAT (C12:0, C14:0, C16:0) are de novo synthesized in the mammary

gland from blood precursors and regulated by two enzymes. Other FA with long chain, for example C18:1 or C18:2cis9cis12, are provided by the cow's diet and are hydrogenated in the rumen by bacteria and transported by the blood. Some SAT and MONO could have both origins such as C16:0. Differences in diet nature have more consequences on long-chain FA than short-chain FA.

Heritability estimates also depend on the definition of the traits and on the model. Considering measures at individual test-day or averaged over the lactation also leads to different heritability estimates. The mean has a smaller residual variance and, therefore, a higher heritability. Their values could not be directly compared.

Genetic determinism along the lactation

Model 2 was used to assess if the different measures over the lactation are repeated expression of the same trait, or expression of different traits. Heritability estimates generally increased with DIM and were highest between 201 and 350 days compared to the beginning of the lactation, mostly because of a decrease in residual variance. This difference between T1 and T4 was less important for FA content in milk than in fat. MO and HO were very different for SAT but the genetic determinism seemed to change for these two breeds during the lactation.

Genetic correlations between traits for MO and HO varied according to traits and units of expression. Some of them are described in the table 3 . For example total content of MONO had higher correlations when expressed in milk than in fat. In contrast, all correlations for C14:0 were very high, whatever the breeds and the definition of the trait. Total of POLY in fat or milk also presented high correlations in most cases.

Table 3: genetic correlations over the lactation

	T1-T2	T2-T3	T3-T4
C14:0 in g/100g milk			
MO	0.657	0.927	0.917
HO	0.685	0.747	0.964
C14:0 in g/100g fat			
MO	0.966	0.967	0.965
HO	0.684	0.913	0.963

The genetic correlations across periods were always positive and generally high, reflecting a strong common genetic determinism. Nevertheless, the beginning of the lactation appeared to be different, with lower correlations with the rest of the lactation. This reflects a different determinism, associated to fat mobilization in adipose tissue, particularly important in the beginning of the lactation and limited thereafter. And fat mobilization is not genetically associated to fat synthesis in milk.

Genetic correlations between fatty acids

The table 4 presents genetic correlation estimates between the main FA with milk yield and fat content (MO breed). Results showed moderate correlations between milk and FA in fat. Estimates were a little more pronounced when FA were expressed relative to milk. Negative genetic correlations between several SAT in milk and milk yield were in agreement with Soyeurt et al. (2007).

Table 4: genetic correlations with milk production and fat content (milk: kg/test day, fat content: g/100g milk).

	SAT		UNSAT	
	%fat	% milk	%fat	%milk
Milk yield	-0.12	-0.15 to -0.40	+0.33	-0.65
Total Fat	+0.68	+0.77 to +0.95	-0.64	+0.51

Tables 5 and 6 present genetic correlations between SAT and UNSAT for the two types of expression. Genetic correlations appeared to be strongly positive across SAT. These results were in agreement with the literature (Soyeurt, 2007). Genetic correlations were also found to be positive across UNSAT, in fat as well as in milk. This is also in agreement with the literature. Due to the heterogeneous origins of the UNSAT, these correlations could not be easily interpreted. However, many of them are products of the same enzyme (the Stearoyl-CoA desaturase) and logically are correlated. These correlations were higher when FA were expressed in fat than in milk, probably due to the negative relationship between UNSAT and fat content.

Table 5: genetic correlations (g/100g milk)

	SAT	UNSAT
SAT	0.19 to 0.95	
UNSAT	0.16 to 0.78	0.69

Table 6: genetic correlations (g/100g fat)

	SAT	UNSAT
SAT	0.33 to 0.90	
UNSAT	-0.12 to -0.75	0.10 to 0.66

The correlation between SAT and UNSAT is found to be negative in the literature. Our results support this conclusion when FA are expressed in g/100g fat. But it should be emphasized that the mode of expression has a big effect. When expressed in % fat, genetic correlations are inflated by the fact that SAT and UNSAT sum to 100% fat. When expressed in % milk, these correlations are much lower.

We expect from our results on correlation that selecting for milk yield or against fat content in milk should result in a decrease in fat content, a decrease in

SAT, an increase in UNSAT in fat content, but probably no significant change in UNSAT expressed in g/100g milk. This hypothesis should be confirmed with additional correlations estimates between individuals UNSAT in milk and milk yield.

Conclusions and Perspectives

SAT in milk and fat were more heritable than other FA, with values ranging from 0.18 to 0.38 for SAT in milk and from 0.16 to 0.44 for SAT in fat. These values are for individual test-day data. Globally, higher heritability estimates were found for FA with greatest content in fat. This reflects higher true values, particularly for final product FA but also more accurate MIR predictions. In agreement with the literature data, MONO and POLY had lower heritability. This general summary of the results could be explained partially by some biological aspects. SAT with short and medium chains are synthesized by the mammary gland, while other FA with long chain, as MONO and POLY, are mainly provided by the cow's diet. Results showed limited correlations between milk and FA in fat. Estimated correlations between fat content and SAT were positive and negative for UNSAT, with the strongest negative estimates observed for C18:2,cis9,cis12.

To summarize, increasing milk yield would induce a decrease of fat content and SAT content in fat and milk, and an increase of UNSAT in fat. In agreement with the literature data, this study found lower heritability values for FA expressed in fat than in milk, particularly for SAT in MO and HO. Our results on these two types of expression show we have to consider them as two different traits.

The genotyping work of 8,000 cows with the bovine 50k chip is being completed and QTL detection is going to start.

The advancements of the PhénoFinlait program are available on http://www.phenofinlait.fr/.

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