An equation based on MIR spectra to explore the genetic determinism of spontaneous lipolysis in dairy cows

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Abstract

Mid infrared (MIR) spectra have been used since the late 2000s to phenotype new traits at large scale through prediction equations. It has been used in France since 2012 to quantify free fatty acids on herd milks in the framework of quality-based milk payment system. In addition to the loss of income, a high lipolysis level leads to a degradation of organoleptic (rancid taste) and technological (processing inability) properties of milk. Monitoring lipolysis is required to maintain the quality of milk and dairy products. Lipolysis depends on many factors: genetics, farming practices, milking equipment, transformation process. Regarding genetics, Vanbergue suggested in 2017 the existence of a genetic susceptibility to spontaneous lipolysis.

The present study aims to investigate this issue further, by studying the genetic determinism of spontaneous lipolysis in a larger number of cows. This work was carried out within the framework of the LIPOMEC project which aims to better understand the molecular mechanisms controlling the degradation of milk fat in dairy species. Studying the genetic determinism of lipolysis requires the phenotyping of many cows. As the equation initially developed on herd milk was not fully appropriate for individual milks (range, precision), a new prediction equation was estimated from the MIR spectra of individual milks. For this purpose, 432 milk samples were collected in 4 experimental farms in 2018 (approximately 40 cows per farm sampled 2 or 3 times a year) to maximize the variability of breeds (Holstein, Normande, Montbéliarde, Jersey) and diets.

A joint analysis of lipolysis according to ISO/TS 22113 standard (BDI method) and by MIR spectrometry was carried out on each sample. Lipolysis measured by BDI method averaged 0.53 mmol/100 g fat (sd=0.41 mmol/100 g fat). The equation was developed by Partial Least Square regression after LOG transformation. Its coefficient of determination R^2 reached 0.72, with a residual standard deviation $S_{y,x}$ of 0.19 mmol/100 g of fat. The equation was then applied to obtain phenotypes on more than 300,000 MIR milk spectra from Holstein, Normande and Montbéliarde breeds. Genetic parameters were estimated using a repeatability animal model. Heritability and repeatability estimates were moderate in both Normande and Holstein breeds but higher in Montbéliarde breed. This work opens the opportunity to new uses of MIR spectra to improve the control of lipolysis in farm, by a closer management of the herd, or even by a selection plan. The LIPOMEC project was funded both by APIS-GENE and the French National Agency.

Keywords: Lipolysis, dairy cattle, mid infrared spectroscopy, cow genetics.

Milk lipolysis is the breakdown of milk fat by an enzyme, the lipoprotein lipase, resulting in the release of free fatty acids in milk. This may lead to the development of a rancid flavor in milk considered unacceptable by the consumer beyond a certain threshold. Moreover, the presence of partial glycerides in milk interferes with technological processes in the industrial processing of dairy foods. In some French regions, lipolysis is therefore part of the criteria of the quality-based milk payment system, which leads to penalties when the level of lipolysis is above national standards (0.89 mEq/100g of fat).

Since 2012, lipolysis levels in milk are routinely measured in France by inter-professional milk analysis laboratories accredited under the milk quality payment scheme according to an instrumental method based on mid-infrared spectroscopy (MIR). However, this method has two drawbacks. First, the equation used was constructed from herd milks, and is not fully adapted to individual milk samples. Second, the anchor method used to check and adjust the calibration equation is the copper soap method, which is calibrated in the range from 0.4 to 1.2 meq/100 g fat, does not thus allow the identification of extreme values of lipolysis in milk.

Lipolysis results from a complex interplay between animal physiology, farming practices, milking equipment and technological process (Vanbergue, 2017; De Marchi et al. 2017). Recently, Vanbergue (2017) suggested the existence of a genetic susceptibility of dairy cows to lipolysis that needs to be confirmed.

The objective of this study was therefore to develop a new equation based on MIR spectra to predict the level of lipolysis in individual milk samples, with the goal of using this tool to phenotype the high number of milk samples required to further explore the genetic determinism of lipolysis. This work was carried out within the framework of the LIPOMEC project, the first integrative project aimed to better understand lipolysis mechanisms in dairy species and granted both by APIS-GENE and the French National Agency.

Material and methods

Four hundred and thirty-two milk samples have been collected to meet the above objectives. A joint analysis of lipolysis according to ISO/TS 22113 standard (BDI method) and by MIR spectrometry has been carried out on each sample.

Data collection

Collection of milk samples

Four hundred and thirty-two milk samples were collected from four experimental farms, located in several regions in France, between March and October 2018 (approximately 40 cows per farm sampled two or three times per year) to maximise variability in dairy breeds (Holstein, Normande, Montbéliarde, Jersey) and diets.

During sampling, vials containing 0.02% bronopol preservative (wt/vol) were filled to capacity (100 mL) to avoid “churning” of the milk that could damage fat globules and activate lipolysis during transport. After collection, milk samples were stored at 4°C to limit bacterial proliferation and lipase-associated activities. Milk samples were sent at 4°C to ACTALIA CECALAIT (39800 Poligny, France) for subsequent analyses.

Table 1 shows the distribution of these samples across farms and time periods.

Table 1. Distribution of samples collected between farms and sampling periods.

	Number of milk samples			
Experimental farm	March/April 2018	June 2018	October 2018	Total
Grignon	41	40	36	117
UE du Pin	40	36	40	116
UE Herbipôle	40	38	40	118
IE PL Le Rheu	41	0	40	81
Total	162	114	156	432

Recording of MIR spectra.

MIR spectra were recorded at ACTALIA CECALAIT using MilkoScan™ FT+ spectrometer (Foss, Hillerød, Denmark).

Measurement of lipolysis in milk.

Reference values for lipolysis in milk were determined using the ISO/TS 22113|IDF/RM 204 BDI (Bureau of Dairy Industry) method which specifies a method for determining the titratable acidity of milk fat. This analysis was carried out by ACTALIA CECALAIT within 36 hours of sampling. Lipolysis measured by BDI method averaged 0.53 mmol/100 g fat (sd=0.41 mmol/100 g fat).

Data processing

Development of the lipolysis prediction equation.

Four hundred and thirty individuals were retained after removal of two outliers for the development of the milk lipolysis prediction equation. The reference lipolysis values obtained by BDI method were log transformed. The equation was developed by partial least squares (PLS) regression using R software.

Calculation of genetic parameters of lipolysis

The lipolysis prediction equation was applied to 348,000 spectra from a database created in the PhenofinLait programme (2008-2013). After exclusion of outliers (very high values), 333,862 individuals were used for phenotypic description.

The estimation of genetic parameters was performed within breed using an animal model with repeated data over the first 3 lactations, i.e., 73,000 data for the Holstein breed, 71,000 for the Normande breed and 55,000 for the Montbéliarde breed. The following fixed effects were considered: herd x test day, analysis laboratory and time between sampling and analysis, month of calving within the season, age at calving in first lactation, stage of lactation (intra parity) and stage of pregnancy.

Results and discussion

Accuracy of the prediction equation of milk lipolysis

The developed milk lipolysis prediction equation has a coefficient of determination (R^2) of 0.72 and an error ($S_{y,x}$) of 0.19 meq/100 g fat. Figure 1 shows the prediction results obtained using this equation, compared with the reference values obtained using the BDI method.

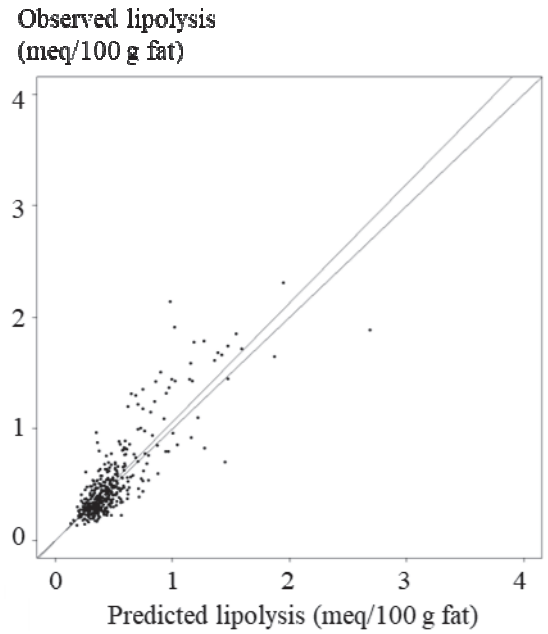

Figure 1. Lipolysis predicted by the developed equation compared with the values observed by BDI method.

The developed milk lipolysis prediction equation is sufficiently accurate to be immediately used for breeding advice or genetic research but is intended to be improved with the inclusion of new data and new samples for others purposes linked to the milk quality payment scheme.

Phenotypic variability of milk lipolysis

Data obtained from the PhenoFinlait database showed that milk lipolysis fluctuates during lactation with a more significant amplitude in first lactation.

The effect of breed was highlighted, with Normande displaying a lower lipolysis rate in milk than Montbéliarde, while Prim'Holstein being intermediate.

Genetic parameters of milk lipolysis

The heritability of the milk lipolysis trait is moderate to high, which makes it possible to consider genomic selection in the future. In addition, for all three breeds, several regions of the genome related to the trait 'milk lipolysis' have been identified, some of which are genes or gene regulatory regions whose involvement remains to be confirmed.

Conclusion

The newly developed equation can be used as a new precision breeding tool to monitor lipolysis in farms for a closer management of the herd. It opens the road to genetic selection to monitor lipolysis at the animal level.

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