



## Prediction of fatty acid profiles in cow, ewe, and goat milk by mid-infrared spectrometry

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### ABSTRACT

Mid-infrared (MIR) spectrometry was used to estimate the fatty acid (FA) composition in cow, ewe, and goat milk. The objectives were to compare different statistical approaches with wavelength selection to predict the milk FA composition from MIR spectra, and to develop equations for FA in cow, goat, and ewe milk. In total, a set of 349 cow milk samples, 200 ewe milk samples, and 332 goat milk samples were both analyzed by MIR and by gas chromatography, the reference method. A broad FA variability was ensured by using milk from different breeds and feeding systems. The methods studied were partial least squares regression (PLS), first-derivative pretreatment + PLS, genetic algorithm + PLS, wavelets + PLS, least absolute shrinkage and selection operator method (LASSO), and elastic net. The best results were obtained with PLS, genetic algorithm + PLS and first derivative + PLS. The residual standard deviation and the coefficient of determination in external validation were used to characterize the equations and to retain the best for each FA in each species. In all cases, the predictions were of better quality for FA found at medium to high concentrations (i.e., for saturated FA and some mono-unsaturated FA with a coefficient of determination in external validation >0.90). The conversion of the FA expressed in grams per 100 mL of milk to grams per 100 g of FA was possible with a small loss of accuracy for some FA.

**Key words:** milk, fatty acid, mid-infrared spectrometry

### INTRODUCTION

Over the last few years, the FA content and quality of human foods has become a major nutritional topic. Evidence of this can be found in the latest French nutritional guidelines that indicate the recommended intake of individual FA in a diversified diet (ANSES, 2011a). The dairy industry has to face 2 major issues regarding these new recommendations: (1) identifying methods to adapt milk FA composition to the consumers' demands and (2) finding tools to precisely characterize the FA composition of milk.

With regard to the first issue, previous studies have shown that feeding management (Chilliard et al., 2007; Raynal-Ljutovac et al., 2008; Coppa et al., 2013) and genetic selection (Arnould et Soyeurt., 2009; Schennink et al., 2009; Stoop et al., 2009) can be efficient tools to alter the FA composition of milk. However, such strategies can only effectively be organized for entire populations if reliable large-scale measuring techniques are available.

As to finding tools to determine the precise FA composition of milk, mid-infrared (MIR) spectrometry can be classically and efficiently used to analyze food products. This technology is already used in dairy farming to measure the fat and protein contents for purposes of milk payment, herd management, and genetic selection. In France, these analyses are performed frequently (once per month for all animals of a herd in dairy cattle or goats managed with the usual milk recording design) at a large scale by laboratories located throughout the country. More recently, this process has been extended to include the analysis of milk FA (Soyeurt et al., 2006, 2011), especially in dairy cattle. Most often, FA composition is predicted using MIR spectra with partial least squares regression (PLS). Although it has been reported that preprocessing before PLS regression im-

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proves calibration equations (Soyeurt et al., 2011), the possible benefit of wavelength selection before applying PLS regression has not been addressed. It has been suggested that wavelength selection before PLS regression might improve calibration equations and provide good results in various situations (Leardi et al., 1992; Spiegelman et al., 1998). It can be performed using different methods: genetic algorithms (Leardi et al., 1992), wavelet decomposition (Mallat, 2008), or penalization methods such as the elastic net, which is often used on genomic data (Croiseau et al., 2011). However, no comparison of these methods is currently available.

Equations for goat milk were developed using near-infrared spectroscopy (NIRS; Andueza et al., 2013), but no calibration equations for predicting milk FA using MIR spectrometry have been published so far for ewe and goat milk. Within the framework of the PhénoFinlait program (Faucon-Lahalle et al., 2009), a large-scale French research and development project aiming at monitoring the composition of cattle, sheep, and goat milk, calibration equations were developed using different mathematical approaches to estimate milk FA composition in French herds using MIR spectrometry.

The objectives of this study were (1) to compare different statistical approaches with wavelength selection for the prediction of milk composition using MIR spectra, (2) to establish whether the use of MIR spectrometry for predicting the FA composition of cow milk could be put into practice in French breeding and feeding systems, and (3) to develop the first referenced equations for FA in goat and ewe milk.

## MATERIALS AND METHODS

### *Experimental Design*

Samples were mainly collected from the Institut National de la Recherche Agronomique (INRA) experimental farms (Table 1). These experimental farms breed ruminants of the most widespread breeds in France in conditions representative of French breeding systems, with at least 2 different feeding systems depending on the season (winter and summer) for each breed. Besides providing more practical technical conditions for collecting samples, these farms apply controlled breeding conditions and, in some cases, shelter populations with strong genetic variability. When this genetic or feeding variability was not sufficiently representative of the national situation, additional samples were collected from commercial herds. Two samples were collected per animal at each milking. Bronopol was added to one of the samples, which was then analyzed by MIR spectrometry; the other was frozen at  $-20^{\circ}\text{C}$  immedi-

ately after collection for future analysis using GC, the reference method.

**Cow Milk Samples.** A first set of 249 milk samples was collected from 127 Holstein  $\times$  Normande crossbred dairy cows in 2008 and 2009 at the INRA "Domaine expérimental du Pin" experimental farm. The cows were a part of a QTL detection experiment (Larroque et al., 2002), and were produced after 2 generations of crosses (F2) between Normande and Holstein breeds that display numerous differences, in particular those pertaining to milk fat and protein content.

Milk samples were collected twice during the first lactation, in the winter and summer. The average stage of lactation was 160 DIM in the winter, and 209 DIM in the summer. During the winter (November–April), all cows were given the same diet, formulated to cover their requirements (INRA, 1989) and based on corn silage given ad libitum and completed with grass silage (4 kg of DM/d), beet pulp (1 kg of DM/d), and soybean meal (2 kg of DM/d). During the summer, cows grazed herbage with high nutritive value in a rotational grazing system (35  $\text{hm}^2$  per cow) and were supplemented with corn silage (1.5 kg/d). When the daily milk yield exceeded 21 kg/d in the winter and 23 kg/d in the summer, cows received 1 kg of energy concentrate per 2.5 kg of milk produced over the limit (maximum 7 kg of concentrate in winter and 5 kg in summer).

A second set of 153 milk samples were collected from 42 Montbéliardes and 35 Holsteins (primiparous or multiparous) in 2009 at the INRA experimental farm in Mirecourt (France), which implements an organic dairy farming system. Depending on calving dates, the cows belonged either to a grazing system or to a mixed-crop dairy system without concentrate. Milk samples were collected twice during their lactation: in the winter (March 2009) at 29 and 184 DIM, or in the summer (June 2009) at 120 and 274 DIM, respectively, for the grazing and mixed-crop dairy systems. In the winter, cows from the grazing system received ad libitum hay from the first cut of permanent grassland, which was completed with a second cut of permanent grassland (5.6 kg of DM/d) and a mineral complement. At the same period, cows from the mixed-crop dairy system received a diet dominated by hay from a first cut of alfalfa and orchard grass offered ad libitum and completed by flattened triticale (1.7 kg of DM/d), a second cut of temporary grassland (6.7 kg of DM/d), and a mineral complement. In the summer, cows from the grazing system grazed a new enclosed pasture of permanent grassland (orchard grass, white clover, and fescue). Cows from the mixed-crop dairy system grazed alternatively (night and day) 2 enclosed pastures of permanent grassland (based on ryegrass, white clover, and orchard grass). Milk samples were collected using

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