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## Genetic and environmental variation in bovine milk infrared spectra

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### ABSTRACT

Fourier transform infrared (FTIR) spectroscopy is widely used to determine milk composition. In this study, 1,060 milk infrared wavenumbers ranging from 925 to 5,008  $\text{cm}^{-1}$  of 1,748 Holstein Friesian cows on 371 herds in the Netherlands were available. The extent to which infrared wavenumbers are affected by genetic and environmental factors was investigated. Inter-herd heritabilities of 1,060 infrared wavenumbers ranged from 0 to 0.63, indicating that the genetic background of infrared wavenumbers differs considerably. The majority of the wavenumbers have moderate to high inter-herd heritabilities ranging from 0.20 to 0.60. The diacylglycerol O-acyltransferase 1 (*DGAT1*), stearoyl-CoA desaturase (*SCD1*),  $\kappa$ -casein (*CSN3*), and  $\beta$ -lactoglobulin (*LGB*) polymorphisms are known to have a large effect on milk composition, and therefore we studied the effects of these polymorphisms on infrared wavenumbers. The *DGAT1* polymorphism had highly significant effects on many wavenumbers. In contrast, the *SCD1* polymorphism did not significantly affect any of the wavenumbers. The *SCD1* is known to have a strong effect on the content of C10:1, C12:1, C14:1, and C16:1 fatty acids. Therefore, these results suggest that FTIR spectra contain little direct information on these monounsaturated fatty acids. The *CSN3* and *LGB* polymorphisms had significant effects on a few wavenumbers. Differences between herds explained 10 to 25% of the total variance for most wavenumbers. This suggests that the wavenumbers of milk FTIR spectra are indicative for differences in feeding and management between herds. The wavenumbers between 1,619 and 1,674  $\text{cm}^{-1}$  and between 3,073 and 3,667  $\text{cm}^{-1}$  are strongly influenced by water absorption and usually excluded when setting up prediction equations. However, we found that some of the wavenumbers in the water absorption region are affected by the *DGAT1* polymorphism and lactation stage. This suggests that these wavenumbers contain useful information regarding milk composition.

**Key words:** bovine milk, FTIR wavenumbers, heritability, herd, genetic polymorphisms

### INTRODUCTION

Milk produced by dairy cows is a complex product consisting of many components (Jelen, 2007). However, at present, only fat and protein content are routinely recorded and considered in most dairy cattle breeding programs (Miglior et al., 2005). Despite this, both from a nutritional and a manufacturing perspective, detailed fat and protein composition are of interest. Several studies suggested protein composition is related to milk coagulation and cheese yield (e.g., Wedholm et al., 2006), since a greater casein content is preferable for cheese making. Due to its relatively high concentration of SFA, the consumption of bovine milk fat has been associated with negative effects on human health (e.g., German and Dillard, 2006), and therefore changing milk fat composition by means of selective breeding might be of interest. Moreover, milk composition can serve as an indicator for the cow's health status (e.g., Vlaeminck et al., 2006; Van Haelst et al., 2008) and methane emission (e.g., Chilliard et al., 2009). For breeding and management purposes, large-scale routinely collected measurements are needed and therefore traits should be easy to measure at relatively low costs. Analytical methods such as gas chromatography to quantify milk fat composition, or HPLC and capillary zone electrophoresis to quantify milk protein composition, are expensive and time-consuming. Therefore, these methods are less suited for large-scale routine measurements.

Fourier transform infrared (FTIR) spectroscopy is a fast and cost-effective method widely used to determine milk composition. It is the standard method for routine quantification of fat, protein, and lactose content of milk (ICAR, 2012). Several studies showed that FTIR also can be used to determine milk fat composition (e.g., Soyeurt et al., 2006; Rutten et al., 2009). Other studies investigated possibilities to predict milk protein composition based on FTIR (Bonfatti et al., 2011; Rutten et al., 2011a). Furthermore, recent research reported the ability of infrared spectroscopy to predict traits such as milk coagulation, ketone bodies, and energy status of dairy cows (De Marchi et al., 2014).

Some studies analyzed the genetic background of infrared wavenumbers (Soyeurt et al., 2010; Bittante and Cecchinato, 2013). Soyeurt et al. (2010) analyzed

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infrared spectra of 1,594 first parity Holstein cows and found substantial differences in heritability between wavenumbers and concluded that not all infrared wavenumbers are of genetic interest. Bittante and Cecchinato (2013) studied the infrared spectra of 1,064 Brown Swiss cows and reported that approximately 30% of the wavenumbers have heritability larger than 0.10.

Besides quantifying the combined action of all genes on infrared wavenumbers, it might be of interest to study the effects of individual genes with known and large effect on milk composition. Polymorphisms in diacylglycerol O-acyltransferase 1 (*DGAT1*), stearoyl-CoA desaturase 1 (*SCD1*),  $\kappa$ -casein (*CSN3*), and  $\beta$ -lactoglobulin (*LGB*) have been shown to have important effects on milk composition (e.g., Schennink et al., 2008; Heck et al., 2009). Their effects on individual infrared wavenumbers can provide insight in the information that is captured by the FTIR spectra. Furthermore, it has been shown that FTIR can be used to predict *LGB* genotypes (Rutten et al., 2011b) and *CSN1S1* haplotypes (Berge et al., 2010). Quantifying the effects of *DGAT1*, *SCD1*, and *CSN3* polymorphisms on individual infrared wavenumbers can give insight in the possibilities of predicting genotypes for these polymorphisms based on FTIR.

It is well known that milk composition is also affected by feed and management strategies, for example, feed composition influences milk fat content and fat composition (e.g., Chilliard et al., 2007) and dietary energy intake influences milk protein content (Emery, 1978). Consumers have increasing interest in the authenticity of milk as they purchase biological and organic products at a higher price. Infrared spectroscopy might be one of the methods that enable discriminating milk samples produced by cows fed different diets (Valenti et al., 2013). Quantifying herd effects will give insight in the extent to which feed and management differences are reflected by individual wavenumbers. Herd effects for individual infrared wavenumbers of bovine milk have not been quantified before.

The aim of this study was to quantify the contribution of genetic and environmental effects to the variation in milk infrared wavenumbers. Furthermore, we aimed at quantifying the effects of polymorphisms in *DGAT1*, *SCD1*, *CSN3*, and *LGB* on milk infrared wavenumbers.

## MATERIALS AND METHODS

### Data

In this study, one morning milk sample from 1,748 first-parity Holstein Friesians cows located in 371 herds was collected for analysis. The data were collected from

February to March 2005. All cows had at least 87.5% Holstein Friesian genes. The population consisted of 5 large paternal half-sib families from proven sires (98–196 daughters per sire), and 50 small paternal half-sib families from test sires (8–23 daughters per sire), as well as 168 cows descending from 44 other proven sires (1–25 daughters per sire) to ensure at least 3 cows per herd. The pedigree of the cows was provided by CRV (cooperative cattle improvement organization, Arnhem, the Netherlands).

Milk samples were conserved using sodium azide (0.03% wt/wt) at 4°C. Subsequently, FTIR spectra were recorded in a 10-mL milk sample using the MilkoScan FT 6000 equipment (Foss, Hillerød, Denmark) at the certified laboratory of the Milk Control Station (Zutphen, the Netherlands). All milk samples used in this study were analyzed on the same MilkoScan FT 6000. The FTIR spectra consisted of the transmittance values measured at 1,060 wavenumbers ranging from 925 to 5,008  $\text{cm}^{-1}$ .

### Genotypes

Blood samples were collected for DNA isolation. The genotyping procedure for *DGAT1* K232A and *SCD1* A239V polymorphisms were described by Schennink et al. (2008). Genotypes of *CSN3* were determined as described by Heck et al. (2009). The polymorphisms associated with the known protein variants for *LGB* were genotyped using a SNaPshot assay as described by Visker et al. (2011).

Among the 1,748 cows with FTIR data, 1,625 cows had *DGAT1* genotypes, 1,579 cows had *SCD1* genotypes, 1,534 cows had *CSN3* genotypes, and 1,542 cows had *LGB* genotypes. For some cows the genotypes were missing because either no DNA sample was available or the sample could not be genotyped unambiguously. The allele frequencies were 60.0% for A allele and 40.0% for K allele of *DGAT1*, 73.0% for A allele and 27.0% for V allele of *SCD1*, 58.3% for A allele and 41.7% for B allele of *LGB*, and 60.4, 30.1, and 9.5% for *CSN3* A, B, and E allele, respectively.

### Statistical Analysis

A series of analyses were performed to quantify the effects of several factors on the 1,060 infrared wavenumbers. The following model was used:

$$y_{ijklm} = \mu + \beta_1 \times lactst_{ijklm} + \beta_2 \times afc_{ijklm} + season_i + sirecode_j + date_k + herd_l + a_m + e_{ijklm}, \quad [1]$$

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