Genetic variation of milk fatty acid composition between and within dairy cattle breeds

Myrthe Maurice – Van Eijndhoven

Thesis committee

Promotors
Prof. Dr J.A.M. van Arendonk
Professor of Animal Breeding and Genetics
Wageningen University

Prof. Dr R.F. Veerkamp
Professor of Numerical Genetics and Genomics
Wageningen University

Co-promotor
Dr M.P.L. Calus
Senior researcher, Animal Breeding and Genomics Centre
Wageningen UR Livestock Research

Other members
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Prof. Dr E. Verrier, INRA/AgroParisTech, Paris, France

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Abstract

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Fat is one of the main components in bovine milk and comprises a large number of individual fatty acids (FA). The composition of FA in milk varies considerably due to differences in the genetics and nutrition of cows and an increasing interest in the possibilities for modifying FA composition can be noticed nowadays. In this thesis two fields of interest were combined, namely: production of milk with specific milk fat composition and conservation of native cattle breeds. Therewith, the overall objective of this thesis was to investigate the variability of detailed milk FA composition between and within different dairy cattle breeds, including the mainstream Holstein Friesian (HF) and Jersey, and the native dual purpose breeds Meuse-Rhine-Yssel (MRY), Groningen White Headed (GWH) and Dutch Friesian (DF) in the Netherlands. For this study the accuracy of mid-infrared (MIR) spectrometry was evaluated for predicting FA composition in different breeds. Differences of milk FA composition within and between breeds were investigated using MIR and Gas Chromatography (GC) information. Finally, similarities in genomic variation associated with detailed milk fat composition between the mainstream HF breed and native dual purpose breeds were studied. Results show that MIR is an accurate method for predicting FA composition among different breeds and countries. Evaluating the FA composition in different breeds, differences were found in milk FA composition among herds using different cattle breeds in the Netherlands, based on detailed milk FA measurements using GC. Evaluating the FA composition in milk between and within breeds using a large dataset that included MIR spectra of milk from cows from a range of farms using one or more breeds, in general, only minor breed differences in FA composition were found and HF showed more genetic variation in FA composition compared to MRY. Furthermore, differences were detected between the native breeds MRY, DF and GWH in genomic variations of regions that are associated with FA composition in HF, while most variation in these main regions was clearly observed in HF. Overall, it was concluded that no large differences existed in milk FA composition among the native Dutch dual purpose breeds and the mainstream HF breed. It is suggested, however, that selecting specific FA composition differences in farms using different breeds in the Netherlands can attribute to modifying the FA composition in bovine milk production.

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General introduction

1.1 Bovine milk

Bovine milk and other dairy products like cheese and yoghurt have been common elements in the diet of humans in many countries for centuries. Bovine milk is a major source of fat, protein, amino acids, minerals and vitamins (Haug et al., 2007). In a number of Western countries, however, the consumption of dairy products has decreased in recent years, partly due to the debate concerning the effect of the consumption of dairy products on human health. This debate is a consequence of a number of studies reporting that the consumption of saturated fat, one of the main components in milk, is negatively related to human health, manifesting through increased blood cholesterol levels and cardiovascular disease in humans (e.g., German and Dillard, 2004, Mensink et al., 2003). Regardless of the suggested negative effects on human health, however, milk also contains components that are suggested to have positive effects on human health, such as its relatively high oleic acid content (C18:1*cis*9), which is important for protection against atheromatosis (e.g., Astrup et al., 2011, Haug et al., 2007). In addition to associations with human health, the composition of milk and especially milk fat is also related to milk processability (e.g., Smet et al., 2009). Therefore, the ability to modify the composition of milk fat is of major interest for the dairy industry (Lock and Bauman, 2004, Palmquist et al., 2006).

1.2 Variation in milk fat composition

'Bovine milk fat' is a generic term that comprises a large number of individual fatty acids (FA). These FAs can be distinguished by their chemical structure and primarily arise in milk through three different pathways: from de novo synthesis within the mammary epithelial cells, by direct uptake from blood circulation, or both of these methods (e.g., Bauman and Griinari, 2003, Palmquist et al., 2006). Dairy cows generally produce between 3% and 6% fat in their milk: around 70% of the total milk FA has no double bounds, i.e., saturated FA (SFA); around 25% of FA has one double bound, i.e., mono unsaturated FA (MUFA) and around 5% of FA have multiple double bounds, i.e., poly unsaturated FA (PUFA). The detailed FA composition in milk can differ between cows, herds and breeds (e.g., Beaulieu and Palmquist, 1995, Soyeurt et al., 2011, Stoop et al., 2008). In several studies, it has been found that detailed milk fat composition is influenced by cows' diet (e.g., Bauman and Griinari, 2003, Grummer, 1991, Walker et al., 2004), as well as by genetic factors (e.g., Mele et al., 2009, Palmquist et al., 1993, Stoop et al., 2008). The genetic variability is a parameter that determines the ability for modifying, for example, milk's FA composition in a defined population through breeding. An

important measure for indicating the extent of a specific trait's genetic variability (one that has been used for several decades), for example, the amount of an individual FA in milk within a defined population, is heritability. Heritabilities for individual FAs have been reported as ranging from low to moderate (up to 0.50), where low heritabilities are mostly found for long-chain UFA, while moderate heritabilities are mainly found for short- and medium-chain FAs (e.g., Soyeurt et al., 2007, Stoop et al., 2008). Genetic differences are also reflected by the results of different studies through different breeds; however, the majority of studies reporting these variabilities within and/or between breeds only include the globally mainstream cow breeds HF and JER (e.g., Palladino et al., 2010, White et al., 2001).
To better understand the effect of genetics on milk FA composition, several studies have been carried out to find locations or regions on the genome that are related to variations in FA composition in milk, i.e., quantitative trait loci (QTL). Furthermore, underlying genes in the identified QTL regions have been reported in a number of studies. Currently, two genes have been identified in multiple studies to have a major effect on FA composition, namely diacylglycerol acyltransferase 1 (*DGAT*1) and stearoyl-CoA desaturase 1 (*SCD*1) (e.g., Moioli et al., 2007, Schennink et al., 2008). The allele frequencies of the causal mutations in the genes *DGAT*1 and *SCD*1 have been studied for several cow populations, the majority of which belong to mainstream dairy breeds (e.g., Kgwatalala et al., 2007, Spelman et al., 2002). In general, milk FA composition can be modified by breeding; however, knowledge concerning the opportunities for using different and numerically small cattle breeds is scarce.

1.3 Cattle breeds in the Dutch dairy industry

Significant changes have occurred during the past number of decades within global dairy cattle breeding. In the Netherlands, these changes have become clearly visible, for example, when examining the type of cows used for milk production. To obtain better insight into the type of cows used for milk production in the Netherlands, this section presents a short overview of the history and the current status of the country's cattle population. The current Dutch dairy cattle population is clearly dominated by the high-yielding Holstein Friesian (HF) breed. In the past, up to the beginning of the 1980s, the native dual purpose breeds dominated Dutch milk production (Figure 1.1). Circa 1980, the Netherlands counted approximately 3000 purebred Groningen White Headed (GWH) cows born per year, 100 000 purebred Meuse-Rhine-Yssel (MRY) cows born per year and 185 000 purebred Dutch Friesian (DF) cows born per year which were registered by the Dutch Cattle

Syndicate (nowadays CRV BV, Arnhem, The Netherlands). Within the Netherlands, developments in animal husbandry (e.g., more intensive feed production and the introduction of milking machines) and developments within the milk processing industry (e.g., the overall ability of higher throughput) has led to the use and further development of specialized breeding for high milk production (Groen et al., 1993). The change in use from mainly native dual purpose breeds to mainly specialized dairy breeds for high input/high output production systems during the past few decades has been observed in many highly industrialized countries like the Netherlands (Hiemstra et al., 2010, Oldenbroek, 2007). The HF breed has become

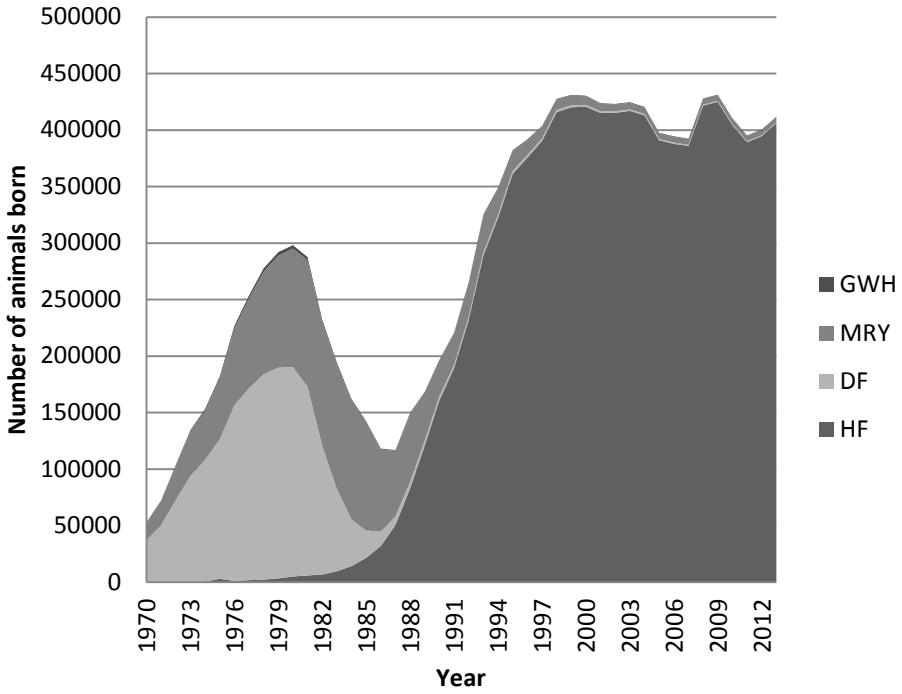

Figure 1.1 The number of purebred animals born per cattle breed per year in the Netherlands[1,2]

[1] Cattle breeds with breeding goal 'dairy' and with at least 10 000 animals born and registered from 1970-2013 by CRV BV (Arnhem, The Netherlands);

[2] GWH = Groningen White Headed; MRY = Meuse-Rhine-Yssel; DF = Dutch Friesian; HF = Holstein Friesian.

the dominating breed today, counting around 400 000 purebred animals born per year (Figure 1.1). Although some farmers have also begun using some other dairy breeds like Jersey (JER), Montbéliarde (MON) and Fleckvieh (FLV), the number of purebred animals born per year among these breeds are low, altogether only around 2000 (registered yearly from 2008 to 2013 by CRV BV, Arnhem, the Netherlands). It is not surprising that the dairy breeding industry in a highly industrialized and small country like the Netherlands is dominated by a single breed. There are several reasons for this: 1) it is easier to focus on a single breeding programme that has been particularly compiled for a specific breed; 2) breeding programmes that focus on a single breed are more likely to yield better genetic progress within that breed, compared to breeding programmes that focus on multiple breeds; 3) when the same mainstream breed is used in different countries, the exchange of, for instance, genetic material, estimated breeding values and genotypes can take place under specific conditions; 4) highly developed farming systems in countries like the Netherlands can easily be adjusted to the need of the cows of the specific breed. In summary, the ability for high production according to the Dutch selection index – in which higher fat and protein yield lead to higher economic value – the highly adjusted and professionalized worldwide HF breeding programme and advanced Dutch farming systems has made the HF breed very suitable for intensive milk production in the Netherlands. The interest in breeds other than HF for dairy production nonetheless remains substantial, as more than 13 per cent of all artificial inseminations in the year 2013 facilitated by CRV BV can be attributed to non-HF breeds. For dairy purposes, in total, 2 160 187 HF inseminations (both, black-and-white and red-and-white) and in total 323 225 inseminations of non-HF bulls (of which 52 875 were of MRY, FH or GWH origin) were facilitated by CRV BV in the year 2013 (CRV, 2014).

1.4 Aim and outline of this thesis

To modify the detailed FA composition in bovine milk, it is important to provide insight into the existing variances and factors influencing FA composition in milk. In general, different cattle breeds have different genetic make-up. Thus, different breeds might also differ in genetic variation related to the production of detailed FA composition. The overall objective in this thesis was therefore to investigate whether native dual purpose breeds comprise different genetic variations for milk fat composition among each other and compared to mainstream dairy breeds. In this thesis, three native Dutch dual purpose cattle breeds (MRY, DF and GWH) and two mainstream dairy breeds (HF and JER) in the Netherlands were

studied. MRY, DF and GWH were chosen because these were the main breeds found in Dutch dairy production in the past; HF had been chosen because it is the major breed in the Netherlands today, while JER was chosen due to the typically high fat percentage and FA composition produced by this breed.

The first aim of this thesis was to explore whether systematic differences in FA composition in milk exist among farms using different breeds in the Netherlands (Chapter 2). Therefore, milk samples of the MRY, DF, GWH and JER breeds were collected at 12 farms (three farms per breed) and analysed by gas chromatography (GC). GC is the most implemented and accurate method for measuring the detailed FA composition in milk. However, the method is expensive and time consuming, and therefore less suitable for regular milk-recording and application to non-mainstream breeds that are of less economic importance. A possible alternative method for analysing milk samples is mid-infrared spectrometry (MIR), which has a high-throughput and is much cheaper when used extensively. Therefore, predictions of FA composition in milk using MIR based on HF were validated across cattle breeds (Chapter 3). Building on the validations in Chapter 3, possible breed differences were investigated for the breeds MRY, DF, GWH, HF and JER using a large dataset containing MIR profiles, which were collected during regular milk recording from a range of farms with different (combinations of) breeds (Chapter 4).

To be able to modify FA composition in bovine milk by breeding, both the extent of between-breed and within-breed variation is important. The genetic variances and heritabilities of a number of individual FA and groups of FA were therefore estimated for the breeds MRY and HF (Chapter 5). To better understand the between- and within-breed variations, the relation of phenotypic variation and differences at the genomic level can be studied. Similarities in genomic variation associated with detailed milk-fat composition between the Holstein Friesian (HF) breed and native dual purpose breeds MRY, DF and GWH are identified in Chapter 6. Finally, in Chapter 7, the genetic variability among cattle associated with milk-fat composition, including genomic differences between breeds related to the well-known gene *DGAT*1 and the perspectives for native and numerically small cattle breeds in the dairy sector is discussed.

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2

Milk fat composition of 4 cattle breeds in the Netherlands

M. H. T. Maurice – Van Eijndhoven[1,2], S. J. Hiemstra[1,3], M. P. L. Calus[1]

[1] Animal Breeding and Genomics Centre, Wageningen UR Livestock Research, P.O. Box 338, 6700 AH Wageningen, The Netherlands; [2] Animal Breeding and Genomics Centre, Wageningen University, P.O. Box 338, 6700 AH Wageningen, The Netherlands; [3] Centre for Genetic Resources (CGN), P.O. Box 338, 6700 AH Wageningen, The Netherlands

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Abstract

Milk fatty acid (FA) composition was compared among 4 cattle breeds in the Netherlands: Dutch Friesian (DF; 47 animals/3 farms), Meuse-Rhine-Yssel (MRY; 52/3), Groningen White Headed (GWH; 45/3), and Jersey (JER; 46/3). Each cow was sampled once between December 2008 and March 2009 during the indoor housing season, and samples were analyzed using gas chromatography. Significant breed differences were found for all traits including fat and protein contents, 13 major individual FA, 9 groups of FA, and 5 indices. The saturated fatty acid proportion, which is supposed to be unfavorable for human health, was smaller for GWH (68.9%) compared with DF (74.1%), MRY (72.3%), and JER (74.3%) breeds. The proportion of conjugated linoleic acid and the unsaturation index, which are associated positively with human health, were both highest for GWH. Differences in milk fat composition can be used in strategies to breed for milk with a FA profile more favorable for human health. Our results support the relevance of safeguarding the local Dutch breeds.

Key words: milk , fatty acid , cattle breed

Within the Netherlands different dairy cattle breeds are used for milk production. High emphasis in selection for milk yield, however, has led to an enormous reduction in breed variability. Today, more than 97% of the milk-recorded population belongs to Holstein-Friesian (**HF**; CRV, 2009). In general, individual cattle breeds comprise unique genetic variation (European Cattle Genetic Diversity Consortium, 2006); thus, it can be hypothesized that local Dutch breeds comprise some genetic variation that is not present in the HF breed. An important question is to understand if these genetic resources are related to any unique and valuable characteristics that could be important now or in the future. Unique characteristics can influence directions in selection or allow the assignment of a breed to a special brand product, which is an important tool to maintain native genetic resources characterized by low production levels (Dalvit et al., 2007; Pretto et al., 2009).

Recently, milk quality traits have become increasingly relevant as consumer awareness of healthy diets is growing. In this context, bovine milk is being increasingly recognized as an important source of energy, high quality protein, and essential minerals and vitamins (Heaney, 2000; Neumann et al., 2003; German and Dillard, 2006). However, several studies report negative effects on human health from the consumption of bovine milk (Lock and Bauman, 2004; German and Dillard, 2006; Uauy, 2009). Results from those studies have led to an ongoing debate on the role of milk and dairy products in human health (Palmquist et al., 2006). The diet used in the herd plays a central role in determining the variation of milk fat composition (Palmquist, 2006). Also, a significant part of the variability in fatty acid (**FA**) composition is genetically determined (e.g., Beaulieu and Palmquist, 1995; Stoop et al., 2008).

The aim of this study was to investigate the differences in individual FA composition in milk of the local cattle breeds Dutch Friesian (**DF**), Meuse-Rhine-Yssel (**MRY**), and Groningen White Headed (**GWH**), and imported Jersey (**JER**) within the Netherlands.

A total of 190 cows was sampled once during morning milking between December 2008 and March 2009. Samples were treated immediately with 0.03% (wt/wt) sodium azide to prevent microbiological growth. Cows belonged to 4 breeds: DF (47 samples from 3 farms), MRY (52 samples from 3 farms), GWH (45 samples from 3 farms), and JER (46 samples from 3 farms). The selected farms were of general size ranging from 35 to 120 cows, and the number of sampled animals per herd ranged from 6 to 24. For each breed farmers were asked to select cows that varied in terms of age at calving, parity, stage of lactation, and ancestors. On all farms cows were kept indoors during the studied period and milked twice a day

with conventional milking systems. From the 3 farms for each breed, 1 or 2 were organic and the other 1 or 2 were conventional (DF, MRY, and GWH 1 organic and 2 conventional, and JER 2 organic and 1 conventional).

Fatty acid composition of milk samples was obtained using GC at the laboratory of Qlip N.V. (Leusden, the Netherlands). The GC outputs were generated by analyzing methyl esters. Fatty acid methyl esters were prepared using fat fractions extracted from the milk, as described in ISO Standard 15884 (ISO-IDF, 2002b). Methyl esters were analyzed, as described in ISO Standard 15885 (ISO-IDF, 2002a), according to the 100% FA methyl ester method with a 100-m polar column (Varian Fame Select CP 7420, Varian Inc., Palo Alto, CA). The percentages of total fat and total protein were obtained from standard mid-infrared spectrometry using a Fourier-transformed interferogram (MilkoScan FT 6000, Foss Electric, Hillerød, Denmark) at the laboratory of Qlip N.V. (Zutphen, the Netherlands).

In the current study, only the major FA were considered. In total 29 traits were studied: 13 individual major FA, 9 groups of FA, 5 indices, and 2 milk production traits. The 13 individual FA were C4:0, C6:0, C8:0, C10:0, C12:0, C14:0, C16:0, C18:0, C18:1 *trans*-6, C18:1 *trans*-9, C18:1 *trans*-10, C18:1 *trans*-11, and C18:2 *cis*-9,*trans*-11 (conjugated linoleic acid; **CLA**). The 9 groups and 5 indices of FA are reported in Table 2.1. The 2 milk production traits were fat and protein contents.

Breed differences were estimated using the GLM procedure (SAS Institute Inc., Cary, NC) with the Bonferroni adjustment for multiple comparisons of means. The model, for all traits, was as follows:

$$y_{ijklmno} = \mu + dim_i + parity_j + age_k(parity_j) + breed_l + system_m + farm_n(breed_l \times system_m) + e_{ijklmno},$$

where $y_{ijklmno}$ is observation *ijklmno* for the studied variable; μ is the overall intercept of the model; dim_i is the fixed effect of the *i*th class of stage of lactation (6 classes of 60 d each, except for the last, which was an open class of >300 d); $parity_j$ is the fixed effect of the *j*th lactation (4 classes: first, second, third, and fourth and later parities); $age_k(parity_j)$ is the fixed effect of the *k*th class of age at calving within the *j*th parity (within each parity, 3 classes were defined containing an equal number of cows); $breed_l$ is the fixed effect of the *l*th breed (DF, MRY, GWH, and JER); $system_m$ is the fixed effect of the *m*th farming system (conventional and organic); $farm_n(breed_l \times system_m)$ is the fixed effect of the *n*th farm nested within the *l*th breed and the *m*th system; and $e_{ijklmno}$ is the random

Table 2.1 Groups of fatty acids and indices.

Group	Fatty acids
Saturated fatty acids	C4:0; C5:0; C6:0; C7:0; C8:0; C9:0; C10:0; C11:0; C12:0; C14:0 *iso*; C14:0; C15:0 *iso*; C15:0 *ante iso*; C15:0; C16:0 *iso*; C16:0; C17:0 *iso*; C17:0 *ante iso*; C17:0; C18:0; C19:0; C20:0
Unsaturated fatty acids	C10:1; C12:1; C14:1; C16:1; C17:1; C20:3 *cis*-8-11-14; C18unsat
C6-12	C6:0; C8:0; C10:0; C12:0
C14-16	C14:0; C16:0
C18 unsaturated (unsat)	C18:1 *trans*-6; C18:1 *trans*-9; C18:1 *trans*-10; C18:1 *trans*-11; C18:1 *trans*-12; C18:1 *cis*-9; C18:1 *cis*-11; C18:1 *cis*-12; C18:2 *cis*-9-12; C18:3 *cis*-9-12-15; C18:2 *cis*-9; *trans*-11 (CLA)
C18 *trans*	C18:1 *trans*-6; C18:1 *trans*-9; C18:1 *trans*-10; C18:1 *trans*-11; C18:1 *trans*-12
n-3	All omega 3 fatty acids
n-6	All omega 6 fatty acids
Branched	C14:0 *iso*; C15:0 *iso*; C15:0 *ante iso*; C16:0 *iso*; C17:0 *iso*; C17:0 *ante iso*
Unsaturation index	(C10:1 + C12:1 + C14:1 + C16:1 + C17:1 + C18:1 cis-9 + C18:2 cis-9, trans-11) / (C10:0 + C10:1 + C12:0 + C12:1 + C14:0 + C14:1 + C16:0 + C16:1 + C17:0 + C17:1 + C18:0 + C18:1 cis-9 + C18:2 cis-9, trans-11)
Unsaturation index C12	C12:1 / (C12:0 + C12:1)
Unsaturation index C14	C14:1 / (C14:0 + C14:1)
Unsaturation index C16	C16:1 / (C16:0 + C16:1)
Unsaturation index C18	C18unsat / (C18:0 + C18unsat)

residual for observation *ijklnmo*. The nested effect was introduced because only one breed was present on each farm and each farm was either conventional or organic. Breed and system were tested on the error term of *farm*(*breed* × *system*) to verify whether the variation attributed to these effects was significant. Although the distributions of the data of several traits showed a certain level of skewness

(range: −1.32 to 1.19) and kurtosis (range: −0.51 to −3.04), log-transforming the *y* variable, which reduced the level of skewness, had no or only a minor effect on the significance of breed differences. To be consistent for all traits, only results of the untransformed data are shown. Furthermore, checking the residuals of the model for the untransformed traits revealed that they were normally distributed, independent, and had equal variances across the range of predicted values (results not shown).

Table 2.2 Means and standard deviations (SD) of calving age, parity, and days in milk (DIM) for each breed[1].

	DF (n = 47)		**MRY** (n = 52)		**GWH** (n = 45)		**JER** (n = 46)	
Item	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Calving age (d)	1,566	919	1,497	731	1,717	958	1,805	927
Parity (n)	2.9	2.5	2.8	1.9	3.2	2.5	3.6	2.4
DIM (d)	144	112	174	130	160	93	211	132

[1] DF = Dutch Friesian; MRY = Meuse-Rhine-Yssel; GWH = Groningen White Headed; JER = Jersey.

Means and standard deviations of the factors included in the model are reported in Table 2.2. On average, the MRY animals were somewhat younger (calving age 1,497 d) and in an earlier parity (2.8) compared with the other breeds, and the JER animals were oldest (1,805 d). Dutch Friesian cows were, on average, shortest in milk (144 d) and JER longest (211 d). The variability of DIM was smallest for the GWH breed (SD: 93 d) compared with MRY (SD: 130 d) and JER (SD: 132 d).

The variation attributed to breed was significant for 15 traits ($P < 0.05$; Table 2.3), and the variation due to system was significant for 5 traits ($P < 0.05$; data not shown). The effects of DIM, parity, age at calving, and farm nested within breed and system were not significant for some traits, but they were always included to keep the model consistent. Coefficients of determination ranged from 0.45 (unsaturation index C18; data not shown) to 0.94 (n-3; data not shown). Surprisingly, the coefficient of determination for n-3 was much higher than that for other traits, but no clear explanation could be identified.

Least squares means of individual FA, groups of FA, indices, and production traits for the different breeds are shown in Table 2.3. An important question is whether these are purely breed effects or are breed-herd effects, because only one breed was sampled per farm. This design of the data may be problematic if (1) the

management (e.g., level of feeding) on farms within one or more breeds deviates systematically from that in the average herd (e.g., all herds with breed A feed concentrates, whereas herds with breed B do not feed any concentrates); or (2) within a particular breed, the selected farms are not representative for the breed. Checking the data revealed that the differences in raw averages of milk production traits among breeds within the data set used were comparable to the differences among breeds shown in national milk production statistics, in which JER produces roughly 1.5% more fat and around 0.5% more protein than the other breeds. This supports the premise that the selected farms are representative for the different breeds. Because, within breed, at least one conventional and one organic farm were included, which implies a large difference in management and feeding level, a systematic deviation in management among breeds was partly avoided. Although it was not possible to correct completely for rearing conditions, analyzing the variances revealed that the model was at least able to differentiate to a certain level between herd and breed effects for most traits.

Table 2.3 Least squares means of individual fatty acids, groups of fatty acids, indices, and milk production traits for each breed.

	Breed[1]					
Trait	**DF**	**MRY**	**GWH**	**JER**	**SE**	***P*-value[2]**
Individual FA (g/100g fat)						
C4:0	3.82 [a]	3.65 [a, b]	3.63 [a, b]	3.59 [b]	0.051-0.058	
C6:0	2.64 [a]	2.56 [a]	2.33 [b]	2.44 [b]	0.030-0.035	*
C8:0	1.59 [a]	1.52 [a]	1.32 [b]	1.39 [b]	0.024-0.027	**
C10:0	3.87 [a]	3.66 [a, b]	2.98 [c]	3.36 [b]	0.078-0.090	*
C12:0	4.74 [a]	4.75 [a]	3.69 [b]	4.05 [b]	0.107-0.124	*
C14:0	12.73 [a]	12.79 [a]	11.61 [b]	11.65 [b]	0.169-0.194	*
C16:0	30.90 [b]	29.54 [b, c]	28.98 [c]	33.64 [a]	0.386-0.446	
C18:0	10.43 [a]	10.32 [a]	10.82 [a]	10.93 [a]	0.216-0.250	
C18:1 trans 6	0.212 [a]	0.213 [a]	0.190 [b]	0.173 [b]	0.005-0.006	
C18:1 trans 9	0.148 [a]	0.148 [a]	0.146 [a]	0.118 [b]	0.003-0.004	†
C18:1 trans 10	0.234 [a]	0.218 [a]	0.172 [b]	0.153 [b]	0.007-0.008	*
C18:1 trans 11	0.704 [c]	0.975 [b]	1.25 [a]	0.898 [b]	0.028-0.032	**
C18:2 cis9 trans11 (CLA)	0.297 [c]	0.413 [b]	0.570 [a]	0.313 [c]	0.011-0.013	***

Trait	Breed[1] DF	MRY	GWH	JER	SE	*P*-value[2]
Groups of FA (g/100g fat)						
SFA	74.06 [a]	72.32 [a]	68.86 [b]	74.27 [a]	0.498-0.574	*
UFA	23.18 [b]	24.26 [b]	26.73 [a]	22.68 [b]	0.467-0.540	†
C6-12	12.83 [a]	12.50 [a]	10.33 [b]	11.24 [b]	0.217-0.251	**
C14-16	43.63 [a, b]	42.33 [b, c]	40.58 [c]	45.29 [a]	0.478-0.553	
Omega 3	0.580 [d]	0.965 [b]	1.38 [a]	0.873 [c]	0.020-0.023	
Omega 6	1.62 [a]	1.41 [b]	1.16 [c]	1.08 [c]	0.037-0.043	*
C18unsat	20.31 [b]	21.29 [b]	23.71 [a]	19.64 [b]	0.450-0.520	
C18trans	1.61[c]	1.86 [b]	2.06 [a]	1.59[c]	0.036-0.042	†
Branched	1.78[a, b]	1.87 [a]	1.74 [b]	1.56 [c]	0.024-0.028	†
Indices						
Unsaturation index	0.228 [b]	0.239 [b]	0.268 [a]	0.224 [b]	0.005-0.006	†
Unsat index C12	0.021 [a]	0.022 [a]	0.020 [a, b]	0.018 [b]	0.001	
Unsat index C14	0.069 [b]	0.078 [a]	0.078 [a]	0.069 [b]	0.002	†
Unsat index C16	0.039 [b]	0.038 [b]	0.045 [a]	0.044 [a, b]	0.001-0.002	*
Unsat index C18	0.658 [b, c]	0.674 [a, b]	0.690 [a]	0.640 [c]	0.005-0.006	**
Production traits						
Fat (%)	4.73 [b]	4.63 [b]	4.53 [b]	6.16 [a]	0.111-0.124	*
Protein (%)	3.59 [c]	3.82 [b]	3.70 [b, c]	4.23 [a]	0.048-0.053	***

[a, b, c, d] different superscripts within a row indicate significance differences of LSM values at $P < 0.05$.

[1] DF = Dutch Friesian; MRY = Meuse-Rhine-Yssel; GWH = Groningen White Headed; JER = Jersey.

[2] † $P < 0.10$, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

The proportion of most FA and groups of FA was significantly different ($P < 0.05$) among breeds (Table 2.3). Jersey cows produced a significantly higher proportion of saturated FA (**SFA**; 74.3%) compared with GWH (68.9%). The latter breed produced the highest proportion of C18:1 *trans*-11 (1.25%) and CLA (0.570%), and had the highest C18 unsaturation index (0.690). Dutch Friesian cows produced the highest proportion of C6–12 (12.8%) and JER the highest proportion of C14–16 (45.3%).

Grazing- or nongrazing-based feeding largely influence milk FA composition (Palmquist et al., 1993; Beaulieu and Palmquist, 1995; Heck et al., 2009). In particular, grazing-based feeding has a negative effect on the proportion of SFA and a positive effect on the proportion of unsaturated FA (Heck et al., 2009). The diet of the sampled cows contained no grazed grass because all cows were kept indoors. This was also the case in the studies of Stoop et al. (2008), Bobe et al. (2007), and Beaulieu and Palmquist (1995).

The average production of C14–16 of JER cows was 45.3% (Table 2.3), which is higher than results from other researches on the same breed: 41.8% (White et al., 2001), 43.3% (Stull and Brown, 1964), and 42.8% (Beaulieu and Palmquist, 1995). In those studies, JER produced a higher proportion of C14–16 than HF cows, except in the study of Beaulieu and Palmquist (1995), which reported higher proportions of C14–16 in HF milk. The average production of C14–16 in JER was in the range of 37.45 to 49.47% that was reported by Carroll et al. (2006). No detailed information on milk fat composition in DF and GWH breeds could be found in other studies. Soyeurt et al. (2006) studied milk fat composition of MRY and JER in Belgium, mainly using data of crossbred animals. In that study, FA profile was based on mid-infrared analysis and the authors reported a lower proportion of total SFA for JER compared with MRY cows, whereas in the current study JER showed the highest proportion of SFA in milk. However, in the same study, the difference in unsaturation index for C16:0 was in agreement with that in the current study. Although the total amount of CLA, which is associated with positive health effects, was generally low, the breed effect was found significant ($P < 0.001$). Significant breed differences for CLA were also reported in Lawless et al. (1999), where the Montbéliarde cows produced higher proportions of CLA (1.99%) compared with the Dutch HF cows (1.76%). In another study including 1,918 Dutch HF cows (Stoop et al., 2008), the proportion of CLA was reported to be, on average, 0.39%. The samples of this large study were taken during the indoor season between February and March, which is comparable with the current study, but the cows were all within first lactation. For C14–16, Stoop et al. (2008) reported an average proportion of 44.2%, which is almost as high as the JER in current study. Other studies including the HF breed reported C14–16 proportions of 32.4% (Mele et al., 2009), 39.1 to 39.5% (Bobe et al., 2007), 44.0% (Beaulieu and Palmquist, 1995), and 36.5% (Lawless et al., 1999).

In conclusion, our results suggest that the Dutch cattle breeds have some favorable milk composition characteristics. First, the local MRY and GWH breeds produced smaller proportions of C14–16 than the mainstream JER. Second, the proportion of n-3 was higher in MRY and GWH. Third, the GWH cows produced

higher proportions of CLA and unsaturated FA. Therefore, the study seems to reveal characteristics of the local Dutch breeds that support the need to conserve them. These results can be used to promote the use of particular breeds and suggests that the genetic features of a population in favor of human health can be modified permanently by making use of the different breeds in the dairy cattle population. However, the results should be confirmed in a larger study in which the breeds compared are held on the same farms and thus exposed to the same production environment.

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3

Validation of fatty acid predictions in milk using mid-infrared spectrometry across cattle breeds

M.H.T. Maurice – Van Eijndhoven[1,2], H. Soyeurt[3,4], F. Dehareng[5], M. P. L. Calus[1]

[1] Animal Breeding and Genomics Centre, Wageningen UR Livestock Research, P.O. Box 338, 6700 AH Wageningen, The Netherlands; [2] Animal Breeding and Genomics Centre, Wageningen University, P.O. Box 338, 6700 AH Wageningen, The Netherlands; [3] Animal Science Unit, Gembloux Agro-Bio Tech, University of Liege, 5030 Gembloux, Belgium; [4]National Fund for Scientific Research, 1000 Brussels, Belgium; [5]Valorisation of Agricultural Products, Walloon Agricultural Research Centre, 5030 Gembloux, Belgium

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Abstract

The aim of this study was to investigate the accuracy to predict detailed fatty acid (FA) composition of bovine milk by mid-infrared spectrometry, for a cattle population that partly differed in terms of country, breed and methodology used to measure actual FA composition compared with the calibration data set. Calibration equations for predicting FA composition using mid-infrared spectrometry were developed in the European project RobustMilk and based on 1236 milk samples from multiple cattle breeds from Ireland, Scotland and the Walloon Region of Belgium. The validation data set contained 190 milk samples from cows in the Netherlands across four breeds: Dutch Friesian, Meuse-Rhine-Yssel, Groningen White Headed (GWH) and Jersey (JER). The FA measurements were performed using gas–liquid partition chromatography (GC) as the gold standard. Some FAs and groups of FAs were not considered because of differences in definition, as the capillary column of the GC was not the same as used to develop the calibration equations. Differences in performance of the calibration equations between breeds were mainly found by evaluating the standard error of validation and the average prediction error. In general, for the GWH breed the smallest differences were found between predicted and reference GC values and least variation in prediction errors, whereas for JER the largest differences were found between predicted and reference GC values and most variation in prediction errors. For the individual FAs 4:0, 6:0, 8:0, 10:0, 12:0, 14:0 and 16:0 and the groups' saturated FAs, short-chain FAs and medium-chain FAs, predictions assessed for all breeds together were highly accurate (validation $R^2 > 0.80$) with limited bias. For the individual FAs *cis*-14:1, *cis*-16:1 and 18:0, the calibration equations were moderately accurate (R^2 in the range of 0.60 to 0.80) and for the individual FA 17:0 predictions were less accurate ($R^2 < 0.60$) with considerable bias. FA concentrations in the validation data set of our study were generally higher than those in the calibration data. This difference in the range of FA concentrations, mainly due to breed differences in our study, can cause lower accuracy. In conclusion, the RobustMilk calibration equations can be used to predict most FAs in milk from the four breeds in the Netherlands with only a minor loss of accuracy.

Key words: milk, fatty acid, mid-infrared spectrometry, cattle breeds

3.1 Implications

Measurement of detailed milk fat composition at individual cow level is of major interest for the dairy industry because of the expected relation with human health. Therefore, the method of analyzing milk fat composition needs to be rapid and suitable for extensive recording. Our study shows that mid-infrared spectrometry (MIR) can be used to accurately predict detailed milk fat composition from different cattle breeds in the Netherlands.

3.2 Introduction

Bovine milk fat consists of a range of different fatty acids (FAs), both unsaturated fatty acids (UFAs) and saturated fatty acids (SFAs), and its relatively large amount of SFA causes some debate about the role of bovine milk in a healthy diet (Palmquist et al., 2006). Clear variation in fat content and milk fat composition can be found among cows (Soyeurt and Gengler, 2008). Milk fat composition varies with both environmental factors (e.g. feed regime; Palmquist, 2006) and genetics (Soyeurt and Gengler, 2008; Stoop et al., 2008). Changing FA composition through the feed regime or genetic selection requires a precise and regular measurement.

To measure the FA composition in milk, several methods can be used, which differ in throughput level, accuracy, workload and costs. The most accurate method is gas–liquid partition chromatography (GC). This largely implemented and regularly used approach quantifies the concentration of individual FAs in fat (Gander et al., 1962; Christie, 1998). The major advantage of GC is the possibility of measuring the individual FA proportions with high accuracy (Smith, 1961; Christie, 1998) even if the content of this FA is low. This method, however, is expensive and time consuming and therefore less suitable for extensive and regular recording. Another method of analyzing milk fat composition, MIR, is rapid and less expensive in case of extensive use (Wilson and Tapp, 1999; Soyeurt et al., 2006). MIR is routinely used in milk recording schemes to measure lactose, urea, total fat and protein percentages in bovine milk (Etzion et al., 2004; Bobe et al., 2007). MIR was used by Soyeurt et al. (2006 and 2011) and Rutten et al. (2009) to estimate calibration equations predicting the FA concentrations in milk (g/dl of milk) and milk fat (g/100 g of fat), and these equations were subsequently validated. In these studies, the predictions have low accuracy for FAs that are present in low concentrations, such as the trans and unsaturated 14, 16 and 18 FAs.

Accuracy and bias in calibration equations may also be affected when there are differences between the samples used to estimate the calibration equations and the samples for which FA composition is predicted using the prediction

equations. In Rutten et al. (2009), calibration equations were based on milk samples only from Holstein–Friesian (HF) cows. In this latter study, analysis of milk samples collected in both winter and summer indicated that season has a limited effect on prediction accuracy but generally a large effect on prediction bias. This indicates that factors causing structural differences between FA composition of groups of animals such as season and breed can affect predictability of calibration equations.

The aim of this study was to investigate the accuracy and bias in predicting detailed FA composition from MIR spectra of milk from four cattle breeds in the Netherlands, using calibration equations based on milk samples collected from Belgian, Irish and Scottish cattle of partly different breeds.

3.3 Materials and Methods

Calibration equations

In this study, prediction of the composition of 11 individual FAs and 3 groups of FAs using MIR spectrometry using calibration equations was validated. These calibration equations were developed in the EU FP 7 project RobustMilk, using a data set with MIR spectra and GC results of 1236 milk samples. The methodology used to develop the calibration equations is explained by Soyeurt et al. (2011) for the calibration equations, but it should be noted that in our study updated versions of the calibrations were used, which are based on 1236 instead of 517 milk samples.

For all 1236 milk samples, the MIR analysis was performed using a Fourier-transformed interferogram with a region of 1000 to 5000/cm (MilkoScan FT 6000, Foss Electric, Hillerod, Denmark). The detailed FA composition of these 1236 milk samples was obtained using GC realized at the milk laboratory of the Walloon Agricultural Research Centre (Gembloux, Belgium). The GC outputs were generated by analyzing methyl esters prepared from milk fat as described in ISO Standard 15 884 (ISO–IDF (International Organization for Standardization–International Dairy Federation), 2002) and the GC was equipped with a CPSil-88 column (Varian Inc., Palo Alto, CA, USA) with a length of 100m and an internal diameter of 0.25 mm.

The 1236 milk samples were collected from herds in Ireland, Scotland and the Walloon Region of Belgium with purebred and crossbred cows from different breeds, that is, HF, Jersey (JER), Red and White, Normande, Montbeliarde and dual-purpose Belgian Blue. This multiple breed and multiple country composition of the data set was chosen to cover a wide range of the variability of FA in bovine milk in order to improve the robustness of the developed calibration equations. The

calibration equations were developed from three MIR regions located between 926 and 1600/cm, 1712 and 1809/cm and 2561 and 2989/cm. The method used to relate MIR spectra to FA data was partial least square regression after a first derivative pre-treatment on spectral data to correct the baseline drift. A T-outlier test was also used during the calibration process to delete potential GC outliers. Therefore, the final number of samples included in each calibration equation varied following the considered FA. Descriptive statistics of the RobustMilk calibration equations are given in Table 3.1. Note that this is an updated version of the prediction equations described by Soyeurt et al. (2011), in the sense that the current prediction equations are based on ~ 4.5 times more samples. In addition to the number of samples included in the calibration data set, the mean and the standard deviation (s.d.) of the FA content measured by GC, the standard error of calibration (SEC), the calibration coefficient of determination (R^2c), standard error of cross-validation (SECV), cross-validation coefficient of determination (R^2cv) and the ratio of s.d. to SECV (RPD) are shown. The R^2c is the square of the correlation coefficient between the predicted and the reference GC values.

During the development of the updated calibration equations, a first assessment of the robustness of the predictions was done by a cross-validation approach to calculate the R^2cv and SECV using the same approach as described by Soyeurt et al. (2011).

Validation data set

Between December 2008 and March 2009 in the Netherlands, that is, in the winter season, a total of 190 cows were sampled once during morning milking. Samples were treated immediately with 0.03% (w/w) sodium azide to avoid microbiological growth. Cows belonged to four breeds: Dutch Friesian (DF; 47 samples from 3 farms), Meuse-Rhine- Yssel (MRY; 52 samples from 3 farms), Groningen White Headed (GWH; 45 samples from 3 farms) and JER (46 samples from 3 farms). The cows were selected by farmers to reflect variations in age, parity, stage of lactation and ancestry. On all farms, cows were kept indoors in the studied period and milked twice a day with conventional milking systems.

The number of sampled cows per herd ranged from 6 to 24, and the selected farms each had between 35 and 120 cows. The cows were either located at organic or conventional farms. For each breed samples were collected at one or two organic farms and the remainder farms were conventional. Differences in FA composition in milk between the four breeds in this data set are presented by Maurice-Van Eijndhoven et al. (2011). Briefly, ranges of individual FA content

generally overlapped between breeds, apart from several FAs and groups of FAs of JER and GWH.

Each milk sample was analyzed using both GC and MIR. The mean and standard deviation of the FA content of each of the 11 individual FAs and the 3 groups of FAs obtained using the GC are given in Table 3.2. The relative variability, which was examined by calculating the coefficient of variation (results not shown), between the different FAs was highest for the cis-14:1 (range 31.1 to 35.8) and cis-16:1 (range 26.2 to 39.1) and lowest for the 4:0, 6:0, 8:0 and total group of short-chain FA (SCFA; range 14.6 to 25.0). GC analysis was performed at the laboratory of Qlip N.V. (Leusden, The Netherlands). The GC outputs were generated by analyzing methyl esters prepared from milk fat as described in ISO Standard 15 884 (ISO–IDF, 2002) and the GC was equipped with a Varian Fame Select CP 7420 column (Varian Inc., Palo Alto, CA, USA) with a length of 100m and an internal diameter of 0.25 mm. The MIR analysis was performed using a Fourier-transformed interferogram with a region of 1000 to 5000/cm (MilkoScan FT 6000, Foss Electric, Denmark) at the laboratory of Qlip N.V. (Zutphen, The Netherlands). The validation data set was independent of the calibration set developed in the RobustMilk project (i.e. different labs for GC and MIR analysis).

Validation

RobustMilk calibration equations (Table 3.1) were used to predict detailed milk composition of the samples recorded in the validation data set for 11 individual FAs 4:0, 6:0, 8:0, 10:0, 12:0, 14:0, *cis*-14:1, 16:0, *cis*-16:1, 17:0, 18:0 and the 3 groups of FAs, that is, total SFA (SFA 4:0 to 22:0 including *iso*- and *ante-iso* FAs), short-chain FA (SCFA; 4:0 to 10:0) and medium-chain FA (MCFA; 12:0 to 16:0). SFA=the saturated fatty acids 4:0 to 22:0 including *iso*- and *ante-iso* FAs; SCFA=4:0 to 10:0; MCFA=12:0 to 16:0. Owing to the lack of agreement between the GC analyses of the calibration and validation data set methods for the long-chain unsaturated FAs and their related FA groups (i.e. total unsaturated, monounsaturated, polyunsaturated and long-chain FAs), these FAs and groups of FAs were considered in this study. This lack of agreement was due to differences in separation of the long-chain unsaturated FAs during the GC analyses of the calibration and validation data sets because the capillary columns used were different. For the other FAs, of which the calibration equations are validated in this study, the separation during the GC analysis was similar. The FA traits were predicted on the basis of milk (g/dl) because these predictions are more accurate than on the basis of fat (g/100 g; Soyeurt et al., 2006; Rutten et al., 2009; Soyeurt et al., 2011).

Table 3.1 Descriptive statistics of RobustMilk FA calibration equations and the data used to derive the equations.

Trait (g/dl of milk)	*N*	Mean	s.d.	SEC	R^2c	SECV	R^2cv	RPD
4:0	1186	0.101	0.030	0.008	0.93	0.008	0.93	3.68
6:0	1189	0.074	0.023	0.005	0.96	0.005	0.96	4.81
8:0	1180	0.048	0.015	0.003	0.96	0.003	0.96	5.00
10:0	1183	0.112	0.036	0.007	0.96	0.008	0.96	4.72
12:0	1180	0.134	0.044	0.009	0.96	0.010	0.95	4.61
14:0	1184	0.448	0.130	0.027	0.96	0.028	0.95	4.70
***cis*-14:1**	1180	0.040	0.015	0.007	0.80	0.007	0.78	2.13
16:0	1179	1.206	0.424	0.066	0.98	0.068	0.97	6.20
***cis*-16:1**	1179	0.067	0.023	0.010	0.79	0.011	0.78	2.14
17:0	1167	0.028	0.008	0.002	0.90	0.003	0.89	3.04
18:0	1173	0.375	0.145	0.043	0.91	0.045	0.90	3.24
SFA	1176	2.689	0.785	0.050	1.00	0.051	1.00	15.34
SCFA	1185	0.349	0.104	0.020	0.96	0.020	0.96	5.10
MCFA	1187	2.056	0.645	0.082	0.98	0.086	0.98	7.53

FA = fatty acid; *N* = number of samples included in the calibration equation; Mean = mean of gas chromatographic data; s.d. = standard deviation of gas chromatographic data; SEC = standard error of calibration; R^2c = calibration coefficient of determination; SECV = standard error of cross-validation; R^2cv = crossvalidation coefficient of determination; RPD = the ratio of s.d. to SECV; SFA = the saturated FAs 4:0 to 22:0 including *iso*- and *ante-iso* FAs; SCFA = short-chain FAs 4:0 to 10:0; MCFA = medium-chain FAs 12:0 to 16:0.

Table 3.2 The mean and standard deviation of gas chromatographic measurements of the validation data for all traits of the individual breeds.

Trait (g/dl milk)	GWH (Mean ± s.d.)	MRY (Mean ± s.d.)	DF (Mean ± s.d.)	JER (Mean ± s.d.)
4:0	0.131 ± 0.020	0.124 ± 0.024	0.130 ± 0.023	0.171 ± 0.028
6:0	0.093 ± 0.014	0.098 ± 0.019	0.104 ± 0.017	0.133 ± 0.024
8:0	0.059 ± 0.011	0.072 ± 0.015	0.072 ± 0.011	0.088 ± 0.018
10:0	0.138 ± 0.031	0.174 ± 0.046	0.186 ± 0.033	0.224 ± 0.057
12:0	0.184 ± 0.051	0.244 ± 0.066	0.230 ± 0.047	0.273 ± 0.076
14:0	0.563 ± 0.094	0.637 ± 0.140	0.617 ± 0.114	0.782 ± 0.151
***cis*-14:1**	0.052 ± 0.019	0.055 ± 0.019	0.044 ± 0.014	0.061 ± 0.019
16:0	1.483 ± 0.275	1.472 ± 0.305	2.260 ± 0.442	1.522 ± 0.353
***cis*-16:1**	0.065 ± 0.017	0.057 ± 0.019	0.058 ± 0.018	0.098 ± 0.028
17:0	0.027 ± 0.008	0.024 ± 0.006	0.023 ± 0.005	0.037 ± 0.007
18:0	0.495 ± 0.150	0.517 ± 0.109	0.524 ± 0.100	0.697 ± 0.118
SFA	3.332 ± 0.503	3.522 ± 0.671	3.563 ± 0.632	4.876 ± 0.817
SCFA	0.436 ± 0.069	0.482 ± 0.101	0.507 ± 0.074	0.637 ± 0.119
MCFA	2.500 ± 0.439	2.621 ± 0.546	2.619 ± 0.537	3.674 ± 0.709

GWH = Groningen White Headed; MRY = Meuse-Rhine-Yssel; DF = Dutch Friesian; JER = Jersey; FA = fatty acid; SFA = the saturated FAs 4:0 to 22:0 including *iso*- and *ante-iso* FAs; SCFA = short-chain FAs 4:0 to 10:0; MCFA = medium-chain FAs 12:0 to 16:0.

The accuracy of the RobustMilk predictions was evaluated using the root mean squared error of prediction (SEV), the coefficient of determination (validation R^2) and the ratio of the s.d. of the validation data set to the SEV (RPD_v). Calibration equations with RPD_v above 3.0 can be considered as good predictors (Williams and Sobering, 1993).

The SEV was calculated as

$$SEV = \sqrt{\frac{\sum_{i=1}^{N}(\hat{y}_i - y_i)^2}{n}},$$

where $\hat{y}_i$ is the predicted value obtained for the sample *i*; y_i is the reference GC value of sample *i*; *n* is the number of samples in the validation set. The approach to calculate SEV is in line with the approach to calculate SEC and SECV, which are described by Soyeurt et al. (2011).

The prediction bias was assessed using the average prediction error ($\hat{y}_i$ - y_i) and slope (β_1) of the linear regression, with the GC values as dependent and the predicted values as independent variable. To be able to compare the average prediction error of the calibration equations across traits and breeds, this measure is expressed as a percentage of the mean of the gas chromatography values.

3.4 Results

For most traits, the SEV was lowest for the predicted FA contents in GWH milk, except for the individual FA *cis*-14:0 and the group of FA SCFA (Table 3.3). The SEV for the predicted FA contents in JER milk was highest for all groups of FAs and the individual FAs 6:0, 14:0, *cis*-14:0 and 16:0, except for the individual FAs 4:0, 12:0, cis-16:1, 17:0 and 18:0, of which the SEV was highest for MRY. The validation R^2 of the predictions of the individual FAs 4:0, 6:0, 8:0, 10:0, 12:0, 14:0, 16:0 and for the groups of FAs SFA, SCFA and MCFA for all breeds were above 0.80 (Table 3.4). The validation R^2 for the individual FA 17:0 was lowest over all breeds (0.43). The FA composition of milk from DF cows was based on the calculated validation R^2 predicted most accurately with an average R^2 of 0.84. The average validation R^2 of the predicted FA composition of milk from GWH was generally lowest (0.81). The long-chain FAs 17:0 and 18:0 and medium-chain FAs *cis*-14:1 and *cis*-16:1 showed the largest variation in validation R^2 between the breeds. The RPD_v was in general

lower than the RPD of the cross-validation; however, a similar trend was observed (Table 3.5). The RPD_v is above 3.0 across all breeds (breeds total) for 6:0; 8:0; 14:0 and all groups of FAs.

Table 3.3 The SEV of 11 FAs and 3 groups of FAs for different dairy breeds.

	Breed				
Trait (g/dl milk)	**GWH**	**MRY**	**DF**	**JER**	**All breeds[1]**
4:0	0.009	0.017	0.011	0.011	0.012
6:0	0.005	0.006	0.006	0.007	0.006
8:0	0.004	0.005	0.006	0.006	0.005
10:0	0.012	0.016	0.025	0.022	0.019
12:0	0.027	0.048	0.036	0.028	0.036
14:0	0.033	0.042	0.037	0.045	0.039
***cis*-14:1**	0.011	0.010	0.014	0.022	0.015
16:0	0.122	0.201	0.215	0.219	0.192
***cis*-16:1**	0.023	0.040	0.033	0.028	0.032
17:0	0.010	0.013	0.012	0.010	0.012
18:0	0.106	0.145	0.139	0.137	0.132
SFA	0.061	0.047	0.050	0.130	0.078
SCFA	0.022	0.028	0.028	0.033	0.028
MCFA	0.105	0.171	0.207	0.253	0.190

SEV = standard error of validation; FA = fatty acid; GWH = Groningen White Headed; MRY = Meuse-Rhine-Yssel; DF = Dutch Friesian; JER = Jersey; SFA = the saturated FAs 4:0 to 22:0 including *iso*- and *ante-iso* FAs; SCFA = short-chain FAs 4:0 to 10:0; MCFA = medium-chain FAs 12:0 to 16:0.

[1] Breeds total is the SEV of the predictions across the breeds GWH, DF, MRY and JER.

Table 3.4 The validation R2 of prediction of 11 FAs and 3 groups of FAs for different dairy breeds.

	Breed				
Trait (g/dl milk)	**GWH**	**MRY**	**DF**	**JER**	**All breeds[1]**
4:0	0.92	0.92	0.89	0.88	0.92
6:0	0.90	0.92	0.88	0.91	0.93
8:0	0.88	0.90	0.88	0.91	0.92
10:0	0.85	0.94	0.89	0.93	0.93
12:0	0.85	0.86	0.80	0.90	0.85
14:0	0.93	0.97	0.93	0.92	0.95
***cis*-14:1**	0.70	0.76	0.79	0.80	0.64
16:0	0.89	0.90	0.93	0.86	0.93
***cis*-16:1**	0.56	0.67	0.48	0.59	0.65
17:0	0.15	0.17	0.73	0.24	0.43
18:0	0.80	0.64	0.65	0.58	0.72
SFA	0.99	1.00	1.00	0.98	0.99
SCFA	0.91	0.93	0.93	0.93	0.95
MCFA	0.95	0.97	0.97	0.92	0.96

FA = fatty acid; GWH = Groningen White Headed; MRY = Meuse-Rhine-Yssel; DF = Dutch Friesian; JER = Jersey; SFA = the saturated FAs 4:0 to 22:0 including *iso*- and *ante-iso* FAs; SCFA = short-chain FAs 4:0 to 10:0; MCFA = medium-chain FAs 12:0 to 16:0.
[1]Breeds total is the R^2 of the predictions across the breeds GWH, DF, MRY and JER.

Table 3.5 The RPD_v[1] of 11 FAs and 3 groups of FAs for different dairy breeds.

	Breed				
Trait (g/dl milk)	**GWH**	**MRY**	**DF**	**JER**	**All breeds[2]**
4:0	2.29	1.44	2.01	2.46	2.41
6:0	3.06	2.94	2.71	3.24	3.86
8:0	2.96	3.14	1.77	3.23	3.53
10:0	2.53	2.89	1.32	2.62	2.76
12:0	1.90	1.36	1.30	2.69	1.91
14:0	2.84	3.30	3.09	3.33	3.80
***cis*-14:1**	1.74	1.84	1.01	0.85	1.28
16:0	2.25	1.52	2.06	1.61	2.50
***cis*-16:1**	0.74	0.47	0.55	1.00	0.85
17:0	0.79	0.44	0.40	0.67	0.68
18:0	1.42	0.75	0.72	0.86	1.09
SFA	8.18	14.20	12.72	6.30	11.55
SCFA	3.14	3.66	2.64	3.59	4.29
MCFA	4.16	3.19	2.59	2.80	3.87

RPD_v = ratio of the standard deviation of the validation samples to the standard error of prediction of the validation; FA = fatty acid; GWH = Groningen White Headed; MRY = Meuse-Rhine-Yssel; DF = Dutch Friesian; JER = Jersey; SFA = the saturated FAs 4:0 to 22:0 including *iso*- and *ante-iso* FAs; SCFA = short-chain FAs 4:0 to 10:0; MCFA = medium-chain FAs 12:0 to 16:0.

[1]Calibration equations with RPD_v above 3.0 can be considered as good predictors (Williams and Sobering, 1993. Journal of Near Infrared Spectroscopy 1, 25–32).

[2]Breeds total is the RPD_v across the breeds GWH, DF, MRY and JER.

Bias was examined by calculating the average prediction error and the slope of the linear regression with the GC values as dependent and the predicted values as independent variable (Tables 3.6 and 3.7). To be able to compare the average prediction error of the calibration equations between traits and breeds, the values were expressed as a percentage of the mean of the gas chromatography absolute value (Table 3.6).

Table 3.6 The average prediction error[1] of the predictions of 11 FAs and 3 groups of FAs for different dairy breeds.

Trait (g/dl milk)	Breed				
	GWH	**MRY**	**DF**	**JER**	**All breeds[2]**
4:0	-4.60	-10.70	26.10	-3.30	-6.40
6:0	-0.40	-2.90	2.60	0.50	-0.10
8:0	-0.80	-0.40	6.70	-0.40	1.50
10:0	0.70	4.90	12.10	7.50	6.30
12:0	6.60	16.80	12.40	4.30	10.30
14:0	3.30	5.10	3.20	-1.40	2.60
***cis*-14:1**	-5.50	-8.30	-23.00	-35.90	-17.90
16:0	-4.50	-10.40	-11.30	-8.50	-8.80
***cis*-16:1**	-28.30	-54.40	-42.50	-30.40	-39.50
17:0	-24.60	-42.50	-43.50	-28.60	-35.10
18:0	12.10	22.90	22.10	19.90	19.40
SFA	1.20	0.80	0.90	0.60	0.90
SCFA	-0.90	-1.80	3.70	0.60	0.40
MCFA	-1.10	-5.00	-6.40	-5.40	-4.50
Mean[3]	-3.40	-6.10	-4.90	-5.70	-5.10
s.d.[3]	10.40	19.70	18.80	15.00	15.60

FA = fatty acid; GWH = Groningen White Headed; DF = Dutch Friesian; MRY = Meuse-Rhine-Yssel; JER = Jersey; SFA = the saturated FAs 4:0 to 22:0 including *iso*- and *ante-iso* FAs; SCF = short-chain FAs 4:0 to 10:0; MCFA = medium-chain FAs 12:0 to 16:0.

[1]The average prediction error calculated as the predicted value minus the reference gas chromatography values and expressed as percentage of the mean of the gas chromatography values: (average prediction error/mean) x 100.

[2]All breeds means the average prediction errors across all predictions for GWH, DF, MRY and JER.

[3]The mean and s.d. of all average prediction errors for each breed and all breeds together.

The bias in terms of average prediction error for individual breeds is largest for MRY with on average - 6.1% followed by JER (- 5.7%) and smallest for GWH with on average 2.4%. For all breeds together, the average prediction error is - 5.1% with an s.d. of 15.6, which means that the average difference between the

predicted content using MIR and the reference GC values was - 5.1% (normalized to the mean). The average prediction error were highest for the predicted contents of the individual FAs *cis*-16:1 and 17:0. The β_1, which is clearly related to the R^2, does not show unexpected results as β_1 is generally closer to 1 when the R^2 is also closer to 1. With a β_1 value of 1.55, the variance of the predicted content of 18:0 for GWH milk showed the largest underestimation (Table 3.7). With a β_1 value of 0.37, the variance of the predicted content of 17:0 for MRY showed the largest overestimation, which indicated a lack of relation between the true and predicted values also shown by the R^2 calculated to be 0.17.

Table 3.7 The slope (β_1) of the linear regression with the gas chromatography values as dependent and the predicted values as independent variable of 11 FAs and 3 groups of FAs for different dairy breeds.

	Breed				
Trait (g/dl milk)	**GWH**	**MRY**	**DF**	**JER**	**All breeds[1]**
4:0	0.95	0.95	0.99	1.16	1.06
6:0	0.90	0.92	0.95	1.07	0.99
8:0	0.97	0.96	1.00	1.06	0.99
10:0	1.04	1.16	1.12	1.14	1.13
12:0	1.27	1.26	1.10	1.14	1.09
14:0	0.93	1.04	1.01	0.91	0.93
***cis*-14:1**	1.22	1.10	0.91	1.11	0.85
16:0	0.92	0.95	1.03	1.00	0.99
***cis*-16:1**	0.77	0.71	0.71	0.89	0.88
17:0	0.76	0.37	0.83	0.63	0.80
18:0	1.55	0.90	0.84	0.82	0.98
SFA	0.99	1.00	1.00	1.01	1.00
SCFA	0.93	1.00	0.97	1.08	1.02
MCFA	0.96	0.97	1.01	0.99	0.97

FA = fatty acid; GWH = Groningen White Headed; DF = Dutch Friesian; MRY = Meuse-Rhine-Yssel; JER = Jersey; SFA = the saturated FAs 4:0 to 22:0 including *iso*- and ante-iso FAs; SCFA = short-chain FAs 4:0 to 10:0; MCFA = medium-chain FAs 12:0 to 16:0.

[1]All breeds means the average prediction errors across all predictions for GWH, DF, MRY and JER.

Comparing the descriptive statistics of the GC data, the FA content in the milk of the validation data set is generally higher than in the milk of the calibration data set. Especially JER milk in the validation data set showed higher FA contents, as the mean contents of 6:0, 8:0, 10:0, 12:0, 14:0, SFA, SCFA and MCFA were outside the 95% confidence interval of the mean of the calibration data of the calibration data set (i.e. larger than 2.5 times the standard deviation above the mean contents).

The performance of the calibration equations to predict the content of the FAs 16:0 and *cis*-16:1 is also visualized in Figures 3.1 and 3.2. For 16:0, a clear linear pattern is shown in Figure 3.1, which result in the high validation R^2 ranging from 0.86 to 0.93. For 16:1, Figure 3.2 clearly shows relatively more deviation of the predicted values. In both figures, especially predictions for JER are located in a different direction.

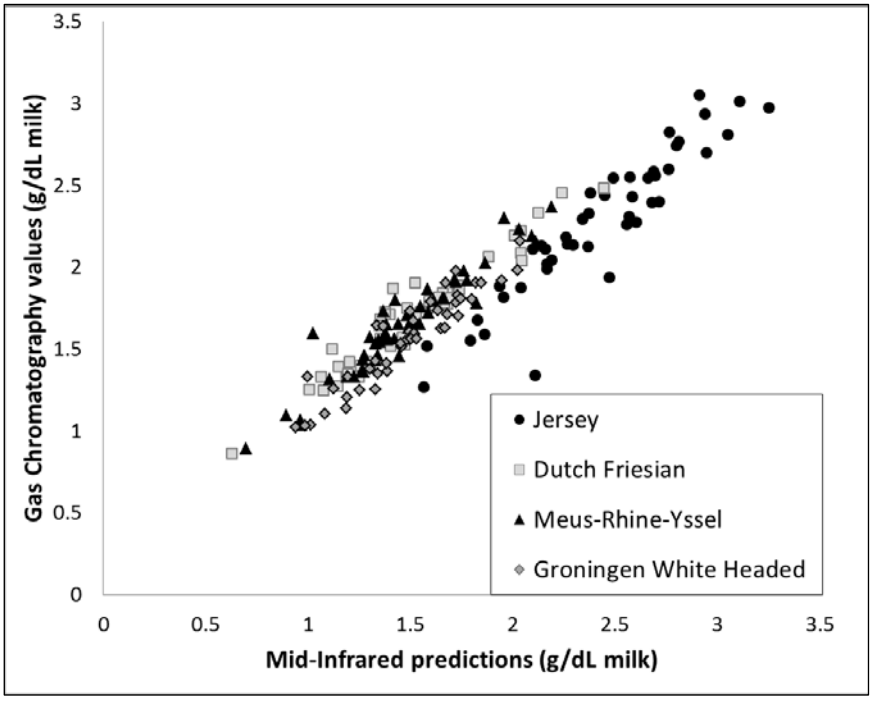

Figure 3.1 The predicted content of the individual fatty acid 16:0 based on mid-infrared spectometry plotted against the reference gas chromatography values.

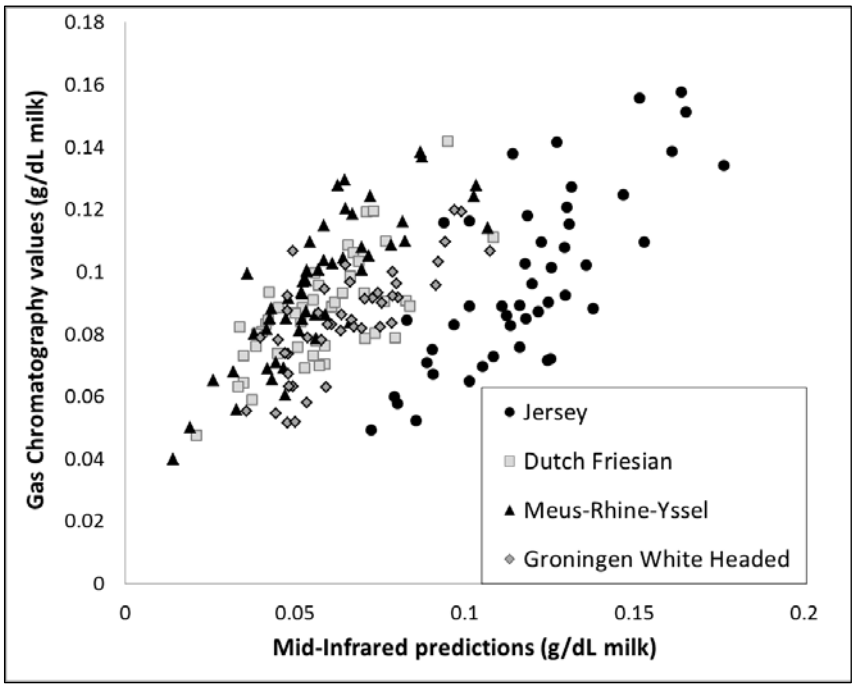

Figure 3.2 The predicted content of the individual fatty acid cis-16:1 based on mid-infrared spectometry plotted against the reference gas chromatography values.

3.5 Discussion

The aim of this study was to investigate the accuracy of calibration equations based on milk samples collected from a population with different origin in terms of country, breed and methodology used to measure actual FA composition. In general, FAs with higher content in milk can be predicted more accurately than milk with a lower FA content (Soyeurt et al., 2006 and 2011; Rutten et al., 2009). In this study, predictions of FA with high content in milk (> 1 g/dl milk) were also highly accurate (validation R^2 > 0.80); however, 7 of the total 11 FAs with lower content in milk (< 1 g/dl milk) were predicted to be highly accurate by means of validation R^2. Differences in performance of the calibration equations between breeds were mainly found by evaluating the SEV and the average prediction error. Results showed on average for GWH the smallest difference between predicted and reference values and least variation in prediction errors, whereas for JER on average the largest differences were found between predicted and reference values and most variation in prediction errors.

The RobustMilk calibration equations validated in our study were updated versions of the calibration equations reported in Soyeurt et al. (2011), in that the calibration data set was enlarged. Despite this increase in size of the calibration data set, the predictions in our study were in general less accurate than those of Soyeurt et al. (2011). Comparing both studies, the FA composition in the validation data set of Soyeurt et al. (2011) was generally closer to the FA composition of the calibration data, whereas FA concentrations in the validation data set of our study were generally higher than those in the calibration data. This difference in range of FA concentrations, mainly due to differences in breed, is the most likely reason for this lower accuracy. A comparable difference in accuracy was found by Rutten et al. (2009) when predicting FA composition in winter or summer, using a calibration equation that was based on winter samples only. This indicates that differences in FA composition due to differences in season (in which the feeding regime differs) are as important as differences due to breed (Rutten et al., 2009). When winter milk samples were used in the calibration data set to predict FA composition of summer samples, differences in concentration ranges between the calibration data set and the validation data set especially affected the bias (i.e. relative difference in means; Rutten et al., 2009).

In our study, the FAs that showed the largest difference in mean between our validation and the calibration data were not necessarily the same FAs as those that showed the largest bias. For instance, despite a relatively small difference in concentration of 14:1, *cis*-16:1, 17:0 and 18:0 between validation and calibration data, those FAs showed the largest bias (i.e. average prediction error and β_1). As *cis*-14:1, 16:1 and 17:0 are present in very low concentrations (< 0.01 g/dl milk), this is the most likely cause of their high bias. Differences between means of validation and calibration data were largest for the concentrations of short and medium FAs in JER milk, which generally had a higher concentration in JER milk compared with the other breeds. Remarkably, despite the large difference in concentration, these FAs generally had accurate predictions. Therefore, it seems that differences in accuracy and bias are not only caused by differences in concentration of the individual FAs, but perhaps also by spectral variability of the milk samples. As indicated by Soyeurt et al. (2011), adding milk samples to the calibration data to maximize the spectral variability of the samples in the calibration data set is an effective method to optimize calibration equations.

The suitability of calibration equations depends on the application of the predictions. If the primary interest is in predicting individual FA composition, then highly accurate and unbiased predictions are important. When the interest is in predicting differences between individuals or populations (e.g. for breeding

purposes), accurate and unbiased predictions are important; however, less accurate or biased predictions can still be suitable, especially when multiple measurements are available per individual. Suitability of calibration equations, which are to some extent derived under different conditions, can be evaluated by means of an external validation as presented in this study.

As the dairy breeding industry is interested in selecting cows producing milk with a specific FA composition, the suitability of the calibration equations depends on the reduction in genetic gain when using MIR information instead of GC information. As Rutten et al. (2010) found, the possible genetic gain estimated using FA composition determined by predictions based on MIR was almost equal to the possible genetic gain estimated using FA composition determined by GC, in dairy breeding schemes with progeny testing. The latter result was reached with even moderate and quite low validation R^2's ranging from 0.53 to 0.77. The genetic gain estimated by Rutten et al. (2010) assumed the availability of information on large groups of daughters per sire. Reaching similar gain could be difficult for the Dutch breeds in our study, as bulls in these breeds have generally smaller daughter groups. For these Dutch breeds, therefore, calibration equations that give highly accurate predictions are necessary to obtain genetic gains similar to the mainstream cattle breeds.

3.6 Conclusion

In conclusion, the RobustMilk calibration equations can be used to predict the content of most saturated FA in milk using MIR spectrometry for the breeds GWH, MRY, DF and JER in the Netherlands with only a minor loss of accuracy compared with predictions for Holstein cows.

3.7 Acknowledgments

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4

Differences in milk fat composition predicted by mid-infrared spectrometry among dairy cattle breeds in the Netherlands

M.H.T. Maurice – Van Eijndhoven[1,2], H. Bovenhuis[2], H. Soyeurt [3,4], M. P. L. Calus[1]

[1] Animal Breeding and Genomics Centre, Wageningen UR Livestock Research, P.O. Box 338, 6700 AH Wageningen, The Netherlands; [2] Animal Breeding and Genomics Centre, Wageningen University, P.O. Box 338, 6700 AH Wageningen, The Netherlands; [3] Animal Science Unit, Gembloux Agro-Bio Tech, University of Liege, 5030 Gembloux, Belgium, [4] National Fund for Scientific Research, 1000 Brussels, Belgium

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Abstract

The aim of this study was to estimate breed differences in milk fatty acid (FA) profile among 5 dairy cattle breeds present in the Netherlands: Holstein-Friesian (HF), Meuse-Rhine-Yssel (MRY), Dutch Friesian (DF), Groningen White Headed (GWH), and Jersey (JER). For this purpose, total fat percentage and detailed FA contents in milk (14 individual FA and 14 groups of FA) predicted from mid-infrared spectra were used. Mid-infrared spectrometry profiles were collected during regular milk recording from a range of herds with different combinations of breeds, including both purebred and crossbred cows. The data set used for the analyses contained 41,404 records from a total of 24,445 cows. In total 7,626 cows were crossbreds belonging to the breeds HF, MRY, DF, GWH, and JER; 1,769 purebreds (≥87.5%) belonging to the breeds MRY, DF, GWH, and JER; and the other 15,050 cows were HF. Breed effects were estimated using a single-trait animal model. The content in milk of short-chain FA C4:0, C6:0, C8:0, C10:0, C12:0, C14:0, and C16:0 was higher for JER and the content in milk of C16:0 was lower for GWH compared with the other breeds; when adjusting for breed differences in fat percentage, however, not all breed differences were significant. Breed differences were also found for *cis*-9 C14:1, *cis*-9 C16:1, C18:0, and a number of C18 unsaturated FA. In general, differences in fat composition in milk between HF, MRY, and DF were not significant. Jerseys tended to produce more saturated FA, whereas GWH tended to produce relatively less saturated FA. After adjusting for differences in fat percentage, breed differences in detailed fat composition disappeared or became smaller for several short- and medium-chain FA, whereas for several long-chain unsaturated FA, more significant breed differences were found. This indicates that short- and medium-chain FA are for all breeds more related to total fat percentage than long-chain FA. In conclusion, between breed differences were found in detailed FA composition and content of individual FA. Especially, for FA produced through de novo synthesis (short-chain FA, C12:0, C14:0, and partly C16:0) differences were found for JER and GWH, compared with the breeds HF, MRY, and DF.

Key words: milk , fatty acid , mid-infrared spectrometry, cattle breed

4.1 Introduction

Bovine milk fat is composed of a wide range of FA, which can be distinguished based on their number of carbons, the saturation of their carbon chain, and the conformation of double bonds. These different FA can roughly be divided into SFA with no double bounds, which make up around 70% of the total milk fat, and unsaturated FA (UFA) with 1 (25% MUFA) or multiple double bounds (5% PUFA). The detailed FA composition in milk is variable and can differ between cows and herds (e.g., Stoop et al., 2008). Extending the knowledge on variation in detailed FA composition is of major interest for the dairy industry because of the expected effects of dairy fat intake on human health (Mensink et al., 2003; Palmquist et al., 2006) and associations between FA composition with milk processability (e.g., Smet et al., 2009) and individual methane emission (Dijkstra et al., 2011). The variation in FA composition in milk can be partly explained by differences in the diet of the cows (e.g., Baumgard et al., 2001; Sterk et al., 2011). Besides diet, a considerable part of the variation also has a genetic origin. For instance, Mele et al. (2009) reported heritabilities for individual FA in milk of Italian Holstein-Friesians (HF) ranging from 0.03 to 0.17 and Stoop et al. (2008) reported heritabilities for individual FA in milk of Dutch HF ranging from 0.22 to 0.71. This indicates that a considerable part of the variation in FA composition is due to genetics. Breeding, therefore, can be a tool to change the FA composition in bovine milk. In addition to genetic variation within breeds, difference between dairy breeds in FA composition might be relevant. Furthermore, identification of specific breed characteristics could provide arguments for breed conservation. Differences in FA composition between herds with different dairy breeds in the Netherlands were reported by Maurice-Van Eijndhoven et al. (2011). Breed differences in FA composition were also found by DePeters et al. (1995) in which differences were reported between HF, Jersey (JER), and Brown Swiss Beaulieu and Palmquist (1995), in which differences were reported between HF and JER, and Lawless et al. (1999) found differences for several individual FA between Irish HF, Dutch HF, Montbeliardes, and Normandes in Ireland. In the first study (Maurice-Van Eijndhoven et al., 2011), however, the structure of the data did not allow separation of breed and herd effects.

To accurately disentangle breed and herd effects, data across a range of herds with multiple combinations of breeds are needed. The latter is a major challenge if the majority of herds only have purebred cows from 1 breed. To be able to identify breed differences, a large number of records including detailed milk FA profiles are needed. Unfortunately, the most commonly used method to determine FA

composition in milk is gas chromatography (GC). Gas chromatography is relatively expensive and time consuming and, therefore, less suitable to assess the detailed milk fat composition for large numbers of milk samples. An alternative method to predict FA composition is mid-infrared spectrometry (MIRS) as described by Soyeurt et al. (2007b), Rutten et al. (2009), and De Marchi et al. (2011). Mid-infrared spectrometry is less expensive and time consuming and commonly used by milk laboratories to analyze the major milk components such as fat and protein content, which makes MIRS attractive for routine prediction of FA and for large-scale experiments. For example, Soyeurt et al. (2007a) reported heritabilities calculated using individual FA predicted using MIRS profiles in milk of dairy cattle in the Walloon region of Belgium, ranging from 0.05 to 0.38. In another study of Soyeurt et al. (2006b), using predicted FA databased on MIRS, some breed differences in FA composition were reported among the dairy breeds dual-purpose Belgian Blue, HF, JER, Montbeliarde, and Meuse-Rhine-Yssel (MRY) participating in the Walloon milk recording in Belgium.

The aim of this paper is to identify breed differences in FA composition among the dairy cattle breeds HF, MRY, Dutch Friesian (DF), Groningen White Headed (GWH), and JER. This was achieved by comparing the predicted FA composition for those different cattle breeds in the Netherlands using a data set with MIRS profiles from regular milk recording, including a range of herds with different combinations of breeds, considering both purebred and crossbred animals.

4.2 Materials and Methods

Collection and Data Editing

Mid-infrared spectrometry profiles of milk samples were collected via the Dutch milk recording system of CRV BV (Arnhem, the Netherlands) between October and December 2006. Samples were treated immediately with 0.03% (wt/wt) sodium azide to avoid microbiological growth. The MIRS profiles were obtained using 3 Fourier-transformed interferogram machines (MilkoScan FT 6000; Foss Electric A/S, Hillerod, Denmark) at the laboratory of Qlip N.V. (Leusden, the Netherlands). The sampled herds were a random
representation of all herds participating in the milk recording system of CRV BV.

The initial data set contained 372,429 test-day records of 230,995 cows. Data-editing steps included the deletion of records and cows for the following reasons: less than 75% of the breed composition known, unknown sire, incomplete milk recording data (e.g., unknown birthdate or DIM), 2 records from the same cow on the same sample date, cows with records in more than 1 herd, cows reported sick at sampling date, cows in parity 11 or higher, cows before 5 or after 365 d in

lactation, and cows in herds with less than 5 purebred cows of the same breed (HF, MRY, DF, or GWH) per herd. To detect records with possible errors, due to, for example, swapped samples, fat content recorded via the regular milk control (predicted by QLIP N.V.) was compared with the values obtained using the RobustMilk prediction equations (Soyeurt et al., 2011). The correlation coefficient between fat content predicted by QLIP N.V. and fat content predicted using the RobustMilk prediction equations was 0.996. When the absolute difference in both predictions for fat percentage was more than 0.35 the record was removed. Finally, complete records with extreme outliers in at least 1 of all predicted traits (± 5 SD of the mean) were deleted. After these editing steps, the data set contained 307,656 records.

A large number of these records were from HF animals from herds without crossbreds or animals from breeds other than HF. Because these records do not contribute to the breed estimates and makes the data set heavily unbalanced, only animals from herds with at least 3 animals with >25% genes from MRY, DF, GWH, or JER were kept in the data set. The final data set used for the analyses contained 41,404 records of 24,445 cows from 445 farms. A total of 7,626 cows were crossbreds belonging to the breeds HF, MRY, DF, GWH, and JER; 1,769 purebreds (≥87.5%) belonging to the breeds MRY, DF, GWH, and JER; and the other 15,050 cows were HF.

Predicting FA Composition

The MIRS profiles were used to predict the total fat percentage and detailed milk fat composition of 14 individual FA (C4:0, C6:0, C8:0, C10:0, C12:0, C14:0, *cis*-9 C14:1, C16:0, *cis*-9 C16:1, C18:0, *cis*-9 C18:1, *cis*-9,12 C18:2, *cis*-9,12,15 C18:3, and *cis*-9,*trans*-11 C18:2) and the 14 groups of FA [total *trans* C18:1, total *cis* C18:1, total C18:2, total trans C18, total SFA, total MUFA, total PUFA, total UFA, short-chain FA (SCFA), medium-chain FA (MCFA), long-chain FA (LCFA), total n-3 FA, total n-6 FA, and total branched-chain FA (BCFA)]. The calibration equations used to predict these FA were updated versions of the calibration equations of Soyeurt et al. (2011) based on 1,236 milk samples. The method used to relate MIRS spectra to FA data was partial least square regression after a first-derivative pretreatment on spectral data to correct the baseline drift. An external validation of the calibration equations used to predict FA in this study was published previously (Maurice-Van Eijndhoven et al., 2013). Results from that validation study indicate that the FA C4:0 to C16:0 and the groups SFA, SCFA, and MCFA can be predicted with high accuracy (R^2 >0.80); *cis*-9 C14:1, *cis*-9 C16:1, and C18:0 can be predicted with

Table 4.1 Definition of the groups of FA and the validation coefficient of determination of the prediction.

Group[1]	FA[2]	*n* and R^2 calibration equations[3]
SFA	C4:0; C6:0; C8:0; C10:0; C12:0; *iso* C13:0; *ante-iso* C13:0; C14:0; *iso* C14:0; C15:0; *iso* C15:0; *ante-iso* C15:0; C16:0; *iso* C16:0; C17:0; *iso* C17:0; *ante-iso* C17:0; C18:0; *iso* C18:0; C19:0; C20:0; C22:0	*n*: 1176 R^2: 0.98-1.0[4]
UFA with 1 double bound (MUFA)	C10:1; *cis* C12:1; *cis* C14:1; *cis* C16:1; *trans* C16:1; C17:1; *cis*-9 C18:1; *cis*-11 C18:1; *cis*-12 C18:1; *trans*-6-11 C18:1; *trans*-12-14 C18:1; *cis*-13 C18:1; *cis*-14 C18:1; *trans*-16 C18:1; *cis*-9 C20:1; *cis*-11 C20:1	*n*: 1180 R^2: 0.98[5]
UFA with 2 or more double bounds (PUFA)	C18:2 ∑ ttNMID; *cis*-9,*trans*-13 C18:2; *trans*-8,*cis*-12 C18:2; *cis*-9,*trans*-12 C18:2; *trans*-8,*cis*-13 C18:2; *trans*-11,*cis*-15 C18:2; *trans*-9,*cis*-12 C18:2; *cis*-9,*cis*-12 C18:2; *cis*-9,*cis*-12,*cis*-15 C18:3; *cis*-9,*trans*-11 C18:2 (CLA); C20:3n-6; C20:4n-6; C20:5n-3 EPA; C22:5 DPA	*n*: 1180 R^2: 0.85[5]
UFA	MUFA; PUFA	*n*: 1179 R^2: 0.98[5]
SCFA	C4-C10	*n*: 1185 R^2: 0.91-0.95[4]
MCFA	C12-C16	*n*: 1187 R^2: 0.92-0.97[4]
LCFA	C17-C22	*n*: 1188 R^2: 0.97[5]
n-3	*cis*-9,*cis*-12,*cis*-15 C18:3; C20:5 (EPA); C22:5 (DPA)	*n*: 1172 R^2: 0.77[5]
n-6	C18:2 ∑ ttNMID; *cis*-9,*trans*-13 C18:2; *trans*-8,*cis*-12 C18:2; *cis*-9,*trans*-12 C18:2; *trans*-8,*cis*-13 C18:2; *trans*-11,*cis*-15 C18:2; *trans*-9,*cis*-12 C18:2; *cis*-9, *cis*-12 C18:2; C20:3n-6; C20:4n-6	*n*: 1167 R^2: 0.76[5]
BCFA	*iso* C13:0; *ante-iso* C13:0; *iso* C14:0; *iso* C15:0; *ante-iso* C15:0; *iso* C16:0; *iso* C17:0; *ante-iso* C17:0; *iso* C18:0	*n*: 1166 R^2: 0.85[5]
Total *trans* C18:1	*trans*-6-11 C18:1; *trans*-12-14 C18:1	*n*: 1176 R^2: 0.92[5]

Total *cis* C18:1	*cis*-9 C18:1; *cis*-11 C18:1; *cis*-12 C18:1; *cis*-13 C18:1; *cis*-14 C18:1; 1 *trans*-16 C18:	*n*: 1189 R^2: 0.97[5]
Total C18:2	C18:2 ∑ ttNMID ; *cis*-9,*trans*-13 C18:2; *trans*-8,*cis*-12 C18:2; *cis*-9,*trans*-12 C18:2; *trans*-8,*cis*-13 C18:2; *trans*-11,*cis*-15 C18:2; *trans*-9,*cis*-12 C18:2; *cis*-9,*cis*-12 C18:2	*n*: 1166 R^2: 0.75[5]
Total *trans* C18	*trans*6-11 C18:1; *trans*12-14 C18:1; C18:2 ∑ ttNMID; *cis*-9,*trans*-13 C18:2; *trans*-8,*cis*-12 C18:2; *cis*-9,*trans*-12 C18:2; *trans*-8,*cis*-13 C18:2; *trans*-11,*cis*-15 C18:2; *trans*-9,*cis*-12 C18:2	*n*: 1181 R^2: 0.92[5]

[1]UFA = unsaturated FA; SCFA = short-chain FA; MCFA = medium-chain FA; LCFA = long-chain FA; BCFA = branched-chain FA.

[2]CLA = conjugated linoleic acid; EPA = eicosapentaenoic acid; DPA = docosapentaenoic acid; *tt*NMID = *trans*,*trans* non-methylene-interrupted diene.

[3]n = the number of samples included in the calibration equation; R^2 = the cross validation coefficient of determination of the calibration equation.

[4]Range of validation R^2 of the calibration equations for the group of FA based on separate validation data sets of the different breeds Groningen White Headed, Dutch Friesian, Meuse-Rhine-Yssel, and Jersey (Maurice-Van Eijndhoven et al., 2013).

[5]The cross-validation R^2 of the calibration equations. The cross-validation approach to calculate the R^2 is described by Soyeurt et al. (2011).

moderate accuracy (R^2 = 0.48–0.80); and C17:0 can be predicted with lower accuracy (R^2 = 0.15–0.73). Only FA were evaluated when the coefficient of determination was at least 0.60 over all breeds in this external validation, and for those not validated in that study, the cross-validation coefficient of determination of the calibration equations used was required to be at least 0.70 [the approach to calculate the cross-validation coefficient of determination was described by Soyeurt et al. (2011)]. The definition of the groups of FA and the predictability of the calibration equations by means of the (cross-validation) coefficient of determination are given in Table 4.1.

Statistical Analysis

Breed effects were estimated in ASReml 3.0 (Gilmour et al., 2009) using the following animal models:

$$y_{ijklmnopqrstu} = \mu + b_1 \times DIM_i + b_2 \times exp^{-0.05 \times DIM}{}_i + parity_j + b_3 \times age_k(parity_j) + htd_l + b_4 \times HF_m + b_5 \times MRY_n + b_6 \times DF_o + b_7 \times GWH_p + b_8 \times JER_q + b_9 \times HET_r + b_{10} \times REC_s + cow_t + e_{ijklmnopqrst},$$

in this study referred to as the FAT− model, and

$$y_{ijklmnopqrstuv} = \mu + b_1 \times DIM_i + b_2 \times exp^{-0.05 \times DIM}{}_i + parity_j + b_3 \times age_k(parity_j) + htd_l + b_4 \times HF_m + b_5 \times MRY_n + b_6 \times DF_o + b_7 \times GWH_p + b_8 \times JER_q + b_9 \times HET_r + b_{10} \times REC_s + b_{11} \times fatperc_t(HF_m) + b_{12} \times fatperc_t(MRY_n) + b_{13} \times fatperc_t(DF_o) + b_{14} \times fatperc_t(GWH_p) + b_{15} \times fatperc_t(JER_q) + cow_u + e_{ijklmnopqrstu},$$

in this study referred to as the FAT+ model, where *yijklmnopqrstu(v)* was the dependent variable for cow *t*(*u*) in DIM *i*, with parity *j*, calving age *k*, producing at herd test date *l*, and having a breed composition *mnopq*; *μ* was the overall mean of the model; b1 was the fixed regression coefficient on DIM*i* and b2 was the fixed regression coefficient on DIM*i* modeled with a Wilmink curve (Wilmink, 1987); parity*j* was a fixed effect with 4 classes for corresponding lactation numbers of parity 1, 2, and 3 and the fourth class including parities 4 to 10; b3 was the fixed regression coefficient on age*k*, which was calving age in days, within the *j*th parity; htd*l* was a fixed effect defining 841 groups of cows sampled in the same herd on the same sample date; b4, b5, b6, b7, and b8 were the fixed regression coefficients on, respectively, HF*m*, MRY*n*, DF*o*, GWH*p*, and JER*q*, which were the estimated percentages of genes belonging to each of those breeds; b9 was the fixed regression on HET*r*, which was the estimated percentage of heterosis; b10 was the fixed regression on REC*s*, which was the estimated percentage of recombination loss effect; b11 to b15 were the fixed regression coefficients of fatperc*t*, which was the total fat percentage in milk, within the breeds HF, MRY, DF, GWH, and JER; cow*t*(*u*) was a random permanent environmental effect (no genetic relationships included) of cow *t*(*u*); and *eijklmnopqrst(u)* was the random residual effect. Heterosis was calculated as function of the degree of heterozygosity of animals and REC was derived from the heterozygosity of parental gametes, whose calculations are both described by Van Der Werf and De Boer (1989).

For each trait model, assumptions were checked for equal variances, independency of the phenotypic values, and normality of the residuals. Only for the

trait *cis*-9 C18:1 did the residuals substantially deviate from normality. To avoid problems in the comparison of results due to differences in scales resulting from, for example, log transforming the values, and transforming results back to the original scale, this trait was simply analyzed on the original scale. Reporting *cis*-9 C18:1 on the original scale is in line with others who analyzed the same trait (e.g., Stoop et al., 2008; Rutten et al., 2009).

Breed effects were estimated by calculating the predicted means of all traits for each breed in the third parity and 156 d in lactation. For the FAT+ model, means were predicted at the average fat percentage of 4.70. To test if the observed differences in FA content between breeds were significant a Student's *t*-test was performed. For the calculation of the *t*-test statistic, the standard error of difference was obtained from ASReml 3.0 software (Gilmour et al., 2009). The *P*-values were obtained using a 2-tailed Student's *t*-distribution with *n* degrees of freedom, where *n* was conservatively chosen to be the number of sires of the smallest breed of the 2 breeds that were compared (n = 596 MRY; 156 FH; 66 G; and 80 JER sires).

The data included crossbred animals; therefore, heterosis and recombination effects were included in the model. To test whether these effects were significantly different from zero, a Student's *t*-test was performed using the same method as described above to test the breed differences and with 24,445 (total number of cows) degrees of freedom.

4.3 Results

Production Traits

The unadjusted production data shows that HF produced the highest daily milk yield, followed by JER, DF, MRY, and GWH (Table 4.2). When assessing the results based on the FAT−, model only the milk yield of HF differed significantly ($P < 0.05$) from milk yields of MRY, GWH, and JER (Table 4.3). The results of the FAT− model show that the total fat percentage in milk for JER was significantly higher and for GWH significantly lower compared with the other breeds, which was in line with differences observed in the unadjusted data. Jerseys also produced a significant higher protein percentage.

Individual FA C4:0 to C18:0

The predicted means for C4:0 to C18:0 are shown in Table 4.3. When assessing the results based on the FAT− model, JER produced significantly higher contents for all of these individual FA than the other breeds. Using the FAT+ model, thus adjusting

Table 4.2 The number of cows and herds, and means ± SD of parity, calving age, DIM, production traits, individual FA, and groups of FA for the crossbred and purebred cows (≥50%) in the unadjusted data.

	Breed[2]				
Item[1]	**HF**	**MRY**	**DF**	**GWH**	**JER**
Number of cows[3]					
87.5-100% [4]	15,050	1,389	259	105	16
50-75% [4]	6,820	660	138	40	273
<50% [4]	945	7,515	2,383	97	214
Number of herds[5]	410	58	11	5	43
Fixed effects[5]					
Parity	2.77 ± 1.75	3.08 ± 1.97	3.00 ± 1.84	3.00 ± 1.82	3.11 ± 1.51
Calving age days	1510 ± 712	1621 ± 764	1618 ± 745	1545 ± 686	1607 ± 605
DIM	155 ± 98	156 ± 95	154 ± 103	152 ± 92	161 ± 97
Production trait[5]					
Milk yield (kg/d)	26.7 ± 8.4	21.3 ± 7.4	22.0 ± 7.7	19.9 ± 6.5	24.7 ± 8.1
Fat (%)	4.70 ± 0.75	4.77 ± 0.72	4.81 ± 0.62	4.32 ± 0.73	5.13 ± 0.90
Protein (%)	3.64 ± 0.42	3.83 ± 0.42	3.76 ± 0.43	3.69 ± 0.42	3.87 ± 0.45
Fat yield (kg/d)	1.23 ± 0.36	0.99 ± 0.31	1.04 ± 0.33	0.84 ± 0.23	1.23 ± 0.37
Protein yield (kg/d)	0.95 ± 0.25	0.80 ± 0.24	0.81 ± 0.24	0.72 ± 0.19	0.93 ± 0.27

FA (g/dL milk)[5]					
C4:0	0.128 ± 0.022	0.131 ± 0.021	0.131 ± 0.018	0.127 ± 0.021	0.143 ± 0.025
C6:0	0.092 ± 0.016	0.096 ± 0.016	0.095 ± 0.013	0.086 ± 0.016	0.105 ± 0.020
C8:0	0.059 ± 0.012	0.063 ± 0.012	0.062 ± 0.010	0.053 ± 0.012	0.068 ± 0.014
C10:0	0.140 ± 0.033	0.151 ± 0.033	0.149 ± 0.031	0.119 ± 0.034	0.161 ± 0.037
C12:0	0.171 ± 0.044	0.184 ± 0.044	0.182 ± 0.042	0.141 ± 0.044	0.196 ± 0.048
C14:0	0.533 ± 0.111	0.556 ± 0.112	0.551 ± 0.104	0.468 ± 0.106	0.603 ± 0.123
***cis*-9 C14:1**	0.053 ± 0.015	0.054 ± 0.015	0.054 ± 0.015	0.049 ± 0.012	0.059 ± 0.015
C16:0	1.48 ± 0.34	1.48 ± 0.32	1.51 ± 0.29	1.16 ± 0.30	1.68 ± 0.38
***cis*-9 C16:1**	0.089 ± 0.021	0.092 ± 0.021	0.092 ± 0.02	0.082 ± 0.016	0.093 ± 0.022
C18:0	0.451 ± 0.115	0.444 ± 0.109	0.443 ± 0.111	0.432 ± 0.098	0.495 ± 0.133
***cis*-9 C18:1**	0.919 ± 0.119	0.926 ± 0.199	0.945 ± 0.194	0.927 ± 0.181	0.936 ± 0.201
Total *cis* C18:1	0.957 ± 0.210	0.965 ± 0.208	0.981 ± 0.205	0.979 ± 0.191	0.974 ± 0.213
Total *trans* C18:1	0.124 ± 0.047	0.122 ± 0.047	0.128 ± 0.060	0.168 ± 0.048	0.128 ± 0.046
***cis*-9,12 C18:2**	0.076 ± 0.016	0.079 ± 0.015	0.076 ± 0.014	0.068 ± 0.022	0.078 ± 0.016
***cis*-9,*trans*-11 C18:2**	0.033 ± 0.017	0.032 ± 0.017	0.037 ± 0.022	0.052 ± 0.022	0.031 ± 0.016
Total C18:2	0.100 ± 0.018	0.102 ± 0.018	0.103 ± 0.018	0.104 ± 0.016	0.102 ± 0.018
***cis*-9,12,15 C18:3**	0.023 ± 0.006	0.024 ± 0.006	0.024 ± 0.007	0.026 ± 0.004	0.026 ± 0.006
Total *trans* C18	0.146 ± 0.057	0.143 ± 0.058	0.150 ± 0.071	0.198 ± 0.061	0.149 ± 0.055

	Breed[2]				
Item[1]	HF	MRY	DF	GWH	JER
UFA	1.45 ± 0.27	1.47 ± 0.27	1.50 ± 0.29	1.53 ± 0.26	1.49 ± 0.28
MUFA	1.27 ± 0.25	1.29 ± 0.24	1.31 ± 0.25	1.32 ± 0.23	1.30 ± 0.26
PUFA	0.170 ± 0.046	0.170 ± 0.047	0.173 ± 0.053	0.207 ± 0.044	0.175 ± 0.044
SCFA	0.435 ± 0.079	0.458 ± 0.078	0.454 ± 0.067	0.399 ± 0.078	0.498 ± 0.093
MCFA	2.50 ± 0.52	2.54 ± 0.51	2.55 ± 0.47	2.08 ± 0.49	2.81 ± 0.59
LCFA	1.77 ± 0.38	1.77 ± 0.38	1.80 ± 0.40	1.85 ± 0.34	1.84 ± 0.41
n-3	0.029 ± 0.007	0.030 ± 0.008	0.030 ± 0.008	0.032 ± 0.006	0.033 ± 0.008
n-6	0.114 ± 0.020	0.116 ± 0.020	0.116 ± 0.021	0.118 ± 0.018	0.116 ± 0.020
BCFA	0.088 ± 0.015	0.086 ± 0.017	0.090 ± 0.017	0.090 ± 0.016	0.091 ± 0.016

[1] UFA = unsaturated FA; SCFA = short-chain FA; MCFA = medium-chain FA; LCFA = long-chain FA; BCFA = branched-chain FA.

[2] HF = Holstein-Friesian; MRY = Meuse-Rhine-Yssel; DF = Dutch Friesian; GWH = Groningen White Headed; JER = Jersey.

[3] Note that crossbred animals are counted twice (i.e., an animal that is 50% HF and 50% MRY appears in the row 50–75% for both HF and MRY).

[4] Recorded breed percentages are expressed as multiples of 1/8.

[5] Values include animals with ≥50% genes from HF, MRY, DF, GWH, or JER.

for differences in fat content, the predicted means of *cis*-9 C16:1 for JER were significantly lower compared with those for HF, MRY, and DF and tended to be lower compared with GWH (P = 0.08), and the predicted means of C18:0 for JER tended to be lower compared with those for HF, MRY, and GWH. The predicted means of GWH for C4:0 to C16:0 and *cis*-9 C16:1 were significantly lower ($P < 0.05$) when assessing the results of the FAT− model compared with all other breeds. When assessing the results of the FAT+ model, GWH also showed the lowest predicted means for C4:0 to C16:0, although not all comparisons with the other breeds were significant. The GWH breed produced the highest content of C18:0 when adjusting for differences in fat content.

C18 UFA

The predicted means of the individual C18 UFA and groups of these FA are in Table 4.4. Assessing the predicted means, whether adjusting for differences in fat percentage (FAT+) or not (FAT−), JER showed a significantly higher predicted mean for *cis*-9,12,15 C18:3. Jerseys produced a higher content of *trans* C18 FA compared with HF and MRY; however, this effect disappeared when adjusting for fat percentage in the model. Evaluating the results of the FAT+ model, GWH generally showed the highest predicted means for the C18 UFA; however, these predicted means were only in several comparisons significantly different from those of the other breeds.

Groups of FA

The predicted means of the groups of FA are shown in Table 4.5. Assessing the results of the FAT− model, JER showed significantly higher predicted means for all traits except for the group of MUFA and n-6 FA. When adjusting for differences in fat percentage (the FAT+ model), predicted means of SFA, SCFA, and MCFA for JER were significantly higher compared with all other breeds and predicted means of PUFA (for JER) were significantly higher compared with HF and MRY. For the FAT+ model, predicted means of UFA, MUFA, LCFA, and n-6 for JER were significantly lower than those for the other breeds. The predicted means of all groups of FA for GWH, except for n-3 and LCFA, were significantly lower compared with the other breeds using the FAT− model, whereas for the FAT+ model, this was only still the case for SCFA compared with MRY, DF, and JER and for MCFA compared with HF, DF, and JER.

Table 4.3 Predicted means of 5 production traits and 10 individual FA for each breed[1].

	Breed[2]					
Item	**HF**	**MRY**	**DF**	**GWH**	**JER**	**SE[3]**
FAT- model[4] trait						
Fat (%)	4.57^{b}	4.61^{b}	4.68^{b}	4.27^{c}	5.06^{a}	0.014-0.185
Protein (%)	3.60^{b,c}	3.80^{b,c}	3.65^{b}	3.58^{c}	3.95^{a}	0.007-0.086
Fat yield (kg/d)	1.27^{a}	1.07^{c,d}	1.14^{a,b,c}	1.00^{d}	1.17^{b}	0.005-0.072
Protein yield (kg/d)	1.00^{a}	0.88^{a,b}	0.90^{a,b}	0.84^{b}	0.91^{b}	0.004-0.050
Milk yield (kg/d)	28.5^{a}	24.0^{b,c}	25.3^{a,b}	24.0^{b,c}	23.2^{c}	0.115-1.521
FA (g/dL milk)						
C4:0	0.124^{b}	0.123^{b}	0.127^{b}	0.112^{c}	0.144^{a}	0.000-0.005
C6:0	0.091^{b}	0.092^{b}	0.094^{b}	0.083^{c}	0.108^{a}	0.000-0.004
C8:0	0.059^{b}	0.061^{b}	0.062^{b}	0.054^{c}	0.071^{a}	0.000-0.003
C10:0	0.143^{b}	0.149^{b}	0.149^{b}	0.134^{c}	0.169^{a}	0.001-0.008
C12:0	0.175^{b}	0.183^{b}	0.183^{b}	0.162^{c}	0.206^{a}	0.001-0.010
C14:0	0.542^{b}	0.553^{b}	0.559^{b}	0.504^{c}	0.631^{a}	0.002-0.024
***cis*-9C14:1**	0.055^{b}	0.055^{b}	0.057^{b}	0.048^{c}	0.064^{a}	0.000-0.003
C16:0	1.46^{b}	1.44^{b}	1.50^{b}	1.33^{c}	1.68^{a}	0.006-0.077
***cis*-9 C16:1**	0.085^{b}	0.087^{b}	0.088^{a,b}	0.078^{c}	0.090^{a}	0.000-0.004
C18:0	0.417^{b}	0.424^{b}	0.419^{b}	0.419^{a,b}	0.460^{a}	0.002-0.026

FAT+ model[4] *trait* *(g/dL of milk)*						
C4:0	0.127[b]	0.125[b]	0.127[b]	0.124[b]	0.135[a]	0.001-0.004
C6:0	0.093[b,c]	0.094[b,c]	0.094[b]	0.092[c]	0.101[a]	0.001-0.002
C8:0	0.061[b,c]	0.062[b]	0.062[b,c]	0.060[c]	0.065[a]	0.001-0.002
C10:0	0.147[b,c]	0.152[b]	0.150[b,c]	0.146[c]	0.157[a]	0.001-0.005
C12:0	0.180[b,c]	0.187[a,b]	0.183[b,c]	0.177[c,d]	0.190[a,d]	0.001-0.007
C14:0	0.556[b]	0.564[b]	0.562[b]	0.549[b]	0.588[a]	0.001-0.014
***cis*-9C14:1**	0.056[b]	0.056[b]	0.057[b]	0.052[c]	0.060[a]	0.001-0.002
C16:0	1.52[b]	1.49[b,c]	1.52[a,b]	1.47[a,c]	1.54[a]	0.002-0.036
***cis*-9 C16:1**	0.087[a]	0.089[a]	0.088[a]	0.086[a,b]	0.082[b]	0.001-0.002
C18:0	0.433[b]	0.436[b]	0.423[b,c]	0.475[a]	0.421[c]	0.001-0.017

[a–d] Values with different superscripts within a row indicate significant differences of predicted means at $P < 0.05$.

[1] Predicted means of all traits for each breed at the third parity and at 156 DIM in both models, and for the FAT+ model, traits were predicted at a fat percentage of 4.70.

[2] HF = Holstein-Friesian; MRY = Meuse-Rhine-Yssel; DF = Dutch Friesian; GWH = Groningen White Headed; JER = Jersey.

[3] Range of SE of the predicted means.

[4] Using the FAT− model, means were predicted regardless of the level of total fat percentage, and using the FAT+ model, means were predicted including a fixed regression coefficient of the total fat percentage in milk within the breeds HF, MRY, DF, GWH, and JER.

Table 4.4 Predicted means of 4 individual C18 unsaturated FA and 4 groups of C18 unsaturated FA for each breed[1].

	Breed[2]					
Item	**HF**	**MRY**	**DF**	**GWH**	**JER**	**SE[3]**
FAT- model[4] trait (g/dL of milk)						
***cis*-9 C18:1[5]**	0.925[a]	0.927[a]	0.928[a]	0.902[b]	0.918[a,b]	0.001-0.020
Total *cis* C18:1	0.878[a]	0.887[a]	0.883[a,b]	0.840[a,b]	0.858[b]	0.003-0.047
Total *trans* C18:1	0.120[b]	0.119[b]	0.125[b]	0.117[a,b]	0.133[a]	0.001-0.009
***cis*-9,12C18:2**	0.073[a,b]	0.077[a]	0.073[a,b]	0.074[a,b]	0.072[b]	0.001-0.003
***cis*-9,*trans*-11 C18:2**	0.033[a,b]	0.031[b,c]	0.035[a,b]	0.029[c,d]	0.035[a,d]	0.001-0.003
Total C18:2	0.098[a,b]	0.099[a]	0.099[a,b]	0.094[b]	0.097[a,b]	0.001-0.004
***cis*-9,12,15 C18:3**	0.023[b]	0.023[b]	0.023[b,c]	0.022[c]	0.026[a]	0.000-0.001
Total *trans* C18	0.143[b]	0.139[b]	0.149[a,b]	0.137[a,b]	0.157[a]	0.001-0.011
FAT+ model[4] trait (g/dL of milk)						
***cis*-9 C18:1[5]**	0.934[a]	0.935[a]	0.930[a]	0.944[a]	0.893[b]	0.001-0.015
Total *cis* C18:1	0.903[a,b]	0.906[a,b]	0.887[b]	0.939[a]	0.792[c]	0.003-0.037
Total *trans* C18:1	0.123[b,c]	0.121[c]	0.125[a,c]	0.133[a]	0.126[a,b]	0.001-0.008
***cis*-9,12C18:2**	0.075[b]	0.078[a,b]	0.073[b]	0.080[a]	0.068[c]	0.001-0.003
***cis*-9,*trans*-11 C18:2**	0.033[b]	0.031[b]	0.035[a,b]	0.032[a,b]	0.035[a]	0.001-0.003

Total C18:2	0.100[a,b]	0.100[a,b]	0.099[b]	0.103[a]	0.092[c]	0.001-0.003
***cis*-9,12,15 C18:3**	0.023[b]	0.024[b]	0.023[b]	0.024[a,b]	0.024[a]	0.000-0.001
Total *trans* C18	0.146[b,c]	0.142[c]	0.149[a,c]	0.156[a]	0.149[a,b]	0.001-0.010

[a–d] Values with different superscripts within a row indicate significant differences of predicted means at $P < 0.05$.

[1] Predicted means of all traits for each breed at the third parity and at 156 DIM in both models, and for the FAT+ model, traits were predicted at a fat percentage of 4.70.

[2] HF = Holstein-Friesian; MRY = Meuse-Rhine-Yssel; DF = Dutch Friesian; GWH = Groningen White Headed; JER = Jersey.

[3] Range of SE of the predicted means.

[4] Using the FAT− model, means were predicted regardless of the level of total fat percentage, and using the FAT+ model, means were predicted including a fixed regression coefficient of the total fat percentage in milk within the breeds HF, MRY, DF, GWH, and JER.

[5] To test the possible breed differences, the dependent variable was transformed using the natural logarithm. The predicted means given are transformed back to the original scale and the SE are on the scale of the natural logarithm.

Table 4.5 Predicted means of 10 groups of FA for each breed[1].

	Breed[3]					
Item[2]	**HF**	**MRY**	**DF**	**GWH**	**JER**	**SE[4]**
FAT- model[5] trait (g/dL of milk)						
SFA	3.19^{b}	3.22^{b}	3.28^{b}	2.96^{c}	3.68^{a}	0.011-0.145
UFA	1.37^{a}	1.38^{a}	1.38^{a}	1.30^{b}	1.37^{a}	0.004-0.062
MUFA	1.19^{a}	1.20^{a}	1.21^{a}	1.12^{b}	1.19^{a}	0.004-0.056
PUFA	0.168^{b}	0.168^{b}	0.172^{b}	0.159^{c}	0.184^{a}	0.001-0.009
SCFA	0.433^{b}	0.442^{b}	0.448^{b}	0.397^{c}	0.512^{a}	0.001-0.019
MCFA	2.49^{b}	2.50^{b}	2.58^{b}	2.28^{c}	2.87^{a}	0.009-0.119
LCFA	1.64^{b}	1.66^{a,b}	1.65^{a,b}	1.60^{a,b}	1.69^{a}	0.006-0.084
n-3	0.029^{b}	0.030^{b}	0.029^{b}	0.028^{b}	0.033^{a}	0.000-0.002
n-6	0.111^{a}	0.112^{a}	0.112^{a}	0.106^{b}	0.111^{a}	0.000-0.004
BCFA	0.089^{b}	0.086^{b}	0.091^{a,b}	0.081^{c}	0.093^{a}	0.000-0.003
FAT+ model[5] trait (g/dL of milk)						
SFA	3.29^{b}	3.29^{b}	3.30^{b}	3.28^{b}	3.40^{a}	0.003-0.046
UFA	1.40^{a}	1.40^{a}	1.39^{a}	1.44^{a}	1.28^{b}	0.003-0.043
MUFA	1.22^{a}	1.22^{a}	1.21^{a}	1.25^{a}	1.10^{b}	0.003-0.039
PUFA	0.171^{b}	0.171^{b}	0.173^{a,b}	0.177^{a,b}	0.176^{a}	0.001-0.007

SCFA	0.445^{b,c}	0.450^{b}	0.450^{b}	0.437^{c}	0.476^{a}	0.001-0.010
MCFA	2.57^{b}	2.56^{b,c}	2.60^{b}	2.51^{c}	2.65^{a}	0.004-0.051
LCFA	1.69^{b}	1.69^{b}	1.66^{b}	1.80^{a}	1.57^{c}	0.004-0.058
n-3	0.030^{c}	0.030^{b,c}	0.030^{c}	0.032^{a}	0.031^{a,b}	0.000-0.001
n-6	0.114^{a}	0.114^{a}	0.112^{a}	0.116^{a}	0.105^{b}	0.001-0.003
BCFA	0.091^{a}	0.087^{a}	0.092^{a}	0.089^{a,b}	0.089^{b}	0.001-0.002

[a–d] Values with different superscripts within a row indicate significant differences of predicted means at $P < 0.05$.

[1] Predicted means of all traits for each breed at the third parity and at 156 DIM in both models, and for the FAT+ model, traits were predicted at a fat percentage of 4.70.

[2] UFA = unsaturated FA; SCFA = short-chain FA; MCFA = medium-chain FA; LCFA = long-chain FA; BCFA = branched-chain FA.

[3] HF = Holstein-Friesian; MRY = Meuse-Rhine-Yssel; DF = Dutch Friesian; GWH = Groningen White Headed; JER = Jersey.

[4] Range of SE of the predicted means.

[5] Using the FAT− model, means were predicted regardless of the level of total fat percentage, and using the FAT+ model, means were predicted including a fixed regression coefficient of the total fat percentage in milk within the breeds HF, MRY, DF, GWH, and JER.

Heterosis and Recombination

The estimates of the heterosis and recombination effects are shown in Table 4.6. Heterosis was shown to be significant ($P < 0.05$) in both FAT− and FAT+ models for the individual FA C4:0 to C10:0 and *cis*-9,*trans*-11 C18:2, and the group of FA SCFA. The recombination effect within the FAT− model was highly significant for most FA and groups of FA, except for the conjugated linoleic acid *cis*-9,*trans*-11 C18:2, total *trans* C18:1, and total *trans* C18. Within the model, adjusting for the differences in fat content (FAT+), the recombination effect was significant for 15 of the traits including C4:0 to C14:0. For almost all traits, both heterosis and recombination had positive effects when estimated with the FAT− model. When using the FAT+ model, heterosis showed slightly more positive than negative effects, and recombination had negative effects in most cases, and was in fact negative in all situations where it had a significant effect.

4.4 Discussion

In this discussion section, first the use of MIRS profiles and the data structure is discussed. Thereafter, our results for different groups of FA are discussed and compared with published results. In published studies, either FA were evaluated as proportion of the total fat (g/100 g of total fat), or as milk content (g/dL of milk). In our study, results are published as content in milk. Although predicted means of studies in which FA are evaluated as proportion of the total fat cannot directly be compared with our results, the direction of breed differences in other studies evaluating proportions in fat can be compared with the direction of the breed differences of the FAT+ model in our study. Comparisons to published results are performed for 3 groups of FA. The first group comprises FA that are formed through de novo synthesis (all SCFA and from all MCFA only C12:0 and C14:0), the second comprises the C16:0 FA that arises in milk through de novo synthesis and uptake from blood circulation, and the third group comprises FA that arise in milk by uptake from blood circulation (FA longer than 16 carbons in length).

MIRS Profiles

The detailed milk fat composition was predicted based on MIRS profiles. The MIRS profiles were directly obtained from milk. In our study, results are, therefore, given on milk basis (g of fat/dL of milk). Recalculation to fat basis leads to a considerable decrease in the accuracy, presumably due to an accumulation of bias. The decrease in accuracy when predicting results on fat basis from MIRS profiles was shown by Soyeurt et al. (2006a) and Rutten et al. (2009). The predictive ability of the calibration equations in milk of MRY, DF, GWH, and JER in the Netherlands has

been investigated previously using an independent data set (Maurice- Van Eijndhoven et al., 2013). In that study, the majority of FA and groups of FA were predicted with moderate to high accuracy (based on the coefficient of determination > 0.60) for all breeds. In the study of Soyeurt et al. (2011), the predictability of calibration equations was also evaluated using, for example, the coefficients of determination, which ranged from 0.68 up to 1 for the FA evaluated in our study. Other studies that evaluated the use of calibration equations to predict FA composition from MIRS profiles, which are not directly linked to the equations used in our study, also showed that MIRS can be a useful method for predicting FA composition for breeding purposes (Rutten et al., 2009; De Marchi et al., 2011). To obtain accurate estimates of differences between groups, predicted individual FA composition does not have to be 100% accurate. Because the objective of our study was to compare FA composition of different breeds, which involves estimation of average effects for large groups of animals, the results of the validation studies mentioned above suggest that using predicted FA composition is appropriate for the range of FA presented here. For instance, the content of the FA group total C18:2 is predicted to be smaller than the content of the 2 individual C18:2 FA together, which is most likely due to the low accuracy of the predictions for the FA group total C18:2.

Estimates of Breed Effects, Heterosis, and Recombination

Breed effects were estimated using a data set from commercial herds including purebred and crossbred cows. Because only a very limited number of herds included purebred cows of multiple breeds, data of crossbreds was needed to disentangle breed and herd effects. Table 4.2 shows the number of cows and herds. All herds apart from 2 (1 MRY and 1 DF herd) had crossbred cows. Thus far, a comparison between breeds based on data across a range of commercial herds with different combinations of breeds was only published by Soyeurt et al. (2006b), which included 7 herds with a total of 275 purebred and crossbred cows belonging to 6 different breeds in the Walloon region of Belgium. Between breed differences were estimated on milk basis, giving results expressed as content in milk (g of fat/dL of milk). Using the FAT− model, the average individual FA contents for each breed were estimated. As individual FA contents are correlated with fat percentage, individual FA contents were also estimated for each breed, accounting for differences in fat percentage using the FAT+ model (i.e., in Tables 4.3–4.5, for the FAT+ model, predicted means at an average fat percentage of 4.7 are shown). As all predictions were performed for FA contents, it may have been expected that

Table 4.6 Estimates of the heterosis and recombination effects.

	Heterosis				Recombination			
	FAT- [1]		FAT+ [1]		FAT- [1]		FAT+ [1]	
Trait	**estimate**	***P*-value**	**estimate**	***P*-value**	**estimate**	***P*-value**	**estimate**	***P*-value**
Fat (%)	0.0634	*			0.2001	***		
Protein (%)	0.0660				0.0782	***		
Fat yield (kg/d)	0.0378	**			0.0005			
Protein yield (kg/d)	0.0175	*			-0.0170	*		
Milk yield (kg/d)	0.4978	†			-1.0012	***		
FA[2] *(g/dL milk)*								
C4:0	0.0030	***	0.0017	***	0.0039	***	-0.0011	*
C6:0	0.0022	***	0.0012	***	0.0030	***	-0.0010	***
C8:0	0.0016	***	0.0009	***	0.0019	***	-0.0008	***
C10:0	0.0035	**	0.0018	*	0.0043	***	-0.0020	*
C12:0	0.0042	*	0.0021	†	0.0053	***	-0.0025	*
C14:0	0.0083	*	0.0023		0.0170	***	-0.0059	**
***cis*-9 C14:1**	0.0005		0.0000		0.0016	***	-0.0004	
C16:0	0.0218	†	0.0022		0.0809	***	0.0014	
***cis*-9 C16:1**	0.0007		-0.0005		0.0049	***	0.0005	
C18:0	0.0059		-0.0011		0.0252	***	0.0001	
***cis*-9 C18:1**	0.0088		-0.0017		0.0408	***	0.0021	

Total *cis* C18:1	0.0104		-0.0006		0.0418	***	0.0010	
Total *trans* C18:1	0.0008		-0.0004		0.0022	†	-0.0022	*
***cis*-9,12 C18:2**	0.0018	**	0.0010	*	0.0015	**	-0.0013	***
***cis*-9,*trans*-11 C18:2**	-0.0006		-0.0006		-0.0001		-0.0001	
Total C18:2	0.0014	*	0.0005		0.0018	**	-0.0016	***
***cis*-9,12,15 C18:3**	0.0003		0.0000		0.0007	***	-0.0003	*
Total *trans* C18	0.0008		-0.0007		0.0021		-0.0033	*
SFA	0.0479	*	0.0070		0.1480	***	-0.0104	
UFA	0.0144		-0.0009		0.0528	***	-0.0037	
MUFA	0.0139		-0.0002		0.0488	***	-0.0034	
PUFA	0.0008		-0.0006		0.0037	***	-0.0015	
SCFA	0.0109	***	0.0060	***	0.0136	***	-0.0052	***
MCFA	0.0359	†	0.0052		0.1139	***	-0.0079	
LCFA	0.0151		-0.0063		0.0805	***	0.0025	
n-3	0.0004		0.0000		0.0010	***	-0.0004	**
n-6	0.0016	*	0.0005		0.0021	**	-0.0018	***
BCFA	0.0006		-0.0002		0.0015	**	-0.0014	***

[1]Using the FAT− model, means were predicted regardless of the level of total fat percentage, and using the FAT+ model, means were predicted including a fixed regression coefficient of the total fat percentage in milk within the breeds Holstein-Friesian, Meuse-Rhine-Yssel, Dutch Friesian, Groningen White Headed, and Jersey.

[2]UFA = unsaturated FA; SCFA = short-chain FA; MCFA = medium-chain FA; LCFA = long-chain FA; BCFA = branched-chain FA.

†$P < 0.10$; *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

individual milk production affected the predictions, due to diluting the FA content. A well-known measure of cow milk production is fat- and protein-corrected milk (FPCM), which is calculated using fat percentage, protein percentage, and milk yield. Breed-specific correlations between FPCM and UFA and SFA based on the unadjusted animal FA profiles ranged from −0.13 to −0.40, however, whereas correlations between fat percentage and UFA and SFA ranged from 0.54 to 0.96. When analyzing FA expressed as content in milk, fat percentage generally had a significant effect on FA content of individual FA and groups of FA. Given the difference in correlation with FA content, FPCM is expected to have a much smaller effect on FA content than fat percentage.

In our study, highly significant effects of heterosis were found for several SCFA. These effects were estimated to be highest for the model without adjusting for differences in fat content. Positive effects of heterosis for fat percentage and fat yield in our study were in line with results found by Ahlborn-Breier and Hohenboken (1991). Recombination is generally expected to have a negative effect, but positive estimates have been reported for fat percentage (Van Der Werf and De Boer, 1989). We found the same result for fat percentage and all FA in the FAT− model, which was expected because FA content is generally positively related with fat percentage. Our results of the FAT+ model seem to indicate that when correcting for fat percentage, recombination effects are negative, following the general expectation.

FA Generated Through De Novo Synthesis (SCFA, C12:0, and C14:0)

The SCFA, C12:0, and C14:0 arise in milk mainly from de novo synthesis within the mammary epithelial cells (Bauman and Griinari, 2003). A considerable part of the variation in production of these FA is, therefore, expected to have a genetic origin. Among the breeds HF, MRY, and DF, no significant differences in the detailed FA composition were found in our study using the FAT+ model. These results are in agreement with Maurice-Van Eijndhoven et al. (2011), who reported no significant differences for SCFA, C12:0, and C14:0 between MRY and DF in the Netherlands and Soyeurt et al. (2006b), who reported no significant differences for C12:0 and C14:0 between HF and MRY participating in the Walloon milk recording in Belgium. In our study, JER produced higher contents of SCFA and C14:0 than the other breeds. This was also found by Beaulieu and Palmquist (1995) for C4:0 to C14:0, by White et al. (2001) for C6:0 to C14:0, and by Palladino et al. (2010) for C4:0 to C12:0, although the differences found in the latter study were not significant. Soyeurt et al. (2006b) reported significantly higher contents (g/dL of

milk) of C12:0 and C14:0 for JER compared with HF, but differences were not significant when expressed as proportion of total fat. Our results for SCFA and C14:0 for JER were not in line with results reported by Maurice-Van Eijndhoven et al. (2011), who found lower proportions for JER compared with DF and MRY. This might be due to confounding of herd and breed effects in the study by Maurice-Van Eijndhoven et al. (2011). Higher contents of SCFA and C14:0 in JER suggest that this breed has a relatively higher production of FA through de novo synthesis.

C16:0

The FA C16:0 is one of the major SFA in bovine milk. The FA C16:0 arises in milk through either de novo synthesis or uptake from blood circulation (Bauman and Griinari, 2003). As SFA such as C16:0 are associated with a higher risk of coronary artery disease (e.g., Mensink et al., 2003), possibilities to alter the content of C16:0 in milk is of interest to the dairy industry. In general, our results showed higher contents of C16:0 for JER and lower contents for GWH. The results for C16:0 in our study are in line with results found by Maurice- Van Eijndhoven et al. (2011). Similar results for C16:0 in JER compared with HF were reported by Soyeurt et al. (2006b) and Palladino et al. (2010). Contrasting results were only reported by Beaulieu and Palmquist (1995) who reported a higher proportion C16:0 for HF and no difference was found by White et al. (2001).

LCFA

FAs with more than 16 carbons are obtained by uptake from circulation, which implies that the cow diet has a large influence on the secretion of these FA in milk. Within the cow, however, 2 important systems affect the final milk fat composition: rumen biohydrogenation and Δ9-desaturase activity (Neville and Picciano, 1997). Two essential FA in the human diet that arise in bovine milk directly from the cow diet are *cis*-9,12,15 C18:3 and *cis*-9,12 C18:2, together comprising the main fraction of the groups n-3 and n-6, respectively. Although these essential FA are present in milk in very low amounts (<2% of total fat), some significant differences were found between breeds. Jerseys produced a higher content of the C18:3 compared with HF, MRY, and DF and a lower content of *cis*-9,12 C18:2 compared with all other breeds when adjusting for differences in fat content. The lower production of *cis*-9,12 C18:2 for JER compared with HF, when analyzed as grams per 100 g of fat, was also reported by Palladino et al. (2010). For the FA *cis*-9,12,15 C18:3, contrasting results were found in several other studies (Drackley et al., 2001; Palladino et al. 2010). No differences for these FA were found by Beaulieu and Palmquist (1995) and White et al. (2001).

The FA C18:0 is produced mainly by biohydrogenation in the rumen of PUFA from dietary fat. The *trans* C18 FA are intermediate products in the biohydrogenation process of C18 PUFA in the rumen and, thus, a result of incomplete biohydrogenation (Bauman and Griinari, 2003). When accounting for differences in fat content, the content of C18:0 was significantly higher for GWH compared with JER; however, contrasting results were found without accounting for differences in fat content, showing higher contents for JER and lower for GWH. Soyeurt et al. (2006b) also found a higher C18:0 content for JER compared with HF. Other studies that evaluated proportions of the total fat, however, reported no significant differences between JER and other breeds such as HF and GWH (Beaulieu and Palmquist, 1995; White et al., 2001; Palladino et al., 2010; Maurice-Van Eijndhoven et al., 2011). Evaluating the total *trans* C18 FA in our study, only using the model without regression on fat percentage, a significantly higher content was found for JER compared with HR and MRY. Thus, the higher content of most LCFA produced by JER is directly related to its higher fat percentage in milk.

The conversion of *trans*-11 C18:1 into *cis*-9,*trans*-11 C18:2 via Δ9-desaturase in the udder is the major source of *cis*-9,*trans*-11 C18:2 in milk. Although GWH seems to produce a higher content of *cis*-9,*trans*-11 C18:2*,* based on the unadjusted data in the current study, no significant difference among breeds was found following the FAT+ model and even a significant lower mean was predicted for the FAT− model compared with HF and DF. Maurice-Van Eijndhoven et al. (2011) reported a higher proportion of *cis*-9,*trans*-11 C18:2 for GWH and a lower proportion for JER. The predicted means of *cis*-9,*trans*-11 C18:2 for JER in current study were significantly higher compared with HF and MRY when evaluating the results of the FAT+ model. Contrasting results were found by White et al. (2001) who reported a lower proportion of *cis*-9,*trans*-11 C18:2 for JER compared with HF. Δ9-Desaturase plays an important role in determining the total proportion of UFA in milk by desaturation of FA, resulting in, for example, *cis*-9,*trans*-11 C18:2, C14:1, C16:1, and C18:1 with a double bond at *cis*-9. Especially these UFA with a *cis* double bound are reported as having an altering effect on the risk of development of coronary artery disease (Mensink et al., 2003). In our study, the results of the FAT+ model for JER showed significantly higher content of *cis*-9 C14:1 and lower content of *cis*-9 C16:1 and *cis*-9 C18:1. In agreement, White et al. (2001) reported lower proportions *cis*-9 C16:1 and *cis*-9 C18:1 for JER compared with HF. For *cis*-9 C18:1, both, Beaulieu and Palmquist (1995) and Palladino et al. (2010) also found lower proportions for JER compared with HF. The differences between the models reveal that the higher content of *cis*-9 16:1 and *cis*-9 C18:1 produced by JER is also directly related to the higher fat percentage in milk of JER.

Major Genes Affecting FA Composition

Despite potential differences between cow diets in our and other studies, our results were generally in agreement with breed differences reported in other studies. This confirms that differences in FA are partly determined by genetics. Several studies reported that polymorphisms in the genes encoding the acyl CoA:diacylglycerol acyltransferase 1 (DGAT1) and stearoyl-CoA desaturase 1 (SCD1) enzymes explain an important part of the variation in milk fat composition (e.g., Grisart et al., 2002; Schennink et al., 2007; Schennink et al., 2008). Potential differences in allele frequencies between breeds for these genes might explain part of the observed breed differences. For some Jersey and several Holstein populations, the frequencies of the *DGAT1* 232K and *SCD1* 293A alleles have been reported in the literature. The frequency of the *DGAT1* 232K allele in Holsteins was reported as ranging from 0.27 to 0.60 (Spelman et al., 2002; Thaller et al., 2003; Kaupe et al., 2004; Lacorte et al., 2006; Schennink et al., 2007) and somewhat higher in JER, ranging from 0.69 to 0.88 (Spelman et al., 2002; Kaupe et al., 2004). For example, Grisart et al. (2002) and Winter et al. (2002) reported an association of the *DGAT1* 232K allele with a higher fat percentage in milk and Schennink et al. (2007) reported an association with a higher proportion C16:0. Thus, these reported differences infrequencies for the *DGAT1* 232K allele in HF and JER, and the reported effects of this allele, are in line with the results of our study.

The frequency of the *SCD1* 293A allele in Holsteins was reported as ranging from 0.56 to 0.73 (Kgwatalala et al., 2007; Mele et al., 2007; Macciotta et al., 2008; Milanesi et al., 2008; Schennink et al., 2008), whereas it was reported to be higher in JER, ranging from 0.94 to 0.95 (Kgwatalala et al., 2007; Moioli et al., 2007). The *SCD1* 293A allele was reported to be associated with a higher proportion of C14:0, *cis*-9 C14:1, and C18:0 and a lower proportion of *cis*-9 C16:1 (Schennink et al., 2008), which is in line with the differences found between JER and HF in our study, except for C18:0.

The above-described differences in allele frequencies and the associations of the alleles with the FA composition indicate that the differences between HF and JER, to a large extent, may be the result of differences in allele frequencies at the *DGAT1* and *SCD1* genes. Whether or not, for instance, differences between JER and GWH in our study can also be attributed to differences in allele frequencies at those genes is currently unknown, as the allele frequencies for GWH, as well as for DF and MRY, are currently not known.

4.5 Conclusion

Breed differences were found in the detailed FA composition and content of individual FA. Especially for FA content in milk produced through de novo synthesis (SCFA, C12:0, C14:0, and partly C16:0), differences were found for JER and GWH compared with the breeds HF, MRY, and DF. For FA having more than 16 carbons, breed differences in content in milk were found for FA that arise in milk directly from the cow diet (*cis*-9,12 C18:2 and *cis*-9,12,15 C18:3) of which the total amount is influenced by biohydrogenation in the rumen. Breed differences were also found for FA content in milk in which Δ9-desaturase plays a role (*cis*- 9 C14:1, *cis*-9 C16:1, and *cis*-9 C18:1). No significant differences were found between the predicted means of any individual FA or group of FA among the breeds HF, MRY, and DF. Comparing predicted means from both models, including and excluding a regression on fat percentage, clearly indicated that the detailed FA composition in content in milk, especially SCFA and MCFA, is related to the total fat percentage in all studied breeds.

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5

Heritability of milk fat composition is considerably lower for Meuse-Rhine-Yssel compared to Holstein Friesian cattle

M.H.T. Maurice – Van Eijndhoven[1,2], R.F. Veerkamp[1], H. Soyeurt[3,4], M.P.L. Calus[1]

[1] Animal Breeding and Genomics Centre, Wageningen UR Livestock Research, P.O. Box 338, 6700 AH Wageningen, The Netherlands; [2] Animal Breeding and Genomics Centre, Wageningen University, P.O. Box 338, 6700 AH Wageningen, The Netherlands; [3] Animal Science Unit, Gembloux Agro-Bio Tech, University of Liege, 5030 Gembloux, Belgium; [4]National Fund for Scientific Research, 1000 Brussels, Belgium

Submitted

Abstract

The aim of this paper is to identify differences in genetic variation of fatty acid (FA) composition in milk in different breeds. Data used included Meuse-Rhine-Yssel (MRY) and Holstein Friesian (HF) cattle breeds. Both populations were kept in the Netherlands and participated in the same milk recording system. The populations did differ, however, in selection history, where in the MRY there has been relatively very little emphasis on selection for high-input high-output production systems compared to HF. Differences in genetic variation were investigated by estimating breed specific additive genetic variances and heritabilities for FA contents in milk of MRY and HF. Mid Infrared Spectrometry spectra were used to predict total fat percentage and detailed FA contents in milk (14 individual FA and 14 groups of FA in g of fat/dL of milk). The dataset for MRY contained 2 916 records from 2 049 registered cows having at least 50% genes of MRY origin and the dataset used for HF contained 155 319 records from 96 315 registered cows having at least 50% genes of HF origin. Variance components of individual FA content in milk for the different breeds were estimated using a single trait animal model. Additive genetic variances for FA produced through *de novo* synthesis (short chain FA, C12:0, C14:0, and partly C16:0), C14:1 *c*-9 and C16:1 *c*-9 were significantly higher ($P<0.001$) for HF compared to MRY. Heritabilities of the individual FA C4:0 to C18:0 for HF ranged from 0.28 to 0.52 and for MRY from 0.17 to 0.34. Heritabilities of the individual C18 unsaturated FA for HF ranged from 0.11 to 0.34 and for MRY from 0.10 to 0.26. Although the mean content in milk for the FA C18:2 *c*-9, *t*-11 was low in both breeds, the additive genetic variance in our dataset was significantly higher for MRY ($P<0.05$) compared to HF. Heritabilities of the groups of FA for HF ranged from 0.19 to 0.53 and for MRY from 0.11 to 0.28. For the majority of the FA, the additive genetic variances for HF were significantly higher compared to MRY, except for most of the poly unsaturated FA. The results for the poly unsaturated FA, however, may be affected by the lower accuracy of the predictions for these FA. In conclusion, our results show that the HF breed has substantially larger genetic variance for most FA compared to MRY.

Key words: milk, fatty acids, infrared spectrometry, genetic variability, cattle breeds

5.1 Introduction

Fat is one of the major components in bovine milk. Bovine milk fat is composed of a wide range of fatty acids (FA) which content and composition in the milk vary between cows. Extending the knowledge on variation in detailed milk fat composition among cows is of interest for the dairy industry because fat composition is associated with processability (e.g. Smet et al., 2009), human health (e.g. Mensink et al., 2003, Palmquist et al., 2006) and also methane emission (Dijkstra et al., 2011). The variation in FA composition in milk between cows is partly due to environmental effects, mainly differences in cows diet (e.g. Baumgard et al., 2001, Sterk et al., 2011), lactation stage (e.g. Stull et al., 1966), and also a considerable part of the variation has a genetic origin within and across lactation (e.g. Stoop et al., 2008, Bastin et al. 2011). For example, Soyeurt et al. (2007) reported heritabilities for individual FA in milk of dairy cattle in the Walloon region of Belgium ranging from 0.05 to 0.38 and Stoop et al. (2008) reported heritabilities for individual FA in milk of the intensively selected dairy breed Holstein Friesian (HF) in the Netherlands ranging from 0.22 to 0.71. Considering the heritabilities reported by Stoop et al. (2008), there are possibilities for the dairy industry to modify FA composition in milk of Dutch HF cows using breeding strategies. An important unanswered question is whether the same applies for other Dutch local breeds like the Meuse-Rhine-Yssel (MRY), in which there has been relatively very little emphasis on selection for high-input high output production systems used in the dairy sector. Differences for the FA profile of milk fat between breeds have been described by e.g. Maurice – Van Eijndhoven et al. (2013) reporting higher content of saturated FA (SAT) produced by Jersey cows compared to a number of local Dutch breeds. Although Maurice-Van Eijndhoven et al. (2013) reported no differences in the level of FA composition between the Dutch MRY and HF, differences in within breed variability for both breeds need to be known to assess whether alternative breeds like MRY can contribute to breeding strategies to change FA composition.

The aim of this paper is to identify differences in genetic variation within the MRY and HF cattle breeds in the Netherlands. This was achieved by estimating breed specific additive genetic variances and heritabilities for FA composition of MRY and HF. Analyses were based on predicted FA composition in milk using Mid Infrared Spectrometry (MIRS) spectra on a large data set.

5.2 Materials and Methods

Data collection and data editing

MIRS spectra of milk samples were collected via the Dutch milk recording system of CRV BV (Arnhem, The Netherlands) between October and December 2006. Samples were treated immediately with 0.03% (wt/wt) sodium azide to avoid microbiological growth. The MIRS spectra were obtained using 3 Fourier-transformed interferogram machines (MilkoScan FT 6000, Foss Electric, Denmark) at the laboratory of Qlip N.V. (Leusden, The Netherlands). The 1 886 sampled herds were a random representation of all herds participating in the milk recording system of CRV BV.

The initial dataset contained 372 429 test-day records of 230 995 cows. Data-editing steps included the deletion of records and cows for the following reasons: less than 75% of the breed composition known, unknown sire, incomplete milk recording data (e.g. unknown birthdate or DIM), multiple records from the same cow on the same sample date, cows with records in multiple herds, cows reported sick at sampling date, cows in parity 11 or higher, cows before 5 or after 365 days in lactation, and records from herds with less than 5 purebred cows of the same breed (HF, MRY, Dutch Friesian (DF), or Groningen White Headed (GWH)) per herd. To detect records with possible errors, due to, for example, swapped samples, fat content recorded via the regular milk control (predicted by QLIP N.V.) was compared to fat content obtained using the RobustMilk prediction equations that were developed by Soyeurt et al. (2011). The correlation coefficient between fat content predicted by QLIP N.V. and fat content predicted using the RobustMilk prediction equations was 0.996. When the absolute difference in both predictions for fat percentage was more than 0.35 the record was removed. Finally, complete records with extreme outliers in at least 1 of all predicted traits (+/- 5 SD of the mean) were deleted. After these editing steps the dataset contained 307 656 records. Because of computational limitations this dataset was reduced by randomly eliminating ~50% of the herds with only HF cows (at least 75% HF, i.e. herds without any pure- or crossbred MRY, DF, or GWH cows). The dataset used for MRY contained 2 916 records of in total 2 049 cows registered having at least 50% genes of MRY origin with a pedigree of 13 506 animals and the dataset used to estimate heritabilities for HF contained 155 319 records of in total 96 315 cows registered having at least 50% genes of HF origin with a pedigree of 405 968 animals. Pedigree files for both data sets included all known ancestors as far back as possible. Ancestors with unknown parents and only 1 offspring in the pedigree were removed.

Measuring fatty acid composition

Detailed milk composition on milk basis (g of FA/dL milk) of the 14 individual FA (C4:0, C6:0, C8:0, C10:0, C12:0, C14:0, C14:1 *c*-9, C16:0, C16:1 *c*-9, C18:0, C18:1 *c*-9, C18:2 *c*-9, 12, C18:3 *c*-9, 12, 15, and C18:2 *c*-9, *t*-11) and the 14 groups of FA [total trans C18:1, total cis C18:1, total C18:2, total trans C18, total SFA, total mono-unsaturated FA (MUFA), total poly-unsaturated FA (PUFA), total UFA, short-chain FA (SCFA), medium-chain FA (MCFA), long-chain FA (LCFA), total *n*-3 FA, total *n*-6 FA, and total branched-chain FA (BCFA); group definitions are given in Table 5.1] were predicted from the MIRS spectra. For those predictions, updated versions of the RobustMilk calibration equations published by Soyeurt et al. (2011) were used, that were based on 1 236 milk samples from multiple breeds and countries (calibration equations were updated by expanding the number of samples used in the calibration data set from 570 to 1 236). The method used to relate MIRS spectra to FA data was partial least square regression after a first derivative pre-treatment on spectral data to correct the baseline drift. A T-outlier test was also used during the calibration process to delete potential outliers based on the gas chromatographic measurements. Therefore the final number of samples included in each calibration equation varied following the considered FA. More detailed information about the methodology used to develop the calibration equations is given by Soyeurt et al. (2011). Some descriptive statistics of the calibration equations, which are described by Soyeurt et al. (2011), are given in Table 5.2. More detailed descriptive statistics of the calibration equations are published by Maurice – Van Eijndhoven et al. (2012) including an external validation for the MRY breed.

Next to the detailed FA composition, 5 production traits were analysed (milk yield, fat%, protein%, fat yield and protein yield). Fat content was predicted using the RobustMilk calibration equations published by Soyeurt et al. (2011) and protein content was predicted by QLIP N.V.

Table 5.1 Definition of the groups of fatty acids.

Group	Fatty acids
Total *t* C18:1	C18:1 *t*-6-11; C18:1 *t*-12-14
Total *c* C18:1	C18:1 *c*-9; C18:1 *c*-11; C18:1 *c*-12; C18:1 *c*-13; C18:1 *c*-14; C18:1 *t*-16
Total C18:2	C18:2 ∑ ttNMID; C18:2 *c*-9, *t*-13; C18:2 *t*-8, *c*-12; C18:2 *c*-9, *t*-12; C18:2 *t*-8, *c*-13; C18:2 *t*-11, *c*-15; C18:2 *t*-9, *c*-12; C18:2 *c*-9, *c*-12

Group	Fatty acids
Total *t* C18	C18:1 *t*6-11; C18:1 *t*12-14; C18:2 ∑ ttNMID; C18:2 *c*-9, *t*-13; C18:2 *t*-8, *c*-12; C18:2 *c*-9, *t*-12; C18:2 *t*-8, *c*-13; C18:2 *t*-11, *c*-15; C18:2 *t*-9, *c*-12
Saturated fatty acids (SFA)	C4:0; C6:0; C8:0; C10:0; C12:0; C13:0 *iso*; C13:0 *ante-iso*; C14:0; C14:0 *iso*; C15:0; C15:0 *iso*; C15:0 *ante-iso*; C16:0; C16:0 *iso*; C17:0; C17:0 *iso*; C17:0 *ante-iso*; C18:0; C18:0 *iso*; C19:0; C20:0; C22:0
Unsaturated fatty acids (UFA)	MUFA; PUFA
Unsaturated fatty acids with 1 double bound (MUFA)	C10:1; C12:1 *cis*; C14:1 *cis*; C16:1 *cis*; C16:1 *trans*; C17:1; C18:1 *c*-9; C18:1 *c*-11; C18:1 *c*-12; C18:1 *t*-6-11; C18:1 *t*-12-14; C18:1 *c*-13; C18:1 *c*-14;C18:1 *t*-16; C20:1 *c*-9; C20:1 *c*-11
Unsaturated fatty acids with 2 or more double bounds (PUFA)	C18:2 ∑ ttNMID; C18:2 *c*-9, *t*-13; C18:2 *t*-8, *c*-12; C18:2 *c*-9, *t*-12; C18:2 *t*-8, *c*-13; C18:2 *t*-11, *c*-15; C18:2 *t*-9, *c*-12; C18:2 *c*-9,*c*-12; C18:3 *c*-9, *c*-12, *c*-15; C18:2 *c*-9, *t*-11 (CLA); C20:3 (*n*-6); C20:4 (*n*-6); C20:5 EPA (*n*-3); C22:5 DPA
Short chain fatty acids (SCFA)	C4-C10
Medium chain fatty acids (MCFA)	C12-C16
Long chain fatty acids (LCFA)	C17-C22
***n*-3**	C18:3 *c*-9 ,*c*-12, *c*-15; C20:5 (EPA); C22:5 (DPA)
***n*-6**	C18:2 ∑ ttNMID; C18:2 *c*-9, *t*-13; C18:2 *t*-8, *c*-12; C18:2 *c*-9, *t*-12; C18:2 *t*-8, *c*-13; C18:2 *t*-11, *c*-15; C18:2 *t*-9, *c*-12; C18:2 *c*-9, *c*-12; C20:3 (*n*-6); C20:4 (*n*-6)
BCFA	C13:0 *iso*; C13:0 *ante-iso*; C14:0 *iso*; C15:0 *iso*; C15:0 *ante-iso*; C16:0 *iso*; C17:0 *iso*; C17:0 *ante-iso*; C18:0 *iso*

Table 5.2 Descriptive statistics of the fatty acid calibration equations and data used to derive the equations.

Traits	N[a]	R^2cv[b]	RPD[c]
Production traits			
Fat percentage	1166	1.00	33.53

Traits	N[a]	R^2cv[b]	RPD[c]
Traits g/dL milk			
C4:0	1186	0.93	3.68
C6:0	1189	0.96	4.81
C8:0	1180	0.96	5.00
C10:0	1183	0.96	4.72
C12:0	1180	0.95	4.61
C14:0	1184	0.95	4.70
C14:1 *c*-9	1180	0.78	2.13
C16:0	1179	0.97	6.20
C16:1 *c*-9	1179	0.78	2.14
C18:0	1173	0.90	3.24
C18:1 *c*-9	1194	0.96	5.06
Total cis C18:1	1189	0.97	5.55
Total trans C18:1	1176	0.92	3.57
C18:2 *c*-9, 12	1172	0.81	2.30
C18:2 *c*-9, *t*-11	1154	0.85	2.59
Total C18:2	1166	0.75	2.00
C18:3 *c*-9,12,15	1169	0.77	2.11
Total trans C18	1181	0.92	3.59
SFA	1176	1.00	15.34
UFA	1179	0.98	7.62
MUFA	1180	0.98	7.18
PUFA	1180	0.85	2.56
SCFA	1185	0.96	5.10
MCFA	1187	0.98	7.53
LCFA	1188	0.97	5.96
***n*-3**	1172	0.77	2.11
***n*-6**	1167	0.76	2.03
BCFA	1166	0.85	2.61

[a] The number of samples included in the calibration equation .

[b] Cross validation coefficient of determination.

[c] The ratio of SD to SECV.

Statistical analysis

Genetic variances were estimated in separate analyses for HF and MRY in ASReml 3.0 (Gilmour et al., 2009) using the following animal model:

$$y_{ijklmnopqrstuv} = \mu + b_1 \times DIM_i + b_2 \times \exp^{-0.05\times dim}{}_i + parity_j + b_3 \times age_k\,(parity_j) + htd_l + b_4 \times HF_m + b_5 \times MRY_n + b_6 \times DF_o + b_7 \times GWH_p + b_8 \times JER_q + b_9 \times HET_r + b_{10} \times REC_s + a_t + pe_t + e_{ijklmnopqrstu},$$

where $y_{ijklmnopqrstuv}$ was the dependent variable for cow *t* in days in milk (DIM) *i*, with parity *j*, calving age *k*, producing at herd test date (htd) *l*, and having a breed composition *mnopq* for HF (*m*), MRY (*n*), DF (*o*), GWH (*p*), and JER (*q*). The μ was the overall mean of the model; b_1 was the fixed regression coefficient on DIM_i and b_2 was the fixed regression coefficient on DIM_i modeled with a Wilmink curve (Wilmink, 1987); $parity_j$ was a fixed effect with 4 classes for corresponding lactation numbers of parity 1, 2, and 3 and the 4th class included parity 4 - 10; b_3 was the fixed regression coefficient on age_k, which was calving age in days, within the *j*th parity; htd_l was a fixed effect defining groups of cows sampled in the same herd on the same sample date; b_4, b_5, b_6, b_7, and b_8 were the fixed regression coefficients on, respectively, HF_m, MRY_n, DF_o, G_p, JER_q, which were the expected percentages of genes belonging to each of those breeds; b_9 was the fixed regression on HET_r, which was the estimated percentage of heterosis; b_{10} was the fixed regression on REC_s, which was the estimated percentage of recombination loss effect; a_t was the random additive genetic effect of cow *t*; pe_t was the random permanent environmental effect of cow *t*, and $e_{ijklmnopqrstu}$ is the random residual effect. HET was calculated as function of the degree of heterozygosity of animals and REC was derived from the heterozygosity of parental gametes which calculations are both described by Van der Werf and de Boer (1989).

Heritabilities for all traits were calculated separately for MRY and HF using the obtained estimated variance components. The heritability was calculated as:

$$h^2 = \frac{\sigma_a^2}{\sigma_a^2 + \sigma_{pe}^2 + \sigma_e^2}.$$

To evaluate the difference between the additive genetic variance components of HF and MRY a log likelihood ratio test was performed using following formula:

$$D = -2\ln\left(\ell R2/\ell R1\right),$$

where ℓR1 is the likelihood for the model to estimate the genetic variance for MRY and ℓR2 is the likelihood for the same model for MRY, except in this case the additive genetic variance was fixed at the value of the additive genetic variance of HF. The additive genetic variance for MRY was considered to be significantly different from the value for HF when the test statistic was above the 5% critical value of 2.71 from a mixture of the χ^2 distribution with 0 and 1 degrees of freedom (Self and Liang, 1987). Significance was assessed from the χ^2 distribution with 1 degrees of freedom, which was used for convenience, instead of *P*-values from the required mixture of χ^2 distribution with 0 and 1 degrees of freedom. The results, however, gives a correct representation of the additive genetic variances which are significantly different from each other ($P \leq 0.5$) and which are not ($P > 0.5$).

5.3 Results

Production traits

Additive genetic variances and heritabilities of 5 production traits were estimated for a Dutch MRY population and a Dutch HF population (Table 5.3). The estimated additive genetic variances of the milk fat percentage and the milk protein percentage were significantly higher (both $P<0.001$) for the Dutch HF population compared to the Dutch MRY population. In addition, the heritabilities of the milk fat percentage and the milk protein percentage were highest too for the Dutch HF population. The relative differences of the additive genetic variances of the milk fat percentage between both populations was highest, of which the additive genetic variance of HF was with an estimated value of 0.210 66% higher than those of MRY with 0.072.

Individual FA C4:0 to C18:0

For all individual FA C4:0 to C18:0 including the unsaturated FA C14:1 *c*-9 and C16:1 *c*-9 the additive genetic variances and heritabilities were estimated for MRY and HF (Table 5.3). For both, the additive genetic variances as well as the heritabilities, the estimates were lower for MRY compared to HF. Except for the FA 18:0 ($P=0.1681$), the differences of the estimated additive genetic variances between MRY and HF were significant (C4:0 $P<0.01$; C6:0-C16:0 $P<0.001$), where the differences ranged from 42% to 65% relative to the additive genetic variances estimated for HF.

Table 5.3 The heritability and additive genetic variance of 5 production traits, 14 individual fatty acids and 14 groups of fatty acids for the breeds MRY and HF and the t-values of the differences between the MRY and HF additive genetic variances.

	MRY[a]		HF[a]		
Traits	**$h^{2,b}$**	**Var A**	**$h^{2,c}$**	**Var A**	**% of diff[d,e]**
Production traits					
Milk yield (kg)	0.22	4.2284	0.21	6.4129	34
Fat%	0.22	0.0719	0.49	0.2104	66 ***
Protein%	0.27	0.0208	0.48	0.0429	52 ***
Fat yield (kg)	0.17	0.0078	0.16	0.0106	27
Protein yield (kg)	0.16	0.0038	0.17	0.0054	30
Traits g/dL milk					
C4:0	0.28	7.4E-05	0.39	0.000126	42 **
C6:0	0.27	4.4E-05	0.50	0.000100	56 ***
C8:0	0.28	2.3E-05	0.49	0.000051	54 ***
C10:0	0.29	0.00018	0.48	0.000352	50 ***
C12:0	0.28	0.00027	0.47	0.000571	52 ***
C14:0	0.27	0.00162	0.52	0.003813	58 ***
C14:1 *c*-9	0.27	1.9E-05	0.48	0.000045	59 ***
C16:0	0.34	0.01842	0.51	0.038513	52 ***
C16:1 *c*-9	0.17	3.6E-05	0.41	0.000104	65 ***
C18:0	0.23	4E-06	0.28	0.000010	29 ***
C18:1 *c*-9	0.1	0.00167	0.17	0.002339	42
Total cis C18:1	0.1	0.00246	0.17	0.004235	40
Total trans C18:1	0.2	0.0028	0.16	0.004646	-18
C18:2 *c*-9, 12	0.26	0.00019	0.26	0.000159	18
C18:2 *c*-9, *t*-11	0.21	0.00346	0.11	0.005174	-78 *
Total C18:2	0.22	0.00003	0.34	0.000037	39 *
C18:3 *c*-9,12,15	0.17	0.00002	0.31	0.000011	47 *
Total trans C18	0.21	4.1E-05	0.16	0.000067	-27
SFA	0.28	2E-06	0.53	0.000004	61 ***
UFA	0.11	0.00029	0.22	0.000229	51 *
MUFA	0.11	0.05544	0.22	0.141201	51 *
PUFA	0.21	0.00496	0.19	0.010186	1

SCFA	0.27	0.00407	0.50	0.008307	57 ***
MCFA	0.28	0.00017	0.53	0.000172	62 ***
LCFA	0.14	0.00099	0.21	0.002300	37
***n*-3**	0.21	0.0363	0.33	0.094703	44 *
***n*-6**	0.22	0.011	0.35	0.017551	34 *
BCFA	0.25	5E-06	0.36	0.000009	36 *

[a] MRY = Meuse-Rhine-Yssel and HF = Holstein Friesian.

[b] With a SE of on average 0.5 (which was between 0.03 and 0.06) for all estimated heritabilities.

[c] With a SE of 0.01 for all estimated heritabilities.

[d] The difference of the additive genetic variance of MRY compared to the additive genetic variance of HF as percentage of the additive variance of HF.

[e] The *P*-values obtained from the χ^2 distribution with 1 degrees of freedom: **P*<0.05; ***P*<0.01; ****P*<0.001.

C18 UFA

The estimated additive genetic variances and heritabilities of the individual C18 unsaturated FA and groups of these FA are also shown in Table 5.3. The additive genetic variances and heritabilities of the traits C18:1 *c*-9, Total cis C18:1, Total C18:2, and C18:3 *c*-9, 12, 15 were estimated to be lower for MRY compared to HF. For Total C18:2 and C18:3 *c*-9, 12, 15 the differences of the additive genetic variances between MRY and HF were significant (both *P*<0.05) with a difference of respectively 39% and 47% relative to the additive genetic variance of HF. The additive genetic variances and heritabilities of the traits Total *trans* C18:1, C18:2 *c*-9, *t*-11, and Total trans C18 were, however, estimated to be higher for MRY compared to HF. For C18:2 *c*-9, *t*-11 the differences of the additive genetic variances between MRY and HF were significant (*P*<0.05) with a difference of -78% relative to the additive genetic variance of HF.

Groups of FA

The estimated additive genetic variances and heritabilities of 10 groups of FA are shown in the bottom part of Table 5.3 (group definitions are given in Table 5.1). For both, the additive genetic variances as well as the heritabilities, the estimates were lower for MRY compared to HF, except for the group of FA PUFA. For PUFA, the heritability of MRY was estimated to be 0.21 and for HF 0.19, however, the additive genetic variance was almost similar. For all other groups of FA, except LCFA, the additive genetic variances were significant higher for HF (*P*<0.05) with a difference ranging from 34% to 62% relative to the additive genetic variance of HF.

5.4 Discussion

This paper reports estimates of the additive genetic variances and the heritabilities of the detailed FA composition in milk for the Dutch MRY in comparison with the estimates for the HF breed. Analyses were based on predicted FA composition, using MIRS spectra collected from a large number of milk samples. The FA predictions used to predict the additive genetic variances and heritabilities were expressed on milk basis (g of FA/dL of milk) thus the estimated variance components indicate to what extent selection is possible on FA composition as contents of individual FA in milk. We used prediction on milk basis, because the accuracy of prediction is considerably higher compared to fat basis (g/100g fat), as shown by De Marchi et al. (2011), Soyeurt et al. (2011), and Rutten et al. (2009). For our study we used the RobustMilk calibration equations because these equations were developed using data of different breeds including MRY and HF in the Walloon region of Belgium and including multiple countries (Soyeurt *et al.,* 2011) to enlarge the data variability which is essential for the application of MIRS (De Marchi et al. 2014). The predictive ability of these calibration equations in milk of different cattle breeds in the Netherlands, including MRY, has been investigated previously using an independent dataset with both MIRS spectra and gas chromatography measurements (Maurice-Van Eijndhoven et al., 2012). In that study, the predictive ability was evaluated for 10 individual FA and 3 groups of FA which are also included in the current study, and the coefficient of determination of the predictions ranged from 0.64 to 1.00. Some desciptive statistics of the calibration equations used in current study are also given in Table 5.2. Highest predictability, e.g. coefficient of determination close to one and highest RPDs, is especially shown for the predictions of the saturated short and medium FA. To use variability of FA composition within breeds for breeding purposes at the population level, predicted individual FA composition does not necessarily have to be 100% accurate. Indeed, Rutten et al. (2010) showed that the MIRS calibration equations have to be based on a large number of calibration samples, roughly 1 000 samples or more, to optimize the variability of calibration data in order to minimize the loss in potential genetic gain when using predicted FA from MIRS. The calibration equations used in our study were based on 1 236 samples from multiple breeds and countries to cover a wide range of FA variation. Lowest accuracies for the calibration equations were found for the poly unsaturated FA (ranging from 0.75 to 0.97) which can lead to bias of the prediction, therefore, results of these FA have to be interpreted carefully. For most of the FA considered in our study, however, it is expected that our results are hardly affected due to the use of MIRS FA predictions,

which is confirmed by the fact that the obtained results for HF are generally in line with results published in the literature.

The additive genetic variances and heritabilities were estimated using a dataset from commercial herds including purebred and crossbred cows. The crossbred cows included animals that are registered having at least 50% genes of MRY or of HF origin. Crossbred animals were included to have as many farms using the MRY breed in the analyses as possible. The HF breed is dominating in the Dutch dairy industry which is the main reason explaining the difference in number of records used in this study between MRY and HF. An important question is whether the difference in numbers of records for the MRY versus HF breeds could have contributed to the observed differences in estimated variance components. To examine this, the data of the HF herds were randomly divided in 75 subsets each having approximately the same size as the MRY dataset used in our study. Of these 75 subsets, 6 randomly chosen subsets were used to estimate the heritabilities and additive genetic variances for fat percentage, 4 individual FA, and 1 group of FA (SFA) (Table 5.4). Estimates for those individual subsets were on average clearly different from the estimates obtained using the MRY dataset, while they were close to the estimates obtained using the large HF dataset. Based on these results of the HF subsets, it is concluded that the differences in results observed between MRY and HF were not due to the differences in the size of the datasets.

It is well known that the FA composition in milk is affected by both genetics as well as the cows diet. Lowest additive genetic variances are found for FA with lower average contents in milk, which are mainly the unsaturated FA. The FA C4:0-C14:0 arise in milk mainly from *de novo* synthesis (Bauman and Griinari, 2003). This means that a considerable part of the variation in production of these FA is expected to have a genetic origin. In this study the heritabilities for HF range from 0.39 to 0.52 for the traits C4:0-C14:0 while the heritabilities for MRY range from 0.27 to 0.29. The relative differences between the estimated additive genetic variances of HF and the additive genetic variances of MRY were even larger, and those differences were also highly significant. Soyeurt et al. (2007) reported similar heritabilities for C12:0 and C14:0 compared to the estimates for MRY in our study. In their study, the dataset contained 7 breeds, including animals of the HF (45.39% of the studied population) and MRY (4.31% of the studied population) breeds in Belgium, and also MIRS spectra were used to predict FA composition although they were expressed on fat basis (g/100g fat). Heritabilities for the Dutch HF population were also estimated by Stoop et al. (2008), they reported heritabilities of HF

Table 5.4 The heritabilities with standard errors and additive genetic variances estimated based on 6 subgroups of HF data.

	HF Group[a,b]						
Traits	**1**	**2**	**3**	**4**	**5**	**6**	**Average**
Fat%							
h^2	0.51	0.38	0.48	0.42	0.49	0.45	0.46
SE	0.06	0.07	0.07	0.07	0.07	0.07	0.07
Var A	0.209042	0.154326	0.195856	0.178338	0.200369	0.208496	0.191071
C6:0							
h^2	0.48	0.36	0.47	0.46	0.55	0.46	0.46
SE	0.06	0.07	0.07	0.07	0.07	0.07	0.07
Var A	0.000094	0.000070	0.000092	0.000092	0.000110	0.000099	0.000093
C16:0							
h^2	0.49	0.47	0.46	0.41	0.47	0.46	0.46
SE	0.07	0.07	0.07	0.07	0.07	0.07	0.07
Var A	0.033271	0.033076	0.033156	0.030624	0.034186	0.036033	0.033391
C16:1 *c*-9							
h^2	0.53	0.31	0.38	0.34	0.43	0.42	0.40
SE	0.06	0.06	0.07	0.06	0.07	0.07	0.07
Var A	0.000138	0.000074	0.000090	0.000087	0.000108	0.000108	0.000101

C18:1 *c*-9							
h^2	0.28	0.07	0.13	0.13	0.12	0.08	0.14
SE	0.06	0.04	0.06	0.05	0.05	0.05	0.05
Var A	0.007265	0.001499	0.003050	0.003315	0.002962	0.002217	0.003385
SFA							
h^2	0.50	0.46	0.51	0.47	0.53	0.50	0.50
SE	0.07	0.07	0.07	0.07	0.07	0.07	0.07
Var A	0.122625	0.114805	0.129838	0.123965	0.132874	0.141525	0.127605

[a] HF = Holstein Friesian.

[b] Each group is a random representation of the total dataset with record of HF cows and the number of records within each group is ranging from 1 823 to 2 214.

ranging from 0.42 to 0.71, which is somewhat higher than in our study (range from 0.39 to 0.52). Compared to C4:0-C14:0, C16:0 is different in the sense that it arises in milk both through *de novo* synthesis and by uptake of blood circulation (Bauman and Griinari, 2003). The heritabilities for C16:0 were estimated to be (almost) highest compared to the other heritabilities of the same breed in our study, which was in line with Soyeurt et al. (2007). For the individual FA C4:0 –C16:1 our results were not in agreement with those of Bobe et al. (2008), however, for these FA their results were generally in disagreement with other results in the literature. The long chain (more than 16 carbons) unsaturated FA in milk are mainly obtained by uptake from blood circulation, however, also the rumen biohydrogenation and the Δ^9-desaturase activity contribute to the milk content of these FA (Neville and Picciano, 1997). Estimated heritabilities for these FA were lower for both the HF as well the MRY breed (heritabilities ranging respectively from 0.11 to 0.34 for HF and 0.10 to 0.26 for MRY) than previously discussed FA. The heritabilities and additive genetic variances were higher for HF compared to MRY except for Total trans C18:1, C18:2 *c*-9, *t*-11, and Total trans C18. However, only the differences in additive genetic variances for C18:2 *c*-9, *t*-11 was significant. Karijord et al. (1982) and Stoop et al. (2008) also reported somewhat lower heritabilities for the long chain and unsaturated FA, which implies a relatively larger influence of environmental aspects compared to genetics on the differences between individuals. In conclusion, although in the other studies FA composition was analysed on fat basis, the relative differences in heritability across FA within other studies tended to be the same as those found in our study.

A possible explanation of (a part of) the differences in additive genetic variances and heritabilities between the MRY and HF breed suggests that the genetic architecture differs between those breeds. The lower genetic variance for MRY was nevertheless unexpected, as there has been relatively very little emphasis on selection for high-input high-output production systems in MRY compared to HF over the last decades. The FA composition of bovine milk is known to be effected by several genes with a moderate to large effect. Two genes, having polymorphisms with reported effects on FA composition, are DGAT1 and SCD1 (e.g. Thaller et al., 2003; Mele et al., 2007; Schennink et al., 2008). Polymorphisms in those genes affect the production of FA and, thus, also the genetic variances within breeds as there are different genotypes. Especially the DGAT1 K232A polymorphism is reported having significant effects on the milk production traits and some medium chain SFA and long chain UFA (Schennink et al., 2007). Percentages of the genetic variances of FA composition in milk explained by the

DGAT1 K232A polymorphism were reported ranging from 1% up to 53% (Schennink et al., 2007; Schennink et al., 2008). Those high percentages of explained genetic variance, are due to the high minor allele frequency of DGAT1, i.e. Schennink et al. (2007) reported a frequency of 0.40 for the 232K allele in the Dutch HF population. Considering what's known about the *DGAT1* K232A polymorphism, the lower genetic variances in MRY for a number of FA may be because one of the alleles at the *DGAT1* locus has an extreme frequency such that the contribution of *DGAT1* to the genetic variance in MRY is limited. Because the allele frequency of the *DGAT1* K232A polymorphism is currently not known for the MRY breed, this hypothesis will be tested in future research.

5.5 Conclusion

For both, MRY and HF, additive genetic variances and heritabilities were estimated for detailed FA composition in milk. The additive genetic variances as well as the heritabilities for the SCFA and MCFA, which mainly arise in milk by *de novo* synthesis, were generally lower for MRY than for HF. Lower heritabilities and less significant differences in heritability between the breeds were estimated for the long chain C18 FA that are mainly obtained by uptake from blood circulation. Lower variances in MRY may be because of a difference of their genetic architecture compared to HF. In conclusion, our results show that the HF breed has substantially larger genetic variance for most FA compared to MRY, despite its stronger selection for milk yield traits in the past. As the estimated genetic variances for MRY were clearly lower, and because it is know that the *DGAT1* locus has an intermediate allele frequency in HF, it is hypothesized that the *DGAT1* locus has a more extreme minor allele frequency in the Dutch MRY population, which will be tested in future research.

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6

Genomic differences associated with milk fat composition in Holstein versus Meuse-Rhine-Yssel, Dutch Friesian, and Groningen White Headed cattle breeds

M.H.T. Maurice – Van Eijndhoven[1,2], H.Bovenhuis[2], R.F. Veerkamp[1,2], M.P.L. Calus[1]

[1] Animal Breeding and Genomics Centre, Wageningen UR Livestock Research, P.O. Box 338, 6700 AH Wageningen, The Netherlands; [2] Animal Breeding and Genomics Centre, Wageningen University, P.O. Box 338, 6700 AH Wageningen, The Netherlands

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Abstract

The aim of this study was to identify if the genomic variation associated with detailed milk fat composition is similar between the Holstein Friesian (HF) and native dual purpose breeds in the Dutch dairy industry. Phenotypic and genotypic information was available for the breeds HF, Meuse-Rhine-Yssel (MRY), Groningen White Headed (GWH), and Dutch Friesian (DF). First the reliability of genomic breeding values of the native Dutch dual purpose cattle breeds MRY, DF, and GWH was evaluated using SNP effects estimated in HF. Secondly, similarities between the breeds across the whole genome were investigated by calculating the correlations between allele frequencies in the breeds MRY, GWH, DF, and HF. Finally, the genomic differences of the regions associated with milk fat composition (regions on BTA 5, 14, and 26), were studied in the different breeds. Comparing the reliability of genomic prediction for the native Dutch dual purpose breeds, the GEBV for the MRY breed were clearly least reliable (reliabilities were on average 0.022 when only including the 8-22 SNPs with the strongest association in HF). For both DF and GWH, the estimated SNP effects based on HF data did have substantial predictive ability for several traits, being highest in GWH with reliabilities for the different FA of on average 0.158 when including only the 8-22 SNPs with the strongest association in HF. These correlations between allele frequencies clearly showed differences between the breeds, albeit that there was no consistent relationship between both statistics. The comparison of different regions associated with milk fat composition (regions on BTA 5, 14, and 26) showed that breeds also clearly differ in genomic variation within these regions. It can be concluded that the minor allele frequencies of genes having an effect on milk FA composition in HF are much smaller in the breeds MRY, GWH and DF. This is especially the case for the MRY breed.

Key words: milk, fatty acid, genomic variation, single nucleotide polymorphism, cattle breed

6.1 Introduction

Over the last decades a clear change in the dairy industry has taken place, towards high production, especially in industrialized countries. High emphasis on selection for milk yield and the consolidation and globalization of breeding companies has led to professional breeding programs for specialized dairy cattle breeds (Brotherstone and Goddard, 2005, Shook, 2006). This development has led directly to a decline in use of native dual purpose breeds which are used for milk and beef production. For example in The Netherlands the percentage dairy cows in the dairy industry belonging to Dutch native dual purpose breeds declined from 91.3 to 1.4 percent in the last 30 years (CRV, 2013). This reduction in breed variability is associated with a decrease of genetic variability in the total cattle population as individual cattle breeds comprise unique genetic variation (European Cattle Genetic Diversity Consortium, 2006). In this perspective, safeguarding existing genetic variation is important to be able to anticipate on unexpected calamities and to exploit currently unknown genetic variation in the future (Oldenbroek, 2007). Therefore and for selection purpose , for the dairy cattle industry it is of interest to know whether the genetic variation in native dual purpose breeds differs from the Holstein Friesian (HF) breed. One trait, which is extensively studied in the HF breed and increasingly more important for the dairy industry, is the detailed fatty acid (FA) composition in milk (e.g. Soyeurt et al., 2006, Stoop et al., 2008). The interest in the possibilities to modify detailed FA composition in milk by the dairy industry arises from supposed associations of milk FA composition with human health (e.g. Astrup et al., 2011, Palmquist et al., 2006) and milk processability (e.g. Smet et al., 2009). In addition to genetic variance for milk FA composition observed within breeds, differences in milk FA composition among dairy cattle breeds are described in several studies (e.g. Maurice-Van Eijndhoven et al., 2013, Soyeurt et al., 2011).

Studying the relation between the bovine genome and the FA composition in bovine milk is an important step towards understanding the genetic variability of milk FA composition. For example on the bovine genome in 2 genes, namely diacylglycerol acyltransferase 1 (*DGAT*1) and stearoyl-CoA desaturase 1 (*SCD*1), polymorphisms are reported to have large associations with milk FA composition of HF cattle (e.g. Bernard et al., 2013, Bouwman et al., 2011). To investigate the genetic variability in native dual purpose breeds, one possible approach is to compare whether different genomic regions have a similar contribution to genetic variation in different cattle breeds. Differences in allele frequencies of mutations in the well-recognized *DGAT*1 and *SCD*1 polymorphisms have been reported also in

several studies between dairy cattle breeds. For example, different allele frequencies of the *DGAT*1 232K allele in New Zealand HF (0.6), Ayrshire (0.22) and Jersey (0.88) are reported by Spelman et al. (2002) and of the *SCD*1 293A allele in Canadian HF (0.83) and Jersey (0.95) are reported by Kgwatalala et al. (2007). Therefore an appropriate and unanswered question is whether effects of different regions in the genome on the milk fat composition in milk of HF cows are similar for the native dual purpose breeds.

The aim of this study is to identify if there are similarities in genomic variation associated with detailed milk fat composition between the HF breed and native dual purpose breeds in the Dutch dairy industry. This will be achieved by evaluating the reliability of predicted genomic breeding values for fat composition in milk of the native Dutch dual purpose cattle breeds Meuse-Rhine-Yssel (MRY), Groningen White Headed (GWH), and Dutch Friesian (DF) using SNP effects estimated in HF and by comparing the genomic variability in regions on the genome showing large effects.

6.2 Materials and Methods

Data collection

In current study phenotypic data of in total 2500 cows from 4 different breeds was used and genotypes of 1,867 cows. In Table 6.1 an overview is given of the data for each of the 3 sampling periods including: the number of cows sampled per breed and the average and standard deviation of the days in milk (DIM), parity, and age at calving (age) of the cows at time of sampling, which are used as fixed effect in the model which is explained below. Milk samples of the first sample period were collected within the project of the Dutch Milk Genomics Initiative between May and June 2005. Gas Chromatography (GC) profiles were obtained from morning milk samples from 1811 cows. From these cows also blood samples were collected for genotyping. All cows belong to the HF breed and were sampled during the 1st parity between 97 and 355 DIM. The data collected in 2005 was used and described in earlier studies where this data is referred to as summer milk (Bouwman et al., 2012, Duchemin et al., 2013, Rutten et al., 2009). Data of the second sample period was collected during December 2008 and March 2009. GC profiles of morning milk samples were collected from 137 cows and 79 of these cows were also genotyped. Cows belonged to 3 breeds: DF (44 samples from 3 herds), MRY (50 samples from 3 herds), and GWH (43 samples from 3 herds). Parity of the cows varied between 1 and 9 and DIM varied between 5 and 535. The GC data collected during winter time in 2008-2009, was earlier used and described by

Table 6.1 Overview of the number of animals per breed and fixed effects per sample period.

	Data			
	Data 2005[1]	**Data 2008-2009[1]**	**Data 2011[1]**	**Data 2011[2]**
N	1811	137	319	233
Breed				
MRY	0	50	144	98
GWH	0	43	105	31
DF	0	44	70	24
HF	1811	0	0	80
Total with genotypes	1,544	79	280	0
Fixed effects	*average (std)*	*average (std)*	*average (std)*	*average (std)*
DIM[3]	274 (42)	144 (88)	158 (94)	162 (98)
parity[4]	1.0 (0)	2.8 (2.0)	2.7 (1.8)	3.2 (1.9)
age[5]	779 (57)	1575 (841)	1508 (704)	1696 (758)

[1] FA composition based on GC.
[2] FA composition based on MIR.
[3] DIM means days in milk.
[4] parity was divided in 4 classes for corresponding lactation numbers of parity 1, 2, and 3 and the 4th class included parity 4 – 10.
[5] age at calving in days.

Maurice-Van Eijndhoven et al. (2011). Data of the third sample period was collected during the end of August 2011 and the beginning of November 2011. GC profiles or Mid Infrared Spectrometry (MIR; this data is not used for the genomic analyses, but only in the step to correct phenotypes, see the paragraph '*Statistical analysis: correcting phenotypes for fixed effects'* below) profiles of morning milk samples were collected from 552 cows and 280 of these cows were also genotyped (including only purebred MRY, GWH, and DF). Cows were sampled once, had access to pasture during day time, and belonged to 4 breeds: MRY (242 cows from 3 herds), GWH (136 cows from 3 herds), DF (94 cows from 2 herds), and HF (80 cows from 3 herds). Parity of the cows varied from 1 to 10 and DIM varied from 5 to 365. All cows were purebreds belonging to the breeds HF, MRY, GWH or DF (i.e. they were registered with at least 87.5% of the genes of any of the breeds mentioned). The data collected in 2011 contains three herds with milk samples of multiple

breeds: one herd with 28 MRY and 15 HF cows; one herd with 6 GWH and 36 HF cows; and one herd with 5 MRY, 4 GWH, and 33 DF cows. Overall, the complete dataset used contains 1 milk sample of each of the 2500 cows which was analysed using GC or MIR and each herd test day (htd) comprises a minimum of 3 records, and of these cows 1,876 were also genotyped.

Measuring fatty acid composition

Collected milk samples during all sample periods were treated immediately with 0.03% (wt/wt) sodium azide to avoid microbiological growth. To obtain detailed FA compositions, all milk samples obtained in the different sample periods were analysed at the laboratory of Qlip N.V. (Zutphen, The Netherlands) by GC or by Fourier-transformed interferogram machines (MilkoScan FT 6000, Foss Electric, Hillerød, Denmark) to obtain MIR profiles. The GC outputs were generated by analyzing methyl esters. Fatty acid methyl esters were prepared using fat fractions extracted from the milk, as described in ISO Standard 15884 (ISO-IDF, 2002b). Methyl esters were analyzed, as described in ISO Standard 15885 (ISO-IDF, 2002a), according to the 100% FA methyl ester method with a 100-m x 0.25-mm polar column (Select Fame Varian CP 7420, Varian Inc., Palo Alto, CA) for samples collected within the 2005 and 2008-2009 and for samples collected in 2011 a new 100-m x 0.25-mm polar column was used.

For the final analyses fat percentage, protein percentage, 1 group of FA, and 9 individual FA were chosen: Group of short chain FA (SCFA) (C4:0-C10:0); C12:1cis9; C14:0; C14:1cis9; C16:0; C18:0; C18:1trans6,9,11; C18:1cis9; C18:2cis9,12; C18:2cis9,trans11). The group of FA and individual FA were analyzed in g/dL milk. The FA were chosen to have reasonably high correlations between summer and winter milk (Duchemin et al., 2012) which allowed us to combine data sampled in different seasons. Other selection criteria were biological background (because of the clear genetic architecture of the FA: generated through de novo synthesis or by delta9-desaturases) (Schennink et al., 2008, Soyeurt et al., 2008) and (lack of) relation with DGAT1 or SCD1 (Schennink et al., 2008). The last two criteria were chosen to investigate whether similarity of genomic variance of FA composition between different breeds depends on biological background and genetic architecture of the FA.

Genotype data

In total, 359 animals were genotyped for the breeds DF, GWH and MRY, using the Illumina HD SNP chip (Ilumina Inc., San Diego, CA). Genotypes with a GC score < 0.2 were set to missing. After this initial editing step SNPs were removed with: a

call rate < 85%, a minor allele frequency < 0.02, unknown map position, or known position on the sex chromosomes. Further edits included removing animals with a call rate < 85% (n=8), parent-offspring inconsistencies (n=2) and because based on principal component analysis of the genotype data animals clustered with a different breed than registered by the herd book registration (n=1). Finally, missing genotypes were imputed using Beagle (Browning and Browning, 2007). After these edits, 619,056 segregating SNPs were available on 79 DF, 120 GWH, and 149 MRY cows. Of those genotyped animals, 327 animals also had data on FA composition; 70 DF, 113 GWH and 144 MRY.

The HF data contained 1,684 animals with imputed HD genotypes for 734,393 SNPs. Those animals were initially genotyped with a custom 50k SNP chip. The HD genotypes were imputed using HD genotypes of all 55 sires of the HF cows, combined with HD genotypes of another 1,278 HF bulls available at CRV. More details on these (imputed) genotypes are provided by Bouwman et al. (2014). Before combining them with the other dataset, SNP were removed with a minor allele frequency < 0.02 or when not all three genotypes were observed, leaving 602,445 SNP. Of the genotyped HF animals, 1,544 also had data on FA composition.

The imputed HD SNP data for the HF animals was combined with the HD SNP data of the other breeds, yielding a combined dataset consisting of 571,275 SNPs which met the editing criteria in both datasets. In the combined dataset, from each pair of neighbouring SNP with an r^2 (LD) value of 1.0, one of the SNPs was removed. After this final step, 350,207 SNP were left in the dataset used for analysis.

Statistical analysis: correcting phenotypes for fixed effects

Before estimating SNP effects the record of each cow was corrected for DIM, parity, age at calving, herd test date (htd), breed, and method of measuring the FA using the following animal model (using ASReml 3.0, Gilmour et al., 2009):

$$y_{ijklmno} = \mu + b_1 \times DIM_i + b_2 \times \exp^{-0.05 \times DIM_i} + parity_j + b_3 \times age_k\ (parity_j) + htd_l + breed_m + method_n + e_{ijklmno},$$

where $y_{ijklmno}$ was the dependent variable for cow *o* in days in milk (DIM) *i*, with parity *j*, age at calving *k*, producing at herd test date (htd) *l*, belonging to breed *m*, and with FA composition obtained using method *n*. The μ was the overall mean of the model; b_1 was the fixed regression coefficient on DIM_i and b_2 was the fixed regression coefficient on DIM_i modeled with a Wilmink curve (Wilmink, 1987); $parity_j$ was the fixed effect with 4 classes for corresponding lactation numbers of parity 1, 2, and 3 and the 4th class included parity 4 - 10; b_3 was the fixed regression coefficient on age_k, which was age at calving in days, within the *j*th parity; htd_l was the fixed effect defining groups of cows sampled in the same herd on the same sample date; $breed_m$ was the fixed effect defining groups of cows belonging to the same breed (HF, MRY, GWH, or DF); $method_n$ was the fixed effect with 2 classes corresponding for the method used to obtain the FA composition (GC or MIR); and $e_{ijklmno}$ is the random residual effect. Predicted FA composition based on MIR profiles was included for 272 animals without genotypes as *y* variate in the analysis to obtain more precise correction of the phenotypes for fixed effects. The residuals of this model for the GC was taken as trait for the subsequent genomic analyses. Table 6.2 shows an overview of the corrected phenotypes per sample period.

Statistical analysis: estimation of SNP effects

To identify if there are similarities in genomic variation associated with milk FA composition the fat composition in milk of Dutch cattle breeds MRY, GWH, and DF was predicted using SNP effects estimated based on the 1,544 HF cows. For this part of the analysis genotype data and the residuals obtained from the model to pre-correct the phenotypes were used. The model used is commonly known as BayesC (Habier et al., 2011), and is described by:

$$\mathrm{y} = 1\mu + \mathrm{Zu} + \mathrm{X}\alpha + \mathrm{e}$$

where y contains pre-corrected phenotypes, 1 is a vector of ones, μ is the overall mean, Z is an incidence matrix that links records to individuals, u contains random polygenic effects of all individuals, X is a matrix that contains the scaled and

Table 6.2 Overview of the corrected phenotypes per sample period.

	Data			
	Data 2005[1]	**Data 2008-2009**[1]	**Data 2011**[1]	**Data 2011**[2]
N	1811	137	319	233
Production traits[3]	*average (std)*	*average (std)*	*average (std)*	*average (std)*
Fat%	4.26 (1.25)	4.56 (1.29)	4.55 (1.39)	4.36 (1.54)
Protein%	3.53 (0.48)	3.60 (0.59)	3.68 (0.70)	3.61 (0.65)
Traits in g/dL milk[4]	*average (std)*	*average (std)*	*average (std)*	*average (std)*
C4:0-C10:0	0.4219 (0.1498)	0.5085 (0.1698)	0.4667 (0.1470)	0.4349 (0.1556)
C12:1	0.0046 (0.0024)	0.0043 (0.0029)	0.0047 (0.0031)	0.0056 (0.0024)
C14:0	0.4738 (0.1447)	0.5651 (0.1895)	0.5292 (0.1926)	0.4419 (0.1542)
C14:1 *cis*9	0.0491 (0.0233)	0.0464 (0.0245)	0.0489 (0.0253)	0.0601 (0.0273)
C16:0	1.2494 (0.4813)	1.3812 (0.4852)	1.3336 (0.4852)	0.9825 (0.6584)
C18:0	0.4234 (0.1903)	0.4782 (0.1877)	0.4406 (0.2314)	0.3446 (0.1891)
C18:1 *cis*9	0.0680 (0.1333)	0.0589 (0.1399)	0.0755 (0.1889)	0.0873 (0.1807)
C18:1 *trans*6-9-11	0.8596 (0.1783)	0.7596 (0.1739)	0.8732 (0.2328)	0.9456 (0.2413)
C18:2 *cis*9-12	0.0472 (0.0170)	0.0593 (0.0264)	0.0538 (0.0259)	0.0591 (0.0206)
C18:2 *cis*9-*trans*11	0.0232 (0.0142)	0.0182 (0.0086)	0.0273 (0.0228)	0.0350 (0.0161)

[1] Milk samples analysed using GC.

[2] Milk samples analysed using MIR.

[3] Corrected phenotypes are showed and both production traits are analyzed using MIR for all data.

[4] Corrected phenotypes are showed.

centered genotypes of all individuals, α contains the (random) allele substitution effects for all loci, and e contains the random residuals. Polygenic effects were included in the model because the HF data contained only a limited number of sires with relative large groups of daughters. Omitting a polygenic effect with such a data structure, is expected to lead to spurious associations (e.g. Balding, 2006), that will not have any predictive value for the other breeds.

The posterior value of α_j, the allele substitution effect of locus *j*, is sampled from:

$N\left(\widehat{\alpha_j};\ \frac{\widehat{\sigma}_e^2}{\mathrm{x}_j'\mathrm{x}_j+\lambda}\right)$ if $I_j = 1$

0 if $I_j = 0$

where $\lambda = \frac{\widehat{\sigma}_e^2}{\widehat{\sigma}_\alpha^2}$.

σ_α^2 has a prior distribution of:

$p(\sigma_\alpha^2) = \chi^{-2}(\nu, S_\alpha^2)$

where ν is the degrees of freedom, set to 4.2 following (de los Campos et al., 2013) and the scale parameter S_α^2 is calculated as $S_\alpha^2 = \frac{\tilde{\sigma}_\alpha^2(\nu-2)}{\nu}$ where $\tilde{\sigma}_\alpha^2$ is the prior value of σ_α^2, that is computed as $\tilde{\sigma}_\alpha^2 = \left(\frac{1}{1-\pi}\right)\frac{\sigma_a^2}{n}$ (de los Campos et al., 2013) where π is the prior probability that a SNP has zero effect (given a value resembling 50 loci with non-zero effects) and *n* is the total number of SNP loci. Genetic variances for HF (σ_a^2), used to compute prior SNP variances, were obtained from Maurice-Van Eijndhoven et al. (submitted) except for C12:1 and C18:1*trans*6_9_11 for which values of respectively 0.000045 and 0.0046 were used. The posterior value of σ_α^2 is drawn from the following inverse-χ^2 distribution with $\nu + n$ degrees of freedom:

$\sigma_\alpha^2|\alpha \sim \chi^{-2}(\nu + n, S_\alpha^2 + \widehat{\alpha}^2)$

where $\widehat{\alpha}^2$ is a vector with squares of the current estimates of the allele substitution effects of all loci.

The posterior distribution of the quantitative trait loci (QTL)-indicator I_j was:

$$\Pr(I_j = 1) = \frac{f(r_j|I_j = 1)(1-\pi)}{f(r_j|I_j = 0)\pi + f(r_j|I_j = 1)(1-\pi)}$$

where $r_j = \mathrm{x}_j'\mathrm{y}^* + \mathrm{x}_j'\mathrm{x}_j\widehat{\alpha}_j$ where y^* contains the conditional phenotypes (i.e. the y-values minus the current estimates for the mean, the polygenic effects and the SNP effects at all other loci), and $f(r_j|I_j = \delta)$ where δ is either 0 or 1, is proportional to

$\frac{1}{\sqrt{v_\delta}}\mathrm{e}^{-\frac{r_j^2}{2v_\delta}}$,

where $v_0 = \mathrm{x}_j'\mathrm{x}_j\sigma_e^2$ and $v_1 = \left(\mathrm{x}_j'\mathrm{x}_j\right)^2\sigma_{\alpha_j}^2 + \mathrm{x}_j'\mathrm{x}_j\sigma_e^2$.

For the polygenic effect, the conditional posterior density of σ_u^2 is an inverse-χ^2 distribution:

$\sigma_u^2|u \sim \chi^{-2}(q-2, \mathrm{u}'\mathrm{A}^{-1}\mathrm{u})$

where q is the number of animals in the pedigree (26300), and u is a vector with the current polygenic effects, and A^{-1} is the inverse of the numerator relationship matrix derived from the pedigree.

Finally, the conditional posterior density of σ_e^2 is an inverse-χ^2 distribution:

$\sigma_e^2|e \sim \chi^{-2}(m-2, \mathrm{e}'\mathrm{e})$

where m is the number of animals with records, and e is a vector with the current residuals.

More details on the implementation of the model can be found in Calus (2014).

For each SNP, a Bayes Factor (BF) was calculated as:

$$BF = \frac{Pr(H_1|y)}{1-Pr(H_1|y)} \div \frac{Pr(H_1)}{1-Pr(H_1)}$$

where H_1 is the hypothesis that the marker has a non-zero effect, $Pr(H_1|y)$ is the posterior probability of the hypothesis and $Pr(H_1)$ is the prior probability of the hypothesis. (1 - $Pr(H_1|y)$) and (1 - $Pr(H_1)$) represent, the posterior and prior probability for the alternative hypothesis, respectively. The Bayes Factors are an indication of the effects that SNPs have on the trait analyzed. Higher Bayes Factors indicate that it is more likely that a SNP is indeed associated with a QTL affecting the trait. To give an overview of SNPs having an effect on the different milk traits in HF a Manhattan plot for the Bayes Factors for each SNP was produced. Plotted Bayes Factors were "smoothed", by calculating for each SNP the average of its own Bayes Factor, and those of the four SNPs located on either side.

The SNP effects estimated using the dataset of the HF population sampled in 2005 were used to calculate genomic estimated breeding values (GEBV) of the analyzed traits for the genotyped cows of the breeds MRY, GWH, and DF sampled in 2008-2009 and 2011. To check how valid the GEBV's are using SNP effects estimated in HF, reliabilities (i.e. accuracies squared) of the predicted breeding values were calculated. The reliability can be expressed as:

$r_{g\hat{g}}^2 = r_{y\hat{g}}^2 / h^2$, following e.g. Verbyla et al. (2010).

where $r_{y\hat{g}}^2$ is the squared correlation between the observed phenotype in MRY, GWH, or DF and their GEBV, and h^2 is the heritability of the trait. The h^2 used were estimated for a Dutch MRY population (Maurice-Van Eijndhoven et al., submitted), and used for all three breeds (MRY, GWH, and DF) because no h^2 estimates were available for GWH and DF. For C12:1 and C18:1*trans*6_9_11 no estimates were available for h^2, and therefore values of respectively 0.3 and 0.16 were used,

resembling h^2 estimates obtained in MRY for other FA that were shown to have similar variance as for C12:1 and C18:1*trans*6_9_11 in HF. GEBV were calculated either using all SNPs, the SNPs having an estimated effect in HF with Bayes Factor > 10, the SNPs having a Bayes Factor > 100, and the SNPs having a Bayes Factor > 1000. Using increasingly higher thresholds for the Bayes Factor of the included SNPs, allowed to investigate the impact of regions with more pronounced effects on FA composition in HF on the accuracy of prediction in the other breeds. Thus, this allowed us to evaluate whether or not the genomic regions affecting FA composition in HF have a similar effect on FA composition in the other breeds. To further evaluate similarities between breeds in those genomic regions, allele frequencies for the different scenarios were compared, as well as variance explained per SNP. The extent to which the SNPs explained the genetic variation of the traits per breed was evaluated. This was achieved by calculating the percentage of the genetic variation explained by the SNP for each breed as $2pq\hat{\alpha}^2/var(a)$ where $var(a)$ was the genetic variance for HF calculated by $var(a) = h^2 * var(y)$ where y were pre-corrected phenotypes and the h^2 for HF were obtained from Maurice-Van Eijndhoven et al. (submitted). For trait C18:2*cis*9-*trans*11 no SNPs were found with a Bayes Factor >1000 and therefore this trait was not included in this last evaluation.

6.3 Results

Corrected data

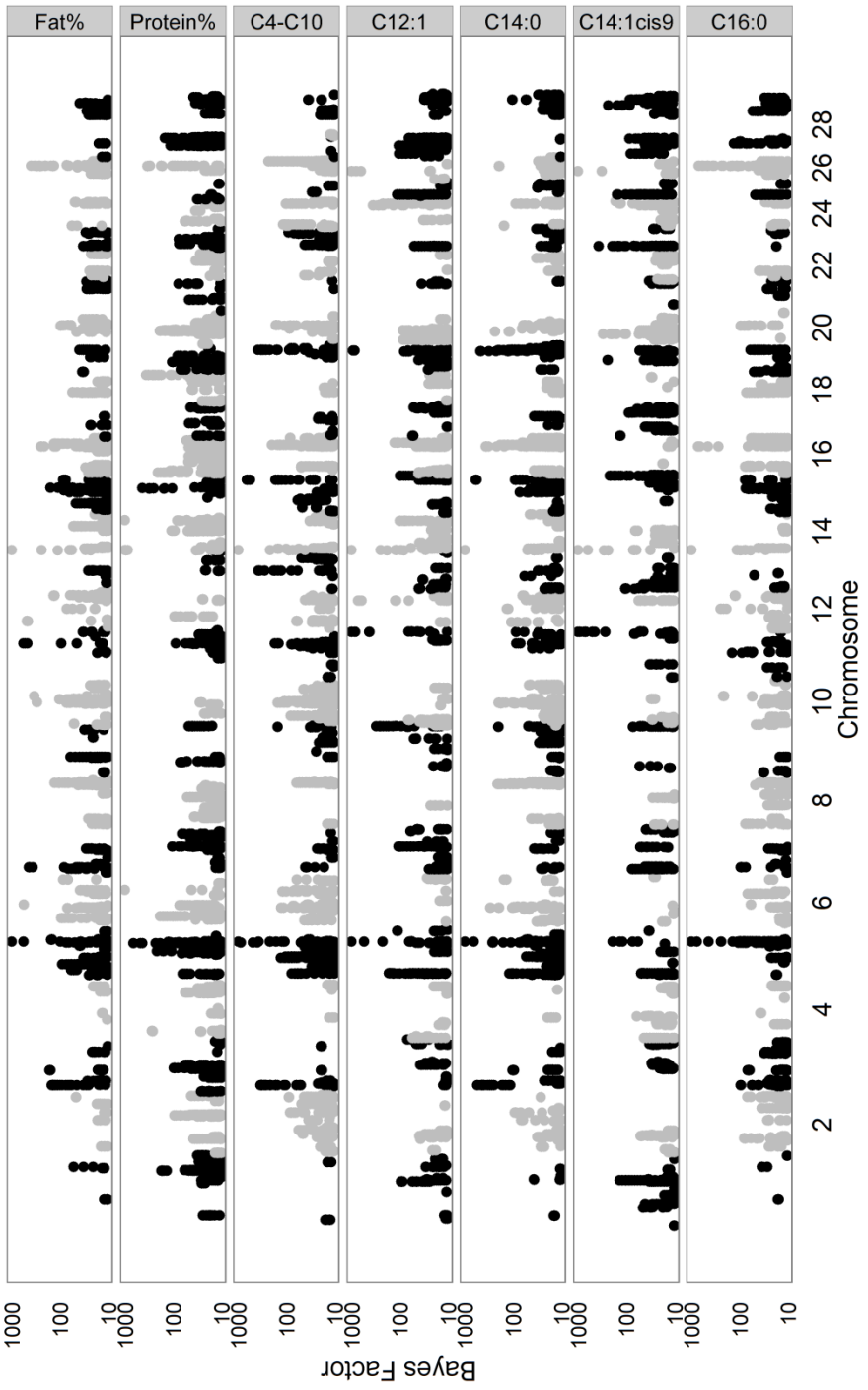
Cows sampled in 2005 were on average 779 days old at calving, 274 DIM, and were all in their first parity (Table 6.1). Cows sampled in 2008-2009 and 2011 were somewhat older, being on average 1508-1696 days old at calving, at 144-162 DIM, and in their third parity. An overview of the data, after correction for fixed effects, is given in Table 6.2. Fat percentage was on average highest for data collected in 2008-2009 (4.56%) and lowest for data collected in 2005 (4.26%) and most variable for data collected in 2011 (SD 1.39-1.54). The average contents in milk of all saturated FA (C4:0-C10:0; C14:0; C16:0; and C18:0) of the samples collected in 2008-2009 were higher and all unsaturated FA contents, except C18:2*cis*9-12, were lower compared to the samples collected in 2005 and 2011. The average contents of unsaturated FA, except C18:2*cis*9-12, were generally lowest in the data collected in 2011 which was analyzed using MIR.

SNP effects: reliability of GEBV

Figure 6.1 gives an overview of the Bayes Factors of all SNPs across the genome for each of the considered traits. The Bayes Factors indicate whether or

not SNPs have a strong association with the traits investigated. Several peaks are observed across multiple traits, indicating that there are several QTL that are associated with the FA traits included in our study. In Table 6.3 the number of SNPs are presented that showed an effect above the thresholds of a Bayes Factor of 10 (fifth column), 100 (ninth column), and 1000 (thirteenth column). Of the 350,207 examined SNPs for the individual traits between 2405 and 4734 SNPs showed a Bayes Factor above the threshold of 10, between 46 and 311 showed a Bayes Factor above the threshold of 100, and between 0 and 22 showed a Bayes Factor above the threshold of 1000.

Table 6.3 shows the reliabilities of the GEBV estimated for the three breeds MRY, GWH, and DF calculated either using all (350,207) examined SNPs, or using only SNPs with a Bayes Factor above thresholds of 10, 100, or 1000 in the HF population. The GEBV of fat percentage showed a reliability ranging between 0.158 and 0.193 for GWH and between 0.237 and 0.256 for DF when SNP effects were used with a Bayes Factor > 10. For MRY the reliabilities for fat percentage were clearly lower (between 0.034 and 0.046). For protein percentage the reliabilities were for all breeds below 0.1 when including SNPs with a Bayes Factor > 10. The GEBV of the FA C12:1 showed relatively high reliabilities for GWH (0.397 - 0.492) and for DF around 0.150 when SNP effects were used with a Bayes Factor > 10. For the FA C14:0 and C16:0 the reliabilities of the GEBV for GWH were all above 0.2 when including SNPs with a Bayes Factor > 10. For the FA C14:1*cis*9 the reliability to calculate the GEBV for GWH using all 350,207 SNPs was highest (0.758), however, the reliability dropped almost to zero when SNPs were included with a Bayes Factor > 10, and the correlation ($r_{y\hat{g}}$) in fact became negative. For the FA C18:1*cis*9 the reliability of the GEBV for DF were relatively high (0.182 – 0.185), regardless the possible threshold based on Bayes Factors for the SNPs included, whereas for MRY and GWH the reliabilities were almost zero. The reliabilities of the GEBV for MRY were below 0.1 for all traits, except for C14:1*cis*9 using all 305,207 SNP effects to calculate the GEBV (0.178), however, when including SNPs with a Bayes Factor >10 this reliability dropped towards zero. Overall, the reliabilities of GEBV clearly differed between using all 350,207 SNPs and when including SNPs with a Bayes Factor > 10, > 100, or > 1000, whereas between subsets of SNPs with the different Bayes Factor thresholds the reliabilities of the GEBV were very similar.


Fat%
Protein%
C4-C10
C12:1
C14:0
C14:1cis9
C16:0
Bayes Factor
1000
100
10
Chromosome
2
4
6
8
10
12
14
16
18
20
22
24
26
28

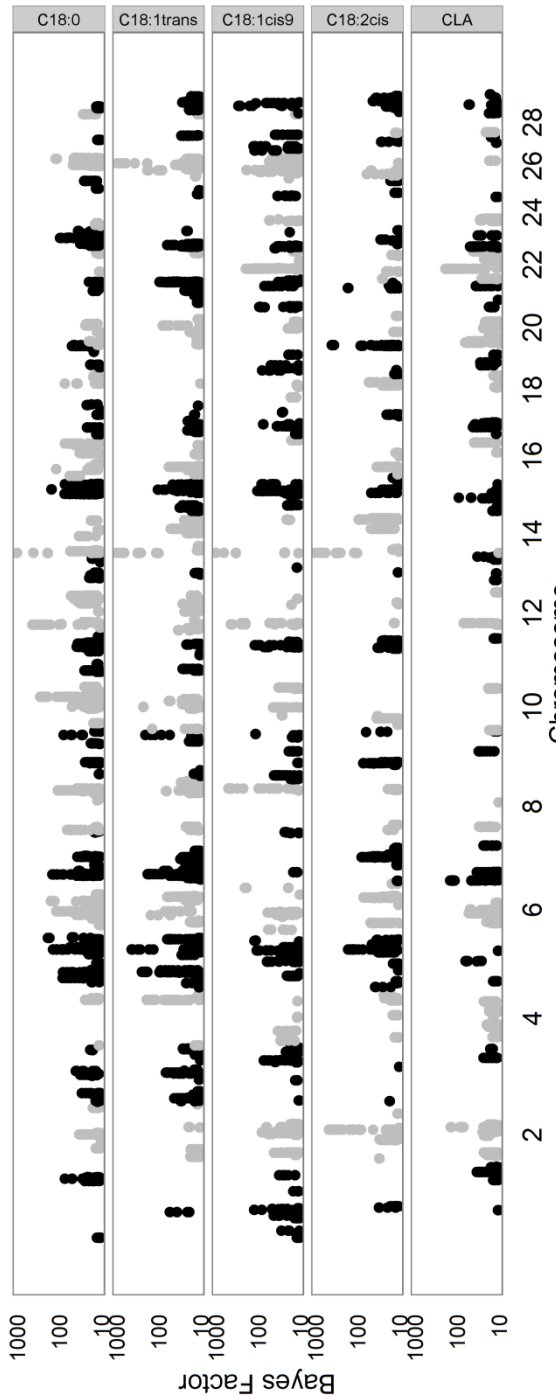


Figure 6.1 Manhattan plot showing the Bayes Factors for each SNP having an effect on the bovine milk fatty acids in HF.

C18:1 trans is the sum of the fatty acids C18:1 trans-6, C18:1 trans-9, and C18:1 trans-11
C18:2 is the fatty acid C18:2 cis-9, 12
CLA is the fatty C18:2 cis-9, trans-11

Table 6.3 The reliabilities[1] of the GEBV[2] in native Dutch dual purpose breeds and the number of segregating SNPs[3] used for GEBV[2] for the prediction.

	All 350,207 SNPs			Bayes factors > 10				Bayes factors > 100				Bayes factors > 1000			
	MRY[4]	GWH[4]	DF[4]	#SNPs[3]	MRY	GWH	DF	#SNPs	MRY	GWH	DF	#SNPs	MRY	GWH	DF
Production traits															
Fat%	0.041	0.050	0.072	3292	0.046	0.193	0.245	211	0.046	0.174	0.256	14	0.034	0.158	0.237
Protein%	0.027	0.229	0.059	4734	0.057*	0.056*	0.007	311	0.058*	0.065*	0.004	16	0.029*	0.086*	0.009
Traits in g/dL milk															
C4:0-C10:0	0.077	0.011*	0.121	3942	0.000	0.060	0.043	301	0.000	0.054	0.042	13	0.000	0.056	0.068
C12:1	0.001	0.492	0.030	3527	0.002	0.397	0.152	255	0.003	0.411	0.162	15	0.001*	0.445	0.147
C14:0	0.006	0.049	0.005	3852	0.004*	0.265	0.045	260	0.002*	0.268	0.051	22	0.004*	0.240	0.082
C14:1*cis*9	0.178	0.758	0.041	3140	0.000*	0.003*	0.001*	198	0.000*	0.002*	0.000*	16	0.001*	0.009*	0.008*
C16:0	0.046	0.098	0.143	3328	0.008*	0.244	0.075	172	0.009*	0.245	0.082	14	0.011*	0.203	0.081
C18:0	0.062	0.006	0.015*	3622	0.001	0.000*	0.033	170	0.001	0.000	0.036	9	0.000	0.001	0.018
C18:1 *trans*6-9-11	0.039*	0.000*	0.110*	3593	0.064	0.003*	0.013*	132	0.064	0.003*	0.014*	14	0.076	0.002*	0.011*
C18:1*cis*9	0.049*	0.039*	0.264*	2405	0.005	0.000	0.182	201	0.002	0.000	0.255	13	0.000	0.001	0.285

C18:2*cis*9-12	0.004*	0.005*	0.005	2552	0.011*	0.019*	0.035*	80	0.034*	0.030*	0.042*	8	0.006*	0.024*	0.026*			
C18:2 *cis*9-*trans*11	0.003*	0.034*	0.011	2707	0.000*	0.002*	0.065*	46	0.005*	0.004*	0.113*	0	0	0	0			

[1] Reliabilities, defined as $r^2_{\hat{g}\hat{g}}$, of the genomic estimates breeding values (GEBV).

[2] GEBV of cows of Dutch native dual purpose breed origin were estimated using SNP effects estimated in an Dutch Holstein Friesian population.

[3] Number of SNPs used to estimate the GEBVs depends on if the SNPs are segregating in the breeds MRY, GWH or DF.

[4] MRY = Meuse-Rhine-Yssel, GWH = Groningen White Headed, and DF = Dutch Friesian.

* The underlying correlation ($r_{y\hat{g}}$), between the predicted breeding values ($\hat{g}$) and the phenotypes (y), was negative.

Correlations of allele frequencies

To assess similarities between the breeds across the whole genome, versus in regions associated with FA composition, the correlations of allele frequencies between the breeds MRY, GWH, DF, and HF were calculated (Table 6.4). Across all 350,207 SNPs, this correlation was between 0.64 (GWH&DF) and 0.71 (DF&HF and MRY&HF) (Table 6.4). When including per trait only SNPs with a Bayes Factor above one of the thresholds, across all traits the correlations ranged from 0.63 (GWH & DF) to 0.70 (DF&HF and MRY&HF) for Bayes Factors > 10; from 0.68 (GWH&DF) to 0.72 (DF&HF; MRY&HF; and MRY&DF) for Bayes Factors > 100; and from 0.50 (MRY&HF) to 0.83 (MRY&DF) for Bayes Factors > 1000. For the FA C18:1*trans*6,9,11 the correlations of the allele frequencies between the breeds were relatively high (on average 0.80) when including SNPs with a Bayes Factor > 100, however, the reliabilities of the GEBV for this trait were low. A similar trend was found for the FA C14:1*cis*9 showing allele frequencies of on average 0.79 when including SNPs with a Bayes Factor > 1000. When including SNPs with a threshold based on the Bayes Factor > 1000 it was remarkable that the correlations of the allele frequencies between HF and the other Dutch breeds were relatively low (on average 0.50-0.53), while between the other Dutch breeds MRY, DF, and GWH, the correlation was relatively high (on average 0.80-0.83). In summary, both the GEBV reliabilities and the correlations between allele frequencies clearly showed differences between the breeds, albeit that there was no consistent relationship between both statistics.

Table 6.4 The correlations of allele frequencies between breeds[1] across all SNPs, or based only on SNPs with a Bayes Factor > 1000.

	#SNPs[2]	HF &MRY	HF &DF	HF &GWH	MRY &DF	MRY &GWH	DF &GWH
Trait							
All	350,207	0.71	0.71	0.66	0.68	0.65	0.64
Production traits[3]							
Fat%	14	0.67	0.50	0.66	0.88	0.88	0.87
Protein%	16	0.87	0.88	0.92	0.84	0.84	0.92
Traits in g/dL milk[3]							
C4:0-C10:0	13	-0.21	0.29	0.44	0.79	0.63	0.94
C12:1	15	0.62	0.22	0.22	0.83	0.86	0.90
C14:0	22	0.40	0.57	0.41	0.65	0.62	0.76
C14:1*cis*9	16	0.63	0.81	0.64	0.91	0.91	0.84
C16:0	14	0.49	0.36	0.41	0.76	0.88	0.85

C18:0	9	0.34	0.61	0.54	0.74	0.83	0.55
C18:1*trans*6-9-11	14	0.39	0.42	0.54	0.90	0.94	0.89
C18:1*cis*9	13	0.63	0.40	0.54	0.87	0.59	0.54
C18:2*cis*9-12	8	0.60	0.71	0.46	0.97	0.82	0.71
C18:2*cis*9-*trans*11	-	-	-	-	-	-	-
average correlation (BF>1000)		0.50	0.52	0.53	0.83	0.80	0.80

[1] MRY = Meuse-Rhine-Yssel, GWH = Groningen White Headed, and DF = Dutch Friesian.
[2] Number of SNPs used to estimate the EBVs depends on if the SNPs are segregating in the breeds MRY, GWH or DF.
[3] For these traits the correlations between breeds are given of allele frequencies of SNPs with an effect above the thresholds of a Bayes Factor of 1000.

SNP variances

Table 6.5 shows the variances explained per SNP for the SNPs whose variance in at least one of the breeds was ≥ 1% relative to the total genetic variation in the HF breed. The region of *DGAT1* on *Bos Taurus* autosome (BTA) 14 was found having the largest effect on most of the traits. This region explained large parts of the genetic variation in HF for the traits fat%, protein%, C4:0-C10:0, C12:1, C14:0, C14:1*cis*9, C16:0, C18:0, and C18:2*cis*9_12 ranging from 1.1% (C18:2cis9_12) to 19.0% (C16:0). For the breeds GWH and DF the region of *DGAT*1 was also found having an effect for a number of traits (ranging from 0.2% for C18:2cis9_12 to 3.3% for C16:0 for GWH and from 0.0% for 6 traits to 2.2% for C16:0 for DF), although heavily reduced in comparison with HF. Almost no effect of the genomic region on BTA14 was found for the traits in MRY. On BTA 26 a genomic region was found having an effect on the genetic variation of the traits C12:1 and C14:1*cis*9 for all breeds, which was largest for HF and DF (explaining 2.8% of C12:1 in HF milk; 8.0% of C12:1 in DF; 2.6% of C14:1*cis*9 in HF; 7.4% of C14:1*cis*9 in DF of the genetic variation for the whole region, sum of both SNPs, respectively). For GWH this region explained 1.8% of the variation of the trait C12:1 and 5.1% of C14:1*cis*9 and for MRY 1.5% of C12:1 and 4.2% of C14:1*cis*9. On BTA 5 one SNP was found with an effect on the genetic variation of the traits fat%, C4:0-C10:0, C12:1, C14:0, and C16:0, this effect was estimated to explain between 1-2 % of the genetic variance for HF, between 0.3-0.6% for DF and MRY, and almost negligible for GWH. On BTA 6 one SNP was found having an effect on the genetic variation of protein% between 2-3 % for HF, DF, and MRY and 0.7% for GWH.

Table 6.5 The variances explained per SNP with position on the genome for the SNPs which explained ≥ 1 percent of the genetic variation in at least one of the breeds.

Trait	SNP name	BTA[1]	Position (bp)	SNP var[2]	HF[3,4]	DF[3,4]	GWH[3,4]	MRY[3,4]
Fat%	ARS-BFGL-NGS-4939	14	1801116	0.48567	13.3	1.5	2.3	0.0
Fat%	BovineHD1400000206	14	1679844	0.04544	1.2	0.0	0.3	0.2
Protein%	ARS-BFGL-NGS-4939	14	1801116	0.14934	2.8	0.3	0.5	0.0
Protein%	BovineHD1400000204	14	1667797	0.15778	3.0	0.0	0.7	0.5
C4:0-C10:0	ARS-BFGL-NGS-4939	14	1801116	0.06546	13.5	1.5	2.4	0.0
C4:0-C10:0	BovineHD1400000206	14	1679844	0.00654	1.3	0.0	0.3	0.2
C12:1	ARS-BFGL-NGS-4939	14	1801116	0.00082	10.8	1.2	1.9	0.0
C14:0	ARS-BFGL-NGS-4939	14	1801116	0.04398	9.0	1.0	1.6	0.0
C14:1*cis*9	ARS-BFGL-NGS-4939	14	1801116	0.00399	3.4	0.4	0.6	0.0
C14:1*cis*9	BovineHD1400000206	14	1679844	0.00451	3.8	0.0	0.9	0.6
C14:1*cis*9	BovineHD1400000199	14	1638045	0.00192	1.6	0.2	0.9	0.3
C16:0	ARS-BFGL-NGS-4939	14	1801116	0.99652	19.0	2.2	3.3	0.0
C18:0	ARS-BFGL-NGS-4939	14	1801116	0.03338	7.5	0.9	1.3	0.0
C18:0	BovineHD1400000206	14	1679844	0.00751	1.7	0.0	0.4	0.3
C18:0	BovineHD1400000204	14	1667797	0.00586	1.3	0.0	0.3	0.2
C18:2*cis*9-12	ARS-BFGL-NGS-4939	14	1801116	0.00030	1.1	0.1	0.2	0.0

C12:1	BovineHD2600005462[5]	26	21141280	0.00026	2.8	2.6	1.8	1.4
C14:1*cis*9	BovineHD2600005462[5]	26	21141208	0.01150	8.0	7.3	5.1	4.1
Fat%	BovineHD0500026666	5	93951064	0.05577	1.5	0.4	0.2	0.6
C4:0-C10:0	BovineHD0500026666	5	93951064	0.00761	1.5	0.4	0.2	0.6
C12:1	BovineHD0500026666	5	93951064	0.00010	1.3	0.4	0.2	0.6
C14:0	BovineHD0500026666	5	93951064	0.00540	1.1	0.3	0.2	0.4
C16:0	BovineHD0500026666	5	93951064	0.06288	1.2	0.3	0.2	0.5
Protein%	BovineHD0600023925	6	87416816	0.15468	2.5	2.6	0.7	2.4

[1] Bos taurus autosome (BTA) number where SNP is located.

[2] The genetic variation explained by the SNP.

[3] HF = Holstein Friesian, MRY = Meuse-Rhine-Yssel, GWH = Groningen White Headed, and DF = Dutch Friesian.

[4] The percentage of the total genetic variation explained by the SNP for each breed, i.e. $(2pq\hat{\alpha}^2)$ / var(a), where var(a) is the variance estimated for HF.

[5] SNP was in complete linkage disequilibrium with the SNP's located on the base pair positions 21140458 and 21146794, thus published SNP effect is the combined effect of the whole region in and around the SCD1 gene.

6.4 Discussion

The aim of this study was to identify similarities in genomic variation associated with detailed milk fat composition between the HF breed and native dual purpose breeds in the Dutch dairy industry. This was achieved by calculating GEBV for fat composition in the native dual purpose breeds using SNP effects estimated for HF. The extent to which the genetic variation associated with milk fat composition in HF was different from the native dual purpose breeds was evaluated by calculating the reliabilities of the predicted GEBV for the native dual purpose breeds. Reliabilities of GEBV across breeds in general are reported to be low because of lower levels of linkage disequilibrium compared to within breeds (LD) (Goddard and Hayes, 2009, Hayes et al., 2009, Hozé et al., 2014). The high-density SNP-chip (HD chip; Illumina Inc., San Diego, CA), also used in this study, is characterized by short-distance LD that is expected to be maintained across breeds (de Roos et al., 2008). The reliability of the GEBV of an animal in general is expected to be higher when this animal is more related to the reference population used to estimate SNP effects (Habier et al., 2010, Pszczola et al., 2012). Nevertheless, the GEBV reliabilities based on across-breed genomic prediction are expected to be higher for traits with few QTL with large effects, such as FA composition. Thus when the genomic variation associated with detailed milk fat composition in HF was very similar to the genomic variation of the native dual purpose breeds, then their GEBV reliabilities were expected to be high, whereas for traits where the genomic variation in HF was very different compared to the other breeds their GEBV reliabilities were expected to be low. To get a better indication of the impact of individual SNPs that have an increasingly larger effect on the traits in HF, GEBV were calculated using different subsets of SNPs based on their Bayes Factors. The highest Bayes Factors used in this study were >1000 which resulted in a subset of SNPs which are very likely to have a strong association with the milk fat composition in HF. The reliabilities of GEBV for the milk production and milk fat composition traits in this study were ranging from 0.000 to 0.758, however, reliabilities for the majority of traits were just above zero indicating large differences in genomic variation associated with detailed milk fat composition between HF and native Dutch dual purpose breeds. Comparing the native Dutch dual purpose breeds, the GEBV calculated for the MRY breed were clearly least reliable while for GWH it was on average most reliable, indicating that the genetic variation associated with milk fat composition of MRY differed most from HF and differed least of GWH compared to HF.

SNP effects

The SNP effects used for calculating the GEBV in Dutch native dual purpose breeds in this study were estimated using genotype and phenotype data of a Dutch HF population. A number of SNPs and genomic regions were identified to have an effect on the milk FA composition in HF. Using increasingly higher thresholds for the Bayes Factors of the included SNPs, regions or SNPs were selected which are more likely to be associated with a QTL affecting the trait.

In this study, 4 regions were identified having most pronounced effect on the FA composition and showing variances explained per SNP, in at least one of the breeds, which are ≥ 1% relative to the total genetic variation in the HF breed per trait. Largest effect was found for a region on BTA 14, which includes also a SNP in the *DGAT*1 gene, for fat%, a number of individual FA and the group of FA C4:0-C10:0. Several genome-wide association studies (GWAS) and QTL mapping studies based on the HF breed report clear associations of the region of *DGAT*1 on BTA 14 with fat% and FA composition in the milk of HF cows (e.g. Bouwman et al., 2011, Grisart et al., 2002). BTA 26 harbors the *SCD*1 gene, which is also reported having an effect on the milk FA composition (e.g. Bouwman et al., 2011, Mele et al., 2007, Schennink et al., 2008), this region is also identified having an effect on the variability of the content of the FA C12:1 and C14:1*cis*9 in current study. A third region with clear effect on fat%, the group of FA C4:0-C10:0 and some individual FA was found on BTA 5, where a peak on the Manhattan plot was shown at a SNP at base pair position 93951064. This position on BTA 5 is close to the Microsomal glatathione S-transferase 1 (*MGST*1) gene which was found by Wang et al. (2012) to be associated with milk fat content. The fourth region with an effect explaining ≥ 1% relative to the total genetic variation in the HF breed was found for the trait protein% in milk on BTA 6. This position on BTA 5 is close to the k-casein (CSN3) gene which is described in several studies to be associated with protein content in milk (Bonfatti et al., 2010, Hallén et al., 2008).

GEBV, allele frequencies and haplotypes

Obtaining the results in our study, it is hypothesized that the allele substitution effects estimated in HF can also be used for the other breeds. As for a number of traits in the other breeds the reliability is clearly above '0', while only a small number of SNPs are used with high Bayes Factors, this hypothesis is likely to be valid at least in those cases. Between the allele frequencies of HF and the other breeds and the reliabilities of the estimated GEBV no relation was observed, except for the SNPs in the *DGAT*1 region where for several traits both, the reliabilities of

Table 6.6a Observed haplotypes in all different breeds for three different SNPs.

		BTA number[3] and SNP name		
		BTA 5, BovineHD 0500026666	BTA 6, BovineHD 0600023925	BTA 26, BovineHD 2600005462
Breed[1]	Genotype[2]	n	n	n
DF	0	63	31	38
DF	1	7	32	26
DF	2	0	7	6
GWH	0	108	94	79
GWH	1	5	17	29
GWH	2	0	2	5
HF	0	188	720	856
HF	1	768	705	590
HF	2	588	119	98
MRY	0	123	14	119
MRY	1	20	58	25
MRY	2	1	72	0

[1] DF = Dutch Friesian, GWH = Groningen White Headed, HF = Holstein Friesian and MRY = Meuse-Rhine-Yssel.

[2] For each SNP two polymorphisms could be observed. Genotype '0' is homozygote for the minor allele; genotype '1' is heterozygote; genotype '2' is homozygote for the major allele.

[3] Bos taurus autosome (BTA) number where SNP is located.

GEBV were clearly above '0' and the SNP was estimated to explain ≥ 1 percent of the genetic variation. One explanation for the reliabilities near '0', especially for the MRY breed, could be low allele frequencies of the causal mutations as suggested by Kemper and Goddard (2012). When investigating the region on BTA 26, however, complete LD was identified between a number of markers over all breeds. When counting the genotypes of the SNPs on BTA 5, 6, and 26 discussed before (Table 6.6a) and the haplotypes on the region of *DGAT*1 on BTA 14 (Table 6.6b) the variation in genotypes and haplotypes is clearly smaller in MRY, GWH, and DF compared to HF. Moreover, the variances explained by the haplotypes are estimated and it is notable that the total variance explained by the haplotypes present in MRY is smallest (Table 6.6c). It could be reasonable that because the regions, which have a relative large influence on the fat %, FA composition, and protein %, in the Dutch HF breed show less genomic variation in the other breeds, reliabilities for a number of traits become near to '0'. It was earlier found by Maurice-Van Eijndhoven et al. (submitted) that the heritabilities for milk FA composition of the MRY breed were clearly smaller than those estimated for HF. A reasonable explanation for these results could be the small variation in genotypes and haplotypes as shown in Table 6.6a and 6.6b.

Table 6.6b Observed haplotypes in all different breeds for SNP's in the area of the *DGAT*1 gene on *Bos Taurus* autosome 14.

Haplotype number[1]	Breed[1]	Haplotype[2]				n
		SNP 1	SNP 2	SNP 3	SNP 4	
1	**DF**	0	0	2	0	66
2	**DF**	1	0	2	1	4
3	**GWH**	0	0	2	0	58
4	**GWH**	1	0	2	0	39
5	**GWH**	2	0	2	0	2
6	**GWH**	1	1	1	0	2
7	**GWH**	2	1	1	0	2
8	**GWH**	1	1	1	1	9
9	**GWH**	2	1	1	1	1
10	**HF**	0	0	2	0	552
11	**HF**	1	0	2	0	2
12	**HF**	1	1	1	0	6
13	**HF**	0	0	2	1	3
14	**HF**	0	1	1	1	1
15	**HF**	1	1	1	1	719
16	**HF**	2	1	1	1	3
17	**HF**	2	2	0	1	3
18	**HF**	1	1	1	2	2
19	**HF**	2	2	0	2	253
20	**MRY**	0	0	2	0	135
21	**MRY**	1	1	1	0	7
22	**MRY**	1	0	2	1	1
23	**MRY**	1	1	1	1	1

[1] DF = Dutch Friesian, GWH = Groningen White Headed, HF = Holstein Friesian and MRY = Meuse-Rhine-Yssel.

[2] Official SNP names: SNP 1 = BovineHD1400000199; SNP 2 = BovineHD1400000204; SNP 3 = BovineHD1400000206; SNP 4 = ARS-BFGL-NGS-4939 and for each SNP two polymorphisms could be observed: '0' is homozygote for the minor allele; '1' is heterozygote; '2' is homozygote for the major allele.

Table 6.6c Numbers of the observed haplotypes for SNP's in the area of the *DGAT*1 gene on *Bos Taurus* autosome 14 with explained haplotype variances.

Haplotype number[1,2]	"Haplotype" variances per trait[3]								
	Fat%	Protein%	C4:0-C10:0	C12:1	C14:0	C14:1cis9	C16:0	C18:0	C18:2 cis9-12
1	1.13E-03	1.95E-05	1.47E-05	1.91E-09	1.12E-05	8.14E-08	2.71E-04	6.35E-06	3.93E-09
	8.3	2.4	8.1	8.4	10.5	3.3	10.7	5.7	3.4
3	2.26E-03	1.46E-04	3.02E-05	3.72E-09	1.78E-05	4.67E-07	4.14E-04	1.87E-05	2.06E-08
	16.5	18.0	16.6	16.2	16.6	18.7	16.4	16.8	17.7
10	1.36E-02	8.13E-04	1.81E-04	2.29E-08	1.07E-04	2.49E-06	2.52E-03	1.11E-04	1.17E-07
	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
20	3.60E-04	3.81E-05	4.75E-06	6.41E-10	2.93E-06	1.39E-07	6.99E-05	3.25E-06	5.44E-09
	2.6	4.7	2.6	2.8	2.7	5.6	2.8	2.9	4.7

[1] The haplotypes can be seen in Table 6.6b.

[2] The haplotypes are sorted on their effect on fat% per breed, with an increasingly effect from the upper to the lowest haplotype mentioned per breed.

[3] The variances estimated per breed for the whole genomic area, thus over all different haplotypes. And for each trait, per breed: on the first row the absolute estimated variance over all haplotypes and on the second row the variance expressed as percentage of the variance estimated for HF.

6.5 Conclusion

Estimated SNP effects for FA composition based on HF data had low to no predictive ability in MRY, while they did have substantial predictive ability for several traits for DF and GWH. This indicates that SNP effects estimated in HF does explain less of the genetic variation of the FA composition in milk of MRY compared to the genetic variation of the milk fat composition in GWH and DF. The frequencies of the alleles having an effect on milk FA composition in HF are more extreme (close to 0 and 1) in the breeds MRY, GWH and DF. The latter is most extreme for the MRY breed. In summary, the similarities in genomic variation associated with detailed milk fat composition between the HF breed and the native Dutch dual purpose breeds are greatest in GWH and substantially lowest for MRY.

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7

General discussion

The aim of this thesis was to investigate the variability of detailed milk fatty acid (FA) composition between and within different dairy cattle breeds, including the mainstream Holstein Friesian (HF) and Jersey (JER), and the native dual purpose breeds Meuse-Rhine-Yssel (MRY), Groningen White Headed (GWH) and Dutch Friesian (DF) in the Netherlands. In Chapter 2, differences in FA composition were found among herds using different cattle breeds in the Netherlands, based on detailed milk FA composition measurements using gas chromatography (GC). In Chapter 3, mid-infrared (MIR) spectrometry was evaluated to be an accurate method for predicting FA composition in different breeds. In a large dataset that included MIR spectra of milk from cows from a range of farms using one or more breeds, in general, only minor breed differences in FA composition were found (Chapter 4) and HF showed more genetic variation in FA composition compared to MRY (Chapter 5). In Chapter 6, some similarities in genomic variation associated with detailed milk FA composition were found between the mainstream HF and the native dual purpose breeds MRY, GWH and DF. Furthermore, it was found that the frequencies of the alleles having an effect on milk FA composition in HF were more extreme (close to 0 or 1) in the breeds MRY, GWH and DF than in HF (Chapter 6).

In this final chapter, first, the genetic variability in cattle associated with milk fat composition is discussed. A short overview of what is currently known will be given, which is mostly based on cattle belonging to one of the globally mainstream breeds HF and JER. Following on, the milk fat composition investigated in this thesis for different cattle breeds in the Netherlands is put into perspective. As *DGAT*1 is a gene known for having a significant effect on milk fat composition, the region on the genome and FA composition in relation with this gene are evaluated. This thesis combines two fields of interest: i) modification of milk fat composition by breeding; ii) conservation of native cattle breeds. As such, in the second part of the discussion, the future perspectives of numerically small cattle breeds in the Dutch dairy sector are discussed. In this final section, attention is paid to the diversification within the use of numerically small cattle breeds in the Netherlands, as well as the diversity among farmers using these different cattle breeds.

7.1 Genetic variability in cattle associated with milk fat composition

In this section, a short overview will first be given of the current status of the existing knowledge on the variation of detailed FA composition among dairy cows. Phenotypic differences of fat percentage and detailed FA composition in bovine milk have been described in numerous studies (e.g., Grummer, 1991, Palmquist et al., 1993). Detailed milk fat composition is influenced by environmental factors such as cows' diet (e.g., Bauman and Griinari, 2003, Palmquist et al., 1993) and by genetic factors (e.g., Mele et al., 2009, Stoop et al., 2008). For breeding purposes and also for safeguarding existing variations in milk fat composition, it is important to know whether phenotypic differences between cows are based on underlying genetic differences. Heritabilities for individual FA are reported to range from low to moderate (up to 0.50), where low heritabilities are mostly found for long-chain unsaturated FA (UFA), with moderate heritabilities mainly found for short- and medium-chain saturated FA (SFA) (e.g., Chapter 5, Soyeurt et al., 2007, Stoop et al., 2008).

In research, increasing attention is being paid to unravelling the underlying genetic background of detailed FA composition. Associations between trait variations and variations among genes provides information on the genetic background of the trait, which can be used for genomic selection in the desirable direction. Selection based on genomic information can be more efficient, as it reduces generational intervals. In several mapping studies and genome wide association studies (GWAS), quantitative trait loci (QTL) have been found for different milk FAs on specific locations or regions on the bovine genome. On the Bos Taurus autosome (BTA) 5, BTA 14, BTA 19 and BTA 26 regions are present and have a major effect on milk FA composition (e.g., Chapter 6, Bouwman et al., 2014, Morris et al., 2007, Stoop et al., 2009). Based on the estimated heritabilities and the evidence that individual QTL are found, it can be concluded that differences in FA composition between cows are at least partly genetically determined; as such, it is therefore possible to modify milk FA composition by selection and breeding.

7.1.1 Differences between breeds

The majority of studies investigating genetic variation in the production of different individual FAs in milk between cows have focussed on mainstream breeds like HF and JER. As more cattle breeds continue to be used for milk production, it is of interest to investigate whether these other breeds, with relatively small population sizes, comprise unique characteristics in terms of FA composition in

milk. In this thesis, three Dutch native dual purpose breeds were studied: MRY, DF and GWH. In Chapters 2 and 4, the breed averages for a number of individual FAs in milk produced by these breeds were studied. Chapter 2 analysed FA composition according to fat source (g/100g fat), while Chapter 4 analysed FA composition according to milk source (g/dL milk). Although JER is neither a mainstream nor a native breed in the Netherlands, this breed was included in the research in Chapters 2, 3 and 4, as it is known from previous research conducted in other countries that the JER produces in general a higher fat percentage and FA composition, which clearly differs from HF (e.g., Beaulieu and Palmquist, 1995, Palladino et al., 2010, White et al., 2001). Both HF and JER are especially bred for milk production, though they clearly differ in terms of their production of FAs in milk. Considering that differences exist between specialized dairy breeds, it might be expected that milk produced by dual purpose breeds, i.e., breeds that are kept to produce both milk and meat, might differ in terms of detailed FA composition among one another and compared to the specialized dairy breeds HF and JER. Therefore, in Chapter 2, cows were sampled on 12 farms using a single breed only (MRY, FH, GWH or JER), while Chapter 4 employed data from multiple farms using one or more breeds. Considering the results of Chapters 2 and 4 together, however, no significant differences among levels of FA composition were found between DF and MRY or between DF and MRY on one side and HF on the other (Chapter 4). Figure 7.1 shows the produced levels of SFA and UFA of the breeds DF, MRY, GWH, HF and JER, studied in Chapters 2 and 4 of this thesis. A similar trend in production among the groups of SFA and UFA can be observed when comparing the produced levels of SFA and UFA to the different breeds MRY, DF, GWH and JER (i.e., GWH produced the least SFA and most UFA, followed by MRY and DF and JER producing the most SFA and least UFA). Only a few of the observed differences were significant; these are mainly discussed in Chapter 2 and reports the results of the analyses of farms using a single breed. For GWH, some significant differences in levels of FA composition were found compared to MRY, DF and JER (Chapter 2). On farms using GWH, relatively higher proportions of UFA and lower SFA were reported; in particular, the proportion of the individual FA C16:0 was found to be lower. When evaluating differences between breeds and correcting for herd effects rather than differences between herds with different breeds, it can be concluded that there are no or only minor differences between the mainstream HF and some native dual purpose breeds where FA composition in milk is concerned.

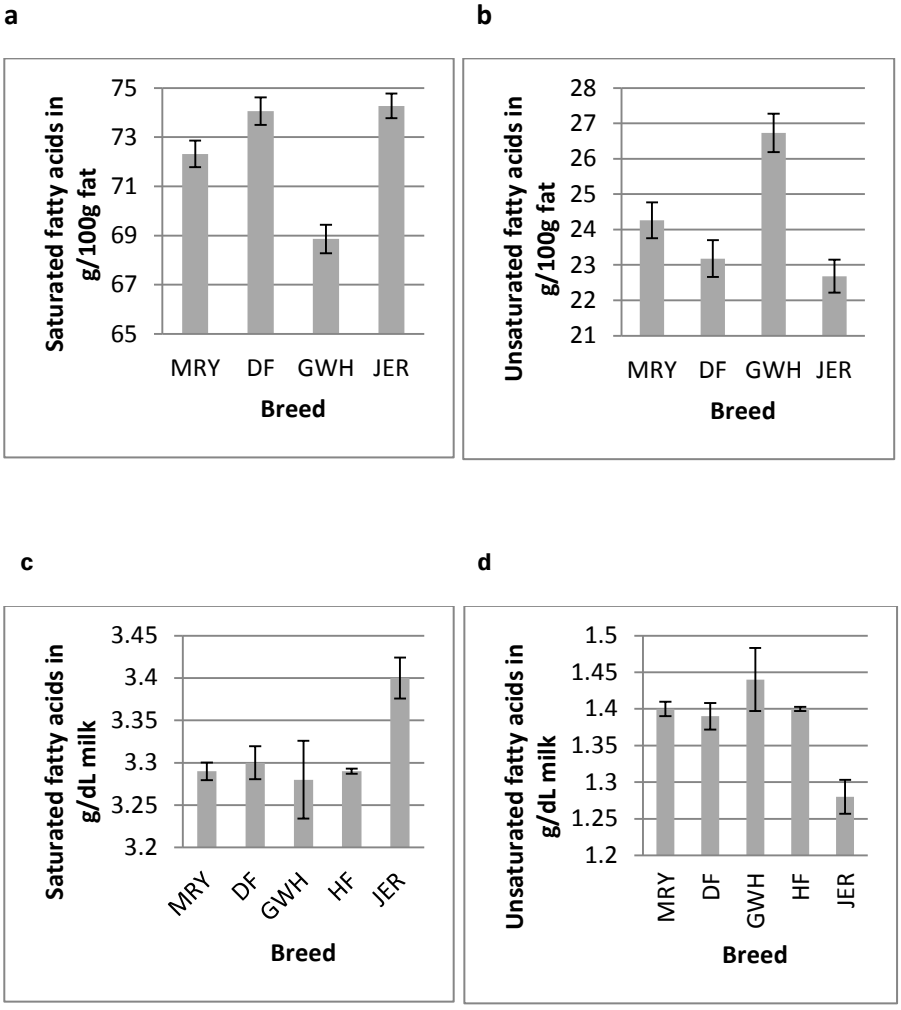


Figure 7.1 For each breed the predicted LS-means in g/100g fat for the groups (**a**) saturated fatty acids and (**b**) unsaturated fatty acids (both described in Chapter 2), as well as the predictions in g/dL milk for the groups (**c**) saturated fatty acids and (**d**) unsaturated fatty acids from a model with adjustment for fat percentage (described in Chapter 4); bars indicate the standard error.

7.1.2 Genetic and genomic differences between and within breeds

To examine the possibilities for modifying detailed FA composition by selection and breeding, the genetic variability of milk FA composition in different native dual purpose breeds was studied. In Chapter 5, the within-breed variance for detailed FA composition (using milk FA composition data in g/dL milk) was investigated for HF and MRY using phenotypic and pedigree data, which gave the remarkable result that the genetic variation and heritability in MRY was considerably lower than in HF. In terms of animal breeding, this means that within the MRY breed, the expected selection response will be lower, even when the same selection intensity would be possible. Therefore, there is less potential for selection concerning detailed FA content in the MRY breed compared to HF.

The relation between the bovine genome and variations in FA composition may provide insight as to why MRY has lower genetic variation for FA composition compared to HF. Therefore, in Chapter 6, phenotypic and genotypic information was used to identify similarities in genomic variation associated with detailed milk FA composition for cows using the three Dutch native dual purpose breeds MRY, DF and GWH, as well as a relatively large number (1544) of HF cows. The high-density SNP-chip (HD chip; Illumina Inc., San Diego, CA) containing almost 800k SNPs was used, as it is expected to capture short-distance LD, which is expected to be maintained across breeds (de Roos et al., 2008). This increased the probably for finding a SNP in linking disequilibrium (LD) with a causal mutation across breeds, compared to using the commonly employed 50k SNP chip.

The gene diacylglycerol acyltransferase 1 (*DGAT*1) on BTA 14 is known to have a large effect on milk fat composition. When zooming in on this gene's region on BTA 14, four SNPs within a 163071 bp region (base pair 1 638 045 up to 1 801 116, which is a region prior to and up to the start of the *DGAT*1 gene) were identified in this thesis as having an effect on detailed milk FA composition in HF. Different combinations of genotypes were identified for these SNPs across the different breeds. Table 7.1 shows the different observed combinations of genotypes per breed, including the counts of number of cows observed for each combination of genotypes per breed. When taking into account the history of selection of the different breeds, it can be hypothesized that HF comprised less genetic variation related to milk traits compared to other breeds. This hypothesis is based on the fact that the HF breed has undergone more intensive selection for milk production traits related to milk FA composition. The within-breed variances of HF and MRY, however, show the opposite estimated genetic variances, as the individual milk FA

composition for MRY was clearly lower than for HF. On the other hand, as the native Dutch dual purpose breeds account for relatively small numbers (maximum 1% per breed of all cows in the national milk recording), it can also be hypothesized that inbreeding or drift has reduced the genetic variation in these breeds. The latter proposition can explain the lower genetic variation in MRY compared to HF, as described above. However, this hypothesis also seems unlikely, because the same genotype based on 4 SNPs (number 3 in Table 7.1) is the major genotype in all three breeds (MRY, DF and GWH). Studying the genotypes in the region of *DGAT*1 in more detail provided an alternative explanation for the lower variability in MRY compared to HF: most different genotypes were identified for HF (10), followed by GWH (7) and MRY (4), while for DF, only two different genotypes were identified (Table 7.1). Taking into account the number of occurrences, the HF breed counted three main genotypes, GWH contained two and MRY and DF both contained only one. In total, three genotypes were found in the native Dutch dual purpose breeds that were not found in HF; of these genotypes, two (genotype number 2 and 5; see Table 7.1) were present in GWH, while one was found in DF and MRY (genotype number 11; see Table 7.1). However, these genotypes were also in those breeds present in low frequencies (maximum 6%). Thus, when comparing the native Dutch dual purpose breeds, GWH showed the most genomic variation in the investigated region of *DGAT*1, but overall, variability was highest in HF; for HF and MRY, this finding was in agreement with the estimates for the within-breed genetic variations found in Chapter 5. The fact that the largest variation in genotypes in the DGAT1 region was found for GWH could be the reason for the genomic estimated breeding values (GEBV) for GWH (using SNP effects) estimated from HF data were comparatively most accurate (Chapter 6). Nevertheless, this does not mean that GWH is most similar to HF, as the frequencies of the genotypes were very different, as can be seen in Table 7.1.

Table 7.1 Observed genotypes in all different breeds for SNPs in a region on the *Bos Taurus* autosome 14, with counts per breed and the *DGAT*1 genotype in Holstein Friesian.

Genotype[2]					Counts per breed[1]				DGAT1 genotype in HF		
Genotype number	**SNP1**	**SNP2**	**SNP3**	**SNP4**	**HF**	**MRY**	**DF**	**GWH**	**AA**	**AK**	**KK**
1	1	1	1	0	6	7	0	2	6		
2	2	1	1	0	0	0	0	2			
3	0	0	2	0	552	135	66	58	549	3	
4	1	0	2	0	2	0	0	39	2		
5	2	0	2	0	0	0	0	2			
6	2	2	0	1	3	0	0	0		3	
7	0	1	1	1	1	0	0	0		1	
8	1	1	1	1	719	1	0	9	7	709	3
9	2	1	1	1	3	0	0	1		3	
10	0	0	2	1	3	0	0	0	1	2	
11	1	0	2	1	0	1	4	0			
12	2	2	0	2	253	0	0	0	3		250
13	1	1	1	2	2	0	0	0		2	
				total	**1544**	**144**	**70**	**113**			

[1] DF = Dutch Friesian; GWH = Groningen White Headed; HF = Holstein Friesian; MRY = Meuse-Rhine-Yssel.

[2] Official SNP names: SNP 1 = BovineHD1400000199; SNP 2 = BovineHD1400000204; SNP 3 = BovineHD1400000206; SNP 4 = ARS-BFGL-NGS-4939. For each SNP, '0' is homozygote for the minor allele; '1' is heterozygote; '2' is homozygote for the major allele.

[3] The genotype of the *DGAT*1 K232A polymorphism for all HF cows in combination with the genotypes of the 4 SNPs.

An interesting question for further exploring knowledge regarding the genetic differences between breeds in FA composition is whether the frequencies of the causal mutation in the *DGAT*1 gene itself, i.e., the A232K allele, differ between breeds. For all HF cows, in addition to the genotypes in the area of *DGAT*1, the *DGAT*1 A232K genotypes were also known (*DGAT*1 A232K genotypes were obtained from the Dutch Milk Genomic Initiative; the genotypes are shown in Table 7.1). For native breeds, the *DGAT*1 A232K genotypes were not known; however, the four SNPs in the region at the start of the *DGAT*1 gene were available. These four SNPs were used to infer the *DGAT*1 A232K genotypes in the other breeds. Based on the data on HF (Table 7.1), it can be concluded that the region from the start of the *DGAT*1 gene (SNP called ARS-BFGL-NGS-4939) up to the A232K polymorphism is almost in complete LD within HF, which can also be assumed for the breeds MRY, DF and GWH. Based on this assumption, it can be concluded that in these breeds, the *DGAT*1 K-allele has a very low allele frequency. In particular, cows from the breeds MRY and DF included mainly (94% in both breeds) the *DGAT*1 genotype AA. Additionally, for the GWH cows, this was the major genotype (51%) (Table 7.2). Thus, lower variability within the native dual purpose breeds is likely and at least partly the result of lower allelic variance at the *DGAT*1 A232K locus.

A logical next question is whether the genetic composition of the different breeds not only causes lower variability within the MRY breed, but can also explain the between-breed differences. According to the *DGAT*1 A232K effects in HF reported by Schennink et al. (2007), it might be expected that on average, the fat percentage in the native dual purpose breeds is lower compared to HF, as the *DGAT*1 KK genotype in HF is associated with a higher fat percentage. This expectation is based on estimations of the different *DGAT*1 genotypes in HF where the AA genotype in HF cows yields a 0.68% (Table 7.2, this thesis) to 0.98% (Schennink et al. 2007) lower milk fat percentage, compared to the KK genotype. Between breeds, including HF and the native dual purpose breeds, hardly any difference for fat percentage was found, despite the differences in frequencies of the DGAT1 A232K genotypes. Thus, it can be concluded that the *DGAT*1 A232K allele does not determine the level of fat percentage or FA composition in milk among different breeds. The *DGAT*1 AA genotype is, according to Schennink et al. (2007), also associated with a higher milk yield in HF (increasing effect of 1.46 kg milk). Comparing HF with the breeds DF, MRY and GWH, no increasing effect of the *DGAT*1 genotype AA was observed (see also the results for the average milk yield according to breed in Chapter 4); in fact, the opposite was observed, as HF produced a clearly higher milk yield than other native Dutch dual purpose breeds.

Table 7.2 The effect the *DGAT*1 K232A polymorphisms on fat percentage estimations, suggested genotypes and frequencies among different breeds.

		DGAT1 genotypes			
		AA	**KA**	**KK**	**Total[7]**
Estimated effects	**Schennink et al. 2007[1]**	-0.53	0	0.45	
	This thesis[2]	-0.34	0	0.34	
(Suggested) genotypes[3,4]	**SNP1**	0	1	2	
	SNP2	0	1	2	
	SNP3	2	1	0	
	SNP4	0	1	2	
(Suggested) frequencies[5,6]	**HF**	0.36	0.47	0.16	0.99
	DF	0.94	0	0	0.94
	GWH	0.51	0.08	0	0.59
	MRY	0.94	<0.01	0	0.94

[1] The effects of the *DGAT*1 genotypes reported by Schennink et al. (2007), which were in the analysis treated as fixed (class) effects.

[2] The genotype effects were estimated using the allele substitution effects estimated for the HF population and were estimated using a regression analyses.

[3] The genotypes that were assumed to be in linkage disequilibrium with the *DGAT*1 polymorphism, based on the frequencies in HF and in combination with the estimated effects on fat percentage.

[4] Official SNP names: SNP 1 = BovineHD1400000199; SNP 2 = BovineHD1400000204; SNP 3 = BovineHD1400000206; SNP 4 = ARS-BFGL-NGS-4939. For each SNP '0' is homozygote for the minor allele; '1' is heterozygote; '2' is homozygote for the major allele.

[5] Counts of the specific genotype divided by all genotyped animals of the breed of interest.

[6] HF = Holstein Friesian; DF = Dutch Friesian; GWH = Groningen White Headed; MRY = Meuse-Rhine-Yssel.

[7] Indicates the sum of the allele frequencies across the three main haplotypes. Deviations from 1.0 indicate the summed frequency of "minor" haplotypes.

Schennink et al. (2007) and De Roos et al. (2007) showed that *DGAT*1 A232K polymorphism has a major effect on the variability in many milk- production and milk fat-related traits in HF. Although the DGAT1 A232K polymorphism does affect the variability of milk fat composition (at least in HF), it does not explain the differences in the observed level of production among different breeds.

Selection for traits was expected to influence the allele frequencies of the underlying causal mutations. From this perspective, the overall economic effect of the *DGAT*1 A232K genotypes on the traits underlying the Dutch selection index INET (http://www.gesfokwaarden.eu/nl/fokwaarden/pdf/E_9.pdf, 2012) was evaluated. The INET formula (as derived in 2012) is as follows: INET = -0.03 * milk yield + 2.2 * fat yield + 5.0 * protein yield. Using estimates provided by Schennink et al. (2007), the *DGAT*1 genotype AA had an effect on milk yield of 1.46, on fat yield of -0.07 and on protein yield of 0.02 when setting the effect of the genotype KK to zero. The estimated effects using the HF data in Chapter 6 were about 2/3 in size compared to the estimates of Schennink et al. (2007) and similar to the estimates of De Roos et al. (2007), where a similar model was used for the estimation of effects. Following the INET formula and the estimated effects provided by Schennink et al. (2007), the economic effect of the different *DGAT*1 genotypes was calculated per lactation, assuming population genotype frequencies of 25% for AA, 50% for KA and 25% for KK. In this case, the use of a bull with the genotype AA had an average economic effect for milk production per produced cow of ~-15 Euro and the use of a bull with the genotype KK had an average economic effect for milk production of ~40 Euro. Since the data presented in Table 7.2 suggest that the native Dutch dual purpose breeds had a high frequency for the *DGAT*1 A-allele, it can be hypothesized that the common ancestors of these breeds (as well as HF) had a high frequency for the A-allele. The positive economic impact of the KK-genotype, considering its association with the INET index, may have caused an increase in the frequency of the K-allele in HF in recent history, due to selection towards the currently observed allele frequencies. In other countries, intermediate allele frequencies for the DGAT1 A232K allele have also been reported (Schennink et al. 2009) and although INET is the Dutch selection index, in other countries, breeding goals have been fairly comparable.

7.2 Perspectives for native and numerically small cattle breeds in the dairy sector

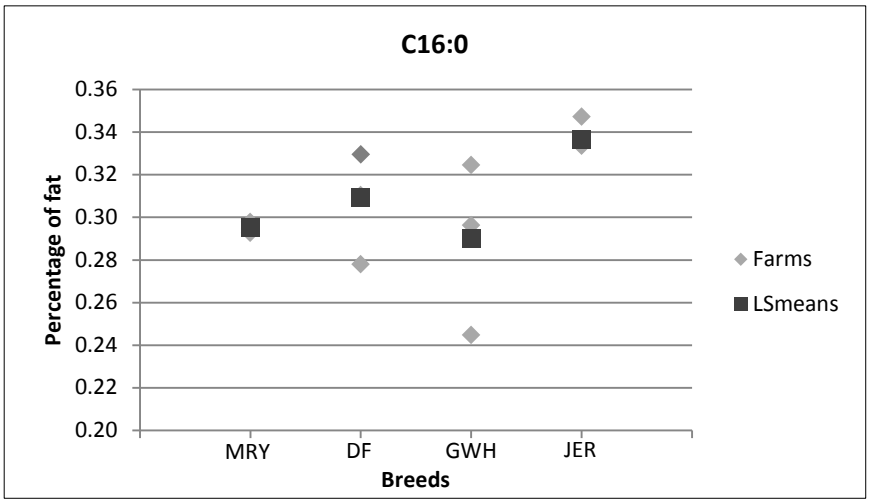
From the results in this thesis it can be concluded that FA composition is not significantly different between native Dutch dual purpose cattle breeds and mainstream dairy breeds. Based on these results, the genetic variation in the native and numerically small breeds is not essential for modifying milk FA production by selection and breeding. This does not mean, however, that these breeds comprise no unique genetic variability in general or present no added value to (dairy) cattle husbandry.

7.2.1 The diversity among farmers using different cattle breeds

Although the HF breed is generally well-suited to the Dutch dairy industry, it is not necessarily always a first choice among farmers. This is substantiated by the observation that although the majority of farms and farmers are adapted to and prefer the HF breed, there are still farmers that traditionally or recently have chosen to use another breed or a combination of breeds (e.g., Hiemstra et al., 2010). During data collection on farms applied in this thesis, a remarkably large variety of farming systems and farmers keeping the native dual purpose breeds was observed. Dairy farms differed, for example, in production intensity, degree of automation, degree of entrepreneurship of the farmer and education level of the farmer. This large variety in farming systems associated with the use of different native dual purpose breeds was also described in a report of the European project EURECA (Hiemstra et al., 2010), which was based on a farmer survey concerning different breeds. The results of this report showed, for example, that native dual purpose breeds were especially used on more extensive farms such as organic farms and/or farms with secondary activities, like social- care farming, on-farm selling, tourism or nature management. The variety of farmers and farming systems, combined with different breeding goals, were also described by Groen et al. (1993). One of the main conclusions was that farmers generally had a clear idea of what type of cows (i.e., which breed) best fit their farming system. When comparing the results of Chapters 2 and 4 of this thesis, it is worth noting that in Chapter 2, larger differences in FA composition between breeds were found, which were in general also significant. However, as the farms sampled for Chapter 2 were only using one single breed, the observed differences represented the combined effect of farm and breed, because farm and breed were confounded within the data. The level of the group UFA and the individual FA C16:0 on different farms

using MRY, DF, GWH or JER is shown in Figures 7.2a and 7.2b. These figures illustrate that the combination of herds and breeds considerably added to the observed variations.

a



b

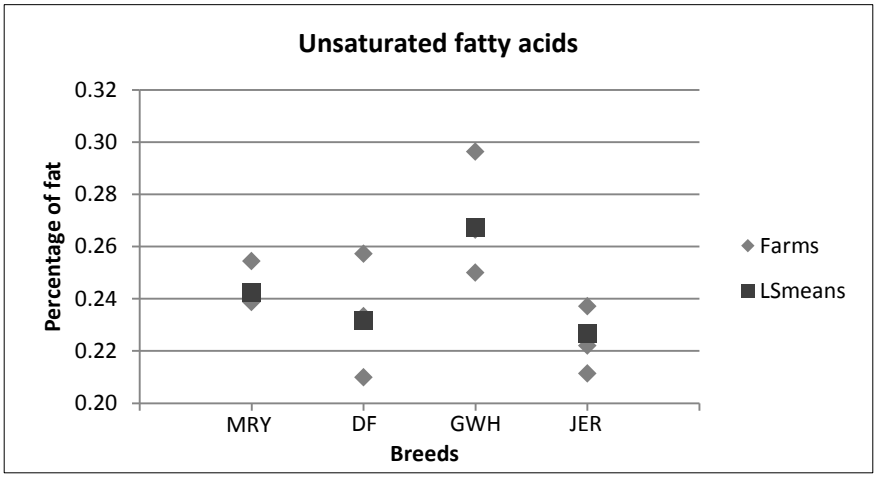


Figure 7.2 The LS means for three different native Dutch dual purpose breeds and the Jersey breed in the Netherlands, and the uncorrected means of the individual farms using different breeds for the (a) percentage of C16:0 and (b) unsaturated fatty acids in milk.

The latter suggests that for the selection of specific FA composition, differences in herd environment had to be taken into account. Finally, the continued use of a variety of cattle breeds on dairy farms might contribute to the diversity in farming systems and enable farmers to continue or to develop their desired or "ideal" way of farming and products, which stresses the relevance of strategies and policies for supporting the conservation of native dual purpose cattle breeds in future.

7.2.2 Using numerically small cattle breeds in the Dutch dairy sector

It is not surprising that the dairy breeding industry - in a country that plays a major role in food production, but where land availability is a major limiting factor like the Netherlands - is dominated by a single breed, for a number of reasons, for example, 1) it is easier and/or more efficient to focus on a single breeding programme specifically compiled for a specific breed; 2) breeding programmes focusing on a single breed are more likely to result in more genetic progress within that breed, compared to breeding programmes that focus on multiple breeds; 3) a mainstream breed can benefit from the exchange of genetic material and estimated breeding values between countries; 4) specialized farming systems in countries like the Netherlands can easily be adjusted for the needs of cows within a specific breed. A combination of the factors described above, along with strong selection for high production using the Dutch INET index rendered the HF breed extremely suitable for intensive milk production in the Netherlands. Within the commercial milk production industry, it is difficult for dairy cattle breeds with a relatively small number of animals, e.g., MRY, DF and GWH to compete with mainstream breeds like HF. However, these numerically small native dual purpose breeds are nonetheless perceived to be particular suitable for extensive farming systems like organic farming (Hiemstra et al., 2010, Nauta et al., 2003, Nauta et al., 2009). Nevertheless, it remains challenging for these native, numerically small breeds to find (other) ways for becoming self-sustainable. Unique genetic variations of specific traits of interest can potentially support conservation strategies for native Dutch dual purpose cattle breeds. Investigating the FA composition in milk produced by native Dutch dual purpose cattle breeds MRY, DF and GWH appears to indicate that these breeds do not add to the genetic variability of the major fatty acids in milk observed in the mainstream HF breed (this thesis). These results therefore do not urge expansion of the use of native Dutch dual cattle breeds for the purpose of changing the FA composition in milk within current regular dairy cattle milk production breeding programmes. As these

native breeds clearly differ in breeding background and appearance it is, however, likely that they do comprise unique genetic variations (Medugorac et al., 2009, Oldenbroek, 2007), although this was not directly detected for FA composition in milk in this thesis. However, relative large variances in milk FA composition were observed among farms using different cattle breeds (MRY, DF, GWH or JER).

To investigate the genetic variation of specific traits such as milk fat composition, this required large datasets and sometimes expensive research methods. These requirements were generally difficult to achieve for native breeds, because of the small number of animals and lack of direct economic prospects. The difficulty of restrictions due to expensive research methods can in some cases be addressed by the introduction of new research methods, for example, the use of relatively cheap and quick mid-infrared spectrometry (MIR) for analysing the detailed FA composition in milk, instead of the expensive and time-consuming gas chromatography (this thesis). It is expected that these new, relatively cheap and high throughput analysis methods for the collection of phenotypes like MIR (Chapter 2) – and which can already be used to measure FA composition in the milk of different cattle breeds – will also become available for measuring other traits. For example, the possibility of using MIR for analysing the methane emissions of individual cows is currently under investigation (Dehareng et al., 2012). To investigate whether numerically small native breeds can contribute to reaching new breeding goals, the phenotypic and genotypic information of a minimum of ~100 cows (as was used in Chapter 6) provided an impression of the potential opportunities that may arise from using these breeds in breeding in instances where information was also available for a reference population, as was the case for the mainstream HF in Chapter 6.

As described in section 7.2.1, the match between farmer and the type of cow or breed can also play an important role in the choice of breed on a farm. For some farmers, using a specific breed can mean more pleasure in working with the cows, because of the animals' specific personalities and/or appearance (Hiemstra et al., 2010), which can, for example, result in extreme early recognition of sickness among cows or the optimization of their circumstances, which can in turn save or even generate money. The special appearance of native breeds – in the case of this thesis, particularly the GWH (a black or red cow, typically with a white head and coloured blisters around the eyes, white socks, belly and udder) – is important for a number of secondary breed functions and has added value, e.g., social-care farming, on-farm selling, tourism or nature management (e.g., Gandini and Villa, 2003, Hiemstra et al., 2010, Oldenbroek, 2007). The development of special

products linked to these native breeds can also help to realize the self-sustainability of these breeds (e.g., Hiemstra et al., 2010, Oldenbroek, 2007).

Finally, in case consequences of possible climate change become visible in future, for example more extreme temperatures or lower quality diets, breeding goals may have to be adjusted and native breeds might comprise the genetic variability required to meet these new breeding goals (Hoffmann, 2010). In animal husbandry, for example, concerns regarding the impact of livestock emissions on climate change has already led to several studies such those investigating the emission of methane by dairy cows, the likes of which differs among cows and is affected by genetics (e.g., de Haas et al., 2011, Johnson and Johnson, 1995).

In summary, to keep numerically small dairy cattle breeds self-sustainable, it is important to understand, measure and use their unique characteristics for the optimization of specific farming systems (e.g., in organic farming) and to add value to the native breed by developing special and recognizable products or other specific functions (e.g., in social-care farming). Furthermore, conservation of genetic variations in cattle through the preservation of different breeds could be important for adaptation to new production environments or currently unknown product or market requirements needed in future.

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Summary

Summary

Bovine milk is a major source of nutrients in the human diet and contains between 3% and 6% fat. 'Milk fat' is the collective term used for a large number of individual fatty acids (FA), which can roughly be divided into saturated FA (SFA) and unsaturated FA (UFA). The composition of FA in milk varies considerably between cows and herds, mainly due to differences in the genetics and nutrition of cows. Since FA composition in milk is related to the processability of milk and also estimated to be related to human health, there is an increasing interest in the possibilities for modifying FA composition. As FA composition in milk is influenced by genetic factors, possible breed differences might be employed for modifying FA composition in the desired direction. In worldwide dairy cattle breeding, since the 1980s, a change has been observed from the use of native breeds toward the use of large and internationally-used mainstream breeds. In the Netherlands, this change has become clearly visible as native dual purpose breeds like Meuse-Rhine-Yssel (MRY), Dutch Friesian (DF) and Groningen White Headed (GWH) are to a large extent being replaced by the globally mainstream breed Holstein Friesian (HF). As a result, these native breeds are now numerically small. Therefore, in this thesis, two fields of interest were combined: the production of milk with a specific milk fat composition and the conservation of native cattle breeds. The overall objective of the thesis was to investigate whether native dual purpose breeds comprise different genetic variations for milk fat composition among one another and compared to the mainstream HF breed.

Chapter 2 describes the detailed FA composition in milk produced on farms using the native dual purpose breeds MRY, DF, GWH and the globally mainstream Jersey (JER) breed. In total, milk samples were collected on 12 farms and for each breed, around 50 cows were sampled on three farms. All milk samples were analysed by gas chromatography (GC). For all studied individual FA (13), groups of FA (9) and unsaturation indices (5), differences in FA composition were found among the groups of farms using different cattle breeds. The proportion of the UFA group relative to the total FA was largest for GWH (26.7%) compared with MRY (24.3%) and DF (23.2%), and smallest for JER (22.7%). This suggests that selecting specific FA composition differences in farms using different breeds in the Netherlands can attribute to modifying the FA composition in bovine milk production.

Because GC is an expensive and time-consuming method for analysing FA composition in milk, the use of mid-infrared spectrometry (MIR) for analysing FA composition in milk of different breeds was validated (Chapter 3). Calibration

equations used to predict FA composition using MIR were based on a dataset containing 1236 milk samples from multiple cattle breeds from Ireland, Scotland and the Walloon region of Belgium. These calibration equations were used to predict 11 individual (mainly short- and medium-chain) FA and three groups of FA of milk from 190 cows in the Netherlands across the breeds MRY, DF, GWH and JER. The FA composition of these 190 milk samples from Dutch cows were also analysed by GC and used as the gold standard. For the majority of FA composition, the predictions were highly accurate (validation $R^2 > 0.80$). This implies that MIR can be a suitable method for predicting FA composition among different breeds and countries.

In Chapter 4, FA composition in milk was predicted using MIR for a large number of cows, including different breeds in the Netherlands. The data contained MIR spectra of in total 1769 purebred cows belonging to the breeds MRY, DF, GWH and JER, 15 050 purebred HF cows and 7626 crossbred cows belonging to the breeds HF, MRY, DF, GWH and JER. Using MIR, FA content in milk was predicted. Analyses were conducted either by adjusting or not adjusting for fat percentage in order to be able to compare different breeds, independent of the level of fat percentage in milk. After adjusting for differences in fat percentage, differences among breeds in detailed fat composition disappeared or became smaller for several short- and medium-chain FAs, whereas for several long-chain unsaturated FAs, more significant breed differences were found. This indicated that short- and medium-chain FA content were for all breeds stronger related to total fat percentage than it was for long-chain FA content. In conclusion, the observed differences in fat composition in milk between HF, MRY and DF were insignificant. JER cows tended to produce a relatively higher SFA content, whereas GWH tended to produce a relatively higher UFA content and especially less short-chain SFA and more long-chain FA when adjusting for differences in fat percentage per breed.

Besides the variation in FA composition between breeds, the within-breed variation for 14 individual FAs and 14 groups of FAs in milk of the breeds HF and MRY was also investigated (Chapter 5). Additive genetic variances and heritabilities were estimated using records of 96 315 HF cows, as well as a MRY population containing 2049 cows. Heritabilities of the groups of FAs for HF ranged from 0.19 to 0.53 and for MRY from 0.11 to 0.28. For the majority of the individual FAs, the additive genetic variances for HF were on average 1.9 times higher compared to the MRY population, except for most of the polyunsaturated FAs. This implied that there was less potential for selection according to detailed FA content in the MRY breed compared to HF cows.

To gain a better insight into the background of genetic variations in FA composition between and within breeds, in Chapter 6, similarities in genomic variation associated with detailed milk fat composition between the Holstein Friesian (HF) breed and native dual purpose breeds MRY, DF and GWH were investigated. The genotypic and phenotypic information of 1867 cows were used in this investigation. Genomic breeding values (GEBV) were estimated for the milk composition of MRY, DF and GWH breeds, using single nucleotide polymorphism (SNP) effects estimated in HF. Comparing the native Dutch dual purpose breeds, the GEBV calculated for the MRY breed were clearly least reliable, indicating that the genetic variation associated with milk fat composition of MRY differed most from HF. For both DF and GWH, the estimated SNP effects for FA composition based on HF data showed a substantial predictive ability for several traits and were highest in GWH. In addition, correlations between the allele frequencies of the breeds MRY, GWH, DF and HF were calculated. These correlations proved similar between any pair of the native Dutch dual purpose breeds and HF when considering all SNPs (on average 0.68). Focussing on SNPs that had a large effect on milk FA composition in HF (at regions on BTA 5, 14 and 26), however, showed strong differences in allele frequencies when comparing the native Dutch dual purpose breeds with HF (average correlation 0.50-0.53). Nevertheless, there was no consistent relationship between differences in GEBV reliability and allele frequencies when using target subsets of SNPs with increasingly larger effects in HF. In conclusion, differences were detected between the native breeds MRY, DF and GWH in genomic variations of regions that are associated with FA composition in HF, while most variation in these main regions was clearly observed in HF. The similarity between the native dual purpose breeds and HF in variances in FA associated with genomic variation was visibly lowest for the native MRY breed.

In the general discussion (Chapter 7), the genetic variability in cattle associated with milk FA composition was first discussed. Overall, it was concluded that no large differences existed in milk FA composition among the native Dutch dual purpose breeds and the mainstream HF breed. The main observed difference was that the GWH breed seemed to produce relatively less SFA and more UFA, especially long-chain UFA. Concerning the *DGAT*1 gene, which is highly related to FA composition in HF, the native Dutch dual purpose breeds had less genetic variation compared to HF and appeared to mainly carry the genotype AA. However, limited differences in levels of FA composition in milk were found among breeds when comparing different breeds on different farms; however, this did not indicate any obvious variations. It is suggested that this was partly the result of the substantial diversity of farmers using different native dual purpose cattle breeds.

In the second part of the discussion, attention was paid to the diversity of farmers using different cattle breeds and the use of numerically small cattle breeds in the Dutch dairy sector. Opportunities for native dual purpose breeds lie in the use of these breeds in specific farming systems (e.g., organic and social-care farming) and at a cultural level (e.g., the cultural heritage of local use and craft animal products), rather than in competing with the HF breed. Finally, in the long-term, it is important to conserve breeds, as they may possibly be needed for adaptation to unknown future production environments.

Samenvatting

Samenvatting

Melk van koeien is een belangrijke bron van voedingstoffen. Melk bevat normaliter tussen de 3 en 6% vet wat bestaat uit een groot aantal verschillende vetzuren (FA) welke grofweg onder te verdelen zijn in verzadigde (SFA) en onverzadigde vetzuren (UFA). De exacte samenstelling van melkvet varieert niet alleen tussen melkveebedrijven, maar ook tussen individuele koeien. Deze variatie is met name het gevolg van verschillen in het rantsoen en genetische verschillen tussen de koeien. Omdat de vetzuursamenstelling in melk van invloed is op de verwerking van melk en tevens geassocieerd wordt met humane gezondheid, is er een groeiende vraag naar mogelijkheden om de vetzuursamenstelling in melk aan te passen. Het is bekend dat de vetzuursamenstelling in de melk wordt beïnvloed door een (onbekend) aantal genen, oftewel door veel verschillende specifieke stukken op het DNA van de koe. Het zou dan ook mogelijk kunnen zijn dat er duidelijke verschillen zijn in de vetzuursamenstelling van verschillende rundveerassen.

Wereldwijd, vanaf ongeveer de jaren ’80, is er een duidelijke verschuiving geweest van het gebruik van inheemse en lokale rundveerassen naar het gebruik van rassen welke internationaal worden ingezet. In Nederland is deze verschuiving duidelijk te herkennen aan het feit dat de inheemse dubbeldoelrassen zoals de Maas-Rijn-IJssel koe (MRY), de Fries Hollandse koe (DF) en de Groninger Blaarkop koe (GWH) op groot deel van de melkveebedrijven is vervangen door de internationaal veel ingezette Holstein Friesian koe (HF). Door deze verschuiving zijn de inheemse dubbeldoelrassen in aantal sterk teruggelopen tot relatief kleine populaties op dit moment.

De interesse in de achtergrond van de vetzuursamenstelling in melk en het feit dat inheemse rassen steeds minder worden gebruikt in de melkveehouderij zijn de reden dat in dit proefschrift de vetzuursamenstelling in de melk van verschillende Nederlandse rundveerassen is onderzocht en vergeleken met het internationale HF ras.

In hoofdstuk 2 wordt de gedetailleerde vetzuursamenstelling van melk beschreven welke is geproduceerd op bedrijven waar gewerkt wordt met de rassen MRY, DF, GWH en de internationaal veel gebruikte Jersey koe (JER). In totaal zijn melkmonsters genomen op een 12-tal melkveebedrijven, waarbij voor ieder ras melkmonsters zijn genomen van ongeveer 50 koeien op 3 verschillende bedrijven. Deze melkmonsters zijn geanalyseerd met behulp van gas chromatografie (GC). In totaal zijn 13 individuele vetzuren, 9 vetzuurgroepen en 5 indexen (onverzadigd/totaal) gemeten en bestudeerd waarbij verschillen zijn gevonden

tussen bedrijven die verschillende rundveerassen melken. Het aandeel onverzadigd vet in de melk ten opzichte van het totaal aan vet was het grootst voor het GWH ras (26,7%) in vergelijking met MRY (24,3%) en DF (23,2%), en was het kleinst voor JER (22,7%). Deze bevindingen suggereren dat de combinatie van ras en bedrijf van belang kan zijn voor een specifieke/aangepaste productie van de vetzuursamenstelling in melk.

Omdat GC-analyse relatief kostbaar is en veel tijd kost is in hoofdstuk 3 een validatiestudie beschreven waarvoor is onderzocht of de analysemethode mid-infraroodspectroscopie (MIR) ook gebruikt kan worden voor het bepalen van de vetzuursamenstelling in de melk van verschillende rundveerassen. Voor het bepalen van de vetzuursamenstelling middels MIR zijn kalibratie vergelijkingen gebruikt die ontwikkeld waren met behulp van een dataset van een 1236-tal melkmonsters die waren verzameld van verschillende rundveerassen in Ierland, Schotland en België (Wallonië). De vergelijkingen werden gebruikt voor het schatten van de hoeveelheid in de melk van 11 individuele vetzuren (voornamelijk vetzuren met korte en middellange ketenlengtes) en 3 vetzuurgroepen van een 190-tal Nederlandse koeien behorende tot de rassen MRY, DF, GWH en JER. De melkmonsters van deze koeien waren tevens geanalyseerd met behulp van GC waarvan bekend is dat hiermee de vetzuursamenstelling nauwkeurig kan worden gemeten. Met behulp van MIR konden de meeste onderzochte vetzuren zeer nauwkeurig worden voorspeld (validatie R^2 > 0,80). Dit wijst erop dat MIR een geschikte methode is voor het bepalen van de vetzuursamenstelling in de melk van verschillende rundveerassen over verschillende landen.

In hoofdstuk 4 is de vetzuursamenstelling in de melk van een zeer grote groep koeien, van verschillende rassen en bedrijven, geanalyseerd met behulp van MIR. De data bevatte MIR gegevens (spectra) van in totaal 1769 zuivere MRY, DF, GWH en JER koeien, 15 050 zuivere HF koeien en 7626 kruislingen van de rassen HF, MRY, DF, GWH en JER. De vetzuursamenstelling van de verschillende rundveerassen is met elkaar vergeleken. Die vergelijking is twee keer gedaan waarbij de ene keer wel en de andere keer geen rekening is gehouden met het totale vetpercentage in de melk. Uit de resultaten bleek dat de vetzuren met korte en middellange ketenlengtes een sterke relatie hebben met het totale vetzuurpercentage in de melk. Geconcludeerd kan worden dat de waargenomen verschillen in vetzuursamenstelling tussen de rundveerassen niet of nauwelijks significant zijn. Wel lijkt JER iets meer verzadigde vetzuren te produceren en GWH iets meer onverzadigde vetzuren wanneer werd gecorrigeerd voor vetpercentage in de melk.

Naast de verschillen tussen de rundveerassen is de variatie in vetzuursamenstelling in de melk van koeien van hetzelfde ras (binnen ras variatie) voor HF en MRY onderzocht en beschreven in hoofdstuk 5. Hiervoor zijn van een 14-tal individuele vetzuren en een 14-tal vetzuurgroepen de hoeveelheden in de melk bepaald met behulp van MIR. De additief genetische varianties en de erfelijkheidsgraden van deze vetzuren en vetzuurgroepen zijn afzonderlijk geschat voor HF en MRY. Hiervoor is MIR data gebruikt van 96 315 HF en 2049 MRY koeien. De erfelijkheidsgraden voor de vetzuurgroepen varieerden van 0,19 tot 0,53 voor het HF ras en varieerden van 0,11 tot 0,28 voor het MRY ras. Voor de meeste individuele vetzuren waren de geschatte genetische varianties gemiddeld 1,9 keer hoger voor het HF ras in vergelijking met het MRY ras, uitgezonderd de meeste meervoudig onverzadigde vetzuren. Dit impliceert dat er minder ruimte is voor selectie op vetzuursamenstelling binnen het MRY ras in vergelijking met het HF ras.

In hoofdstuk 6 is getracht om meer inzicht te verkrijgen in de genetische achtergrond van variatie in vetzuursamenstelling binnen en tussen verschillende rundveerassen. Hiervoor zijn mogelijke overeenkomsten onderzocht in genomische variatie geassocieerd met vetzuursamenstelling in melk tussen het internationale HF ras en de inheemse dubbeldoelrassen MRY, DF en GWH. Fenotypische (geanalyseerde vetzuursamenstelling) en genotypische (DNA-profielen) gegevens van in totaal 1867 koeien zijn gebruikt voor dit onderzoek. Genomische fokwaarden (GEBV) waren geschat voor de vet- en eiwitpercentage en de gedetailleerde vetzuursamenstelling in de melk van MRY, DF en GWH waarbij gebruik was gemaakt van geschatte genetische effecten in HF. De GEBV schattingen voor het MRY ras waren duidelijk het minst betrouwbaar wat een indicatie is dat de genetische variantie geassocieerd met vetzuursamenstelling in melk in MRY het meest verschilt van die van HF. Voor de beide rassen DF en GWH waren de schattingen beter, met name voor een aantal vetzuren van het GWH ras. Hiernaast is ook naar de gemiddelde overeenkomsten en verschillen (correlaties) op het DNA (op basis van SNP's) gekeken tussen de rassen MRY, DF, GWH en HF. Deze correlaties waren gemiddeld 0.68 tussen alle rassen. Wanneer op de SNP's werd gefocust waarvan bekend is dat ze een effect hebben op de vetzuursamenstelling in de melk van HF was de correlatie tussen de rassen aanmerkelijk lager (0,50-0,53). Hieruit kan worden geconcludeerd dat er duidelijke genomische verschillen zijn tussen de inheemse rassen MRY, DF en GWH als wordt gekeken naar de variatie die is geassocieerd met vetzuursamenstelling in de melk, de meeste variatie echter is gevonden in het HF ras.

In de algemene discussie (hoofdstuk 7) is als eerste de genetische variatie die is geassocieerd met vetzuursamenstelling in melk in rundvee en rundveerassen

uiteengezet. Globaal kan worden geconcludeerd dat er geen grote verschillen in vetzuursamenstelling in de melk van de verschillende inheemse dubbeldoelrassen en HF zijn. De belangrijkste waargenomen verschillen zijn gevonden voor de koeien van het GWH ras die grofweg minder verzadigde vetzuren en meer onverzadigde vetzuren lijken te produceren. Wanneer expliciet het voorkomen van het *DGAT*1 gen (van dit gen is het bekend dat het een aanzienlijk effect heeft op vetzuursamenstelling in melk van HF koeien) werd onderzocht is het duidelijk dat de inheemse dubbeldoelrassen voor dit gen minder genetische variatie vertonen.

De verschillen in vetzuursamenstelling die zijn gevonden tussen de rundveerassen kunnen op basis van dit onderzoek niet los worden gezien van de verschillen tussen de bedrijven waar deze rassen worden gemolken. Op veel van de bedrijven waar de inheemse dubbeldoelrassen worden gemolken valt de “eigen manier” van bedrijfsvoering op en daardoor is de melk verzameld op een grote variëteit aan bedrijven. De direct in de melk gemeten verschillen in vetzuursamenstelling zijn waarschijnlijk deels een gevolg van de combinatie van rundveeras en bedrijf.

In het tweede deel van de algemene discussie is aandacht besteed aan de diversiteit tussen veehouders die de verschillende rundveerassen gebruiken en aan het gebruik van (in aantallen kleine) inheemse dubbeldoelrassen in het algemeen. Kansen voor de inheemse dubbeldoelrassen liggen waarschijnlijk vooral op het gebied van alternatieve veehouderijsystemen (zoals biologische veehouderij en zorgfuncties op het bedrijf), meer dan in het concurreren tegen HF op het gebied van melkproductie. Ten slotte, voor de langere termijn is het van belang om verschillende rundveerassen te behouden, omdat deze mogelijk van belang zullen zijn in de nog onbekende toekomst als er aanpassingen nodig zijn door veranderende productieomstandigheden.

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‘to the world you may be one person, but to one person you are the world’

Myrthe

Curriculum Vitae

About the author

Myrthe Hendrika Teunie Maurice – Van Eijndhoven was born on January 23 1984 in Meeuwen (Aalburg), the Netherlands. In 2001, she graduated from high school Willem van Oranje College, Waalwijk. In the same year she started her BSc Animal Husbandry at HAS Den Bosch. During her BSc, she spend 2.5 months abroad for DCA-VET, a non-governmental veterinary orientated organisation helping to rebuild Afghanistan, where she monitored and evaluated an education programme for Afghan women at the country side. In 2005, she received her BSc diploma and started the MSc Animal Sciences at Wageningen University, with the specializations "Animal Breeding and Genetics" and "Quantitative Veterinary Epidemiology". For her specialization in Animal Breeding and Genetics she performed her thesis at the Centre for Genetic Resources (CGN), the Netherlands, on genetic diversity within the Dutch cattle breeds Meus-Rhine-Yssel and Dutch Friesian. For her specialization in Quantitative Veterinary Epidemiology she performed her thesis at ForFarmers on the improvement of biosecurity on Dutch pig farms. After finishing her MSc in 2007 she started working at CGN. In 2008, she started her PhD study at CGN / the Animal Breeding and Genomics Centre, Wageningen Livestock Research. The results of this research are described in this thesis. Since 2013, she is working at Stichting Zeldzame Huisdierrassen (SZH) where she supports Dutch rare breed organisations and studbooks to execute good breeding practice to conserve the genetic diversity in their breed.

Over de auteur

Myrthe Hendrika Teunie Maurice – Van Eijndhoven is geboren op 23 januari 1984 te Meeuwen (gemeente Aalburg). In 2001 behaalde ze haar HAVO diploma aan het Willem van Oranje College te Waalwijk, waarna ze begon aan de BSc studie Dier- en Veehouderij aan de HAS Den Bosch. Tijdens deze studie deed ze onder andere een buitenlandstage van 2,5 maand bij DCA-VET, een organisatie die gespecialiseerd is in veterinaire ontwikkelingshulp, waarbij ze een educatieproject voor plattelandsvrouwen heeft gemonitord in Afghanistan. In 2005 ontving ze haar BSc diploma en is ze begonnen aan de MSc studie Dierwetenschappen aan Wageningen Universiteit. Voor haar specialisatie 'Fokkerij en Genetica' heeft ze een afstudeeropdracht volbracht bij het Centrum voor Genetische Bronnen (CGN) waar ze de genetische diversiteit in de Nederlandse rundveepopulaties Maas-Rijn-IJssel en Fries Hollands heeft onderzocht. Voor haar specialisatie 'Kwantitatieve Veterinaire Epidemiologie' heeft ze een afstudeeropdracht uitgevoerd bij veevoederbedrijf ForFarmers, waarbij een plan is ontwikkeld voor het verbeteren van de hygiëne-status op Nederlandse varkensbedrijven. In 2007 studeerde ze af waarna ze is gaan werken voor het CGN. In 2008 is ze begonnen met haar promotieonderzoek bij het Animal Breeding and Genomics Centre van Wageningen Livestock Research, waarvan de resultaten beschreven zijn in dit proefschrift. Sinds 2013 is ze werkzaam bij de Stichting Zeldzame Huisdierrassen waar ze o.a. stamboeken en organisaties van (zeldzame) Nederlandse rassen begeleidt bij het ontwikkelen van hun fokkerijbeleid ter behoud van de genetische diversiteit in hun populaties.

Publications

Peer reviewed publications

Maurice-Van Eijndhoven, M. H. T., H. Bovenhuis, H. Soyeurt, and M. P. L. Calus. 2013. Differences in milk fat composition predicted by mid-infrared spectrometry among dairy cattle breeds in the Netherlands. Journal of Dairy Science 96, 2570-2582.

Hulsegge, B., M. P. L. Calus, J. J. Windig, A. H. Hoving, M. H. T. Maurice – Van Eijndhoven, and S. J. Hiemstra. 2013. Selection of SNP from 50k and 777k arrays to predict breed of origin in dairy cattle. Journal of Animal Science 91, 5128-5134

Maurice-Van Eijndhoven, M. H. T., H. Soyeurt, F. Dehareng, and M. P. L. Calus. 2013. Validation of fatty acid predictions in milk using mid-infrared spectrometry across cattle breeds. Animal 7, 348-354.

Maurice-Van Eijndhoven M. H. T., S. J. Hiemstra and M. P. L. Calus. 2011. Short communication: milk fat composition of 4 cattle breeds in the Netherlands. Journal of Dairy Science 94, 1021–1025.

Gandini, G., L. Avon, D. Bohte-Wilhelmus, E. Bay, F. G. Colinet, Z. Choroszy, C. Diaz, D. Duclos, J. Fernández, N. Gengler, R. Hoving, F Kearney, T. Lilja, Mäki Tanila, D Martín-Collado, M. Maurice-van Eijndhoven, M. Musella, F. Pizzi, K. Soini, M. Toro, F. Turri, H. Viinalas, the EURECA Consortium and Hiemstra, S. J. 2010. Motives and values in farming local cattle breeds in Europe: a survey on 15 breeds. Animal Genetic Resources 47, 45-58.

Conference proceedings

Maurice-Van Eijndhoven, M. H. T., H. Bovenhuis, H. Soyeurt, and M. P. L. Calus. Differences in bovine milk fat composition among dairy breeds in the Netherlands. The 63th Annual Meeting of the European Association of Animal Production, Bratislava, Slovakia, 27 - 31 August 2012.

Maurice-Van Eijndhoven, M. H. T., H. Soyeurt, F. Dehareng, and M. P. L. Calus. Across breed and country validation of mid-infrared calibration equations to predict milk fat composition. The 62th Annual Meeting of the European Association of Animal Production, Stavanger, Norway, 29 August – 2 September 2011.

Maurice-Van Eijndhoven, M. H. T., S. J. Hiemstra, and M. P. L. Calus. Proportion saturated and unsaturated fatty acids in milk of four cattle breeds in the Netherlands. Proceedings of the 9th World Congress on Genetics Applied to Livestock Production, Leipzig, Germany, 1 -7 August 2010.

Maurice-Van Eijndhoven, M. H. T., M. J. M. Rutten, and M. P. L. Calus. Using mid-infrared spectrometry to investigate milk fatty acid composition of different Dutch cattle breeds. The 60th Annual Meeting of the European Association of Animal Production, Barcelona, Spain, 24 - 27 August 2009.

De Haas, Y., S. J. Hiemstra, D. Bohte-Wilhelmus, J. J. Windig, A. H, Hoving, and M. H. T. Maurice-Van Eijndhoven. How to maintain declining Dutch local cattle breeds? The 60th Annual Meeting of the European Association of Animal Production, Barcelona, Spain, 24 - 27 August 2009.

Maurice-Van Eijndhoven, M. H. T., S. J. Hiemstra, and P. A. Oliehoek. Genetic diversity in Dutch cattle breeds by means of pedigree analysis. The 59th Annual Meeting of the European Association of Animal Production, Vilnius, Lithuania, 24 -27 August 2008.

Training and supervision plan

Training and supervision plan

Basic Package (3 ECTS)	**Year**
WIAS Introduction Course	2009
Ethics and Philosophy of Animal Science	2009

Scientific Exposure (16 ECTS)

International conferences (7 ECTS)

Cattle Breeders Round Table, Cork, Ireland	2008
59th annual meeting EAAP, Vilnius, Lithuania	2008
60th annual meeting EAAP, Barcelona, Spain	2009
Cattle Breeders Round Table, Soenderborg, Denmark	2010
9th WCGALP, Leipzig, Germany	2010
62th annual meeting EAAP, Stavanger, Norway	2011

Seminars and workshops (3 ECTS)

WIAS Science Day, Wageningen, the Netherlands (4x)	2008-2011
F&G connectiedagen, Vught, the Netherlands (2x)	2008-2010
QTL MAS workshop, Wageningen, the Netherlands	2009
Genetics of Milk Quality, Wageningen, the Netherlands	2009

Presentations (6 ECTS)

Cattle Breeders Round Table, Cork, Ireland	2008
59th annual meeting EAAP, Vilnius, Lithuania	2008
60th annual meeting EAAP, Barcelona, Spain	2009
Cattle Breeders Round Table, Soenderborg, Denmark	2010
9th WCGALP, Leipzig, Germany	2010
62th annual meeting EAAP, Stavanger, Norway	2011

In-Dept Studies (7 ECTS)

Disciplinary and interdisciplinary courses (7 ECTS)

Introduction to R, Wageningen, the Netherlands	2008
Nutrient Density of Milk, Wageningen, the Netherlands	2009
Animal Breeding and Genetics Short Course, Iowa, USA	2009
Quantitative Genetics of Selection Response, Wageningen, the Netherlands	2010
WIAS Advanced Statistics Design of Experiments, Wageningen, the Netherlands	2010

Professional Skills Support Courses (6 ECTS)	**Year**
Techniques for Writing and Presenting Scientific Papers, Wageningen, the Netherlands	2008
PhD Competence Assessment or Job Assessment, Wageningen, the Netherlands	2008
Project- and Time Management, Wageningen, the Netherlands	2008
Supervising MSc thesis work, Wageningen, the Netherlands	2010
Writing for Academic Publication, Wageningen, the Netherlands	2010
Career Perspectives, Wageningen, the Netherlands	2011
Research Skills Training (5 ECTS)	
Preparing own PhD research proposal	2008
Didactic Skills Training (2 ECTS)	
Supervising theses (2 ECTS)	
MSc Thesis Animal Breeding and Genetics, Wageningen, the Netherlands	2009
Management Skills Training (13 ECTS)	
Organisation of seminars and courses (1 ECTS)	
WIAS Science Day	2009
Membership of boards and committees (12 ECTS)	
Young ASG	2009
WAPS Council secretary and Education committee	2009
WAPS Chairman and Education committee	2010
WAPS Council Accie	2011
Education and Training Total	**52 ECTS**

Colophon

The research described in this thesis was funded by the Dutch Ministry of Economic Affairs (The Hague, the Netherlands), the Dutch Milk Genomics Initiative, the project 'Melk op Maat' and the cooperative cattle improvement organisation CRV (Arnhem, the Netherlands).

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