Genes involved in bovine milk-fat composition

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Contents

1

General introduction

1.1 Milk

Milk is a fluid secreted by the mammary glands of female mammals, and provides a primary source of nutrition for the neonate. Raw milk contains fat, protein, lactose, vitamins, minerals and, of course, water. In addition to a natural source of nutrition for infant mammals, milk and dairy products are major components of the human diet in many parts of the world. Recorded milk production today is about 560 billion tons per year, about 85% of which is bovine, 11% is buffalo and about 2% each is ovine and caprine, with small amounts produced by mares, camels, yaks and reindeer (Fox, 2003). For millennia, milk has been processed into dairy products such as butter, cream, yogurt, kefir, ice cream, and cheese.

1.2 Milk-fat

This thesis will focus on milk-fat. About 98% of the total fat in milk is present in the form of triacylglycerols (Jensen, 2002). A triacylglycerol is formed from a single molecule of glycerol, combined with three fatty acids. In addition to the triacylglycerols, milk contains small amounts of diacylglycerols, monoacylglycerols, free (unesterified) fatty acids and phospholipids. The triacylglycerols have a major effect on the chemical properties of milk-fat, which in turn influences the functional properties of a number of dairy products, such as the spreadibility of butter.

Fatty acids are named and categorized according to their length, which is determined by the number of carbon atoms, and according to their degree of saturation, which is determined by the number of double bonds. Fatty acids without double bonds are called saturated, fatty acids with one double bond are called monounsaturated, and fatty acids with two or more double bonds are called polyunsaturated. Bovine milk-fat is regarded as one of the most complex fats. About 400 fatty acids have been identified in bovine milk-fat, but the vast majority of fatty acids are present in very small quantities (Jensen, 2002).

1.3 Origin of fatty acids in bovine milk

The fatty acids in milk arise from two sources: *de novo* synthesis in the mammary gland and plasma lipids. Short and medium chain fatty acids,

C4:0 to C14:0 and also some C16:0, are synthesized *de novo* in the mammary gland. The long chain fatty acids, with 18 and more carbon atoms, and, to a lesser extent, C16:0 arise from the cow's plasma (Palmquist, 2006). The long chain fatty acids in the mammary gland are derived from circulating plasma lipids, and originate from the diet, from microbial fatty acid synthesis in the rumen, and from endogenous lipids. Lipids entering the rumen are first lipolyzed by microbial lipases, causing the release of fatty acids. After lipolysis, unsaturated fatty acids are isomerized and hydrogenated by ruminal microbes, in a process called biohydrogenation (Harfoot and Hazlewood, 1997; Jenkins et al., 2008). Both short/medium and long chain fatty acids can be further metabolized in the mammary gland.

1.4 Aim and outline of this thesis

The research described in this thesis is part of the Dutch Milk Genomics Initiative (more information on the Dutch Milk Genomics Initiative can be found in Text box 1.1). The aim of the research was to identify genes that underlie the genetic variation in bovine milk-fat composition. In Chapter 2 is shown that large genetic variation in milk-fat composition is present. A dual approach to identify genes has been pursued: a candidate gene approach and a genome scan or quantitative trait loci (QTL) mapping approach. In Chapter 2, DGAT1, which was known to have a major effect on milk-fat percentage (Grisart et al., 2002; Winter et al., 2002), is identified as a major gene for milk-fat composition as well. Chapter 3 describes genetic variation in unsaturation of milk-fat, and reveals SCD1 and DGAT1 as two genes with large impact on the unsaturation indices. To map regions of the bovine genome influencing milk-fat composition without prior knowledge on functions of genes, a genome-wide scan was conducted. Results of this genome-wide scan are described in Chapters 4 and 5. In Chapter 6, the effects of polymorphisms in a number of other genes previously related to milk production are reported. Furthermore, it is discussed whether these genes could underlie the QTL identified in Chapters 4 and 5. Finally, Chapter 7 examines the results from previous chapters from two other perspectives. First, differences in milk-fat composition within and between species are illustrated by polymorphisms in DGAT1 and SCD1. Second, knowledge on

the physiological functions of DGAT1 and SCD1 is reviewed, and effects of mutations in these genes on conformation traits of dairy cattle are discussed.

Text box 1.1 Framework: the Dutch Milk Genomics Initiative

The aim of the Dutch Milk Genomics Initiative is to identify the possibilities to use breeding strategies for improving milk-quality characteristics and to identify genes that contribute to natural genetic variation in milk-quality traits. The initiative started in 2004 and is funded by Wageningen University, breeding organization CRV, the Dutch Dairy Association (NZO) and the Dutch Technology Foundation (STW). The initiative was designed to have approximately 2,000 cows descending from a number of selected bulls; 50 young bulls were aimed to have 20 daughters each, and 5 proven bulls were aimed to have 200 daughters each. Over 600 farmers were invited to participate in the study. Positive reactions were received from 535 of them, and finally 400 farmers were selected. Three milk samples per cow were collected during February – July 2005, and one blood sample was collected for DNA isolation. Information on herd management, rations, mastitis, etc. was inventoried. Meanwhile, phenotypes are available for milk-fat composition, milk-protein composition, mineral composition and immunological parameters. Taking this all together makes the Dutch Milk Genomics Initiative resource a unique and valuable biobank.

2

DGAT1 underlies large genetic variation in milk-fat composition of dairy cows

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Abstract

Dietary fat may play a role in the etiology of many chronic diseases. Milk and milk-derived foods contribute substantially to dietary fat, but have a fat composition that is not optimal for human health. We measured the fat composition of milk samples in 1,918 Dutch Holstein Friesian cows in their first lactation and estimated genetic parameters for fatty acids. Substantial genetic variation in milk-fat composition was found: heritabilities were high for short and medium chain fatty acids (C4:0-C16:0) and moderate for long chain fatty acids (saturated and unsaturated C18). We genotyped 1,762 cows for the DGAT1 K232A polymorphism, which is known to affect milk-fat percentage, to study the effect of the polymorphism on milk-fat composition. We found that the DGAT1 K232A polymorphism has a clear influence on milk-fat composition. The *DGAT1* allele that encodes lysine (K) at position 232 (232K) is associated with more saturated fat; a larger fraction of C16:0; and smaller fractions of C14:0, unsaturated C18, and CLA ($P<0.001$). We conclude that selective breeding can make a significant contribution to change the fat composition of cow's milk.

2.1 Introduction

The impact of dietary fat on chronic disease, such as coronary heart disease, has been a topic of interest for decades (Hu and Willett, 2002). For almost 50 years, effects of fatty acid intake on serum lipids have been investigated. It is now clear that intake of saturated fatty acids increases total and low-density lipoprotein (LDL) cholesterol levels, whereas intake of polyunsaturated fatty acids decreases LDL cholesterol (Keys et al., 1957; Hegsted et al., 1965; Mensink et al., 2003). Not all saturated fatty acids affect cholesterol concentrations to the same extent. Lauric acid (C12:0), for example, reduces the ratio of total to high-density lipoprotein (total:HDL) cholesterol; reduction in this ratio is associated with reduction in the risk of coronary heart disease. Myristic acid (C14:0) and stearic acid (C18:0), furthermore, reduce total:HDL cholesterol less than C12:0, whereas palmitic acid (C16:0) has the unfavorable effect of increasing the ratio (Mensink et al., 2003). More recently, *trans* fatty acids have been reported to increase total:HDL cholesterol, hence increasing the risk of coronary heart disease (Mozaffarian et al., 2006). A high intake of saturated and *trans* fatty acids has also been related to insulin resistance and, subsequently, to type 2 diabetes (Hu et al., 2001; Parillo and Riccardi, 2004). It has been suggested, furthermore, that dietary fatty acids play a role in the development of obesity and cancer (Bartsch et al., 1999; Zock, 2001; Willett and Leibel, 2002). These findings suggest that an alteration of the dietary fat composition could have a major impact on public health.

Hulshof et al. (1999) showed that milk and milk-derived foods (including cheese and butter) were the main source of dietary saturated fatty acids across Europe; milk and milk-derived foods contributed for 27% to 58% to the intake of saturated fatty acids in the diet. The contribution from milk and milk-derived foods to dietary *trans* fatty acids was between 17% and 72%. Milk-fat is relatively high in saturated fatty acids, especially C14:0 and C16:0, and low in polyunsaturated fatty acids.

Milk-fat composition can be altered through the nutrition of dairy cows (Palmquist, 2006), and possibly by selective breeding, although prospects for the latter have not been studied extensively. The major prerequisite for

selective breeding is existence of genetic variation in milk-fat composition among cows. For milk-fat percentage, around half the observed variation is estimated to be due to genetic variation (Fuerst and Solkner, 1994; Ikonen et al., 1999; Calus et al., 2005; Hinrichs et al., 2005). Phenotypic variation in milk-fat composition has been reported as well, both between and within breeds, although the number of reports on genetic variation is limited and recent publications are lacking (Stull and Brown, 1964; Renner and Kosmack, 1974; Karijord et al., 1982; Lawless et al., 1999; Soyeurt et al., 2006). In the Netherlands, selection on milk-production traits has contributed to an increase in milk-fat percentage from 3.66% in 1950 to 4.42% in 2005 (NRS, 2006). Consequences of this increase in milk-fat percentage on milk-fat composition are unknown.

Traditional selective breeding requires extensive recording of phenotypes. Conversely, direct handle on the genes conferring merit enables faster genetic progress. Recently, a quantitative trait locus (QTL) mapping study in cattle resulted in the identification of a polymorphism (K232A) in the gene coding for acyl CoA:diacylglycerol acyltransferase 1 (DGAT1), which is a key enzyme in triglyceride synthesis (Cases et al., 1998) and has a strong effect on milk-fat percentage and other milk-production characteristics (Grisart et al., 2002; Winter et al., 2002). Female mice deficient in DGAT1 do not produce milk (Smith et al., 2000) and show an altered fatty acid composition in adipose tissue and skeletal muscle: less monounsaturated C16:1 and C18:1, and more saturated C16:0 and C18:0 fatty acids (Chen et al., 2002a). Using a mathematical model of fatty acid synthesis and triglyceride assembly, Shorten et al. (2004) predicted that increase in milk yield due to the DGAT1 232K allele would lead to a more saturated fat composition. The effect of the DGAT1 mutation on milk-fat composition, however, has not been tested. In this study, we investigated the genetic variation in bovine milk-fat composition and examined the effect of the DGAT1 K232A mutation on milk-fat composition.

2.2 Materials and methods

Animals

This study is part of the Dutch Milk Genomics Initiative, which focuses on the genetic background of detailed milk composition. As part of this study, morning milk samples and blood samples were collected from 1,918 first-lactation cows on 398 commercial herds in the Netherlands. At least three cows per herd were sampled and cows were milked twice a day. Cows descended from one of 50 young bulls (843 cows), from one of five proven bulls (888 cows), or from other proven bulls (187 cows). The NRS (Arnhem, the Netherlands) provided pedigrees of the cows and milk yield records. Each cow was over 87.5% Holstein Friesian and was in lactation between day 63 and day 263.

Phenotypes

A 0.5 liter milk sample was collected from each cow at one morning milking between February and March 2005. Milk-fat composition was measured at the COKZ laboratory (Netherlands Controlling Authority for Milk and Milk Products, Leusden, the Netherlands). Milk-fat was extracted from the milk samples, and fatty acid methyl esters were prepared from fat fractions, as described in ISO Standard 15884 (ISO-IDF, 2002b). Methyl esters were analyzed according to ISO Standard 15885 (ISO-IDF, 2002a) on a Trace GC Ultra chromatograph (Thermo Electron Corporation, Waltham, MA), using a Varian Fame Select column (100m x 0.25mm ID, Varian Inc., Palo Alto, CA). The initial temperature was held at 70ºC for 1 min, raised to 225ºC at 3ºC/min, and held at 225ºC for 5 min. A volume of 1 µl was injected. Each peak was identified and quantified using pure methyl ester samples (Sigma-Aldrich, Zwijndrecht, the Netherlands; Larodan, Malmö, Sweden). The fatty acids were expressed as weight-proportion of total fat weight. The fatty acids included in this study were grouped according to their relevance to human nutrition and health. Fat and protein percentages were measured by infrared spectroscopy, using a MilkoScan FT6000 (Foss Electric, Hillerod, Denmark) at the Milk Control Station (Zutphen, the Netherlands). Fat and protein yields were calculated by multiplying each percentage by the milk yield. Yield data were missing for 135 cows.

Genotypes

Blood samples for DNA isolation were collected between April and June 2005. Genotyping of the DGAT1 polymorphism was performed using a Taqman allelic discrimination method in an Applied Biosystems 7500 Real-Time PCR System (Applied Biosystems, Foster City, CA). The primers and labeled oligonucleotide probes for this reaction were designed based on the *DGAT1* sequence (AY065621): forward, 5'- CGCTTGCTCGTAGCTTTGG -3'; reverse, 5'- CGCGGTAGGTCAGGTTGTC -3'; VIC probe (detects the allele encoding 232K), 5'- CGTTGGCCTTCTTAC -3'; FAM probe (detects the allele encoding 232A), 5'-TTGGCCGCCTTAC-3'. PCR cycling conditions were 94°C for 5 min, followed by 40 cycles of 92°C for 15 s and 60°C for 1 min. About 7% of samples were genotyped in duplicate, and repeatability was 100%. In total, 1,762 animals were genotyped. Genotypes were missing for 156 animals, because either no DNA sample was available (n=144) or the sample could not be genotyped unambiguously (n=12).

Statistical analysis

Analyses were performed first using SAS 9.1 (2002) procedures to determine fixed effects. The model included stage of lactation (days in milk = time between calving and date of sample), age at first calving, season of calving, and an effect of the differences in genetic level between groups of proven bull daughters and young bull daughters. Variance components and genetic parameters were estimated using an Animal Model in ASReml (Gilmour et al., 2002):

$$y_{ijklmn} = \mu + b_1*dim_i + b_2*e^{-0.05*dim} + b_3*afc_j + b_4*afc_j^2 + season_k + scode_l + herd_m + U_n + e_{ijklmn} ,$$

where y was the dependent variable, μ was the general mean, dim was the covariate describing the effect of days in milk modelled with a Wilmink curve (Wilmink, 1987), afc was the covariate describing the effect of age at first calving, season was the fixed effect of the class of calving season (June-August 2004, September-November 2004, or December 2004-February 2005), scode was the fixed effect of the differences in genetic level between groups of proven bull daughters and young bull daughters, herd was the

random effect of groups of animals sampled in the same herd, U_n was the random additive genetic effect of animal n, and e was the random residual effect. Effects of the DGAT1 K232A mutation were estimated using the same model, but extended with effect g: the fixed effect of the DGAT1 genotype (KK, KA, AA). Ungenotyped individuals were included as a separate group, and appeared to be random.

The variance-covariance structure of the additive genetic effects was $Var(U)=A\sigma_U^2$, where A was a matrix of additive genetic relationships between individuals and σ_U^2 was the additive genetic variance. Heritabilities were estimated using univariate analyses, and phenotypic and genetic correlations were estimated using bivariate analyses.

2.3 Results

The fat composition of winter milk samples was measured in 1,918 Dutch Holstein Friesian cows in their first lactation. The average milk-fat percentage was 4.36 (Table 2.1). The most abundant fatty acid was C16:0, which accounted for about 33% of total fat (Table 2.2). The unsaturated C18 fatty acids (C18u), of which oleic acid (C18:1*cis*9) was the principal one, account for more than 21% of total fat. *Trans* fatty acids contributed 1.54%. The ratio of saturated to unsaturated fatty acids (SFA/UFA) averaged 2.80, meaning that saturated fatty acids accounted for about 70% of total fat. The coefficient of variation (CV) was highest (28%) for conjugated linoleic acid (CLA) and *trans* fatty acids, and lowest (about 9%) for C4:0-C12:0, C14:0, and C16:0.

Table 2.1 Mean, coefficient of variation (CV), heritability (h^2) and additive genetic standard deviation (σ_U) of milk-production traits, measured on a single morning milk sample of 1,918 first-lactation Dutch Holstein Friesian cows

Trait	Mean	CV (%)	h^2 (SE)	σ_U
Milk yield (kg)	13.47	20	0.41 (0.10)	1.43
Fat yield (kg)	0.58	19	0.39 (0.10)	0.06
Protein yield (kg)	0.47	19	0.23 (0.08)	0.03
Fat (%)	4.36	16	0.51 (0.10)	0.49
Protein (%)	3.51	9	0.65 (0.12)	0.22

Table 2.2 Mean, coefficient of variation (CV), heritability (h^2) and additive genetic standard deviation (σ_U) of groups of fatty acids, measured on a single morning milk sample of 1,918 first-lactation Dutch Holstein Friesian cows

Trait	Mean (% w/w)	CV (%)	h^2 (SE)	σ_U
C4:0-C12:0[1]	14.24	9	0.59 (0.11)	0.83
C14:0	11.62	8	0.59 (0.11)	0.66
C16:0	32.61	9	0.43 (0.11)	1.57
C18:0	8.73	16	0.23 (0.07)	0.61
C18u[2]	21.58	11	0.26 (0.09)	1.02
CLA[3]	0.39	28	0.42 (0.09)	0.05
Trans[4]	1.54	28	0.20 (0.08)	0.15
SFA/UFA[5]	2.80	13	0.28 (0.09)	0.16

[1] C4:0-C12:0 includes saturated fatty acids C4:0, C6:0, C8:0, C10:0 and C12:0.
[2] C18u includes unsaturated C18 fatty acids: C18:1*trans*6, C18:1*trans*9, C18:1*trans*11, C18:1*cis*9, C18:1*cis*11, C18:2*cis*9,12, C18:3*cis*9,12,15.
[3] CLA: C18:2*cis*9,*trans*11.
[4] *Trans* includes C16:1*trans*9, C18:1*trans*4-8, C18:1*trans*9, C18:1*trans*10, C18:1*trans*11, C18:1*trans*12.
[5] SFA (saturated fatty acids): C4:0, C5:0, C6:0, C7:0, C8:0, C9:0, C10:0, C11:0, C12:0, C13:0, C14:0, C15:0, C16:0, C17:0, C18:0; UFA (unsaturated fatty acids): C10:1, C12:1, C14:1, C16:1, C18u, CLA.

We computed heritabilities to estimate what proportion of total phenotypic variation is additive genetic variation, i.e. heritable. Heritability for fat percentage was high (0.51), meaning that about half of the total variation was additive. Short and medium chain fatty acids (C4-C16) also had high heritabilities, ranging from 0.43 to 0.59. Saturated and unsaturated C18 showed lower heritabilities (around 0.25), as did the *trans* fatty acids (0.20).

Heritabilities indicate that genetic variation for milk-fat composition is present and, therefore, that selective breeding to alter composition is possible. We computed genetic correlations to estimate to what extent different traits are influenced by the same genes (Table 2.3). Fat percentage showed a positive genetic correlation with C16:0 (0.65) and a negative

Table 2.3 Phenotypic[1] (below diagonal) and genetic (above diagonal, SE in parentheses) correlations between groups of fatty acids, fat percentage, and fat yield

Trait	C4:0-C12:0	C14:0	C16:0	C18:0	C18u	CLA	Fat %	Fat yield
C4:0-C12:0		0.63 (0.09)	-0.48 (0.15)	-0.22 (0.19)	-0.37 (0.17)	0.07 (0.17)	0.20 (0.16)	0.35 (0.17)
C14:0	0.66		-0.84 (0.08)	-0.34 (0.18)	0.23 (0.19)	0.33 (0.16)	-0.43 (0.13)	-0.11 (0.18)
C16:0	-0.22	-0.35		0.27 (0.23)	-0.53 (0.15)	-0.59 (0.13)	0.65 (0.11)	0.18 (0.20)
C18:0	-0.08	-0.22	-0.28		-0.41 (0.23)	-0.58 (0.16)	0.01 (0.20)	0.18 (0.22)
C18u	-0.45	-0.23	-0.66	0.06		0.71 (0.12)	-0.72 (0.12)	-0.35 (0.20)
CLA	-0.22	-0.02	-0.34	-0.35	0.58		-0.58 (0.12)	-0.30 (0.18)
Fat %	0.10	-0.27	0.43	0.08	-0.42	-0.32		0.51 (0.14)
Fat yield	0.23	0.01	0.22	-0.05	-0.29	-0.22	0.45	

[1] Standard errors of phenotypic correlations were between 0.02 and 0.03.

genetic correlation with unsaturated C18 (-0.72), CLA (-0.58), and C14:0 (-0.43). These results imply that selection for an increased milk-fat percentage will lead to a correlated response in fat composition; increased fat percentage will lead to a correlated increase in the fraction of C16:0 and a correlated decrease in the fractions of unsaturated C18, CLA and C14:0. Genetic correlations for fat yield showed similar directions as genetic correlations for fat percentage. C16:0, which was the most abundant and most unfavorable fatty acid from a nutritionist's point of view, showed a high negative genetic correlation with C14:0 (-0.84), CLA (-0.59), and unsaturated C18 (-0.53).

Table 2.4 Effect of the DGAT1 K232A polymorphism on milk production traits

Trait	KK (n=289)	KA[1] (SE) (n=829)	AA[2] (SE) (n=644)	P value[3]	$r^2_{genetic}$%[4]
Milk yield (kg)	0	0.84 (0.16)	1.46 (0.18)	<0.001	22
Fat yield (kg)	0	-0.02 (0.01)	-0.07 (0.01)	<0.001	22
Protein yield (kg)	0	0.02 (0.01)	0.02 (0.01)	<0.001	14
Fat (%)	0	-0.45 (0.04)	-0.98 (0.04)	<0.001	50
Protein (%)	0	-0.10 (0.02)	-0.25 (0.02)	<0.001	22

[1] contrast of KA-KK genotypes.
[2] contrast of AA-KK genotypes.
[3] statistical significance of the DGAT1 K232A effect.
[4] percentage of the genetic variance explained by the DGAT1 K232A polymorphism.

To study the effect of the DGAT1 K232A polymorphism, a total of 1,762 cows was genotyped for this polymorphism. The frequency of 232K was 0.40. The estimated effects of this polymorphism on milk-production traits are in Table 2.4. 232K was associated with increased fat percentage, protein percentage and fat yield, whereas it was associated with decreased milk yield and protein yield. Interestingly, 232K led to an increase in the fraction of C16:0 and the ratio SFA/UFA, whereas it led to a decrease in the fractions of C14:0, unsaturated C18 and CLA (Table 2.5). The DGAT1 K232A polymorphism explained large proportions of the genetic variance: 50% for fat percentage, 53% for unsaturated C18, 40% for C16:0, and 36% for SFA/UFA. We also estimated correlations between fat composition and fat

percentage based on the effects of the DGAT1 K232A polymorphism. This showed that the effects of the DGAT1 K232A polymorphism were in line with the genetic correlations from Table 2.3.

Table 2.5 Effect of the DGAT1 K232A polymorphism on fatty acid composition

Trait	KK (n=289)	KA[1] (SE) (n=829)	AA[2] (SE) (n=644)	P value[3]	$r^2_{genetic}$%[4]
C4:0-C12:0	0	0.16 (0.07)	0.03 (0.08)	0.05	1
C14:0	0	0.43 (0.06)	0.79 (0.06)	<0.001	23
C16:0	0	-1.02 (0.16)	-2.52 (0.17)	<0.001	40
C18:0	0	-0.18 (0.09)	-0.10 (0.10)	0.18	1
C18u	0	0.80 (0.14)	2.12 (0.15)	<0.001	53
CLA	0	0.02 (0.01)	0.05 (0.01)	<0.001	16
Trans	0	-0.01 (0.02)	0.04 (0.03)	0.03	2
SFA/UFA	0	-0.11 (0.02)	-0.27 (0.02)	<0.001	36

[1] contrast of KA-KK genotypes.

[2] contrast of AA-KK genotypes.

[3] statistical significance of the DGAT1 K232A effect.

[4] percentage of the genetic variance explained by the DGAT1 K232A polymorphism.

2.4 Discussion

We have demonstrated the existence of substantial genetic variation in milk-fat composition within a dairy cattle population. Heritabilities were high for the short chain saturated fatty acids C4:0-C12:0 (0.59), and for the medium chain fatty acids C14:0 (0.59) and C16:0 (0.43). Heritabilities for the long chain C18 fatty acids were lower, around 0.25. This contrast may be explained by the dual origins of fatty acids in the milk. Short and medium chain fatty acids, C4:0 to C14:0 and also some C16:0, are synthesized *de novo* in the mammary gland and may be influenced by genetics. The C18 fatty acids and, to a lesser extent, C16:0 arise from the cow's plasma lipids and have a dietary origin (Palmquist, 2006); they are, therefore, likely to be under less genetic control. Heritability for *trans* fatty acids was also low (0.20). This low heritability may be because *trans* fatty acids are produced by microbial biohydrogenation in the rumen of the cow (Chilliard et al., 2000), which may be under less genetic control as well. Heritabilities for milk, protein and fat yield and protein and fat percentage are generally in line with previous studies (Fuerst and Solkner, 1994; Goddard and Wiggans,

1999; Ikonen et al., 1999; Kadarmideen et al., 2000; Mulder et al., 2004; Calus et al., 2005; Hinrichs et al., 2005). Genetic parameters for fat composition were reported only previously by Karijord et al. (1982), who estimated lower heritabilities (0.11-0.17 for short chain, 0.07 for C14:0, 0.15 for C16:0 and 0.06-0.15 for long chain fatty acids).

Estimated effects of the DGAT1 K232A polymorphism on fat percentage, protein percentage, and yield traits are consistent with previous studies (Grisart et al., 2002; Spelman et al., 2002; Thaller et al., 2003a). 232K increases fat percentage, protein percentage, and fat yield, whereas it decreases milk yield and protein yield. Ours is the first report on the effects of the DGAT1 K232A polymorphism on milk-fat composition. We show that 232K is associated with a larger fraction of C16:0; smaller fractions of C14:0, unsaturated C18, and CLA; and a higher ratio SFA/UFA. DGAT1 catalyzes the last step in triglyceride synthesis: the esterification of a fatty acyl-CoA to the *sn*-3 position of a diacylglycerol. The effect of the DGAT1 K232A polymorphism on fat composition may have different causes: a higher activity of DGAT1 and alteration of specificity of DGAT1. For the first, using a baculovirus expression system, 232K has been shown to have a higher V_{max} than 232A in producing triglycerides, which is consistent with the *in vivo* effect of the K232A polymorphism (Grisart et al., 2004). Furthermore, the mathematical model of Shorten et al. (2004) predicted that an increase in fat yield because of 232K corresponds with a 120% increase in the DGAT1 acylation rate and, consequently, is associated with a more saturated fatty acid composition. For the second, the specificity of the DGAT1 enzyme could be altered by the K232A polymorphism. Fatty acids in milk-fat are not distributed randomly on the glycerol backbone. The *sn*-1 position is occupied predominantly by C16:0 and C18:1; the *sn*-2 position predominantly by saturated fatty acids; and the *sn*-3 position predominantly by short chain and unsaturated fatty acids (Parodi, 1982). This non-random distribution is related to the actions of the acyltransferases. Glycerol-3-phosphate acyltransferase (GPAT), which catalyzes the esterification of fatty acyl-CoA to the *sn*-1 position of glycerol-2-phosphate, utilizes saturated and unsaturated fatty acyl-CoA with equivalent affinity. However, lysophosphatidic acid acyltransferase (LPAT) from cells from the bovine

mammary gland, which catalyzes the esterification of fatty acyl-CoA to the *sn*-2 position, has greater affinity for saturated than unsaturated fatty acyl-CoA. DGAT1 is suggested to prefer short chain and unsaturated fatty acids (Kinsella, 1976; Morand et al., 1998; Mistry and Medrano, 2002). This preference suggests specificity of the DGAT1 enzyme, which could be altered by the K232A polymorphism resulting in a change in fat composition.

Genetic correlations show that an increase in fat percentage implies an increase in the fraction of C16:0, while decreasing the fractions of unsaturated C18, CLA, and C14:0. It is likely that selection in the past decades in the Dutch dairy has resulted not only in increased fat percentage and fat yield, but also in a more saturated fat composition, with more C16:0, less unsaturated C18, less CLA, and less C14:0. Our results show that it is possible to change fat composition of milk-fat by selective breeding, and that efficiency of selective breeding can be improved using the K232A polymorphism in DGAT1. From a public health point of view, increasing the frequency of the DGAT1 232A variant is desirable because of its association with more unsaturated milk-fat, less C16:0, and more unsaturated C18. An increase of the frequency of 232A could lead to a decrease of the C16:0 fraction and an increase in the unsaturated C18 fraction of approximately 5% to 10%. Such a change in milk-fat composition can be relevant because milk and milk-derived foods are large contributors to saturated fatty acid intake.

2.5 Acknowledgments

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3

Milk fatty acid unsaturation: genetic parameters and effects of SCD1 and DGAT1

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Abstract

With regard to human health aspects of milk-fat, increasing the amount of unsaturated fatty acids in milk is an important selection objective. The cow's diet has an influence on the degree of unsaturation, but literature suggests that genetics also plays a role. To estimate genetic variation in milk fatty acid unsaturation indices, milk fatty acid composition of 1,933 Dutch Holstein Friesian heifers was measured and unsaturation indices were calculated. An unsaturation index represents the concentration of the unsaturated product proportional to the sum of the unsaturated product and the saturated substrate. Intraherd heritabilities were moderate, ranging from 0.23±0.07 for CLA index to 0.46±0.09 for C16 index. We genotyped the cows for the SCD1 A293V and DGAT1 K232A polymorphisms, which are known to alter milk fatty acid composition. Both genes explain part of the genetic variation in unsaturation indices. The SCD1 V allele is associated with lower C10, C12 and C14 indices, and with higher C16, C18 and CLA indices in comparison to the SCD1 A allele, with no differences in total unsaturation index. In comparison to the DGAT1 K allele, the DGAT1 A allele is associated with lower C10, C12, C14 and C16 indices, and with higher C18, CLA and total indices. We conclude that selective breeding can contribute to higher unsaturation indices, and that selective breeding can capitalize on genotypic information of both the SCD1 A293V and the DGAT1 K232A polymorphism.

3.1 Introduction

Recent studies show that bovine milk fatty acid composition is determined to a large extent by genetics, indicating that selective breeding can be an effective means to alter the composition of milk-fat (Chapter 2; Soyeurt et al., 2007). Milk-fat is characterized by a high amount of saturated fatty acids, especially myristic acid (C14:0) and palmitic acid (C16:0), and by a low amount of (poly)unsaturated fatty acids. With regard to human health aspects, increasing the amount of unsaturated fatty acids in milk is an important selection objective.

The cow's diet plays a role in determining the degree of unsaturation of milk-fat (Baumgard et al., 2000; Perfield et al., 2006; Perfield et al., 2007). Dietary fatty acids are hydrogenated in the rumen by bacteria and transported via the blood. In the mammary gland fatty acids originating from the blood or from *de novo* fatty acid synthesis can be desaturated. Eventually, the fatty acids that are secreted into the milk determine the degree of unsaturation of milk-fat. This degree of unsaturation is often addressed by a so-called index: the concentration of the unsaturated product proportional to the sum of the unsaturated product and the saturated substrate.

Studies demonstrating a significant variation in unsaturation among breeds and cows on the same diet suggest that also genetics plays a role (Beaulieu and Palmquist, 1995; DePeters et al., 1995; Lawless et al., 1999; Sol Morales et al., 2000; Drackley et al., 2001; Lock and Garnsworthy, 2002; Kelsey et al., 2003; Lock and Garnsworthy, 2003). For example, Kelsey et al. (2003) found that the milk-fat content of C18:2*cis*9,*trans*11 (CLA) and the CLA index varied largely among individual cows on the same diet, namely over threefold. Lock and Garnsworthy (2002 and 2003) reported that the C14:1/C14:0 index differed significantly between cows. However, genetic parameters for milk fatty acid unsaturation indices are scarce in literature (Royal and Garnsworthy, 2005).

Animals are capable of desaturating saturated fatty acids to Δ9 unsaturated fatty acids by the stearoyl-CoA desaturase (SCD) enzyme, which catalyzes

the insertion of a double bond between carbon atoms 9 and 10 of a fatty acid (Pereira et al., 2003). Two SCD isoforms have been identified in cattle, SCD1 and SCD5. SCD1 is located on chromosome 26 and expressed in a variety of tissues among which are adipose and mammary tissue, and SCD5 is located on chromosome 6 and expressed primarily in the brain (Chung et al., 2000; Lengi and Corl, 2007). A non-synonymous SNP in exon 5 of *SCD1*, causing the substitution of valine with alanine (A293V), has been associated with carcass fatty acid composition in Japanese Black cattle (Taniguchi et al., 2004) and with milk fatty acid composition in Italian Holstein, Piedmontese and Valdostana cattle (Mele et al., 2007; Moioli et al., 2007). The SCD1 A allele was associated with a higher monounsaturated fatty acids (MUFA) content.

Another candidate gene that may affect unsaturation is acyl CoA:diacylglycerol acyltransferase 1 (DGAT1), which is located on chromosome 14 (Grisart et al., 2002). The DGAT1 enzyme plays a key role in triacylglycerol synthesis; it catalyzes the esterification of a fatty acyl-CoA to the *sn*-3 position of a diacylglycerol. A lysine to alanine polymorphism in DGAT1 (K232A) explains 50% of the genetic variation in milk-fat percentage and also has a strong effect on milk fatty acid composition. The DGAT1 K allele was associated with a larger fraction of C16:0; and smaller fractions of C14:0, unsaturated C18 and CLA (Chapter 2).

Our study aims to estimate genetic variation for milk fatty acid unsaturation indices for specific fatty acids and their phenotypic and genetic correlations. Furthermore, we study the effects of polymorphisms in 2 candidate genes, namely SCD1 A293V and DGAT1 K232A, on milk fatty acid unsaturation indices.

3.2 Materials and methods

Animals

This study is part of the Dutch Milk Genomics Initiative, which focuses on the genetic background of detailed milk composition. As part of this study, morning milk samples and blood samples were collected from 1,933 first-lactation cows on 398 commercial herds in the Netherlands. A 0.5 liter milk

sample was collected from each cow at 1 morning milking between February and March 2005. At least 3 cows per herd were sampled and cows were milked twice a day. Cows descended from 1 of 50 young bulls (845 cows), from 1 of 5 proven bulls (897 cows), or from other proven bulls (191 cows). The NRS (Arnhem, the Netherlands) provided pedigrees of the cows and the milk yield records. Total pedigree size was 26,300 records. Each cow was over 87.5% Holstein Friesian and was in lactation between day 63 and day 282.

Phenotypes

Fat and protein percentages were measured by infrared spectroscopy, using a MilkoScan FT6000 (Foss Electric, Hillerod, Denmark) at the Milk Control Station (Zutphen, the Netherlands). Fat and protein yields were calculated by multiplying fat or protein percentage by the morning milk yield. Milk yield (kg) data were missing for 145 cows. Milk fatty acid composition was measured by gas chromatography at the COKZ laboratory (Netherlands Controlling Authority for Milk and Milk Products, Leusden, the Netherlands) as described in Chapter 2. With this method, the C18:1*cis*9 peak was probably slightly overestimated due to coelution (Jensen, 2002). The *cis* double bond of C10:1 and C12:1 could not be ascertained at the carbon-9 position. The fatty acids were expressed as weight-proportion of total fat weight. Fatty acid unsaturation indices were calculated by expressing each product as a proportion of the product plus substrate, multiplied by 100 (Kelsey et al., 2003):

$$\frac{\text{unsaturated}}{\text{unsaturated} + \text{saturated}} * 100, \text{ e.g. C14 index} = \frac{\text{C14:1}cis9}{\text{C14:1}cis9 + \text{C14:0}} * 100$$

We calculated indices for the following product and substrate pairs: C10:1 and C10:0 (C10 index), C12:1 and C12:0 (C12 index), C14:1*cis*9 and C14:0 (C14 index), C16:1*cis*9 and C16:0 (C16 index), C18:1*cis*9 and C18:0 (C18 index), C18:2*cis*9,*trans*11 (CLA) and C18:1*trans*11 (CLA index).

Genotypes

Blood samples for DNA isolation were collected between April and June 2005. Genotypes for the SCD1 A293V polymorphism were assayed by the SNaPshot single base primer extension method (Applied Biosystems, Foster City, CA). The primer designs were based on the Genbank sequence (AY241932): forward PCR primer, 5'- TCATTTAACCCCTCATTACCTCA -3'; reverse PCR primer, 5'- TGTAAAATACTAGGCTTTCTGG -3'; genotyping primer, 5'- TGGTTTCCCTGGGAGCTG - 3'. To amplify the SCD1 fragment, 12 µl PCR reactions were set up containing 20 ng of genomic DNA, 0.2 µM of each primer and 2X AccuPrime Supermix II (Invitrogen, Carlsbad, CA). PCR cycling conditions were 94°C for 5 min, 36 cycles of 94°C for 30 s, 55°C for 45 s, 68°C for 90 s, followed by an extension cycle of 68°C for 10 min. PCR products were purified by incubation with shrimp alkaline phosphatase (SAP) (USB, Cleveland, OH) and Exo I (USB) at 37°C for 1 h and 72°C for 15 min. Extension reactions, using 3 µl of purified PCR product and 5 pmol of genotyping primer and SNaPshot multiplex Ready reaction mix (Applied Biosystems), were performed using 40 cycles of 96°C for 10 s, 50°C for 5 s, and 60°C for 30 s. The extension products were incubated with SAP at 37°C for 1 h and 72°C for 15 min. Two microliters of extension product were added to 8 µl of Hi-Di formamide and electrophoresed on an ABI 3730 DNA analyzer. Results were analyzed using the GeneMapper Software v4.0 (Applied Biosystems). In total, 1,725 animals were genotyped for the SCD1 A293V polymorphism. Genotypes were missing for 208 animals because either no DNA sample was available (n=141) or the sample could not be genotyped unambiguously (n=67). Genotyping of the DGAT1 K232A dinucleotide polymorphism was performed as described in Chapter 2. In total, 1,779 animals were genotyped for the DGAT1 polymorphism. Genotypes were missing for 154 animals because either no DNA sample was available (n=141) or the sample could not be genotyped unambiguously (n=13).

Statistical analysis

Analyses were performed first using SAS 9.1 (2002) procedures to determine significance of fixed effects. The model included days in milk (days between calving and date of sample), age at first calving, season of

calving, and an effect of the differences in genetic level between proven bull daughters and young bull daughters. Variance components and genetic parameters were estimated using an Animal Model in ASReml (Gilmour et al., 2002):

$$y_{ijklmn} = \mu + b_1 * dim_i + b_2 * e^{-0.05 * dim} + b_3 * afc_j + b_4 * afc_j^2 + season_k + scode_l + herd_m + a_n + e_{ijklmn},$$

where y was the dependent variable, μ was the general mean, dim was the covariate describing the effect of days in milk modelled with a Wilmink curve (Wilmink, 1987), afc was the covariate describing the effect of age at first calving, season was the fixed effect of the class of calving season (June-August 2004, September-November 2004, or December 2004-February 2005), scode was the fixed effect of the differences in genetic level between proven bull daughters and young bull daughters, herd was the random effect of groups of animals sampled in the same herd, a_n was the random additive genetic effect of animal n, and e was the random residual effect. Effects of the polymorphisms were estimated using the same model, but extended with effect g: the fixed effect of the SCD1 genotype (AA, AV or VV) or the DGAT1 genotype (KK, KA or AA). Ungenotyped individuals were included as a separate group, and appeared to be randomly distributed across other effects in the model.

The variance-covariance structure of the additive genetic effects was $Var(a)=A\sigma_a^2$, where A was a matrix of additive genetic relationships between individuals and σ_a^2 was the additive genetic variance. Heritabilities were estimated using univariate analyses. Heritability was defined as:

$$h^2_{IH} = \frac{\sigma^2_a}{\sigma^2_a + \sigma^2_e},$$

where σ^2_a was the additive genetic variation, and σ^2_e was the residual variation. Heringstad et al. (2006) referred to this heritability as the intraherd heritability. The intraherd heritability is the parameter that is required to predict selection responses of alternative breeding strategies. The proportion of variance due to herd (h_{herd}) was defined as:

$$h_{herd} = \frac{\sigma^2_{herd}}{\sigma^2_{herd} + \sigma^2_a + \sigma^2_e},$$

where σ^2_{herd} was the herd variance. The proportion of additive genetic variance explained by the polymorphism was defined as:

$$r^2_{genetic} = \frac{\sigma^2_{polymorphism}}{\sigma^2_a},$$

where $\sigma^2_{polymorphism}$ was calculated by estimating the variance of the polymorphism based on the estimated genotype effects and the genotype frequencies. Corrected means for the reference group in Tables 3.3-3.5 were estimated by the PREDICT function in ASReml. Genetic correlations were estimated using bivariate analyses. Estimates of skewness and kurtosis for the fatty acid indices are in the same range as the estimates for milk production traits.

3.3 Results and discussion

Table 3.1 Mean, coefficient of variation (CV), 5% and 95% quantiles for fatty acid unsaturation indices, measured on 1 morning milk sample of 1,933 first-lactation Dutch Holstein Friesian cows

Trait	Mean	CV (%)	5% quantile	95% quantile
C10 index[1]	10.9	17	6.6	14.9
C12 index[1]	2.7	20	1.6	3.9
C14 index[1]	10.5	17	7.0	14.6
C16 index[1]	4.2	19	2.9	6.3
C18 index[1]	67.6	6	59.5	75.3
CLA index[2]	33.7	12	25.4	42.8
Total index[3]	26.4	10	21.5	33.1

[1] Indices are calculated according to the following example: C14 index = C14:1*cis*9/(C14:1*cis*9 + C14:0) *100.

[2] CLA index = CLA*cis*9,*trans*11/(CLA*cis*9,*trans*11 + C18:1*trans*11)*100.

[3] Total index = (C10:1 + C12:1 + C14:1*cis*9 + C16:1*cis*9 + C18:1*cis*9 + CLA*cis*9,*trans*11)/(C10:1 + C12:1 + C14:1*cis*9 + C16:1*cis*9 + C18:1*cis*9 + CLA*cis*9,*trans*11 + C10:0 + C12:0 + C14:0 + C16:0 + C18:0 + C18:1*trans*11)*100.

Means and coefficients of variation

Fatty acid unsaturation indices were calculated from milk fatty acid profiles of 1,933 Dutch Holstein Friesian heifers (Table 3.1). Unsaturation indices of the short and medium chain fatty acids C10, C12, C14 and C16 were between 2.7 and 10.9. Indices of the long chain fatty acids C18 and CLA were higher, 67.6 and 33.7 respectively. These values are in line with those reported for dairy cattle in other studies (Royal and Garnsworthy, 2005; Perfield et al., 2006; Mele et al., 2007; Perfield et al., 2007). Almost all C10, C12, C14 and approximately 50% of C16 fatty acids are synthesized in the mammary gland, whereas the longer chain fatty acids as well as a proportion of the unsaturated long chain fatty acids are derived from the blood. The dual origin of the long chain fatty acid unsaturation may play a role in the contrast between short/medium and long chain fatty acid unsaturation indices. An alternative explanation for this contrast may be that long chain fatty acids are unsaturated to a larger extent. The coefficient of variation was lowest for C18 index (6%) and highest for C12 and C16 indices (about 20%).

Table 3.2 Intraherd heritability (h_{IH}^2), additive genetic variance estimate (σ^2_a), herd effect (h_{herd}), and ratio between additive genetic variance and herd variance (σ^2_a/ σ^2_{herd}) for fatty acid unsaturation indices, measured on 1 morning milk sample of 1,933 first-lactation Dutch Holstein Friesian cows

Trait	h_{IH}^2 (SE)	h_{herd} (SE)	σ^2_a	$\sigma^2_a/\sigma^2_{herd}$
C10 index	0.37 (0.09)	0.06 (0.02)	1.23	5.5
C12 index	0.37 (0.09)	0.06 (0.02)	0.09	5.6
C14 index	0.45 (0.09)	0.06 (0.02)	1.35	6.6
C16 index	0.46 (0.09)	0.07 (0.02)	0.30	6.2
C18 index	0.33 (0.08)	0.06 (0.02)	4.36	5.1
CLA index	0.23 (0.07)	0.09 (0.02)	3.49	2.5
Total index	0.30 (0.09)	0.26 (0.02)	1.58	0.8

Heritabilities and herd effects

Intraherd heritabilities range from 0.23 for CLA index to 0.46 for C16 index, and demonstrate a significant genetic effect on the variation in fatty acid unsaturation indices (Table 3.2). The heritability for total index was 0.30. Repeatabilities for C14, C16 and C18 indices, which are considered to be the upper limit of heritabilities, were estimated between 40 and 45% by Soyeurt

et al. (2006), suggesting moderate heritabilities as well. Only Royal and Garnsworthy (2005) reported heritabilities for fatty acid unsaturation indices, based on 1,520 Holstein Friesian cows, and reported similar values for C14 (0.30), C18 (0.19) and CLA (0.29) indices, but much lower values for C16 (0.01) and total (0.02) indices. The proportion of total variance explained by herd was small, ranging from 0.06 to 0.09 of total variance in individual unsaturation indices. For total unsaturation index the herd effect was larger (0.26). The ratio of genetic variance to herd variance showed that for all indices the genetic variance was much larger than the herd variance, except for total index, for which the herd variance was slightly larger than the genetic variance. The total unsaturation index, in fact, mainly represents the ratio of C18:1*cis*9 to C16:0, because these fatty acids are the largest saturated and unsaturated fatty acid fractions in milk. The proportion of variance explained by herd was 0.28 for C18:1*cis*9 fraction and 0.29 for C16:0 fraction in milk, which explains the relative large herd variance of the total unsaturation index (Stoop et al., 2008). The moderate to high heritabilities for unsaturation indices in combination with the

Table 3.3 Effect of the SCD1 A293V polymorphism on milk fatty acid composition (g/100g of total fatty acids)

Trait	Predicted mean of AA group	AA (n=919)	VA[1] (n=689)	VV[2] (n=117)	P value [3]
C10:0	2.95	0	0.10 (0.02)	0.16 (0.04)	<0.001
C10:1	0.38	0	-0.03 (0.00)	-0.06 (0.01)	<0.001
C12:0	4.09	0	0.09 (0.03)	0.15 (0.06)	0.003
C12:1	0.12	0	-0.01 (0.00)	-0.02 (0.00)	<0.001
C14:0	11.38	0	0.22 (0.04)	0.42 (0.09)	<0.001
C14:1*cis*9	1.46	0	-0.17 (0.01)	-0.33 (0.02)	<0.001
C16:0	32.67	0	-0.12 (0.13)	-0.25 (0.25)	0.58
C16:1*cis*9	1.38	0	0.17 (0.02)	0.34 (0.03)	<0.001
C18:0	8.83	0	-0.30 (0.07)	-0.43 (0.13)	<0.001
C18:1*cis*9	18.28	0	0.08 (0.09)	0.17 (0.18)	0.55
C18:1*trans*11	0.77	0	-0.02 (0.01)	-0.03 (0.02)	0.01
CLA*cis*9,*trans*11	0.38	0	0.01 (0.00)	0.02 (0.01)	<0.003

[1] Contrast of VA-AA genotypes.

[2] Contrast of VV-AA genotypes.

[3] Statistical significance of the SCD1 A293V polymorphism.

moderate to high coefficients of variation indicate that the unsaturation indices can be changed by means of selection.

Effects of the SCD1 A293V polymorphism

To study the effect of the SCD1 A293V polymorphism, 1,725 cows were genotyped. The frequency of 293A was 0.73 and the genotypes were in Hardy-Weinberg equilibrium. A higher frequency of the A allele is also reported for Italian Holsteins (0.57), Valdostana (0.65), Jerseys (0.94) and Japanese Black cattle (0.59) (Taniguchi et al., 2004; Mele et al., 2007; Moioli et al., 2007). The SCD1 genotype did not significantly affect fat or protein percentage, nor fat, protein or milk yield (results not shown). Effects of the SCD1 genotype on milk fatty acid composition are in Table 3.3. In comparison to the A allele, the V allele was associated with a higher proportion of C10:0, C12:0, C14:0, C16:1 and CLA, and with a lower proportion of C10:1, C12:1, C14:1, C18:0 and C18:1*trans* 11. Moioli et al. (2007), who studied 27 Piedmontese and 27 Valdostana cows, found similar results, but no significant effects on C10:0 and C12:0. Mele et al. (2007), who studied 297 Italian Holstein Friesian cows, only found significant effects on C14:1 and on total monounsaturated fatty acids (MUFA), similar to ours, and on C18:1*cis*9, on which we found no significant effect.

Table 3.4 Effect of the SCD1 A293V polymorphism on milk fatty acid unsaturation indices

Trait	Predicted mean of AA group	AA (n=919)	VA[1] (n=689)	VV[2] (n=117)	P value[3]	$r^2_{genetic}$ (%)[4]
C10 index	11.68	0	-1.18 (0.09)	-2.16 (0.17)	<0.001	41
C12 index	2.92	0	-0.29 (0.02)	-0.56 (0.05)	<0.001	34
C14 index	11.42	0	-1.34 (0.08)	-2.61 (0.16)	<0.001	52
C16 index	4.05	0	0.48 (0.04)	0.98 (0.08)	<0.001	31
C18 index	67.57	0	0.87 (0.19)	1.43 (0.37)	<0.001	6
CLA index	33.56	0	1.32 (0.20)	2.39 (0.40)	<0.001	18
Total index	26.63	0	0.05 (0.13)	0.10 (0.25)	0.85	<1

[1] Contrast of VA-AA genotypes.

[2] Contrast of VV-AA genotypes.

[3] Statistical significance of the SCD1 A293V polymorphism.

[4] Percentage of genetic variance explained by the SCD1 A293V polymorphism.

The SCD1 A293V polymorphism had significant effects on the unsaturation indices for all individual fatty acids, but not on the total unsaturation index (Table 3.4). The V allele was associated with lower indices of C10, C12 and C14, and higher indices of C16, C18 and CLA. The negative effect of the V allele on C10 and C14 index is in agreement with Moioli et al. (2007); however, they reported no significant effects on the other indices, which could be due to their limited sample size. The negative effect on C14 index was also found by Mele et al. (2007), but they didn't find a significant effect on other indices.

The finding that the SCD1 polymorphism has highly significant effects on individual indices, but not on the total index suggests that the activity of the enzyme is not affected. An altered substrate specificity, however, ís supported by the differential effects on the individual fatty acid indices. Substrate specificity of SCD enzymes has been demonstrated for SCD isoforms in knockout studies in mice (Miyazaki and Ntambi, 2003). *In vitro* studies in rat liver microsomal preparations have shown that acyl-CoA derivatives with 12 to 19 carbon atoms were required for activity of the SCD1 enzyme and that the enzyme has substrate specificity with preference for longer chain fatty acids (Enoch et al., 1976). Based on Enoch et al. (1976) it would be unlikely that C10:1 is desaturated from C10:0, which is, however, suggested by our finding that the C10 index is affected by the SCD1 A293V polymorphism. The enzyme function of SCD1 may be affected by the polymorphism, since it causes a valine to alanine substitution on position 293, which is located in the third histidine-rich region of the enzyme. These histidine-rich regions are important for catalytic activity (Shanklin et al., 1994). The SCD1 A293V polymorphism contributed considerably to the genetic variance (Table 3.4): it explained 31% to 52% of the genetic variance for C10, C12, C14 and C16 indices, but only 18% for CLA and 6% for C18 index (Mosley and McGuire, 2007).

Effects of the DGAT1 K232A polymorphism

A total of 1,779 cows was genotyped for the DGAT1 K232A polymorphism. The frequency of 232K was 0.40 and the genotypes were in Hardy-Weinberg equilibrium. The DGAT1 A allele was associated with lower indices of C10,

C12, C14 and C16, and with higher indices of C18 and CLA (Table 3.5). The A allele was also associated with a higher total unsaturation index. The vast majority of fatty acids in milk are present in the form of triacylglycerols. DGAT1 catalyzes the last step in triacylglycerol synthesis: the esterification of a fatty acyl-CoA to the *sn*-3 position of a diacylglycerol. The DGAT1 K232A polymorphism was reported to have an effect on, among others, C14:0, C16:0 and unsaturated C18 fatty acids in milk. Given these effects of the polymorphism on milk fatty acid composition, an effect on milk fatty acid unsaturation indices was conceivable. The effects on composition and unsaturation may be explained by different causes: a higher activity of DGAT1 and an alteration of specificity of DGAT1 (Chapter 2).

Table 3.5 Effect of the DGAT1 K232A polymorphism on milk fatty acid unsaturation indices

Trait	Predicted mean of KK group	KK (n=293)	KA[1] (n=837)	AA[2] (n=649)	P value[3]	$r^2_{genetic}$ (%)[4]
C10 index	11.54	0	-0.29 (0.12)	-0.54 (0.13)	<0.001	3
C12 index	2.92	0	-0.09 (0.03)	-0.20 (0.04)	<0.001	5
C14 index	11.47	0	-0.47 (0.11)	-0.97 (0.12)	<0.001	9
C16 index	4.57	0	-0.25 (0.05)	-0.56 (0.06)	<0.001	14
C18 index	66.46	0	1.23 (0.24)	2.31 (0.26)	<0.001	15
CLA index	32.87	0	1.15 (0.27)	1.90 (0.29)	<0.001	12
Total index	25.67	0	0.62 (0.16)	1.80 (0.17)	<0.001	29

[1] Contrast of KA-KK genotypes.

[2] Contrast of AA-KK genotypes.

[3] Statistical significance of the DGAT1 K232A polymorphism.

[4] Percentage of genetic variance explained by the DGAT1 K232A polymorphism.

Joint effects of the SCD1 and DGAT1 polymorphisms

The contribution of the DGAT1 K232A polymorphism to the genetic variance of unsaturation indices was lower than the contribution of the SCD1 A293V polymorphism for all indices, except for C18 and total index (Table 3.4, 3.5). When the statistical model was extended to include both the SCD1 genotype and the DGAT1 genotype as fixed effects, the effects of DGAT1 on the unsaturation indices did not change considerably, meaning that the two polymorphisms do not explain the same part of the genetic variation (results

not shown). In other words, the genetic variation explained by DGAT1 and the genetic variation explained by SCD1 is additive. Cows with the SCD1 VV genotype and the DGAT1 AA genotype (n=41) have 9%, 6% and 14% higher indices of C16, C18 and CLA, respectively, compared with cows with the SCD1 AA and DGAT1 KK genotype (results not shown, frequencies of combined genotypes in Table 3.6). This shows that selective breeding can improve when both the polymorphisms in SCD1 and DGAT1 are used in genetic selection.

Table 3.6 Number of animals per combined genotype of the SCD1 A293V and DGAT1 K232A polymorphisms

		DGAT1 K232A genotype			
		KK	**KA**	**AA**	**Total**
SCD1 A293V genotype	**AA**	159	439	316	914
	AV	106	313	264	683
	VV	16	59	41	116
	Total	281	811	621	1,713[1]

[1] 220 animals could not be genotyped for one or both polymorphisms.

Correlations

Phenotypic and genetic correlations were high and positive among the medium chain fatty acid unsaturation indices C10, C12 and C14 and among the long chain fatty acid indices C18 and CLA, ranging from 0.86 through 0.98 (Table 3.7). Genetic correlations were low between the medium chain and the long chain fatty acid indices (0.05-0.26). C16 index showed low to moderate genetic correlations with C10 (0.15), C12 (0.37) and C14 (0.24) indices. Genetic correlations of C16 index with C18 and CLA indices were also moderate, although slightly higher (0.45 and 0.60). Our observations are similar to the phenotypic correlations reported in previous studies (Peterson et al., 2002; Kelsey et al., 2003). Genetic correlations of fat percentage with C10 (0.25), C12 (0.26), C14 (0.31) and C16 (0.17) indices were low, but positive, whereas genetic correlations of fat percentage with C18 (-0.35) and CLA (-0.48) indices were negative. Accordingly, an increase in fat percentage will lead to slightly higher C10, C12, C14 and C16 indices, but to lower C18 and CLA indices. Fat percentage also showed a negative genetic correlation with total index (-0.52). This might partly reflect the

Table 3.7 Phenotypic (below diagonal) and genetic (above diagonal, SE in parentheses) correlations[1] between fatty acid unsaturation indices and milk production traits

Trait	C10 index	C12 index	C14 index	C16 index	C18 index	CLA index	Total index	Fat %	Fat yield	Protein yield	Milk yield
C10 index		0.87 (0.05)	0.92 (0.03)	0.15 (0.17)	0.14 (0.18)	0.07 (0.20)	0.33 (0.18)	0.25 (0.17)	-0.05 (0.19)	-0.14 (0.23)	-0.22 (0.19)
C12 index	0.87		0.95 (0.02)	0.37 (0.15)	0.24 (0.17)	0.26 (0.18)	0.39 (0.18)	0.26 (0.17)	-0.21 (0.19)	-0.25 (0.22)	-0.39 (0.18)
C14 index	0.86	0.94		0.24 (0.15)	0.08 (0.17)	0.05 (0.19)	0.30 (0.17)	0.31 (0.16)	-0.13 (0.18)	-0.29 (0.20)	-0.39 (0.17)
C16 index	0.34	0.48	0.44		0.45 (0.13)	0.60 (0.12)	0.44 (0.16)	0.17 (0.16)	-0.21 (0.18)	-0.32 (0.21)	-0.37 (0.17)
C18 index	0.50	0.58	0.49	0.63		0.98 (0.02)	0.83 (0.08)	-0.35 (0.16)	-0.36 (0.18)	0.17 (0.22)	0.01 (0.20)
CLA index	0.47	0.58	0.46	0.63	0.90		0.83 (0.09)	-0.48 (0.16)	-0.44 (0.19)	0.10 (0.25)	0.05 (0.22)
Total index	0.38	0.35	0.39	0.47	0.66	0.53		-0.52 (0.15)	-0.43 (0.18)	0.09 (0.25)	0.14 (0.21)
Fat %	-0.07	-0.03	0.02	0.07	-0.29	-0.28	-0.37		0.51 (0.14)	-0.29 (0.20)	-0.58 (0.13)
Fat yield	-0.03	-0.04	-0.06	-0.04	-0.13	-0.12	-0.29	0.45		0.59 (0.15)	0.38 (0.16)
Protein yield	0.09	0.11	0.03	0.01	0.20	0.20	0.01	-0.26	0.67		0.88 (0.05)
Milk yield	0.04	0.00	-0.07	-0.09	0.14	0.13	0.08	-0.47	0.57	0.89	

[1] Standard errors of phenotypic correlations were between 0.004 and 0.03.

effects of the DGAT1 K232A polymorphism, for which the K allele is known to have a very large effect on fat percentage. In this study, it has been shown that the K allele is also associated with higher C10, C12, C14 and C16 indices, and lower C18 and CLA indices. It is likely that breeding in the Dutch dairy in the past years has resulted not only in increased fat percentage and fat yield, but also in higher C10 to C16 indices, and lower C18 and CLA indices.

The moderate genetic correlation between C14 and C16 index can be partly explained by the SCD1 polymorphism (Figure 3.1). Three groups can be identified in this figure, which almost completely coincide with the 3 genotypes of SCD1 A293V (AA, AV and VV). Correction for the SCD1 polymorphism, by adding the SCD1 genotype to the model as a fixed effect, results in a higher genetic correlation between C14 and C16 index (0.93

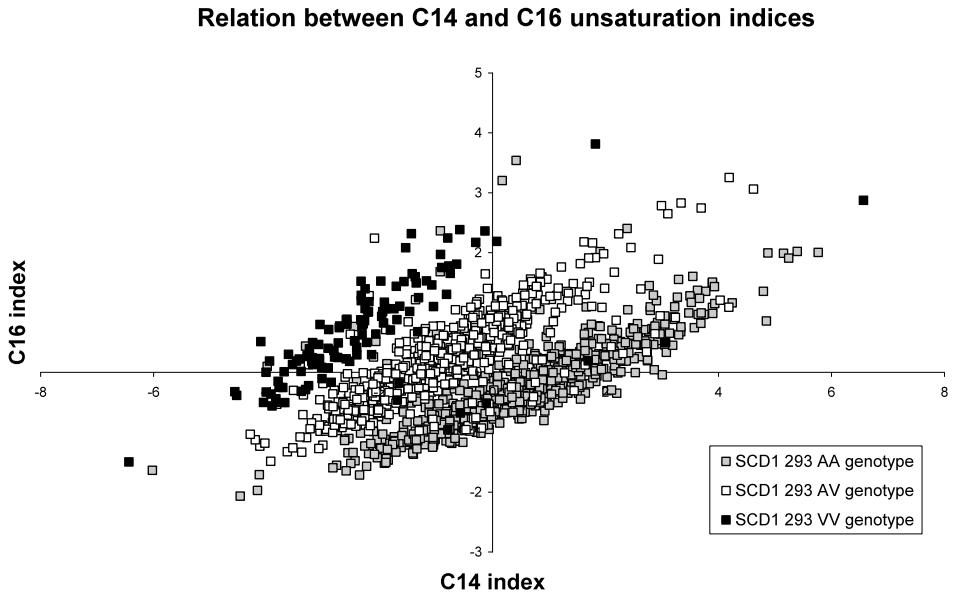

Figure 3.1 Relation between C14 and C16 unsaturation indices of individual cows. Phenotypes are corrected for DIM modeled with a Wilmink curve, age at first calving, calving season, differences in genetic level between groups of proven bull daughters and young bull daughters, and herd.

with vs. 0.24 without correction, Table 3.7), but also in higher correlations between C10 index and C16 index (0.69 vs. 0.15) and between C12 index and C16 index (0.91 vs. 0.37), and between the medium chain C10, C12 and C14 indices and the long chain C18 and CLA indices (0.2 to 0.4 higher after correction; data not shown). Thus, the correlation due to the SCD1 A293V polymorphism is negative for these indices, whereas the correlation due to other genetic effects is positive.

Impact

We have demonstrated that milk fatty acid unsaturation indices have a substantial genetic component, indicating that it is possible to change unsaturation indices by selective breeding.

Our results also show that both the SCD1 A293V and the DGAT1 K232A polymorphism explain part of the genetic variation in the unsaturation indices of milk-fat. Even though the SCD1 A293V polymorphism does not affect the overall degree of unsaturation, its effects on the individual fatty acid indices offer opportunities for improving milk fatty acid composition. Human trial studies show that not all saturated fatty acids affect cholesterol concentrations to the same extent and that some are more unfavorable than others (Mensink et al., 2003). C16:0 is the most unfavorable, hypercholesterolemic fatty acid. Therefore, an increase of certain fatty acid indices (i.e. a shift from saturated to unsaturated) at the expense of others would be beneficial.

Our study gives more insight into the process of unsaturation. Not only SCD1 plays a significant role by desaturating saturated fatty acids into unsaturated fatty acids, but also DGAT1 is important by influencing the composition of the triacylglycerols. The entire pathway of lipogenesis, which next to SCD1 and DGAT1 involves other enzymes like fatty acid synthase and acetyl-coenzyme A carboxylase, is regulated by the transcription factor sterol regulatory element-binding protein (SREBP)-1c and activated by dietary fatty acids. We made an important start to unravel the genetics of unsaturation and this could be the start of other studies looking into the interaction between genetics and feeding.

3.4 Acknowledgments

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4

Genome-wide scan for bovine milk-fat composition I. QTL for short and medium chain fatty acids

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Abstract

A genome-wide scan was performed to identify quantitative trait loci (QTL) for short and medium chain fatty acid w/w percentages. Milk samples were available from 1,905 cows from 398 commercial herds in the Netherlands, and milk-fat composition was measured by gas chromatography. DNA was available from 7 of the paternal half-sib families: 849 cows and their 7 sires. A genetic map was constructed comprising 1,341 SNP and 2,829 cM with an average information content of 0.83. Multi-marker interval mapping was used in a weighted across-family regression on corrected phenotypes for the 7 half-sib families. Twenty-three traits showed significant evidence for linkage ($P_{genome} \leq 0.05$) on 4 chromosomes: C6:0 and C8:0 on BTA6; fat %, all uneven chain fatty acids, and C14:0, C16:0, and C16:1 and their unsaturation indices on BTA14; C14:0 on BTA19; and the monounsaturated fatty acids and their unsaturation indices on BTA26. The QTL explained 3 to 19 percent of phenotypic variance. Furthermore, 49 traits with suggestive evidence for linkage ($P_{chromosome} \leq 0.05$) were found on 21 chromosomes. Additional analyses revealed that the QTL on BTA14 was most likely caused by a mutation in DGAT1, whereas the QTL on BTA26 was most likely caused by a mutation in the SCD1 gene. QTL that affect specific fatty acids might increase our understanding of physiological processes regarding fat synthesis and the position of the causal genes. Selection might be used to decrease proportions of unfavorable fatty acids in bovine milk, like C14:0 and C16:0.

4.1 Introduction

Changing milk fatty acid proportions to a milk-fat composition that contributes to improved human health has received considerable attention in the last years (Jensen, 2002; German and Dillard, 2006; Soyeurt et al., 2006; Vlaeminck et al., 2006; Soyeurt et al., 2007; Stoop et al., 2008). For short and medium chain fatty acids focus is on decreasing the relative proportions of mainly C14:0 and C16:0, as these two fatty acids have been associated with an increase in low-density lipoprotein cholesterol and increased risk of cardio-vascular diseases (German and Dillard, 2006).

Short and medium chain C4 to C17 saturated and monounsaturated milk fatty acids are largely synthesized *de novo* in the mammary gland. Saturated even chain C4:0 to C16:0 fatty acids are synthesized from C2 and C4 precursors. For C16:0, about 50% comes from *de novo* synthesis in the mammary gland and 50% from blood. Uneven chain fatty acids are mainly derived from rumen bacteria and partly from *de novo* synthesis from C3 precursors. Monounsaturated fatty acids are suggested to originate from Δ^9-desaturase enzyme activity (MacGibbon and Taylor, 2006). Several studies showed genetic variation in milk fatty acid proportions (Soyeurt et al., 2007; Bobe et al., 2008; Stoop et al., 2008). Stoop et al. (2008) showed substantial genetic variation for the saturated even fatty acids C4:0 to C16:0, with heritabilities ranging from 0.43 to 0.71. Identifying the genes responsible for this genetic variation is expected to greatly contribute to our understanding of milk fatty acid synthesis and to enhance opportunities to improve milk-fat composition through selective breeding.

Several studies have looked at the effects of genes that were expected to play a role in milk-fat synthesis (Clarke, 1993; Falaki et al., 1997; Stephens et al., 1999; Brym et al., 2004; Cohen et al., 2004; Schnabel et al., 2005; Roy et al., 2006b; Viitala et al., 2006; Khatib et al., 2007b; Morris et al., 2007). Although many of these studies found an effect on fat percentage or fat yield, only Morris et al. (2007) reported the effects on milk-fat composition and individual fatty acids. Recent studies found significant effects of the diacylglycerol acyltransferase 1 (DGAT1) K232A mutation on C4:0 to C12:0, C14:0, and C16:0 (Chapter 2) and of the stearoyl-CoA

desaturase 1 (SCD1) A293V mutation on C10:0, C12:0, and C14:0 (Chapter 3). However, these two genes explain only part of the genetic variation in milk-fat composition and it is expected that more genes are involved.

We performed a genome-wide scan to identify QTL for fatty acid composition of bovine milk. This paper presents results for short and medium chain fatty acids. The accompanying paper in Chapter 5 presents results for long chain fatty acids.

4.2 Materials and methods

Phenotypes

This study was part of the Dutch Milk Genomics Initiative, which focuses on genetic aspects of milk composition. To study milk-fat composition, milk samples were available from 1,905 cows from 398 commercial herds in the Netherlands. Cows descended either from 1 of 5 proven bulls (n=871), 1 of 50 young bulls (n=844), or 1 of 46 other proven bulls (n=190). The latter group ensured 3 sampled cows per farm. Each cow was over 87.5% Holstein Friesian, between day 63 and 282 of first lactation, and was milked twice a day. One morning milk sample of 500 ml per cow was collected in the winter of 2005. Sample bottles contained sodium azide (0.03 w/w%) for conservation. Milk-fat composition was measured by gas chromatography at the laboratory of the Netherlands Controlling Authority for Milk and Milk Products (Leusden, the Netherlands) as described in Chapter 2 and by Stoop et al. (2008). The fatty acids were identified and quantified by comparing the fatty acid methyl ester chromatograms of the milk-fat samples to chromatograms of pure fatty acid methyl ester standards. The fatty acids were measured as weight-proportion of total fat weight. In this study data are presented for 24 short and medium chain fatty acids traits: saturated C4 to C17 fatty acids, *cis*9 monounsaturated C10:1, C12:1, C14:1, C16:1 and C17:1, and the unsaturation indices of these fatty acids. The unsaturation index is an indication for the activity of the Δ^9-desaturase enzyme and was calculated as (Kelsey et al., 2003):

$$\frac{\text{unsaturated}}{\text{unsaturated} + \text{saturated}} * 100, \text{ e.g. C14 index} = \frac{\text{C14:1}cis9}{\text{C14:1}cis9 + \text{C14:0}} * 100$$

Table 4.1 shows the average weight proportion (w/w%) and heritability of the included fatty acids and indices.

Table 4.1 Mean proportion, phenotypic standard deviation (σ_P), and heritability (h^2) of short and medium chain fatty acids and unsaturation indices, measured on 1,905 cows

Trait	Mean (w/w%)[2]	σ_P	h^2 (SE)
C4:0	3.50	0.24	0.44 (0.09)
C5:0	0.03	0.01	0.14 (0.05)
C6:0	2.22	0.14	0.47 (0.10)
C7:0	0.03	0.01	0.17 (0.07)
C8:0	1.37	0.12	0.61 (0.11)
C9:0	0.04	0.02	0.25 (0.08)
C10:0	3.03	0.35	0.72 (0.12)
C11:0	0.08	0.03	0.34 (0.09)
C12:0	4.11	0.46	0.64 (0.11)
C13:0	0.11	0.04	0.19 (0.07)
C14:0	11.61	0.78	0.62 (0.11)
C15:0	1.17	0.16	0.30 (0.08)
C16:0	32.59	2.15	0.43 (0.11)
C17:0	0.45	0.04	0.33 (0.09)
C10:1	0.37	0.06	0.34 (0.09)
C12:1	0.12	0.02	0.38 (0.09)
C14:1	1.36	0.23	0.34 (0.08)
C16:1	1.44	0.30	0.44 (0.09)
C17:1	0.18	0.03	0.43 (0.10)
C10 index[1]	10.89	1.76	0.37 (0.09)
C12 index	2.74	0.49	0.36 (0.09)
C14 index	10.51	1.68	0.45 (0.09)
C16 index	4.24	0.76	0.47 (0.09)
C17 index	28.29	2.58	0.47 (0.10)
Fat %	4.36	0.64	0.49 (0.10)

[1] Unsaturation index calculated as unsaturated/(unsaturated + saturated) *100.

[2] Individual fatty acids in this table sum to 63.8 w/w%. Other fatty acids include branched fatty acids (no results shown), and long chain fatty acids (see Chapter 5).

Genotypes, markers and linkage map

Blood and semen samples were collected for DNA isolation. For the genome scan genotypes were available from 7 paternal half-sib families of in total 849 cows and 7 sires. The 7 sires were the 5 proven bulls and 2 of the young bulls of which daughter phenotypes were collected. The half-sib families consisted of 193, 179, 170, 166, 91, 29, and 21 cows respectively. A total of 1,536 SNP was selected either based on heterozygosity in the sires as known from previous experiments in the Dutch Holstein Friesian population by CRV (Arnhem, the Netherlands), or from dbSNP (http://www.ncbi.nlm.nih.gov/projects/SNP). Genotyping was performed by the GoldenGate assay (Illumina). Additionally, genotypes for SCD1 and DGAT1 were available from previous studies; genotypes for SCD1 were obtained by a SNaPshot assay (Chapter 3) and genotypes for DGAT1 were obtained by an allelic discrimination method (Chapter 2). Genotypes of DGAT1 and SCD1 were available for 1,687 out of 1,905 cows.

Of all SNP, 1,341 SNP were successfully incorporated in the linkage map of 2,829 cM (Table 4.2) that was calculated with Crimap (Green et al., 1990). Full details on the genotypings and construction of the linkage map are reported by Schopen et al. (2008). When markers mapped at the same position they were placed 0.1 cM apart to ensure all markers were included in the analysis by the QTL program. The resulting map of 2,831 cM was used in the QTL analysis.

QTL mapping

Phenotypes were pre-corrected for the systematic effects days in milk, age at first calving, season of calving, and herd. The effects were estimated using an Animal Model in ASReml (Gilmour et al., 2002) on all 1,905 cows with phenotypes, as described in Chapter 2 and by Stoop et al. (2008). Multi-marker interval mapping was used in a weighted across-family regression on the corrected phenotypes for the 7 half-sib families that were genotyped (Knott et al., 1996). The regression was performed for each trait and each chromosome using the following regression model (Spelman et al., 1996):

$y_{ijk} = \mu + s_i + b_{ik}X_{ijk} + e_{ijk}$,

where y_{ijk} is the corrected phenotypic observation for daughter j nested within sire i at position k, μ is the overall mean, s_i is the fixed effect of sire i, b_{ik} is the regression coefficient for sire i at position k, X_{ijk} is the probability of daughter j inheriting gamete 1 from sire i at position k and e_{ijk} is the random residual effect for daughter j nested within sire i at position k.

Significance thresholds were calculated by permutation testing (Churchill and Doerge, 1994). Chromosome-wise significance levels (P_{chr}) were obtained by 10,000 permutations. Genome-wise significance levels (P_{genome}) were derived from the chromosome-wise significance levels using a Bonferroni correction as De Koning et al., (1998): $P_{genome} = 1 - (1 - P_{chr})^{1/r}$, where r is ratio of the chromosome length over the genome length. Significant linkage was declared at the 5% genome-wise significance threshold and suggestive linkage was declared at the 5% chromosome-wise significance threshold (Lander and Kruglyak, 1995). The confidence interval for the most likely QTL position was based on 1,000 bootstraps (Visscher et al., 1996). Allele substitution effects were estimated for the significant QTL within each of the 7 families at the most likely QTL position using SAS 9.1.

Additional analyses were done to investigate whether the effects of polymorphisms in DGAT1 and SCD1 genes could explain the QTL detected in this study. For this analysis, phenotypes were additionally corrected for the DGAT1 K232A as well as the SCD1 A293V genotype by including these as fixed effect in the Animal Model. Subsequently, the QTL regression was re-run for all adjusted trait values.

4.3 Results

Linkage map

The genetic map comprised a total of 2,829 cM, with individual autosomes ranging in length from 44 cM (BTA27) to 145 cM (BTA1). An overview is given in Table 4.2. Average information content was high: 0.83, ranging from 0.70 (BTA19) to 0.88 (BTA2).

Table 4.2 Chromosome length (cM), number of markers per chromosome, and average and range of information content (IC) for all 29 *Bos taurus* autosomes

BTA	Length (cM)	# Markers	IC average	IC min	IC max
1	145	75	0.86	0.69	0.95
2	129	63	0.88	0.79	0.96
3	126	78	0.86	0.66	0.96
4	124	55	0.82	0.66	0.96
5	123	70	0.84	0.70	0.97
6	122	63	0.83	0.59	0.96
7	126	57	0.84	0.64	0.93
8	118	53	0.86	0.74	0.95
9	103	46	0.82	0.58	0.92
10	114	66	0.86	0.77	0.93
11	123	47	0.79	0.62	0.95
12	78	42	0.86	0.80	0.95
13	108	40	0.84	0.70	0.93
14	103	47	0.79	0.57	0.94
15	94	46	0.87	0.70	0.95
16	105	40	0.78	0.63	0.91
17	98	35	0.80	0.65	0.95
18	83	33	0.84	0.70	0.95
19	104	35	0.70	0.54	0.83
20	77	31	0.81	0.72	0.88
21	92	37	0.78	0.64	0.96
22	82	37	0.81	0.64	0.97
23	76	46	0.85	0.70	0.94
24	68	37	0.86	0.77	0.94
25	64	33	0.81	0.69	0.93
26	68	34	0.79	0.61	0.93
27	44	22	0.85	0.79	0.90
28	66	37	0.86	0.75	0.94
29	66	36	0.83	0.76	0.91
Total	2,829	1,341	0.83	0.54	0.97

Quantitative Trait Loci

QTL for short and medium chain fatty acids were identified on 21 chromosomes in this study and are summarized in Table 4.3. All QTL that exceeded the threshold for suggestive linkage (P_{chr}<0.05) are reported. Significant statistical evidence (P_{genome}<0.05) for QTL was found for 23 traits, which point to 4 distinct chromosomal regions on BTA6, BTA14, BTA19, and BTA26. Figures 4.1 to 4.4 show the graphs with test statistic distributions for these 4 chromosomes; for BTA14 and BTA26 only a selection of the significant traits is shown (see Table 4.3 for all traits). Allele substitution effects for significant QTL are shown in Table 4.4. Results are reported and discussed per chromosome.

On BTA6, a significant QTL near 55 cM affected C6:0 and C8:0 (Table 4.3, Figure 4.1). The QTL on BTA6 significantly segregated within family 1,

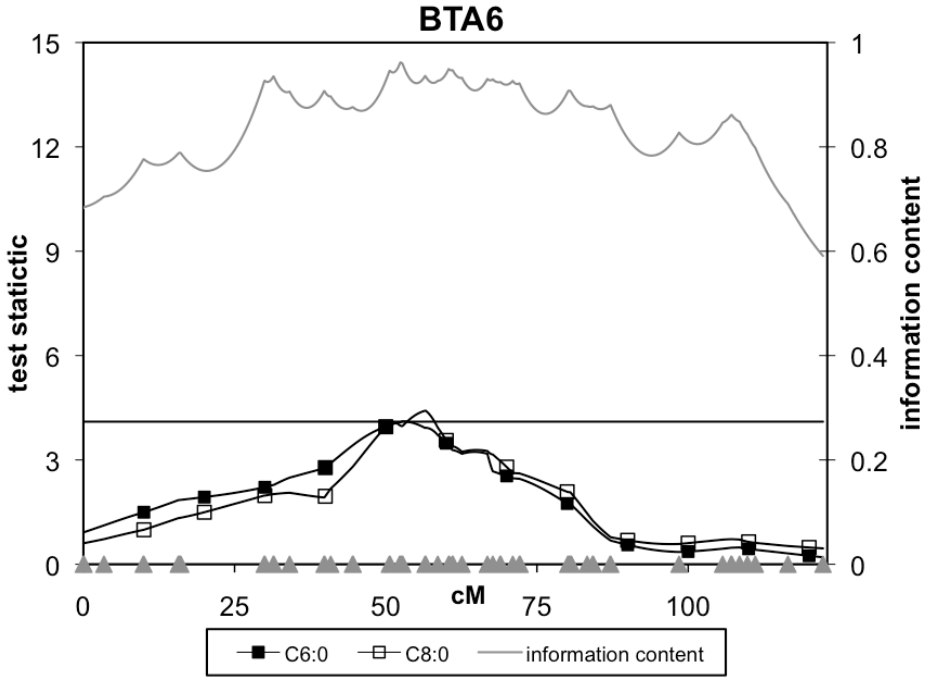

Figure 4.1 QTL mapping across families for C6:0 and C8:0 on BTA6. Triangles on the x-axis represent the location of the markers. The solid black line represents the genome-wise significance threshold. Although this threshold is slightly different for each trait, only the line of the trait with the lowest genome-wise significance threshold is shown.

Table 4.3 Location and characteristics of suggestive and significant QTL affecting short and medium chain fatty acids, unsaturation indices, and fat percentage

								DGAT1 and SCD1 corrected			
BTA	Trait	F-stat	Pos. (cM)	95% CI (cM)	P_{chr}	P_{genome}	[1]	Pos. (cM)	P_{chr}	P_{genome}	[1]
1	C16:0	3.32	64	25-112	0.034	0.492		-	-	-	
1	C16:1	-	-	-	-	-		71	0.048	0.621	
1	C17:0	3.84	77	28-145	0.010	0.185		132	0.039	0.544	
2	C4:0	3.74	112	0-129	0.010	0.195		112	0.017	0.314	
2	C6:0	3.80	110	14-127	0.006	0.120		110	0.014	0.270	
2	C15:0	3.17	53	0-78	0.043	0.620		-	-	-	
3	C17:1	2.94	94	0-126	0.045	0.646		-	-	-	
4	C7:0	-	-	-	-	-		85	0.009	0.194	
4	C13:0	-	-	-	-	-		86	0.047	0.665	
4	C16:0	-	-	-	-	-		64	0.022	0.405	
4	C17 index	3.79	31	0-79	0.006	0.125		-	-	-	
5	C10 index	4.81	95	49-114	0.003	0.071		96	0.010	0.208	
5	C12 index	3.19	94	44-114	0.033	0.538		-	-	-	
5	C14 index	3.17	82	43-114	0.040	0.609		-	-	-	
6	C6:0	4.11	53	16-71	0.002	0.050	*	53	0.005	0.105	
6	C8:0	4.42	57	19-72	0.002	0.034	*	57	0.003	0.067	
6	C10:0	-	-	-	-	-		57	0.050	0.694	
6	C16:1	-	-	-	-	-		57	0.036	0.574	
6	C17:1	3.15	57	8-72	0.025	0.446		-	-	-	
6	C17 index	2.96	57	2-107	0.042	0.627		-	-	-	
6	Fat %	3.07	81	3-109	0.033	0.538		-	-	-	
7	C4:0	3.51	0	0-126	0.014	0.276		0	0.022	0.390	
8	C15:0	3.10	67	23-105	0.048	0.697		65	0.029	0.504	
8	Fat %	3.23	82	45-113	0.022	0.410		-	-	-	
9	C5:0	2.95	48	13-87	0.039	0.667		36	0.033	0.604	
9	C16:0	-	-	-	-	-		38	0.047	0.730	
10	C16 index	-	-	-	-	-		103	0.029	0.520	
11	C5:0	3.76	97	0-124	0.006	0.127		97	0.010	0.200	
11	C16:0	3.06	91	49-124	0.050	0.689		91	0.007	0.145	
11	C10:1	4.03	49	23-118	0.017	0.320		49	0.007	0.147	
11	C12:1	3.11	49	11-124	0.042	0.629		43	0.038	0.586	
11	C10 index	3.88	75	49-124	0.047	0.665		80	0.015	0.284	
11	C12 index	-	-	-	-	-		124	0.048	0.674	
11	Fat %	-	-	-	-	-		124	0.024	0.421	
13	C6:0	3.00	78	0-108	0.036	0.614		77	0.041	0.663	
13	C12:1	2.99	59	0-101	0.050	0.739		-	-	-	
13	C14:1	3.05	60	0-108	0.047	0.717		-	-	-	

Table 4.3 Continued

								DGAT1 and SCD1 corrected			
BTA	Trait	F-stat	Pos. (cM)	95% CI (cM)	P_{chr}	P_{genome}	[1]	Pos. (cM)	P_{chr}	P_{genome}	[1]
13	C16:1	3.47	75	17-108	0.011	0.258		75	0.006	0.146	
13	C17:1	3.16	108	35-108	0.020	0.409		108	0.008	0.194	
13	C12 index	2.98	56	0-108	0.045	0.698		101	0.043	0.684	
13	C14 index	-	-	-	-	-		107	0.047	0.716	
13	C16 index	2.94	101	0-108	0.035	0.607		104	0.026	0.502	
13	C17 index	3.03	102	0-108	0.031	0.565		107	0.017	0.361	
13	Fat %	3.63	53	11-69	0.008	0.197		53	0.016	0.352	
14	C5:0	8.95	7	0-11	≤0.001	≤0.001	***	-	-	-	
14	C7:0	8.01	1	0-16	≤0.001	≤0.001	***	-	-	-	
14	C8:0	3.03	0	0-87	0.039	0.660		-	-	-	
14	C9:0	9.29	1	0-8	≤0.001	≤0.001	***	-	-	-	
14	C11:0	7.97	1	0-5	≤0.001	≤0.001	***	-	-	-	
14	C13:0	5.56	3	0-88	≤0.001	≤0.008	**	-	-	-	
14	C14:0	9.84	0	0-0	≤0.001	≤0.001	***	-	-	-	
14	C15:0	6.28	2	0-25	≤0.001	≤0.001	***	-	-	-	
14	C16:0	13.86	0	0-1	≤0.001	≤0.001	***	-	-	-	
14	C10:1	-	-	-	-	-		49	0.028	0.540	
14	C12:1	-	-	-	-	-		51	0.024	0.480	
14	C14:1	-	-	-	-	-		58	0.045	0.714	
14	C16:1	18.17	0	0-2	≤0.001	≤0.001	***	-	-	-	
14	C12 index	-	-	-	-	-		73	0.050	0.755	
14	C14 index	4.55	0	0-99	0.001	0.032	*	-	-	-	
14	C16 index	10.19	0	0-16	≤0.001	≤0.000	***	-	-	-	
14	C17 index	3.36	0	0-73	0.014	0.325		0	0.049	0.749	
14	Fat %	28.84	0	0-0	≤0.001	≤0.001	***	-	-	-	
16	C11:0	-	-	-	-	-		49	0.039	0.656	
16	C10:1	3.35	19	1-88	0.047	0.727		18	0.025	0.486	
16	C12:1	-	-	-	-	-		49	0.031	0.565	
16	C12 index	-	-	-	-	-		52	0.029	0.551	
17	C8:0	2.88	50	1-89	0.046	0.740		50	0.048	0.756	
17	C10:1	3.53	28	0-95	0.025	0.513		-	-	-	
19	C4:0	-	-	-	-	-		54	0.042	0.688	
19	C7:0	-	-	-	-	-		0	0.040	0.669	
19	C14:0	4.97	59	38-83	0.001	0.037	*	57	0.002	0.061	
19	C14:1	-	-	-	-	-		96	0.045	0.716	
19	C16:1	2.85	54	14-96	0.044	0.702		-	-	-	
19	C10 index	-	-	-	-	-		58	0.050	0.752	

Table 4.3 Continued

								DGAT1 and SCD1 corrected			
BTA	Trait	F-stat	Pos. (cM)	95% CI (cM)	P_{chr}	P_{genome}	[1]	Pos. (cM)	P_{chr}	P_{genome}	[1]
19	C12 index	-	-	-	-	-		96	0.003	0.079	
19	C14 index	-	-	-	-	-		96	0.004	0.111	
19	C16 index	-	-	-	-	-		96	0.035	0.622	
21	C5:0	3.18	29	0-92	0.021	0.475		29	0.004	0.108	
21	C7:0	3.22	29	0-92	0.018	0.423		29	0.001	0.042	*
22	C10:0	-	-	-	-	-		39	0.044	0.789	
22	C14:0	-	-	-	-	-		40	0.037	0.724	
22	C15:0	3.44	52	10-76	0.014	0.390		55	0.015	0.407	
22	C17:0	3.38	52	1-82	0.017	0.435		-	-	-	
22	C17 index	-	-	-	-	-		61	0.024	0.558	
23	C6:0	-	-	-	-	-		70	0.045	0.822	
25	C4:0	3.16	56	8-63	0.018	0.555		56	0.018	0.549	
26	C10:1	10.61	29	22-31	≤0.001	≤0.001	***	-	-	-	
26	C12:1	4.25	28	18-44	0.002	0.092		-	-	-	
26	C14:1	9.52	28	22-37	≤0.001	≤0.001	***	-	-	-	
26	C16:1	6.04	31	19-35	≤0.001	≤0.001	***	-	-	-	
26	C17:1	3.82	32	17-64	0.002	0.084		-	-	-	
26	C10 index	6.98	28	0-37	≤0.001	≤0.001	***	-	-	-	
26	C12 index	5.91	28	0-37	≤0.001	≤0.001	***	-	-	-	
26	C14 index	9.90	28	22-37	≤0.001	≤0.001	***	-	-	-	
26	C16 index	8.35	30	22-33	≤0.001	≤0.001	***	-	-	-	
26	C17 index	10.25	27	23-32	≤0.001	≤0.001	***	-	-	-	
27	C4:0	2.89	34	8-44	0.029	0.848		34	0.026	0.812	
27	Fat %	2.58	33	15-44	0.049	0.959		-	-	-	
29	C8:0	3.69	17	5-46	0.006	0.211		17	0.005	0.184	
29	C10:0	3.35	17	6-66	0.019	0.570		17	0.016	0.490	
29	C12:0	3.15	17	7-66	0.042	0.840		19	0.034	0.778	

[1] * = P_{genome}≤0.05, ** = P_{genome}≤0.01, *** = P_{genome}≤0.001. All listed QTL have P_{chr}≤0.05.

family 5 and family 6, with an effect of about 0.5 phenotypic SD (Table 4.4). In family 1, the difference between the two daughter groups was 0.08 w/w% for C6:0, and 0.07 w/w% for C8:0. In family 5, the difference for C6:0 was 0.05 w/w%, but not significant, whereas the difference for C8:0 was also 0.07 w/w%. In family 6, the difference for C6:0 was 0.10 w/w%. The QTL explained around 3% of phenotypic variation for both C6:0 and C8:0.

Table 4.4 Allele substitution effects[1] and SE within 7 paternal half-sib families for QTL on BTA6, 14, 19 and 26, and approximate phenotypic variation explained by QTL

Trait	Family (no of daughters)							Phenotypic variation explained by QTL (%)
	1 (193)	2 (179)	3 (170)	4 (166)	5 (91)	6 (29)	7 (21)	
BTA6								
C6:0	$-0.08_{0.02}^{*}$	$0.03_{0.02}$	$-0.03_{0.02}$	$-0.01_{0.02}$	$-0.05_{0.03}$	$-0.10_{0.05}^{*}$	$0.04_{0.06}$	3
C8:0	$-0.07_{0.02}^{*}$	$0.02_{0.02}$	$-0.01_{0.02}$	$-0.01_{0.02}$	$-0.07_{0.02}^{*}$	$-0.03_{0.04}$	$0.05_{0.05}$	3
BTA14								
Fat %	$-0.53_{0.09}^{*}$	$-0.69_{0.09}^{*}$	$-0.70_{0.10}^{*}$	$-0.68_{0.10}^{*}$	$-0.06_{0.18}$	$0.50_{0.33}$	$-0.93_{0.26}^{*}$	19
C5:0	$-0.01_{0.00}^{*}$	$0.00_{0.00}^{*}$	$-0.01_{0.00}^{*}$	$-0.01_{0.00}^{*}$	$-0.01_{0.00}$	$0.00_{0.01}$	$-0.01_{0.01}$	7
C7:0	$-0.01_{0.00}^{*}$	$-0.01_{0.00}^{*}$	$-0.01_{0.00}^{*}$	$-0.01_{0.00}^{*}$	$-0.01_{0.00}$	$0.01_{0.01}$	$0.00_{0.01}$	6
C9:0	$-0.01_{0.00}^{*}$	$0.00_{0.00}$	$-0.01_{0.00}^{*}$	$-0.01_{0.00}^{*}$	$-0.01_{0.01}$	$0.02_{0.01}$	$-0.01_{0.01}$	7
C11:0	$-0.01_{0.00}^{*}$	$-0.01_{0.00}$	$-0.02_{0.01}^{*}$	$-0.02_{0.01}^{*}$	$-0.01_{0.01}$	$0.02_{0.02}$	$-0.01_{0.01}$	6
C14:0	$0.43_{0.11}^{*}$	$0.59_{0.11}^{*}$	$0.35_{0.12}^{*}$	$0.35_{0.12}^{*}$	$-0.01_{0.23}$	$0.65_{0.41}$	$0.92_{0.32}^{*}$	7
C15:0	$-0.09_{0.03}^{*}$	$-0.03_{0.03}$	$-0.11_{0.03}^{*}$	$-0.11_{0.03}^{*}$	$-0.10_{0.05}$	$0.05_{0.10}$	$-0.07_{0.08}$	5
C16:0	$-1.63_{0.33}^{*}$	$-1.57_{0.34}^{*}$	$-1.34_{0.36}^{*}$	$-1.67_{0.35}^{*}$	$0.03_{0.66}$	$-1.39_{1.19}$	$-3.25_{0.92}^{*}$	10
C16:1	$-0.21_{0.04}^{*}$	$-0.30_{0.04}^{*}$	$-0.13_{0.05}^{*}$	$-0.32_{0.05}^{*}$	$0.13_{0.09}$	$-0.19_{0.16}$	$-0.20_{0.12}$	13
C16 index	$-0.38_{0.11}^{*}$	$-0.63_{0.12}^{*}$	$-0.16_{0.13}$	$-0.61_{0.12}^{*}$	$0.39_{0.23}$	$-0.44_{0.42}$	$-0.15_{0.32}$	8
C13:0	$-0.01_{0.01}^{*}$	$-0.01_{0.01}$	$-0.02_{0.01}^{*}$	$-0.02_{0.01}^{*}$	$-0.01_{0.01}$	$0.02_{0.02}$	$-0.01_{0.02}$	4
C14 index	$-0.65_{0.25}^{*}$	$-0.83_{0.26}^{*}$	$-0.40_{0.28}$	$-0.87_{0.27}^{*}$	$0.51_{0.52}$	$-0.70_{0.93}$	$-0.80_{0.72}$	3
BTA19								
C14:0	$0.09_{0.10}$	$0.72_{0.22}^{*}$	$-0.20_{0.11}$	$-0.28_{0.11}^{*}$	$0.08_{0.15}$	$-0.32_{0.30}$	$1.14_{0.33}^{*}$	4
BTA26								
C10:1	$0.05_{0.01}^{*}$	$0.04_{0.01}^{*}$	$0.01_{0.01}$	$0.01_{0.01}$	$0.01_{0.01}$	$0.03_{0.02}$	$-0.03_{0.02}$	8
C14:1	$0.18_{0.03}^{*}$	$0.16_{0.03}^{*}$	$0.00_{0.04}$	$0.03_{0.04}$	$0.08_{0.05}$	$0.21_{0.09}^{*}$	$0.04_{0.09}$	6
C16:1	$-0.22_{0.04}^{*}$	$-0.15_{0.04}^{*}$	$0.03_{0.05}$	$0.04_{0.05}$	$0.08_{0.06}$	$-0.04_{0.11}$	$0.06_{0.12}$	5
C10 index	$1.26_{0.23}^{*}$	$0.93_{0.24}^{*}$	$0.04_{0.26}$	$0.30_{0.26}$	$0.44_{0.34}$	$0.87_{0.64}$	$-0.24_{0.69}$	5
C12 index	$0.30_{0.07}^{*}$	$0.26_{0.07}^{*}$	$0.01_{0.08}$	$0.09_{0.08}$	$0.15_{0.10}$	$0.34_{0.19}$	$0.22_{0.20}$	4
C14 index	$1.29_{0.23}^{*}$	$1.14_{0.23}^{*}$	$0.03_{0.25}$	$0.30_{0.25}$	$0.69_{0.32}^{*}$	$1.51_{0.62}^{*}$	$0.45_{0.67}$	6
C16 index	$-0.62_{0.11}^{*}$	$-0.45_{0.11}^{*}$	$0.07_{0.12}$	$0.17_{0.11}$	$0.29_{0.15}$	$-0.14_{0.29}$	$0.17_{0.31}$	6
C17 index	$-2.31_{0.36}^{*}$	$-1.69_{0.37}^{*}$	$0.19_{0.39}$	$0.79_{0.39}^{*}$	$0.93_{0.51}$	$-1.04_{0.97}$	$0.79_{1.06}$	8

[1] Significantly segregating QTL (P≤0.05, calculated by a t-test) are marked with an asterisk.

On BTA14, a significant QTL at the centromeric end of the chromosome affected all uneven chain fatty acids, and C14:0, C16:0, and C16:1 with their unsaturation indices (Table 4.3, Figure 4.2). Size of the QTL effect was generally 0.5 to 1 phenotypic SD. This QTL mapped to the approximate location of the DGAT1 gene. Sires of family 1, 2, 3, 4, and 7 were heterozygous for the QTL. The QTL explained between 3% and 19% of phenotypic variance for the different traits. This QTL also affected several long chain fatty acids and the total proportion of saturated and unsaturated fatty acids (Chapter 5).

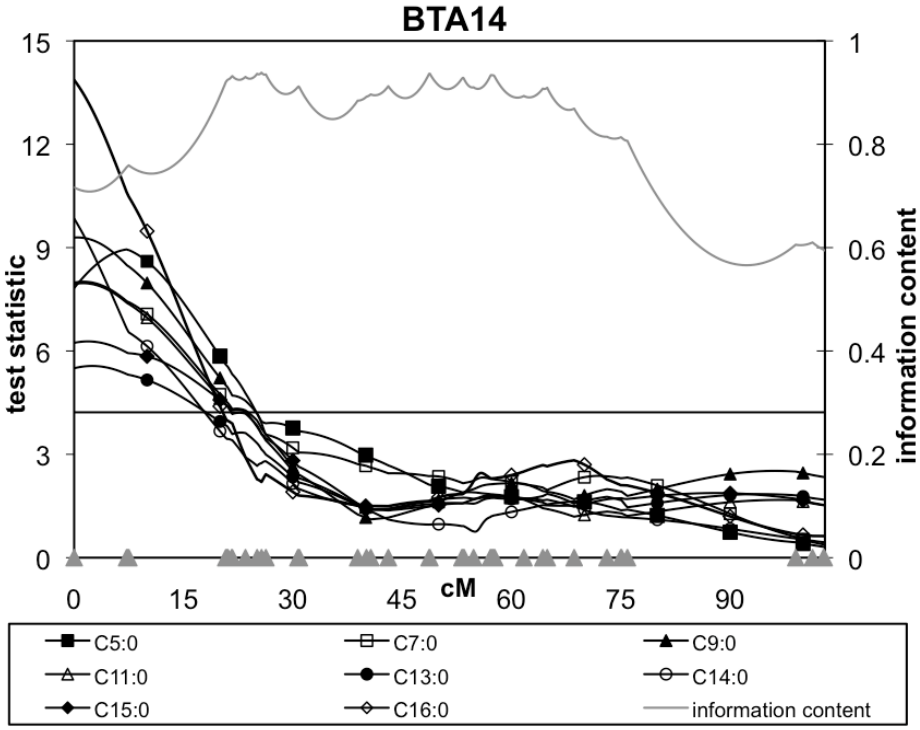

Figure 4.2 QTL mapping across families for saturated short and medium chain fatty acids on BTA14. Triangles on the x-axis represent the location of the markers. The solid black line represents the genome-wise significance threshold. Although this threshold is slightly different for each trait, only the line of the trait with the lowest genome-wise significance threshold is shown.

On BTA19, a significant QTL was found for C14:0 (60 cM, Table 4.3, Figure 4.3). The QTL on BTA19 significantly segregated within family 2, 4, and 7, with an average effect of 0.7 SD. The difference between the two daughter groups for C14:0 was 0.72 w/w% in family 2, 0.28 w/w% in family 4, and 1.14 w/w% in family 7. This QTL explained 4% of phenotypic variance in C14:0.

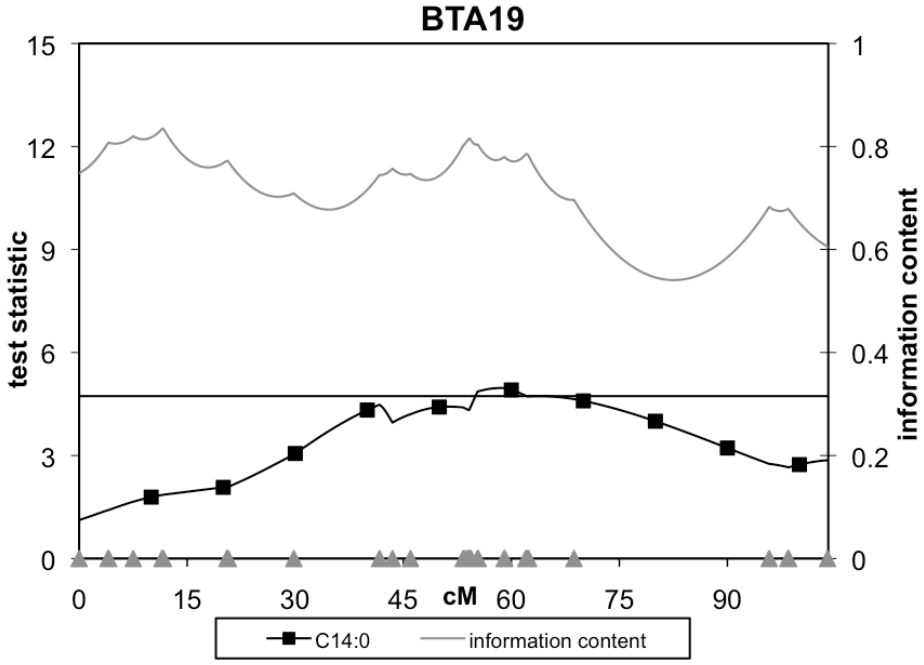

Figure 4.3 QTL mapping across families for C14:0 on BTA19. Triangles on the x-axis represent the location of the markers. The solid black line represents the genome-wise significance threshold.

On BTA26, a QTL near the location of the SCD1 gene (28 cM) affected all monounsaturated fatty acids and their unsaturation indices (Table 4.3, Figure 4.4). Size of the QTL effect was about 0.7 phenotypic SD for all traits. Sires of family 1, 2, and 6 were heterozygous. In family 1 and 2, the QTL significantly affected all monounsaturated fatty acids and unsaturation indices. Family 6 has only 29 offspring; therefore, not all QTL effects were significant. This QTL explained between 4% and 8% of phenotypic variance.

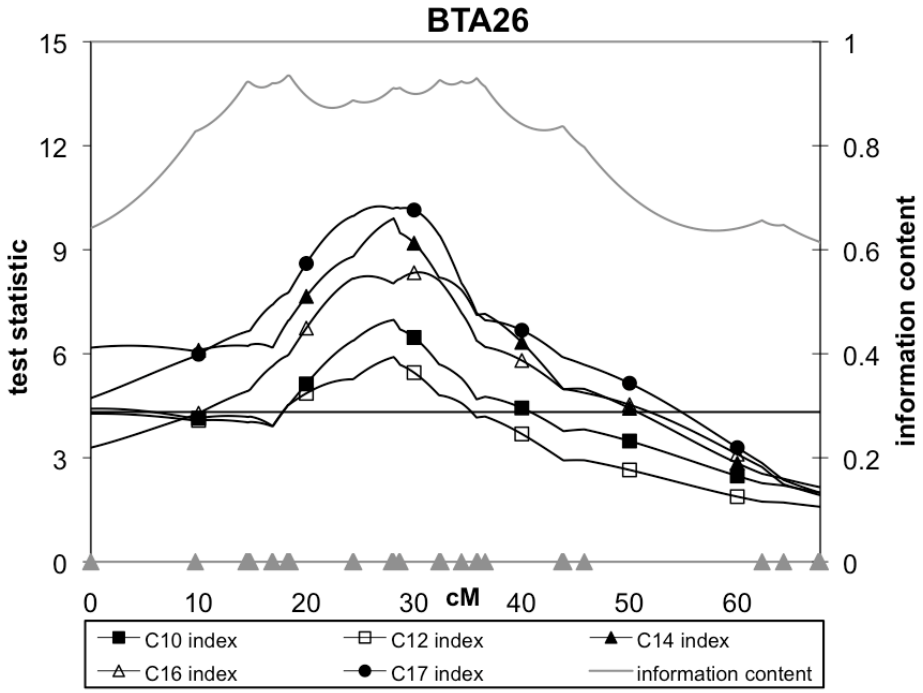

Figure 4.4 QTL mapping across families for unsaturation indices on BTA26. Triangles on the x-axis represent the location of the markers. The solid black line represents the genome-wise significance threshold. Although this threshold is slightly different for each trait, only the line of the trait with the lowest genome-wise significance threshold is shown.

Beside the significant QTL, 49 traits with suggestive QTL were found on 21 autosomes (Table 4.3). No QTL was found on BTA10, 12, 15, 18, 20, 23, 24, and 28. Although QTL were suggestive, some of these chromosomal regions were found for multiple traits that are known to overlap in synthesis pathways. For even chain fatty acids suggestive QTL were found on two chromosomes: on BTA2 for C4:0 and C6:0, and on BTA29 for C8:0, C10:0, and C12:0. For uneven chain fatty acids suggestive QTL were found on two other chromosomes: on BTA21 for C5:0 and C7:0, and on BTA22 for C15:0 and C17:0. For several monounsaturated fatty acids and unsaturation indices there were suggestive QTL on BTA5, BTA11, and BTA13.

Effect of DGAT1 and SCD1 genotype polymorphisms

In a second analysis data was adjusted for the effects of DGAT1 K232A and SCD1 A293V genotypes, which resulted in complete disappearance of all linkage previously found on BTA14 and BTA26, except for the suggestive QTL for C17 index on BTA14 (Table 4.3). This means that the detected QTL are most likely caused by the known mutations in DGAT1 and SCD1. After accounting for known mutations, however, we found a new suggestive QTL on BTA14 for C10:1, C12:1 and C14:1 at approximately 50 cM, and for C12 index at 73 cM. This suggestive QTL was also detected for several long chain monounsaturated fatty acids (see Chapter 5). Furthermore, the pre-correction for DGAT1 and SCD1 genotypes resulted in additional suggestive QTL for the unsaturation indices of C10 to C16 on BTA19, and a significant QTL for C7:0 on BTA21. In total, 39 traits no longer showed significant linkage when correcting for DGAT1 and SCD1, of which 23 traits were located on BTA14 and BTA26, whereas 29 new traits with suggestive QTL were identified.

4.4 Discussion

Power calculations

In this study, we reported suggestive QTL with $P_{chr} \leq 0.05$ and significant QTL with $P_{genome} \leq 0.05$. Based on power calculations on a daughter design of this size, there was a high (>80%) power to identify QTL that explain at least 5% of phenotypic variance and a medium (about 50%) power to identify QTL that explain 2.5% to 4.2% of phenotypic variance. The significant QTL found in this study explained 3% to 19% of phenotypic variance, which is in accordance with this expectation.

Adjusting the phenotypic data for effects of known genes reduces the residual variation in the traits and is therefore expected to result in a higher power to detect QTL (De Koning et al., 2001). In a second analysis the phenotypic data was pre-corrected for the DGAT1 K232A and SCD1 A293V mutations. Several new suggestive QTL and 1 new significant QTL were detected. For some fatty acids, including the previously found QTL on BTA6 and BTA19, the test statistic decreased below the threshold for suggestive QTL. In general, the change in test statistic was rather small (<0.3).

Fat percentage

Contrary to the limited research on fatty acid proportions, fat percentage and fat yield have been extensively studied. A review by Khatkar et al. (2004) reported significant QTL for fat percentage and fat yield on BTA2, 3, 4, 6, 9, 10, 12, 14, 16, 20, and 26. The current study found one highly significant QTL for fat percentage on BTA14 and 4 suggestive QTL on BTA6, 8, 13, and 27. The suggestive QTL on BTA6 is also mentioned in the review by Khatkar et al. (2004). In addition, in the current study 4 significant QTL for short and medium chain fatty acids were found on BTA6, 14, 19, and 26, and numerous suggestive QTL, which do not match with the locations of the QTL for fat percentage that we detected.

Finding different QTL for fat percentage and milk-fat composition is partly due to more detailed phenotyping of individuals, resulting in new potential linkage regions being identified. Moreover, the genetic map was of good quality, with extensive covering of the bovine genome, high rates of heterozygosity for the sires and consequently high information content throughout the genetic map. Finally, milk-fat composition is not the same trait as milk-fat percentage. Although there is obviously a clear relation between fat percentage and milk-fat composition, the genetic correlations between individual fatty acid proportions and fat percentage differ largely between fatty acid and range between 0.00 and 0.78 (Stoop et al., 2008). Fatty acid proportions increase with increasing fat percentage, whereas others decrease. These differences in size and sign of genetic correlations imply several genetic and physiological mechanisms with opposing effects, which might explain the presence of QTL for milk-fat composition that do not affect fat percentage.

Fatty acid synthesis

Several other studies have looked at candidate genes that were expected to play a role in milk-fat synthesis, such as peroxysome proliferator-activated receptor-γ coactivator-1α (PPARGC1A, Khatib et al., 2007b), FAM13A1 (Cohen et al., 2004) and osteopontin (OPN, Khatib et al., 2007b; Schnabel et al., 2005) on BTA6, DGAT1 (Chapter 2) on BTA14, growth hormone (GH, Viitala et al., 2006), fatty acid synthase (FASN, Roy et al., 2006b; Morris et

al., 2007) and signal transducer and activator of transcription 5A (STAT5A, Stephens et al., 1999; Brym et al., 2004) on BTA19, and SCD1 (Chapter 3) on BTA26. Although most studies reported effects on fat percentage, data on milk-fat composition was not available, and only Chapter 2 and 3 looked at milk-fat composition. In fact, there are only very few studies reporting effects on fat composition, mainly in adipose tissue of beef cattle (Reh et al., 2004; Taniguchi et al., 2004; Morris et al., 2007; Zhang et al., 2008).

Strong evidence was found for significant QTL on BTA14 and BTA26. These QTL effects are likely caused by DGAT1 (BTA14) and SCD1 (BTA26), because these QTL disappear after correction for the DGAT1 and SCD1 genotypes. The C10 to C18 fatty acids can be desaturated by SCD1 (Chung et al., 2000; Bernard et al., 2001; Keating et al., 2005) and sequentially all present individual fatty acids can be esterified to glycerol via glycerol-3-phosphate acyltransferase (GPAT, Roy et al., 2006a), lysophosphatidic acid acyltransferase (LPAT, Mistry and Medrano, 2002), and DGAT1 (Winter et al., 2002) to end up as triacylglycerols in the milk. For more details regarding the DGAT1 and SCD1 gene effects, see Chapters 2 and 3.

We reported significant QTL for C6:0 and C8:0 on BTA6 at approximately 55 cM, which is in near proximity of the PPARGC1A gene. PPARGC1A is one of several major transcription factors that have been proposed to play a central role in regulation of milk-fat synthesis activity in the mammary gland (Khatib et al., 2007b; Bernard et al., 2008). It is involved in up- and down regulation of several genes of the acetyl-CoA cycle, such as acetyl-CoA carboxylase (ACC) – which activates acetyl-CoA - (Barber et al., 1997; Mao et al., 2001) and FASN (Smith, 1994).

We also reported significant QTL for C14:0 on BTA19. Morris et al. (2007) reported a significant effect of the candidate gene FASN (located on BTA19) on C14:0. Within the acetyl-CoA cycle, chain elongation is initiated by acyltransferase, one of the six enzymes associated with FASN, which loads mainly acetyl and malonyl substrates to β-ketoacylsynthase (Roy et al., 2005). Regulation of chain termination for C14:0 and C16:0 is done by thioesterase I, another enzyme associated with FASN (Smith, 1994). FASN,

in particular the thioesterase I enzyme, might be a good candidate for the detected QTL on BTA19 in the present study. However, at least two other genes in the proximity of the QTL have been associated with fat synthesis, and could also be responsible for the observed QTL effect: GH and STAT5A (Brym et al., 2004; Viitala et al., 2006). STAT5 mediates a prolactin signal and stimulates promoter III of acetyl-CoA carboxylase (Rosen et al., 1999; Mao et al., 2002), and can in turn be stimulated by GH and IGF1 (Yang et al., 2000).

Several QTL were identified for short or medium chain fatty acids: for C4:0 and C6:0 we find a suggestive QTL on BTA2, for C6:0 and C8:0 on BTA6, for C14:0 on BTA19, and for C8:0, C10:0, and C12:0 on BTA29 (suggestive). Although these chromosomes harbor genes that have been related to milk-fat synthesis, additional mapping at higher resolutions will be necessary to reduce size of confidence intervals and identify possible candidate genes.

4.5 Implications

Four distinct significant QTL were identified that affected short and medium chain fatty acids. DGAT1 (BTA14) and SCD1 (BTA26) are two major genes that are known to affect milk-fat composition. DGAT1 affected mainly uneven chain fatty acids and C14:0, C16:0, and C16:1 with their unsaturation indices, and several long chain fatty acids (Chapter 5). SCD1 affected all monounsaturated medium chain fatty acids and their unsaturation indices. The two other significant QTL affected C6:0 and C8:0 (BTA6), and C14:0 (BTA19). These 4 QTL explain 3 to 19% of phenotypic variance in the affected traits. In total 49 suggestive QTL were reported that affect either even chain fatty acids, uneven chain fatty acids, or monounsaturated fatty acids and unsaturation indices.

QTL that affect specific fatty acids might increase our understanding of physiologic processes involved in fat synthesis and may aid in locating the underlying genes. Selection might be used to decrease proportions of unfavorable fatty acids in bovine milk, like C14:0 and C16:0, and reduce fat melting point for better spreadable butter.

4.6 Acknowledgments

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5

Genome-wide scan for bovine milk-fat composition II. QTL for long chain fatty acids

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Abstract

Previous research has shown substantial genetic variation between dairy cows in milk-fat composition, with low to moderate heritabilities for long chain fatty acids (18 and more carbon atoms). In this paper we present the results of the first genome-wide scan to identify quantitative trait loci (QTL) that contribute to genetic variation in long chain milk fatty acids. Milk fatty acid composition phenotypes were available on 1,905 Dutch Holstein Friesian cows. A total of 849 cows representing 5 large and 2 small paternal half-sib families, and their 7 sires were genotyped for 1,341 SNP across all autosomes. QTL analyses were performed using a weighted across-family regression on phenotypes, which were pre-adjusted for systematic environmental effects. We detected significant QTL ($P_{genome} \leq 0.05$) on *Bos taurus* autosomes (BTA) 14, 15 and 16: for C18:1*cis*9, C18:1*cis*12, C18:2*cis*9,12, C18:2*cis*9,*trans*11 (CLA), C18:3*cis*9,12,15, C18 index, total index, total saturated fatty acids (SFA), total unsaturated fatty acids (UFA) and ratio SFA/UFA on BTA14, for C18:1*trans* fatty acids on BTA15, and for C18 and conjugated linoleic acid (CLA) indices on BTA16. The QTL explained 3% to 19% of the phenotypic variance. Suggestive QTL ($P_{chromosome} \leq 0.05$) were found on 16 other chromosomes. The DGAT1 K232A polymorphism on BTA14 is known to influence fatty acid composition. This polymorphism most likely explains the QTL that we detected on BTA14. QTL mapping for milk-fat composition is an important step in the unraveling of regulation of lipogenesis of long chain fatty acids.

5.1 Introduction

Milk-fat is characterized by a high amount of saturated fatty acids, especially medium chain fatty acids C14:0 and C16:0, and by a low amount of (poly)unsaturated fatty acids. Whereas the medium chain fatty acids C14:0 and C16:0 are commonly considered to have a negative effect on human health due to their cholesterol-raising properties, long chain fatty acids of 18 and more carbon atoms are considered to have neutral or positive effects (Mensink et al., 2003). Of the long chain fatty acids, special attention is paid to conjugated linoleic acids (CLA), because of their supposed role in modulation of plasma lipid concentrations, and their anti-carcinogenic and anti-inflammatory effects, shown in studies mostly performed in cell lines and animal models (Haug et al., 2007).

Long chain fatty acids in the mammary gland are derived from circulating plasma lipids, and originate from the diet, from microbial fatty acid synthesis in the rumen, and from endogenous lipids. Lipids entering the rumen are first lipolyzed by microbial lipases, causing the release of fatty acids. After lipolysis, unsaturated fatty acids are isomerized and hydrogenated by ruminal microbes.

Genetic variation in bovine milk-fat composition has been shown in a number of recent studies (Soyeurt et al., 2007; Bobe et al., 2008; Stoop et al., 2008). Although estimated heritabilities for the individual fatty acids vary between studies, all reveal substantial genetic variation between cows and suggest opportunities to improve composition of milk-fat by selective breeding. Quantitative trait locus (QTL) mapping for long chain milk fatty acids can provide insight into long chain fatty acid metabolism and will allow more efficient selective breeding strategies. Studies have revealed a number of QTL affecting milk-fat percentage and milk-fat yield in several cattle populations (Khatkar et al., 2004). However, a genome-wide scan for QTL affecting milk-fat composition is lacking. Morris et al. (2007) performed QTL mapping for fat composition of milk and adipose tissue on a single chromosome, *Bos taurus* autosome (BTA) 19, and identified a QTL with significant effects on, among others, C18 fatty acids. They identified fatty acid synthase (FASN) as a candidate gene. Genotyping of 5 single nucleotide

polymorphisms (SNP) in this gene showed association between SNP in FASN and C18:0 and C18:1*cis*9 in milk. Association studies showed that mutations in candidate genes DGAT1 and SCD1 were also associated with milk-fat composition (Chapters 2 and 3). The DGAT1 232A allele was associated with more C18:1*cis*9 and more CLA*cis*9,*trans*11, and the SCD1 293V allele was associated with less C18:0 and C18:1*cis*11, and more CLA*cis*9,*trans*11. Although these genes have shown major effects, a large proportion of the genetic variation in milk-fat composition still cannot be attributed to specific chromosomal regions or genes.

In this paper, we present the results of the first genome-wide scan to map QTL contributing to the genetic variation in long chain fatty acid composition of bovine milk. The accompanying paper in Chapter 4 presents results for short and medium chain fatty acids.

5.2 Materials and methods

Phenotypes

This study was part of the Dutch Milk Genomics Initiative, which focuses on genetic aspects of milk composition. Phenotypic data were available from 1,905 cows from 398 commercial herds in the Netherlands. Cows descended from 1 of 5 proven bulls representing 5 large half-sib families (871 cows), from 1 of 50 young bulls representing 50 small half-sib families (844 cows), or from other proven bulls (190 cows). The last group was added to ensure that at least 3 cows were sampled per herd. The NRS (Arnhem, the Netherlands) provided pedigrees of the cows. Each cow was over 87.5% Holstein Friesian, between day 63 and 282 of first lactation, and was milked twice a day. One morning milk sample of 500 ml per cow was collected between February and March 2005, which is the winter period. Sample bottles contained sodium azide (0.03% wt/wt) for conservation.

Fat percentage was measured by infrared spectroscopy, using a MilkoScan FT6000 (Foss Electric, Hillerod, Denmark) at the Milk Control Station (Zutphen, the Netherlands). Milk-fat composition was measured by gas chromatography at the COKZ laboratory (Netherlands Controlling Authority for Milk and Milk Products, Leusden, the Netherlands) as described in

Chapter 2. The fatty acids were expressed as weight-proportion of total fat weight. In the present study, 21 traits were analyzed: the individual fatty acids C18:0, C18:1*cis*9, C18:1*cis*11, C18:1*cis*12, C18:1*trans*4-8, C18:1*trans*9, C18:1*trans*10, C18:1*trans*11, C18:2*cis*9,12, C18:2*cis*9,*trans*11 (CLA), C18:3*cis*9,12,15, C19:0 and C20:0, the group C18:1*trans* fatty acids, the group saturated fatty acids (SFA: C4:0-C18:0 and C20:0), the group unsaturated fatty acids (UFA: mono- and polyunsaturated C10-C18), the ratio SFA/UFA, and the unsaturation indices for C18, CLA and total fatty acids. Unsaturation indices were calculated by expressing each product as a proportion of the product plus substrate, multiplied by 100 (Kelsey et al., 2003), e.g.

$$\text{C18 index} = \frac{\text{C18:1}cis9}{\text{C18:1}cis9 + \text{C18:0}} * 100 .$$

Means, standard deviations and heritabilities of the traits are given in Table 5.1.

Markers and genotypes

Blood and semen samples were collected for DNA isolation. A total of 849 cows, representing the 5 large and 2 small paternal half-sib families (of 193, 179, 170, 166, 91, 29 and 21 cows), and their 7 sires, were included in the genome-wide scan and successfully genotyped for 1,341 SNP. The 7 sires were the 5 proven bulls and 2 of the young bulls of which daughter phenotypes were collected. Their genotypes were in agreement with their pedigree. The vast majority of SNP were genotyped by GoldenGate assay (Illumina, San Diego, CA) according to manufacturer's protocol. SCD1 A293V and DGAT1 K232A genotypes were available from previous studies and genotyped by SNaPshot assay (Chapter 3) and allelic discrimination method (Chapter 2), respectively. The SCD1 A293V and DGAT1 K232A genotypes were available for 1,687 out of 1,905 animals. The linkage map was constructed using Crimap (Green et al., 1990). The number of markers per chromosome and information content is available in Chapter 4 and full details on the genotypings and construction of the linkage map are reported by Schopen et al. (2008). Briefly, a linkage map was constructed of the total of 1,341 SNP, which covered 2,829 cM on the Haldane-scale, representing all 29 autosomes. The size of the chromosomes varied between 44.3 and

Table 5.1 Mean, phenotypic standard deviation (σ_P) and heritability (h^2) of all traits included in the analysis, measured on 1,905 cows

Trait	Mean (w/w%)	σ_P	h^2_{SE}
C18:0	8.72	1.18	$0.24_{0.07}$
C18:1*cis*9	18.18	1.57	$0.26_{0.09}$
C18:1*cis*11	0.41	0.07	$0.21_{0.09}$
C18:1*cis*12	0.20	0.03	$0.21_{0.07}$
C18:1*trans*4-8[1]	0.21	0.03	$0.36_{0.10}$
C18:1*trans*9	0.15	0.02	$0.22_{0.08}$
C18:1*trans*10	0.23	0.15	$0.10_{0.06}$
C18:1*trans*11	0.78	0.12	$0.28_{0.09}$
C18:1*trans*[2]	1.37	0.24	$0.31_{0.09}$
CLA*cis*9,*trans*11	0.39	0.07	$0.42_{0.10}$
C18:2*cis*9,12	1.20	0.18	$0.27_{0.08}$
C18:3*cis*9,12,15	0.41	0.06	$0.26_{0.08}$
C19:0[3]	0.08	0.01	$0.23_{0.09}$
C20:0	0.13	0.02	$0.24_{0.08}$
C18 index[4]	67.62	3.49	$0.33_{0.08}$
CLA index[5]	33.72	3.73	$0.23_{0.07}$
Total index[6]	21.48	1.66	$0.30_{0.09}$
SFA[7]	70.76	2.11	$0.30_{0.09}$
UFA[8]	25.69	1.93	$0.29_{0.09}$
Ratio SFA/UFA	2.79	0.28	$0.29_{0.09}$
Fat %	4.36	0.64	$0.49_{0.10}$

[1] C18 :1*trans*4-8 may comprise C18:1 fatty acids with *trans* double bonds at position 4, 5, 6, 7, and 8. We were not able to separate these fatty acids by our method.

[2] C18:1*trans* comprises C18:1*trans*4-8, C18:1*trans*9, C18:1*trans*10, C18:1*trans*11.

[3] For C19:0 only 1,401 observations were available.

[4] C18 index = C18:1*cis*9/(C18:1*cis*9 + C18:0) * 100.

[5] CLA index = CLA*cis*9,*trans*11/(CLA*cis*9,*trans*11 + C18:1*trans*11) * 100.

[6] Total index = (C10:1 + C12:1 + C14:1*cis*9 + C16:1*cis*9 + C18:1*cis*9 + CLA*cis*9,*trans*11) / (C10:1 + C12:1 + C14:1*cis*9 + C16:1*cis*9 + C18:1*cis*9 + CLA*cis*9,*trans*11 + C10:0 + C12:0 + C14:0 + C16:0 + C18:0 + C18:1*trans*11)*100.

[7] SFA comprises saturated C4, C5, C6, C7, C8, C9, C10, C12, C13, C14, C15, C16, C17, C18 and C20.

[8] UFA comprises C10:1, C12:1, C14:1*cis*9, C16:1*cis*9, C16:1*trans*9, C18:1*cis*9, C18:1*cis*11, C18:1*cis*12, C18:1*trans*4-8, C18:1*trans*9, C18:1*trans*10, C18:1*trans*11, CLA*cis*9,*trans*11, C18:2*cis*9,12, and C18:3*cis*9,12,15.

144.7 cM, and the number of markers varied between 22 and 78 per chromosome. When markers mapped at the same position they were manually placed 0.1 cM apart to ensure that information of closely linked markers was used in the QTL analysis.

QTL analysis

Phenotypes of all 1,905 animals were used to estimate systematic effects of days in milk, age at first calving, season of calving and herd using an Animal Model in ASReml (Gilmour et al., 2002), according to Chapter 2. Phenotypes of the 849 cows used in the QTL analysis were pre-corrected using these estimates.

Half-sib QTL mapping was performed for each trait separately by a multi-marker regression method across all families (Knott et al., 1996). The regression was performed for each trait on each chromosome using the following model:

$$Y_{ijk} = \mu + s_i + b_{ik}X_{ijk} + e_{ijk},$$

where y_{ijk} is the adjusted phenotype for daughter j nested within sire i at position k, μ is the overall mean, s_i is the fixed effect of sire i, b_{ik} is the regression coefficient for sire i at position k, X_{ijk} is the probability of daughter j inheriting gamete 1 from sire i at position k and e_{ijk} is the random residual effect. Significance thresholds were calculated by permutation testing (Churchill and Doerge, 1994). Chromosome-wise significance levels were obtained by 10,000 permutations. Genome-wise significance levels were derived from the chromosome-wise significance levels as follows: $P_{genome} = 1 - (1 - P_{chr})^{1/r}$, where r is ratio of the chromosome length over the genome length. Significant linkage is indicated by a 5% genome-wise significance threshold and suggestive linkage by a 5% chromosome-wise significance threshold (Lander and Kruglyak, 1995). To define a 95% confidence interval for QTL location, 1,000 bootstraps were performed (Visscher et al., 1996). Allele substitution effects within each of the 7 families were calculated for significant QTL at the most likely QTL position. To study the effect of the two major genes DGAT1 and SCD1 on the QTL

profile, a second analysis was performed in which the combined DGAT1 K232A and SCD1 A293V genotype was also included as systematic effect in the Animal Model. The QTL regression was re-run for all traits and all 29 autosomes using the phenotypes adjusted for DGAT1 K232A and SCD1 A293V genotypes.

5.3 Results

QTL for long chain milk fatty acids were identified on several chromosomes in this study and are summarized in Table 5.2. All the QTL that exceeded the threshold for suggestive linkage are reported. There was significant statistical evidence for QTL on BTA14, BTA15 and BTA16. Figures 5.1-5.4 give graphical presentations of these QTL. Suggestive QTL were found on 16 other chromosomes. Allele substitution effects for the significant QTL on BTA14, 15 and 16 are shown in Table 5.3. Results of significant QTL will be discussed per chromosome.

On BTA14, a QTL for fat percentage was found at the centromeric end of the chromosome. At the same position significant evidence for QTL for C18:1*cis*9, C18:1*cis*12, C18:2*cis*9,12, CLA*cis*9,*trans*11, C18:3*cis*9,12,15, C18index, total index, SFA, UFA and ratio SFA/UFA was detected (Table 5.2, Figure 5.1). Families 1, 2, 3, 4 and 7 segregated for the QTL for fat percentage, C18:1*cis*9, and C18:3*cis*9,12,15 on BTA14 (Table 5.3). These families, but not necessarily all five, also contributed to the QTL for the other traits. Family 5 and 6 did not segregate for any of the QTL on BTA14. The difference in fat percentage between the two daughter groups inheriting alternative sire alleles was 0.53, 0.69, 0.70, 0.68 and 0.93 in families 1, 2, 3, 4, and 7, respectively. The difference in C18:1*cis*9 between the two daughter groups was 1.10, 0.60, 1.37, 1.18, and 1.83 %w/w in families 1, 2, 3, 4, and 7, respectively. This QTL explained 19% of the phenotypic variance for fat percentage, and 10% for C18:1*cis*9. For the other traits this QTL explained 4-7% of the phenotypic variance. At the centromeric end of BTA14, QTL for several short and medium fatty acids were detected as well (see Chapter 4).

Table 5.2 Location and characteristics of suggestive and significant QTL affecting long chain fatty acids, unsaturation indices and fat percentage

								DGAT1 and SCD1 corrected			
BTA	Trait	F-stat	Pos. (cM)	95% CI (cM)	P_{chr}	P_{genome}[1]		Pos. (cM)	P_{chr}	P_{genome}[1]	
1	C18:0	3.40	125	32-134	0.025	0.392		103	0.004	0.066	
1	C18:1*trans*11	3.21	79	0-129	0.028	0.425		-	-	-	
1	C19:0	5.60	87	28-129	0.012	0.206		87	0.005	0.086	
1	C20:0	-	-	-	-	-		107	0.034	0.494	
1	C18 index	3.65	124	10-135	0.011	0.193		129	<0.001	0.008	**
1	CLA index	3.75	125	8-134	0.008	0.139		129	0.013	0.227	
3	C18:3*cis*9,12,15	3.09	126	5-126	0.048	0.669		-	-	-	
6	Fat %	3.07	81	3-109	0.033	0.538		-	-	-	
6	C18 index	2.96	57	10-117	0.042	0.621		-	-	-	
6	CLA index	2.97	57	10-107	0.041	0.62		-	-	-	
7	C18:2*cis*9,12	3.62	125	45-126	0.01	0.21		125	0.015	0.280	
8	Fat %	3.23	82	45-113	0.022	0.41		-	-	-	
8	C18:1*cis*11	3.58	63	0-98	0.009	0.186		64	0.021	0.404	
8	C18:1*trans*4-8	2.97	105	19-111	0.034	0.569		-	-	-	
8	SFA	3.11	87	17-108	0.028	0.489		87	0.048	0.691	
8	UFA	3.06	85	0-108	0.027	0.481		-	-	-	
11	Fat %	-	-	-	-	-		124	0.024	0.421	
11	C18:0	3.19	99	8-124	0.034	0.542		97	0.048	0.678	
11	C18:1*trans*11	3.10	123	0-124	0.026	0.452		123	0.042	0.625	
11	C18:2*cis*9,12	3.17	97	33-124	0.029	0.495		96	0.027	0.466	
11	CLA*cis*9,*trans*11	3.36	79	0-122	0.022	0.398		79	0.026	0.450	
12	C18:2*cis*9,12	3.28	26	10-57	0.017	0.453		29	0.049	0.835	
13	Fat %	3.63	53	11-69	0.008	0.197		-	-	-	
13	C19:0	6.37	78	52-83	0.002	0.059		78	0.002	0.044	*
14	Fat %	28.84	0	0-0	<0.001	<0.001	***	-	-	-	
14	C18:1*cis*9	13.85	0	0-0	<0.001	<0.001	***	-	-	-	
14	C18:1*cis*11	-	-	-	-	-		41	0.043	0.700	
14	C18:1*cis*12	4.57	0	0-76	<0.001	0.008	**	-	-	-	
14	C18:1*trans*4-8	3.23	0	0-76	0.023	0.47		42	0.043	0.701	
14	C18:1*trans*9	3.57	50	0-76	0.008	0.198		47	0.013	0.306	
14	C18:1*trans*10	3.55	45	21-60	0.008	0.194		44	0.008	0.200	
14	C18:1*trans*	2.94	52	0-102	0.039	0.661		46	0.041	0.682	
14	C18:2*cis*9,12	6.00	0	0-76	<0.001	0.003	**	-	-	-	
14	CLA*cis*9,*trans*11	4.99	0	0-74	<0.001	0.011	**	-	-	-	
14	C18:3*cis*9,12,15	9.43	0	0-4	<0.001	<0.001	***	-	-	-	
14	C19:0	5.36	55	9-101	0.013	0.293		55	0.011	0.271	
14	C18 index	5.32	0	0-101	<0.001	<0.001	***	-	-	-	

Table 5.2 Continued

								DGAT1 and SCD1 corrected			
BTA	Trait	F-stat	Pos. (cM)	95% CI (cM)	P_{chr}	P_{genome}[1]		Pos. (cM)	P_{chr}	P_{genome}[1]	
14	CLA index	3.54	0	0-103	0.009	0.222		-	-	-	
14	Total index	7.83	0	0-49	<0.001	<0.001	***	-	-	-	
14	SFA	9.11	0	0-3	<0.001	<0.001	***	-	-	-	
14	UFA	8.50	0	0-4	<0.001	<0.001	***	-	-	-	
14	Ratio SFA/UFA	9.03	0	0-2	<0.001	<0.001	***	-	-	-	
15	C18:1*cis*12	2.85	57	1-94	0.032	0.624		57	0.038	0.684	
15	C18:1*trans*4-8	3.92	91	34-94	0.007	0.185		90	0.014	0.349	
15	C18:1*trans*9	3.78	80	28-94	0.005	0.135		80	0.007	0.183	
15	C18:1*trans*10	3.50	82	36-90	0.008	0.224		81	0.011	0.287	
15	C18:1*trans*	4.53	80	34-92	0.001	0.021	**	80	0.003	0.080	
15	C18:2*cis*9,12	-	-	-	-	-		57	0.039	0.696	
15	C19:0	4.52	33	5-86	0.04	0.707		-	-	-	
16	C18:0	3.61	42	10-88	0.01	0.23		42	0.013	0.289	
16	C20:0	3.37	29	13-88	0.016	0.35		29	0.026	0.500	
16	C18 index	4.35	45	26-73	0.001	0.037	*	45	0.001	0.027	*
16	CLA index	4.33	52	23-105	0.001	0.032	*	41	0.001	0.027	*
17	CLA*cis*9,*trans*11	3.05	66	24-95	0.037	0.66		-	-	-	
18	C18:1*trans*11	2.86	51	1-82	0.037	0.722		51	0.025	0.577	
19	C18:0	2.89	54	30-101	0.049	0.746		54	0.044	0.710	
19	C18 index	3.05	96	42-100	0.029	0.545		-	-	-	
19	CLA index	3.02	96	21-96	0.03	0.563		-	-	-	
19	Total index	3.20	98	42-103	0.017	0.378		-	-	-	
19	UFA	2.87	98	40-104	0.033	0.597		-	-	-	
21	C18:1*cis*9	3.22	29	5-86	0.021	0.483		28	0.004	0.102	
21	C18:1*trans*4-8	2.72	38	0-92	0.037	0.691		-	-	-	
21	C18:1*trans*9	3.27	26	13-89	0.014	0.344		26	0.016	0.393	
21	C18:1*trans*10	2.88	39	17-92	0.028	0.576		39	0.040	0.717	
21	C18:2*cis*9,12	3.23	19	0-83	0.015	0.364		19	0.032	0.636	
21	C20:0	2.90	39	7-92	0.042	0.729		39	0.042	0.734	
21	Total index	-	-	-	-	-		27	0.034	0.652	
21	SFA	-	-	-	-	-		27	0.031	0.623	
21	UFA	2.77	27	4-89	0.04	0.712		27	0.022	0.499	
21	Ratio SFA/UFA	-	-	-	-	-		27	0.048	0.781	
22	C18:0	-	-	-	-	-		61	0.014	0.379	
22	C18:3*cis*9,12,15	3.57	12	0-83	0.009	0.272		-	-	-	
22	C20:0	-	-	-	-	-		61	0.004	0.131	

Table 5.2 Continued

							DGAT1 and SCD1 corrected		
BTA	Trait	F-stat	Pos. (cM)	95% CI (cM)	P_{chr}	P_{genome}[1]	Pos. (cM)	P_{chr}	P_{genome}[1]
22	C18 index	-	-	-	-	-	61	0.042	0.774
26	UFA	2.58	36	10-68	0.05	0.881	-	-	-
26	Ratio SFA/UFA	2.72	36	10-59	0.037	0.797	-	-	-
27	Fat %	2.58	33	15-44	0.049	0.959	-	-	-
27	C18:1*trans*11	2.56	44	0-44	0.044	0.944	44	0.031	0.867
27	Total index	2.62	44	6-44	0.043	0.941	-	-	-
29	C19:0	4.18	1	0-64	0.046	0.87	-	-	-

[1] * $P_{genome} \leq 0.05$, ** $P_{genome} \leq 0.01$, *** $P_{genome} \leq 0.001$. All listed QTL have $P_{chr} \leq 0.05$.

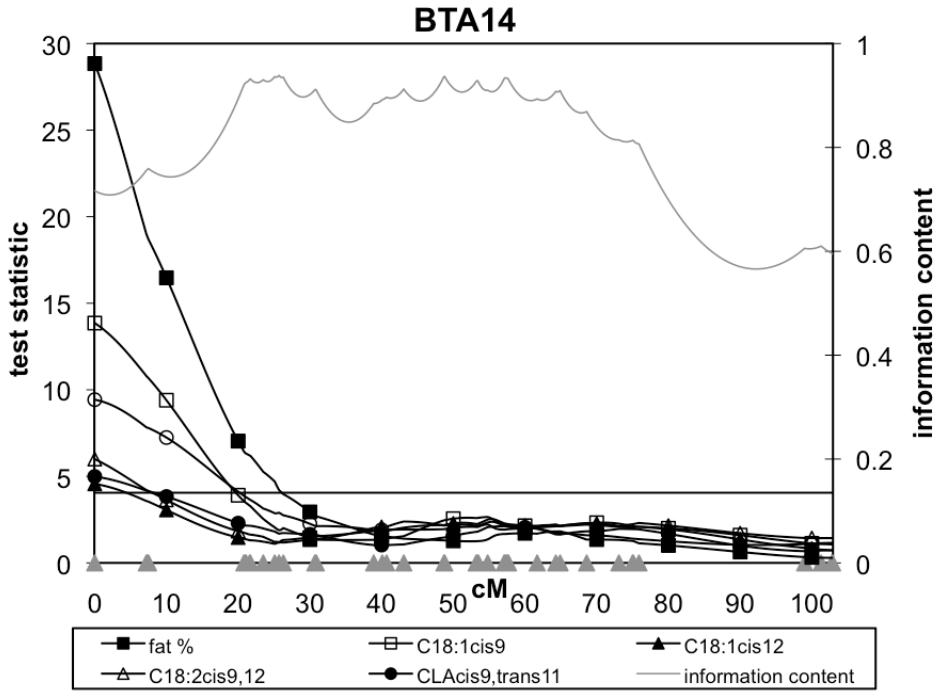

Figure 5.1 QTL mapping across families for long chain fatty acids on BTA14. Triangles on the x-axis represent the location of the markers. The solid black line represents the genome-wise significance threshold. Although this threshold is slightly different for each trait, only the line of the trait with the lowest genome-wise significance threshold is shown.

The centromeric region of BTA14 has been studied in detail in previous studies on fat percentage (Farnir et al., 2002; Grisart et al., 2002; Winter et al., 2002), which revealed the role of DGAT1, which is mapped to BTA14 at 0 cM. A nonsynonymous mutation in the DGAT1 gene has been shown to have a large effect on milk-fat composition in this material (Chapter 2). The QTL at 0 cM was most likely caused by, or very closely linked to this DGAT1 mutation. The sires from segregating families 1, 2, 3, 4 and 7 were all heterozygous KA for the DGAT1 K232A mutation, whereas sire 5 was homozygous KK, and sire 6 was homozygous AA. To validate that this QTL on BTA14 was caused by DGAT1, the combined DGAT1 K232A and SCD1 A293V genotype was included as an additional fixed effect in the model in a second analysis. This correction resulted in the disappearance of the QTL effect on the centromeric region of BTA14 on all above reported traits (Table 5.2), indicating that the DGAT1 genotype was responsible for this QTL

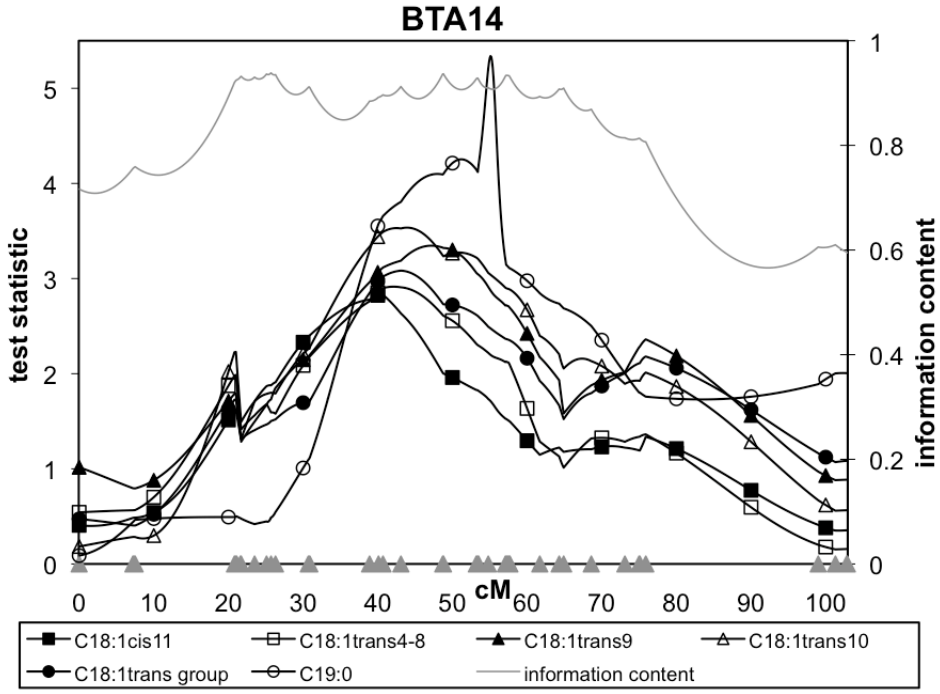

Figure 5.2 QTL mapping across families for long chain fatty acids pre-corrected for DGAT1 K232A and SCD1 A293V genotypes on BTA14. Triangles on the x-axis represent the location of the markers. All shown QTL are suggestive.

effect. Furthermore, a suggestive QTL at approximately 50 cM became clear for the C18:1*trans* fatty acids as a group, and for the individual fatty acids C18:1*trans*4-8, C18:1*trans*9, C18:1*trans*10, C18:1*cis*11 and C19:0 (Figure 5.2). This position also showed suggestive QTL for C10:1, C12:1 and C14:1 fatty acids after the DGAT1/SCD1 correction (see Chapter 4).

On BTA15, a QTL for the group of C18:1*trans* fatty acids was found at position 80 cM. This QTL region also affected the individual C18:1*trans* fatty acids C18:1*trans*4-8, C18:1*trans*9 and C18:1*trans*10, although not genome-wise significant, but suggestive (Table 5.2). The shape of the confidence interval and approximate position correspond to the observed similar shape of the QTL profile across families for the C18:1*trans* fatty acids (Figure 5.3).

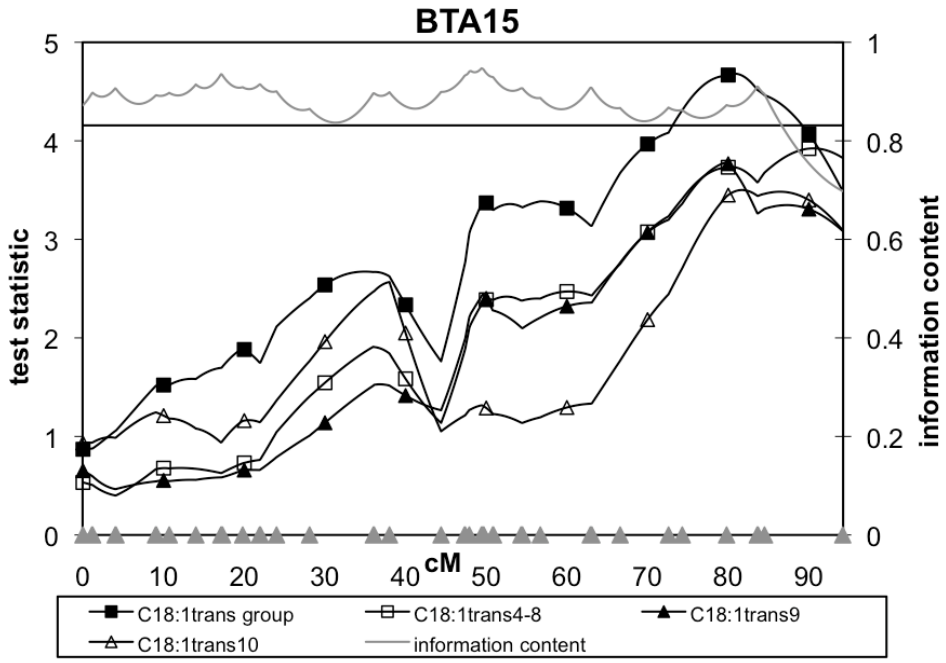

Figure 5.3 QTL mapping across families for long chain fatty acids on BTA15. Triangles on the x-axis represent the location of the markers. The dotted black line presents the genome-wise significance threshold for the C18:1*trans* group. The QTL for individual C18:1*trans* fatty acids did not reach the genome-wise significance threshold.

Family 1 and 2 contributed to the QTL (Table 5.3), and the difference between the daughter groups in the fraction of the C18:1*trans* group fatty acids was 0.19 and 0.08 %w/w, respectively. The QTL explained 4% of the phenotypic variance of C18:1*trans*. Pre-correction for DGAT1/SCD1 slightly lowered the test statistic, as a result of which the genome-wise P-value did not exceed the 5% significance level (P=0.08).

On BTA16, QTL for C18 index and CLA index were found at positions 45 and 52 cM, within the same confidence interval (Table 5.2, Figure 5.4). A suggestive QTL within the same interval is also found for C18:0 and C20:0. Family 1, 3 and 4 were segregating for the QTL for C18 index; for CLA index only family 1 and 3 segregated significantly (Table 5.3). The QTL explained 3% of the phenotypic variance of both C18 and CLA index.

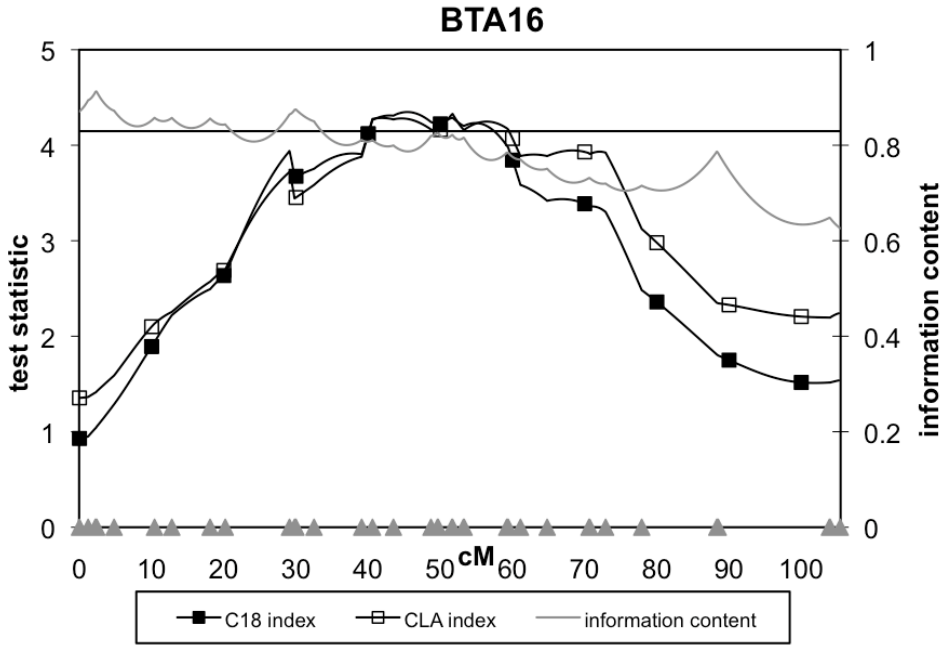

Figure 5.4 QTL mapping across families for long chain fatty acids on BTA16. Triangles on the x-axis represent the location of the markers. The solid black line represents the genome-wise significance threshold. Although this threshold is slightly different for each trait, only the line of the trait with the lowest genome-wise significance threshold is shown.

Removal of the effects of DGAT1 and SCD1 genotypes resulted furthermore in 2 new significant linkage positions. On BTA1, a QTL at 129 cM for C18 index was detected. Suggestive QTL within the same confidence interval on BTA1 were found for C18:0, C19:0, C20:0 and CLA index. On BTA13, a QTL for C19:0 was found at 78 cM.

Table 5.3 Allele substitution effects[1] and SE within 7 paternal half-sib families for QTL on BTA14, 15 and 16, and approximate phenotypic variation explained by the QTL

Trait	Family (no of daughters)							Phenotypic variation explained by QTL (%)
	1 (193)	2 (179)	3 (170)	4 (166)	5 (91)	6 (29)	7 (21)	
BTA14								
Fat %	$-0.53_{0.09}^{*}$	$-0.69_{0.10}^{*}$	$-0.70_{0.11}^{*}$	$-0.68_{0.10}^{*}$	$-0.06_{0.19}$	$0.50_{0.34}$	$-0.93_{0.26}^{*}$	19
C18:1*cis*9	$1.10_{0.23}^{*}$	$0.60_{0.23}^{*}$	$1.37_{0.26}^{*}$	$1.18_{0.24}^{*}$	$0.50_{0.47}$	$-0.26_{0.83}$	$1.83_{0.65}^{*}$	10
C18:1*cis*12	$0.01_{0.01}^{*}$	$0.01_{0.01}^{*}$	$0.01_{0.01}$	$0.02_{0.01}^{*}$	$0.00_{0.01}$	$0.02_{0.02}$	$0.02_{0.01}$	4
C18:2*cis*9,12	$0.06_{0.03}^{*}$	$0.07_{0.03}^{*}$	$0.06_{0.03}$	$0.13_{0.03}^{*}$	$0.00_{0.06}$	$0.19_{0.10}$	$0.18_{0.08}^{*}$	5
CLA*cis*9,*trans*11	$0.02_{0.01}^{*}$	$0.01_{0.01}$	$0.03_{0.01}^{*}$	$0.04_{0.01}^{*}$	$0.04_{0.02}$	$-0.04_{0.04}$	$0.07_{0.03}^{*}$	4
C18:3*cis*9,12,15	$0.03_{0.01}^{*}$	$0.02_{0.01}^{*}$	$0.03_{0.01}^{*}$	$0.06_{0.01}^{*}$	$0.01_{0.02}$	$0.01_{0.03}$	$0.05_{0.02}^{*}$	7
C18 index	$1.55_{0.56}^{*}$	$0.36_{0.56}$	$2.43_{0.63}^{*}$	$0.78_{0.59}$	$1.47_{0.14}$	$-1.45_{2.00}$	$4.19_{1.56}^{*}$	4
Total index	$0.83_{0.25}^{*}$	$-0.32_{0.25}$	$1.27_{0.28}^{*}$	$0.82_{0.26}^{*}$	$0.77_{0.51}$	$-0.51_{0.90}$	$1.60_{0.70}^{*}$	6
SFA	$-1.18_{0.32}^{*}$	$-0.51_{0.33}$	$-1.58_{0.37}^{*}$	$-1.36_{0.34}^{*}$	$-0.95_{0.66}$	$0.29_{1.17}$	$-2.42_{0.91}^{*}$	7
UFA	$1.04_{0.30}^{*}$	$0.48_{0.30}$	$1.42_{0.33}^{*}$	$1.17_{0.31}^{*}$	$0.84_{0.60}$	$-0.30_{1.10}$	$2.03_{0.83}^{*}$	6
Ratio SFA/UFA	$-0.15_{0.04}^{*}$	$-0.09_{0.04}$	$-0.21_{0.05}^{*}$	$-0.18_{0.05}^{*}$	$-0.12_{0.09}$	$0.03_{0.16}$	$-0.33_{0.12}^{*}$	7
BTA15								
C18:1*trans*	$-0.19_{0.04}^{*}$	$-0.08_{0.04}^{*}$	$0.01_{0.04}$	$0.02_{0.04}$	$-0.01_{0.06}$	$-0.01_{0.09}$	$0.10_{0.11}$	4
BTA16								
C18 index	$1.60_{0.54}^{*}$	$0.22_{0.53}$	$2.61_{0.76}^{*}$	$-1.41_{0.56}^{*}$	$0.66_{0.76}$	$0.10_{1.30}$	$-0.12_{3.07}$	3
CLA index	$1.41_{0.53}^{*}$	$-0.01_{0.54}$	$3.46_{0.87}^{*}$	$-1.09_{0.58}$	$0.26_{0.82}$	$-1.41_{1.39}$	$-0.74_{3.13}$	3

[1] Significantly segregating QTL (P≤0.05, calculated by a t-test) are marked with an asterisk.

5.4 Discussion

In this study, QTL were mapped for long chain milk fatty acids in the Dutch Holstein Friesian cattle population. Given the design and size of our study, power calculations showed that QTL that explain at least 5% of the phenotypic variation could be detected with a probability of 0.8 for traits with a heritability of 0.25. Also smaller QTL can be detected, however, with a lower probability (more on the power of the study in Chapter 4).

The identification of QTL for long chain fatty acids strongly supports the hypothesis of a genetic component that influences variation for these fatty acids - as for the short to medium chain fatty acids -, which was already posed by the low to moderate heritabilities. Long chain fatty acids are not *de novo* synthesized by the cow herself, but are derived from circulating plasma lipids, and originate from the diet, from microbial fatty acid synthesis in the rumen, and from endogenous lipids. Therefore, the most obvious candidate genes for long chain fatty acid proportions, would be those that are involved in the uptake, desaturation, esterification, biohydrogenation, and elongation of long chain fatty acids.

The QTL on the centromeric end of BTA14 is most likely caused by the DGAT1 K232A mutation, which has a known effect on milk-fat composition (Chapter 2), or by a closely linked mutation. This is confirmed by the elimination of this QTL by the adjustment of phenotypes for the DGAT1 genotype. The DGAT1 enzyme plays a role in the formation of triacylglycerols, by the esterification of a fatty acyl-CoA to the *sn*-3 position of the glycerol backbone. In an association study (Chapters 2 and 3), the DGAT1 232A allele was shown to be associated with more C18:1*cis*9 and more CLA*cis*9,*trans*11 in milk, which is in agreement with findings in the present study. The allele substitution effect for C18:1*cis*9 on BTA14 ranged between 0.60 and 1.83 for the different segregating sires, and the allele substitution effect in the association study was 1.11 (calculated from contrasts between KK and KA, and KK and AA genotypes for the C18 unsaturated fatty acids, which predominantly consists of C18:1*cis*9). Allele substitution effects for CLA*cis*9,*trans*11, C18 index, total index and ratio SFA/UFA are also in line with the genotype contrasts reported in the

association study. Moreover, allele substitution effects show that a lower fat percentage is correlated with more C18:1*cis*9, more CLA*cis*9,*trans*11, a higher C18 and total index, and a lower ratio SFA/UFA, a result which confirms effects of the DGAT1 232A allele in the association study. This result is also reflected by the genetic correlation of -0.63 between fat percentage and C18:1*cis*9 in our population (Stoop et al. 2008). A suggestive QTL, positioned in the middle of BTA14, was not affected by pre-correction of phenotypes for DGAT1/SCD1 genotypes. This position showed suggestive linkage for both long chain and short/medium chain fatty acids, and suggests the presence of another QTL for milk-fat composition on BTA14.

A QTL for C18:1*trans* fatty acids is detected on BTA15. In the rumen, dietary unsaturated fatty acids are metabolized by ruminal microbes, and via intermediates reduced to C18:0 as final end product, in a process called biohydrogenation. The final reduction to C18:0, with C18:1*trans* fatty acids as common intermediates, is considered to be the rate-limiting step. When this metabolism is incomplete, C18:1*trans* fatty acids will accumulate. Among the C18:1*trans* intermediates, the *trans*11 isomer is the main one, but also other isomers are produced (Harfoot and Hazlewood, 1997; Shingfield et al., 2003; Loor et al., 2005). The rumen microbial population consists of, among others, several, genetically very diverse, bacteria, which metabolize fatty acids by several possible routes (Edwards et al., 2004). Some kinds of C18:1*trans* isomers that are produced have been reported to be specific to particular bacterial populations. We hypothesize that between animal variation in rumen bacterial populations and/or biohydrogenating activity of ruminal fluid might partly be explained by genetic differences between cows (Wasowska et al., 2006; Paillard et al., 2007). The QTL on BTA15 may harbor a gene that is involved in differences in ruminal populations or ruminal activity, and thereby influence C18:1*trans* fatty acids. The route that long chain fatty acids undergo - from intake or adipose tissue release to triacylglycerol formation in the udder and secretion into the milk - is complex and involves many processes, e.g. lipolysis, transport, esterification, which can be influenced by different genes and affect C18:1*trans* fatty acids.

The presence of a QTL on BTA15 for C18:1*trans*4-8, C18:1*trans*9, and C18:1*trans*10, but not for C18:1*trans*11 might be explained by the actions of the SCD1 enzyme in the mammary gland. Positional isomers of C18:1*trans*, with double bonds at positions other than 8, 9 and 10, can be converted by SCD1, as shown by studies in rat liver microsomal systems (Mahfouz et al., 1980; Pollard et al., 1980). Because C18:1*trans*11 can be converted to C18:2*cis*9,*trans*11 by SCD1, and thus involves different metabolism and maybe also different transport, no QTL for C18:1*trans*11 on BTA15 could possibly be detected.

QTL for C18 index and CLA index were found on BTA16. An index reflects a ratio between a saturated fatty acid and its *cis*9-monounsaturated counterpart, which is influenced (among others) by SCD1 conversion. About 40% of the C18:0 taken up by the mammary gland is converted to C18:1*cis*9, and about 26% of C18:1*trans*11 to CLA*cis*9,*trans*11 (Chilliard et al., 2000; Mosley et al., 2006). Although we previously showed a significant association between the SCD1 A293V genotype, located on BTA26, and C18 index and CLA index (Chapter 3), we were not able to detect significant or suggestive linkage for these indices on BTA26. This would suggest QTL for these indices were below detection. The QTL on BTA16 might harbor a gene involved in the complex regulation of SCD1, however, no obvious candidate genes are known on BTA16.

QTL mapping for milk-fat composition has only been reported by Morris et al. (2007), who restricted their study to BTA19, and identified FASN as a candidate gene. Although Morris et al. (2007) detected a QTL for C18 fatty acids on BTA19, and found an association between SNP in FASN and C18:0 and C18:1*cis*9 in milk, we were not able to confirm these findings. Different production circumstances might explain this: a pasture-based system in New Zealand vs. indoor winter period in the Netherlands. A significant effect of FASN genotype on C18 fatty acids was also found in tissue of the *longissimus* muscle of beef cattle (Abe et al., 2008b; Zhang et al., 2008). Another candidate gene for fat composition that has been put forward is sterol regulatory element binding protein-1 (SREBP-1). Hoashi et al. (2007)

reported association of a 84-bp insertion in SREBP-1 with monounsaturated fatty acids (MUFA) proportion in adipose tissue of Japanese black cattle.

This is the first genome-wide scan for milk-fat composition, which makes comparison to literature impossible. However, (partial) genome scans to detect QTL for carcass-fat composition have been performed in beef cattle, pigs and sheep (Clop et al., 2003; Karamichou et al., 2006; Alexander et al., 2007; Sanchez et al., 2007; Abe et al., 2008a). Alexander et al. (2007) analyzed the fat composition of the *longissimus* muscle of Wagyu x Limousin cattle, and found significant QTL on BTA2 (a.o. for MUFA, SFA, CLA and ratio of C18:1 to C18:0), and BTA7 (for MUFA). Abe et al. (2008a) mapped QTL for fat composition of the *longissimus* muscle of Japanese Black x Limousin cattle, and detected QTL on BTA2 (among others for C18:2) and on BTA19 (among others for C18:1).

The study described in this and the previous chapter is the first, to our knowledge, to present results of a genome-wide scan for milk-fat composition, and is an important step in the unraveling of regulation of lipogenesis of fatty acids. For short and medium chain fatty acids, we detected 4 significant QTL on BTA6, 14, 19 and 26, and for long chain fatty acids, we detected 3 significant QTL on BTA14, 15, and 16. Only BTA14 is involved in both short/medium and long chain milk fatty acids, whereas the other significant QTL are detected for either one of them. This finding indicates that short/medium chain fatty acids on one hand, and long chain fatty acids on the other hand, undergo distinct processes of synthesis and metabolism. The improvement of the mapping resolution is an essential step towards positional cloning of mapped QTL and understanding of fatty acids metabolism.

5.5 Acknowledgments

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6

Effect of polymorphisms in the FASN, OLR1, PPARGC1A, PRL, and STAT5A genes on bovine milk-fat composition

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Animal Genetics, accepted with minor modifications

Abstract

The aim of our study was to estimate effects of polymorphisms in the ABCG2, FASN, OLR1, PPARGC1A, PRL, and STAT5A genes on detailed milk-fat composition. Milk-fat composition phenotypes were available for 1,905 Dutch Holstein Friesian cows. First, the presence of each SNP in the Dutch Holstein Friesian population was evaluated by direct sequencing of the PCR product surrounding the SNP in 22 proven Dutch Holstein Friesian bulls. The SNP in ABCG2 appeared to be monomorph in all bulls. Second, we genotyped the cows for the $FASN_{16024}$, $FASN_{17924}$, $OLR1_{8232}$, $PPARGC1A_{1790+514}$, $PPARGC1A_{1892+19,}$ PRL_{8398} and $STAT5A_{9501}$ polymorphisms, and estimated genotype effects on milk production traits and milk-fat composition. $FASN_{17924}$ and OLR_{8232} had a significant effect on milk-fat percentage. However, we were not able to confirm results reported in literature, which showed effects of all evaluated polymorphisms on milk-fat percentage or milk-fat yield. All polymorphisms showed significant effects on milk-fat composition. The polymorphisms in FASN and STAT5A, which had an effect on C14:0 and were located on chromosome 19, could not fully explain the quantitative trait locus (QTL) for C14:0 detected on chromosome 19 in a previous genome-wide scan using linkage analysis.

6.1 Introduction

Recent studies have revealed large genetic variation in bovine milk-fat composition (Soyeurt et al., 2007; Stoop et al., 2008). Candidate genes underlying this variation may be found in fat synthesis and metabolism pathways, which are under the control of multiple genes. Information on effects of DNA polymorphisms on milk-fat composition is scarce, because milk-fat composition data is, unlike milk-fat percentage and milk-fat yield, not routinely collected in milk recording schemes. Polymorphisms in the diacylglycerol acyltransferase 1 (DGAT1) and stearoyl-CoA desaturase 1 (SCD1) genes have been shown to affect the composition of bovine milk-fat in different cattle populations (Chapters 2 and 3; Mele et al., 2007; Moioli et al., 2007). Single nucleotide polymorphisms (SNP) in several other genes that play a role in fat synthesis or metabolism pathways have been associated with milk-fat percentage or milk-fat yield. In the current study, we evaluated associations between SNP in those genes and bovine milk-fat composition. The genes under study are ATP-binding cassette G2 (ABCG2), fatty acid synthase (FASN), oxidized low-density lipoprotein receptor 1 (OLR1), peroxysome proliferator-activated receptor-γ coactivator-1α (PPARGC1A), prolactin (PRL), and signal transducer and activator of transcription 5A (STAT5A).

The multidrug transporter encoded by ABCG2 is shown to be strongly induced in the mammary gland of mice, cows and humans during lactation, and is responsible for the secretion of clinically and toxicologically important substrates into mouse milk (Jonker et al., 2005). Cohen-Zinder et al. (2005) proposed a non-synonymous mutation in ABCG2 as the causative polymorphism underlying a quantitative trait locus (QTL) on *Bos taurus* autosome (BTA) 6 affecting milk yield, milk-fat percentage and milk-protein percentage.

FASN is a multifunctional enzyme complex that catalyzes *de novo* fatty acid synthesis and has been put forward as a candidate gene for milk-fat percentage as well as fat composition of milk and beef. SNP in different exons of the FASN gene, and different functional domains of the FASN protein have been associated with milk-fat percentage (Roy et al., 2006b),

and with medium and long chain fatty acid content of milk (Morris et al., 2007) and beef (Zhang et al., 2008).

OLR1 is involved in fatty acid transport and binds and degrades the oxidized form of low-density lipoprotein. Khatib et al. (2006) identified OLR1 as a functional and positional candidate gene for milk-fat percentage and milk-fat yield, and showed association of a SNP in the 3'-UTR of OLR1 with milk-fat percentage and milk-fat yield in a population of North American Holstein cattle.

PPARGC1A plays a central role in the activation of nuclear hormone receptors and transcription factors regulating energy homeostasis. Weikard et al. (2005) identified PPARGC1A as a candidate gene for milk production traits and found an association between a SNP in this gene and milk-fat yield in German Holstein cattle. However, this finding could not be confirmed in an American cattle population (Khatib et al., 2007b).

PRL is a hormone secreted by the anterior pituitary with multiple functions, among which a critical role in mammary gland development, lactogenesis and galactopoiesis. A synonymous SNP in exon 4 of PRL has been genotyped in various dairy cattle populations, but association analyses with milk-fat percentage or milk-fat yield did not show consistent results (Dybus, 2002; Brym et al., 2005; Dybus et al., 2005).

STAT are transcription factors known to play an important role in cytokine signaling. The STAT5A protein was initially identified as mammary gland factor and mediates the action of PRL on, among others, milk protein gene expression. SNP in the STAT5A gene have been associated with milk production traits, among which milk-fat percentage, in Jersey, Polish Black and White, and US Holstein cattle (Brym et al., 2004; Flisikowski et al., 2004; Khatib et al., 2008).

The objective of the present study was to estimate effects of polymorphisms in the ABCG2, FASN, OLR1, PPARGC1A, PRL, and STAT5A genes on detailed milk-fat composition.

6.2 Materials and methods

Animals

This study is part of the Dutch Milk Genomics Initiative, which focuses on the genetic background of detailed milk composition. Phenotypic data were available from 1,905 cows from 398 commercial herds in the Netherlands. Cows descended from 1 of 5 proven bulls (871 cows), from 1 of 50 young bulls (844 cows), or from other proven bulls (190 cows). The last group was added to ensure that at least 3 cows were sampled per herd. CRV (Arnhem, the Netherlands) provided pedigrees of the cows. Each cow was over 87.5% Holstein Friesian (HF), between day 63 and 282 of first lactation, and was milked twice a day. Blood or semen samples for DNA analysis were collected from the cows, the 5 proven and 50 young bulls. To test SNP frequencies in a small group of animals representative of the Dutch HF cattle population, semen was collected from 17 other frequently used proven HF bulls.

Phenotypes

One morning milk sample of 500 ml per cow was collected between February and March 2005, which is the winter period. Milk-fat composition was measured by gas chromatography at the COKZ laboratory (Netherlands Controlling Authority for Milk and Milk Products, Leusden, the Netherlands) as described in Chapter 2. The fatty acids were expressed as weight-proportion of total fat weight. Fat and protein percentages were measured by infrared spectroscopy, using a MilkoScan FT6000 (Foss Electric, Hillerod, Denmark) at the Milk Control Station (Zutphen, the Netherlands). Fat and protein yields were calculated by multiplying each percentage by the morning milk yield. Yield data were missing for 138 cows. In the present study, 26 traits were analyzed: fat and protein percentage, fat, protein and milk yield, the individual fatty acids C10:0, C10:1, C12, C12:1, C14:0, C14:1*cis*9, C16:0, C16:1*cis*9, C18:0, C18:1*cis*9, C18:2*cis*9,12, C18:2*cis*9,*trans*11 (CLA), C18:3*cis*9,12,15, the group C18:1*trans* fatty acids, and the unsaturation indices for C10, C12, C14, C16 and C18, CLA and total fatty acids. Unsaturation indices were calculated by expressing each product as a proportion of the product plus substrate, multiplied by 100 (Kelsey et al., 2003), e.g.

$$\text{C18 index} = \frac{\text{C18:1}cis9}{\text{C18:1}cis9 + \text{C18:0}} * 100$$

The overall means, standard deviations and heritabilities (as calculated in Chapters 4 and 5) of the traits analyzed in this study are listed in Table 6.1.

Table 6.1 Means, standard deviations (SD) and heritabilities[1] (h^2) of traits under study, as measured on test-day morning milk samples from 1,905 first-lactation Holstein Friesian cows

Trait	Mean	SD	h^2
	Milk production traits		
Fat %	4.36	0.70	0.49
Protein %	3.51	0.30	0.65
Fat yield (kg)[2]	0.58	0.11	0.39
Protein yield (kg)[2]	0.47	0.09	0.23
Milk yield (kg)[2]	13.46	2.73	0.41
	Milk-fat composition[3]		
C10:0	3.03	0.43	0.72
C10:1	0.37	0.07	0.34
C12:0	4.11	0.69	0.64
C12:1	0.12	0.03	0.38
C14:0	11.61	0.92	0.62
C14:1*cis*9	1.36	0.26	0.34
C16:0	32.59	2.83	0.43
C16:1*cis*9	1.44	0.32	0.44
C18:0	8.72	1.42	0.24
C18:1*cis*9	18.18	2.04	0.26
C18:1*trans*[4]	1.37	0.37	0.31
C18:2*cis*9,12	1.20	0.29	0.27
C18:2*cis*9,*trans*11 (CLA)	0.39	0.11	0.42
C18:3*cis*9,12,15	0.41	0.11	0.26
C10 index	10.89	2.74	0.37
C12 index	2.74	0.54	0.36
C14 index	10.51	1.84	0.45
C16 index	4.24	0.82	0.47
C18 index	67.62	3.74	0.33
CLA index	33.72	4.06	0.23
Total index	21.48	2.10	0.30

[1] Heritabilities are taken from previous studies (Chapter 4 and 5).
[2] Based on 1,766 morning milk samples.
[3] Expressed as w/w percentage, except for the indices.
[4] C18:1*trans* contains C18:1*trans*4-8, C18:1*trans*9, C18:1*trans*10, and C18:1*trans*11.

Genotypes

Genomic DNA was isolated from blood samples using Puregene (Gentra, Qiagen) and from semen samples according to Ganai et al. (2008). The presence of the SNP in the Dutch HF population was evaluated by direct sequencing of the PCR product surrounding the SNP in the 22 proven bulls: the 5 proven bulls that have daughters in the Milk Genomics Initiative population, and 17 other frequently used proven bulls. The primer designs (Table 6.2) were based on the Genbank sequences AJ871176 (ABCG2), AF285607 (FASN), NW_215807 (OLR1), AY321517 and NC_007304 (PPARGC1A), AF426315 (PRL), and AJ237937 (STAT5A). The ABCG2 g.62569A>C SNP was monomorph AA in the 22 proven bulls, as well as in the 50 young bulls, and therefore excluded from further analysis. The cows were genotyped by one of 3 methods described below. Genotypes for FASN g.16024A>G, OLR1 g.8232A>C, PPARGC1A c.1790+514G>A, PPARGC1A c.1892+19C>T and PRL g.8398A>G were assayed by the SNaPshot single base primer extension method (Applied Biosystems, Foster City, CA) as previously described (Chapter 3). Genotyping primers are in Table 6.2. The STAT5A g.9501A>G polymorphism was genotyped by GoldenGate assay (Illumina, San Diego, CA) according to manufacturer's protocol. The FASN g.17924A>G polymorphism was genotyped by a custom Infinium assay (Illumina, San Diego, CA) according to manufacturer's protocol.

Linkage disequilibrium

Haploview software (Barrett et al., 2005) was used to calculate linkage disequilibrium (r^2) between the SNP that were located on the same chromosomes: the two SNP in FASN and $STAT5A_{9501}$, located on BTA19, and the two SNP in PPARGC1A, located on BTA6. Calculations in Haploview are based on genotypic data without considering the pedigree structure.

Statistical analysis

Phenotypes of all 1,905 animals were used to estimate variance components and systematic environmental effects as described in Chapter 2. Genotypes were not available for all 1,905 animals and this caused problems in adjusting for herd effects as some herds had less than 3 observations. Therefore we used pre-corrected data to estimate genotype effects. Data

Table 6.2 PCR and extension primers[1] for direct sequencing and genotyping using SNaPshot assay

Primer	Sequence
FASN_F	CCAGACCTTAATTTGCCAATC
FASN_R	CAAGGGCTGTGCAGGGAGAA
FASN g.16024G>A	CCGGGTGCCATCCAGTG
OLR1_F	GCTTTAAGACAAGGTGGCAGT
OLR1_R	CCTTGAGTTAGGCAACAAGTTC
OLR1 g.8232A>C	CTAACTTGTTCCAAGTCCTCCC
PPARGC1A_F	GCCGGTTTATGTTAAGACAG
PPARGC1A_R	GGTATTCTTCCCTCTTGAGC
PPARGC1A c.1790+514G>A	GCTACTCAGTCATGCTGATAAACTG
PPARGC1A c.1892C>T	CAGGTAATGATGCACGTTCGC
PRL_F	GGTCAATCACTCTGAGCAAAA
PRL_R	CCTGTGGTTTGAGGAGAATA
PRL g.8398A>G	GGCTCCTTTCATACCCCG
STAT5A_F	ATGAGGAAAGACAGCCCAGA
STAT5A_R	CAGTCTCTGGCTTTCCCAAG
ABCG2_F	ACGAGACTGTCAGGGACTTA
ABCG2_R	TCTTTGTCATACCAAAGCAA

[1] Length of a poly-T tail was used to modify the size of each extension primer.

was pre-corrected for days in milk, age at calving, season of calving and herd. Genotype effects were estimated using the following Animal Model in ASReml, with the variance of additive genetic effects at fixed values (Gilmour et al., 2002):

$$y_{ij} = \mu + SNP_i + a_j + e_{ij},$$

where y_{ij} = dependent variable adjusted for systematic environmental effects of days in milk, age at calving, season of calving and herd; SNP_i = effect of SNP genotype i; a_j = random additive genetic effect of animal j; e_{ij} = random residual effect. Additive genetic relations between animals were accounted for.

In a previous study, we performed a genome-wide scan to detect QTL for bovine milk-fat composition. This genome-wide scan consisted of 849 cows representing 5 large and 2 small paternal half-sib families in a weighted across-family regression. Significant QTL were detected on BTA6, 14, 15, 16, 19 and 26 (Chapters 4 and 5). Both FASN and STAT5A are located on BTA19 and to investigate whether the SNP in FASN or STAT5A contributed to the QTL detected on BTA19 for C14:0, the QTL analysis was repeated using phenotypes corrected for these SNP effects in FASN or STAT5A (see Chapters 4 and 5 for details).

6.3 Results and discussion

In the current study, SNP in FASN, OLR1, PPARGC1A, PRL and STAT5A were genotyped in Dutch HF cattle, and their effects on milk production traits and detailed milk-fat composition were estimated. Effects on milk-fat percentage were only found for SNP in FASN and OLR1, whereas effects on milk-fat composition were found for all SNP.

Allele frequencies

Allele frequencies are shown in Table 6.3. All genotypes, except for $PPARGC1A_{1892+19}$, were in Hardy-Weinberg equilibrium. Because the sampled population is not a random sample, but a selection of a number of families, there is no reason to exclude $PPARGC1A_{1892+19}$ from further analyses. The $ABCG2_{62569}$ SNP (or p.581Y>S) appeared to be monomorph AA in the 22 proven and the 50 young bulls. This result is in line with results from Ron et al. (2006), who investigated the allele frequencies of this SNP in 35 cattle breeds, including Israeli, German and US Holstein, and found the A allele to be predominant in all populations, with frequencies ranging from 0.80 to 1. The ABCG2 A allele, which decreases milk yield, and increases protein and fat percentage (Cohen-Zinder et al., 2005), is economically favorable for most selection indexes, which might explain a fixation on this allele.

The allele frequency of $FASN_{16024}$ G of 0.89 is the same as found by Roy et al. (2006), who named this SNP_{16009}, but refers to the same polymorphism. Morris et al. (2007) identified 5 SNP in FASN, which included $FASN_{17924}$, but

not $FASN_{16024}$. The $FASN_{17924}$ A allele frequency of 0.53 in our population is higher than the frequencies found by Morris et al. (2007) in Friesian and Jersey cattle (0.31 and 0.13, respectively), but lower than the frequency of 0.62 reported in Angus beef cattle (Zhang et al., 2008). Allele frequencies of $OLR1_{8232}$ (Khatib et al., 2006; Khatib et al., 2007a), $PPARGC1A_{1892+19}$ (Weikard et al., 2005; Khatib et al., 2007b), PRL_{8398} (allele frequencies of several studies are reviewed by Brym et al. 2005), and $STAT5A_{9501}$ (Brym et al., 2004) are in line with previous reports. $PPARGC1A_{1790+514}$ was newly identified in this study.

Table 6.3 Allele frequencies of the genotyped polymorphisms

Gene locus	BTA	SNP[1]	Number of genotyped animals[2]	Allele frequency[3]
			Bulls	
ABCG2	6	g.62569A>C	72	1.00
			Cows	
FASN	19	g.16024G>A	1,724	0.89
		g.17924A>G	1,688	0.53
OLR1	5	g.8232C>A	1,724	0.71
PPARGC1A	6	c.1790+514G>A	1,724	0.80
		c.1892+19C>T	1,722	0.75
PRL	23	g.8398G>A	1,722	0.80
STAT5A	19	g.9501G>A	815[4]	0.57

[1] SNP location is numbered according to the Genbank sequence in case of FASN (AF285607), PRL (AF426315), STAT5A (AJ237937) and ABCG2 (AJ871176), and according to reference in case of OLR1 (Khatib et al., 2006), and PPARGC1A (Weikard et al., 2005).

[2] Number of genotyped animals indicates how many of the 1,905 cows were genotyped for the polymorphism. Genotypes were missing for part of the animals because either no DNA sample was available or the polymorphism could not be genotyped unambiguously. The ABCG2 g.62569A>C SNP was sequenced in 22 proven and 50 young bulls, but appeared to be monomorph in these animals.

[3] The allele frequency of the first allele is given, e.g. the frequency of ABCG2 g.62569A is 1.

[4] For the STAT5A g.9501G>A SNP only the daughters of the 5 proven bulls and 2 young bulls were genotyped.

Effects on milk production traits

All studied genes were shown to be associated with milk-fat percentage, milk-fat yield, or both in previous studies (for references see the introduction of this chapter). In the present study, we evaluated these associations in the Dutch Holstein Friesians. Only $FASN_{17924}$ and $OLR1_{8232}$ showed significant effects on milk-fat percentage in our cattle population (Table 6.4). No association of $FASN_{17924}$ with milk-fat percentage is reported in literature, however, an association with the other SNP in FASN, $FASN_{16024}$, has been reported (Roy et al., 2006b). Association of the OLR1 A allele with lower milk-fat percentage is in concordance with Khatib et al. (2006), although an effect on milk-fat yield was not shown in the present study. The majority of reported associations between other SNP and milk-fat percentage or milk-fat yield could not be confirmed in this study, nor could effects on other milk production traits, e.g. protein percentage or milk yield, be confirmed. This could be explained by the generally smaller number of animals used in the other studies. The fact that we are not able to confirm the results of previous studies suggests that these SNP are not the causal mutations.

Effects on milk-fat composition

All genotyped SNP showed a significant effect on milk-fat composition at the 5% level (Table 6.4). We are aware of the fact that we performed multiple tests and that some of the effects might be significant simply by chance: based on the total number of performed tests, the expected number of false positives would be 9.1 when using a 5% significance threshold, and 1.6 considering a 1% significance threshold. However, we presented all effects with a P value smaller than 5%, in order to show suggestive effects as well. Furthermore, our true Type I errors will be slightly higher than the ones we reported as we used pre-corrected data in our analyses.

Both SNP in FASN affected C14:0, whereas $FASN_{16024}$ also affected C18:2*cis*9,12, and $FASN_{17924}$ also affected C18:1*cis*9 and the total index. Effect of SNP in FASN on milk-fat composition has been shown in a New Zealand study (Morris et al., 2007). Although Morris *et al.* (2007) did not

Table 6.4 Effects (with SE) of the polymorphisms in FASN, OLR1, PPARGC1A, PRL and STAT5A on milk production traits and fat composition

$FASN_{16024}$	CC (n=1350)	CT (n=358)	TT (n=16)	P value[1]
C14:0	0	-0.21 (0.05)	-0.38 (0.19)	**
C18:2*cis*9,12	0	0.02 (0.01)	0.08 (0.04)	
$FASN_{17924}$	AA (n=465)	AG (n=849)	GG (n=374)	
Fat %	0	-0.07 (0.04)	-0.13 (0.05)	
C14:0	0	-0.14 (0.04)	-0.23 (0.06)	**
C18:1*cis*9	0	0.10 (0.09)	0.36 (0.12)	
Total index	0	0.11 (0.10)	0.36 (0.13)	
$OLR1_{8232}$	CC (n=847)	CA (n=744)	AA (n=133)	
Fat %	0	-0.03 (0.03)	-0.16 (0.06)	
C18:0	0	-0.08 (0.06)	-0.29 (0.11)	
C18 index	0	0.31 (0.18)	0.89 (0.33)	
CLA index	0	0.27 (0.19)	0.94 (0.34)	
$PPARGC1A_{1790+514}$	GG (n=1084)	GA (n=574)	AA (n=66)	
C16:1*cis*9	0	0.05 (0.02)	0.10 (0.04)	*
C16 index	0	0.10 (0.04)	0.21 (0.10)	
$PPARGC1A_{1892+19}$	CC (n=937)	CT (n=692)	TT (n=93)	
C14:1*cis*9	0	-0.04 (0.01)	-0.04 (0.03)	*
C12 index	0	-0.09 (0.03)	-0.07 (0.06)	*
C14 index	0	-0.31 (0.09)	-0.22 (0.19)	*
C18 index	0	-0.39 (0.19)	-0.81 (0.40)	
PRL_{8398}	CC (n=1097)	CT (n=560)	TT (n=65)	
C10:0	0	-0.04 (0.02)	-0.07 (0.04)	
C12:1	0	$-0.33 \cdot 10^{-2}$ $(0.01 \cdot 10\text{-}2)$	$-0.33 \cdot 10^{-2}$ $(0.30 \cdot 10\text{-}2)$	
$STAT5A_{9501}$	GG (n=267)	GA (n=400)	AA (n=148)	
C10:0	0	0.03 (0.03)	0.11 (0.04)	
C14:0	0	0.06 (0.06)	0.32 (0.09)	**
C14:1	0	-0.01 (0.02)	-0.07 (0.03)	
C16:1*cis*9	0	$0.02 \cdot 10^{-2}$ (0.02)	-0.09 (0.03)	*
C10 index	0	-0.09 (0.14)	-0.55 (0.20)	
C12 index	0	-0.03 (0.04)	-0.19 (0.06)	*
C14 index	0	-0.12 (0.13)	-0.73 (0.19)	**
C16 index	0	0.04 (0.06)	-0.18 (0.09)	*
C18 index	0	-0.17 (0.28)	-0.95 (0.40)	

[1] All listed effects have a P value <0.05; * indicates P value <0.01; ** indicates P value <0.001.

find an effect of these particular SNP, they showed association of two other SNP with C14:0 and C18:1*cis*9, but only in Jersey cattle and not in Holstein cattle. Association of the $FASN_{17924}$ G allele with higher C14:0 and lower C18:1*cis*9 was also reported in beef cattle (Zhang et al., 2008).

The SNP in OLR1 showed association with the long chain fatty acid C18:0, and with C18 and CLA indices. Long chain milk fatty acids originate from blood lipids, whereas short and medium chain milk fatty acids originate from *de novo* fatty acid synthesis in the mammary gland. OLR1 mRNA was initially identified in bovine aortic endothelial cells (Sawamura et al., 1997), where it is highly expressed compared with other tissues. The association of the $OLR1_{8232}$ with long chain fatty acids might reflect the high expression of OLR1 in heart tissue and the origin of these long chain fatty acids, i.e. the blood stream. This SNP in the 3' UTR of OLR1, or another SNP that is in linkage disequilibrium, might influence the expression level of OLR1 and therefore affect long chain fatty acid composition of milk. This hypothesis is supported by the finding that the expression of OLR1 transcripts was lower in AA cows than in CC cows (Khatib et al., 2006).

Both SNP in PPARGC1A showed significant effects on milk-fat composition, however, not on the same fatty acids and unsaturation indices. The effect on the unsaturation indices might reflect the role of PPARG transcription factors in the complex regulation of fat synthesis and metabolism. The finding that PPAR agonists are able to increase SCD1 mRNA levels in humans, mice and rats, suggests that PPAR are able to regulate SCD1 (Popeijus et al., 2008). Because the SCD1 enzyme is involved in the desaturation of saturated fatty acids into *cis*9-unsaturated fatty acids, PPAR might have an effect on unsaturation indices by its regulation of SCD1.

PRL_{8398} shows an effect on C10:0 and C12:1. PRL has a critical role in mammary gland development, lactogenesis and galactopoiesis. How PRL exerts its effects on milk-fat composition is not known, but this could be via STAT5A, which is shown to be regulated by PRL (Welte et al., 1994).

$STAT5A_{9501}$ is associated with medium chain fatty acids and indices, where the A allele is associated with more saturated and less unsaturated medium chain fatty acids, and lower indices. STAT5 proteins are implicated in a wide variety of signaling events (Hennighausen and Robinson, 2008). Studies with transgenic mice have shown that STAT5 proteins have a function in the regulation of mammary tissue development (Teglund et al., 1998), and several *in vitro* and *in vivo* studies have also indicated a role for STAT5A in adipogenesis and fat cell function (Floyd and Stephens, 2003; Richter et al., 2003).

Linkage disequilibrium

Two of the analyzed SNP are located in the FASN gene, and 2 SNP in the PPARGC1A gene. Both FASN and STAT5A genes are located on BTA19, about 8 Mb apart from each other. To indicate if the effects of the SNP in FASN and STAT5A, and of the SNP in PPARGC1A were partly explaining the same variation, r^2 was calculated. The r^2 for the SNP in FASN was 0.14, and the r^2 for the SNP in PPARGC1A was 0.09. For the SNP in FASN and STAT5A, r^2 was below 0.01. These low r^2 suggest that the effect of one SNP explained variation in the other SNP only to a small extent.

QTL for C14:0 on BTA19

In a previous study, we performed a genome-wide scan to detect QTL for bovine milk-fat composition using linkage analysis (Chapters 4 and 5). This genome-wide scan consisted of 849 cows representing 5 large and 2 small paternal half-sib families in a weighted across-family regression. The cows included in the genome-wide scan were part of the 1,905 animals used in the present study. Significant QTL were detected on BTA6, 14, 15, 16, 19 and 26. On BTA6, where PPARGC1A is located, a significant QTL was detected for C6:0 and C8:0. However, SNP in PPARGC1A were not associated with C6:0 or C8:0 (results not shown). On BTA19, where FASN and STAT5A are located, a significant QTL was detected for C14:0. Both SNP in FASN and STAT5A showed significant association with C14:0 in the current association study. Both FASN and STAT5A are located within the confidence interval for the QTL, at 58 and 62 cM, respectively. To investigate whether the SNP in FASN or STAT5A were contributing to the

QTL for C14:0 on BTA19, the QTL analysis was repeated using phenotypes corrected for the SNP effect in FASN or STAT5A. Correction of the phenotypes did not eliminate the QTL, suggesting that the SNP cannot (fully) explain the QTL detected. Subsequently, the genotypes of the 7 bulls that sired the daughters in the genome-scan were analyzed (Table 6.5). All 7 sires were homozygous for $FASN_{16024}$, implying that this SNP could not contribute to the QTL detected in the linkage study. Three out of the 7 sires were heterozygous for $FASN_{17924}$ (sire 5, 6 and 7) and $STAT5A_{9501}$ (sire 4, 5 and 6), indicating that these SNP could be contributing to the QTL. However, sire 2, which is significantly segregating for the QTL, is homozygous for both $FASN_{17924}$ and $STAT5A_{9501}$. This last observation shows that although $FASN_{17924}$ and $STAT5A_{9501}$ might contribute to the QTL for C14:0 on BTA19, the QTL cannot solely be explained by these 2 SNP. An alternative explanation could be that the SNP in FASN and STAT5A are in linkage disequilibrium with a causal mutation and that these SNP are no causal SNP.

Table 6.5 FASN and STAT5A genotypes of the 7 sires from which daughters were included in the genome-wide scan for milk-fat composition[1]

Sire	$FASN_{16024}$	$FASN_{17924}$	$STAT5A_{9501}$	# daughters in genome-wide scan	QTL allele substitution effect[1]	Segregating significantly[1]
1	GG	AA	GG	193	0.09 $_{0.10}$	
2	GG	AA	AA	179	0.72 $_{0.22}$	*
3	GG	AA	GG	170	-0.20 $_{0.11}$	
4	GG	GG	AG	166	-0.28 $_{0.11}$	*
5	GG	AG	AG	91	0.08 $_{0.15}$	
6	GG	AG	AG	29	-0.32 $_{0.30}$	
7	GG	AG	GG	21	1.14 $_{0.33}$	*

[1] From Chapter 4.

In conclusion, we showed effect of SNP in FASN, OLR1, PPARGC1A, PRL and STAT5A on milk-fat composition in Dutch HF. However, we were not able to confirm effect of these SNP on milk production traits, except for FASN and OLR1. This finding stresses the need for replication of association studies in different cattle populations.

6.4 Acknowledgments

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7

General discussion

In this thesis, the genetic background of bovine milk-fat composition was investigated. Substantial genetic variation in milk-fat composition and degree of unsaturation are described in Chapters 2 and 3. Two approaches to identify genes or chromosomal regions underlying this genetic variation were used: the candidate gene approach and the genome scan or quantitative trait loci (QTL) approach. Polymorphisms in the genes DGAT1 and SCD1 were shown to explain a large part of the genetic variation in milk-fat composition (Chapters 2 and 3). In addition to these two major genes, other candidate genes with smaller effects, such as FASN and STAT5A were identified, as described in Chapter 6. A genome-wide scan for milk-fat composition resulted in the detection of significant QTL on bovine chromosomes 6, 14, 15, 16, 19 and 26, as described in Chapters 4 and 5.

In this general discussion, differences in milk-fat composition within and between species will be reviewed. The potential role of polymorphisms in the DGAT1 and SCD1 genes will be discussed and the presence of the DGAT1 and SCD1 polymorphisms will be evaluated in different dairy cattle breeds, and in buffalo, goat and sheep. Furthermore, I will give an overview of the knowledge on the physiological functions of the DGAT1 and SCD1 enzymes, mainly based on mice studies. Inactivation of these genes in mice has a large impact on the body characteristics of the mice. This finding has lead to an analysis of the effects of DGAT1 and SCD1 genotypes on conformation traits in the Dutch Holstein Friesian cattle population.

7.1 Differences in milk-fat composition between dairy cattle breeds

Whereas previous chapters have focused on variation in milk-fat composition within the Dutch Holstein Friesian breed, variation between dairy cattle breeds has been reported in literature as well. Breed differences are generally studied using a limited number of cows. Differences between Holsteins and Jerseys are the most pronounced, and the most studied.

Results on breed differences in C12:0 and C18:1 content from several studies are depicted in Figure 7.1. The figure shows that the differences are large: the studied breeds have 6% to 43% more C12:0 than the Holsteins in

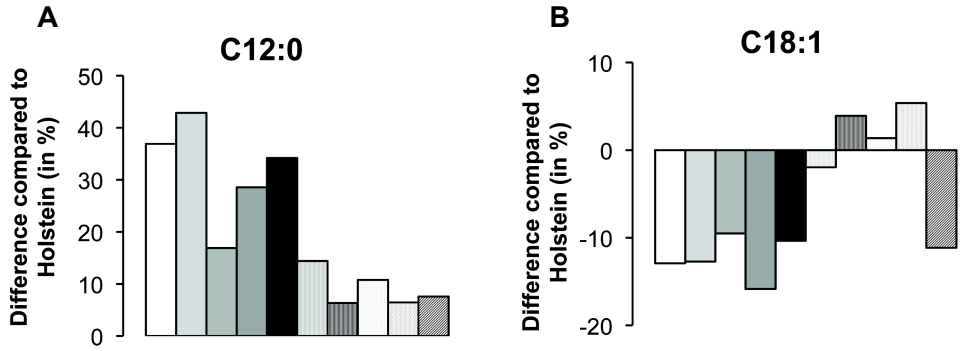


Figure 7.1 Breed differences in milk-fat composition. From left to right, the vertical bars represent the following breeds: Jersey 1 (Stull and Brown, 1964), Jersey 2 (Beaulieu and Palmquist, 1995), Jersey 3 (DePeters et al., 1995), Jersey 4 (Drackley et al., 2001); Jersey 5 (White et al., 2001), Brown Swiss 1 (Kelsey et al., 2003), Brown Swiss 2 (DePeters et al., 1995), Normande (Lawless et al., 1999), Montbeliarde (Lawless et al., 1999), Guernsey (Stull and Brown, 1964). **A.** Difference in C12:0 content compared to Holstein in the same study; **B.** Difference in C18:1 content compared to Holstein in the same study.

the same study, and 16% less to 5% more C18:1 fatty acids. The large differences between studies in fat composition of Jerseys most likely reflect differences in feeding. In general, milk-fat from Jerseys is found to contain more short and medium chain fatty acids, and less C18:1 fatty acids (Stull and Brown, 1964; Beaulieu and Palmquist, 1995; DePeters et al., 1995; Drackley et al., 2001; White et al., 2001). This general observation is in line with our within-breed genetic correlations between milk-fat percentage and fatty acid composition in the Dutch Holstein Friesian breed (Chapter 2). Jerseys are known to have a higher milk-fat percentage than Holsteins, e.g. 5.11 vs. 3.85 (Bitman et al., 1996) and 4.81 vs. 3.74 (DePeters et al., 1995). Within a breed, a higher milk-fat percentage is correlated with more saturated C4-C12 and C16 fatty acids, and less unsaturated C18 fatty acids. The between-breed differences are in line with the expectations based on within-breed correlations. The different milk-fat composition of Jerseys might point at a higher *de novo* fat synthesis in Jerseys than in Holsteins.

The ratio of C18:1*cis* to C18:0 (C18 ratio) was found to be lower in Jerseys than in Holsteins in some (Beaulieu and Palmquist, 1995; DePeters et al.,

1995; Drackley et al., 2001; Carroll et al., 2006), but not all studies (Bitman et al., 1996). This difference in the C18 ratio might be due to a differential expression or activity of SCD1. The A allele of SCD1 is associated with a lower C18 index. The lower C18 index for Jerseys could be explained by a higher frequency of the A allele.

Data on milk-fat composition of other breeds is scarce, but shows breed differences in fat composition as well (Brown Swiss, Jersey and Holstein studied by DePeters et al., 1995; Montbeliarde, Normande and Holstein studied by Lawless et al., 1999; Brown Swiss and Holstein studied by Kelsey et al., 2003; Brown Swiss, Jersey and Holstein studied by Carroll et al., 2006; see also Figure 7.1). Differences in milk-fat composition between breeds were also reported by Soyeurt et al. (2006), who used mid-infrared spectrometry to determine a number of fatty acids in milk of 5 different breeds, i.e. Holstein Friesian, Jersey, Montbeliarde, dual purpose Belgian Blue and non-Holstein Meuse-Rhine-Yssel type Red and White. Breed differences in milk-fat composition indicate the presence of a genetic component, and might imply different allele frequencies of alleles in genes involved in milk-fat synthesis, e.g. DGAT1 and SCD1.

7.2 Differences in milk-fat composition between species

Milk-fat composition differs greatly between species. Here, the focus is on ruminant species producing milks that are consumed by humans, namely cattle, buffalo, goat and sheep. Fat percentage of goat milk is comparable to or slightly higher than fat percentage of cow milk (which is 4.36% in our study), whereas fat percentage of buffalo and sheep milk can be about twice as high (reviews by Raynal-Ljutovac et al., 2008 and Pandya and Khan, 2006; Rosati and Van Vleck, 2002). If relationships between milk-fat percentage and milk fatty acids that we found for cattle hold across species, sheep and buffalo milk is expected to have higher saturated C4-C12 and C16 contents and a lower unsaturated C18 content. The number of studies on fat composition of goat and sheep milk is limited compared to cows and the available studies have mainly focused on dietary sources of variation in fat composition. In general, milk of sheep and goat contained higher contents of short chain fatty acids (C4-C10, especially C10:0, see also Table

Table 7.1 Major milk fatty acids from cow, goat and sheep

	Fatty acid content (% w/w)		
Fatty acid	Cow[1]	Goat[2]	Sheep[3]
C4:0	3.5	2.2	3.6
C6:0	2.2	2.4	2.4
C8:0	1.4	2.7	1.9
C10:0	3.0	10.0	9.2
C12:0	4.1	5.0	4.5
C14:0	11.6	9.8	9.6
C16:0	32.6	28.2	18.1
C18:0	8.7	8.9	8.5
C18:1	19.6	19.3	19.3
C18:2	1.6	3.2	1.7
C18:3	0.4	0.4	1.0

[1] Based on 1,918 samples from Holstein Friesian cows (Stoop et al., 2008).

[2] Based on 35 samples collected from 5 Spanish herds (Alonso et al., 1999).

[3] Based on 1,454 samples from 727 Sardinian x Lacaune ewes (Carta et al., 2008).

7.1). Interestingly, C16:0 content is lower in milk of sheep and goat (32.6% in cow, 28.2% in goat, and 18.1% in sheep), and C18:1 content is comparable between cow, goat and sheep. This is not in line with the expectations based on within-breed genetic correlations, which implies that we cannot predict the milk-fat composition of goat and sheep on basis of the relationships that we found for cattle. This could be due to species differences in the genes that are involved in fat synthesis and metabolism. In addition, it should be kept in mind that differences in diet, lactation stage, season, breed and other factors are sources of variation within and between species (Alonso et al., 1999; Addis et al., 2005; Matsushita et al., 2007; Signorelli et al., 2008). Reports on fat composition of buffalo milk are scarce and differences with cow milk are not consistent (MacGibbon and Taylor, 2006; Pandya and Khan, 2006).

Not only the fat composition differs between species, but also the composition of the triacylglycerols, i.e. the fatty acids esterified at each position of the triacylglycerol, differs between cow, goat and sheep (Ruiz-Sala et al., 1996; Blasi et al., 2008). These differences in composition of triacylglycerols may indicate a different activity or specificity of the enzymes

that catalyze the esterification of fatty acyl-CoA to the glycerol backbone, among which DGAT1. The DGAT1 enzyme might have a different activity or fatty acid specificity in different species, which might be due to variation in stability or amino acid sequence of the enzyme.

In the next paragraphs (7.3 and 7.4) the potential role of polymorphism in DGAT1 and SCD1 will be investigated. From the above it is clear that information, e.g. on genetic correlations, cannot be used across species. Studying the variation between species will help to increase our understanding of the role of genetic variation in milk-fat composition.

7.3 Reported polymorphisms in DGAT1 and SCD1 in cattle, buffalo, goat and sheep

Polymorphisms in DGAT1

Differences in milk-fat composition within and between breeds and species may partly have the same genetic background. We have shown effects of the DGAT1 K232A and the SCD1 A293V polymorphisms on milk-fat composition in the Dutch Holstein Friesian cattle population. Below, I will summarize the literature on polymorphisms in DGAT1 and SCD1 in cattle, buffalo, goat and sheep.

Since the identification of the DGAT1 K232A polymorphism and its effect on milk-fat percentage, the frequencies and effects of this polymorphism on milk production traits have been described in several cattle populations. An overview of the estimated frequencies is shown in Table 7.2. Up till now, the DGAT1 K232A polymorphism has only been detected in cattle. The frequencies of the alleles represent a large range, from fixation of the A allele to fixation of the K allele. A generally higher frequency of the A allele is found in *Bos taurus* breeds, whereas a generally higher frequency of the K allele - fixed or nearly fixed - is found in *Bos indicus* breeds. These results suggest that the high frequency of the K allele in *Bos indicus* is due to the occurrence of the K232A mutation after separation of *Bos indicus* and *Bos taurus*. The fact that the K allele frequency is high, but not fixed at 1, in *Bos indicus*, could be explained by introgression from *Bos taurus*, which implies that the A allele is from taurine origin. Also buffalo (*Bubalus bubalis*) and

Table 7.2 Frequency of the DGAT1 232K allele in cattle breeds, yak and buffalo

Breed	n	K	Breed	n	K
Bos taurus			*Bos indicus*		
Dutch HF[1,2]	1,762	0.40	Nellore[7]	62	1
New Zealand HF[1,3]	1,527	0.60	Nellore[6]	46	0.99
French Holstein[5]	2,086	0.37	Banyo Gudali[6]	72	0.88
German Holstein[5]	858	0.55	White Fulani[6]	44	0.92
German Holstein[6]	79	0.42	Guzerat[7]	53	1
Brazilian Holstein[7]	50	0.27	Red Sindhi[7]	60	0.98
Polish Black and White[8]	502	0.48-0.68	Gyr[7]	53	0.96
Ayrshire[3]	113	0.22	Sahiwal[10]	20	1
Ayrshire[6]	41	0.02	Rathi[10]	20	1
German Brown Swiss[6]	48	0.02	Deoni[10]	20	1
German Simmental[6]	126	0.06	Tharparkar[10]	20	1
Fleckvieh[5]	833	0.07	Red Kandhari[10]	20	1
Jersey[3]	1,053	0.88	Punganur[10]	20	1
Jersey[6]	47	0.69	Hariana[11]	7	1
German Angeln[9]	749	0.61	Sahival[11]	5	1
Normande[4]	535	0.13	Tharparkar[11]	4	1
Montbeliarde[4]	384	0.04			
N'Dama[6]	25	0.52	*Bos grunniens* (yak)		
Aberdeen Angus[6]	43	0.13	[11]	2	1
Charolais[6]	31	0.08	*Bubalus bubalis* (buffalo)		
Chianina[6]	44	0.34	[12]	117	1
Hereford[6]	50	0	[10]	100	1
			[11]	2	1

[1] HF = Holstein Friesian [2] Chapter 2 [3] Spelman et al., 2002 [4] Gautier et al., 2007 [5] Thaller et al., 2003a [6] Kaupe et al., 2004 [7] Lacorte et al., 2006 [8] Pareek et al., 2005 [9] Sanders et al., 2006 [10] Tantia et al., 2006 [11] Winter et al., 2002 [12] Yuan et al., 2007

yak (*Bos grunniens*), although not extensively studied, show fixation of the K allele. Within the *Bos taurus* breeds, the frequencies show a large range. The frequency of the K allele in Holsteins is around 0.4, whereas the frequency of K in Jerseys is higher: 0.69 in a German study of 47 animals and 0.88 in a New Zealand study of 1,053 animals. This higher frequency of the K allele in Jerseys agrees with the higher fat percentage generally found in Jerseys (Bitman et al., 1995; DePeters et al., 1995; Bitman et al., 1996). The beef breeds Aberdeen Angus, Charolais, Chianina, Hereford show low

frequencies of the K allele. The positive effect of the K allele on milk-fat percentage might also hold for intermuscular fat content. In that case, the low frequencies of K in these beef breeds might be the result of selection on lower fat content in meat. Alternatively, the K allele is shown to be associated with more intramuscular fat (also referred to as marbling; (Thaller et al., 2003b), which might favor the K allele in beef breeds, because more intramuscular fat makes the beef more tender and juicier. However, the number of genotyped animals is low for most of the breeds, and it is unknown whether the genotyped animals are a representative sample from the population because no information on the background of these animals is available. For example, it is unknown whether they originated from one herd or whether they are genetically related.

Yuan et al. (2007) screened all exons and introns of the buffalo DGAT1 gene for sequence variation in 117 animals and identified 7 single nucleotide polymorphisms (SNP). Six SNP were located in introns, and one was within exon 17 (A484V), which has not been reported before in the mammalian DGAT1 gene. Angiolillo et al. (2007) reported sequencing of the caprine DGAT1 cDNA in 9 goats, but detected no SNP in this way. The DGAT1 K232A in exon 8 showed fixation of the K allele in these goats. Angiolillo et al. (2007) also sequenced 1 kb of genomic DNA comprising exons 12–17 in 21 goats of different breeds and identified 1 SNP in intron 16. No sequence variation in the DGAT1 gene has been reported for sheep. However, a QTL for milk-fat percentage was detected on ovine chromosome 9, which is homologous to bovine chromosome 14, where DGAT1 is located (Barillet et al., 2005).

In view of the available literature it is likely that the DGAT1 K232A polymorphism is only present in *Bos taurus* breeds, and it is less likely that this polymorphism is present in other species. The differences in milk-fat composition between buffalo, goat and sheep are probably caused by other polymorphisms in the DGAT1 gene or by polymorphisms in other genes.

Polymorphisms in SCD1

Sequence variation in the SCD1 gene has been studied in cattle and goat only. Taniguchi et al. (2004) identified a polymorphism in exon 5 of the SCD1 cDNA (c.878C>T), which resulted in an amino acid change at position 293 (A293V), and showed the association of this polymorphism with carcass-fat composition. As described in Chapter 3 and in a number of other studies, the SCD1 A293V polymorphism is associated with differences in milk-fat composition (Mele et al., 2007; Moioli et al., 2007). Table 7.3 gives an overview of the frequencies of this polymorphism in different breeds of cattle. Frequency of the A allele ranges from 0.34 to 0.95. Jerseys show a higher A allele frequency, 0.94 and 0.95 in two different studies, than Holsteins (0.56 to 0.83). This result is in line with the lower C18 index found in Jerseys, because the SCD1 A allele is associated with a lower C18 index (Chapter 3). The caprine SCD1 cDNA has been sequenced by Bernard et al. (2001), who studied sequence variation in 4 goats. A TGT deletion in the 3' UTR was the only detected polymorphism. Yahyaoui et al. (2003) genotyped a synonymous SNP in exon 5 in 99 goats from 5 different breeds, but did not study effects on milk-fat composition. Although no reports on SCD1 sequence variation in sheep are available, a QTL study for milk-fat composition shows promising results: in a genome-wide scan for ovine milk-fat composition, QTL for C14, C16, C18 and CLA ratios were detected on ovine chromosome 22, where SCD1 is located (Carta et al., 2008). Given

Table 7.3 Frequency of the SCD1 293A allele in cattle

Breed	n	A	Breed	n	A
Dutch HF[1,2]	1,725	0.73	Cabannina[5]	21	0.74
Italian Holstein[3]	313	0.56	Grey Alpine[5]	18	0.89
Italian Holstein[4]	297	0.57	Italian Brown[5]	26	0.85
Italian Holstein[5]	28	0.71	Rendena[5]	24	0.62
Canadian Holstein[6]	44	0.83	Romagnola (beef)[5]	28	0.66
Jersey[6]	48	0.95	Chianina (beef)[5]	26	0.83
Jersey[7]	25	0.94	Marchigiana (beef)[5]	30	0.57
Piedmontese[7]	27	0.42	Piedmontese (beef)[5]	27	0.52
Valdostana[7]	27	0.65	Podolica (beef/work)[5]	28	0.45
Japanese Black (beef)[8]	1,003	0.59	Italian Red Pied (beef/dairy)[5]	25	0.34

[1] HF = Holstein Friesian [2] Chapter 3 [3] Macciotta et al., 2008 [4] Mele et al., 2007 [5] Milanesi et al., 2008 [6] Kgwatalala et al., 2007 [7] Moioli et al., 2007 [8] Taniguchi et al., 2004

our finding on the effects of SCD1 in cattle, SCD1 is a good candidate to explain the observed QTL effects.

Summarizing, limited information is available for the SCD1 A293V polymorphism, since it is only studied in Japanese Black, Holstein, Jersey and local Italian cattle breeds, and in a very limited sample of goats.

7.4 SCD1 sequence variation in cattle, buffalo, goat and sheep

The DGAT1 K232A polymorphism is likely to be present only in *Bos taurus* breeds, and less likely to be present in other species. This conclusion is based on allele frequencies from several studies in *Bos taurus*, *Bos indicus* and buffalo. Therefore, we have not further studied the DGAT1 polymorphism. Limited information is available for the SCD1 A293V polymorphism, since it is only studied in Japanese Black, Holstein, Jersey and local Italian cattle breeds, and in a very limited sample of goats. Therefore, our aim was to evaluate the presence of the SCD1 A293V polymorphism in different dairy cattle breeds, buffalo, goat and sheep. Furthermore, we aimed to identify new polymorphisms in the coding region of the SCD1 gene.

Semen, blood or hair samples were collected from 6 dairy cattle breeds, buffalo (*Bubalus bubalis*), goat (*Capra hircus*) and sheep (*Ovis aries*) for DNA isolation (Table 7.4). Samples were provided by CRV, GGI Holland, Semex, Geiten KI Nederland, and 6 Dutch farms. Pedigree information of the animals was checked to make sure that no sibs or half-sibs were included. This does not hold for the buffalo samples, which were collected from 9 paternal half-sib families located on 2 different farms. Genomic DNA of the animals was screened for polymorphisms in the coding regions of the SCD1 gene. All 6 exons plus their flanking intronic regions were sequenced according to the protocol of Ganai et al. (2008) with minor modifications. Primers for PCR and sequencing were designed based on Genbank sequences AY241932 (cow) and AF422166-AF422171 (goat). Due to sequencing problems, results for exon 1 were only available for Holstein Friesian, Brown Swiss and Fleckvieh animals.

Table 7.4 Animals included in SCD1 sequencing

Species	Breed	Number of animals
Cattle	Holstein Friesian	22
	Jersey	17
	Fleckvieh	27
	Brown Swiss	20
	Ayrshire	11
	Guernsey	9
Buffalo		24
Goat	Dutch White	17
	Nubic	5
	mixed[1]	22
Sheep	Dutch milk sheep	31

[1] Included purebreds and crossbreds from Dutch White, Saanen, Nubic, Alpine, and Toggenburger.

In total, 51 polymorphisms were detected within species: 11 SNP in cattle, 6 SNP in buffalo, 31 SNP and a 5-nucleotide deletion in goat, and 2 SNP in sheep (Tables 7.5a-d). Of these polymorphisms, 26 were in exons, of which 11 caused amino acid changes. The SNP g.10153A>G, g.10213T>C, and

Table 7.5a Polymorphisms in the SCD1 gene in cattle, based on 6 cattle breeds

Polymorphism[1]	Location		Frequency	LD[2]
g.6844G>A	exon 3	synonymous	0.99	
g.6926A>G[3]	**intron 3**		**0.64**	
g.8243A>G	intron 3		0.71	a
g.8646A>G	intron 4		0.69	a
g.10153A>G	exon 5	synonymous	0.69	a
g.10213T>C	exon 5	synonymous	0.69	a
g.10329C>T	exon 5	A293V	0.69	a
g.12907G>C	intron 5		0.70	a
g.13101G>A	exon 6	synonymous	0.95	
g.13209G>A	exon 6	synonymous	0.93	
g.13238C>T	intron 6		0.95	

[1] The allele frequency of the first allele is given, e.g. the frequency of g.6844G is 0.99.

[2] Polymorphisms that are in complete linkage disequilibrium are marked with the same letter. Allele frequencies for these SNP may slightly differ, due to differences in the number of successfully genotyped animals.

[3] The polymorphism in bold is present in cattle and goat.

Table 7.5b Polymorphisms in the SCD1 gene in buffalo

Polymorphism[1]	Location		Frequency	LD[2]
g.8489G>A	exon 4	R206K	0.40	a[3]
g.8505G>A	exon 4	synonymous	0.52	
g.8583C>T	intron 4		0.60	a[3]
g.10079C>T	intron 4		0.60	
g.12884T>G	intron 5		0.06	
g.13125C>A	exon 6	synonymous	0.52	

[1] The allele frequency of the first allele is given, e.g. the frequency of g.8489G is 0.40.

[2] Polymorphisms that are in complete linkage disequilibrium are marked with the same letter.

[3] The G allele of SNP g.8489 and the T allele of SNP g.8583 are linked.

g.10329C>T in cattle have also been identified by Taniguchi et al. (2004) who sequenced cDNA from Japanese Black beef cattle. These 3 SNP in exon 5, together with g.8243A>G in intron 3, g.8646A>G in intron 4, and g.12907G>C in intron 5, were in complete linkage disequilibrium in our sample. SNP g.8243A>G and g.8646A>G were also reported in Genbank sequence AY241932. The g.6844G>A SNP was also detected by Kgwatalala et al. (2007), who screened the SCD1 coding region in Holstein and Jersey cattle. They identified, in addition to the 3 SNP in exon 5, the g.6844G>A SNP in exon 3, but only in Holsteins, with a G allele frequency of 0.95. In our study, this g.6844G>A SNP in exon 3 was only detected in Fleckvieh and Ayrshire, with G allele frequencies of 0.96 and 0.95, respectively. This very low minor allele frequency of A might explain the detection of this polymorphism in some, but not all sampled breeds. The g.10210T>G SNP in goat was also reported by Yahyaoui et al. (2003), who found frequencies of the T allele of 0-0.08 in 4 local Spanish goat breeds, and 0.31 in French Saanen goats. In our goat samples, the T allele also had a low frequency (0.11). The other 44 polymorphisms have not been reported before.

In addition to sequence variation within species, sequence variation between species was detected. The coding regions of SCD1 of cow, buffalo, goat and sheep are aligned by ClustalW (http://www.ebi.ac.uk/Tools/clustalw2) in Figures 7.2a-e. In 20 cases, a sequence variation caused an amino acid difference between species. A special case was detected at position 6734 in exon 3, where 3 different amino acids could be predicted. Due to a non-

Table 7.5c Polymorphisms in the SCD1 gene in goat

Polymorphism[1]	Location		Frequency	LD[2]
g.2533delCCTCT	intron 1		0.82	a
g.2585A>G	exon 2	synonymous	0.88	
g.2617G>A	exon 2	p.R26K	0.82	a
g.2778T>G	exon 2	p.S80G	0.82	a
g.2779C>G	exon 2	p.S80G	0.82	a
g.2801A>G	exon 2	synonymous	0.82	a
g.2842A>T	exon 2	p.Y101F	0.82	a
g.2844A>C	exon 2	p.I102L	0.82	a
g.2873T>C	intron 2		0.82	a
g.2876C>T	intron 2		0.82	a
g.6671C>G	intron 2		0.08	b
g.6676G>A	intron 2		0.08	b
g.6680T>C	intron 2		0.08	b
g.6688T>G	intron 2		0.08	b
g.6689C>A	intron 2		0.08	b
g.6734A>G	exon 3	M109V	0.41	
g.6740G>A	exon 3	G111S	0.08	b
g.6765C>T	exon 3	A119V	0.08	b
g.6787A>G	exon 3	synonymous	0.81	
g.6808T>C	exon 3	synonymous	0.08	b
g.6829T>C	exon 3	synonymous	0.08	b
g.6831G>C	exon 3	G141A	0.08	b
g.6853A>G	intron 3		0.08	b
g.6893G>T	intron 3		0.08	
g.6896C>T	intron 3		0.74	
g.6905G>A	intron 3		0.43	
g.6910C>T	intron 3		0.08	c
g.6921C>T	intron 3		0.08	c
g.6926A>G[3]	**intron 3**		**0.08**	**c**
g.6953C>T	intron 3		0.08	c
g.10183C>T	exon 5	synonymous	0.72	
g.10210T>G	exon 5	synonymous	0.11	

[1] The allele frequency of the first allele is given, e.g. the frequency of g.2585A is 0.88.

[2] Polymorphisms that are in complete linkage disequilibrium are marked with the same letter. Allele frequencies for these SNP may slightly differ, due to differences in the number of successfully genotyped animals.

[3] The polymorphism in bold is present in cattle and goat.

Table 7.5d Polymorphisms in the SCD1 gene in sheep

Polymorphism[1]	Location		Frequency	LD[2]
g.10279T>C	exon 5	synonymous	0.03	
g.10341C>T	intron 5		0.98	

[1] The allele frequency of the first allele is given, e.g. the frequency of g.10279T is 0.03.

[2] Polymorphisms that are in complete linkage disequilibrium are marked with the same letter. Allele frequencies for these SNP may slightly differ, due to differences in the number of successfully genotyped animals.

synonymous sequence variation at position 6734, cows and buffalos have leucine (L) at amino acid position 109, sheep have valine (V) at this position, and goats have a valine/methionine (V/M) polymorphism at this position. This amino acid position is non-conserved, humans were found to have phenylalanine (F) at this position, and mouse were found to have methionine (M) (Taniguchi et al., 2004).

```
                                                                    ▲
cow     ATCTCTAGCTCCTACACAACCACCACCACCATCACAGCACCTCCTTCCAGGGTCCTGCAG 2627
buffalo ATCTCTAGCTCCTACACAACCACCACCACCATCACAGCACCTCCTTCCAGGGTCCTGCAG
goat    ATCTCTAGCTCCTACACRACCACCACCACCATCACAGCACCTCCTTCCARGGTCCTGCAG
sheep   ATCTCTAGCTCCTACACAACCACCACCACCATCACAGCACCTCCTTCCAGGGTCCTGCAG
        ********** ********** ********** ********** **********

cow     AATGGAGGGGGCAAATTGGAGAAGACTCCCCTATACTTGGAAGAAGACATCCGCCCTGAA 2687
buffalo AATGGAGGGGGCAAATTGGAGAAGACTCCCCTATACTTGGAAGAAGACATCCGCCCTGAA
goat    AATGGAGGGGGCAAATTGGAGAAGACTCCCCTATACTTGGAAGAAGACATCCGCCCTGAA
sheep   AATGGAGGGGGCAAATTGGAGAAGACTCCCCTATACTTGGAAGAAGACATCCGCCCTGAA
        ************************************************************

cow     ATGAGAGATGACATCTATGACCCAACTTACCAGGATAAGGAGGGCCCAAAGCCCAAGCTT 2747
buffalo ATGAGAGATGACATCTATGACCCAACTTACCAGGATAAGGAGGGCCCAAAGCCCAAGCTT
goat    ATGAGAGATGACATCTATGACCCAACTTACCAGGATAAGGAGGGCCCAAAGCCCAAGCTT
sheep   ATGAGAGATGACATCTATGACCCAACTTACCAGGATAAGGAGGGCCCAAAGCCCAAGCTT
        ************************************************************
                                    ▲
cow     GAGTATGTTTGGAGAAACATCATCCTCATGTCTCTGTTACACTTGGGAGCCCTATATGGG 2807
buffalo GAGTATGTTTGGAGAAACATCATCCTCATGTCTCTGTTACACTTGGGAGCCCTATATGGG
goat    GAGTATGTTTGGAGAAACATCATCCTCATGKSTCTGTTACACTTGGGAGCCCTRTATGGG
sheep   GAGTATGTTTGGAGAAACATCATCCTCATGGGTCTGTTACACTTGGGAGCGCTGTATGGG
        ****************************  ****************** ** ******
                                      ▲ ▲
cow     ATCACATTGATCCCCACCTGCAAGATATACACCTATATCTGGG 2850
buffalo ATCACATTGATCCCCACCTGCAAGATATACACCTATATCTGGG
goat    ATCACATTGATCCCCACCTGCAAGATATACACCTWTMTCTGGG
sheep   ATCACATTGATCCCCACCTGCAAGATATACACCTTTCTCTGGG
        ******************************** * ******
```

Figure 7.2a Sequence alignment of exon 2 of SCD1. Triangles above the sequence represent sequence variation leading to an amino acid change. Single nucleotide polymorphisms within a species are represented by their IUPAC single-letter codes (R = G/A, Y = C/T, S = G/C, M = C/A). The sequence variations at position 2778 and 2779 together lead to an amino acid change S80G. Cows and buffalos have a S at amino acid position 80, sheep have a G at this position, and goats have a S>G polymorphism at this position.

```
                      ▲    ▲▲                          ▲
cow     TGTTATTCTACTATCTGATGGGTGCCCTGGGCATCACAGCAGGGGCCCATCGCCTGTGGA 6779
buffalo TGTTATTCTACTATCTGATGGGTGCCCTGGGCATCACAGCAGGGGCCCATCGCCTGTGGA
goat    TGTTATTCTACTATRTGATGRGTGCCCTGGGCATCACAGCAGGGGYCCATCGCCTGTGGA
sheep   TGTTATTCTACTATGTGATCAGTGCCCTGGGCATCACAGCAGGGGTCCATCGCCTGTGGA
        ************** ****  ********************** **************
                                                     ▲
cow     GTCACCGAACCTACAAAGCTCGGCTGCCTCTGCGGGTCTTCCTGATCATTGGCAACACCA 6839
buffalo GTCACCGAACCTACAAAGCTCGGCTGCCTCTGCGGGTCTTCCTGATCATTGGCAACACCA
goat    GTCACCGRACCTACAAAGCTCGGCTGCCYCTGCGGGTCTTCCTGATCATYGSCAACACCA
sheep   GTCACCGAACCTACAAAGCTCGGCTGCCTCTACGGGTCTTCCTGATCATCGCCAACACCA
        ******* ******************* ** ***************** * ********

cow     TGGCRTTCCAG 6850
buffalo TGGCGTTCCAG
goat    TGGCGTTCCAG
sheep   TGGCGTTCCAG
        **** ******
```

Figure 7.2b Sequence alignment of exon 3 of SCD1. Triangles above the sequence represent sequence variation leading to an amino acid change. Single nucleotide polymorphisms within a species are represented by their IUPAC single-letter codes (R = G/A, Y = C/T, S = G/C, M = C/A). Due to a non-synonymous sequence variation at position 6734, cows and buffalos have a L at amino acid position 109, sheep have a V at this position, and goats have a V/M polymorphism at this position.

```
cow     AATGACGTTTTTGAATGGTCCCGAGATCACCGTGCCCACCACAAGTTTTCAGAAACGGAT 8373
buffalo AATGACGTTTTTGAATGGTCCCGAGATCACCGTGCCCACCACAAGTTTTCAGAAACGGAT
goat    AATGACGTTTTTGAATGGTCCCGAGATCACCGTGCCCACCACAAGTTTTCAGAAACGGAT
sheep   AATGACGTTTTTGAATGGTCCCGAGATCACCGTGCCCACCACAAGTTTTCAGAAACGGAT
        ************************************************************

cow     GCCGACCCCCACAATTCCCGACGTGGCTTTTTCTTCTCTCACGTGGGTTGGCTGCTTGTG 8433
buffalo GCTGATCCCCACAATTCCCGACGTGGCTTTTTCTTCTCTCACGTGGGTTGGCTGCTTGTG
goat    GCTGATCCCCACAATTCCCGACGTGGCTTTTTCTTCTCTCACGTGGGTTGGCTGCTTGTG
sheep   GCTGATCCCCACAATTCCCGACGTGGCTTTTTCTTCTCTCACGTGGGTTGGCTGCTTGTG
        ** ** ******************************************************
                           ▲           ▲     ▲              ▲
cow     CGCAAACACCCAGCTGTCAAAGAAAAGGGTTCCACGCTAAATTTATCCGACCTAAGAGCC 8493
buffalo CGCAAACACCCAGCTGTCATAGAAAAGGGTTCTACGCTAAATTTATCCGACCTAARAGCC
goat    CGCAAACACCCAGCTGTCAGAGAAAAGGGTGCTACGCTAGATTTATCCGACCTAAGAGCT
sheep   CGCAAACACCCAGCTGTCAGAGAAAAGGGTGCTACACTAGATTTATCCGACCTAAGAGCT
        ****************** ********** * ** *** *************** ***

cow     GAGAAGCTGGTGATGTTCCAGAGGAG 8519
buffalo GAGAAGCTGGTRATGTTCCAGAGGAG
goat    GAGAAGCTGGTGATGTTCCAGAGGAG
sheep   GAGAAGCTGGTGATGTTCCAGAGGAG
        *********** **************
```

Figure 7.2c Sequence alignment of exon 4 of SCD1. Triangles represent sequence variation leading to an amino acid change. Single nucleotide polymorphisms within a species are represented by their IUPAC single-letter codes (R = G/A, Y = C/T, S = G/C, M = C/A).

```
cow     GTACTACAAACCTGGTGTCCTGTTGTTGTGCTTCATCCTGCCCACACTCGTGCCRTGGTA 10158
buffalo GTACTACAAACCTGGTGTCCTGTTGTTGTGCTTCATCCTGCCCACACTCGTGCCGTGGTA
goat    GTACTACAAACCTGGTGTCCTGCTGTTGTGCTTCATCCTGCCCACACTCGTGCCGTGGTA
sheep   GTACTACAAACCTGGTGTCCTGCTGTTGTGCTTCATCCTGCCCACACTCGTGCCGTGGTA
        ********************** ******************************** *****
               ▲                                        ▲▲          ▲
cow     TCTGTGGGATGAAACGTTTCAAAACAGCCTGTTTTTTGCCACCTTATTCCGTTAYGCCCT 10218
buffalo TCTGTGGG?TGAAACGTTTCAAAACAGCCTGTTTTTTGCCACCTTATTCCGTTATGCCCT
goat    TCTATGGGGTGAAACGTTTCAAAAYAGCCTTTTTTTTGCCACCCTTCTCCGKTACGCTGT
sheep   TCTATGGGGTGAAACGTTTCAAAACAGCCTATTTTTTGCCACCTTTCTCCGTTACGCTGT
        *** **** *************** ***** ************ *  **** ** **  *
          ▲       ▲
cow     TGGGCTCAACGTCACCTGGCTGGTGAATAGTGCTGCCCATATGTATGGATACCGCCCTTA 10278
buffalo TGGGCTCAACGTCACCTGGCTGGTGAATAGTGCTGCCCATATGTATGGATA?CGCCCTTA
goat    TGTGCTCAATGCCACCTGGCTGGTGAACAGCGCTGCCCATATGTATGGATATCGCCCTTA
sheep   TGTGCTCAACGCCACCTGGCTGGTGAACAGCGCTGCCCATATGTATGGATATCGCCCTTA
        ** ****** * *************** ** ******************** ********
                                                          ▲
cow     TGACAAGACCATCAACCCCCGAGAGAATATTCTGGTTTCCCTGGGAGCTGYGG 10331
buffalo TGACAAGACCATCAACCCCCGAGAGAATATTCTGGTTTCCCTGGGAGCTGTGG
goat    CGACAAGACCATCAACCCCCGAGAGAATATCCTGGTTTCCCTGGGAGCTGTGG
sheep   YGACAAGACCATCAACCCCCGAGAGAATATCCTGGTTTCCCTGGGAGCTGTGG
         **************************** ****************** **
```

Figure 7.2d Sequence alignment of exon 5 of SCD1. Triangles above the sequence represent sequence variation leading to an amino acid change. Single nucleotide polymorphisms within a species are represented by their IUPAC single-letter codes (R = G/A, Y = C/T, S = G/C, M = C/A).

```
cow     GTGAGGGCTTCCACAACTACCACCACACCTTTCCTTATGACTACTCAGCCAGTGAGTACC 13078
buffalo GTGAGGGCTTCCACAACTACCACCACACCTTTCCTTATGACTACTCAGCCAGTGAGTACC
goat    GTGAGGGCTTCCACAACTACCACCACACCTTTCCTTATGACTACTCTGCCAGCGAGTACC
sheep   GTGAGGGCTTCCACAACTACCACCACACCTTTCCTTATGACTACTCTGCCAGCGAGTACC
        ********************************************** ***** *******

cow     GCTGGCACATCAACTTTACCACRTTCTTCATTGATTGCATGGCTGCCATCGGTCTGGCTT 13138
buffalo GCTGGCACATCAACTTTACCACGTTCTTCATTGATTGCATGGCTGCMATCGGTCTGGCTT
goat    GCTGGCACATCAACTTTACCACATTCTTCATTGATTGCATGGCTGCCATCGGTCTGGCTT
sheep   GCTGGCACATCAACTTTACCACATTCTTCATTGATTGCATGGCTGCCATCGGTCTGGCTT
        ********************** ********************** *************
                                        ▲             ▲
cow     ATGACCGGAAGAAAGTATCCAAGGCTGCCATCTTGGCCAGGATAAAAAGAACTGGAGAGG 13198
buffalo ATGACCGGAAGAAAGTATCCAAGGCTGCCATCTTGGCCAGGATGAAAAGAACTGGAGAGG
goat    ATGACCGGAAGAAAGTATCCAAGGCTGCCGTCTTGGCCAGGATGAAAAGAACTGGAGAGG
sheep   ATGACCGGAAGAAAGTATCCAAGGCTGCCGTCTTGGCCAGGATGAAAAGAACTGGAGAGG
        **************************** ************* ****************
           ▲
cow     AAAGCTACAARAGTGGCTGA 13218
buffalo AAAGCTACAAGAGTGGCTGA
goat    AAAGCTGCAAGAGTGGCTGA
sheep   AAAGCTACAAGAGTGGCTGA
        ****** *** *********
```

Figure 7.2e Sequence alignment of exon 6 of SCD1. Triangles above the sequence represent sequence variation leading to an amino acid change. Single nucleotide polymorphisms within a species are represented by their IUPAC single-letter codes (R = G/A, Y = C/T, S = G/C, M = C/A).

The A293V polymorphism (g.10329C>T) in exon 5 was only found in cattle. Frequencies of the A allele (g.10329C) were 0.75 for Holstein Friesian, 0.88 for Jersey, 0.52 for Fleckvieh, 0.83 for Brown Swiss, 0.73 for Ayrshire, and 0.39 for Guernsey. The high frequency of the A allele in Jersey is in line with previous studies (as shown in Table 7.3), and with the lower C18 index generally found in Jerseys. Buffalo, goat and sheep showed fixation at the V allele (g.10329T). Based on these results, the A293V polymorphism, which explains differences within cattle, could not explain differences in milk-fat composition between species. However, the large number of sequence variations in SCD1 between species, as shown in the alignments in Figures 7.2a-e, make it likely that variation in SCD1 contributes to differences in milk-fat composition between species. One SNP, g.6926A>G in intron 3, was present in both cattle and goats.

The newly identified polymorphisms within cattle, buffalo, goat and sheep possibly explain within-species variation in milk-fat composition. Further research is necessary to link phenotypes to these genotypes. In the above described work, only the exons plus their flanking intronic regions of SCD1 were screened. Also the promoter, the 5' and the 3' UTR might harbor additional polymorphisms that influence the expression or stability of SCD1, and these regions deserve further investigation. SNP in the 3' UTR have been identified in Japanese Black cattle (Taniguchi et al., 2004), whereas no polymorphisms were detected in the promoter region (Keating et al., 2005).

7.5 Physiological functions of DGAT1 and SCD1

Insight into the physiological functions of DGAT1 and SCD1 is essential to understand the effects of polymorphisms in these genes. The inactivation of the DGAT1 and SCD1 genes in mice has led to a better understanding of the physiological functions of the enzymes.

Its role in triacylglycerol synthesis and the finding that female mice deficient in DGAT1 did not produce milk made the DGAT1 gene a strong functional candidate for milk production traits in cattle. The mapping of a QTL for milk-fat percentage to the proximal end of BTA14 made DGAT1 a positional candidate for milk-fat percentage as well. Subsequent SNP identification and

association analysis identified showed that this polymorphism has large effect on milk-fat percentage (Grisart et al., 2002; Winter et al., 2002). In Chapter 2 we have shown that the DGAT1 K232A polymorphism has large effects on milk-fat composition.

DGAT1: lessons from mice and man

Two DGAT isoforms, DGAT1 and DGAT2 (encoded by different genes), have been identified in several species, including cattle (Grisart et al., 2002; Winter et al., 2003). Both DGAT enzymes are widely expressed, and highest levels are found in tissues typically associated with triacylglycerol synthesis, namely adipose tissue, liver and small intestine (for review on DGAT genes see Yen et al. 2008). DGAT2-deficient ($DGAT2^{-/-}$) mice pups are smaller than their controls ($DGAT2^{+/+}$) and die within several hours after birth. They show a severe reduction in carcass triacylglycerol content and abnormalities in skin lipids and impaired epidermal barrier function. $DGAT2^{+/-}$-mice are not resistant to diet-induced obesity (Stone et al., 2004; Chen and Farese, 2005). DGAT1-deficient ($DGAT1^{-/-}$) mice are viable and have less adipose mass and smaller adipocytes when adult than their wild type controls (Smith et al., 2000). They are resistant to diet-induced obesity and show increased sensitivity to insulin and leptin. $DGAT1^{+/-}$ mice have an intermediate phenotype. $DGAT1^{-/-}$ mice are also protected from diet-induced hepatic steatosis (fatty liver). The effects on energy metabolism are suggested to result partly from an altered endocrine function of the white adipose tissue. Furthermore, $DGAT1^{-/-}$ mice develop several skin abnormalities such as dry fur and hair loss (Chen et al., 2002b). $DGAT1^{-/-}$ female mice do not produce milk, and show an impaired mammary gland development (Smith et al., 2000; Cases et al., 2004).

It is still not clear whether polymorphisms in DGAT1 or DGAT2 affect body condition in humans. A DGAT1 promoter polymorphism, which modestly affects promoter activity, has been associated with body mass index (BMI) in Turkish women, but not in Turkish men (Ludwig et al., 2002) or in a French population of men and women (Coudreau et al., 2003). DGAT2 is located on human chromosome 11, at a region for extreme early onset obesity. Case-control and family-based association studies did not result in

any association between detected polymorphisms in the DGAT2 gene and obesity phenotypes (Friedel et al., 2007).

Text box 7.1 SCD isoforms

Many animal species are known to have multiple SCD isoforms. In mice, 4 SCD isoforms have been identified (SCD1, SCD2, SCD3, SCD4), encoded by 4 different genes. SCD1 and SCD2 are expressed in a variety of tissues, including liver and adipose tissue, SCD3 is expressed in the skin, Harderian gland and preputial gland, and SCD4 is expressed in the heart (Ntambi et al., 1988; Kaestner et al., 1989; Zheng et al., 2001; Miyazaki et al., 2003). Two isoforms have been found in humans, SCD1 and SCD5. The human SCD1 shares 85% amino acid identity with mouse SCD1-SCD4, and appears to be an ortholog of mouse SCD1 (Zhang et al., 2005). Homologs of mouse SCD1 have been identified in many other mammalian species, such as cow, sheep, goat, pig and hamster, as well as in a non-mammalian species, the chicken. Human SCD5 shares limited identity to the mouse SCD genes and appears to be a distinct SCD isoform. In humans, SCD1 is most highly expressed in adipose tissue and liver, while SCD5 is most abundant in brain and pancreas (Wang et al., 2005). SCD5 has originally been thought to be unique to primates, but Corl and colleagues recently identified a SCD5 homolog in cows, sheep, pigs and chickens as well (Lengi and Corl, 2007; Lengi and Corl, 2008). In addition to the tissue-specific expression, specific substrate preferences have been reported for the different isoforms. Whereas mSCD1, mSCD2 and mSCD4 desaturate both palmitoyl-CoA and stearoyl-CoA, mSCD3 desaturates palmitoyl-CoA but not stearoyl-CoA. The tissue and substrate specificity of SCD isoforms suggests distinct physiological roles.

SCD1 expression

Two SCD isoforms have been identified in cattle, SCD1 and SCD5 (Chung et al., 2000; Lengi and Corl, 2007; see Text box 7.1 for more information on SCD isoforms). SCD1 is located on BTA26 and its mRNA is expressed in a variety of tissues, but predominantly in adipose (Lengi and Corl, 2007) and mammary tissue (data based on one sample only; B.A. Corl, Virginia Tech, personal communication). SCD5 is located on BTA6 and its mRNA is

expressed primarily in the brain (Lengi and Corl, 2007). Based on the finding that SCD5 is expressed primarily in brain and pancreas - neither very lipogenic tissues - in both human and cattle, we expect SCD1 to be the major, if not only, SCD isoform active in the mammary gland. This hypothesis is supported by the fact that we did not detect a QTL for any of the unsaturation indices on BTA6 in our genome-wide scan (Chapters 4 and 5). To elucidate the expression patterns of SCD1 and SCD5 in the mammary gland, quantitative PCR, western blotting and immunohistochemistry techniques could be used to quantify their mRNA and protein levels. This information would be necessary to exclude SCD5 as a candidate desaturase of fatty acids in the mammary gland.

Here I focus on SCD1, because of its expression in the mammary gland and the reported effects of a SCD1 polymorphism on bovine milk-fat composition (Chapters 3 and 4).

Regulation of SCD1

The regulation and role of SCD have been studied in different species and processes. The SCD1 gene is regulated at the transcriptional level by a large number of nutrients (cholesterol, glucose, fructose, fatty acids), hormones (insulin, leptin, thyroid hormone) and environmental factors (temperature, metals). One of the key transcription factors controlling lipid synthesis is the sterol regulatory element binding protein 1c (SREBP-1c). SREBP-1c regulates lipogenic genes, among which SCD1, but also acetyl-CoA carbocylase (ACC), fatty acid synthase (FASN) and glycerol phosphate acyl-CoA transferase (GPAT). The nuclear receptor PPARα is suggested to play a role in the regulation of SCD1 as well. PPARα is a transcriptional regulator of fatty acid oxidation and thermogenic genes (Dobrzyn and Ntambi, 2005). Polymorphisms in genes involved in fat synthesis and metabolism, such as FASN and PPARγ, were shown to affect bovine milk-fat composition (Chapter 6). The high degree of regulation and the specific tissue distribution of SCD suggest that SCD is critical to various biological processes. Apart from being components of milk lipids, the monounsaturated products of SCD are constituents of complex body lipids among which cholesteryl esters and phospholipids, which form the cell membranes. These monounsaturated

fatty acids (MUFA) also play a role in diverse cellular processes such as cell differentiation, signal transduction and apoptosis.

SCD1: lessons from mice and man

Mice with a targeted deletion of SCD1 or with natural *asebia* mutations in SCD1 have provided insights into the metabolic role of SCD1 (Zheng et al., 1999). SCD1-deficient mice ($SCD1^{-/-}$) have a metabolic phenotype that, in many aspects, reflects "protection" against the metabolic syndrome. $SCD1^{-/-}$ mice have higher energy metabolism, increased insulin sensitivity and are resistant to diet-induced obesity and liver steatosis, compared to wild-type mice. Moreover, differential expression analysis revealed that lipid oxidation genes, such as carnitine palmitoyltransferase 1 (CPT1), acyl-CoA oxidase and very long chain acyl-CoA dehydrogenase, were up-regulated, whereas lipid synthesis genes, such as SREBP-1, FASN and GPAT, were down-regulated in $SCD1^{-/-}$ mice (Ntambi et al., 2002). Also normal mice treated with antisense oligos to specifically downregulate SCD1 mRNA were leaner, more insulin sensitive and became resistant to diet-induced obesity (Jiang et al., 2005).

The human SCD1 homologue maps to a region of chromosome 10, which variants are linked to type 2 diabetes (Wiltshire et al., 2001). This, together with the results from the mouse knockout studies, made SCD1 a positional and functional candidate for type 2 diabetes in human, which was tested in association studies. Liew et al. (2004) were not able to detect an association between SNP in SCD1 and type 2 diabetes, body mass index (BMI) and waist-to-hip ratio in a UK case-control study concerning 605 type 2 diabetes cases. However, Warensjo et al. (2007) recently reported 4 SCD1 SNP (different from the SNP studied by Liew et al. 2004) to be associated with BMI, waist circumference and insulin sensitivity in a Swedish longitudinal study comprising 1,143 elderly men.

The knockout studies in mice give insight into the physiological roles of DGAT1 and SCD1, and indicate that both DGAT1 and SCD1 play an important role in lipid metabolism. Of course, the knockout mice and its wild type control are a black-or-white situation: absence or presence of the

enzyme. Polymorphisms, generally, do not have such dramatic effects, but could alter the activity or specificity of the enzyme, with implications for its physiological role.

7.6 Effects of DGAT1 and SCD1 polymorphisms on conformation traits in cattle

The paragraph above describes our knowledge on the physiological roles of DGAT1 and SCD1, indicating that both DGAT1 and SCD1 play an important role in lipid metabolism. The phenotype of the $DGAT1^{-/-}$ mice showed alterations in size, volume of adipose mass, and resistance to diet-induced obesity, compared to $DGAT1^{+/+}$ mice. Also $SCD1^{-/-}$ mice were leaner and had a higher energy metabolism than $SCD1^{+/+}$ mice and were resistant to diet-induced obesity. These findings suggest that DGAT1 and SCD1 have an important role in body mass and body conformation. Polymorphisms in DGAT1 and SCD1 could also affect body mass and body conformation, which is supported by the association studies in human. We can hypothesize that, in addition to their effects on milk-fat composition, DGAT1 K232A and SCD1 A293V also influence conformation traits that reflect differences in body mass in cattle. Furthermore, $DGAT1^{-/-}$ mice did not produce milk and showed impaired mammary gland development. DGAT1 K232A, therefore, might affect udder conformation traits. In this section, the effects of the DGAT1 K232A and SCD1 A293V polymorphisms on conformation traits will be examined in our Dutch Holstein Friesian cattle population.

Known from literature

With regard to DGAT1, limited data is available on the effect of the DGAT1 K232A genotype on conformation traits in cattle. Spelman et al. (2002) did not find an effect of DGAT1 genotype on any non-production trait, including conformation traits in New Zealand cattle. Kaupe et al. (2007) studied the effect of the DGAT1 genotype on conformation traits in a granddaughter design of 1,291 German Holstein sons from 18 paternal half-sib families, and reported significant effects for strength and rump width, which suggests differences in body build between cows of the different genotypes. Banos et al. (2008) showed a marginal effect of DGAT1 genotype on cumulative effective energy balance (a measure of the change in energy status as it

accumulates throughout lactation) in a study of 571 UK Holstein cows from different feed and selection trials. Oikonomou et al. (2008) were able to detect associations of the DGAT1 K allele with higher body condition score and energy content (calculated from body condition score and live weight records) measured throughout the whole lactation of 497 primiparous Greek Holstein cows. To my knowledge, no literature is available on the effect of SCD1 genotypes on body condition or conformation in cattle. In a QTL mapping study for conformation traits in the Dutch Holstein Friesian population, using data from 833 sons in a granddaughter design, no QTL were detected on BTA14, where DGAT1 is located, or BTA26, where SCD1 is located (Schrooten et al., 2000).

Effects in the Dutch Holstein Friesian population

Conformation phenotypes of 1,320 Dutch Holstein Friesian heifers were provided by CRV. All animals were part of the Milk Genomics Initiative resource population, as described in detail in Chapter 2. DGAT1 K232A and SCD1 A293V genotypes were available for 1,213 and 1,171 animals, respectively (Chapters 2 and 3). Effects of DGAT1 and SCD1 genotypes were estimated using an Animal Model in ASReml (Gilmour et al., 2002):

$$y_{ijklmno} = \mu + b_{1*}age_i + b_{2*}dim_j + season_k + scode_l + herd_m + genotype_n + A_o + e_{ijklmno}$$

where y was the dependent variable, μ was the general mean, age_i was the covariate describing the effect of age at first calving, dim_j was the covariate describing the effect of days in milk at the time of inspection, $season_k$ was the fixed effect of the class of calving season (June-August 2004, September-November 2004 or December 2004-February 2005), $scode_l$ was the fixed effect of the differences in genetic level between groups of proven bull daughters and young bull daughters, $herd_m$ was the random effect of groups of animals sampled in the same herd, $genotype_n$ was the fixed effect of the DGAT1 or SCD1 genotype, A_o was the random additive genetic effect and $e_{ijklmno}$ was the random residual effect.

Because DGAT K232A has known effects on milk production traits, effects were also estimated using an alternative model: the same model as above,

Table 7.6 Effect of the DGAT1 K232A polymorphism on conformation traits

	Without FPCM correction			*With FPCM correction*		
Trait	KA[1]	AA[2]	P[3]	KA[1]	AA[2]	P[3]
Stature	0.46	0.03	ns	0.21	-0.41	0.03
Chest width	0.18	-0.01	ns	0.15	-0.07	0.05
Body depth	0.03	-0.14	ns	-0.11	-0.36	<0.01
Angularity	-0.08	-0.24	ns	-0.25	-0.53	<0.01
Body condition	0.06	0.07	ns	0.15	0.22	ns
Rump angle	-0.07	-0.13	ns	-0.12	-0.21	ns
Rump width	0.01	-0.20	ns	-0.07	-0.34	0.01
Udder depth	-0.19	-0.18	ns	-0.02	0.10	ns
Fore udder attachment	-0.23	-0.37	0.04	-0.14	-0.22	ns
Rear udder height	-0.16	-0.13	ns	-0.24	-0.27	ns
Front teat placement	-0.15	-0.19	ns	-0.18	-0.23	ns
Rear teat placement	-0.20	-0.06	ns	-0.22	-0.10	ns
Teat length	0.29	0.24	ns	0.27	0.20	ns
Suspensory ligament	-0.17	0.01	ns	-0.22	-0.07	ns
Rear legs rear	0.09	0.11	ns	0.05	0.04	ns
Rear legs side	0.05	0.08	ns	0.06	0.10	ns
Foot angle	0.17	-0.01	ns	0.15	-0.05	ns
Locomotion	0.02	0.04	ns	-0.08	-0.12	ns
Frame class	0.26	-0.30	ns	-0.08	-0.89	<0.01
Type class	0.10	-0.32	ns	-0.46	-1.18	<0.01
Udder class	-0.42	-0.50	ns	-0.54	-0.69	ns
Feet and legs class	0.17	-0.05	ns	-0.07	-0.46	ns
Final class	-0.01	-0.26	ns	-0.27	-0.70	<0.01

[1] Contrast of KA-KK genotypes.

[2] Contrast of AA-KK genotypes.

[3] Statistical significance of the DGAT1 genotype effect. Ns = not significant.

but extended with the covariate fat-and-protein-corrected-milk (FPCM). FPCM reflects the production of milk at an energy basis and was calculated as (Centraal Veevoederbureau, 2004):

FPCM = (0.337 + 0.116 * milk-fat percentage + 0.06 * milk-protein percentage) * milk yield.

The milk production data were based on 305d data, provided by CRV.

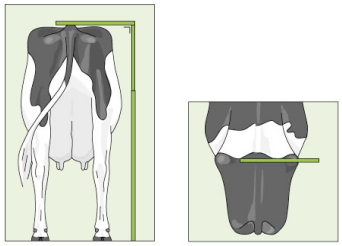
The DGAT1 A allele was associated with higher FPCM (P<0.001): the KA-KK contrast was 143 kg, and the AA-KK contrast was 244 kg. The estimated effects of the DGAT1 polymorphism on conformation traits are shown in Table 7.6. Without correction for FPCM, the DGAT1 polymorphism had a significant effect on fore udder attachment only, where the A allele was associated with a lower score. With correction of FPCM in the model, the DGAT1 polymorphism had significant effects on the linear traits stature, chest width, body depth, angularity, rump width, and on the overall traits frame class, type class and final class (all traits for which a significant effect was found are explained in Text box 7.2). The A allele was associated with lower scores for all traits, which would imply that cows with the AA genotype are smaller, have less body depth and are leaner. This result is consistent with the knockout studies in mice. DGAT1 232A has a lower V_{max} than 232K in producing triacylglycerols *in vitro* (Grisart et al., 2004). A lower activity of DGAT1 (in case of the A allele) or no activity (in case of the $DGAT^{-/-}$ mice) results in smaller and leaner animals. Chest width, body depth and rump width are all linear traits from which, together with body condition, the trait dairy strength is derived. Dairy strength has a direct relationship with longevity. Although $DGAT^{-/-}$ mice showed impaired mammary gland development, DGAT1 genotype did not influence udder traits.

The difference in significance of estimated effects using a model without or with correction for FPCM could partly be explained by a lower residual variance using the model with correction. However, the estimated effects of DGAT1 are also affected by adjusting for FPCM. The effect of FPCM appears to be opposite to the effect of the DGAT1 genotype. Taking angularity as example, the effect of genotype is -0.24 without FPCM correction, and -0.53 with correction for FPCM. This overall effect of DGAT1 on angularity (of -0.24) can be decomposed in an effect of DGAT1 on angularity through FPCM of 0.19 (calculated from estimated regression coefficient and the effect of DGAT1 on FPCM) and an effect of DGAT1 on angularity not working through FPCM of -0.53.

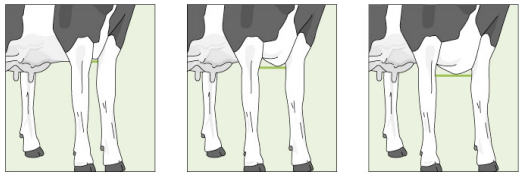
Text box 7.2 Conformation traits

Data on conformation traits were collected by experienced classifiers as part of the national classification system. Heifers were classified once. The conformation traits can be subdivided into 18 linearly scored traits and 5 general characteristics. All linear traits were scored on a 1 to 9 scale, except for stature, which is measured in cm. The general characteristics form a value judgment of the animal and were scored in a range between 71 and 89 points.

Stature
Height from the middle of the rump till the ground.

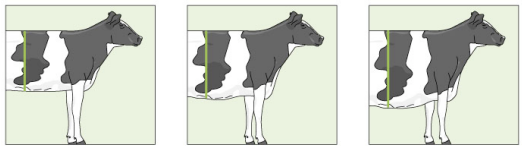


Chest width
Distance between front legs measured at the highest point of the front legs.



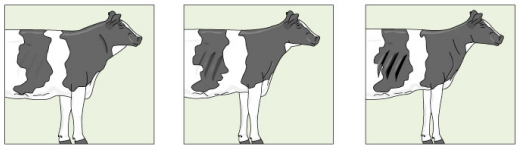
narrow (1) wide (9)

Body depth
Distance between the upper side of the back and the bottom of the belly at the last rib. Score is independent from stature.



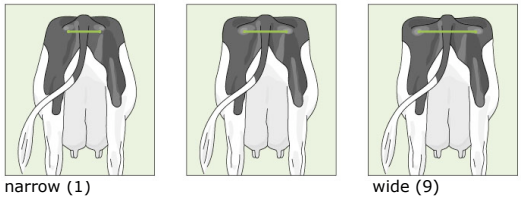
shallow (1) deep (9)

Angularity
The angle of the ribs and the distance between the ribs.



little (1) much (9)

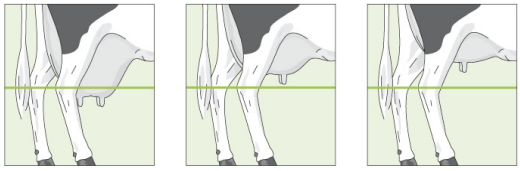
Rump width
Distance between the pin (ischium) bones, at the most posterior end.



narrow (1) wide (9)

Text box 7.2 Conformation traits - continued

Udder depth
Distance from the bottom of the udder till the heel.



deep (1) shallow (9)

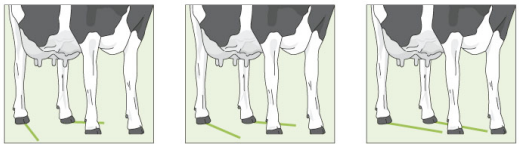
Rear legs side
Angle scored at the anterior side of the hock.

straight (1) curved (9)

Foot angle
Angle at the anterior side of the outer claw of the right hind leg.

little (1) much (9)

Locomotion
Leg use, both the length and the direction of the step.



weak (1) firm (9)

Pictures from CRV

Table 7.7 Effect of the SCD1 A293V polymorphism on conformation traits

	Without FPCM correction			*With FPCM correction*		
Trait	VA[1]	VV[2]	P[3]	VA[1]	VV[2]	P[3]
Stature	0.02	-0.12	ns	0.00	-0.08	ns
Chest width	-0.10	0.07	ns	-0.10	0.08	ns
Body depth	-0.04	0.13	ns	-0.05	0.15	ns
Angularity	0.03	0.23	ns	0.01	0.26	ns
Body condition	-0.05	-0.28	ns	-0.04	-0.28	ns
Rump angle	0.01	0.14	ns	0.00	0.15	ns
Rump width	0.12	0.14	ns	0.11	0.15	ns
Udder depth	-0.06	-0.41	ns	-0.05	-0.44	0.03
Fore udder attachment	-0.17	-0.07	ns	-0.17	-0.06	ns
Rear udder height	-0.09	-0.09	ns	-0.09	-0.11	ns
Front teat placement	0.15	0.01	ns	0.15	0.01	ns
Rear teat placement	-0.06	-0.06	ns	-0.06	-0.05	ns
Teat length	-0.07	0.03	ns	-0.07	0.04	ns
Suspensory ligament	-0.11	-0.29	ns	-0.11	-0.28	ns
Rear legs rear	-0.05	-0.23	ns	-0.06	-0.22	ns
Rear legs side	0.32	0.30	<0.01	0.33	0.30	<0.01
Foot angle	-0.25	-0.32	0.01	-0.25	-0.32	0.01
Locomotion	-0.20	-0.51	0.02	-0.21	-0.50	0.02
Frame class	-0.02	-0.10	ns	-0.05	-0.05	ns
Type class	-0.13	-0.28	ns	-0.21	-0.27	ns
Udder class	-0.36	-0.56	ns	-0.36	-0.54	ns
Feet and legs class	-0.43	-1.07	0.02	-0.46	-1.06	0.01
Final class	-0.22	-0.58	ns	-0.24	-0.55	ns

[1] Contrast of VA-AA genotypes.

[2] Contrast of VV-AA genotypes.

[3] Statistical significance of the SCD1 genotype effect. Ns = not significant.

The estimated effects of the SCD1 polymorphism on conformation traits are shown in Table 7.7. SCD1 genotype affected primarily the trait group feet and legs, where the V allele was associated with a lower score for the overall class feet and legs. Correction for FPCM did not influence the effects on these traits, and resulted in a slightly lower P value for udder depth. It should be kept in mind that we performed multiple tests and that some of the effects might be significant simply by chance: based on the total

number of performed tests, the expected number of false positives would be 2.3 considering a 5% significance threshold, and 0.5 considering a 1% significance threshold. The mechanism by which the SCD1 polymorphism exerts its effects on feet and legs traits is unknown. SCD1 A293V genotype did not affect traits related to body size or leanness, but it cannot be excluded that another SCD1 polymorphism does affect these traits.

In summary, DGAT1 K232A genotype shows significant effects on conformation traits that are related to body size. The DGAT1 A allele, which is associated with less C16:0 and more unsaturated C18:1, is associated with smaller and leaner animals. This result could be related to other economically important traits, such as fertility, health and longevity.

7.7 Integration of approaches

To better understand the effects of polymorphisms, knowledge about the physiological functions of the genes is essential, as well as an insight into the effects of the polymorphisms on the activity of the enzymes. This requires functional studies at the molecular level, but also in cell cultures or whole-animal models. However, results from association studies can provide handhelds to identify genes in pathways of interest. The research described in this thesis revealed DGAT1 and SCD1 as major genes affecting bovine milk-fat composition. The DGAT1 K232A genotype explained about 50% of the genetic variation in the content of unsaturated C18 fatty acids, and about 40% of the genetic variation in C16:0 content. The SCD1 A293V genotype explained 6% to 52% of the genetic variation in the unsaturation indices. With these two major genes identified, it is not expected to find other major genes. The genome-wide scan identified several chromosomal regions involved in bovine milk-fat composition. QTL detected on chromosomes other than BTA14 and 26, where DGAT1 and SCD1 are located, were small and explained a few percent of the phenotypic variation. To narrow down the confidence intervals of these QTL, fine mapping is necessary.

The availability of very-high-dense SNP assays, integrated with pathway knowledge from intensively studied species such as mice and human, will

provide a basis to identify the genes involved in complex traits, such as milk-fat composition. Recently, all cows in our resource population were genotyped using a 60K SNP assay, and a genome-wide association study is being performed.

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Summary

Milk is a fluid secreted by the mammary glands of female mammals, and provides a primary source of nutrition for the neonate. Raw milk contains fat, protein, lactose, vitamins, minerals and, of course, water. About 98% of the total fat in milk is present in the form of triacylglycerols, which are glycerides in which the glycerol is esterified with three fatty acids. About 400 fatty acids have been identified in bovine milk-fat, but the vast majority of fatty acids are present in very small quantities.

The aim of the research described in this thesis was to identify genes that underlie the genetic variation in bovine milk-fat composition. A dual approach to identify genes has been pursued: a candidate gene approach and a genome scan or quantitative trait loci (QTL) mapping approach.

Genetic variation in milk-fat composition
The fat composition of milk samples in approximately 2,000 Dutch Holstein Friesian cows in their first lactation was measured by gas chromatography (Chapter 2). The proportion of total phenotypic variation due to additive genetic variation, i.e. the heritability, was estimated using an Animal Model in ASReml. Substantial genetic variation in milk-fat composition was found: heritabilities were high for short and medium chain fatty acids (C4:0-C16:0, heritabilities ranging from 0.43 to 0.59) and moderate for long chain fatty acids (saturated and unsaturated C18, heritabilities around 0.25).

Candidate gene approach
A non-synonymous polymorphism (K232A) in the diacylglycerol acyltransferase 1 (DGAT1) gene is known to have a large effect on milk-fat percentage. To study the effect of this DGAT1 K232A polymorphism on milk-fat composition, the cows were genotyped for this polymorphism. The DGAT1 K232A polymorphism had a clear influence on milk-fat composition. The K allele of DGAT1, associated with a higher milk-fat percentage, was also associated with more saturated fat, a larger fraction of C16:0, and smaller fractions of C14:0, unsaturated C18, and conjugated linoleic acid (CLA) *cis*9,*trans*11. The DGAT1 K232A polymorphism explained a large part of the genetic variation in milk-fat composition, e.g. 40% of the genetic

variation in C16:0 and 53% of the genetic variation in unsaturated C18 fatty acids.

The unsaturation index represents the concentration of a *cis*9-unsaturated fatty acid proportional to the sum of the *cis*9-unsaturated and the saturated fatty acid. Chapter 3 reported heritabilities for milk-fat unsaturation indices. Heritabilities were moderate to high and ranged from 0.23 to 0.46. We genotyped the cows for a non-synonymous mutation (A293V) in the stearoyl-CoA desaturase 1 (SCD1) gene, which was known to affect the content of mono-unsaturated fatty acids in carcasses from beef cattle. Our analysis revealed that the SCD1 V allele was associated with lower C10, C12 and C14 indices, and with higher C16, C18 and CLA indices in comparison to the A allele, with no differences in total unsaturation index (i.e. an overall index based on C10, C12, C14, C16, C18 and CLA). The SCD1 A293V polymorphism explained 6%-52% of the genetic variation in unsaturation indices. The DGAT1 K232A polymorphism showed effect on the unsaturation indices as well: the DGAT1 A allele was associated with lower C10, C12, C14 and C16 indices, and with higher C18, CLA and total indices. The DGAT1 K232A polymorphism explained 3%-29% of the genetic variation in unsaturation indices.

In Chapter 6, effects of polymorphisms in the candidate genes fatty acid synthase (FASN), oxidized low-density lipoprotein receptor 1 (OLR1), peroxysome proliferator-activated receptor-γ coactivator-1α (PPARGC1A), prolactin (PRL), and signal transducer and activator of transcription 5A (STAT5A) on milk-fat composition were investigated. We found significant effects of FASN and OLR1 on milk-fat percentage, however, we were not able to confirm the effects of other investigated genes on milk-fat percentage or milk-fat yield that were reported in literature. All polymorphisms showed effects on milk-fat composition, with most pronounced effects of FASN and STAT5A polymorphisms on C14:0 and C14 index.

QTL approach

To identify chromosomal regions linked to variation in milk-fat composition, without any prior knowledge on genes or functions, we performed a genome-wide scan. A total of 849 cows representing 5 large and 2 small paternal half-sib families were genotyped for 1,341 single nucleotide polymorphisms (SNP) across all 29 autosomes. We detected significant QTL ($P_{genome} \leq 0.05$) for short/medium chain (Chapter 4) and for long chain (Chapter 5) fatty acids on 6 chromosomes: on *Bos taurus* autosome (BTA) 6 for C6:0 and C8:0; on BTA14 for fat%, all uneven chain fatty acids, C14:0, C16:0, C16:1, C18:1*cis*9 and their unsaturation indices, C18:1*cis*12, C18:2*cis*9,12, CLA*cis*9,*trans*11, C18:3*cis*9,12,15, total index, total saturated fatty acids (SFA), total unsaturated fatty acids (UFA) and ratio SFA/UFA; on BTA15 for C18:1*trans* fatty acids; on BTA16 for C18 and CLA indices; on BTA19 for C14:0; and on BTA26 for monounsaturated C10, C12, C14 and C16, and their unsaturation indices. The QTL explained 3%-19% of the phenotypic variation. The DGAT1 K232A and SCD1 A293V polymorphisms, located on BTA14 and 26, respectively, most likely explained the QTL detected on BTA14 and 26. The polymorphisms in FASN and STAT5A, which had an effect on C14:0 and were located on BTA19, could not fully explain the QTL for C14:0 detected on BTA19.

In conclusion, the candidate gene approach identified 2 major genes for milk-fat composition, DGAT1 and SCD1, and a number of other candidate genes. The QTL approach identified 6 regions on the bovine genome that are associated with milk-fat composition.

In the final chapter (Chapter 7), the presence of the DGAT1 and SCD1 polymorphisms was evaluated in different dairy cattle breeds, and in buffalo, goat and sheep. Literature review showed that the DGAT1 K232A polymorphism is likely to be present only in *Bos taurus* breeds, and less likely to be present in other species. Because limited information was available for the SCD1 polymorphism, the coding regions of this gene were sequenced in a number of animals representing 6 dairy cattle breeds, buffalo, goat and sheep. In total, 51 polymorphisms were detected within species: 11 SNP in cattle, 6 SNP in buffalo, 31 SNP and a 5-nucleotide

deletion in goat, and 2 SNP in sheep. In addition to sequence variation within species, sequence variation between species was detected, causing an amino acid difference in 20 cases. Although further research to link milk-fat composition to these genotypes is necessary, the identified polymorphisms may explain differences in milk-fat composition within and between species.

Furthermore, an overview of the knowledge on the physiological functions of the DGAT1 and SCD1 enzymes, mainly based on mice studies, was presented. Inactivation of these genes in mice has a large impact on the body characteristics of the mice. This finding has lead to an analysis of the effects of DGAT1 and SCD1 genotypes on conformation traits in the Dutch Holstein Friesian cattle population. DGAT1 K232A genotype showed significant effects on conformation traits that are related to body size. The analysis revealed that the DGAT1 A allele, which is associated with less C16:0 and more unsaturated C18:1, is associated with smaller and leaner animals. This result could be related to other economically important traits, such as fertility, health and longevity.

Samenvatting

Melk en zuivelproducten zoals karnemelk, yoghurt, kwark en kaas vormen een belangrijk onderdeel van onze voeding. Verreweg de meeste zuivelproducten worden gemaakt van koemelk. Rauwe koemelk bevat vet, eiwit, koolhydraten, vitamines en mineralen, en natuurlijk water. Maar niet elke koe geeft precies dezelfde melk, de samenstelling ervan verschilt van koe tot koe. Niet alleen geeft de ene koe melk met meer vet of eiwit dan de andere koe, maar ook de samenstelling van het vet of het eiwit kan verschillen. In mijn onderzoek heb ik mij gericht op de gedetailleerde samenstelling van het vet in de melk.

We zijn begonnen met het verzamelen van melkmonsters van ongeveer 2000 koeien van 400 melkveebedrijven in heel Nederland. Dit waren niet zomaar 2000 koeien; ze zijn geselecteerd op basis van hun stamboom, zodat we melkmonsters konden verzamelen van verschillende families melkkoeien van het Holstein Friese ras. De vetsamenstelling van de melkmonsters werd geanalyseerd door middel van gas chromatografie. De melk van de koeien bestond voor gemiddeld 4,4 procent uit melkvet. Dat melkvet was opgebouwd uit een groot aantal vetzuren (wel meer dan 100 verschillende). Vetzuren krijgen hun naam op basis van de lengte van het vetzuur en de verzadiging van het vetzuur. Zo zijn er korte, medium en lange vetzuren, en kunnen vetzuren verzadigd, enkelvoudig onverzadigd, of meervoudig onverzadigd zijn. Uit het onderzoek bleek dat er aanzienlijke verschillen tussen koeien bestaan in vetsamenstelling van de melk.

Met behulp van een statistisch model (waarbij we rekening hebben gehouden met het lactatiestadium van de koe, de leeftijd waarop de koe haar kalf heeft gekregen, etc.) hebben we bepaald hoeveel van die verschillen in vetsamenstelling te verklaren zijn door verschillen in genetische aanleg. Dit aandeel dat terug te voeren is op de genetische aanleg van de koe noemen we een erfelijkheidsgraad. Zo'n erfelijkheidsgraad kan tussen de 0 (niks wordt verklaard door genetische aanleg) en 1 (alle verschillen worden verklaard door genetische aanleg) liggen. We vonden dat de erfelijkheidsgraden voor de korte en medium vetzuren hoog waren, namelijk tussen de 0.43 en 0.59, en dat de erfelijkheidsgraden voor lange vetzuren wat lager waren, namelijk rond de

0.25. We konden dus concluderen dat de vetsamenstelling van melk voor een belangrijk deel erfelijk bepaald is.

De volgende stap in het onderzoek was het vinden van genen die bijdragen aan de erfelijke verschillen tussen de koeien. Naast melkmonsters hadden we ook bloedmonsters verzameld van de koeien. Uit het bloed hebben we DNA geïsoleerd. Dit DNA bevat al het erfelijk materiaal van de koe. Als eerste hebben we gekeken naar het gen DGAT1. Uit eerder onderzoek is gebleken dat een mutatie in dit gen een groot effect heeft op het percentage vet in melk. In het lab hebben we voor elke koe bepaald welke variant van dit gen ze heeft. Door de melk van koeien met de ene variant te vergelijken met de melk van koeien met de andere variant, werd duidelijk dat het DGAT1 gen ook een grote invloed heeft op de samenstelling van het melkvet. Koeien met de ene variant hebben niet alleen een lager melkvetpercentage, (namelijk 4,0 procent, ten opzichte van 4,9 procent voor koeien met de andere variant), maar ook meer onverzadigde vetzuren.

Op dezelfde manier heb ik onderzocht of het gen SCD1 een invloed had op melkvetsamenstelling. Eerder onderzoek had uitgewezen dat dit gen betrokken was bij de hoeveelheid enkelvoudig onverzadigde vetzuren in spierweefsel van vleesvee. Ook dit gen bleek van invloed op melkvetsamenstelling.

We hebben ook voor een andere benadering gekozen om genen op te sporen die bijdragen aan erfelijke verschillen in melkvetsamenstelling tussen koeien. In het geval van de genen DGAT1 en SCD1 wisten we op basis van literatuur dat deze genen betrokken waren bij de aanmaak van vetten. Nu wilden we zonder voorkennis over genen of functies op zoek gaan naar gebieden op het koeiengenoom die iets te maken hebben met melkvetsamenstelling. Het koeiengenoom is, net als dat van mensen, opgedeeld in chromosomen. Het koeiengenoom telt 29 paar chromosomen, plus de geslachtschromosomen. Voor ongeveer 850 koeien is bepaald welke varianten ze hebben voor meer dan 1300 mutaties. Van deze mutaties was niet bekend in welke genen ze lagen, ze dienden alleen als vlaggetjes om een locatie op het koeiengenoom aan te geven. De resultaten tonen 6

gebieden op het koeiengenoom die betrokken zijn bij melkvetsamenstelling aan. Deze gebieden bevatten ook chromosoom 14, waar het gen DGAT1 ligt, en chromosoom 26, waar het gen SCD1 ligt.

In het laatste hoofdstuk heb ik gekeken of de genoemde mutaties in DGAT1 en SCD1 ook voorkomen in andere koeienrassen, en in buffels, geiten en schapen. Het is immers bekend dat er ook verschillen in melkvetsamenstelling zijn tussen koeienrassen, en tussen soorten dieren. Uit literatuuronderzoek bleek dat het niet waarschijnlijk is dat de DGAT1 mutatie voorkomt in andere soorten. Over SCD1 was weinig bekend en heb ik DNA materiaal van verschillende koeienrassen, buffels, geiten en schapen geanalyseerd in het lab. De betreffende mutatie in het SCD1 gen die betrokken is bij melkvetsamenstelling is alleen gevonden in koeien. Wel heb ik andere mutaties in het SCD1 gen gevonden in buffels, geiten en schapen die mogelijk een rol spelen bij melkvetsamenstelling.

Van de DGAT1 en SCD1 genen weten we dat ze een rol spelen bij de aanmaak van vetten. Omdat vetten belangrijk zijn in het hele lichaam van de koe (als brandstof, als onderdeel van celmembranen, als signaalmolecuul, etc.) zouden deze mutaties wel eens invloed kunnen hebben op andere eigenschappen dan alleen melkvetsamenstelling. Daarom heb ik de effecten van deze mutaties op de exterieureigenschappen (uiterlijke kenmerken) van de koeien bestudeerd. De DGAT1 mutatie bleek effect te hebben op eigenschappen die te maken hebben met lichaamsbouw. Dit zou van belang kunnen zijn voor bijvoorbeeld de vruchtbaarheid of gezondheid van de koeien.

De onderzoeksresultaten tonen aan dat het fokken van koeien op een andere, verbeterde, melkvetsamenstelling mogelijk is, en dat informatie over DGAT1 en SCD1 genotypes hierbij gebruikt kan worden. Een verbeterde melkvetsamenstelling kan een gezondere vetsamenstelling zijn, met meer van de gezonde en minder van de ongezonde vetzuren, en kan ook een vetsamenstelling zijn die meer geschikt is voor het maken van bijvoorbeeld boter of kaas.

Nawoord

Ruim 4 jaar geleden ben ik begonnen als AIO Moleculaire genetica binnen het Milk Genomics project. Een hele stap als je totaal onbekend bent in de wereld van de erfelijkheidsgraden, ASReml, caseïnes, residual variance, koeien, QTL, enz. Maar het is allemaal reuze meegevallen, en ik heb een supertijd gehad in het bolwerk van veetelers en fokkers! En daar hebben veel mensen aan bijgedragen.

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Natuurlijk is ook een bedankje aan alle veehouders die hebben meegewerkt aan het verzamelen van monsters – van de koeien, buffels, geiten en schapen – hier op z'n plaats.

De activiteiten binnen het Milk Genomics project worden besproken tijdens vergaderingen van de begeleidingsgroep, met vertegenwoordigers uit de

industrie. Het is fijn om te horen dat er mensen enthousiast worden van je onderzoeksresultaten. Bedankt!

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Proost!

Curriculum Vitae

Curriculum Vitae

Anke Schennink was born on July 23 1980 in Haps, the Netherlands. In 1998, she graduated from high school Stedelijk Gymnasium Nijmegen and started her study Biology at the Radboud University Nijmegen. During her studies, she performed a pharmacobehavioral research thesis at the department of Psychoneuropharmacology of the Radboud University Medical Centre. Another molecular biological research thesis was carried out in the Plant Cell Biology group of Radboud University Nijmegen. In 2003, she started a second MSc study in Bioinformatics, with a thesis at the Centre for Molecular and Biomolecular Informatics (CMBI). In 2004, she obtained her MSc degrees in Biology and Bioinformatics. In that same year, she started her PhD study at the Animal Breeding and Genomics Centre of Wageningen University. The results of the research on the genetic background of milk-fat composition are described in this thesis. Since January 2009, she is working as a postdoc in the group of Dr. Russel C. Hovey, in the Department of Animal Science, University of California Davis, USA.

Curriculum Vitae

Anke Schennink werd geboren op 23 juli 1980 te Haps. In 1998 behaalde ze haar gymnasiumdiploma aan het Stedelijk Gymnasium Nijmegen, waarna ze biologie ging studeren aan de Radboud Universiteit Nijmegen (toenmalige Katholieke Universiteit Nijmegen). Een gedragsfarmacologische stage op de afdeling Psychoneurofarmacologie van het Universitair Medisch Centrum St. Radboud en een moleculair biologische stage bij de groep Celbiologie van de Plant van Radboud Universiteit Nijmegen waren onderdeel van de doctoraalfase. In 2003 startte ze een tweede studie, de MSc opleiding Bioinformatica, en deed ze een onderzoeksproject bij het Centre for Molecular and Biomolecular Informatics (CMBI). In 2004 studeerde ze af in biologie en bioinformatica. In datzelfde jaar begon ze als promovendus bij de leerstoelgroep Fokkerij en Genetica van Wageningen Universiteit. De resultaten van het onderzoek naar de genetische achtergrond van melkvetsamenstelling zijn beschreven in dit proefschrift. Sinds januari 2009 is ze werkzaam als postdoc in de groep van dr. Russel C. Hovey, in het departement Animal Science van de Universiteit van California Davis, VS.

List of publications

Papers in refereed journals

Schennink A., Stoop W. M., Visker M. H. P. W., Heck J. M. L., Bovenhuis H., van der Poel J. J., van Valenberg H. J. F., and van Arendonk J. A. M. (2007) DGAT1 underlies large genetic variation in milk-fat composition of dairy cows. Anim Genet 38:467-473

Schennink A., Heck J. M. L., Bovenhuis H., Visker M. H. P. W., van Valenberg H. J. F., and van Arendonk J. A. M. (2008) Milk fatty acid unsaturation: genetic parameters and effects of stearoyl-CoA desaturase 1 (SCD1) and acyl-CoA:diacylglycerol acyltransferase 1 (DGAT1). J Dairy Sci 91:2135-2143

Heck J. M. L., Olieman C., Schennink A., van Valenberg H. J. F., Visker M. H. P. W., Meuldijk R. C. R., and van Hooijdonk A. C. M. (2008) Estimation of variation in concentration, phosphorylation and genetic polymorphism of milk proteins using capillary zone electrophoresis. Int Dairy J 18:548-555

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Schennink A., Bovenhuis H., Léon-Kloosterziel K. M., van Arendonk J. A. M., and Visker M. H. P. W. (2009) Effect of polymorphisms in the FASN, OLR1, PPARGC1A, PRL, and STAT5A genes on bovine milk-fat composition. Anim Genet, accepted with minor modifications

Papers submitted or in preparation

Stoop W. M., Schennink A., Visker M. H. P. W., Mullaart E., van Arendonk J. A. M., and Bovenhuis H. Genome-wide scan for bovine milk-fat composition I. QTL for short and medium chain fatty acids. Submitted

Schennink A., Stoop W. M., Visker M. H. P. W., van der Poel J. J., Bovenhuis H., and van Arendonk J. A. M. Genome-wide scan for bovine milk-fat composition II. QTL for long chain fatty acids. Submitted

Schennink A., Kinders S. M., Zewoldi E. E., and Visker M. H. P. W. Sequence variation in the coding regions of the SCD1 gene in 6 dairy cattle breeds, buffalo, goat and sheep. In preparation

Conference abstracts

Schennink A., Heck J. M. L., van Valenberg H. J. F., Bovenhuis H., Visker M. H. P. W., van Arendonk J. A. M, and van Hooijdonk, A. C. M. (2008) Protein variants of β-lactoglobulin, β-casein and κ-casein show large effects on the protein composition of bovine milk. 5th International Symposium Milk Genomics and Human Health, Sydney, Australia

Schennink A., Stoop W. M., Bovenhuis H., Heck J. M. L., Koks P. D., Visker M. H. P. W., and van Arendonk J. A. M. (2008) Quantitative trait loci for milk-fat composition in Dutch Holstein Friesians. J Anim Sci 86 E-suppl 2/J Dairy Sci 91 E-suppl 1, p. 611, ADSA-ASAS Joint Annual Meeting, Indianapolis, USA

Schennink A., Stoop W. M., Visker M. H. P. W., Koks P. D., Mullaart E., van Arendonk J. A. M., and Bovenhuis H. (2008) Quantitative trait loci for milk-fat composition in Dutch Holstein Friesians. 16th Conference of the International Society for Animal Genetics, Amsterdam, the Netherlands

Schennink A., Heck J. M. L., Visker M. H. P. W., Bovenhuis H., van Valenberg H. J. F., Groenen M. A. M., and van Arendonk J. A. M. (2008) DGAT1 and SCD1 underlie large genetic variation in composition and unsaturation of milk-fat. Genetica Retraite, Kerkrade, the Netherlands

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Schennink A., Visker M. H. P. W., Bovenhuis H., van der Poel J. J., and van Arendonk J. A. M. (2008) Effect of the DGAT1 K232A polymorphism on milk-fat composition of dairy cows. Plant and Animal Genome XVI, San Diego, USA

Schennink A., Stoop W. M., Visker M. H. P. W., Heck J. M. L., Bovenhuis H., van der Poel J. J., van Valenberg H. J. F., van Arendonk J. A. M. (2007) Milk-fat composition of dairy cows can be improved by use of genetic variation. 58th Annual Meeting of the European Association for Animal Production, Dublin, Ireland

Training and Supervision Plan

The Basic Package (3 credits[1])	
WIAS Introduction Course	2006
Course on Philosophy of Science and/or Ethics	2005
Scientific exposure (20 credits)	
International Conferences (type of presentation)	
European Farm Animal Functional Genomics Symposium, Edinburgh, UK	2005
EAAP annual meeting, Dublin, Ireland *(oral)*	2007
ADSA/ASAS joint annual meeting, Indianapolis, USA *(oral)*	2008
International Conference on Animal Genetics, Amsterdam *(oral, poster)*	2008
Milk Genomics & Human Health symposium, Brussels, Belgium	2006
Milk Genomics & Human Health symposium, Napa, USA *(oral)*	2007
Milk Genomics & Human Health symposium, Sydney, Australia *(poster)*	2008
Seminars (type of presentation)	
WIAS Science Day, Wageningen *(oral in 2008)*	2005-08
Genetica Retraite Rolduc, Kerkrade *(oral in 2008)*	2005/08
KNAW colloquium 'The role of DNA polymorphisms in complex traits and diseases', Amsterdam	2006
CBG symposium 'Genome variation and complex phenotypes', Amsterdam	2007
Fokkerij & Genetica connectiedagen, Vught	2004-08
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VLAG/NZO Masterclass 'Dietary influences on blood pressure'	2006
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Summer School in Statistical Genetics, Liège, Belgium	2007
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Epigenesis and Epigenetics	2008

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Supervising 2 MSc theses	2005/08

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WIAS associated PhD students (WAPS) council chairman	2006/07
Organization WIAS Science Day 2007	2007

Total credits: 64

[1] One credit equals a study load of approximately 28 hours.

Colophon

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