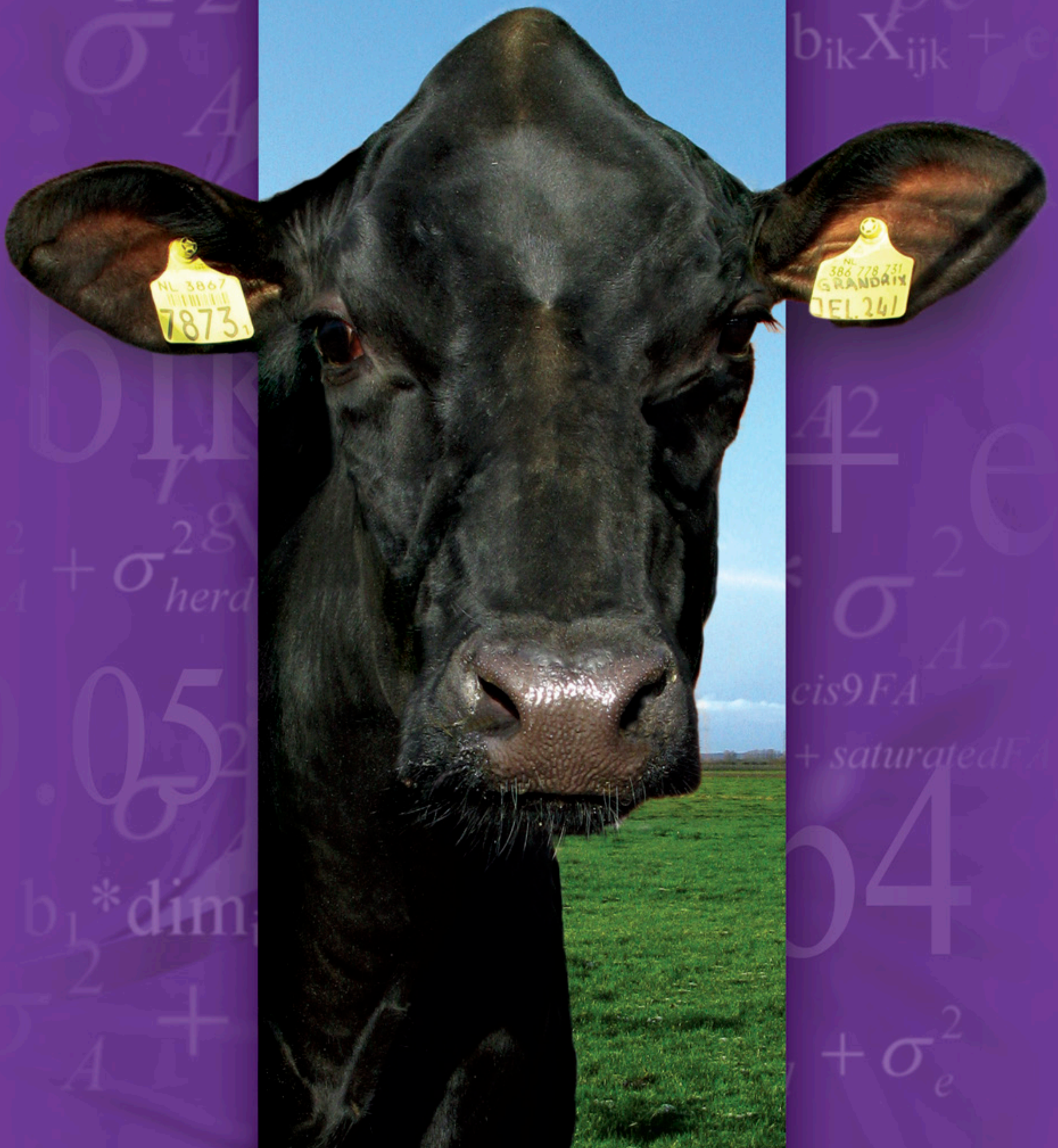
Marianne Stoop

Genetic variation in bovine milk fat composition



Promotor:
Prof. dr. ir. J.A.M. van Arendonk
Hoogleraar Fokkerij en Genetica, Wageningen Universiteit

Co-promotor:
Dr. ir. H. Bovenhuis
Universitair Docent, leerstoelgroep Fokkerij en Genetica,
Wageningen Universiteit

Promotiecommissie:

Prof. dr. ir. E.W. Brascamp	(Wageningen Universiteit)
Dr. P. Dillon	(Teagasc, Moorepark, Ireland)
Prof. dr. J. Keijer	(Wageningen Universiteit)
Prof. dr. ir. G.J. Hiddink	(Wageningen Universiteit)

Dit onderzoek is uitgevoerd binnen de onderzoeksschool WIAS.

W.M. Stoop

Genetic variation in bovine milk fat composition

Proefschrift
ter verkrijging van de graad van doctor
op gezag van de rector magnificus
van Wageningen Universiteit,
Prof. dr. M.J. Kropff,
in het openbaar te verdedigen
op maandag 6 april 2009
des namiddags te vier uur in de Aula.

Stoop, W.M.
Genetic variation in bovine milk fat composition
PhD thesis, Wageningen University, 2009
With summary in English and Dutch
ISBN 978-90-8585-355-8

Abstract

This thesis is part of the Dutch Milk Genomics Initiative and focuses on the genetic background of milk fat composition and on identifying opportunities to change milk fat composition by genetic selection. In this thesis I report estimates of genetic variance in milk fat composition, and the importance of other factors contributing to variation in milk fat composition. In the last part, results of studies to detect genes that affect milk fat composition are reported.

Chapter 2 set the frame-work to which to relate the findings on new traits in later chapters. In chapters 3, 4, and 5, genetic and non-genetic variation in milk fat composition was studied. Results showed considerable genetic variation for all milk fatty acids (**FA**), with C4:0 to C16:0 having higher heritabilities (approximately 0.60) than C18 FA (approximately 0.25). Positive genetic correlations existed within, but not so much between, the groups of short and long chain FA, which coincided with the origin of the FA. Both lactation stage and energy balance significantly contributed to variation in milk fat composition, although their effect was smaller than genetic variation or variation due to herd. Genetic correlations between milk fat composition in winter and summer were approximately 0.8 to 0.9, which indicated that there is no genetic difference between milk fat composition in winter and summer.

Chapters 6 and 7 present QTL for milk fat composition. For short and medium chain FA, 4 significant QTL were detected on BTA6, 14, 19 and 26; and for long chain FA, 3 significant QTL were detected on BTA14, 15, and 16. Each QTL explained 3 to 19 % of phenotypic variance in the affected traits. Results indicated that short and medium chain FA on one hand, and long chain FA on the other hand, undergo distinct processes of synthesis and metabolism.

The final chapter discusses the opportunities for genetic selection. The different selection scenarios presented in the final chapter show that there are opportunities to succesfully breed for improved milk fat composition.

Contents

Chapter 1 Introduction 9

Chapter 2 Genetic parameters for milk urea nitrogen in relation to milk production traits 21

Chapter 3 Genetic parameters for major milk fatty acids and milk production traits of Dutch Holstein-Friesians 35

Chapter 4 Effect of lactation stage and energy balance on milk fat composition 55

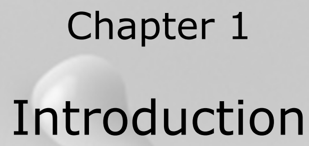
Chapter 5 Genetic correlations between winter and summer milk fat composition 75

Chapter 6 Genome-wide scan for milk fat composition I. QTL for short and medium chain fatty acids 89

Chapter 7 Genome-wide scan for milk fat composition II. QTL for long chain fatty acids 117

Chapter 8 General discussion 141

Summary 159
Samenvatting
Curriculum vitae
List of publications
Training & Supervision Plan
Acknowledgments



Chapter 1

Introduction

Milk as food

Bovine milk is widely recognized as an important food containing many nutrients. It is a major contributor to human welfare and an important base for infant foods (Maijala, 2000; Jensen, 2002). In the European Union, consumption of milk products is high, with about 93 ltr milk, 18 kg cheese and 4 kg butter per capita in 2006. In the USA, these consumption estimates are 84 ltr milk, 16 kg cheese, and 2 kg butter (International Dairy Federation, Bulletin 423/2007). Milk fat makes up about 15 to 25% of total fat in the human diet, but is responsible for 25 to 35% of total saturated fat (Chilliard et al., 2000). The last 40 years milk quality is being increasingly questioned, especially the high amount of saturated fatty acids (**FA**) and relatively low amount of polyunsaturated FA (Maijala, 2000). More information about milk fat properties and the possibilities to change milk fat composition should become available.

Milk fat metabolism

Milk fat contains several hundred different FA. There are two major pathways for these FA: direct transport from rumen to mammary gland through the blood, and de novo synthesis in the mammary gland from precursors, mainly acetate (Bauman & Davis, 1974; Dills, 1986; Neville & Picciano, 1997). Additionally, Δ^9-desaturase enzyme activity in intestines and the mammary gland can transform FA into *cis*-9 unsaturated FA. Figure 1.1 gives a schematic overview of the two major synthesis pathways of milk FA.

It seems not possible to elongate C16:0 to C18:0 through the Acetyl-CoA (**ACC**) cycle (Moore & Christie, 1981). Long chain FA, as well as approximately 50% of C16:0, are suggested to originate entirely from diet and milk quantities depend mainly on rumen outflow of these FA (Chilliard et al., 2000). Dietary FA are hydrolyzed and biohydrogenated in the rumen by microbes. Estimates state that up to 80% of linoleic acid and up to 92% of linolenic acid are hydrogenated in the rumen (Doreau & Ferlay, 1994). The C10 to C18 FA can be desaturated by Δ^9-desaturase, an enzyme that converts FA into *cis*-9 unsaturated FA (Bernard et al., 2001; Keating et al., 2005; Chung et al., 2000). Besides these two main pathways, FA in the mammary gland might originate from body fat reserves, which are mainly long chain C16:0 and C18:0, and some C18:1 *cis*-9. Typically, body fat mobilization accounts for <10% of FA in bovine milk. However, during negative energy balance, body fat mobilization increases in direct proportion to the extend of energy deficit (Bauman & Griinari, 2001).

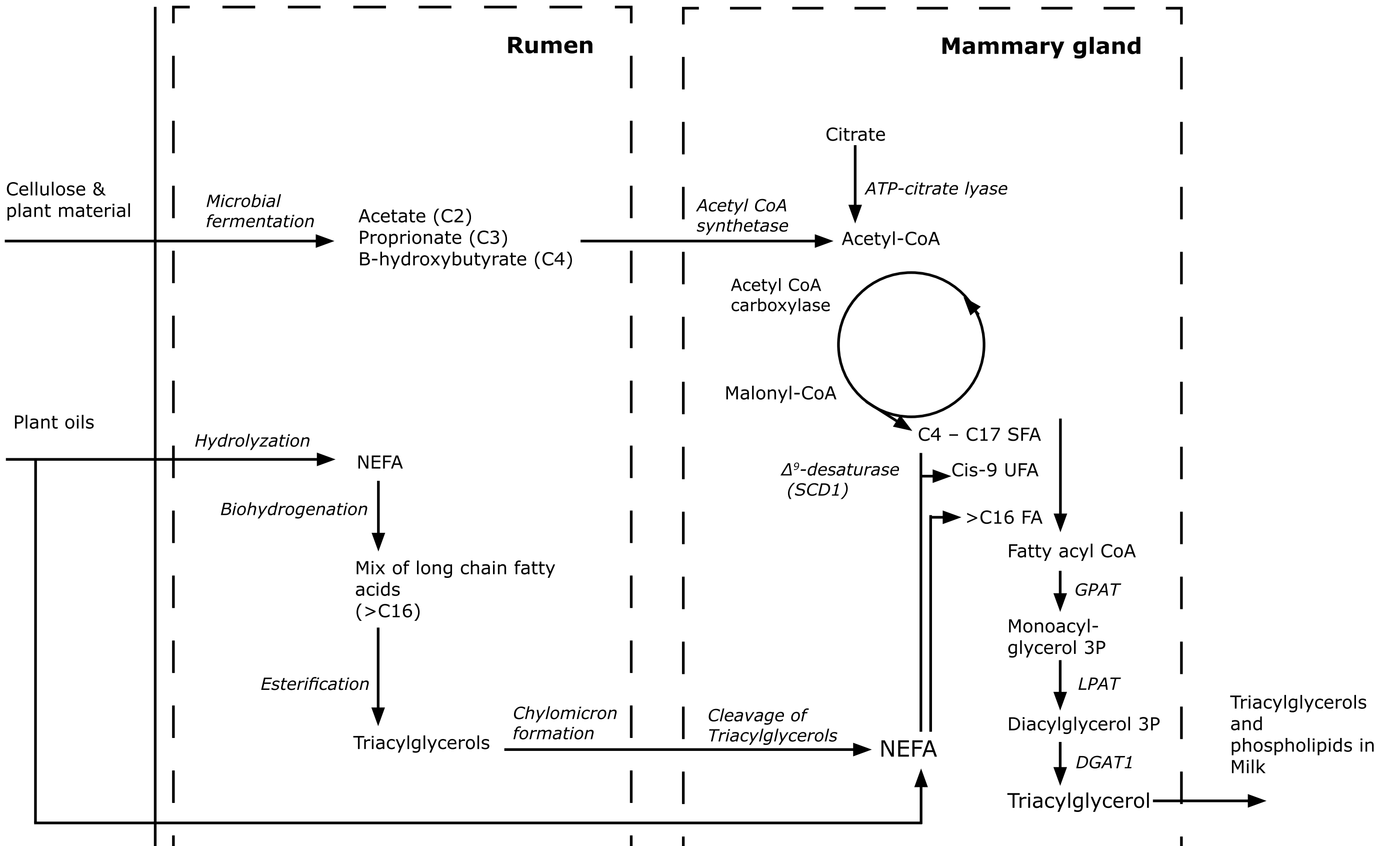


Figure 1.1. Schematic overview of major milk fatty acid synthesis pathways.

Research on milk fat composition

Milk fat composition can be changed in roughly three ways: by nutrition and management, by genetics, and by dairy manufacturing technologies (Walker et al., 2004). The latter is extensively researched within dairy factories. The effect of dietary and management factors on milk fat has been widely studied and is an ongoing topic in current research.

Milk fat depression

Bauman extensively studied the effect of specific conjugated linoleic acid (**CLA)** isomers that cause milk fat depression (e.g. Bauman & Griinari, 2001; Bauman & Griinari, 2003). Feeding starch-rich diets can lead to a decrease in rumen pH, altering rumen biohydrogenation. As a consequence linoleic and linolenic FA are converted to C18:1 *trans*-10 instead of the more common C18:1 *trans*-11. C18:1 *trans*-10 can be transformed to CLA *trans*-10,cis-12. This process is referred to as the biohydrogenation theory of milk fat depression. This CLA *trans*-10,*cis*-12 strongly inhibits de novo synthesis of FA, causing a major reduction in milk fat percentage up to 40 % and reduces saturated fat content of the fat fraction.

It is widely recognized that long chain FA inhibit de novo synthesis of short and medium chain FA (Hansen et al. 1986; Hansen & Knudsen, 1987; Chilliard et al., 1991; Barber et al., 1997; Jayan & Herbein, 2000; Keating et al., 2006). It is suggested that inhibition of de novo synthesis increases from C6:0 to C14:0, as longer chains require more acetyl unit additions from the ACC cycle (Palmquist et al., 1969). Although it has been suggested that long chain FA directly inhibit ACC (Barber et al., 1997), it is likely that several genes in milk fat synthesis in the bovine mammary gland share a common regulatory mechanism, like rodents (Bauman et al., 2001; Peterson et al., 2003). This is supported by the coordinated downregulation of several genes in the milk fat synthesis pathway observed to CLA *trans*-10,*cis*-12 and milk-fat-depression- inducing diets. It seems that several major transcription factors such as SREBP1, PPAR, LXR, and HNF-4ɑ are involved in decreasing de novo synthesis (Bernard et al., 2008). Also hormones may be involved, as STAT5 mediates a prolactin signal and contributes to lactational stimulation of promoter III of ACC (Rosen et al., 1999; Mao et al., 2002). Long chain FA from body fat mobilization seem to predominantly decrease C10:0 to C16:0, and not C4:0 to C8:0 (Storry et al., 1980; Chilliard et al., 1986).

Dietary fat supplementation

Multiple feeding experiments have been performed that add dietary FA, especially polyunsaturated FA (**PUFA**), and measure the duodenal fat flow and the uptake of these added FA in the milk. Although usually the unsaturated fat fraction is increased, the rates in which FA are directly transported to the mammary gland is variable (Steele et al., 1971; McDonald & Scott, 1977; Chilliard et al., 1991; Drackley et al., 1992; Christensen et al., 1994; Lacount et al., 1994; Wu et al., 1994; Ottou et al., 1995; Jenkins et al., 1996; Gulati et al., 1997; Goodridge & Ingalls, 1998). This is caused by the extensive hydrolysis and biohydrogenation of dietary FA in the rumen (Grummer, 1991). Studies that use fish oil to increase PUFA have the further disadvantage of a possible 'fish-off flavor' to the milk (Lacasse et al., 1998).

Health

Studies on cow health and energy balance showed that cows in negative energy balance had an increased level of long chain FA from body fat reserves (Bauman & Griinari, 2001) and a decrease of C5:0 to C15:0 components. The C3 precursor of these FA is, during negative energy balance, reallocated to among others the production of lactose to maintain milk production, resulting in a reduction in C5:0 to C15:0 FA in milk.

Many studies have tried to link milk FA to human health characteristics. Preliminary evidence suggests cholesterol increasing effects of C12:0, C14:0, and C16:0. Lauristic acid, C12:0, seems to increase only the beneficial HDL cholesterol, whereas C14:0 increases both LDL and HDL cholesterol and C16:0 increases mainly LDL cholesterol. This suggests that C16:0 contributes most to the adverse health effect of saturated FA (German & Dillard, 2006). Beneficiary health effects have been attributed to PUFA - which would decrease cholesterol levels (German & Dillard, 2006) - butyric acid, oleic acid, C18-C22 PUFA and CLA (Chilliard et al., 2001). CLA has been associated with anticarcinogenic, antiatherogenic, antidiabetogenic, and immune modulating properties (Parodi, 1997; Maijala, 2000; Jensen, 2002; German & Dillard, 2006).

Genetics

Genetic factors have been identified to affect milk fat composition. For years cows have been genetically selected for milk production traits. Figure 1.2 shows the trend in fat percentage over the last 40 years. Some of the above mentioned studies indicated negative health associations

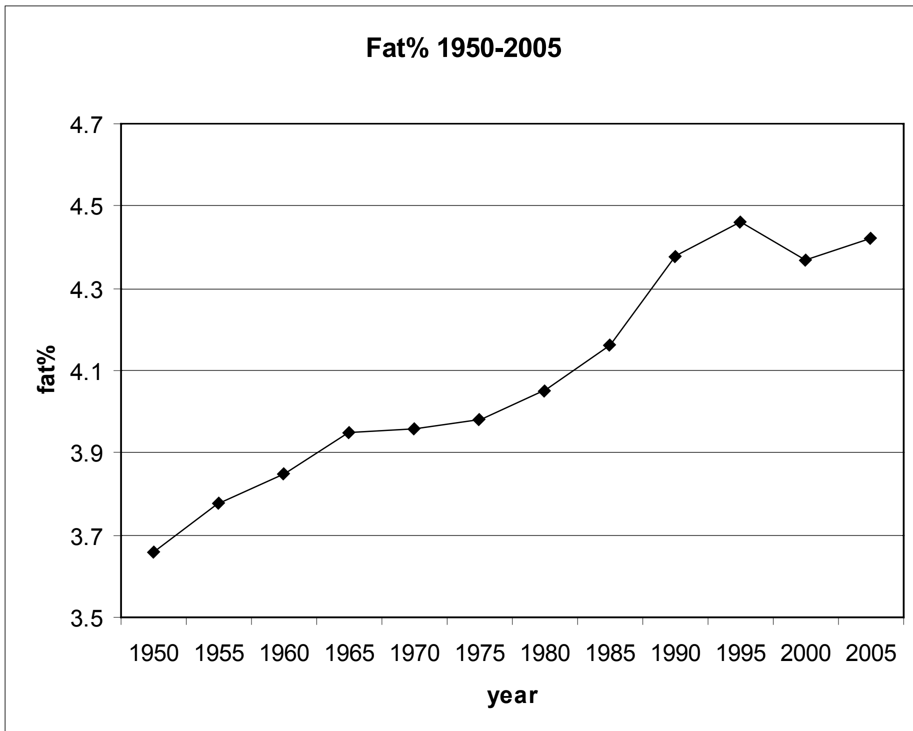


Figure 1.2. Changes in average milk fat percentage from 1950 to 2005 (CRV, 2006)

with milk fat, and a relative decrease of unsaturated fat with increasing fat percentage. Studies on genetic parameters for milk fat composition and the relation between different FA and an increasing milk fat percentage are, however, limited. In 1982, Karijord et al. published heritabilities and genetic correlations for a large number of FA, measured in Norwegian cattle. Smaller studies were done by Everett (1990) and Renner & Kosmack (1974ab). More recent research (Lawless et al., 1999; Soyeurt et al., 2006) has shown differences in milk fat composition between breeds. In conclusion, knowledge on the genetic variation in milk fat composition is rather limited. About 10 years ago some dairy associations studied the possibilities for changes in milk (fat) composition. The initiative was taken, but change seemed impossible at the time; partly because expected human health effects would be small and partly because the required changes in the production chain were predicted to be very large (Gibson, 1991; Maijala, 2000).

Dutch Milk Genomics Initiative

In 2004, a large multidisciplinary study was initiated. The Dutch Milk Genomics Initiative aims to identify possibilities to use breeding strategies to improve milk quality in the desired direction. The project aims to measure detailed milk composition and estimate genetic variances for many fat and protein components and link this variance to specific DNA regions. Additional projects will study economic consequences and necessary adaptations in the dairy industry chain.

Because the multiple questions risen in the Dutch Milk Genomics Initiative, a tight selection of animals was made. Approximately 2,000 first parity cows were selected based on their pedigree: 5 large half-sib families of each 200 daughters were selected for the QTL experiment, and 50 smaller half-sib families of each 20 daughters were selected to contribute in the estimation of genetic parameters and fine-mapping of QTL. Furthermore, at least 5 cows per herd were required to estimate herd effects.

Three morning milk samples were collected from each cow in winter, spring, and early summer 2005, which were conserved by adding 0.03 ww% sodium azide and subsequently frozen. One blood sample was collected from each cow in spring 2005 to extract DNA. DNA of sires was extracted from semen samples. Additional management information on herd size, general feeding practice and health was available from 88% of the farms.

As part of the Dutch Milk Genomics Initiative, this thesis focuses on the genetic background of milk fat composition and the opportunities to change milk fat composition by genetic selection. Aims are to estimate genetic variation in milk fat composition, to identify non-genetic factors contributing to variation in milk fat composition, and to identify QTL that affect milk fat composition.

Outline thesis

In chapter 2 we aimed to estimate heritability for MUN, phenotypic and genetic correlations of MUN with SCS, percentages of fat, protein, and lactose, yields of fat, protein, lactose, and milk, and net energy concentration of the milk, and quantify the effects of herd-test day on these milk production traits to set a frame-work to which to relate the findings on new traits in later chapters. Chapter 3 describes genetic variance in milk fat composition and studies genetic correlations among major milk FA and between FA and milk production traits. Chapter 4 aims to estimate

the effect of lactation stage and energy balance throughout lactation on milk fat composition in cows kept on commercial farms across dietary regimes. In chapter 5 we estimated genetic correlations between winter and summer milk fat composition to infer whether there are genetic differences in milk fat composition throughout the year, and we evaluated the effect of season on heritability estimates. Chapter 6 and 7 present the results of a genome-wide scan to identify QTL for milk fat composition in bovine milk; chapter 6 presents results for short and medium chain FA and chapter 7 presents results for long chain FA. Chapter 8 is the general discussion and focuses on opportunities for genetic selection.

REFERENCES

Barber, M.C., R.A. Clegg, M.T. Travers & R.G. Vernon. 1997. Lipid metabolism in the lactating mammary gland. Biochem Biophys. Acta 1347: 101-126.

Bauman, D.E., P.A. Corl, L.H. Baumgard & J.M. Griinari. 2001. Conjugated Linoleic Acid (CLA) and the dairy cow. In: Recent advances in animal nutrition. Ed.: J. Wisman & P.C. Garnsworthy. Nothingham University Press, Nothingham, UK, pp. 221-250.

Bauman, D.E. & C.L. Davis. 1974. Biosynthesis of milk fat. In: Lactation: A comprehensive treatise Vol.2. Ed.: B.L. Larson & V.R. Smith. New York: Academic, pp. 31-75.

Bauman, D.E. & J.M. Griinari. 2001. Regulation and nutritional manipulation of milk fat: low-fat milk syndrome. Livest. Prod. Sci. 70: 15-29.

Bauman., D.E. & J.M. Griinari. 2003. Nutritional regulation of milk fat synthesis. Annu.Rev.Nutr. 23:203-227.

Bernard, L., C. Leroux & Y. Chilliard. 2008. Expression and nutritional regulation of lipogenic genes in the ruminant lactating mammary gland. In: Bioactive components of milk. Ed.: Z. Bosze. Springer, NY, USA, pp. 67-108.

Bernard, L., C. Leroux, H. Hayes, M. Gautier, Y. Chilliard & P. Martin. 2001. Characterization of the caprine stearoyl-CoA desaturase gene and its mRNA showing an unusually long 3′-UTR sequence arising from a single exon. Gene 281: 53-61.

Chilliard, Y., C. Delouis, M.C. Smith, D. Sauvant & P. Morand-Fehr. 1986. Mammary metabolism in the goat during normal and hormonally-induced lactation. Reprod. Nutr. Dev. 26: 607-615.

Chilliard, Y., A. Ferlay & M. Doreau. 2001. Effect of different types of forages, animal fat or marine oils in cow’s diet on milk fat secretion and composition, especially conjugated linoleic acid (CLA) and polyunsaturated FA. Livest. Prod. Sci. 70: 31-48.

Chilliard, Y., A. Ferlay, R.M. Mansbridge & M. Doreau. 2000. Ruminant milk fat plasticity: nutritional control of saturated, polyunsaturated, trans and conjugated FA. Ann. Zootech. 49: 181-205.

Chilliard, Y., G. Gaglistro, J. Flechet, J. Lefaivre & I. Sebastian. 1991. Duodenal rapeseed oil infusion in early and midlactation cows. 5. Milk FA and adipose tissue lipogenis activities. J. Dairy Sci. 74: 1844-1854.

Christensen, R.A., J.K. Drackley, D.W. Lacount & J.H. Clark. 1994.

Infusion of four long-chain fatty acid mixtures into the abomasums of lactating dairy cows. J. Dairy Sci. 77: 1052-1069.

Chung, M., S. Ha, S. Jeong, J. Bok, K. Cho, M. baik & Y. Choi. 2000. Cloning and characterization of bovine stearoyl CoA desaturase cDNA from adipose tissues. Biosci. Biotechn. Biochem. 64: 1526-1530.

Dills, R.R. 1986. Comparative aspects of milk fat synthesis. J. Dairy Sci. 69:904-910.

Doreau, M. & A. Ferlay. 1994. Digestion and utilization of FA by ruminants. Anim. Feed Sci. Technol. 45: 379-396.

Drackley, J.K., T.H. Klusmeyer, A.M. Trusk & J.H. Clark. 1992. Infusion of long-chain FA varying in saturation and chain length into the abomasums of lactating dairy cows. J. Dairy Sci. 75: 1517-1526.

Everett, R.W. 1990. Dealing with milk fat – genetically. Proc. Cornell. Nutr. Conf. Feed Manuf. Cornell University, Ithaca, NY.

German, J.B. & C.J. Dillard. 2006. Composition, structure and absorption of milk lipids: a source of energy, fat-soluble nutrients and bioactive molecules. Crit. Rev. Food Sci. Nutr. 46: 57-92.

Gibson, J. P. 1991. The potential for genetic change in milk fat composition. J. Dairy Sci. 74:3258–3266.

Goodridge, J. & J.R. Ingalls. 1998. Transfer of omega-3 linolenic acid from flaxseed to milk fat. J. Anim. Sci. 76 (suppl. 1): 353.

Grummer, R.R. 1991. Effect of feed on the composition of milk fat. J. Dairy Sci. 74: 3244-3257.

Gulati, S.K., E.B. Byers, Y.G. Byers, J.R. Ashes & T.W. Scott. 1997. Effect of feeding different fat supplements on the fatty acid composition of goat milk. Anim. Feed Sci. Technol. 66: 159-164.

Hansen, H.O. & J. Knudsen. 1987. Effect of exogenous long-chain FA on lipid biosynthesis in dispersed ruminant mammary gland epithelial cells: esterification of long-chain exogenous FA. J. Dairy Sci. 70: 1344-1349.

Hansen, H.O., D. Tornehave, & J. Knudsen. 1986. Synthesis of milk specific FA and proteins by dispersed goat mammary-gland epithelial cells. Biochem. J. 238: 167-172.

Jayan, G.C. & J.H. Herbein. 2000. "Healthier" dairy fat using trans-vaccenic acid. Nutr. And Food Sci. 30: 304-309.

Jenkins, T.C., H.G. Bateman & S.M. Block. 1996. Butylsoyamide increases unsaturation of FA in plasma and milk of lactating dairy cows. J. Dairy Sci. 79: 585-590.

Jensen, R.G. 2002. Invited review: the composition of bovine milk lip-

ids: January 1995 to December 2000. J. Dairy Sci. 85: 295-350.

Karijord, O.N., N. Standal & O. Syrstad. 1982. Sources of variation in composition of milk fat. Z. Tierz. Zuechtungsbiol. 99: 81.

Keating, A.F., J.J. Kennelly & F.Q. Zhao. 2006. Characterization and regulation of the bovine stearoyl-CoA desaturase gene promoter. Biochem. Biophys. Res. Comm. 344: 233-240.

Keating, A.F., C. Stanton, J.J. Murphy, T.J. Smith, R.P. Ross & M.T. Cairns. 2005. Isolation and characterization of the bovine stearoyl-CoA desaturase promoter and analysis of polymorphisms in the promoter region in dairy cows. Mammalian Genome 16: 184-193.

Lacasse, P., J.J. Kennelly & C.E. Ahnadi. 1998. Feeding protected and unprotected fish oil to dairy cows: II Effect on milk fat composition. J. Anim. Sci. 76 (suppl. 1): 231.

Lacount, D.W., J.K. Drackley, S.O. Laesch & J.H. Clark. 1994. Secretion of oleic acid in milk fat in response to abomasal infusions of canola or high oleic sunflower FA. J. Dairy Sci. 77: 1372-1385.

Lawless, F., C. Stanton, P. L'Escop, R. Devery, P. Dillon, and J.J. Murphy. 1999. Influence of breed on bovine milk cis-9, trans-11-conjugated linoleic acid content. Livest. Prod. Sci. 62: 43-49.

Maijala, K. 2000. Cow milk and human development and well being. Livest. Prod. Sci. 65: 1-18.

Mao, J., A.J. Molenaar, T.T. Wheeler & H.M. Seyfert. 2002. STAT5 binding contributes to lactational stimulation of promoter III expressing the bovine acetyl-CoA carboxylase alpha-encoding gene in the mammary gland. J. Molecular Endocrinology 29: 73-88.

McDonald, I.W. & T.W. Scott. 1977. Foods of ruminant origin with elevated content of polyunsaturated FA. World Rev. Nutr. Diet 26: 144-207.

Moore, J.H. & W.W. Christie. 1981. Lipid metabolism in the mammary gland of ruminant animals. In: lipid metabolism in ruminant animals. Ed.: W.W. Christie. Pergamon Press,pp. 227-277.

Neville, M.C. & M.F. Picciano. 1997. Regulation of milk lipid secretion and composition. Annu. Rev. Nutr. 17: 159-184.

Ottou, J.F., M. Doreau & Y. Chilliard. 1995. Duodenal infusion of rapeseed oil in midlactation cows. 6. Interaction with niacin on dairy performance and nutritional balance. J. Dairy Sci. 78: 1345-1352.

Palmquist, D.L., C.L. Davis, R.E. Brown & D.S. Sachan. 1969. Availability and metabolism of various substrates in ruminants. V. Entry rate into the body and incorporation into milk fat of D(-)β hydroxybutyrate. J. Dairy Sci. 52: 633.

Parodi, P.W. 1997. Cows' milk fat components as potential anticarcinogenic agents. J. Nutr. 127: 1055-1060.

Peterson, D.G., E.A. Matitashvili & D.E. Bauman. 2003. Diet-induced milk fat depression in dairy cows results in increased trans-10, cis-12 CLA in milk fat and coordinate suppression of mRNA abundance for mammary enzymes involved in milk fat synthesis. J. Nutr. 133: 3098-3102.

Renner, E. and U. Kosmack. 1974a. Genetic aspects concerning the composition of FA in milk-fat I. Delimitation to non-genetic factors. Zuchtungskunde 46: 90-99.

Renner, E. and U. Kosmack. 1974b. Genetic aspects concerning fatty acid composition of milk-fat 2. Fatty acid pattern of milk from progeny groups. Zuchtungskunde 46: 217-226.

Rosen, E.D., P. Sarraf, A.E. Troy, G. Bradwin, K. Moore, D.S. Milstone, B.M. Spiegelman & R.M. Mortensen. 1999. PPARγ is required for the differentiation of adipose tissue in vivo and in vitro. Molecular Cell 4: 611-617.

Soyeurt, H., P. Dardenne, A. Gillon, C. Croquet, S. Vanderick, P.Mayeres, C. Bertozzi, and N. Gengler. 2006. Variation in fatty acid contents of milk and milk-fat within and across

breeds. J. Dairy Sci.89: 4858-4865.

Steele, W., R.C. Noble & J.H. Moore. 1971. The effects of 2 methods of incorporating soybean oil into the diet on milk yield and composition in the cow. J. Dairy Sci. 38: 43-48.

Storry, J.E., P.E. Brumby & W.L. Dunkley. 1980. Influence of nutritional factors on the yield and content of milk fat: protected non-polyunsaturated fat in the diet. Int. Dairy Fed. Bull. 125: 105-125.

Walker, G.P., F.R. Dunshea, and P.T. Doyle. 2004. Effects of nutrition and management on the production and composition of milk fat and protein: a review. Australian J. of agricult. Res. 55: 1009-1028.

Wu, Z., J.T. Huber, S.C. Chan, J.M. Simas, K.H. Chen, J.G. Varela, F. Santos, C. Fontes jr. & P. Yu. 1994. Effect of source and amount of supplemental fat on lactation and digestion in cows. J. Dairy Sci. 77: 1644-1651.

Chapter 2

Genetic parameters for milk urea nitrogen in relation to milk production traits

W.M. Stoop, H. Bovenhuis, and J.A.M. van Arendonk

Journal of Dairy Science (2007) 90:1981–1986

ABSTRACT

The aim of this study was to estimate genetic parameters for test-day milk urea nitrogen (**MUN**) and its relationships with milk production traits. Three test-day morning milk samples were collected from 1,953 Holstein-Friesian heifers located on 398 commercial herds in the Netherlands. Each sample was analyzed for somatic cell count, net energy concentration, MUN, and the percentage of fat, protein, and lactose. Genetic parameters were estimated using an animal model with covariates for days in milk and age at first calving, fixed effects for season of calving and effect of young or proven bull, and random effects for herd-test day, animal, permanent environment, and error. Coefficient of variation for MUN was 33%. Estimated heritability for MUN was 0.14. Phenotypic correlation of MUN with each of the milk production traits was low. The genetic correlation was close to zero for MUN and lactose percentage (-0.09); was moderately positive for MUN and net energy concentration of milk (0.19), fat yield (0.41), protein yield (0.38), lactose yield (0.22), milk yield (0.24), percentage of fat (0.18), and percentage of protein (0.27); and was high for MUN and somatic cell score (0.85). Herd-test day explained 58% of the variation in MUN, which suggested that management adjustments at herd-level could reduce MUN. This study shows that it is possible to influence MUN by herd practice and by genetic selection.

INTRODUCTION

Milk urea nitrogen (**MUN**) has become an important trait now that, due to new European legislation, the Netherlands will start using MUN to monitor mineral efficiencies of herds (LNV, 2006). Milk urea is synthesized as a consequence of an imbalance between dietary nitrogen and energy in the rumen, and protein synthesis inefficiency (DePeters & Ferguson, 1992). As the main non-protein source of nitrogen in milk, MUN reflects the efficiency of nitrogen utilization and the nitrogen output towards the environment. The amount of urea in blood, plasma, urine, and milk is related to the crude protein and energy percentage of the feed (Roseler et al., 1993), which suggests a relationship between urea and energy balance (DePeters & Ferguson,1992), and possibly with the energy concentration of the milk. Cows in early lactation have a ruminal flora that is not adapted to the shift to high protein diets after parturition. The consequential mismatch in energy and protein may lead to an increased MUN in the first months of lactation (Jorritsma et al., 2003). The apparent relationship of MUN with nitrogen excretion in milk and urine suggest that decreased MUN will decrease environmental pollution with nitrogen. Milk urea nitrogen might be used as a selection tool, and therefore, information on genetic parameters is needed. Estimates of genetic parameters for MUN have resulted in heritabilities between 0.06 and 0.44, and in low phenotypic correlations with production traits such as fat and milk yield (Wenninger & Distl, 1993; Wood et al., 2003; Mitchell et al., 2005). The range in estimates is broad, as are the numbers of animals and the types of models used, and gives no clear indication of the heritability or genetic correlations of MUN for the Dutch Holstein-Friesian population. Our study aims to estimate heritability for MUN, phenotypic and genetic correlations of MUN with SCS, percentages of fat, protein, and lactose, yields of fat, protein, lactose, and milk, and net energy concentration of the milk (**NEm**), and quantify the effects of herd-test day on MUN.

MATERIALS & 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, milk samples of 1,953 first-lactation cows on 398 commercial herds in the Netherlands were collected. The cows were selected such that at

least 5 selected cows per herd were present at the start of the experiment. Each cow was between 5 and 220 days in milk (**DIM**) of first lactation at the start of the experiment, implying a restricted range in date of calving. Cows were over 87.5% Holstein-Friesian. The cows were sired by 1 of 50 young bulls (857 cows), 1 of 5 proven bulls (909 cows), or other proven bulls (187 cows). The pedigree of each of the 1,953 selected cows was supplied by CRV (Arnhem, the Netherlands).

Milk Samples

Cows were sampled at 3 test-day mornings during February to June 2005. Cows were milked twice daily, but only the morning milk samples were analyzed for milk composition. Sodium azide (0.03 ww%) was added to the milk samples. Time between subsequent samples in the same herd ranged from 4 to 8 weeks. A total of 5,737 samples were collected. A total of 156 records were discarded, mainly because there were fewer than 2 samples per cow, fewer than 3 animals per herd-test day class, or cows were more than 335 DIM. In total, 5,581 test-day records were analyzed for MUN, SCC, and percentage traits. For yield traits, 5,292 records were analyzed because milk yield was missing for 289 records.

Analysis

For each sample, MUN and percentage of fat, protein, and lactose were determined by infrared spectroscopy using a Fourier transform interferogram (MilkoScan FT 6000, Foss Electric, Hillerød, Denmark) at the laboratory of the Milk Control Station (Zutphen, the Netherlands). A calibrated regression curve was used to calculate parameter values from the peak pattern resulting from infrared spectroscopy. For MUN, about 95% of the control samples had a difference between a pair of duplicated samples less than 5 mg/100 g. Yields of MUN, fat, protein, and lactose were calculated by multiplying percentages with milk yield. Somatic cell count was determined using a Fossomatic 5000 (Foss Electric) and analyzed as log-transformed SCS. Net energy concentration of each milk sample (NEm in MJ/kg) was calculated as:

NEm = 0.384 (fat%) + 0.223 (protein%) + 0.199 (lactose%) – 0.108 (Tyrrell & Reid, 1965).

Variance components and genetic parameters were estimated using a repeatability animal model in AS-Reml (Gilmour et al., 2002):

$$y_{ijklmno} = \mu + b_1 * dim_i + b_2 * e^{-0.05 * dim} + b_3 * afc_j + b_4 * afc_j^2 + season_k + scode_l + htd_m + A_n + pe_o + e_{ijklmno} \quad (1)$$

where $y_{ijklmno}$ is the dependent variable corresponding to the o test-day observation of cow n with a sire code of l, calving age of first calving j during season k and at DIM i on herd test day m; μ is the general mean; dim_i is DIM (time between calving and date of sample), modeled with a Wilmink curve (Wilmink, 1987); afc_j is a covariate describing the effect of age at first calving; $season_k$ has 3 classes for season of calving: summer (June–August 2004), autumn (September–November 2004), and winter (December 2004–February 2005); $scode_l$ is a fixed effect accounting for differences between groups of proven bull daughters and young bull daughters; htd_m is a random effect defining groups of animals sampled in the same herd on the same day; A_n is the random additive genetic effect of animal n; pe_o is the random permanent environmental effect cow n; and $e_{ijklmno}$ is the random residual effect.

Heritabilities and repeatabilities were estimated using univariate analyses. Correlations were estimated using model [1] and bivariate analyses. Starting values for variance structures in bivariate analyses were based on results of univariate analysis.

Heritability (h^2) was calculated as:

$$h^2 = \frac{\sigma^2_A}{\sigma^2_A + \sigma^2_{pe} + \sigma^2_e} \quad (2)$$

where σ^2_A = additive genetic variation, σ^2_{pe} = permanent environment variation, and σ^2_e = residual variation.
The repeatability (r) estimates the correlation between consecutive samples of the same cow in time. Repeatability was calculated as:

$$r = \frac{\sigma^2_A + \sigma^2_{pe}}{\sigma^2_A + \sigma^2_{pe} + \sigma^2_e} \quad (3)$$

The proportion of variance due to htd (h_{htd}) was calculated as

$$h_{htd} = \frac{\sigma^2_{htd}}{\sigma^2_{htd} + \sigma^2_A + \sigma^2_{pe} + \sigma^2_e} \quad (4)$$

where σ^2_{htd} = herd-test day variation.

RESULTS

Mean and coefficient of variation

Means and coefficients of variation for MUN and milk production traits, based on 3 test-day morning milk samples, are in Table 2.1. Coefficient of variation for MUN was high (33%) compared with other milk production traits; moderate for yield traits (around 20%) and fat percentage (17%); and low for percentage of protein (8%) and lactose (3%). Coefficient of variation for NEm (10%) was half of the variation of the yield traits and one-third of the variation of MUN.

Heritability and repeatability

Heritabilities for MUN and milk production traits are in Table 2.2. Heritability for MUN was 0.14, which was higher than that for SCS (0.08), but lower than those for milk production traits. The heritability estimate was moderate for yield of MUN (0.28), protein (0.34), fat (0.37), milk (0.44), and lactose (0.47); and high for percentage of protein (0.60), fat (0.52), and lactose (0.64), as well as for NEm (0.56). Repeatabilities are also in Table 2.2. For MUN, the repeatability was relatively low (0.43), which suggests that the correlation of MUN between test-days was lower than for the other traits. For SCS, the difference between repeatability and heritability was large (0.66). Much variation in SCS can therefore be explained by permanent environmental effects.

Effect of Herd-Test Day and DIM

Table 2.2 shows the proportion of total variance explained by herd-test day. For MUN, herd-test day explained 58% of the variation, whereas for SCS this was only 1%. Variation due to herd-test day for percentage traits was 8% for fat, 6% for protein, and 5% for lactose. The ratio of genetic variance to herd-test day variance showed that for all traits, except MUN and MUN yield, genetic effects were much larger than herd-test day effects. Days in milk had significant effects ($P < 0.05$) on all traits, except for NEm, although NEm increased slightly with advancing lactation (result not shown). Mean MUN (Figure 2.1) peaked around the third month of lactation and decreased thereafter.

Table 2.1. Means and coefficients of variation for MUN and milk production traits measured on 3 morning milk samples of 1,953 primiparous Holstein-Friesian cows.

Trait	n	Mean	CV (%)
Urea (mg/ 100 g)	5,576	20.39	33
Urea yield (g)	5,587	2.65	40
Fat (%)	5,581	4.30	17
Protein (%)	5,581	3.52	8
Lactose (%))	5,581	4.63	3
Fat yield (kg)	5,292	0.55	20
Protein yield (kg)	5,292	0.46	20
Lactose yield (kg)	5,292	0.60	22
Milk yield (kg)	5,292	13.00	21
SCS[1]	5,579	3.90	28
NEm[2](MJ/ kg)	5,581	3.25	10

[1] SCS= log-transformed somatic cell count

[2] NEm= net energy concentration of the milk sample (Tyrrell & Reid, 1965)

Table 2.2. Phenotypic variance (σ^2_p), ratio $\sigma^2_A/\sigma^2_{htd}$, heritability ($h^2$), repeatability (r), and proportion of variation due to herd-test day (h_{htd}) for MUN and milk production traits estimated from 3 morning milk samples of 1,953 primiparous Holstein-Friesian cows.[1]

Trait	σ^2_p	ratio	h^2	r	h_{htd}
Urea (mg/ 100 g)	18.64	0.11	$0.14_{0.05}$	$0.43_{0.02}$	$0.58_{0.01}$
Urea yield (g)	0.51	0.30	$0.28_{0.07}$	$0.58_{0.02}$	$0.49_{0.02}$
Fat (%)	0.51	6.81	$0.52_{0.09}$	$0.68_{0.01}$	$0.07_{0.01}$
Protein (%)	0.08	9.04	$0.60_{0.10}$	$0.85_{0.01}$	$0.06_{0.01}$
Lactose (%)	0.02	14.69	$0.64_{0.10}$	$0.72_{0.01}$	$0.04_{0.01}$
Fat yield (kg)	0.01	4.60	$0.37_{0.08}$	$0.69_{0.01}$	$0.08_{0.01}$
Protein yield (kg)	0.01	2.96	$0.34_{0.08}$	$0.79_{0.01}$	$0.10_{0.01}$
Lactose yield (kg)	0.01	8.57	$0.47_{0.09}$	$0.83_{0.01}$	$0.05_{0.01}$
Milk yield (kg)	6.47	8.35	$0.44_{0.09}$	$0.84_{0.01}$	$0.05_{0.01}$
SCS[2]	1.13	5.63	$0.08_{0.04}$	$0.74_{0.01}$	$0.01_{0.00}$
NEm[3] (MJ/ kg)	0.10	9.46	$0.56_{0.10}$	$0.75_{0.01}$	$0.06_{0.01}$

[1] $\sigma^2_p = \sigma^2_A + \sigma^2_{pe} + \sigma^2_e$

[2] SCS= log-transformed somatic cell count

[3] NEm= net energy concentration of the milk sample (Tyrrell & Reid, 1965)

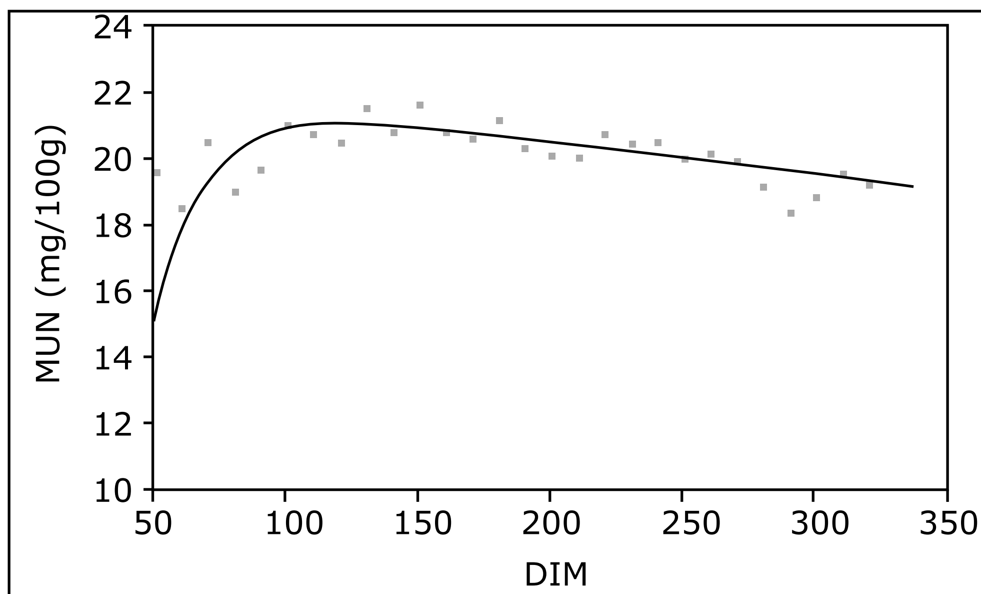


Figure 2.1. Mean MUN during advancing DIM. ■ = mean MUN from data (means are estimated for classes of DIM). Curve is modeled after the following formula, based on model [1]: MUN = 22.389 –0.009 x DIM – 76.7 x $e^{-0.05 \text{x} \text{DIM}}$.

Correlations

The phenotypic and genetic correlations among MUN and percentage traits are in Table 2.3. Phenotypic correlations among MUN and percentage traits were low, ranging from –0.06 to 0.11. Genetic correlation was low to moderate among MUN and percentage of lactose (–0.09), fat (0.19), and protein (0.27).

Genetic correlation of MUN with SCS was high (0.85). Standard errors of the estimates were high. The phenotypic and genetic correlations among MUN and yield traits are in Table 2.4. Phenotypic correlations were low, ranging from –0.05 to 0.06, with an exception for the correlation of MUN with MUN yield, which was 0.84. Genetic correlations were moderate among MUN and yield traits, ranging from 0.22 to 0.41, except for the correlation of MUN with MUN yield, which was 0.77. Genetic correlations among yield traits were consistently high, ranging from 0.58 for fat with lactose yield, to 0.89 for milk with protein yield.

Table 2.3. Phenotypic[1] (below diagonal) and genetic (above diagonal, SE in subscript) correlations among MUN and percentage of fat, protein, and lactose, SCS, and NEm estimated from 3 morning milk samples of 1,953 primiparous Holstein-Friesian cows.

		Percentage (%)				
Trait	MUN	Fat	Protein	Lactose	SCS[2]	NEm[3]
MUN (mg/ 100g)		$0.19_{0.20}$	$0.27_{0.19}$	$-0.09_{0.19}$	$0.85_{0.19}$	$0.21_{0.20}$
Fat (%)	0.07		$0.72_{0.08}$	$-0.18_{0.14}$	$0.39_{0.25}$	$0.99_{0.01}$
Protein (%)	0.11	0.51		$-0.35_{0.04}$	$0.27_{0.26}$	$0.79_{0.06}$
Lactose (%)	-0.06	-0.09	-0.07		$-0.44_{0.21}$	$-0.14_{0.14}$
SCS[2]	0.00	0.06	0.06	-0.24		$0.36_{0.25}$
NEm[3]	0.07	0.98	0.66	0.00	0.04	

[1] Phenotypic correlations had s.e. around 0.02

[2] SCS = log-transformed somatic cell count

[3] NEm = net energy concentration of the milk (Tyrrell and Reid, 1965)

DISCUSSION

In our study, average MUN was 20.39 mg/100 g of milk. Butler et al. (1996) reported a MUN of 22.8 mg/dL for non-pregnant cows, 21.3 mg/dL for cows later identified pregnant, and overall mean values of 22.3 mg/dL. Other studies reported lower means of around 12 to 13 mg/dL (Wood et al., 2003; Mitchell et al., 2005), although the range was similar to our results (1 to 50 mg/dL). In our study, MUN was measured per 100 g, which is slightly less than 1 dL, though it is considered to be approximately the same unit.

The international use of AI bulls makes it unlikely that the large difference in mean is due to different genetic level of Holstein populations. More likely, the difference is due to feed, because Dutch diets are generally high in protein.

Heritability of milk production traits described in this study were in range of those reported in literature (e.g., Hayes et al., 1984; Ikonen et al., 1999; Wood et al., 2003). For test-day MUN, Mitchell et al. (2005) estimated a heritability for first-parity cows of 0.22 when using infrared spectroscopy, and 0.14 when using wet chemistry techniques to determine MUN, which was equal to the estimate found in our study (0.14). Wenninger & Distl (1993) estimated heritabilities for MUN in 2 German breeds: 0.06 for German Simmental and 0.25 for German Brown. Wood

Table 2.4. Phenotypic[1] (below diagonal) and genetic correlations (above diagonal, SE in subscript) among MUN and yield of MUN, fat, protein, lactose and milk, estimated from 3 morning milk samples of 1,953 primiparous Holstein-Friesian cows.

		Yield (kg)				
Trait	MUN	MUN	Fat	Protein	Lactose	Milk
MUN (mg/100 g)		$0.77_{0.09}$	$0.41_{0.19}$	$0.38_{0.20}$	$0.22_{0.21}$	$0.24_{0.22}$
MUN yield (kg)	0.84		$0.66_{0.11}$	$0.85_{0.06}$	$0.82_{0.07}$	$0.87_{0.05}$
Fat yield (kg)	0.06	0.46		$0.75_{0.08}$	$0.58_{0.11}$	$0.59_{0.11}$
Protein yield (kg)	0.05	0.64	0.76		$0.86_{0.04}$	$0.89_{0.03}$
Lactose yield (kg)	0.00	0.60	0.66	0.91		NA[2]
Milk yield (kg)	-0.05	0.68	0.67	0.92	NA[2]	

[1] Phenotypic correlations had SE around 0.02.

[2] NA = not available

et al. (2003) estimated a higher heritability for infrared-determined MUN of 0.44, using random regression analysis of at least 4 test-day samples per cow with heterogeneous variance structures based on DIM.

Results are sometimes presented as lactation yields based on 305-d production. This 305-d production increases the heritability because residual variance is decreased when taking an overall value of 10 test-days. Recalculating heritabilities based on 305-d production led to heritabilities of 0.17 for MUN and 0.32 for MUN yield in this study.

Phenotypic correlations of MUN with the other traits were low, ranging from −0.06 to 0.11. Broderick & Clayton (1997) found negative phenotypic correlations of MUN with milk yield, fat yield, and NEm. Godden et al. (2001) also found a negative correlation of MUN with milk yield but found a correlation near zero for MUN with fat yield. A weak positive phenotypic correlation of MUN with fat and protein percentages was observed in our study as well as in a few others (Wenninger & Distl, 1993; Godden et al., 2001).

We found a strong genetic correlation of milk urea with SCS (0.85). Our genetic correlation was surprising because the phenotypic correlation was weak (0.00). The phenotypic correlation was in line with other studies that demonstrated only a slight increase in nonprotein nitrogen with increasing SCC (Ng-Kwai-Hang et al., 1985), no significant effect of SCC on urea (Eicher et al., 1999), and a negative correlation of −0.01 (Godden et al., 2000). The genetic correlation in our study, however, suggests that the same genetic mechanism is associated with SCS and MUN, e.g., possibly due to changes in protein metabolism during episodes of mastitis.

The very high genetic correlation of fat percentage with NEm (0.99) suggests that these are genetically similar traits. The NEm reflects the energy concentration of the milk and might therefore be related to the energy status of the cow, as is MUN (Jorritsma et al., 2003). The genetic correlation between MUN and NEm, however, seemed low to moderate (0.21) with a high standard error. Results indicate that NEm is almost completely dependent on fat percentage and that protein metabolism and MUN are not strongly related to NEm.

Wood et al. (2003) found a weak genetic correlation of MUN with milk yield (0.11). In our study this correlation was in the same range, although slightly higher (0.24). Wood et al. (2003) found correlations close to zero for MUN with both fat yield (0.01) and protein yield (0.04), whereas in our study the correlations were moderate for MUN with both fat yield (0.41) and protein yield (0.38). The genetic correlation of MUN yield with both fat yield (0.66) and protein yield (0.85) was high, suggesting that yield traits are related.

A number of studies reported significant effects of DIM on MUN, but the direction of the effect was inconsistent. There was an increase of MUN with advancing DIM from around the second month onward (e.g., Wood et al., 2003), leading to a curve for MUN similar to those for fat and protein percentage. Jonker et al. (1998), however, found a decrease of MUN with advancing DIM from around the second month onward, leading to a curve for MUN similar to that for milk yield. Jorritsma et al. (2003) hypothesized that MUN might be increased under a negative energy balance, suggesting a peak in MUN during early lactation like Jonker et al. (1998). Our data suggest a peak between the second and third month of lactation and a slight decrease in MUN with advancing DIM.

As in our study, Wood et al. (2003) found the effect of herd to be the most significant effect on MUN. This effect might be due mainly to feed differences among herds. It has been suggested that energy and total crude protein intake (Roseler et al., 1993) and feeding time (Gustafsson & Palmquist, 1993) affect MUN. In our study, herd-test day had small effects on fat percentage (7%) and fat yield (8%), suggesting small effects of feed on fat, whereas other studies have shown an effect of diet on fat (e.g., Keady et al., 2001). Herd-test day includes not only effects of management and feed but also possible effects of sampler, measurement technique, and season of sampling. Season of sampling has been suggested to affect MUN, with MUN being higher in the summer than in winter (Wattiaux et al., 2005).

In most countries protein yield takes an important place in the national selection index (Miglior et al., 2005) and has been associated with an increase in MUN. The present study shows moderate positive genetic correlations of MUN with yield traits, and strong positive genetic correlations of MUN yield with yield traits, suggesting that selection on protein yield indeed leads to an increase in MUN, but standard errors were high. An increase in MUN might have several causes: inefficient ruminal degradation of protein, less efficient protein synthesis in the mammary gland, or changes in conversion processes elsewhere. The use of MUN in new legislation and the possible relationship between increased MUN and decreased fertility (e.g., Melendez et al., 2000) require a decrease of MUN.

The large coefficient of variation (33%) for MUN in combination with a heritability of 0.14 suggests that there are possibilities to change or control MUN by means of selection. Further research, however, is needed to identify the biological pathways that are affected when selecting for a decreased MUN. The large fraction of the variance due to herd-test day (0.58) indicates that breeding is not the only way to change MUN. Management strategies can play an important role in controlling MUN.

CONCLUSIONS

Our aim was to estimate genetic parameters for MUN and to evaluate its relationships with milk production traits. Heritability of MUN was low. Phenotypic correlations of MUN with milk production traits were close to zero, and genetic correlations were low to moderate and positive. This suggests that selection for milk production traits tends to increase yield of MUN. Results from this study show that it is possible to influence MUN by herd practice (as reflected by the high amount of variation explained by herd-test day) and by genetic selection.

ACKNOWLEDGMENTS

This study is part of the Dutch Milk Genomics Initiative, funded by Wageningen University, NZO (Dutch Dairy Organisation), breeding company CRV, and technology foundation STW. The authors thank the owners of the herds for their help in collecting the data, the Milk Control Station (Zutphen, the Netherlands) for analyzing the milk samples, and CRV (Arnhem, the Netherlands) for supplying pedigrees and milk production data.

REFERENCES

Broderick, G. A., and M. K. Clayton. 1997. A statistical evaluation of animal and nutritional factors influencing concentrations of milk urea nitrogen. J. Dairy Sci. 80:2964–2971.

Butler, W. R., J. J. Calaman, and S. W. Beam. 1996. Plasma and milk urea nitrogen in relation to pregnancy rate in lactating dairy cattle. J. Anim. Sci. 74:858–865.

DePeters, E. J., and J. D. Ferguson. 1992. Nonprotein nitrogen and protein distribution in the milk of cows. J. Dairy Sci. 75:3192–3209.

Eicher, R., E. Bouchard, and A. Tremblay. 1999. Cow level sampling factors affecting analysis and interpretation of milk urea concentrations in 2 dairy herds. Can. Vet. J. 40:487–492.

Gilmour, A. R., B. J. Gogel, B. R. Cullis, S. J. Welham, and R. Thompson. 2002. ASReml User Guide Release 1.0. VSN International Ltd., Hemel Hempstead, UK.

Godden, S. M., K. D. Lissemore, D. F. Kelton, K. E. Leslie, J. S. Walton, and J. H. Lumsden. 2001. Relationships between milk urea concentrations and nutritional management, production, and economic values in Ontario dairy herds. J. Dairy Sci. 84:1128–1139.

Godden, S. M., K. D. Lissemore, D. F. Kelton, J. H. Lumsden, K. E. Leslie, and J. S. Walton. 2000. Analytical validation of an infrared milk urea assay and effects of sample acquisition factors on milk urea results. J. Dairy Sci. 83:435–442.

Gustafsson, A. H., and D. L. Palmquist. 1993. Diurnal variation of rumen ammonia, serum urea, and milk urea in dairy cows at high and low yields. J. Dairy Sci. 76:475–484.

Hayes, J. F., K. F. Ng-Kwai-Hang, and J. E. Moxley. 1984. Heritability of milk casein and genetic and phenotypic correlations with production traits. J. Dairy Sci. 67:841–846.

Ikonen, T., K. Ahlfors, R. Kempe, M. Ojala, and O. Ruottinen. 1999. Genetic parameters for the milk coagulation properties and prevalence of noncoagulating milk in Finnish Dairy cows. J. Dairy Sci. 82:205–214.

Jonker, J. S., R. A. Kohn, and R. A. Erdman. 1998. Using milk urea nitrogen to predict nitrogen excretion and utilization efficiency in lactating dairy cows. J. Dairy Sci. 81:2681–2692.

Jorritsma, R., T. Wensing, T. A. M. Kruip, P. L. A. M. Vos, and J. P. T. M. Noordhuizen. 2003. Metabolic changes in early lactation and impaired reproductive performance in dairy cows (review). Vet. Res. 34:11–26.

Keady, T. W. J., C. S. Mayne, D. A. Fitzpatrick, and M. A. McCoy. 2001. Effect of concentrate feed level in late gestation on subsequent milk yield, milk composition and fertility of dairy cows. J. Dairy Sci. 84:1468–1479.

LNV. 2006. Manure policy 2006: System of utilization norms. Brochure 2006, Regulation Service, Ministry of Agriculture, Nature, and Food Quality, The Hague, the Netherlands.

Melendez, P., A. Donovan, and J. Hernandez. 2000. Milk urea nitrogen and infertility in Florida Holstein cows. J. Dairy Sci. 83:459–463.

Miglior, F., B. L. Muir, and B. J. van Doormaal. 2005. 2005. Selection indices in Holstein cattle of various countries. J. Dairy Sci. 88:1255–1263.

Mitchell, R. G., G. W. Rogers, C. D. Dechow, J. E. Vallimont, J. B. Cooper, U. Sander-Nielsen, and J. S. Clay. 2005. Milk urea nitrogen concentration: Heritability and genetic correlations with reproductive performance and disease. J. Dairy Sci. 88:4434–4440.

Ng-Kwai-Hang, K. F., J. F. Hayes, J. E. Moxley, and H. G. Monardes. 1985. Percentages of protein and nonprotein nitrogen with varying fat and somatic cells in bovine milk. J. Dairy Sci. 68:1257–1262.

Roseler, D. K., J. D. Ferguson, C. J. Sniffen, and J. Herrema. 1993. Dietary protein degradability effects on plasma and milk urea nitrogen and milk nonprotein nitrogen in Holstein cows. J. Dairy Sci. 76:525–534.

Tyrrell, H. F., and J. T. Reid. 1965. Prediction of the energy value of cow's milk. J. Dairy Sci. 48:1215–1223.

Wattiaux, M. A., E. V. Nordheim, and P. Crump. 2005. Statistical evaluation of factors and interactions affecting dairy herd improvement milk urea nitrogen in commercial Midwest dairy herds. J. Dairy Sci. 88:3020–3035.

Wenninger, A., and O. Distl. 1993. Analysis of environmental and genetic influences on urea and acetone content of milk from German Simmental and German Brown. Dtsch. Tierartzl. Wochenschr. 100:405–410. (Abstr.)

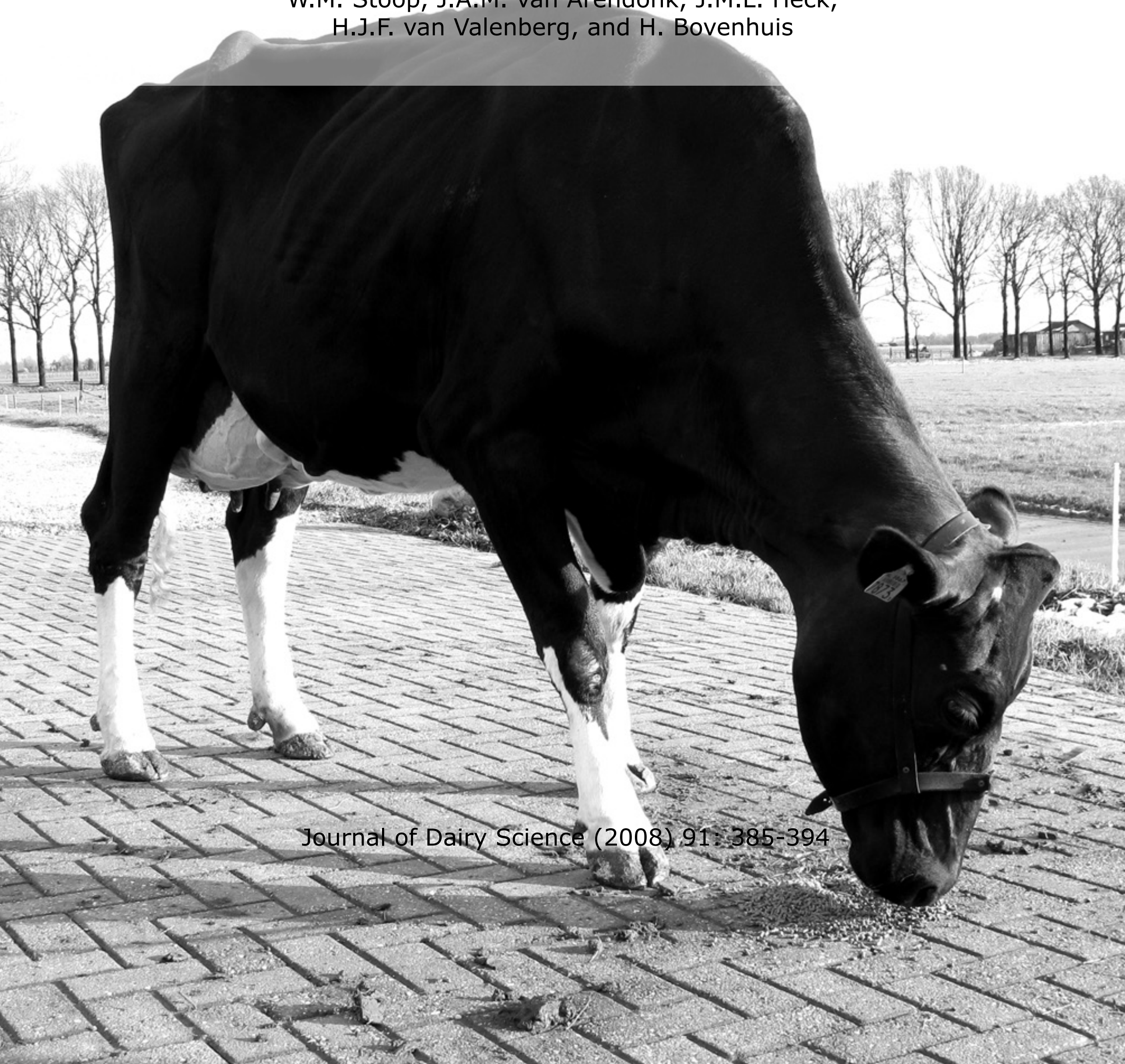
Wilmink, J. B. M. 1987. Adjustment of test-day milk, fat and protein yield for age, season and days-in-milk. Livest. Prod. Sci. 16:335–348.

Wood, G. M., P. J. Boettcher, J. Jamrozik, G. B. Jansen, and D. F. Kelton. 2003. Estimation of genetic parameters for concentrations of milk urea nitrogen. J. Dairy Sci. 86:2462–2469.

Chapter 3

Genetic parameters for major milk fatty acids and milk production traits of Dutch Holstein-Friesians

W.M. Stoop, J.A.M. van Arendonk, J.M.L. Heck,
H.J.F. van Valenberg, and H. Bovenhuis



Journal of Dairy Science (2008) 91: 385-394

ABSTRACT

The objective of this study was to estimate genetic parameters for major milk fatty acids and milk production traits. One morning milk sample was collected from 1,918 Holstein-Friesian heifers located in 398 commercial herds in the Netherlands. Each sample was analyzed for total percentages of fat and protein, and for detailed fatty acid (**FA**) percentages (computed as fatty acid weight as a proportion of total fat weight). Intra-herd heritabilities were high for C4:0 to C16:0, ranging from 0.42 for C4:0 to 0.71 for C10:0. Saturated and unsaturated C18 FA had intra-herd heritability estimates of approximately 0.25, except for C18:2 *cis*-9, *trans*-11, which was 0.42. Standard errors of the heritabilities were between 0.07 and 0.12. Genetic correlations were high and positive among C4:0 to C14:0, as well as among unsaturated C18, but correlations of C4:0 to C14:0 with unsaturated C18 were generally weak. The genetic correlation of C16:0 with fat percentage was positive (0.65), implying that selection for fat percentage should result in a correlated increase of C16:0, whereas unsaturated C18 FA decreased with increasing fat percentage (−0.74). Milk fat composition can be changed by means of selective breeding, which offers opportunities to meet consumer demands regarding health and technological aspects.

INTRODUCTION

Milk fat contains many nutrients necessary for humans, including fat-soluble vitamins, energy, and bio-active lipids (German & Dillard, 2006). During the last decennia, however, milk fat consumption has become negatively associated with human health (Bitman et al., 1995; Jensen, 2002). Cow milk fat contains a relatively low proportion of unsaturated fatty acids (**UFA**) and a relative high proportion of LDL-cholesterol-increasing fatty acids (**FA**), mainly C14:0 and C16:0 (German & Dillard, 2006). Both C14:0 and C16:0 have been associated with increased levels of cholesterol and increased risk of cardiovascular disease (Maijala, 2000; Jensen, 2002). In the past, some studies have questioned whether changing milk fat composition could result in a significant improvement for human health (Gibson, 1991; Maijala, 1995). In recent years, however, market attention has increasingly focused on improving the health aspects of (dairy) products. Possibilities of changing milk fat composition by genetically altering proportions of FA are therefore of interest. To study these possibilities, genetic parameters for FA have to be estimated.

Soyeurt et al. (2006a), in studying 600 milk samples of 275 animals from 5 breeds, found differences in FA proportions among breeds of cattle. Lawless et al. (1999) studied approximately 25 animals per breed and found a relatively high amount of C16:0 and a slightly lower amount of C18:0 in Holstein-Friesians, compared with Normande and Montbeliarde breeds.

Renner & Kosmack (1974b) studied 243 cows originating from 10 sires and found evidence for the existence of within-breed genetic variation in milk fat composition. Karijord et al. (1982) and Famula et al. (1995) also estimated within-breed genetic variation for milk fat composition. The study by Karijord et al. (1982) consisted of approximately 3,000 animals and 7,000 samples, and Famula et al. (1995) studied 523 animals and one sample per animal. Information on within-breed genetic variation in milk fat composition, however, is still limited. The aim of this study was to estimate genetic parameters for major milk FA and to study the relationship of major milk FA with milk production traits in the Dutch Holstein-Friesian population.

MATERIALS & METHODS

Animals and milk samples

This study was part of the Dutch Milk Genomics Initiative, which focuses on genetic aspects of milk composition. The Dutch Milk Genomics 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. The pedigree was the leading reason for the choice of farms; all the farms in the database of CRV (Arnhem, the Netherlands) with at least one proven bull heifer and one young bull heifer were invited to participate in the study. When 2,000 animals were assigned to the study, no further farms were selected.

To study milk fat composition, data were available from 1,918 cows on 398 commercial herds in the Netherlands. Of those cows, 843 cows were sired by the 50 young bulls, 888 cows by the 5 proven bulls, and 187 cows by 46 other proven bulls, to have at least 3 cows per farm. Each cow was more than 87.5% Holstein-Friesian, and was between 63 and 263 days in milk (**DIM**) of first lactation.

One milk sample of 500 mL per cow was collected between February and March 2005. Cows were milked twice daily, but only the morning milk was collected for the study to ensure the quality of the samples. Milk was cooled to 4°C within 3 h after sampling and transported to the laboratory the same morning. Sample bottles contained sodium-azide (0.03 ww%) for conservation.

Analysis of milk samples

Milk fat (butter) was extracted from approximately 400 mL of milk, keeping the remaining 100 mL for other analyses. Fatty acid methyl esters were prepared from milk fat as described in ISO Standard 15884 (International Organization for Standardization - International Dairy Federation, 2002b). The methyl esters were analyzed by gas chromatography according to the 100% FA methyl ester method (International Organization for Standardization-International Dairy Federation, 2002a) with a 100 m polar column (Varian Fame Select CP 7420, Varian Inc., Palo Alto, CA) at the laboratory of the Netherlands Controlling Authority for Milk and Milk Products (Leusden, the Netherlands). The FA were identified and quantified by comparing the methyl ester chromatograms of the milk fat samples with the chromatograms of pure FA methyl ester standards, and

Table 3.1. Trait definition: Groups of fatty acids

Group	Content
C6-12	C6:0, C8:0, C10:0, C12:0
C14-16	C14:0, C16:0
C18u	C18:1 *trans*-4-8, 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
CLA	conjugated C18:2 *cis*-9, *trans*-11
Ratio	Ratio between saturated and unsaturated FA
Saturated	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
Unsaturated	C10:1, C12:1, C14:1, C16:1, C18u, CLA

were measured as the weight proportion of total fat weight. The chromatograms resulted in approximately 130 measurable FA peaks, of which approximately one-third could be identified. Of these, 16 major FA were used for the estimation of genetic parameters in the present study: the even chain FA C4:0 to C18:0; 5 identified C18:1 isomers; C18:2 *cis*-9,12; C18:3 *cis*-9,12,15; and conjugated linoleic acid (**CLA**) *cis*-9, *trans*-11. These 16 FA comprised 89% of total fat. In addition to the individual FA, a number of FA groups were defined based on their potential effect on human health (German & Dillard, 2006; Table 3.1): a "neutral" group (**C6–12**) containing C6:0, C8:0, C10:0, and C12:0; a "negative" group (**C14–16**) containing C14:0 and C16:0; and a "positive" group (**C18u**) containing all unsaturated C18 that were part of the data set. Data were analyzed as weight proportion. To calculate the ratio of SFA:UFA, 11 additional monounsaturated FA and odd-chain FA were included. Percentages of fat and protein were determined from a 10 mL milk subsample by infrared spectroscopy by using a Fourier-transformed interferogram (MilkoScan FT 6000, Foss Electric, Denmark) at the certified laboratory of the Milk Control Station (Zutphen, The Netherlands). In total, 1,918 records were analyzed for fat and protein percentages and fat composition. The CRV supplied the corresponding morning test-day milk yield of the samples; 1,783 CRV records matched our samples. Data for milk yield, fat yield, and protein yield was missing for 135 cows.

Statistical analyses

Variance components and genetic parameters were estimated by an animal model in AS-Reml (Gilmour et al., 2002):

$$y_{ijklmn} = \mu + b_1*dim_i + b_2*e^{-0.05*dim} + b_3*afc_j + b_4*afc_j^2 + season_k + scode_l + herd_m + A_n + e_{ijklmn} \quad (1)$$

where y_{ijklmn} is the dependent variable corresponding to the observation of animal n in herd m, with scode l, age at first calving j in season k, and DIM i; μ is the general mean; dim_i is the covariate for DIM, modeled with a Wilmink curve (Wilmink, 1987); afc_j is the covariate describing the effect of age at first calving; $season_k$ is the fixed effect with 3 classes for season of calving, summer (June to August 2004), autumn (September to November 2004), and winter (December 2004 to February 2005); $scode_l$ is the fixed effect accounting for possible differences between the groups of proven bull daughters and young bull daughters; $herd_m$ is the random effect defining groups of animals sampled in the same herd; A_n is the random additive genetic effect of animal n; and e_{ijklmn} is the random residual effect.

Relationships between individuals were accounted for, and the total pedigree consisted of 26,300 animals and was supplied by CRV (Arnhem, the Netherlands). Heritabilities were estimated by using univariate analyses. We defined two heritabilities:

$$h_{IH}^2 = \frac{\sigma^2_A}{\sigma^2_A + \sigma^2_e} \quad (2)$$

where σ^2_A is the additive genetic variation, and σ^2_e is 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 ratio of the additive genetic over the total phenotypic variance was defined as:

$$h^2 = \frac{\sigma^2_A}{\sigma^2_A + \sigma^2_{herd} + \sigma^2_e} \quad (3)$$

where σ^2_{herd} is the herd test-day variation.

The proportion of variance attributable to herd reflects the relative importance of herd effects such as feed, hygiene, and management, and could be estimated as:

$$h_{herd} = \frac{\sigma^2_{herd}}{\sigma^2_A + \sigma^2_{herd} + \sigma^2_e} \qquad (4)$$

To compare the relative importance of genetic and herd effects, the ratio $\sigma^2_A/\sigma^2_{herd}$ was calculated. Genetic correlations were estimated by using bivariate analyses and model [1] as:

$$r_g = \frac{\sigma^2_{A1,A2}}{\sqrt{(\sigma^2_{A1} * \sigma^2_{A2})}} \qquad (5)$$

where $\sigma^2_{A1,A2}$ is the additive genetic covariance between trait 1 and trait 2, and σ^2_{A1} and σ^2_{A2} are the additive genetic variance of traits 1 and 2.

A principal component analysis PROC VARCLUS, in combination with PROC TREE in SAS (SAS 9.1, SAS Institute, 1999) was used to graphically visualize genetic correlations among major FA.

RESULTS

Mean and coefficient of variation

Mean, coefficient of variation, and 5 and 95% quantiles for individual FA, groups of FA, and milk production traits are shown in Table 3.2. The results showed considerable variation in milk fat composition among cows. Short-chain FA (C4:0 to C12:0) averaged 15% of the total fat, medium-chain FA (C14:0 and C16:0) averaged 44%, and C18:0 averaged 8%. The FA C18:1 *cis*-9, the most prominent among the UFA, averaged 18%, and the other UFA averaged 3.5%. The total FA in Table 3.2 made up approximately 89% of the total fat; the remaining 11% consisted of a large number of FA present in small amounts. These FA included unsaturated medium-chain FA, odd-chain FA, branched-chain FA, and several long-chain FA. The ratio of saturated FA (**SFA**) to UFA averaged 2.8, indicating that approximately 74% of the fat was saturated. This number slightly overestimates the true ratio of SFA:UFA, because the ratio was based on only 27 major FA, whereas trace amounts of some FA, mainly UFA, were not taken into account.

The coefficients of variation for individual FA ranged from 7% for C6:0

to 28% for C18:2. A low coefficient of variation (approximately 10%) was found for most saturated FA (C4:0 to C18:0), and a higher coefficient of variation (approximately 25%) was found for most unsaturated C18 FA. The highest coefficient of variation was found for long-chain polyunsaturated FA: 28% for CLA *cis*-9, *trans*-11, and 27% for C18:3 *cis*-9,12,15. The coefficient of variation for the entire C18u group was only 11%.

Heritability and proportion of variance attributable to herd

Intraherd heritabilities for individual FA, groups of FA, and milk production traits are shown in Table 3.3. High intraherd heritabilities of 0.42 to 0.71 were found for C4:0 to C16:0. Intraherd heritabilities of 0.22 to 0.35 were found for both saturated and unsaturated C18 FA, but for CLA *cis*-9, *trans*-11, heritability was 0.42. Intraherd heritabilities for the groups C6–12 (0.67) and C18u (0.26) were in line with the results for individual FA. For C14–16, intraherd heritability was rather low (0.16) compared with intraherd heritabilities of 0.59 for C14:0 and 0.43 for C16:0. Standard errors of intraherd heritability estimates were between 0.07 and 0.12. Results for the proportion of variance explained by herd are also shown in Table 3.3. Differences among herds most likely represent differences in feeding regimes among herds, but the effects of other management factors also cannot be excluded. Variance attributable to herd was lower for saturated FA (C4:0 to C18:0, approximately 0.20), than for unsaturated C18 FA (approximately 0.50). This difference, however, was not found when comparing different groups of FA: for C6–12, herd explained 27% of the variation, whereas for C18u, herd explained 31%. The ratio of genetic variance to variance attributable to herd ($\sigma_A^2/\sigma_{herd}^2$; Table 3.3) gives the relative importance of genetic vs. herd effects. For C4:0 to C18:0, genetic effects were generally larger than herd effects, whereas for unsaturated C18, herd effects were larger than genetic effects.

Genetic correlations among individual FA

Genetic correlations among individual FA are shown in Table 3.4. The C4:0 had a moderate negative correlation with most other FA. The C6:0 to C14:0 FA were positively correlated (0.34 to 0.96), with a weak correlation of 0.08 for C6:0 with C14:0. The C16:0 showed negative correlations with all studied FA except for C4:0, C6:0, and C18:0.

The C18:0 also showed negative correlations with most other FA, but the correlations were weak. The unsaturated C18 FA were positively correlated (0.25 to 0.99), with a weak correlation of 0.12 for C18:1

Table 3.2. Number (N), mean$_{SD}$[1], CV, and 5 and 95% quantiles for fatty acids, groups of fatty acids[2], and milk production traits, measured on a test-day morning milk sample of 1,918 cows in first lactation.

Trait	N	Mean	CV (%)	5% quantile	95% quantile
Individual fatty acids					
C4:0	1,918	$3.50_{0.27}$	8	3.05	3.96
C6:0	1,918	$2.23_{0.17}$	7	1.95	2.48
C8:0	1,918	$1.37_{0.14}$	10	1.14	1.58
C10:0	1,918	$3.03_{0.43}$	14	2.34	3.73
C12:0	1,918	$4.11_{0.69}$	17	3.02	5.35
C14:0	1,918	$11.62_{0.91}$	8	10.03	13.01
C16:0	1,918	$32.61_{2.81}$	9	28.07	37.11
C18:0	1,918	$8.73_{1.42}$	16	6.55	11.07
C18:1 *cis*-9	1,918	$18.02_{2.09}$	12	14.92	21.76
C18:1 *cis*-11	1,918	$0.41_{0.11}$	27	0.26	0.61
C18:1 *trans*-4-8	1,918	$0.21_{0.05}$	24	0.15	0.29
C18:1 *trans*-9	1,918	$0.15_{0.03}$	20	0.11	0.20
C18:1 *trans*-11	1,918	$0.77_{0.20}$	26	0.49	1.13
C18:2 *cis*-9,12	1,918	$1.20_{0.29}$	24	0.81	1.68
C18:2 *cis*-9,*trans*-11 (CLA)	1,918	$0.39_{0.11}$	28	0.25	0.59
C18:3 *cis*-9,12,15	1,918	$0.41_{0.11}$	27	0.27	0.61
Groups of fatty acids					
C6-12	1,918	$10.74_{1.23}$	11	8.70	12.72
C14-16	1,918	$44.24_{2.78}$	6	39.51	48.47
C18u	1,918	$21.58_{2.43}$	11	18.19	26.16
Ratio SFA/ UFA	1,918	$2.80_{0.37}$	13	2.17	3.38
Milk Production Traits					
Fat percentage (%)	1,918	$4.36_{0.70}$	16	3.33	5.48
Protein percentage (%)	1,918	$3.51_{0.30}$	9	3.04	4.01
Fat yield (kg)	1,783	$0.58_{0.11}$	19	0.40	0.76
Protein yield (kg)	1,783	$0.47_{0.09}$	19	0.33	0.61
Milk yield (kg)	1,783	$13.5_{2.70}$	20	9.0	18.1

[1] For each fatty acid: mean = mean FA as ww proportion of the total fat fraction of 100%.

[2] Groups are defined in Table 3.1.

Table 3.3. Genetic, residual, and herd variation, ratio of genetic over herd variance, heritabilities[1], and proportion of variance explained by herd[2] for individual fatty acids, groups of fatty acids[3], and milk production traits, measured on a test-day morning milk sample of 1,918 cows in first lactation.

Trait	σ^2_A	σ^2_E	σ^2_{herd}	σ^2_A / σ^2_{herd}	h^2_{IH}	h^2	h_{herd}
Individual fatty acids							
C4:0	0.027	0.038	0.013	2.09	0.42	0.35	0.17
C6:0	0.011	0.012	0.004	2.39	0.46	0.39	0.16
C8:0	0.010	0.006	0.004	2.42	0.61	0.48	0.20
C10:0	0.107	0.044	0.047	2.30	0.71	0.54	0.24
C12:0	0.177	0.106	0.217	0.81	0.63	0.35	0.43
C14:0	0.433	0.296	0.157	2.75	0.59	0.49	0.18
C16:0	2.458	3.296	2.298	1.07	0.43	0.31	0.29
C18:0	0.374	1.218	0.382	0.98	0.23	0.19	0.19
C18:1 *cis*-9	0.770	2.340	1.204	0.64	0.25	0.18	0.28
C18:1 *cis*-11	0.001	0.005	0.005	0.29	0.22	0.12	0.43
C18:1 *trans*-4-8	0.0004	0.0008	0.0013	0.35	0.35	0.18	0.49
C18:1 *trans*-9	0.0001	0.0004	0.0005	0.21	0.22	0.11	0.50
C18:1 *trans*-11	0.005	0.013	0.023	0.22	0.28	0.12	0.55
C18:2 *cis*-9,12	0.010	0.029	0.040	0.25	0.26	0.13	0.51
C18:2 *cis*-9,*trans*-11 (CLA)	0.003	0.004	0.006	0.43	0.42	0.21	0.49
C18:3 *cis*-9,12,15	0.001	0.003	0.007	0.14	0.26	0.09	0.64
Groups of fatty acids							
C6-12	0.785	0.385	0.437	1.81	0.67	0.49	0.27
C14-16	0.814	4.142	2.624	0.34	0.16	0.11	0.35
C18u	1.045	2.983	1.770	0.59	0.26	0.18	0.31
Ratio SFA/ UFA	0.027	0.071	0.038	0.71	0.28	0.20	0.28
Milk Production Traits							
Fat percentage (%)	0.239	0.231	0.038	6.25	0.51	0.47	0.07
Protein percentage (%)	0.046	0.025	0.017	2.76	0.65	0.53	0.19
Fat yield (kg)	0.004	0.006	0.003	1.21	0.39	0.29	0.24
Protein yield (kg)	0.001	0.004	0.003	0.39	0.23	0.14	0.37
Milk yield (kg)	2.036	2.951	1.930	1.06	0.41	0.29	0.28

[1] $h^2_{IH} = \sigma^2_A / (\sigma^2_A + \sigma^2_e)$, $h^2 = \sigma^2_A / (\sigma^2_A + \sigma^2_{herd} + \sigma 2e)$, SE between 0.07 and 0.12

[2] $h_{herd} = \sigma^2_{herd} / (\sigma^2_A + \sigma^2_{herd} + \sigma^2_e)$, SE approximately 0.03

[3] Groups are defined in Table 3.1

cis-9 with C18:1 *trans*-11. A clustering tree to visualize the genetic correlations among FA is shown in Figure 3.1. Eight clusters explained more than 90% of the variance. The figure shows the clustering of C6:0 to C14:0 in one group and the clustering of unsaturated C18 in another group. This reflects the high correlations within these groups. The C4:0 and C16:0 did not cluster in one of these 2 main groups.

Genetic correlations among groups of FA

Genetic correlations among the groups of FA [C4:0, C6–12, C18:0, C18u, CLA *cis*-9, *trans*-11, and the ratio of SFA/UFA] are shown in Table 3.5. The C4:0 shows moderate correlations with other FA, as does C6–12. Both C14–16 and C18:0 showed negative correlations with C18u and CLA *cis*-9, *trans*-11, and a positive correlation with the ratio of SFA/UFA. The positive correlation was expected, because C14–16 and C18:0 are the main saturated fractions, so a relative increase will also increase the ratio. The C18u and CLA *cis*-9, *trans*-11 were highly correlated (0.71), and both showed a strong negative correlation with the ratio of SFA/UFA, because they are the main unsaturated fractions.

Genetic correlations of FA with production traits

Genetic correlations of individual FA and groups of FA with milk production traits are shown in Table 3.6. Fat percentage showed a positive correlation with C16:0 (0.65), and negative correlations with C14:0 (−0.43) and all unsaturated C18 FA (−0.43 to −0.78). Results were similar for the correlations of fat percentage with groups of FA: fat percentage showed a positive correlation with C14–16 (0.65) and a strong negative correlation with C18u (−0.74). Protein percentage and fat yield also had positive correlations with C16:0 and C14–16, and negative correlations with all unsaturated C18 FA. For protein yield and milk yield, however, results were opposite: a negative correlation with C14–16 and moderate to strong positive correlations with all unsaturated C18 FA.

DISCUSSION

The results of the current study show that there is considerable genetic variation for proportions of FA, with genetic variation being high for C4:0 to C16:0 and moderate for C18 FA. The moderate coefficient of variation in combination with a moderate to high intraherd heritability indicates that FA proportions can be changed by genetic selection. The ge-

Table 3.4. Genetic correlations[1] between individual fatty acids, measured on a test-day morning milk sample of 1,918 cows in first lactation

	C4:0	C6:0	C8:0	C10:0	C12:0	C14:0	C16:0	C18:0	C18:1 *cis*-9	C18:1 *cis*-11	C18:1 *trans*-4-8	C18:1 *trans*-9	C18:1 *trans*-11	C18:2 *cis*-9, 12	C18:3 *cis*-9, 12, 15
C6:0	0.48														
C8:0	-0.05	0.82													
C10:0	-0.40	0.55	0.92												
C12:0	-0.56	0.34	0.81	0.96											
C12:0	-0.49	0.08	0.50	0.72	0.76										
C12:0	0.52	0.15	-0.33	-0.64	-0.67	-0.84									
C18:0	0.15	-0.01	-0.23	-0.26	-0.29	-0.34	0.27								
C18:1 *cis*-9	-0.11	-0.58	-0.53	-0.35	-0.35	0.13	-0.37	-0.41							
C18:1 *cis*-11	-0.62	-0.31	0.11	0.39	0.39	0.51	-0.79	0.02	0.35						
C18:1 *trans*-4-8	-0.32	-0.38	-0.21	0.01	0.14	0.42	-0.51	0.05	0.25	0.47					
C18:1 *trans*-9	-0.49	-0.36	-0.01	0.20	0.26	0.46	-0.74	-0.44	0.63	0.59	0.66				
C18:1 *trans*-11	-0.12	0.05	0.07	0.09	0.13	0.12	-0.38	0.05	0.12	0.40	0.69	0.73			
C18:2 *cis*-9, 12	-0.23	0.06	0.33	0.42	0.45	0.61	-0.79	-0.27	0.41	0.33	0.52	0.86	0.63		
C18:3 *cis*-9, 12, 15	-0.31	-0.28	-0.12	0.02	0.09	0.31	-0.61	-0.36	0.62	0.55	0.73	0.99	0.94	0.90	
C18:2 *cis*-9, *trans*-11 (CLA)	-0.14	-0.10	0.02	0.08	0.14	0.33	-0.59	-0.58	0.54	0.35	0.55	0.87	0.76	0.68	0.95

[1]SE between 0.01 and 0.26. Most SE were between 0.12 and 0.21, with strong correlations havng SE between 0.01 and 0.10

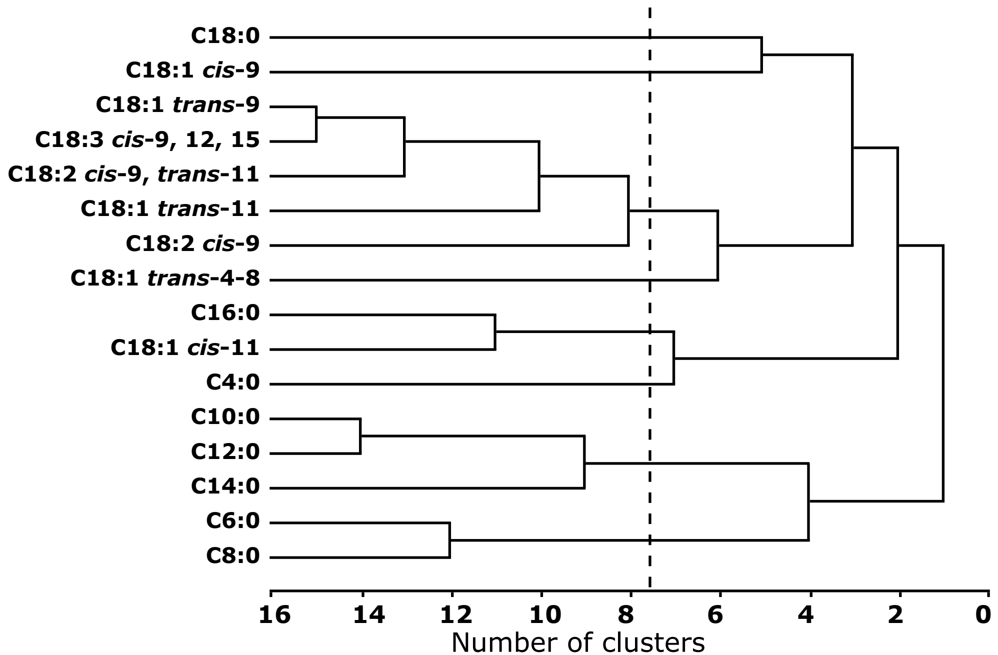


Figure 3.1. Cluster tree based on principal component analysis of genetic correlations among individual fatty acids. Eight clusters explained more than 90% of the variation (dotted line).

Table 3.5. Genetic correlations[1] between 4:0, 18:0, conjugated linoleic acid (CLA) *cis*-9, *trans*-11, ratio of SFA/UFA, and groups of fatty acids[2], measured on a test-day morning milk sample of 1,918 cows in first lactation.

Trait	C4:0	C6-12	C14-16	C18:0	C18u	CLA
C6-12	-0.36					
C14-16	0.42	-0.43				
C18:0	0.15	-0.25	0.21			
C18u	-0.17	-0.30	-0.62	-0.41		
C18:2 *cis*-9,*trans*-11 (CLA)	-0.14	-0.09	-0.60	-0.58	0.71	
Ratio SFA/ UFA	0.19	0.30	0.67	0.45	-0.96	-0.71

[1] SE between 0.02 and 0.28

[2] Groups are defined in Table 3.1

netic correlations suggest that individual FA react differently to selection. Selection on fat percentage seems to have little effect on C6–12, whereas it is expected to lead to an increase in C14–16 and a decrease in C18u.

Samples

In the current study, one morning milk sample per cow was analyzed. Only morning milk was used to ensure a quick transport from the farm to the laboratory. Usually, however, milk production data are analyzed by using a mixed evening-morning sample. This difference could have affected the obtained results. A study by Morris et al. (2005), using an interval of 8 h:16 h, showed high correlations of approximately 0.6 to 0.9 for proportions of FA between the evening and morning samples. Chromatograms of the milk fat samples showed a total of 133 different peaks. About one-third of these peaks could be identified. The current study focused on the identified major FA, constituting 89% of total fat, which were even chain FA (C4:0 to C18:0); 5 monounsaturated C18:1; C18:2 *cis*-9,12; C18:3 *cis*-9,12,15; and the CLA isomer *cis*-9, *trans*-11. Because FA were measured as weight proportion, SFA and UFA were dependent variables and were analyzed as the ratio of SFA/UFA.

Heritabilities

In the current study, the effect of herd was modeled as a random effect, because our interest was also in variation attributable to herd effects. Most studies model herd as a fixed effect. Modeling herd as fixed or random effect has, among others, consequences for the total phenotypic variance, and consequently for the heritability estimates. We also performed an analysis in which we took herd as a fixed effect. The heritabilities that were obtained from those analyses (results not shown) were very similar to what was termed the intraherd heritability (hIH2). This makes the intraherd heritability reported in the current study comparable to the heritabilities reported by other studies, that modeled herd as a fixed effect.

Renner & Kosmack (1974b) and Karijord et al. (1982) estimated heritabilities for short chain FA between 0.13 and 0.26, for medium chain FA between 0.06 and 0.11, and for unsaturated C18 FA at 0.04 (Renner & Kosmack, 1974b), which were lower than estimates found in the current study. Both studies (Renner & Kosmack, 1974b; Karijord et al., 1982) also found low heritabilities for milk yield (0.36 and 0.09) and fat percentage

Table 3.6. Genetic correlations[1] between individual fatty acids, groups of fatty acids[2], and milk production traits, measured on a test-day morning milk sample of 1,918 cows in first lactation.

Trait	Fat (%)	Protein (%)	Fat yield	Protein yield	Milk yield
Individual fatty acids					
C4:0	0.16	-0.11	0.30	0.04	0.09
C6:0	0.46	0.17	0.58	0.12	0.01
C8:0	0.34	0.25	0.45	0.23	0.03
C10:0	0.09	0.19	0.24	0.28	0.10
C12:0	0.00	0.20	0.15	0.25	0.09
C14:0	-0.43	-0.10	-0.11	0.33	0.30
C16:0	0.65	0.32	0.18	-0.49	-0.50
C18:0	0.01	-0.48	0.18	-0.13	0.15
C18:1 *cis*-9	-0.63	-0.32	-0.36	0.23	0.32
C18:1 *cis*-11	-0.45	-0.02	-0.17	0.46	0.29
C18:1 *trans*-4-8	-0.78	-0.35	-0.38	0.33	0.41
C18:1 *trans*-9	-0.76	-0.26	-0.29	0.57	0.54
C18:1 *trans*-11	-0.43	-0.11	-0.13	0.38	0.34
C18:2 *cis*-9,12	-0.70	-0.35	0.04	0.94	0.77
C18:2 *cis*-9, *trans*-11 (CLA)	-0.58	-0.02	-0.30	0.40	0.33
C18:3 *cis*-9,12,15	-0.75	-0.28	-0.28	0.60	0.53
Groups of fatty acids					
C6-12	0.14	0.23	0.26	0.24	0.06
C14-16	0.65	0.38	0.13	-0.57	-0.57
C18u	-0.72	-0.35	-0.35	0.38	0.43
Ratio SFA/ UFA	0.56	0.21	0.37	-0.19	-0.23

[1] SE between 0.08 and 0.29
[2] Groups defined in Table 3.1

(0.28 and 0.09), compared to the current study. The milk production trait heritabilities found in this study were, however, in line with more recent literature (e.g., Vos & Groen, 1998; Ikonen et al., 1999; Ojala et al., 2004).

Short versus long chain FA

Intraherd heritability for C4:0 to C14:0 (approximately 0.60) was higher than for unsaturated C18 FA (approximately 0.25), which is in agreement with the findings of Renner & Kosmack (1974b) and Karijord

et al. (1982). The proportion of variance attributable to herd is smaller for C4:0 to C14:0 (approximately 0.25) than for unsaturated C18 FA (approximately 0.50). There can be many reasons for variance attributable to herd (Jensen, 2002); feed differences among farms, but also other management factors, might play a role. Possible reasons for the difference between C4:0 to C14:0 and unsaturated C18 FA could be that short chain FA are synthesized de novo by the cow. Long chain FA, however, originate predominantly from dietary FA. Because plant material contains mainly long chain FA, differences in diet affect long chain FA more than short chain FA. The difference caused by herd effects, however, was less clear in the FA groups, where herd consistently explained approximately 30% of the variance. The difference between the FA was further evident from genetic correlations between FA. Strong positive genetic correlations were reported for both C6:0 to C14:0 and unsaturated C18 FA. To the contrary, genetic correlations of C6:0 to C14:0 with unsaturated C18 FA were weak, as were genetic correlations of C4:0, C16:0, and C18:0 with the other FA. Few papers have studied genetic correlations between FA. Genetic correlations found in the current study were comparable to the results reported by Karijord et al. (1982).

Explanation for the grouping of the FA can be found in the biological pathways of synthesis. The FA C4:0 (butyric acid) was negatively correlated with almost all other FA. It is formed partly in the rumen by bacterial processes, together with acetate and propionate, and is a precursor for most other short and medium chain FA. Increased de novo synthesis will possibly convert more C4, so less C4 is present in milk, hence the negative correlation. The C6:0 to C16:0 FA are synthesized de novo in a FA cycle starting from C2 and C4 (Bobe et al., 1999). The C16:0, however, is partly synthesized de novo and is partly excreted from blood, which might explain the correlations found for this FA. The unsaturated C18 FA originate mainly from dietary FA, and their proportions are highly dependent on rumen biohydrogenation and on Δ^9-desaturase enzymatic activity in the mammary gland (MacGibbon & Taylor, 2006).

Because of the synthesis pathways, short and medium chain FA are expected to be under stronger genetic control than the long chain FA. This is also reflected in the higher heritability estimates and the smaller influence of herd for short and medium chain FA, compared with long chain FA. Genetic selection is therefore likely to be more effective for short and medium chain FA.

Correlation between fat percentage and FA

Average milk fat percentage in the Netherlands has increased from 3.7% in 1950 to 4.4% in 2005 (NRS, 2006). A positive genetic correlation was found in the present study between fat percentage and C16:0, and a negative genetic correlation was found between fat percentage and unsaturated C18 FA. As a result of the increase in fat percentage, a correlated increase in C16:0 and a decrease in unsaturated C18 FA were expected. In 1974, Renner & Kosmack (1974a) found a fat percentage of 4%, with 25.5% C16:0 and 31.1% unsaturated C18 FA. In the current study, the fat percentage was 4.36%, with 32.6% C16:0 and 21.6% unsaturated C18 FA. Thus, comparing results from this study with those from Renner & Kosmack (1974a), fat percentage has increased, with a strong increase in the proportion of C16:0 and an even stronger decrease in the proportions of unsaturated C18 FA. Although there may be many reasons for these differences, such as breed, season, lactation stage, time of day, or feed, the change in milk fat composition could have been a correlated response to selection for fat, favoring C16:0 rather than increasing all FA simultaneously.

Selection for fat composition

In the past, some studies questioned the need to change milk fat composition for mainly 2 reasons: 1) because large changes in milk fat composition would be required to substantially decrease risks to human health (Maijala, 1995); and 2) because changes that are positive for one product might be detrimental for other products. The latter reason might imply that multiple breeding goals are needed and that the entire production chain would have to be adapted, for example by separately collecting milk for different end uses (Gibson, 1991). In recent years, however, there has been increased awareness among consumers and increased market demands for differentiated, specific, and healthy dairy products, even if these require large adaptations within the current production structure. Even if individual products have small effects because they are only a small part of the total diet, ultimately the sum of all improved products might result in an overall beneficial health effect by, for example, decreasing the intake of C14:0 and C16:0 FA and increasing the intake of unsaturated C18 FA. However, before a breeding program can focus on milk fat composition, several issues need to be resolved. Among others, measuring FA proportions by gas chromatography analysis is too expensive to use in routine analysis, and cheaper methods have to become available, such

as using infrared spectroscopy (Soyeurt et al., 2006b). Farmers in many countries are currently paid based on yields of fat and protein, which tend to increase rather than decrease the amount of C14–16, so FA-based payment should possibly be introduced. Finally, the direction of selection might depend on the purpose of the milk product, because changes that are favorable for one product might be unfavorable for others. In addition to these practical considerations, it would seem worthwhile to study repeated samples from all cows to estimate parameters more accurately and to study physiological changes when selecting for or against certain FA. This study provides the necessary first data to evaluate the possibilities of improving milk fat composition by selective breeding.

CONCLUSIONS

Results have shown considerable genetic variation for all milk FA, with C4:0 to C16:0 having higher intraherd heritabilities (around 0.60) than C18 FA (around 0.25). High genetic correlations exist within the groups of short and long chain FA, which coincide with the origin of the FA and the biological pathways of synthesis. Selection for fat may lead to an increased proportion of C16:0 and a decreased proportion of unsaturated C18 FA because of the high genetic correlations. This study shows that it is possible to change milk fat composition by genetic selection.

ACKNOWLEDGMENTS

This study was part of the Dutch Milk Genomics Initiative, funded by Wageningen University, NZO (Dutch Dairy Organization, Zoetermeer, the Netherlands), breeding company CRV (Arnhem, the Netherlands), and technology foundation STW (Utrecht, the Netherlands). The authors thank the owners of the herds, the Milk Control Station (Zutphen, the Netherlands), and CRV (Arnhem, the Netherlands) for their help in collecting the data.

REFERENCES

Bitman, J., D. L. Wood, R. H. Miller, J. C. Wilk, and E. D. Moore. 1995. Comparison of lipid composition of milk from half-Danish Jersey cows and United States Jersey cows. J. Dairy Sci. 78:655–658.

Bobe, G., D. C. Beitz, A. E. Freeman, and G. L. Lindberg. 1999. Associations among individual proteins and FA in bovine milk as determined by correlations and factor analysis. J. Dairy Sci. 66:523–536.

Famula, T. R., J. F. Medrano, E. J. DePeters, and S. L. Berry. 1995. Estimation of heritability and genetic correlations among fatty acid components of milk in Holstein cows. J. Dairy Sci. 78(Suppl.1):194. (Abstr.)

German, J. B., and C. J. Dillard. 2006. Composition, structure and absorption of milk lipids: A source of energy, fat-soluble nutrients and bio-active molecules. Crit. Rev. Food Sci. Nutr. 46:57–92.

Gibson, J. P. 1991. The potential for genetic change in milk fat composition. J. Dairy Sci. 74:3258–3266.

Gilmour, A. R., B. J. Gogel, B. R. Cullis, S. J. Welham, and R. Thompson. 2002. ASReml User Guide Release 1.0. VSN International Ltd., Hemel Hempstead, UK.

Heringstad, B., D. Gianola, Y. M. Chang, J. Odegard, and G. Klemetsdal. 2006. Genetic associations between clinical mastitis and somatic cell score in early first-lactation cows. J. Dairy Sci. 89:2236–2244.

Ikonen, T., K. Ahlfors, R. Kempe, M. Ojala, and O. Ruottinen. 1999. Genetic parameters for the milk coagulation properties and prevalence of noncoagulating milk in Finnish Dairy cows. J. Dairy Sci. 82:205–214.

International Organization for Standardization-International Dairy Federation. 2002a. Milk fat—Determination of the fatty acid composition by gas-liquid chromatography. ISO 15885-IDF 184. International Dairy Federation, Brussels, Belgium.

International Organization for Standardization-International Dairy Federation. 2002b. Milk fat—Preparation of fatty acid methyl esters. ISO 15884-IDF 182. International Dairy Federation, Brussels, Belgium.

Jensen, R. G. 2002. Invited review: The composition of bovine milk lipids: January 1995 to December 2000. J. Dairy Sci. 85:295–350.

Karijord, O., N. Standal, and O. Syrstad. 1982. Sources of variation in composition of milk fat. Z. Tierz. Zcht. Biol. 99:81–93.

Lawless, F., C. Stanton, P. L'Escop, R. Devery, P. Dillon, and J. J. Murphy. 1999. Influence of breed on bovine milk cis-9, trans-11-conjugated

linoleic acid content. Livest. Prod. Sci. 62:43–49.

MacGibbon, A. K. H., and M. W. Taylor. 2006. Composition and Structure of Bovine Milk Lipids. Pages 1–42 in Advanced Dairy Chemistry. 3rd ed. Vol. 2: Lipids. P. F. Fox and P. L. H. McSweeney, ed. Springer, New York, NY.

Maijala, K. 1995. Current possibilities of breeding to influence milk composition from the viewpoint of human nutrition and health. Page 23 in Proc. NJF/NMR—Seminar No. 252. Milk in Nutrition: Effects of Production and Processing Factors. Turku, Finland. S. Mantere-Alhonen and K. Maijala, University Printing House, Helsinki, Finland.

Maijala, K. 2000. Cow milk and human development and well-being. Livest. Prod. Sci. 65:1–18.

Morris, C. A., S. M. Hickey, and M. P. Agnew. 2005. Repeatability of fatty acid composition in pm:am milk samples from cows. Proc. N. Z. Soc. Anim. Prod. 65:297–299.

NRS. 2006. Year Statistics 2005, NRS, Arnhem, the Netherlands.

Ojala, M., T. Seppanen, A. Tyriseva, and T. Ikonen. 2004. Associations of milk protein polymorphism and milk production traits and udder health traits in Finnish Ayrshire cows. EAAP 2004, GPh1.7. Eur. Assoc. Anim. Prod., Bled, Slovenia.

Renner, E., and U. Kosmack. 1974a. Genetic aspects concerning the composition of FA in milk fat. 1. Delimitation to non-genetic factors. Zuchtungskunde 46:90–99.

Renner, E., and U. Kosmack. 1974b. Genetic aspects concerning fatty acid composition of milk fat. 2. Fatty acid pattern of milk from progeny groups. Zuchtungskunde 46:217–226.

SAS Institute. 1999. SAS OnlineDoc, Version 8. SAS Inst. Inc., Cary, NC.

Soyeurt, H., P. Dardenne, F. Dehareng, G. Lognay, D. Veselko, M. Marlier, C. Bertozzi, P. Mayeres, and N. Gengler. 2006b. Estimating fatty acid content in cow milk using mid-infrared spectrometry. J. Dairy Sci. 89:3690–3695.

Soyeurt, H., P. Dardenne, A. Gillon, C. Croquet, S. Vanderick, P. Mayeres, C. Bertozzi, and N. Gengler. 2006a. Variation in fatty acid contents of milk and milk-fat within and across breeds. J. Dairy Sci. 89:4858–4865.

Vos, H., and A. F. Groen. 1998. Altering milk protein/fat ratio: Results of a selection experiment in dairy cattle. Livest. Prod. Sci 53:49–55.

Wilmink, J. B. M. 1987. Adjustment of test-day milk, fat and protein yield for age, season and days-in-milk. Livest. Prod. Sci. 16:335–348.

Chapter 4

Effect of lactation stage and energy status on milk fat composition of Holstein-Friesian cows

W.M. Stoop, H. Bovenhuis, J.M.L. Heck, and J.A.M. van Arendonk

Journal of Dairy Science (accepted for publication)

ABSTRACT

The effect of lactation stage, negative energy balance (**NEB**) and milk fat depression (**MFD**) on detailed milk fat composition were estimated in primiparous Holstein-Friesian cows. One morning milk sample was collected from each of 1,933 cows from 398 commercial Dutch herds in winter 2005. Milk fat composition was measured using gas chromatography and fat and protein percentage were measured using infra red spectrometry. Each fatty acid (FA) changed 0.5 to 1 phenotypic standard deviation over lactation, except odd-chain C5:0 to C15:0, branched FA, and *trans*-10, *cis*-12 conjugated linoleic acid (CLA). The largest change was an increase from 31.2 to 33.3 ww% for C16:0 in day 80 to 150 of lactation. Energy status was estimated for each cow as the deviation from each cow's average lactation fat-to-protein ratio (FPdev). A high FPdev (>0.12) indicated NEB. Negative energy balance was associated with an increase in C16:0 (0.696±0.178) and C18:0 (0.467±0.093), which suggested mobilization of body fat reserves. Furthermore, NEB was associated with a decrease in odd chain C5:0 to C15:0 (-0.084±0.020), which might reflect a reduced allocation of C3 components to milk fat synthesis. A low FPdev indicated MFD (<-0.12) and was associated with a decrease in C16:0 (-0.681±0.255) and C18:0 (-0.128±0.135), and an increase in total unsaturated FA (0.523±0.227). Our study showed that both lactation stage and energy balance significantly contribute to variation in milk fat composition and alter the activity of different fatty acid pathways.

INTRODUCTION

The dairy industry has focused on improving specific health aspects of dairy products, and recent studies on milk fat have sought to identify factors affecting milk fat composition (German & Dillard, 2006). Milk fat composition changes with lactation stage. Karijord et al. (1982) found on a limited number of fatty acids (**FA**) in 3,000 Norwegian cows that proportions of C6:0 to C14:0 FA peaked around the third month of lactation. Proportions of C18 FA, on the other hand reached a minimum around the third month (Karijord et al., 1982; Syrstad et al., 1982; Palmquist & Beaulieu, 1993). More recent studies on approximately 20 cows showed similar trends for Holstein cows (Kay et al., 2005; Garnsworthy et al., 2006).

Milk FA originate from 4 major pathways: directly from diet, de novo synthesis in the mammary gland, formation in the rumen, by bio-hydrogenation or bacterial degradation, and release from body fat stores (Chilliard et al., 2000; MacGibbon & Taylor, 2006). Changes in milk fat composition over lactation imply shifts in the activity of these pathways, and have been related to changes in energy status of the cow (Van Knegsel et al., 2005). The fat-to-protein ratio (**FPratio**) was proposed as an indicator of energy status (Grieve et al., 1986; Friggens et al., 2007a). A high FPratio indicated low or negative energy balance (**NEB**), the combined result of elevated fat percentage and decreased milk and protein yield. Van Knegsel et al. (2005) suggested that during NEB, de novo synthesis of FA (C6:0 to C14:0) was reduced and body fat reserves addressed. A low FPratio, on the other hand, reflected milk fat depression (**MFD**), a dietary problem causing an energy imbalance and possibly acidosis in the rumen (Bauman & Griinari, 2003; Bauman et al., 2008; Plaizier et al., 2008), the result of decreased milk fat percentage with no effect on milk or protein yield. Bauman et al. (2008) suggested that during diet-induced MFD, levels of conjugated linoleic acid (**CLA**) were increased, which inhibited de novo synthesis resulting in decreased levels of saturated FA.

Studies on NEB and MFD traditionally are limited to small, nutritional studies that focus mainly on the first weeks after calving. Data on the effect of energy status on milk fat composition are scarce. The aim was to estimate the effect of lactation stage and energy status throughout lactation on detailed milk fat composition in a large population of first lactation Holstein-Friesian cows kept on commercial farms across feeding regimes.

MATERIALS & METHODS

Cows

This study was part of the Dutch Milk Genomics Initiative. The Dutch Milk Genomics Initiative was designed to have approximately 2,000 cows descending from a number of selected bulls; 50 young bulls aimed to have 20 daughters each and 5 proven bulls aimed to have 200 daughters each. The pedigree was the main reason for the choice of cows and farms, but at least 3 cows per farm were selected. Selected farms reflected housing and feeding regimes across the Netherlands.

Data were available for 1,933 cows from 398 commercial herds in the Netherlands to study milk fat composition. Each cow was between day 63 and 282 of first lactation and was over 87.5% Holstein-Friesian. Pedigree of each cow was available from the Dutch herd book (CRV, Arnhem, The Netherlands). All cows were housed indoors during the winter period when the milk samples were collected. Cows were milked twice daily, but 1 composite morning milk sample of 500 mL per cow was collected in February or March 2005. Sample bottles contained sodium azide (0.03 ww%) for preservation of milk samples.

Sample analysis

Milk fat (butter) was extracted from approximately 400 mL milk, with the remaining 100 mL milk kept at -40 ºC for other analyses. Fatty acid methyl esters were prepared from milk fat as described in ISO Standard 15884 (ISO-IDF, 2002a). The FA methyl esters were analyzed using gas chromatography according to the 100% FAME method (ISO-IDF, 2002b) with a 100 m polar column (Varian Fame Select CP 7420, Varian Inc., Palo AltoCA.) at the laboratory of the Netherlands Controlling Authority For Milk and Milk Products (Leusden, The Netherlands). The FA methyl ester chromatograms of the milk fat samples were compared to chromatograms of pure FA methyl ester standards to identify and quantify the FA. The FA were measured as weight-proportion of total fat weight.

The following FA and groups of FA were studied (Table 4.1): C4:0; even chain C6:0 to 12:0 (**C6-12**); odd-chain C5:0 to C15:0 (**C5-15**); C14:0; C16:0; C18:0; unsaturated C18 (**C18u**); *trans* C18 (**C18tr**); saturated FA (**SFA**); unsaturated FA (**UFA**); Branched FA; CLA *cis*-9,*trans*-11 and CLA *trans*-10,*cis*-12; and the unsaturation index. The unsaturation index was calculated as (*cis*-9 FA)/ (*cis*-9 FA + substrate). Percentages of fat and protein were determined from a 10 mL milk subsample by infrared

spectroscopy using a Fourier-transformed interferogram (MilkoScan FT 6000, Foss Electric, Hillerød, Denmark) at the certified laboratory of the Milk Control Station (Zutphen, The Netherlands).

Table 4.1. Overview of studied fatty acids and fatty acid groups

Trait	Fatty acids included
C4:0	
C6-12	C6:0, C8:0, C10:0, C12:0
C14:0	
C5-15	C5:0, C7:0, C9:0, C11:0, C13:0, C15:0
C16:0	
C18:0	
C18u	C18:1 *trans*-4-8, C18:1 *trans*-9, C18:1 *trans*-10, C18:1 *trans*-11, C18:1 *trans*-12, C18:1 *cis*-9, C18:1 *cis*-11, C18:1 *cis*-12, C18:2 *cis*-9,12, C18:3 *cis*-9,12,15, CLA *cis*-9,*trans*-11, CLA *trans*-10,*cis*-12
C18tr	C18:1 *trans*-4-8, C18:1 *trans*-9, C18:1 *trans*-10, C18:1 *trans*-11, C18:1 *trans*-12
Branched	C14:0 *iso*, C15:0 *iso*, C15:0 *ante-iso*, C16:0 *iso*, C17:0 *ante-iso*, C17:0 *iso*
Unsaturation index	(C10:1 + C12:1 + C14:1 + C16:1 + C17:1 + C18:1 cis-9 + CLA *cis*-9,*trans*-11) / (C10:0 + C10:1 + C12:0 + C12:1 + C14:0 + C14:1 + C16:0 + C16:1 + C17:0 + C17:1 + C18:0 + C18:1 *cis*-9 + C18:1 *trans*-11 + CLA cis-9, *trans*-11)
SFA	C4:0, C5:0, C6:0, C7:0, C8:0, C9:0, C10:0, C11:0, C12:0, C14:0 *iso*, C14:0, C15:0 *iso*, C15:0, C15:0 *ante-iso*, C16:0 *iso*, C16:0, C17:0 *iso*, C17:0, C17:0 *ante-iso*, C18:0, C19:0, C20:0
UFA	C10:1, C12:1, C14:1, C16:1 *cis*-9, C16:1 *trans*-9, C17:1, C18u, C20:3
CLA *cis*-9,*trans*-11	
CLA *trans*-10,*cis*-12	

Statistical analyses

Data analysis was performed using an animal model in AS-Reml (Gilmour et al., 2002). This analysis enabled a simultaneous estimation of the effect of lactation stage, while correcting for several other environmental and genetic factors. The base model was:

$$y_{ijklmn} = \mu + \text{lactation stage}_i + \text{scode}_j + \text{afc}_k + \text{herd}_l + A_m + e_{ijklm} \quad (1)$$

where y_{ijklmn} is the dependent variable corresponding to the observation of animal m in herd l, with scode j, age at first calving k, and lactation stage i; μ is the general mean; lactation stage_i is a fixed effect for 10 classes of lactation stage; scodej is a fixed effect accounting for a different genetic level between the groups of proven bull daughters and young bull daughters; afc_k is a fixed effect for 9 classes of age at first calving; herdl is a random effect defining groups of animals sampled in the same herd; A_m is the random additive genetic effect of animal m; and e_{ijklm} is the random residual effect.

To study the effect of lactation stage on milk fat composition, 10 classes of lactation stage were modeled: <85 DIM (n = 40); 85 to 104 (n = 83); 105 to 124 (n = 175); 125 to 144 (n = 280); 145 to 164 (n = 325); 165 to 184 (n = 337); 185 to 204 (n = 307); 205 to 224 (n = 247); 225 to 244 (n = 102); and >244 DIM (n = 37).

De Vries & Veerkamp (2000) proposed that a deviation from average fat-to-protein ratio (**FPratio**) might be a better variable for energy balance than simple FPratio, as it avoids that cows with high genetic merit for fat are always defined as having a high FPratio, and consequently NEB. Energy balance for each cow was estimated by **FPdev**, which is the deviation of the FPratio in the milk sample from the average FPratio over each cow's first lactation based on approximately 7 test-days; for each cow, FPdev = FPsample-FPlactation. FPdev reflects a deviation from an individual lactation average and can be seen as an indicator for temporary physiological changes specific to that cow. Three classes of FPdev were defined: <-0.12 (MFD, n = 174); between 0.12 and -0.12 (Normal, n = 1511); and >0.12 (NEB, n = 247). For 1 animal, data for FPdev were missing as fat and protein percentage data were not available. A FPdev of -0.12 corresponded to an average decrease in fat percentage of approximately 0.4%, which was described as threshold for MFD (Calus et al., 2005). A FPdev of 0.12 corresponded to an average FPratio of 1.35, which

approximated values for NEB reported in literature (Grieve et al., 1986; Heuer et al., 1999). To calculate the effect of FPdev on milk fat composition model [2] was used:

$$y_{ijklmn} = \mu + FPdev_i + scode_j + afc_k + herd_l + A_m + e_{ijklm} \qquad (2)$$

where,
$FPdev_i$ = fixed effect for 3 classes of FPdev: MFD, Normal, and NEB.

To study whether FPdev could explain the effects of lactation stage, 3 models were compared: the 'base model' [1] with lactation stage as fixed effect, the 'FPdev model [2]' without lactation stage, but with FPdev as fixed effect, and finally the 'full model [3]' with both lactation stage and FPdev as fixed effects. First, F values for lactation stage (model [1] and [3]) or FPdev (model [2] and [3]) were compared to assess changes in significance of lactation stage or FPdev. Secondly, the size of the estimated effects for lactation stage or FPdev were compared.

RESULTS

Average milk fat composition

The mean composition of milk fat of the 1,933 cows is in Table 4.2. Short chain FA (C4:0 and C6-12) averaged about 14 ww% of fat; medium chain FA (C14:0 and C16:0) were over 44 %, making them the largest contributor; and long chain FA (C18:0 and C18u) were about 30 %. Approximately 71 w/w% of the fat was analyzed as SFA, 26% as UFA, and 3% of the fat was unidentified, but was likely unsaturated. CLA *trans*-10,*cis*-12 occurred only in trace amounts and was below the detection limit in 1,784 out of 1,933 samples.

Changes in milk fat composition with lactation stage

The changes in FA proportions during lactation are shown in Figures 5.1 A to N. The first y-axis shows the ww% for that FA. The second y-axis ranges from -1 to +1 phenotypic SD, which facilitates comparison across FA. For most FA, cows in the last class of lactation stage (>244 DIM) had slightly deviating means and larger SE, which is likely due to the relative low number of 37 cows in this class. Lactation stage significantly affected all FA except for C5-15, Branched, and CLA *trans*-10,*cis*-12. C16:0 increased from day 80 to 150 of lactation, from 31.2 to 33.3 ww% and re-

mained relatively stable thereafter. C18:0 decreased from day 80 to 150 of lactation, from 9.8 to 8.4 ww%. SFA had a large change over lactation, increasing during the first half of lactation and then decreasing from 71.5 to 69.7 ww%. The patterns for C18u, UFA, and the unsaturation index were similar to one another and showed a minimum at mid-lactation, with UFA being 1.5 ww% lower in mid-lactation than in early and late lactation. The *trans* FA (C18:1 *trans*-4-8, C18:1 *trans*-9, C18:1 *trans*-10, C18:1 *trans*-11, and C18:1 *trans*-12 slightly decreased with lactation stage, except C18:1 *trans*-11, which increased in the second half of lactation (day 150 to 300).

Table 4.2. Mean[1]$_{SD}$, coefficient of variation (CV), and 5% and 95% quantiles for fatty acids measured in a test-day morning milk sample from 1,933 cows in first lactation.[2]

Trait	Mean	CV (%)	5% quantile	95% quantile
C4:0	$3.50_{0.27}$	8	3.05	3.96
C6-12	$10.74_{1.23}$	11	8.70	12.72
C14:0	$11.62_{0.91}$	8	10.03	13.01
C5-15	$1.47_{0.32}$	22	1.09	2.02
C16:0	$32.61_{2.81}$	9	28.07	37.11
C18:0	$8.73_{1.42}$	16	6.55	11.07
C18tr	$1.50_{0.43}$	29	1.00	2.14
C18u	$21.58_{2.43}$	11	18.19	26.16
Branched	$0.61_{0.10}$	16	0.48	0.80
Unsaturation index	$0.26_{0.03}$	12	0.22	0.31
UFA	$25.74_{2.51}$	10	22.24	30.39
SFA	$70.84_{2.74}$	4	65.99	74.75
CLA *cis*-9,*trans*-11	$0.39_{0.11}$	28	0.25	0.59
CLA *trans*-10,*cis*-12[3]	$0.00_{0.01}$	NA3	0.00	0.02

[1] For each fatty acid: mean = mean FA as ww proportion of the total fat fraction of 100%.
[2] Groups are defined in Table 4.1.
[3] CLA *trans*-10,*cis*-12 is 0.00 in 1,784 out of 1,933 samples; CV is not available.

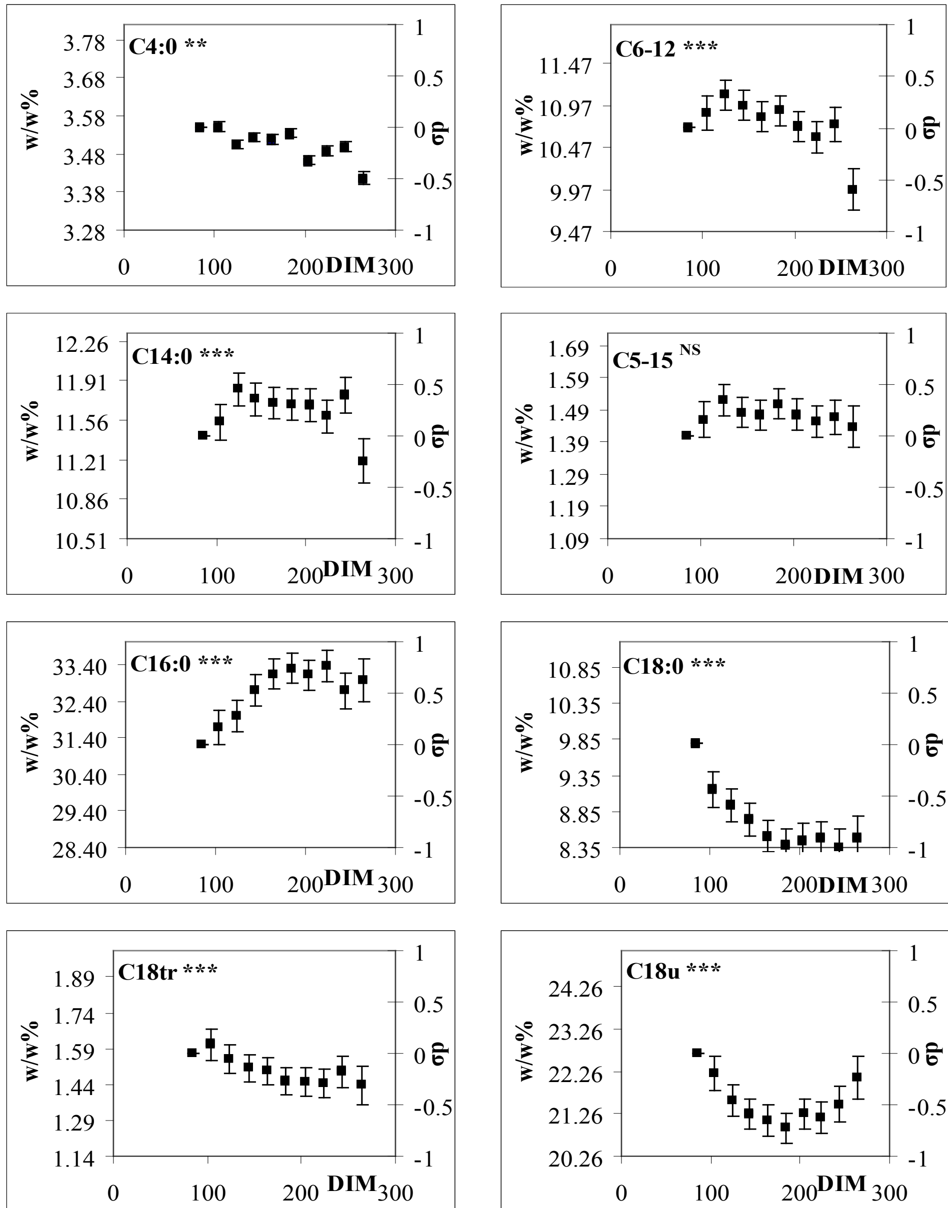


Figure 4.1 A–H. Changes in fatty acid proportions during lactation. X-axis shows days in lactation (DIM). Y-axis shows changes in fatty acid ww%. Second y-axis shows relative change in σP to facilitate comparison across graphs. * = P < 0.05, ** = P < 0.01, *** = P < 0.001, NS = non significant.

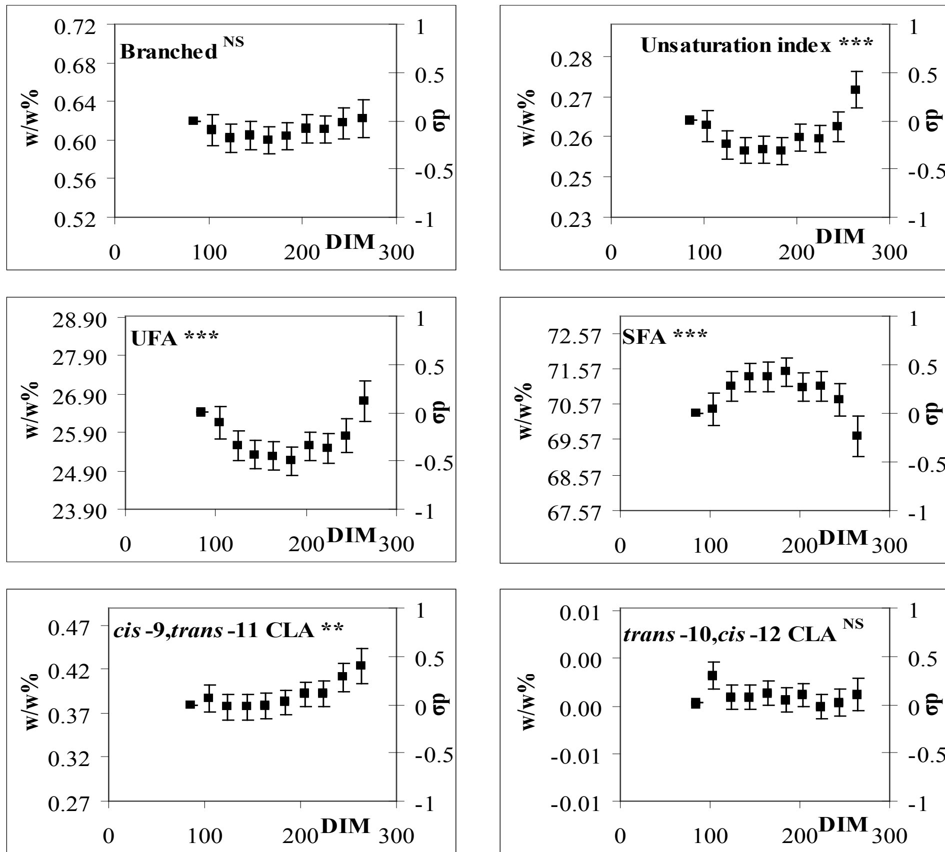


Figure 4.1 I–N. Changes in fatty acid proportions during lactation. X-axis shows days in lactation (DIM). Y-axis shows changes in fatty acid ww%. Second y-axis shows relative change in σP to facilitate comparison across graphs. * = P < 0.05, ** = P < 0.01, *** = P < 0.001, NS = non significant.

Changes in milk fat composition with energy status

Composition of milk production traits for each FPdev class is in Table 4.3. Compared to cows in the Normal group, cows with MFD (FPdev < -0.12) had a decrease in fat percentage of 0.6% without changes in protein or milk yield. Cows with NEB had an increase of 0.6 in fat percentage, no change in fat yield, and decreased protein (0.06 kg) and milk yield (1.2 kg) compared to Normal cows. Average lactation stage was 10 days lower in MFD individuals compared to Normal and NEB animals.

The milk fat composition for each FPdev class is in Table 4.4. Results

Table 4.3. Number of cows in FPdev class, average lactation stage, fat percentage, protein percentage, fat yield, protein yield, milk yield, FPratio, and FPdev, with SD in subscript.[1,2]

Trait	FPdev Class			P-value[5]
	<-0.12 MFD[3]	-0.12 to 0.12 Normal	>0.12 NEB[4]	
Cows (n)	174	1511	247	
Lactation stage Range (d)	66 - 249	63 - 282	66 - 263	
Lactation stage (d)	158_{43}	168_{40}	169_{42}	
FPdev	$-0.21_{0.12}$	$0.00_{0.06}$	$0.21_{0.16}$	<0.001
FPratio	$1.05_{0.19}$	$1.23_{0.13}$	$1.45_{0.21}$	<0.001
Fat %	$3.77_{0.78}$	$4.33_{0.60}$	$4.93_{0.82}$	<0.001
Protein %	$3.60_{0.31}$	$3.52_{0.29}$	$3.40_{0.29}$	<0.001
Fat yield (kg)	$0.51_{0.13}$	$0.58_{0.10}$	$0.60_{0.12}$	<0.001
Protein yield (kg)	$0.49_{0.09}$	$0.48_{0.09}$	$0.42_{0.10}$	<0.001
Milk yield (kg)	$13.6_{2.7}$	$13.6_{2.7}$	$12.4_{2.9}$	<0.001

[1] FPdev is calculated as $FP_{sample} - FP_{lactation}$, where $FP_{lactation}$ is based on approximately 7 test days.
[2] Yield traits are based on 1 morning sample.
[3] MFD = milk fat depression.
[4] NEB = negative energy balance.
[5] P-values are based on t-test.

for MFD (FPdev <-0.12) and NEB (FPdev >0.12) are shown as deviation from Normal (<-0.12 FPdev >0.12). Animals with MFD had 0.52 ww% more UFA as compared to normal cows, mainly because of an increase of 0.44 ww% in C18u. In addition, the unsaturation index was slightly increased, which might reflect increased desaturase activity. MFD animals had 0.62 ww% less SFA, mainly because of a decrease of 0.68 ww% in C16:0 and a decrease of 0.13 ww% in C18:0, whereas the amount of C6-12 was slightly increased (0.14 ww%).

Animals with NEB had less C6-12, C5-15 and C14:0 then normal cows, whereas C16:0 increased 0.70 ww% as compared to normal cows. C18:0 increased 0.47 ww% as compared to normal cows, however this effect was not significant. This resulted in an overall increase in SFA of 0.45 ww%, whereas UFA decreased by 0.37 ww%.

Table 4.4. Differences in milk fat composition during milk fat depression (MFD) and negative energy balance (NEB), relative to "Normal" individuals, with se, estimated on 1,933 cows in first lactation.[1,2]

Trait	FPdev		P-value
	<-0.12 (MFD)[3]	>0.12 (NEB)[4]	
C4:0	$-0.094_{0.025}^{ab}$	$0.031_{0.018}^{ab}$	<0.001
C6-12	$0.143_{0.109}^{b}$	$-0.301_{0.075}^{ab}$	<0.001
C14:0	$0.061_{0.084}^{b}$	$-0.326_{0.058}^{ab}$	<0.001
C5-15	$0.040_{0.029}^{b}$	$-0.084_{0.020}^{ab}$	<0.001
C16:0	$-0.681_{0.255}^{ab}$	$0.696_{0.178}^{ab}$	<0.001
C18:0	$-0.128_{0.135}$b	$0.467_{0.093}^{b}$	<0.001
C18tr	$0.072_{0.036}^{a}$	$0.006_{0.029}$	0.04
C18u	$0.436_{0.221}^{ab}$	$-0.194_{0.153}^{ab}$	0.02
Branched	$-0.001_{0.009}$	$-0.010_{0.006}$	0.20
Unsaturation index	$0.005_{0.003}^{ab}$	$-0.005_{0.002}^{ab}$	<0.001
UFA	$0.523_{0.227}^{ab}$	$-0.365_{0.156}^{ab}$	<0.001
SFA	$-0.617_{0.246}^{ab}$	$0.448_{0.170}^{ab}$	<0.001
CLA *cis*-9,*trans*-11	$0.009_{0.009}^{b}$	$-0.020_{0.006}^{ab}$	<0.001
CLA *trans*-10,*cis*-12	$0.002_{0.001}^{ab}$	$-0.001_{0.001}^{ab}$	<0.001

[a] = significantly different (P < 0.05) from "Normal" FPdev class.
[b] = significant difference between MFD and NEB individuals.
[1] FPdev is calculated as $FP_{sample} - FP_{lactation}$, where $FP_{lactation}$ is based on approximately 7 test days.
[2] Groups are defined in Table 4.1.
[3] MFD = milk fat depression.
[4] NEB = negative energy balance.

MFD cows had higher levels of C18tr then normal cows. NEB cows had 1.38 ww% more C16:0 than MFD cows and 1.07 ww% more SFA. Compared to MFD individuals, NEB animals had 0.60 ww% more C18:0 and 0.12 ww% less C5-15.

Effects of lactation stage and FPdev

F values for lactation stage and FPdev from the 3 different statistical models are in Table 4.5. In general, F values for lactation stage were slightly decreased (0.04 to 0.27 lower) in model [3] compared to model [1], but both significance and size of the estimates were not affected

Table 4.5. F values for lactation stage (Model 1), FPdev (Model 2), or both (Model 3) to compare significance of the effects of lactation stage and FPdev.[1]

Trait	Model 1	Model 2	Model 3	
	lactation stage	FPdev	lactation stage	FPdev
C4:0	2.45	12.93	2.56	13.59
C6-12	5.55	10.20	5.42	9.55
C14:0	3.28	16.83	3.02	16.13
C5-15	1.44	11.48	1.34	11.03
C16:0	9.05	15.03	8.82	14.36
C18:0	8.34	14.14	8.30	14.80
C18tr	3.01	3.15	2.78	2.41
C18u	5.98	4.20	5.71	3.88
Branched	0.93	1.59	0.95	1.75
Unsaturation index	4.06	7.33	4.09	7.90
UFA	4.37	7.67	4.29	7.87
SFA	4.29	9.36	4.25	9.68
CLA *cis*-9,*trans*-11	2.48	7.26	2.58	7.81
CLA *trans*-10,*cis*-12	1.55	7.19	1.51	6.91

[1] Groups are defined in Table 4.1.

(Table 4.5). This suggests that FPdev as energy indicator explained only a minor part of the lactation stage effect.

DISCUSSION

Milk fat composition significantly changed with lactation stage. The size of the effect was typically 0.5 to 1 phenotypic SD. Energy status, as reflected by FPdev, significantly affected milk fat composition. Changes in milk fat composition due to lactation stage cannot be explained by energy status.

In the current study, C16:0 changed a maximum of 2.1 ww% with lactation stage. This change was small compared to differences between

herds or sires (Stoop et al., 2008). For C16:0, the difference in estimated herd effect was 8.4 ww% between the lowest and the highest herd, implying a large effect of feeding differences on C16:0. Likewise, there was a difference in genetic merit for C16:0 between animals of 6.18 ww%, implying that genetic selection on C16:0 could lead to large changes in C16:0 proportion in milk. The same magnitude of difference was found for other FA, where herd (feed) and genetics explain much more variation than lactation stage or energy status.

Changes in milk fat composition with lactation stage

For C6:0 to C14:0, several studies reported an increase during the first 3 months of lactation and a decrease thereafter, whereas C18 FA follow an inverted pattern (Palmquist & Beaulieu, 1993; Kay et al., 2005; Garnsworthy et al., 2006). The current study found similar results. *Trans* FA slightly decreased with lactation stage. CLA *cis*-9,*trans*-11 increased with lactation stage. CLA *trans*-10,*cis*-12, C5-15, and the Branched FA did not significantly change with lactation stage.

Fat percentage linearly increases from day 100 to 300 from 4.24 to 5.02% (results not shown). Short chain FA (C4:0 to C14:0) seem to peak around day 100, whereas long chain FA of more than 16 C peak (or be at a minimum) around day 150 to 200. This suggests that these changes in milk fat composition over lactation are not explained by changes in overall fat percentage.

Changes in milk fat composition with energy status

Several studies have described FPratio as the milk trait best suited to predict energy status (Grieve et al., 1986; Friggens et al., 2007a; Van Knegsel et al., 2007). Nonetheless, accuracy was not very high compared to studies in which dry matter intake and body weight are known (Heuer et al., 2000, 2001; Friggens et al., 2007a). De Vries & Veerkamp (2000) suggested that a deviation of average cow FPratio might be more appropriate to use, as it adjusts FPratio for genetic merit of individual cows. From Table 4.3 it follows that FPdev classified MFD and NEB as expected: the MFD class showed a significant decrease in fat percentage with no changes in other milk production traits, and the NEB class showed an increased fat percentage, and decreased protein and milk yield. Still, a cow that remains in NEB throughout lactation would be wrongly classified as a Normal cow. This would result in smaller differences between the groups of Normal and NEB cows, which would result in underestimation

of the actual effect of energy balance on milk fat composition. The same situation applies to cows that show MFD throughout lactation. The results showed that 13% of animals were classified as NEB and 9% were classified as MFD.

FPdev as an energy indicator could not explain the effect of lactation stage. The largest changes in energy status and most severe NEB were expected in the first few weeks of lactation, mainly in animals of higher parity (Friggens et al., 2007b). In the current study, animals classified as NEB were not limited to early lactation. Possible reasons why these animals had elevated FPratio, compared to their lactation average, could be changes in or problems with feed (Hermansen, 1995), pregnancy, overall body condition, and health (Heuer et al., 1999). Available data on feed, insemination dates, BCS, and fertility were too limited to associate causes for the observed NEB.

FPdev significantly affected milk fat composition. MFD (low FPdev) led to increased levels of *trans* FA (C18tr), CLA *cis*-9, *trans*-11, and CLA trans-10, cis-12 (Table 4.4), which might reflect an altered or incomplete bio-hydrogenation activity in the rumen (Bauman & Griinari, 2003; Bauman et al., 2008; Plaizier et al., 2008). According to Bauman & Griinari (2003) this altered rumen biohydrogenation would lead to more C18:1 trans intermediates, particularly C18:1 *trans*-10, which can then be converted to CLA *trans*-10, *cis*-12, possibly explaining the increased levels of C18tr and CLA *trans*-10,*cis*-12 in the current study. It was expected that the increase in long chain FA in MFD cows would inhibit de novo synthesis of C6:0 to C16:0 FA. Overall fat percentage and C16:0 were decreased, but the proportion of C6:0 to C14:0 were not different from Normal cows. Palmquist and Beaulieu (1993) suggested that inhibition by long chain FA increased from C6:0 to C16:0, as longer chains require more acetyl unit addition via malonyl CoA, which might explain why the inhibiting effect was only observed in C16:0.

NEB (high FPdev) led to higher proportions of SFA, mainly C16:0 and some C18:0, and a decrease in UFA (Table 4.4). This result was in agreement with Stoop et al. (2008) and Karijord et al. (1982), who found positive genetic correlations between fat percentage and SFA and negative genetic correlations between fat percentage and UFA. Additional calculations were performed to study to what extent the effects of high FPdev were due to an increase in fat percentage. Fat percentage was added as a covariate to model [2]. Changes in fat percentage explained only part of the effect for NEB, depending on the size of the genetic correlation.

Clarke (1993) suggested that most fat deposition in body fat reserves in production animals was C16:0 and C18:0. During NEB, cows mobilize these body fat reserves, which consequently leads to increased proportions of C16:0 and C18:0 in milk, as we saw in the current study. NEB resulted in less C5-15. These FA can be synthesized from C3 components. During NEB, C3 components were redirected to the increased production of, among others, lactose. This redirection results in a relative C3 shortage, explaining the decrease in C5-15 (Van Knegsel et al., 2005). Our result showed that a more negative energy status, reflected by high FPdev, led to less favorable milk fat composition: C16:0 was relatively high, while the unsaturated C18 FA were relatively low.

CONCLUSION

Moderate changes 0.5 to 1 phenotypic SD were found in milk fat composition over lactation stage of for most FA. Effect of lactation stage could not be explained by energy status. Cows with MFD generally had increased levels of C18tr and CLA, which might point to physiological disturbances, incomplete bio-hydrogenation in the rumen, and possible acidosis. Cows with NEB generally had decreased levels of C5-15 and increased levels of C16:0 and C18:0, indicating possible energy shortage and reallocation of C3 components in the FA de novo synthesis, as well as body fat reserve mobilization. Our study showed that both lactation stage and energy balance significantly contribute to variation in milk fat composition and alter the activity of different fatty acid pathways.

ACKNOWLEDGMENTS

This study was part of the Dutch Milk Genomics Initiative, funded by Wageningen University, NZO (Dutch Dairy Organisation), breeding company CRV, and technology foundation STW. The authors thank the owners of the herds, the Milk Control Station (Zutphen, The Netherlands), and CRV (Arnhem, The Netherlands) for their help in collecting the data, and Nicolas Friggens for his highly appreciated comments on the manuscript.

REFERENCES

Bauman, D.E. and J.M. Griinari. 2003. Nutritional regulation of milk fat synthesis. Annu. Rev. Nutr. 23: 203-227.

Bauman, D.E., J.W. Perfield II, K.J. Harvatine, and L.H. Baumgard. 2008. Regulation of Fat Synthesis by Conjugated Linoleic Acid: Lactation and the Ruminant Model. J. Nutr. Suppl. 138:403-409.

Calus, M.P.L., M.J. Carrick, R.F. Veerkamp, and M.E. Goddard. 2005. Estimation of Genetic Parameters for Milk Fat Depression in dairy cattle. J. Dairy Sci. 88: 1166-1177.

Chilliard, Y., A. Ferlay, R.M. Mansbridge, and M. Doreau. 2000. Ruminant fat plasticity: nutritional control of saturated, polyunsaturated, trans and conjugated FA. Ann. Zootech. 49: 181-205.

Clarke, S.D. 1993. Regulation of fatty Acid Synthase Gene Expression: An approach for reducing fat accumulation. J. Anim. Sci. 71: 1957-1965.

De Vries, M.J. and R.F. Veerkamp. 2000. Energy balance of dairy cattle in relation to milk production variables and fertility. J. Dairy Sci. 83: 62-69.

Friggens, N.C., C. Ridder, and P. Lovendahl. 2007a. On the use of milk composition measures to predict energy balance in dairy cows. J. Dairy Sci. 90: 5453-5467.

Friggens, N.C., P. Berg, P. Theilgaard, I.R. Korsgaard, K.L. Ingvartsen, P. Lovendahl, and J. Jensen. 2007b. Breed and parity effects on energy balance profiles through lactation: evidence of genetically driven body energy change. J. Dairy Sci. 90: 5291-5305.

Garnsworthy, P.C., L.L. Masson, A.L. Lock, and T.T. Mottram. 2006. Variation of milk citrate with stage of lactation and de novo fatty acid synthesis in dairy cows. J. Dairy Sci. 89: 1604-1612.

German, J.B., and C.J. Dillard. 2006. Composition, Structure and Absorption of milk lipids: A source of energy, fat-soluble nutrients and bioactive molecules. Crit. Rev. in Food Sci. and Nutr. 46: 57-92.

Gilmour, A.R., B.J. Gogel, B.R. Cullis, S.J. Welham, and R. Thompson. 2002. ASReml User Guide Release 1.0, VSN International Ltd, Hemel Hempstead, HP11ES, UK.

Grieve, D.G., S. Korver, Y.S. Rijpkema, and G. Hof. 1986. Relationship between milk composition and some nutritional parameters in early lactation. Livest. Prod. Sci. 14: 239-254.

Hermansen, J.E. 1995. Prediction of milk fattys acid profile in dairy cows fed dietary fat differing in fatty acid composition. J. Dairy Sci. 78:

872-879.

Heuer, C., Y.H. Schukken, and P. Dobbelaar. 1999. Postpartum body condition score and results from the first test day milk as predictors of disease, fertility, yield, and culling in commercial dairy herds. J. Dairy Sci. 82: 295-304.

Heuer, C., W.M. van Straalen, Y.H. Schukken, A. Dirkzwager, and J.P.T.M. Noordhuizen. 2000. Prediction of energy balance in a high yielding dairy herd in early lactation: Model development and precision. Livest. Prod. Sci. 65: 91-105.

Heuer, C., W.M. van Straalen, Y.H. Schukken, A. Dirkzwager, and J.P.T.M. Noordhuizen. 2001. Prediction of energy balance in high yielding dairy cows with test-day information. J. Dairy Sci. 84: 471-481.

ISO 15884. 2002a. Milk-fat – Preparation of fatty acid methyl esters. ISO 15884-IDF 182, International Dairy Federation, Brussels, Belgium.

ISO 15885. 2002b. Milk-fat – Determination of the fatty acid composition by gas-liquid chromatography. ISO 15885-IDF 184, International Dairy Federation, Brussels, Belgium.

Karijord, O., N. Standal, and O. Syrstad. 1982. Sources of variation in composition of milk fat. Z. Tierz. Zcht. Biol. 99: 81-93.

Kay, J.K., W.J. Weber, C.E. Moore, D.E. Bauman, L.B. Hansen, H. Chester-Jones, B.A. Crooker, and L.H. Baumgard. 2005. Effects of week of lactation and genetic selection for milk yield on milk fatty acid composition in Holstein cows. J. Dairy Sci. 88: 3886-3893.

MacGibbon, A.K.H., and M.W. Taylor. 2006. Composition and Structure of Bovine Milk Lipids. Pages 1-42 in Advanced Dairy Chemistry. Vol. 2: Lipids, 3rd edition. Edited by P.F. Fox and P.L.H. McSweeney, Springer, New York.

Palmquist, D.L. and A.D. Beaulieu. 1993. Feed and animal factors influencing milk fat composition. J. Dairy Sci. 76: 1753-1771.

Plaizier, J.C., D.O. Krause, G.N. Gozho, B.W. McBride. 2008. Subacute ruminal acidosis in dairy cows: The physiological causes, incidence and consequences. The Veterinary Journal 176: 21-31.

Stoop, W.M., J.A.M. van Arendonk, J.M.L. Heck, H.J.F. van Valenberg, and H. Bovenhuis. 2008. Genetic parameters for major milk FA and milk production traits of Dutch Holstein Friesians. J. Dairy Sci. 91: 385-394.

Syrstad, O., N. Standal, and O. Karijord. 1982. Concentration of various FA in milk. Z. Tierzuchtg. Zuchtgsbiol. 99: 94-100.

Van Knegsel, A.T.M., H. van den Brand, J. Dijkstra, S. Tamminga, and B. Kemp. 2005. Effect of dietary energy source on energy balance, pro-

duction, metabolic disorders and reproduction in lactating dairy cattle –review-. Reprod. Nutr. Dev. 45: 665-688.

Van Knegsel, A.T.M., H. van den Brand, J. Dijkstra, W.M. van Straalen, M.J.W. Heetkamp, S. Tamminga, and B. Kemp. 2007. Dietary energy source in dairy cows in early lactation: energy partitioning and milk composition. J. Dairy Sci. 90: 1467-1476.

Chapter 5

Genetic correlations between winter and summer milk fat composition

W.M. Stoop, H. Bovenhuis, and J.A.M. van Arendonk

(concept)

ABSTRACT

Aim of our study was to estimate the genetic correlation between winter and summer milk fat composition to infer whether milk fat composition in winter and summer is genetically the same trait, and to evaluate the effect of season on the estimated heritabilities. Two milk samples, one from winter and one from summer were available from 1,689 cows on 378 commercial herds in the Netherlands, and were analyzed for milk fat percentage and for milk fat composition. All FA changed over season. The largest change was a 3.39 ww% reduction in C16:0 from winter to summer. Standard deviations of FA in summer were on average 20% larger than in winter, implying more variation in FA proportions among cows in summer. Heritabilities were very comparable across season. Variation due to herd increased approximately 50% in summer, compared to winter. The increase in variation in summer compared to winter thus seemed for a large part due to increased herd variation. The phenotypic correlations ranged from 0.38 (C18tr) to 0.64 (Fat%), which indicated that observed phenotypes of a cow were moderately repeatable between winter and summer. Genetic correlations were high, ranging from 0.78 (C16:0) to 0.98 (C5-15). An exception was the genetic correlation of 0.58 for C18tr. The results indicated that despite the phenotypic differences, milk fat composition in winter and summer can be considered as the same genetic trait. This offers opportunities for genetic selection on altered milk fat composition.

INTRODUCTION

Milk fat contains many nutrients necessary for humans, including fat soluble vitamins, energy and bio-active lipids (German & Dillard, 2006). During the last decennia, however, milk fat consumption has also been associated with negative health effects (Bitman et al., 1995; Jensen, 2002). Cow-milk fat contains a relatively low proportion of unsaturated fatty acids, and a relative high proportion of LDL-cholesterol-increasing fatty acids (**FA**), mainly C14:0 and C16:0 (German & Dillard, 2006). Both C14:0 and C16:0 have been associated with increased levels of cholesterol and increased risk of cardio-vascular disease (Maijala, 2000; Jensen, 2002). Possibilities to change milk fat composition are, therefore, of interest.

A previous study by Stoop et al. (2008) reported heritabilities and genetic correlations for milk fat composition, based on a winter sample of 1,918 first lactation cows. Several studies have reported variation in milk fat composition over the year (Karijord et al., 1982; Palmquist & Beaulieu, 1993; Heck et al., 2009). In general it seems that proportions of short chain FA, C6:0 to C16:0, are increased during the winter, whereas long chain FA, C18:1 and C18:3, are 1.2 to 2 times higher in summer than in winter. Palmquist & Beaulieu (1993) suggested that all seasonal effects on milk fat composition are presumably caused by dietary changes between seasons, with a large effect of fresh grass availability.

In the Netherlands considerable dietary differences exist between winter and summer. Consequently, different genes might be involved in milk fat synthesis in summer, as compared to winter.

Aim of our study was to estimate the genetic correlation between winter and summer milk fat composition to infer whether milk fat composition in winter and summer is genetically the same trait, and to evaluate the effect of season on the estimated heritabilities.

MATERIALS & METHODS

Animals and milk samples

Data were available from 1,689 cows on 378 commercial herds in the Netherlands that had both a winter and summer record. Of those cows, 734 cows were sired by 50 young bulls, 792 cows by 5 proven bulls, and 163 cows by 44 other proven bulls to have at least 3 cows per farm. Each cow was over 87.5% Holstein-Friesian. Pedigree information was available

from the Dutch Studbook (CRV, Arnhem, The Netherlands) and included a total of 26,300 individuals.

Two milk samples, each of 500 mL, were available from each cow. The first milk sample was collected between February and March 2005 ("winter") and the second milk sample was collected between May and June 2005 ("summer"). Cows were milked twice daily, but only morning milk was collected for the study to ensure quality of the samples: milk was cooled at 4ºC within 3 hours after sampling and transported to the lab the same morning. Sample bottles contained sodium azide (0.03 ww%) for conservation.

Analysis of milk samples

Milk fat (butter) was extracted from about 400 mL milk, keeping the remaining 100 mL for other analyses. Fatty acid methyl esters were prepared from milk fat as described in ISO Standard 15884 (ISO-IDF, 2002a). The methyl esters were analyzed using gas chromatography according to the 100% FAME method (ISO-IDF, 2002b) with a 100 m polar column (Varian Fame Select CP 7420, Varian Inc., U.S.A.) at the laboratory of The Netherlands Controling Authority For Milk and Milk Products (Leusden, The Netherlands). The FA were identified and quantified by comparing the methyl ester chromatograms of the milk-fat samples with the chromatograms of pure fatty acid methyl ester standards, and were measured as weight-proportion of total fat weight. In this study results will be shown of the following FA and FA groups: C4:0, even chain C6:0 to C12:0 (**C6-12**), uneven chain C5:0 to C15:0 (**C5-15**), C14:0, C16:0, C18:0, trans C18 FA (**C18tr**), saturated FA (**SFA**: C4:0-C18:0 and C20:0), the group unsaturated FA (**UFA**: mono- and polyunsaturated C10-C18), ratio SFA/UFA, C18:2 *cis*-9,12, C18:3 *cis*-9,12,15, CLA *cis*-9,*trans*-11, and the total unsaturation index (**Totalindex**). The totalindex is calculated as (C10:1 + C12:1 + C14:1 + C16:1 + C18:1 *cis*-9 + CLA *cis*-9,*trans*-11)/ (C10:0 + C10:1 + C12:0 + C12:1 + C14:0 + C14:1 + C16:0 + C16:1 + C18:0 + C18:1 *cis*-9 + C18:1 *trans*-11 + CLA *cis*-9,*trans*-11). The SFA plus UFA sum to 97 ww% of total fat, the remaining 3 ww% was unidentified.

Percentage of fat was determined from a 10 mL milk subsample by infrared spectroscopy using a Fourier-transformed interferogram (Milko-Scan FT 6000, Foss Electric, Denmark) at the certified laboratory of the Milk Control Station (Zutphen, The Netherlands). In total 3,378 records, 2 from each of 1,689 cows, were analyzed for fat percentage and fat composition.

Statistical analyses

A bivariate analysis was used to estimate heritability in both winter and summer, variation due to herd in winter and summer, and correlations between winter and summer milk fat composition. Variance components and genetic parameters were estimated with an animal model in AS-Reml (Gilmour et al. 2002), following the model used in analysing the winter sample by Stoop et al. (2008):

$$y_{ijklmn} = \mu + b_1*dim_i + b_2*e^{-0.05*dim} + b_3*afc_j + b_4*afc_j^2 + season_k + scode_l + herd_m + A_n + e_{ijklmn} \quad (1)$$

where
y_{ijklmn} is the dependent variable; μ is the general mean; dim_i is a covariate for days in milk, modeled with a Wilmink curve (Wilmink, 1987); afc_j is a covariate describing the effect of age at first calving; $season_k$ is a fixed effect with 3 classes for season of calving; summer (June-August 2004), autumn (September-November 2004) and winter (December 2004-February 2005); $scode_l$ is a fixed effect accounting for possible differences between the groups of proven bull daughters and young bull daughters; $herd_m$ is a random effect defining groups of animals sampled in the same herd; A_n is the random additive genetic effect of animal n; e_{ijklmn} is the random residual effect.

Heritabilities for winter and summer were based on variance components resulting from model (1), and were estimated as:

$$h^2 = \frac{\sigma^2_A}{\sigma^2_A + \sigma^2_e} \quad (2)$$

where σ^2_A = additive genetic variation and σ^2_e = residual variation.

Proportion of variance due to herd reflects the relative importance of herd effects such as feed, hygiene, and management, and were estimated as:

$$h_{herd} = \frac{\sigma^2_{herd}}{\sigma^2_A + \sigma^2_{herd} + \sigma^2_e} \quad (3)$$

where σ^2_{herd} = herd variation.

RESULTS

Mean milk fat composition

Phenotypic means for FA in winter and summer are shown in Table 5.1. All FA changed over season, except for C4:0. Part of the observed phenotypic changes may be due to other effects than season, such as a difference in lactation stage. Average lactation stage was 164 days for the winter sample, and 249 days for the summer sample.

Fat percentage was approximately 4.3% in both winter and summer. The largest change was a 3.39 ww% reduction in C16:0 from winter to summer. C18tr, CLA *cis*-9,*trans*-11, and C18:3 *cis*-9,12,15 also showed large differences between winter and summer. Proportions of C6:0 to C16:0 were lower in summer than in winter, whereas proportions of long chain FA (>18C), with the exception of C18:2 *cis*-9,12, were higher in summer. Standard deviations of FA in summer were on average 20% larger than in winter, implying more variation in FA proportions among cows in summer.

Heritabilities and effect of herd

Heritabilities and variation due to herd for FA in winter and summer are shown in Table 5.2. Heritabilities were very comparable across season. There was, however, a decrease in heritability for C6-12 from 0.67 to 0.51 and for CLA *cis*-9,*trans*-11 from 0.44 to 0.27, and an increase in heritability for C18:2 *cis*-9,12 from 0.26 to 0.39. Heritabilities for short and medium chain FA were higher (0.29 to 0.63) than for long chain FA (0.20 to 0.33).

Variation due to herd (Table 5.2) was much higher in summer than in winter. For all traits, except C6-12 and C18:3 *cis*-9,12,15, variation due to herd increased approximately 50% in summer, compared to winter. The increase in variation in summer compared to winter (Table 5.1, SD and Table 5.4, σ_P^2) thus seemed for a large part due to increased herd variation (Table 5.4). For short and medium chain FA, residual variation seemed slightly increased in summer, compared to winter, whereas the genetic variation was similar for both seasons, explaining the small decrease (on average 0.02) in heritabilities (Table 5.2) observed for these traits in summer. For groups of FA and long chain FA, however, both residual and genetic variation show an increase in summer, leading to slightly increased (on average 0.03) heritabilities when compared to winter samples.

Table 5.1. Phenotypic means for fatty acids and fatty acid groups in winter and summer, based on 3,378 records from 1,689 cows.

Trait	Winter		Summer		Difference
	Mean	SD	Mean	SD	Summer-Winter
Fat %	4.35	0.69	4.27	0.73	-0.08
C4:0	3.50	0.27	3.52	0.35	0.02
C6-12	10.76	1.22	10.15	1.37	-0.61
C14:0	11.63	0.91	11.16	1.05	-0.47
C16:0	32.58	2.83	29.19	3.51	-3.39
C18:0	8.71	1.40	9.88	1.78	1.17
C5-15	1.47	0.33	1.33	0.29	-0.14
C18tr	1.50	0.43	2.14	0.72	0.64
SFA	71.03	2.71	68.08	3.86	-2.95
UFA	26.00	2.51	28.85	3.47	2.85
SFA/UFA	2.77	0.35	2.41	0.42	-0.36
Total index	0.27	0.03	0.30	0.04	0.03
CLA *cis*-9, *trans*-11	0.40	0.11	0.56	0.28	0.16
C18:2 *cis*-9,12	1.21	0.29	1.12	0.25	-0.09
C18:3 *cis*-9,12,15	0.41	0.11	0.50	0.16	0.09

Correlations between milk fat composition in winter and summer

The phenotypic, genetic, herd, and residual correlations between winter and summer FA proportions are shown in Table 5.3. The phenotypic correlations ranged from 0.38 (C18tr) to 0.64 (Fat%), which indicated that observed phenotypes of a cow were moderately repeatable between winter and summer. Genetic correlations were high, ranging from 0.78 (C16:0) to 0.98 (C5-15). An exception was the genetic correlation of 0.58 for C18tr. In general milk fat composition in winter and summer seemed genetically similar traits. Herd correlations were below 0.44, except for a herd correlation of 0.76 for C18:2 *cis*-9,12, indicating that herd effects in winter and summer are very different.

DISCUSSION

In this study we quantified the difference in milk fat composition between winter and summer milk, evaluated the effect of season on the estimated heritabilities, and - based on the genetic correlations – concluded that there is no evidence that there is a genetic difference in milk fat composition between winter and summer.

Table 5.2. Heritability, and herd variation in winter and summer samples, based on 3,378 records of 1,689 cows. SE in subscripts.

Trait	Winter		Summer	
	h^2	herd	h^2	herd
Fat %	$0.51_{0.10}$	$0.07_{0.02}$	$0.60_{0.12}$	$0.12_{0.02}$
C4:0	$0.41_{0.10}$	$0.16_{0.02}$	$0.36_{0.09}$	$0.25_{0.03}$
C6-12	$0.67_{0.12}$	$0.27_{0.03}$	$0.51_{0.11}$	$0.23_{0.02}$
C14:0	$0.60_{0.11}$	$0.19_{0.02}$	$0.60_{0.11}$	$0.34_{0.03}$
C16:0	$0.38_{0.11}$	$0.31_{0.03}$	$0.36_{0.10}$	$0.53_{0.03}$
C18:0	$0.23_{0.08}$	$0.22_{0.03}$	$0.18_{0.07}$	$0.31_{0.03}$
C5-15	$0.28_{0.08}$	$0.28_{0.03}$	$0.30_{0.09}$	$0.31_{0.03}$
C18tr	$0.23_{0.09}$	$0.48_{0.03}$	$0.29_{0.10}$	$0.66_{0.02}$
SFA	$0.30_{0.09}$	$0.30_{0.03}$	$0.31_{0.10}$	$0.45_{0.03}$
UFA	$0.27_{0.09}$	$0.30_{0.03}$	$0.30_{0.10}$	$0.43_{0.03}$
SFA/UFA	$0.28_{0.09}$	$0.31_{0.03}$	$0.29_{0.09}$	$0.45_{0.03}$
Total index	$0.27_{0.09}$	$0.26_{0.03}$	$0.30_{0.09}$	$0.36_{0.03}$
CLA *cis*-9,*trans*-11	$0.44_{0.11}$	$0.51_{0.03}$	$0.27_{0.10}$	$0.61_{0.02}$
C18:2 *cis*-9,12	$0.26_{0.09}$	$0.52_{0.03}$	$0.39_{0.10}$	$0.59_{0.02}$
C18:3 *cis*-9,12,15	$0.24_{0.09}$	$0.64_{0.02}$	$0.22_{0.08}$	$0.65_{0.02}$

Summer milk fat contained a larger proportion of unsaturated and long chain FA, and a lower proportion of short chain FA (Table 5.1), which was in concordance with literature findings (Palmquist & Beaulieu, 1993; Soyeurt et al., 2008; Heck et al., 2009). The observed effects in our study might be partly due to changes in lactation stage, as cows in summer were on average 85 days further in lactation than in winter (249 versus 164 days). Stoop et al. (2009) reported moderate effects of lactation stage in winter samples. However, where C16:0 decreases from winter to summer (Table 5.1), Stoop et al. (2009) reported an increase in C16:0 with increasing lactation stage. Likewise C18tr increases from winter to summer (Table 5.1), but tended to decrease with lactation stage (Stoop et al., 2009). Therefore, observed changes in this study were likely not the effect of lactation stage. Several studies have suggested that seasonal effects are mainly caused by differences in herd management, mainly dietary changes (Palmquist & Bealieu, 1993; Chilliard et al., 2001). Elgers-

Table 5.3. Phenotypic, genetic, herd, and residual correlation between winter and summer samples, based on 3,378 records of 1,689 cows. SE in subscripts.

Trait	r_P	r_A	r_{herd}	r_E
Fat %	$0.64_{0.02}$	$0.97_{0.00}$	$0.22_{0.14}$	$0.60_{0.02}$
C4:0	$0.51_{0.02}$	$0.95_{0.05}$	$0.29_{0.09}$	$0.23_{0.09}$
C6-12	$0.55_{0.02}$	$0.94_{0.04}$	$0.39_{0.07}$	$0.01_{0.21}$
C14:0	$0.57_{0.02}$	$0.93_{0.04}$	$0.36_{0.07}$	$0.03_{0.21}$
C16:0	$0.57_{0.02}$	$0.78_{0.11}$	$0.20_{0.07}$	$0.44_{0.08}$
C18:0	$0.48_{0.02}$	$0.84_{0.13}$	$0.31_{0.08}$	$0.39_{0.05}$
C5-15	$0.56_{0.02}$	$0.98_{0.04}$	$0.42_{0.06}$	$0.39_{0.06}$
C18tr	$0.38_{0.03}$	$0.58_{0.20}$	$0.28_{0.06}$	$0.31_{0.07}$
SFA	$0.51_{0.02}$	$0.79_{0.12}$	$0.22_{0.07}$	$0.38_{0.07}$
UFA	$0.50_{0.02}$	$0.79_{0.12}$	$0.19_{0.07}$	$0.38_{0.06}$
SFA/UFA	$0.51_{0.02}$	$0.78_{0.13}$	$0.17_{0.07}$	$0.41_{0.06}$
Total index	$0.51_{0.02}$	$0.83_{0.11}$	$0.19_{0.07}$	$0.39_{0.06}$
CLA *cis*-9,*trans*-11	$0.42_{0.03}$	$0.82_{0.11}$	$0.29_{0.06}$	$0.21_{0.09}$
C18:2 *cis*-9,12	$0.60_{0.02}$	$0.96_{0.06}$	$0.76_{0.03}$	$0.43_{0.06}$
C18:3 *cis*-9,12,15	$0.48_{0.02}$	$0.83_{0.12}$	$0.40_{0.05}$	$0.37_{0.06}$

ma et al. (2006) showed that the availability of fresh grass leads to milk fat with more polyunsaturated FA and more CLA *cis*-9,*trans*-11. In the current study, data on grazing opportunity was collected from about 90% of the farms using a query. In the current study all cows (100%) were kept inside in the winter season, whereas approximately 50% of cows had the ability for grazing in the summer season (3.5 to 24 hours/ day). Comparing, within the summer samples, grazing versus non-grazing cows, indeed the grazing cows had approximately 4 ww% more UFA than non-grazing cows. However, non-grazing cows in summer also had approximately 2 ww% more UFA than non-grazing cows in winter , indicating that grazing was not the only factor causing the seasonal effects observed. No information was available on possible feeding of freshly mowed grass to stabled cows in summer, which might affect the observed result. Variation in milk fat composition was larger in summer than in winter samples, mainly due to increased herd variation. One of the objectives of this study was to study whether this increase in variance would affect the genetic parameters. If all changes in observed variation are due to herd, then no effect on the heritabilities is expected. If part of this variation is due to other reasons, it might lead to increased residual variation and decreased heritability estimates. And if there is a seasonal effect on cow metabolism,

Table 5.4. Phenotypic, Residual, additive genetic and herd variation in winter and summer, based on 3,378 records of 1,689 cows.

Trait	Winter				Summer			
	V_p	V_e	V_a	V_{herd}	V_p	V_e	V_a	V_{herd}
Fat %	0.508	0.231	0.239	0.038	0.527	0.201	0.297	0.069
C4:0	0.077	0.038	0.026	0.013	0.122	0.059	0.033	0.030
C6-12	1.577	0.380	0.767	0.430	1.896	0.722	0.738	0.436
C14:0	0.868	0.286	0.420	0.162	1.113	0.294	0.442	0.377
C16:0	8.151	3.530	2.135	2.486	12.080	3.660	2.055	6.365
C18:0	1.910	1.140	0.347	0.423	3.074	1.722	0.386	0.966
C5-15	0.104	0.054	0.021	0.029	0.081	0.039	0.017	0.025
C18tr	0.216	0.086	0.026	0.104	0.604	0.145	0.058	0.401
SFA	7.315	3.606	1.520	2.189	14.179	5.347	2.408	6.424
UFA	6.239	3.175	1.174	1.890	11.483	4.556	1.988	4.939
SFA/UFA	0.125	0.062	0.024	0.039	0.168	0.066	0.027	0.075
Total index	0.000	0.000	0.000	0.000	0.001	0.001	0.000	0.000
CLA *cis*-9,trans-11	0.012	0.003	0.003	0.006	0.076	0.022	0.008	0.046
C18:2 *cis*-9,12	0.079	0.028	0.010	0.041	0.064	0.016	0.010	0.038
C18:3 *cis*-9,12,15	0.012	0.003	0.001	0.008	0.025	0.007	0.002	0.016

the increased variation may be partly due to changes in genetic variation. Several studies have shown changes in genetic variation of mastitis, body condition score and body mass over lactation and season (Lund et al., 1998; Reale et al., 1999; Ravagnolo & Misztal, 2000; Mao et al., 2004). For milk fat composition heritability estimates slightly differed between winter and summer. Heritabilities for short and medium chain FA tended to be slightly lower in winter, which was mainly due to increased residual variation, especially for C6-12 (Table 5.4). Groups of FA and long chain FA showed an increase in genetic variation in summer. This might be (in part) related to the availability of fresh grass – or at least dietary changes - , which possibly triggers additional fat metabolism pathways that were less active during winter, thus increasing genetic variation.

The main objective of this paper was to study the phenotypic and genetic correlations between winter and summer FA proportions. Although there was a significant increase in UFA and decrease in short chain FA, there was much individual variation between cows and between farms, which resulted in moderate phenotypic correlations of approximately 0.5. Herds that showed, for example, high levels of UFA in winter were not necessarily the herds that had high UFA levels in summer, which resulted in low herd correlations. This result implied that herd management is not constant over the year. It is currently not possible for farmers to monitor milk fat composition in their herds. Monitoring milk fat composition on a regular basis would help to identify causes for changes in milk fat composition between seasons. This identification of factors could result in management tools to potentially improve milk fat composition throughout the year.

Genetic correlations were generally high, 0.78 to 0.98, which indicated that there was no significant alteration in fat metabolism between winter and summer, and that milk fat composition in winter and summer can be considered as the same genetic trait.

The results of this paper indicate that the differences in milk fat composition between winter and summer are mainly caused by environmental effects and do not influence genetic parameters. This means that genetic selection for milk fat composition does not need to be tailored towards season, and that selection response is independent of season of selection or season of phenotype collection.

CONCLUSION

Milk fat composition in summer is characterized by a more unsaturated composition, with increased levels of UFA and long chain FA and decreased levels of short chain FA, compared to winter milk samples. Milk fat composition in summer was more variable than in winter, and variation due to herd was about 50% higher in summer than in winter, and was likely caused by larger dietary differences between herds. Heritabilities for milk fat composition were comparable for winter and summer milk samples, and genetic correlations between winter and summer FA proportions were high. This indicates that despite the phenotypic differences, milk fat composition in winter and summer can be considered as the same genetic trait. This offers opportunities for genetic selection on altered milk fat composition.

ACKNOWLEDGMENT

This study was part of the Dutch Milk Genomics Initiative, funded by Wageningen University, NZO (Dutch Dairy Organization), breeding company CRV, and technology foundation STW. The authors thank the owners of the herds, the Milk Control Station (Zutphen, The Netherlands), and CRV (Arnhem, The Netherlands) for their help in collecting the data.

REFERENCES

Bitman, J., D. L. Wood, R. H. Miller, J. C. Wilk, and E. D. Moore. 1995. Comparison of lipid composition of milk from half-Danish Jersey cows and United States Jersey cows. J. Dairy Sci. 78:655–658.

Chilliard, Y., A. Ferlay & M. Doreau. 2001. Effect of different types of forages, animal fat or marine oils in cow's diet on milk fat secretion and composition, especially conjugated linoleic acid (CLA) and polyunsaturated FA. Livest. Prod. Sci. 70: 31-48.

Elgersma, A., S. Tamminga, and G. Ellen. 2006. Modifying milk composition through forage. Anim. Feed Sci. and Techn. 131: 207-225.

German, J.B. and C.J. Dillard. 2006. Composition, structure and absorption of milk lipids: a source of energy, fat-soluble nutrients and bioactive molecules. Crit. Rev. Food Sci. Nutr. 46: 57-92.

Gilmour, A. R., B. J. Gogel, B. R. Cullis, S. J. Welham, and R. Thompson. 2002. ASReml User Guide Release 1.0. VSN International Ltd., Hemel Hempstead, UK.

Heck, J.M.L., H.J.F. van Valenberg, J. Dijkstra, A.C.M. van Hooijdonk. 2009. Seasonal variation in the Dutch bovine raw milk composition. Submitted.

International Organization for Standardization-International Dairy Federation. 2002a. Milk fat—Determination of the fatty acid composition by gas-liquid chromatography. ISO 15885-IDF 184. International Dairy Federation, Brussels, Belgium.

International Organization for Standardization-International Dairy Federation. 2002b. Milk fat—Preparation of fatty acid methyl esters. ISO 15884-IDF 182. International Dairy Federation, Brussels, Belgium.

Jensen, R. G. 2002. Invited review: The composition of bovine milk lipids: January 1995 to December 2000. J. Dairy Sci. 85:295–350.

Karijord, O., N. Standal, and O. Syrstad. 1982. Sources of variation in composition of milk fat. Z. Tierz. Zcht. Biol. 99:81–93.

Kelsey, J.A., B.A. Corl, R.J. Collier, and D.E. Bauman. 2003. The effect of breed, parity, and stage of lactation on conjugated linoleic acid (CLA) in milk fat from dairy cows. J. Dairy Sci. 86: 2588-2597.

Lund, M.S., J. Jensen, and P.H. Petersen. 1998. Estimation of genetic and phenotypic parameters for clinical mastitis, somatic cell production deviance, and protein yield in dairy cattle using gibbs sampling. J. Dairy Sci. 82: 1045-1051.

Maijala, K. 2000. Cow milk and human development and well being.

Livest. Prod. Sci. 65: 1-18.

Mao, I.L., K. Sloniewski, P.Madsen, and J. Jensen. 2004. Changes in body condition score and in its genetic variation during lactation. Livest. Prod. Sci. 89: 55-65.

Palmquist, D.L. & A.D. Beaulieau. 1993. Feed and Animal factors influencing milk fat composition. J. Dairy Sci. 76: 1753-1771.

Ravagnolo, O., and I. Misztal. 2000. Genetic component of heat stress in dairy cattle, parameter estimation. J. Dairy Sci. 83: 2126-2130.

Reale, D., M. Festa-Bianchet, and J.T. Jorgenson. 1999. Heritability of body mass varies with age and season in wild bighorn sheep. Heredity 83: 526-532.

Soyeurt, H., P. Dardenne, F. Dehareng, C. Bastin, and N. Gengler. 2008. Genetic parameters of saturated and monounsaturated fatty acid content and the ratio of saturated to unsaturated FA in bovine milk. J. Dairy Sci. 91: 3611-3626.

Stoop, W.M., J.A.M. van Arendonk, J.M.L. Heck, H.J.F. van Valenberg, and H. Bovenhuis. 2008. Genetic parameters for major milk FA and milk production traits of Dutch Holstein Friesians. J. Dairy Sci. 91: 385-394.

Stoop, W.M., H. Bovenhuis, J.M.L. Heck, and J.A.M. van Arendonk. 2009. Effect of lactation stage and energy status on milk fat composition of Holstein-Friesian cows. J. Dairy Sci. 92: in press.

Wilmink, J. B. M. 1987. Adjustment of test-day milk, fat and protein yield for age, season and days-in-milk. Livest. Prod. Sci. 16:335–348.

Chapter 6

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

W.M. Stoop, A. Schennink, M.H.P.W. Visker, E. Mullaart,
J.A.M. van Arendonk, and H. Bovenhuis

(submitted)

ABSTRACT

A genome-wide scan was performed to identify quantitative trait loci (QTL) for short and medium chain fatty acid ww 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}<0.05) on 4 chromosomes: C6:0 and C8:0 on BTA6; fat %, all uneven chain FA, and C14:0, C16:0, and C16:1 and their unsaturation indices on BTA14; C14:0 on BTA19; and the monounsaturated FA and their unsaturation indices on BTA26. The QTL explained 3 to 19 percent of phenotypic variance. Furthermore, 49 traits with suggestive evidence for linkage ($P_{chromosome}$<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 FA 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 FA in bovine milk, like C14:0 and C16:0.

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 (a.o. Jensen, 2002; German & Dillard, 2006; Vlaeminck et al., 2006; Soyeurt et al., 2006 & 2007; Stoop et al., 2008). For short and medium chain fatty acids (**FA**) focus is on decreasing the relative proportions of mainly C14:0 and C16:0, as these two FA have been associated with an increase in low-density lipoprotein cholesterol and increased risk of cardio-vascular diseases (German & Dillard, 2006).

Short and medium chain C4 to C17 saturated and monounsaturated milk FA are largely synthesized de novo in the mammary gland. Saturated even chain C4:0 to C16:0 FA 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 FA are mainly derived from rumen bacteria and partly from de novo synthesis from C3 precursors. Monounsaturated FA are suggested to originate from Δ^9-desaturase enzyme activity (MacGibbon & Taylor, 2006). Several studies showed genetic variation in milk FA 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 FA 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 FA 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 (Stephens et al., 1999; Falaki et al., 1997; Khatib et al., 2007; Viitala et al., 2006; Schnabel et al., 2005; Cohen et al., 2004; Brym et al., 2004; Clarke, 1993; Roy et al., 2006b; 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. Recent studies found significant effects of the diacylglycerol acyltransferase 1 (**DGAT1**) K232A mutation on C4:0 to C12:0, C14:0, and C16:0 (Schennink et al., 2007) and of the stearoyl-Coenzyme A desaturase 1 (**SCD1**) A293V mutation on C10:0, C12:0, and C14:0 (Schennink et al., 2008a). 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 fat composition

of bovine milk. This paper presents results for short and medium chain FA. The accompanying paper by Schennink et al. (2008b) presents results for long chain FA.

MATERIALS & 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 one of 5 proven bulls (n=871), one of 50 young bulls (n=844), or one 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 ww%) for conservation. Milk fat composition was measured by gas chromatography at the laboratory of the Netherlands Controling Authority for Milk and Milk Products (Leusden, The Netherlands) as described by Schennink et al. (2007) and Stoop et al. (2008). The FA were identified and quantified by comparing the FA methyl ester chromatograms of the milk fat samples to chromatograms of pure FA methyl ester standards. The FA were measured as weight-proportion of total fat weight. In this study data are presented for 24 short and medium chain FA traits: saturated C4 to C17 FA, *cis*-9 monounsaturated C10:1, C12:1, C14:1, C16:1 and C17:1, and the unsaturation indices of these FA. The unsaturation index is an indication for the activity of the Δ^9-desaturase enzyme and was calculated as:

$$\frac{cis9\text{FA}}{cis9\text{FA} + saturated\text{FA}} * 100$$ (Kelsey et al., 2003), e.g.

$$\frac{C10{:}1}{C10{:}1 + C10{:}0} * 100$$

Table 6.1 shows the average weight proportion (ww%) and heritability of the included FA and indices.

Table 6.1. 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 (ww%)[a]	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	$0.43^{0.10}$
C10index[b]	10.89	1.76	$0.37_{0.09}$
C12index	2.74	0.49	$0.36_{0.09}$
C14index	10.51	1.68	$0.45_{0.09}$
C16index	4.24	0.76	$0.47_{0.09}$
C17index	28.29	2.58	$0.47_{0.10}$
Fat%	4.36	0.64	$0.49_{0.10}$

[a] Individual fatty acids in this table sum to 63.8 ww%. Other FA include branched FA (no results shown), and long chain FA (see Schennink et al., 2008b)

[b] Unsaturation index calculated as $\frac{cis9FA}{cis9FA + saturatedFA} * 100$

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 (Schennink et al., 2008a) and genotypes for DGAT1 were obtained by an allelic discrimination method (Schennink et al., 2007). 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 6.2) 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 AS-Reml (Gilmour et al., 2002) on all 1,905 cows with phenotypes, as described by Schennink et al. (2007) and 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):

$$Y_{ijk} = \mu + s_i + b_{ik}X_{ijk} + e_{ijk} \quad [1]$$

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 & 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 & 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 from model [1] using SAS 9.1 (SAS Institute Inc., 2004).

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 genotypes by including these as fixed effect in the animal model. Subsequently, the QTL regression was re-run for all adjusted trait values.

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 6.2. Average information content was high: 0.83, ranging from 0.70 (BTA19) to 0.88 (BTA2).

Quantitative Trait Loci

QTL for short and medium chain FA were identified on 21 chromosomes in this study and are summarized in Table 6.3. All QTL that exceeded the threshold for suggestive linkage (P_{chr}<0.05) are reported.

Table 6.2. Chromosome length (cM), number of markers per chromosome, and average and range of information content (IC) for all 29 *Bos taurus* autosomes, based on Crimap.

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

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 1 to 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 6.3 for all traits). Allele substitution effects for significant QTL are shown in Table 6.4.

Table 6.3. Location and characteristics of suggestive and significant QTL affecting short and medium chain fatty acids, unsaturation indices, and fat percentage.

BTA	Trait	F-statistic	Position (cM)	95% CI (cM)	P_{chr}	P_{genome}[a]		DGAT1 and SCD1 corrected: Position (cM)	DGAT1 and SCD1 corrected: P_{chr}	DGAT1 and SCD1 corrected: P_{genome}[a]
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	C17index	3.79	31	0-79	0.006	0.125		-	-	-
5	C10index	4.81	95	49-114	0.003	0.071		96	0.010	0.208
5	C12index	3.19	94	44-114	0.033	0.538		-	-	-
5	C14index	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		-	-	-

Table 6.3. Continued…

BTA	Trait	F-statistic	Position (cM)	95% CI (cM)	P_{chr}	P_{genome}[a]	DGAT1 and SCD1 corrected: Position (cM)	P_{chr}	P_{genome}[a]
6	C17index	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	C16index	-	-	-	-	-	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	C10index	3.88	75	49-124	0.047	0.665	80	0.015	0.284
11	C12index	-	-	-	-	-	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	-	-	-
13	C16:1	3.47	75	17-108	0.011	0.258	75	0.006	0.146

Table 6.3. Continued...

BTA	Trait	F-statistic	Position (cM)	95% CI (cM)	P_{chr}	P_{genome} [a]		DGAT1 and SCD1 corrected: Position (cM)	DGAT1 and SCD1 corrected: P_{chr}	DGAT1 and SCD1 corrected: P_{genome} [a]
13	C17:1	3.16	108	35-108	0.020	0.409		108	0.008	0.194
13	C12index	2.98	56	0-108	0.045	0.698		101	0.043	0.684
13	C14index	-	-	-	-	-		107	0.047	0.716
13	C16index	2.94	101	0-108	0.035	0.607		104	0.026	0.502
13	C17index	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	0.000	0.000	***	-	-	-
14	C7:0	8.01	1	0-16	0.000	0.000	***	-	-	-
14	C8:0	3.03	0	0-87	0.039	0.660		-	-	-
14	C9:0	9.29	1	0-8	0.000	0.000	***	-	-	-
14	C11:0	7.97	1	0-5	0.000	0.000	***	-	-	-
14	C13:0	5.56	3	0-88	0.000	0.008	**	-	-	-
14	C14:0	9.84	0	0-0	0.000	0.000	***	-	-	-
14	C15:0	6.28	2	0-25	0.000	0.000	***	-	-	-
14	C16:0	13.86	0	0-1	0.000	0.000	***	-	-	-
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	0.000	0.000	***	-	-	-
14	C14index	4.55	0	0-99	0.001	0.032	*	-	-	-
14	C16index	10.19	0	0-16	0.000	0.000	***	-	-	-
14	C17index	3.36	0	0-73	0.014	0.325		0	0.049	0.749
14	fat%	28.84	0	0-0	0.000	0.000	***	-	-	-

Table 6.3. Continued...

BTA	Trait	F-statistic	Position (cM)	95% CI (cM)	P_{chr}	P_{genome}[a]		DGAT1 and SCD1 corrected: Position (cM)	DGAT1 and SCD1 corrected: P_{chr}	DGAT1 and SCD1 corrected: P_{genome}[a]	
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	C12index	-	-	-	-	-		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	C10index	-	-	-	-	-		58	0.050	0.752	
19	C12index	-	-	-	-	-		96	0.003	0.079	
19	C14index	-	-	-	-	-		96	0.004	0.111	
19	C16index	-	-	-	-	-		96	0.035	0.622	
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	C17index	-	-	-	-	-		61	0.024	0.558	

Table 6.3. Continued…

BTA	Trait	F-statistic	Position (cM)	95% CI (cM)	P_{chr}	P_{genome} [a]		DGAT1 and SCD1 corrected: Position (cM)	DGAT1 and SCD1 corrected: P_{chr}	DGAT1 and SCD1 corrected: P_{genome} [a]
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	0.000	0.000	***	-	-	-
26	C12:1	4.25	28	18-44	0.002	0.092		-	-	-
26	C14:1	9.52	28	22-37	0.000	0.000	***	-	-	-
26	C16:1	6.04	31	19-35	0.000	0.000	***	-	-	-
26	C17:1	3.82	32	17-64	0.002	0.084		-	-	-
26	C10index	6.98	28	0-37	0.000	0.000	***	-	-	-
26	C12index	5.91	28	0-37	0.000	0.000	***	-	-	-
26	C14index	9.90	28	22-37	0.000	0.000	***	-	-	-
26	C16index	8.35	30	22-33	0.000	0.000	***	-	-	-
26	C17index	10.25	27	23-32	0.000	0.000	***	-	-	-
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

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

Results are reported and discussed per chromosome.

On BTA6, a significant QTL near 55 cM affected C6:0 and C8:0 (Table 6.3, Figure 6.1). The QTL on BTA6 significantly segregated within family 1, family 5 and family 6, with an effect of about 0.5 phenotypic SD (Table 6.4). In family 1, the difference between the two daughter groups was 0.08 ww% for C6:0, and 0.07 ww% for C8:0. In family 5, the difference for C6:0 was 0.05 ww%, but not significant, whereas the difference for C8:0 was also 0.07 ww%. In family 6, the difference for C6:0 was 0.10 ww%. The QTL explained around 3 percent of phenotypic variation for both C6:0 and C8:0.

On BTA14, a significant QTL at the centromeric end of the chromosome affected all uneven chain FA, and C14:0, C16:0, and C16:1 with their unsaturation indices (Table 6.3, Figure 6.1). 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 percent of phenotypic variance for the different traits. This QTL also affected several long chain FA and the total proportion of saturated and unsaturated FA (Schennink et al., 2008b).

On BTA19, a significant QTL was found for C14:0 (60 cM, Table 6.3, Figure 6.1). 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 ww% in family 2, 0.28 ww% in family 4, and 1.14 ww% in family 7. This QTL explained 4 percent of phenotypic variance in C14:0.

On BTA26, a QTL near the location of the SCD1 gene (28 cM) affected all monounsaturated FA and their unsaturation indices (Table 6.3, Figure 6.1). 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 FA and unsaturation indices. Family 6 has only 29 offspring; therefore, not all QTL effects were significant. This QTL explained between 4 and 8 percent of phenotypic variance.

Beside the significant QTL, 49 traits with suggestive QTL were found on 21 autosomes (Table 6.3). 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 short, even chain FA 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 FA 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 FA 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 C17index on BTA14 (Table 6.3). 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 C12index at 73 cM. This suggestive QTL was also detected for several long chain mono-unsaturated FA (see Schennink et al., 2008b). 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.

DISCUSSION

Power calculations

In this study, we reported suggestive QTL with $P_{chr}<0.05$ and significant QTL with $P_{genome}<0.05$. 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 percent of phenotypic variance and a medium (about 50%) power to identify QTL that explain 2.5 to 4.2 percent of phenotypic variance. The significant QTL found in this study explained 3 to 19 percent 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 FA, including the previously found QTL on BTA6 and BTA19, the test statistic decreased below the threshold for suggestive

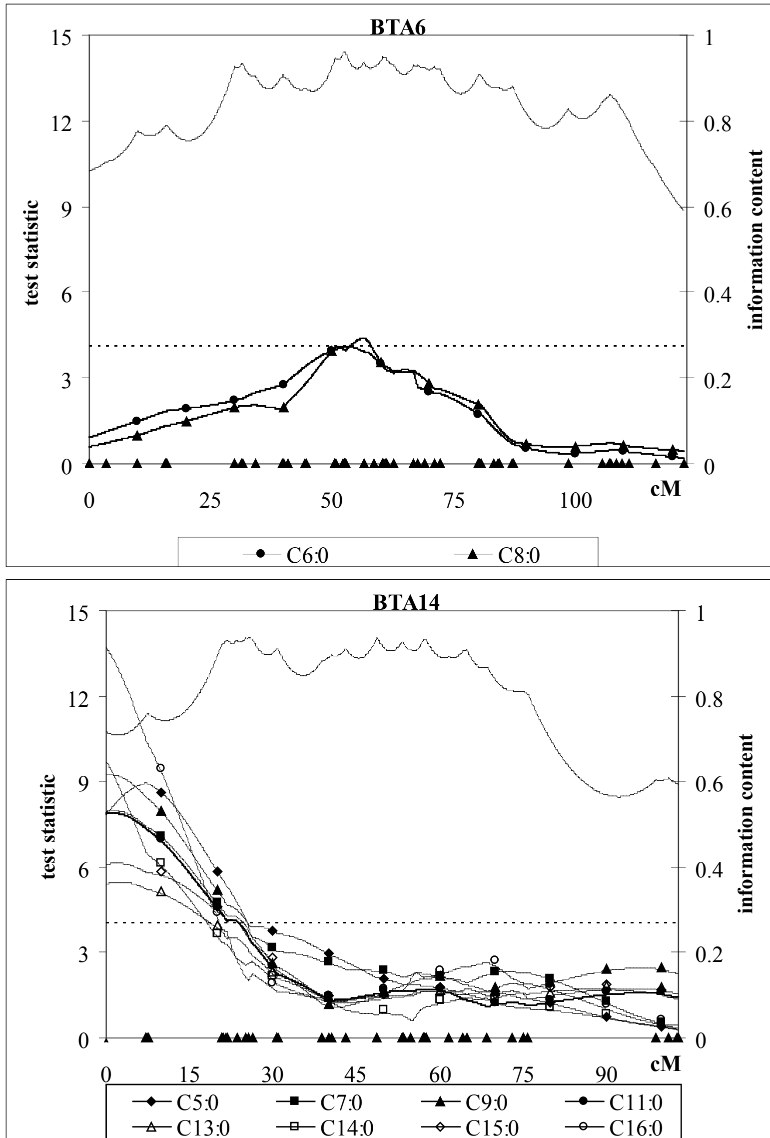


Figure 6.1 A and B. Test statistic for genome-wise significant QTL. The dotted black line represents the genome-wise significance threshold. This threshold may vary slightly between traits and is only shown for the trait with lowest genome-wise significance level. The solid black line represents the information content. The triangles on the x-axis represent the location of the markers.

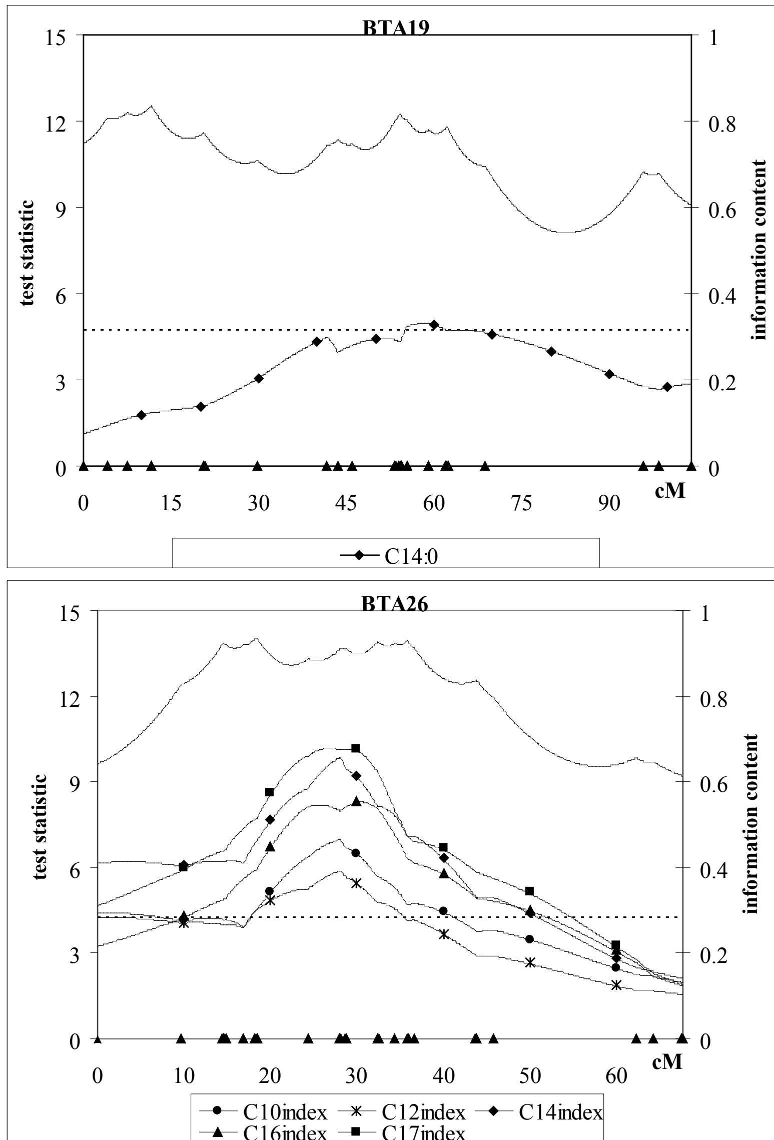


Figure 6.1 C and D. Test statistic for genome-wise significant QTL. The dotted black line represents the genome-wise significance threshold. This threshold may vary slightly between traits and is only shown for the trait with lowest genome-wise significance level. The solid black line represents the information content. The triangles on the x-axis represent the location of the markers.

Table 6.4. Allele substitution effects[a] 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
C16index	$-0.38_{0.11}$*	$-0.63_{0.12}$*	$-0.16_{0.13}$	$-0.61_{0.12}$*	$0.39_{0.23}$	$-0.44_{0.42}$	$-0.15_{0.32}$	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
C14index	$-0.65_{0.25}$*	$-0.83_{0.26}$*	$-0.40_{0.28}$	$-0.87_{0.27}$*	$0.51_{0.52}$	$-0.70_{0.93}$	$-0.80_{0.72}$	3

Table 6.4. Continued...

Trait	Family (no of daughters)							Phenotypic variation explained by QTL (%)
	1 (193)	2 (179)	3 (170)	4 (166)	5 (91)	6 (29)	7 (21)	
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
C10index	$1.26_{0.23}$*	$0.93_{0.24}$*	$0.04_{0.26}$	$0.30_{0.26}$	$0.44_{0.34}$	$0.87_{0.64}$	$-0.24_{0.69}$	5
C12index	$0.30_{0.07}$*	$0.26_{0.07}$*	$0.01_{0.08}$	$0.09_{0.08}$	$0.15_{0.10}$	$0.34_{0.19}$	$0.22_{0.20}$	4
C14index	$1.29_{0.23}$*	$1.14_{0.23}$*	$0.03_{0.25}$	$0.30_{0.25}$	$0.69_{0.32}$*	$1.51_{0.62}$*	$0.45_{0.67}$	6
C16index	$-0.62_{0.11}$*	$-0.45_{0.11}$*	$0.07_{0.12}$	$0.17_{0.11}$	$0.29_{0.15}$	$-0.14_{0.29}$	$0.17_{0.31}$	6
C17index	$-2.31_{0.36}$*	$-1.69_{0.37}$*	$0.19_{0.39}$	$0.79_{0.39}$*	$0.93_{0.51}$	$-1.04_{0.97}$	$0.79_{1.06}$	8

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

QTL. In general, the change in test statistic was rather small (<0.3).

Fat percentage

Contrary to the limited research on FA 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, the current study found four significant QTL for short and medium chain FA 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 a 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 FA proportions and fat percentage differ largely between FA and range between 0.00 and 0.78 (Stoop et al., 2008). FA 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 PPARGC1A (Khatib et al., 2007), FAM13A1 (Cohen et al., 2004), and Osteopontin on BTA6 (OPN, Khatib et al., 2007; Schnabel et al., 2005); DGAT1 on BTA14 (Schennink et al., 2007); Growth Hormone (Viitala et al., 2006), FAS (Smith, 1994), and STAT5A on BTA19 (Stephens et al., 1999; Brym et al., 2004); and SCD1 on BTA26 (Schennink et al., 2008a). Although most studies reported effects on fat percentage, data on milk fat composition was not available,

and only Schennink et al. (2007, 2008a) 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 (Taniguchi et al., 2004; Reh 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 FA can be desaturated by SCD1 (Chung et al., 2000; Bernard et al., 2001; Keating et al., 2005) and sequentially all present individual FA can be esterified to glycerol via GPAT (Roy et al., 2006a), AGPAT (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 Schennink et al. (2007, 2008a).

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 has been proposed to play a central role in regulation of milk fat synthesis activity in the mammary gland (Bernard et al., 2008; Khatib et al., 2007). It is involved in up- and down regulation of several genes of the acetyl-CoA cycle, such as acetyl-CoA carboxylase – which activates acetyl-CoA - (ACC, Barber et al., 1997; Mao et al., 2001) and Fatty Acid Synthase (FAS, Smith,1994).

We also reported significant QTL for C14:0 on BTA19. Morris et al. (2007) reported a significant effect of the candidate gene FAS (BTA19) on C14:0. Within the acetyl-CoA cycle, chain elongation is initiated by acyltransferase, one of the six enzymes associated with FAS, 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 FAS (Smith, 1994). FAS, 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: growth hormone 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 growth hormone and IGF1 (Yang et al., 2000).

Several QTL were identified for short or medium chain FA: 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 (sugges-

tive). 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.

IMPLICATIONS

Four distinct significant QTL were identified that affected short and medium chain FA. DGAT1 (BTA14) and SCD1 (BTA26) are two major genes that are known to affect milk fat composition. DGAT1 affected mainly uneven chain FA and C14:0, C16:0, and C16:1 with their unsaturation indices, and several long chain FA (Schennink et al., 2008b). SCD1 affected all monounsaturated medium chain FA 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 FA, uneven chain FA, or monounsaturated FA and unsaturation indices.

QTL that affect specific FA 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 FA in bovine milk, like C14:0 and C16:0, and reduce fat melting point for better spreadable butter.

ACKNOWLEDGMENTS

This study was part of the Dutch Milk Genomics Initiative, funded by Wageningen University, NZO (Dutch Dairy Association), breeding company CRV, and technology foundation STW. The authors thank the owners of the herds, Richard Spelman for his work on the QTL program, and Patrick Koks for his work on the genetic map.

REFERENCES

Barber, M.C., R.A. Clegg, M.T. Travers, and R.G.Vernon. 1997. Lipid metabolism in the lactating mammary gland. Biochem. Biophys. Acta. 1347: 101-126.

Bernard, L., C. Leroux, and Y. Chilliard. 2008. Expression and nutritional regulation of lipogenic genes in the ruminant lactating mammary gland. In: Bioactive components of milk, editor: Z. Bosze, Springer, New York, USA, pp 67-108.

Bernard, L., C. Leroux, H. Hayes, M. Gautier, Y. Chilliard, and P. Martin. 2001. Characterization of the caprine stearoyl-CoA desaturase gene and its mRNA showing an unusually long 3′-UTR sequence arising from a single exon. Gene 281: 53-61.

Bobe, G., J.A. Minick-Bormann, G.L. Lindberg, A.E. Freeman, and D.C. Beitz. 2008. Short Communication: Estimates of genetic variation of milk-FA in US Holstein cows. J. Dairy Sci. 91: 1209-1213.

Brym, P., S. Kaminski, and A. Rusc. 2004. New SSCP polymorphism within bovine STAT5A gene and its associations with milk performance traits in Black-and-White and Jersey cattle. J. Appl. Genet. 45(4): 445-452.

Chung, M., S. Ha, S. Jeong, J. Bok, K. Cho, M. Baik, and Y. Choi. 2000. Cloning and characterization of bovine stearoyl CoA desaturase cDNA from adipose tissues. Biosci. Biotechn. Biochem. 64: 1526-1530.

Churchill, G.A., and R.W. Doerge. 1994. Empirical threshold values for quantitative trait mapping. Genetics 138: 963-971.

Clarke, S.D. 1993. Regulation of Fatty Acid Synthase Gene Expression: An approach for reducing fat accumulation. J. Anim. Sci. 71: 1957-1965.

Cohen, Mm, M. Reichenstein, A. Everts-van der Wind, J. Heon-Lee, M. Shani, H.A. Lewin, J.I. Weller, M. Ron, and E. Seroussi. 2004. Cloning and characterization of FAM13A1 – a gene near a milk protein QTL on BTA6: evidence for population-wide linkage disequilibrium in Israeli Holsteins. Genomics 84: 374-383.

De Koning, D.J., P.M. Visscher, S.A. Knott, and C.S. Haley. 1998. A strategy for QTL detection in half-sib populations. Anim. Sci. 67: 257-268

De Koning, D.J., N.F. Schulman, K. Elo, S. Moisio, R. Kinos, J. Vilkki, and A. Maki-Tanila. 2001. Mapping of multiple quantitative trait loci by simple regression in half-sib designs. J. Anim. Sci. 79: 616-622.

Falaki, M., A. Prandi, C. Corradini, M. Sneyers, N. Gengler, S. Massart,

U. Fazzini, A. Burny, D. Portetelle, and R. Renaville. 1997. Relationships of growth hormone gene and milk protein polymorphisms to milk production traits in Simmental cattle. J. Dairy Res. 64: 47-56.

German, J.B., and C.J. Dillard. 2006. Composition, structure and absorption of milk lipids: a source of energy, fat-soluble nutrients and bioactive molecules. Crit. Rev. Food Sci. Nutr. 46: 57-92.

Gilmour, A.R., B.J. Gogel, B.R. Cullis, S.J. Welham, and R. Thompson. 2002. ASReml User Guide Release 1.0 (VSN International Ltd, Hemel Hempstead, HP11ES, UK).

Green, P., K. Falls, and S. Crooke. 1990. Documentation for CRI-MAP. Ver 2.4. (Washington School of Medicine, St. Louis, MO).

Jensen, R.G. 2002. Invited review: The composition of bovine milk lipids: January 1995 to December 2000. J. Dairy Sci. 85: 295-350.

Keating, A.F., C. Stanton, J.J. Murphy, T.J. Smith, R.P. Ross, and M.T. Cairns. 2005. Isolation and characterization of the bovine stearoyl-CoA desaturase promoter and analysis of polymorphisms in the promoter region in dairy cows. Mamm. Genome. 16: 184-193.

Kelsey, J.A., B.A. Corl, R.J. Collier, and D.E. Bauman. 2003. The effect of breed, parity, and stage of lactation on conjugated linoleic acid (CLA) in milk-fat from dairy cows. J. Dairy Sci. 86(8): 2588-2597.

Khatib, H., I. Zaitoun, J. Wiebelhaus-Finger, Y.M. Chang, and G.J.M. Rosa. 2007. The association of bovine PPARGC1A and OPN genes with milk composition in two independent Holstein cattle populations. J. Dairy Sci. 90: 2966-2970.

Khatkar, M.S., P.C. Thomson, I. Tammen, and H.W. Raadsma. 2004. Quantitative trait loci mapping in dairy cattle: review and meta-analysis. Genet. Sel. Evol. 36: 163-190.

Knott, S.A., J.M. Elsen, and C.S. Haley. 1996. Methods for multiple-marker mapping of quantitative trait loci in half-sib populations. Theor. Appl. Genet. 93: 71-80.

Lander, E., and L. Kruglyak. 1995. Genetic dissection of complex traits: guidelines for interpreting and reporting linkage results. Nature genetics 11: 241-247.

MacGibbon, A.K.H., and M.W. Taylor. 2006. Composition and structure of bovine milk lipids. In: Advanced Dairy Chemistry, Volume 2: Lipids, 3th ed., P.F. Fox and P.L.H. McSweeney, eds. (Springer, New York).

Mao, J., S. Marcos, S.K. Davis, J. Burzlaff, and H.M. Seyfert. 2001. Genomic distribution of three promoters of the bovine gene encoding acetyl-CoA carboxylase alpha and evidence that the nutritionally regulated pro-

moter I contains a repressive element different from that in rat. Biochem. J. 358: 127-135

Mao, J., A.J. Molenaar, T.T. Wheeler, and H.M. Seyfert. 2002. STAT5 binding contributes to lactational stimulation of promoter III expressing the bovine acetyl-CoA carboxylase alpha-encoding gene in the mammary gland. J. Molecular Endocrinology 29: 73-88.

Mistry, D.H., and J.F. Medrano. 2002. Cloning and localization of the bovine and ovine lysophosphatidic acid acyltransferase (LPAAT) genes that codes for an enzyme involved in triglyceride biosynthesis. J. Dairy Sci. 85: 28-35.

Morris, C.A., N.G. Cullen, B.C. Glass, D.L. Hyndman, T.R. Manley, S.M. Hickey, J.C. McEwan, W.S. Pitchford, C.D.K. Bottema, and M.A.H. Lee. 2007. Fatty acid synthase effects on bovine adipose fat and milk-fat. Mammalian Genome 18: 64-74.

Reh, W.A., E.A. Maga, N.M.B. Collette, A. Moyer, J.S. Conrad-Brink, S.J. Taylor, E.J. DePeters, S. Oppenheim, J.D. Rowe, R.H. BonDurant, G.B. Anderson, and J.D. Murray. 2004. Hot Topic: Using a Stearoyl-CoA desaturase transgene to alter milk-fatty acid composition. J. Dairy Sci. 87: 3510-3514.

Rosen, E.D., P. Sarraf, A.E. Troy, G. Bradwin, K. Moore, D.S. Milstone, B.M. Spiegelman, and R.M. Mortensen. 1999. PPARγ is required for the differentiation of adipose tissue in vivo and in vitro. Molecular Cell 4: 611-617.

Roy, R., L. Ordovas, S. Taourit, P. Zaragoza, A. Eggen, and C. Rodellar. 2006a. Genomic structure and an alternative transcript of bovine mitochondrial glycerol-3-phosphate acyltransferase gene (GPAM). Cytogen. Gen. Res. 112: 82-89.

Roy, R., L. Ordovas, P. Zaragoza, A. Romero, C. Moreno, J. Altarriba, and C. Rodellar. 2006b. Association of polymorphisms in the bovine FASN gene with milk-fat content. Anim. Genet. 37: 215-218.

Roy, R., S. Taourit, P. Zaragoza, A. Eggen, and C. Rodellar. 2005. Genomic structure and alternative transcript of bovine fatty acid synthase gene (FASN): Comparative analysis of the FASN gene between monogastric and ruminant species. Cytogen. Gen. Res. 111: 65-73.

SAS Institute Inc. 2004. SAS ® 9.1 National Language support (NLS): User’s Guide. Cary, NC: SAS Institute Inc.

Schennink, A., W.M. Stoop, M.H.P.W. Visker, J.M.L. Heck, H. Bovenhuis, J.J van der Poel, H.J.F. van Valenberg, and J.A.M. van Arendonk. 2007. DGAT1 underlies large genetic variation in milk-fat composition of

dairy cows. Anim. Genet. 38: 467–473.

Schennink, A., J.M.L. Heck, H. Bovenhuis, M.H.P.W. Visker, H.J.F. van Valenberg, and J.A.M. van Arendonk. 2008a. Milk-fatty Acid Unsaturation: Genetic Parameters and Effects of Stearoyl-CoA Desaturase (SCD1) and Acyl CoA: Diacylglycerol Acyltransferase 1 (DGAT1). J. Dairy Sci. 91: 2135-2143.

Schennink, A., W.M. Stoop, M.H.P.W. Visker, J.J. van der Poel, H. Bovenhuis, and J.A.M. van Arendonk. 2008b. Genome-wide scan for bovine milk-fat composition II. QTL for long chain FA. (submitted).

Schnabel, R.D., J. Kim, M.S. Ashwell, T.S. Sonstegard, C.P. van Tassell, E.E. Connor, and J.F. Taylor. 2005. Fine-mapping milk production quantitative trait loci on BTA6: Analysis of the bovine osteopontin gene. PNAS 102: 6896-6901.

Schopen, G.C.B., M.H.P.W. Visker, J.A.M. van Arendonk, and H. Bovenhuis. 2008. Whole Genome Scan to detect QTLs for detailed bovine milk protein composition. (submitted).

Smith, S. 1994. The animal fatty acid synthase: one gene, one polypeptide, seven enzymes. FASEB J. 8(15): 1248-1259.

Soyeurt, H., P. Dardenne, A. Gillon, C. Croquet, S. Vanderick, P. Mayeres, C. Bertozzi, and N. Gengler. 2006. Variation in fatty acid contents of milk and milk-fat within and across breeds. J. Dairy Sci. 89: 4858-4865.

Soyeurt, H., A. Gillon, S. Vanderick, P. Mayeres, C. Bertozzi, and N. Gengler. 2007. Estimation of heritability and genetic correlations for the major FA in bovine milk. J. Dairy Sci. 90: 4435-4442.

Spelman, R.J., W. Coppieters, L. Karim, J.A.M. van Arendonk, and H. Bovenhuis. 1996. Quantitative trait loci analysis for five milk production traits on chromosome six in the Dutch Holstein-Friesian population. Genetics 144: 1799-1808.

Stephens, J.M., R.F. Morrison, Z. Wu, and S.R. Farmer. 1999. PPARγ Ligand-dependent induction of STAT1, STAT5A, and STAT5B during adipogenesis. Biochem. Biophys. Res. Comm. 262: 216-222.

Stoop, W.M., J.A.M. van Arendonk, J.M.L. Heck, H.J.F. van Valenberg, and H. Bovenhuis. 2008. Genetic parameters for major milk-FA and milk production traits of Dutch Holstein-Friesians. J. Dairy Sci. 91: 385-394.

Taniguchi, M., T. Utsugi, K. Oyama, H. Mannen, M. Kobayashi, Y. Tanabe, A. Ogino, and S. Tsuji. 2004. Genotype of stearoyl-CoA desaturase is associated with fatty acid composition in Japanese Black cattle. Mamm. Genome 15: 142–148.

Viitala, S., J. Szyda, S. Blott, N. Schulman, M. Lidauer, A. Maki-Tanila,

M. Georges, and J. Vilkki. 2006. The role of bovine growth hormone receptor and prolactin receptor genes in milk, fat and protein production in Finnish Ayrshire dairy cattle. Genetics 173: 2151-2164.

Visscher, P.M., R. Thompson, and C.S. Haley. 1996. Confidence Intervals in QTL mapping by bootstrapping. Genetics 143: 1013-1020.

Vlaeminck, B., V. Fievez, A.R.J. Cabrita, A.J.M. Fonseca, and R.J. Dewhurst. 2006. Factors affecting odd- and branched-chain FA in milk: a review. Anim. Feed Sci. Tech. 131: 389-417.

Winter, A., W. Kramer, F.A.Werner, S. Kollers, S. Kata, G. Durstewitz, J. Buitkamp, J.E. Womack, G. Thaller, and R. Fries. 2002. Association of a lysine-232/alanine polymorphism in a bovine gene encoding acyl-CoA:diacylglycerol acyltransferase (DGAT1) with variation at a quantitative trait locus for milk-fat content. Proc. National Academy of Sciences USA 99: 9300-9305.

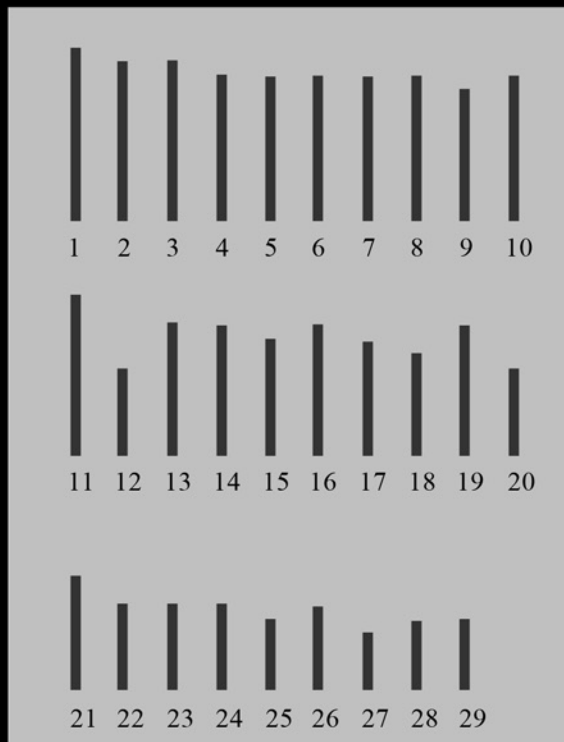
Yang, J., J.J. Kennelly, and V.E. Baracos. 2000. The activity of transcription factor Stat5 responds to prolactin, growth hormone, and IGF-I in rat and bovine mammary explant culture. J. Anim. Sci. 78: 3114-3125.

Zhang, S., T.J. Knight, J.M. Reecy, and D.C. Beitz. 2008. DNA polymorphisms in bovine fatty acid synthase are associated with beef fatty acid composition. Anim. Genet. 39(1): 62-70.

Chapter 7

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

A. Schennink, W.M. Stoop, M.H.P.W. Visker, J.J. van der Poel, H. Bovenhuis, and J.A.M. van Arendonk



(submitted)

ABSTRACT

Previous research has shown substantial genetic variation between dairy cows in milk fat composition, with low to moderate heritabilities for long chain FA (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 FA. 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}<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, CLA *cis*-9,*trans*-11, C18:3 *cis*-9,12,15, C18index, totalindex, total saturated FA (SFA), total unsaturated FA (UFA) and ratio SFA/UFA on BTA14, for C18:1 *trans* FA on BTA15, and for C18 and conjugated linoleic acid (CLA) indices on BTA16. The QTL explained 3 to 19 percent of the phenotypic variance. Suggestive QTL ($P_{chromosome}$<0.05) were found on 16 other chromosomes. The DGAT1 K232A polymorphism on BTA14 is known to influence milk fat 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 FA.

INTRODUCTION

Milk fat is characterized by a high amount of saturated FA (**FA**), especially medium chain FA C14:0 and C16:0, and by a low amount of (poly) unsaturated FA. Whereas the medium chain FA C14:0 and C16:0 are commonly considered to have a negative effect on human health due to their cholesterol-raising properties, long chain FA of 18 and more carbon atoms are considered to have neutral or positive effects (Mensink et al., 2003). Of the long chain FA, 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 FA in the mammary gland are derived from circulating plasma lipids, and originate from the diet, from microbial FA synthesis in the rumen, and from endogenous lipids. Lipids entering the rumen are first lipolyzed by microbial lipases, causing the release of FA. After lipolysis, unsaturated FA 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., 2008b). Although estimated heritabilities for the individual FA 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 FA can provide insight into long chain FA 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 (see review of 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 FA. They identified fatty acid synthase (**FAS**) as a candidate gene. Genotyping of five single nucleotide polymorphisms (**SNP**) in this gene showed association between SNP in FAS 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 (Schennink et al., 2007; Schennink et al., 2008). 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 FA composition of bovine milk. The accompanying paper by Stoop et al. (2008a) presents results for short and medium chain FA.

MATERIALS & 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 CRV (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 ww%) for conservation.

Fat percentage was measured by infrared spectroscopy, using a Milko-Scan 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 by Schennink et al. (2007). The FA were expressed as weight-proportion of total fat weight. In the present study, 21 traits were analyzed: the individual FA 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* FA, the group saturated FA (**SFA**: C4:0-C18:0 and C20:0), the group unsaturated FA (**UFA**: mono- and polyunsaturated C10-C18), the ratio SFA/UFA, and the unsaturation indices for C18, CLA and total

FA. 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. C18index = $\frac{C18{:}1cis9}{C18{:}1cis9 + C18{:}0}$ *100. Means, standard deviations and heritabilities of the traits are given in Table 7.1.

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 Illumina 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 (Schennink et al., 2008) and allelic discrimination method (Schennink et al., 2007), respectively. The SCD1 A293V and DGAT1 K232A genotypes were available for 1,687 out of 1,905 animals. The linkage map was constructed using Cri-map (Green et al., 1990). The number of markers per chromosome and information content is available from Stoop et al. (2008a) 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 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 makers 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 AS-Reml (Gilmour et al., 2002), according to Schennink et al. (2007). Phenotypes of the 849 cows used in the QTL analysis were pre-corrected using these estimates.

Table 7.1. Mean, phenotypic standard deviation (σ_P) and heritability (h^2) of all traits included in the analysis, measured on 1,905 cows.

Trait	Mean (ww%)	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[a]	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*[b]	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[c]	0.08	0.01	$0.23_{0.09}$
C20:0	0.13	0.02	$0.24_{0.08}$
C18index[d]	67.62	3.49	$0.33_{0.08}$
CLAindex	33.72	3.73	$0.23_{0.07}$
Totalindex	21.48	1.66	$0.30_{0.09}$
SFA[e]	70.76	2.11	$0.30_{0.09}$
UFA[f]	25.69	1.93	$0.29_{0.09}$
Ratio SFA/UFA	2.79	0.28	$0.29_{0.09}$
Fat %	4.36	0.64	$0.49_{0.10}$

[a] C18:1 *trans*-4-8 may comprise C18:1 FA with *trans* bonds at position 4, 5, 6, 7, and 8.
[b] C18:1 *trans* comprises C18:1 *trans*-4-8, C18:1 *trans*-9, C18:1 *trans*-10, C18:1 *trans*-11.
[c] For C19:0 only 1,401 observations were available.

[d] Unsaturation index calculated as $\frac{cis9FA}{cis9FA + saturatedFA} * 100$

[e] SFA comprises saturated C4, C5, C6, C7, C8, C9, C10, C12, C13, C14, C15, C16, C17, C18 and C20.
[f] UFA comprises C10:1, C12:1, C14:1, 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.

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, bik 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.

RESULTS

QTL for long chain milk FA were identified on several chromosomes in this study and are summarized in Table 7.2. 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 1-4 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 7.3. Results of significant QTL will be discussed per chromosome.

Table 7.2. Location and characteristics of suggestive and significant QTL affecting long chain fatty acids, unsaturation indices and fat percentage

BTA	Trait	F-statistic	Position (cM)	95% CI (cM)	P_{chr}	P_{genome} [a]	DGAT1 and SCD1 corrected Position (cM)	DGAT1 and SCD1 corrected P_{chr}	DGAT1 and SCD1 corrected P_{genome} [a]	
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	-	-	-	

Table 7.2. Continued...

BTA	Trait	F-statistic	Position (cM)	95% CI (cM)	P_{chr}	P_{genome} [a]		DGAT1 and SCD1 corrected: Position (cM)	DGAT1 and SCD1 corrected: P_{chr}	DGAT1 and SCD1 corrected: P_{genome} [a]	
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	***	-	-	-	
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	

Table 7.2. Continued...

BTA	Trait	F-statistic	Position (cM)	95% CI (cM)	P_{chr}	P_{genome} [a]		DGAT1 and SCD1 corrected Position (cM)	P_{chr}	P_{genome} [a]	
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	

Table 7.2. Continued...

							DGAT1 and SCD1 corrected		
BTA	Trait	F-statistic	Position (cM)	95% CI (cM)	P_{chr}	P_{genome}[a]	Position (cM)	P_{chr}	P_{genome}[a]
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
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	-	-	-

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

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, totalindex, SFA, UFA and ratio SFA/UFA was detected (Table 7.2, Figure 7.1). 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 7.3). 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 ww% 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 ww% 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 FA were detected as well (see accompanying paper by Stoop et al., 2008a).

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 (Schennink et al., 2007). 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 7.2), indicating that the DGAT1 genotype was responsible for this QTL effect. Furthermore, a suggestive QTL at approximately 50 cM became clear for the C18:1 *trans* FA as a group, and for the individual FA C18:1 *trans*-4-8, C18:1 *trans*-9, C18:1 *trans*-10, C18:1 *cis*-11 and C19:0 (Figure 7.2). This position also showed suggestive QTL for C10:1, C12:1 and C14:1 FA after the DGAT1/SCD1 correction (see accompanying paper by Stoop et al., 2008a).

Table 7.3. Allele substitution effects[a] 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
CLAcis9,trans11	$0.02_{0.01}$*	$0.01_{0.01}$	$0.03_{0.01}$*	$0.04_{0.01}$*	$0.04_{0.02}$	$-0.04_{0.04}$	$0.07_{0.03}$*	4
C18:3cis9,12,15	$0.03_{0.01}$*	$0.02_{0.01}$*	$0.03_{0.01}$*	$0.06_{0.01}$*	$0.01_{0.02}$	$0.01_{0.03}$	$0.05_{0.02}$*	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

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

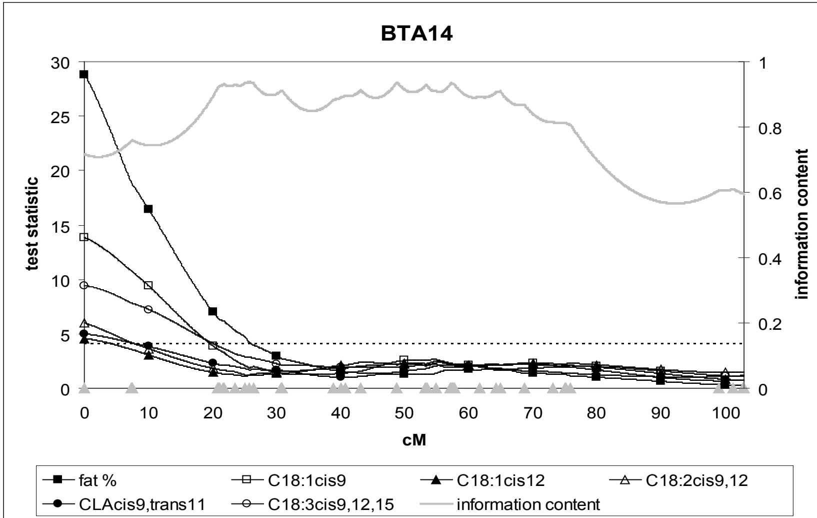


Figure 7.1. QTL mapping across families for long chain FA on BTA14. Triangles on the x-axis represent the location of the markers. The dotted black line presents the genome-wise significance threshold. Although this threshold is slightly different for each trait, only the line of the trait with the lowest genome-wise significance threshold is shown.

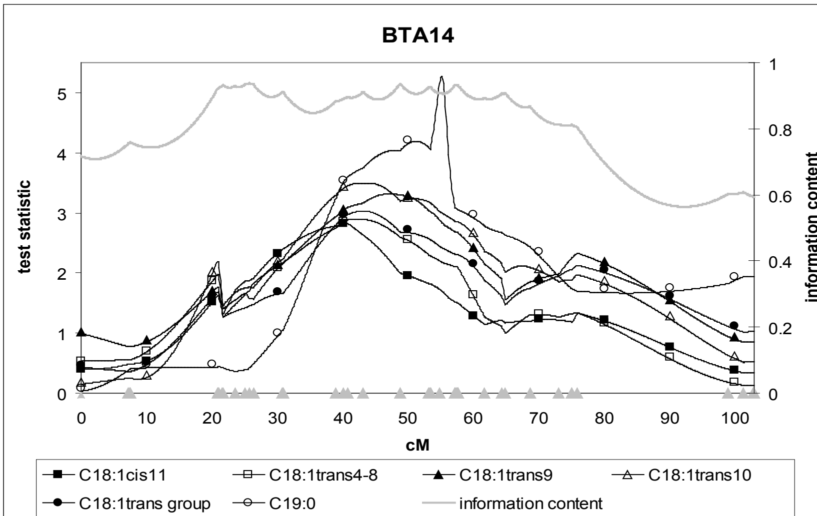
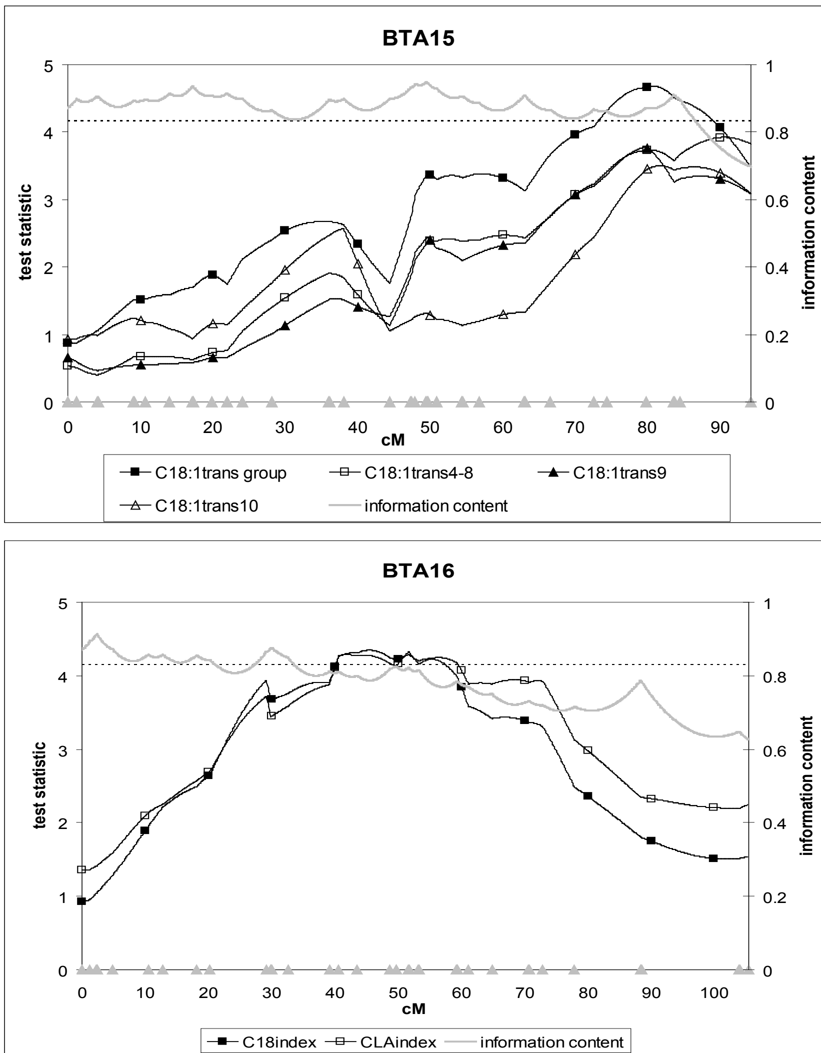


Figure 7.2. QTL mapping across families for long chain FA, pre-corrected for DGAT1 K232A and SCD1 A293V genotypes on BTA14. Triangles on the x-axis represent the location of the markers. All shown QTL are suggestive.



Figures 7.3. and 7.4. QTL mapping across families for long chain FA on BTA15 and BTA16. Triangles on the x-axis represent the location of the markers. The dotted black line presents 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 QTL for individual C18:1*trans* FA did not reach the genome-wise significance threshold on BTA15.

On BTA15, a QTL for the group of C18:1 *trans* FA was found at position 80 cM. This QTL region also affected the individual C18:1 *trans* FA C18:1 *trans*-4-8, C18:1 *trans*-9 and C18:1 *trans*-10, although not genome-wise significant, but suggestive (Table 7.2). 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* FA (Figure 7.3). Family 1 and 2 contributed to the QTL (Table 7.3), and the difference between the daughter groups in the fraction of the C18:1 *trans* group FA was 0.19 and 0.08 ww%, respectively. The QTL explained 4% of the phenotypic variance of C18:1 *trans* FA. 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 C18index and CLAindex were found at positions 45 and 52 cM, within the same confidence interval (Table 7.2, Figure 7.4). 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 C18index; for CLAindex only family 1 and 3 segregated significantly (Table 7.3). The QTL explained 3% of the phenotypic variance of both C18 and CLAindex.

Removal of the effects of DGAT1 and SCD1 genotypes resulted furthermore in two new significant linkage positions. On BTA1, a QTL at 129 cM for C18index was detected. Suggestive QTL within the same confidence interval on BTA1 were found for C18:0, C19:0, C20:0 and CLAindex. On BTA13, a QTL for C19:0 was found at 78 cM.

DISCUSSION

In this study, QTL were mapped for long chain milk FA in the Dutch Holstein-Friesian cattle population. Given the design and size of our study, power calculations showed that QTL which 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 the accompanying paper by Stoop et al., 2008a).

The identification of QTL for long chain FA strongly supports the hypothesis of a genetic component that influences variation for these FA - as for the short to medium chain FA -, which was already posed by the low to moderate heritabilities. Long chain FA are not de novo synthesized by the

cow herself, but are derived from circulating plasma lipids, and originate from the diet, from microbial FA synthesis in the rumen, and from endogenous lipids. Therefore, the most obvious candidate genes for long chain FA proportions, would be those that are involved in the uptake, desaturation, esterification, biohydrogenation, and elongation of long chain FA.

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 (Schennink et al., 2007), 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 (Schennink et al., 2007; Schennink et al., 2008), 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 FA, which predominantly consists of C18:1*cis*9). Allele substitution effects for CLA *cis*-9,*trans*-11, C18index, totalindex 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 totalindex, 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., 2008b).

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 FA, and suggests the presence of another QTL for milk fat composition on BTA14.

A QTL for C18:1 *trans* FA is detected on BTA15. In the rumen, dietary unsaturated FA 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* FA as common

intermediates, is considered to be the rate-limiting step. When this metabolism is incomplete, C18:1 *trans* FA will accumulate. Among the C18:1 *trans* intermediates, the *trans*-11 isomer is the main one, but also other isomers are produced (Harfoot & 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 FA 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 FA. The route that long chain FA 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* FA.

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, possibly no QTL for C18:1 *trans*-11 on BTA15 could be detected.

QTL for C18index and CLAindex were found on BTA16. An index reflects a ratio between a saturated FA and its *cis*-9 mono-unsaturated 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 C18index and CLAindex (Schennink et al., 2008), 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 FAS as a candidate gene. Although Morris et al. (2007) detected a QTL for C18 FA on BTA19, and found an association between SNP in FAS 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 FAS genotype on C18 FA 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 mono-unsaturated FA (**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 (Abe et al., 2008a; Alexander et al., 2007; Clop et al., 2003; Karamichou et al., 2006; Sanchez et al., 2007). 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 (a.o. for C18:2) and on BTA19 (a.o. for C18:1).

The study described in this and the accompanying paper 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 FA. For short and medium chain FA, we detected 4 significant QTL on BTA6, 14, 19 and 26, and for long chain FA, we detected 3 significant QTL on BTA14, 15, and 16. Only BTA14 is involved in both short/medium and long chain milk FA, whereas the other significant QTL are detected of either one of them. This finding indicates that short/medium chain FA on one hand, and long chain FA 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 FA metabolism.

ACKNOWLEDGMENTS

This study is part of the Dutch Milk Genomics Initiative, funded by Wageningen University, the Dutch Dairy Association (NZO), CRV and the Dutch Technology Foundation STW. The authors thank the owners of the herds for their help in collecting the data, and Jeroen M. L. Heck and Hein J. F. van Valenberg for fruitful discussions.

REFERENCES

Abe, T., J. Saburi, H. Hasebe, T. Nakagawa, T. Kawamura, K. Saito, T. Nade, S. Misumi, T. Okumura, K. Kuchida, T. Hayashi, S. Nakane, T. Mitsuhasi, K. Nirasawa, Y. Sugimoto, and E. Kobayashi. 2008a. Bovine QTL analysis for growth, carcass, and meat quality traits in an F2 population from a cross between Japanese Black and Limousin. J. Anim. Sci. doi:10.2527/jas.2007-0676.

Abe, T., J. Saburi, H. Hasebe, T. Nakagawa, S. Misumi, T. Nade, H. Nakajima, N. Shoji, M. Kobayashi, and E. Kobayashi. 2008b. Novel mutations of bovine FASN gene, g.16024A>G and g.16039T>C, effects on the fatty acid composition of Japanese black beef. in 31st Conference of the International Society of Animal Genetics. Amsterdam, the Netherlands.

Alexander, L. J., M. D. Macneil, T. W. Geary, W. M. Snelling, D. C. Rule, and J. A. Scanga. 2007. Quantitative trait loci with additive effects on palatability and fatty acid composition of meat in a Wagyu-Limousin F2 population. Anim. Genet. 38:506-513.

Bobe, G., J. A. Minick Bormann, G. L. Lindberg, A. E. Freeman, and D. C. Beitz. 2008. Short communication: estimates of genetic variation of milk FA in US Holstein cows. J. Dairy Sci. 91:1209-1213.

Chilliard, Y., A. Ferlay, R. M. Mansbridge, and M. Doreau. 2000. Ruminant milk fat plasticity: nutritional control of saturated, polyunsaturated, trans and conjugated FA. Ann. Zootech. 49:181-205.

Churchill, G. A. and R. W. Doerge. 1994. Empirical threshold values for quantitative trait mapping. Genetics 138:963-971.

Clop, A., C. Ovilo, M. Perez-Enciso, A. Cercos, A. Tomas, A. Fernandez, A. Coll, J. M. Folch, C. Barragan, I. Diaz, M. A. Oliver, L. Varona, L. Silio, A. Sanchez, and J. L. Noguera. 2003. Detection of QTL affecting fatty acid composition in the pig. Mamm. Genome 14:650-656.

Edwards, J. E., N. R. McEwan, A. J. Travis, and R. John Wallace. 2004. 16S rDNA library-based analysis of ruminal bacterial diversity. Antonie Van Leeuwenhoek 86:263-281.

Farnir, F., B. Grisart, W. Coppieters, J. Riquet, P. Berzi, N. Cambisano, L. Karim, M. Mni, S. Moisio, P. Simon, D. Wagenaar, J. Vilkki, and M. Georges. 2002. Simultaneous mining of linkage and linkage disequilibrium to fine map quantitative trait loci in outbred half-sib pedigrees: revisiting the location of a quantitative trait locus with major effect on milk production on bovine chromosome 14. Genetics 161:275-287.

Gilmour, A. R., B. J. Gogel, B. R. Cullis, S. J. Welham, and R. Thomp-

son. 2002. ASReml User Guide Release 1.0. VSN International Ltd, Hemel Hempstead, UK.

Green, P., K. Falls, and S. Crooke. 1990. Documentation for CRI-MAP. Ver 2.4. Washington School of Medicine, St. Louis, MO.

Grisart, B., W. Coppieters, F. Farnir, L. Karim, C. Ford, P. Berzi, N. Cambisano, M. Mni, S. Reid, P. Simon, R. Spelman, M. Georges, and R. Snell. 2002. Positional candidate cloning of a QTL in dairy cattle: identification of a missense mutation in the bovine DGAT1 gene with major effect on milk yield and composition. Genome Res. 12:222-231.

Harfoot, C. G. and G. P. Hazlewood. 1997. Lipid metabolism in the rumen. Pages 382-426 in The Rumen Microbial Ecosystem. 2nd ed. P. N. Hobson and C. S. Stewart, ed. Blackie Academic & Professional, London.

Haug, A., A. T. Høstmark, and O. M. Harstad. 2007. Bovine milk in human nutrition-a review. Lipids Health Dis 6:25.

Hoashi, S., N. Ashida, H. Ohsaki, T. Utsugi, S. Sasazaki, M. Taniguchi, K. Oyama, F. Mukai, and H. Mannen. 2007. Genotype of bovine sterol regulatory element binding protein-1 (SREBP-1) is associated with fatty acid composition in Japanese Black cattle. Mamm. Genome 18:880-886.

Karamichou, E., R. I. Richardson, G. R. Nute, K. P. Gibson, and S. C. Bishop. 2006. Genetic analyses and quantitative trait loci detection, using a partial genome scan, for intramuscular fatty acid composition in Scottish Blackface sheep. J. Anim. Sci. 84:3228-3238.

Kelsey, J. A., B. A. Corl, R. J. Collier, and D. E. Bauman. 2003. The effect of breed, parity, and stage of lactation on conjugated linoleic acid (CLA) in milk fat from dairy cows. J. Dairy Sci. 86:2588-2597.

Khatkar, M. S., P. C. Thomson, I. Tammen, and H. W. Raadsma. 2004. Quantitative trait loci mapping in dairy cattle: review and meta-analysis. Genet. Sel. Evol. 36:163-190.

Knott, S. A., J. M. Elsen, and C. S. Haley. 1996. Methods for multiple-marker mapping of quantitative trait loci in half-sib populations. Theor. Appl. Genet. 93:71-80.

Lander, E. and L. Kruglyak. 1995. Genetic dissection of complex traits: guidelines for interpreting and reporting linkage results. Nat. Genet. 11:241-247.

Loor, J. J., A. Ferlay, A. Ollier, K. Ueda, M. Doreau, and Y. Chilliard. 2005. High-concentrate diets and polyunsaturated oils alter trans and conjugated isomers in bovine rumen, blood, and milk. J. Dairy Sci. 88:3986-3999.

Mahfouz, M. M., A. J. Valicenti, and R. T. Holman. 1980. Desatura-

tion of isomeric trans-octadecenoic acids by rat liver microsomes. Biochim Biophys Acta 618:1-12.

Mensink, R. P., P. L. Zock, A. D. M. Kester, and M. B. Katan. 2003. Effects of dietary FA and carbohydrates on the ratio of serum total to HDL cholesterol and on serum lipids and apolipoproteins: a meta-analysis of 60 controlled trials. Am. J. Clin. Nutr. 77:1146-1155.

Morris, C. A., N. G. Cullen, B. C. Glass, D. L. Hyndman, T. R. Manley, S. M. Hickey, J. C. McEwan, W. S. Pitchford, C. D. Bottema, and M. A. Lee. 2007. Fatty acid synthase effects on bovine adipose fat and milk fat. Mamm. Genome 18:64-74.

Mosley, E. E., B. Shafii Dagger, P. J. Moate, and M. A. McGuire. 2006. cis-9, trans-11 conjugated linoleic acid is synthesized directly from vaccenic acid in lactating dairy cattle. J. Nutr. 136:570-575.

Paillard, D., N. McKain, M. T. Rincon, K. J. Shingfield, D. I. Givens, and R. J. Wallace. 2007. Quantification of ruminal Clostridium proteoclasticum by real-time PCR using a molecular beacon approach. J. Appl. Microbiol. 103:1251-1261.

Pollard, M. R., F. D. Gunstone, A. T. James, and L. J. Morris. 1980. Desaturation of positional and geometric isomers of monoenoic FA by microsomal preparations from rat liver. Lipids 15:306-314.

Sanchez, M. P., N. Iannuccelli, B. Basso, J. P. Bidanel, Y. Billon, G. Gandemer, H. Gilbert, C. Larzul, C. Legault, J. Riquet, D. Milan, and P. Le Roy. 2007. Identification of QTL with effects on intramuscular fat content and fatty acid composition in a Duroc x Large White cross. BMC Genet. 8:55.

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

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

Schopen, G. C. B., H. Bovenhuis, M. H. P. W. Visker, and J. A. M. Van Arendonk. 2008. Whole genome scan to detect QTLs for detailed bovine milk protein composition. Submitted.

Shingfield, K. J., S. Ahvenjarvi, V. Toivonen, A. Arola, K. V. V. Nurmela, P. Huhtanen, and J. M. Griinari. 2003. Effect of dietary fish oil on biohydrogenation of FA and milk fatty acid content in cows. Anim. Sci.

77:165-179.

Soyeurt, H., A. Gillon, S. Vanderick, P. Mayeres, C. Bertozzi, and N. Gengler. 2007. Estimation of heritability and genetic correlations for the major FA in bovine milk. J. Dairy Sci. 90:4435-4442.

Stoop, W. M., A. Schennink, M. H. P. W. Visker, E. Mullaart, J. A. M. Van Arendonk, and H. Bovenhuis. 2008a. Genome-wide scan for bovine milk-fat composition. I. Quantitative trait loci for short and medium chain FA. Submitted.

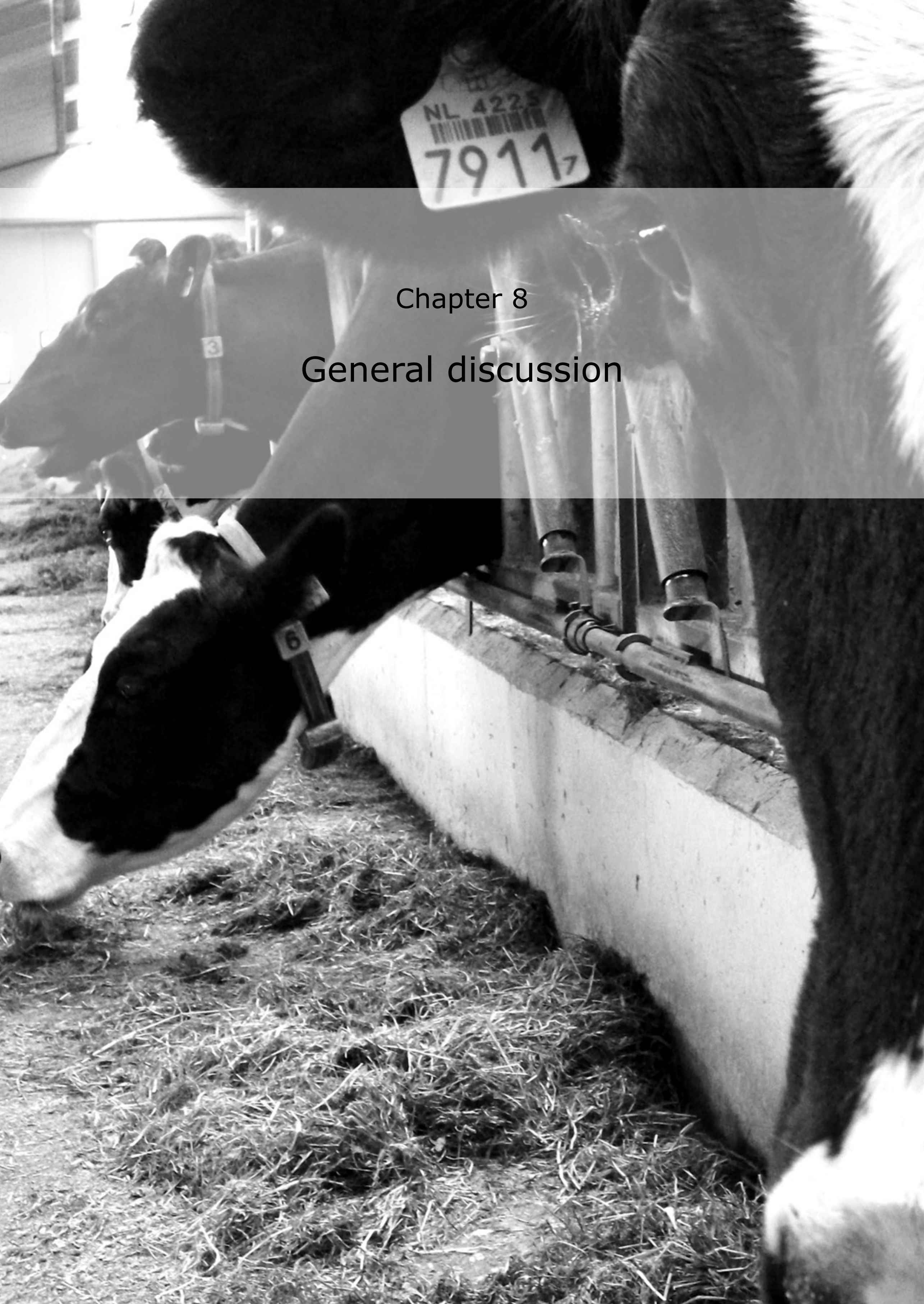
Stoop, W. M., J. A. M. Van Arendonk, J. M. L. Heck, H. J. F. Van Valenberg, and H. Bovenhuis. 2008b. Genetic parameters for major milk FA and milk production traits of Dutch Holstein Friesians. J. Dairy Sci. 91:385-394.

Visscher, P. M., R. Thompson, and C. S. Haley. 1996. Confidence intervals in QTL mapping by bootstrapping. Genetics 143:1013-1020.

Wasowska, I., M. R. Maia, K. M. Niedzwiedzka, M. Czauderna, J. M. Ribeiro, E. Devillard, K. J. Shingfield, and R. J. Wallace. 2006. Influence of fish oil on ruminal biohydrogenation of C18 unsaturated FA. Br. J. Nutr. 95:1199-1211.

Winter, A., W. Kramer, F. A. Werner, S. Kollers, S. Kata, G. Durstewitz, J. Buitkamp, J. E. Womack, G. Thaller, and R. Fries. 2002. Association of a lysine-232/alanine polymorphism in a bovine gene encoding acyl-CoA:diacylglycerol acyltransferase (DGAT1) with variation at a quantitative trait locus for milk fat content. Proc. Natl. Acad. Sci. U S A 99:9300-9305.

Zhang, S., T. J. Knight, J. M. Reecy, and D. C. Beitz. 2008. DNA polymorphisms in bovine fatty acid synthase are associated with beef fatty acid composition. Anim. Genet. 39:62-70.



Chapter 8

General discussion

In this thesis we looked at variation in milk fat composition, with emphasis on the opportunities to use this variation to genetically improve the milk fat composition of Dutch dairy cows. A population of approximately 2,000 first parity cows were selected from 398 commercial farms throughout the Netherlands. In chapter 2 heritabilities and genetic correlations were estimated for milk production traits. These genetic parameters were in line with literature findings and showed we had a representative sample of the Dutch Holstien-Friesian population. In chapters 3,4,5 variation in milk fat composition between cows was analyzed and variation due to genetic effects, herd effects, lactation stage, energy balance, and season were quantified. The analysis revealed considerable genetic variation in milk fat composition. Heritabilities for short and medium chain FA (**FA**) were higher than for long chain FA, whereas variation due to herd were lower for short and medium chain FA than for long chain FA. In general, genetic correlations among C6:0 to C14:0 were positive, as were correlations among unsaturated C18 FA. Correlations of C16:0 with other FA were mostly negative, which might relate to the dual origin of C16:0. A cluster analysis confirmed three clusters of FA: C6:0 to C14:0, C4:0 and C16:0, and C18 FA. These results indicated similar genes and possibly similar synthesis pathways were involved in regulating proportions of C6:0 to C14:0, but (partly) other genes may be involved in regulating proportions of unsaturated C18 FA.

Chapters 6 and 7 describe a genome-wide scan in which several QTL regions were identified that were associated with milk fat composition and could explain part of the genetic variation. Several QTL were identified that significantly affected milk fat composition. These results give us better understanding of the genomic regions and genes involved in milk fat synthesis. This final chapter will explore the opportunities to genetically improve milk fat composition. The first step is to discuss the desired direction of change, after that we will look at extreme cow and herd performance, selection responses, and finally to consequences of selection.

DESIRED DIRECTION OF CHANGE

The Dutch Milk Genomics Initiative focuses on the opportunities to use genetic selection to improve milk quality in the desired direction. In the end of the last century, Gibson (1991) and Maijala (1995) concluded that it was difficult to define the desired direction of change in milk fat composition (Gibson, 1991; Maijala, 1995). From discussions with experts in

The Netherlands it appeared that at present it seems that lower fat percentage, lower proportions of C14:0 and C16:0 and higher proportions of unsaturated FA (**UFA**) are desired. Cows with a more unsaturated milk fat composition seem less prone to severe negative energy balance (**NEB**, Van Knegsel et al., 2007b). A more unsaturated milk fat composition is positively associated with human health, as it might decrease cholesterol levels and decrease risk for diseases as coronary heart disease, obesity, and diabetes (Maijala, 2000; Williams, 2000). Increased proportions of long chain FA reduce melting point of fat which improves spreadability of butter. However, milk fat with increased proportions of long chain and/or unsaturated FA also reduces shelf-life of dairy products and increases the chance of oxidation, which might be problematic for cheese production (Chilliard et al., 2001).

In this discussion I will focus on selection possibilities for 2 traits, which were chosen after discussion with the WEVO, i.e. the discussion group on nutritional research from the Dutch Dairy Association (NZO): C16:0 and unsaturated FA (**UFA**). Palmitic acid (C16:0) has been mentioned as most harmful for human health and hardens butter fat, and the aim is to reduce the proportion in milk fat. The UFA are generally considered beneficial for human health and therefore the aim is to increase the proportion of UFA in milk fat.

CHANGING MILK FAT COMPOSITION

Aiming to improve milk fat composition, a first step is to quantify the phenotypic variation currently present in the population. Table 8.1 shows the average phenotype of the 10% cows with lowest and highest proportions of C16:0 or UFA, both for winter and summer milk fat. Two results

Table 8.1. Extreme phenotypes of cows for C16:0 and UFA, based on 1,905 winter milk records and 1,795 summer milk records, from approximately 398 farms.

Trait		10%lowest	average	10%highest
C16:0	Winter	27.64	32.59	37.53
	Summer	23.44	29.17	35.56
UFA	Winter	21.99	25.75	30.82
	Summer	23.79	29.24	35.99

[1] C16:0 and UFA are given as ww% of total fat fraction, and are not corrected for systemic environmental effects.

are of particular interest. Firstly, the difference in cows with lowest and highest phenotype is approximately 10 ww% for both C16:0 and UFA. Secondly, summer milk has significantly lower levels of C16:0 and higher levels of UFA; with the best cows giving 5 ww% more UFA in summer than in winter. As these are raw means, variation between the cows can be caused by many factors, such as differences in genetic merit, lactation stage, age-at-first-calving, etcetera. Part of the observed differences will be due to herd, which offers opportunities for on-farm separation of milk streams.

Many factors contribute to the observed variation in milk fat composition. However, when interest is in changing milk fat composition, there are three options: 1) nutrition and management, 2) manufacturing technology, and 3) genetic selection (Walker et al., 2004).

The main factor described in literature is nutrition. Feeding studies use diet or dietary supplements to enhance levels of –mainly- long chain polyunsaturated FA, such as conjugated linoleic acid (**CLA**, Palmquist & Beaulieu, 1993; Chilliard et al., 2000; Chilliard et al., 2001; Walker et al., 2004; Lourenco et al., 2005; Elgersma et al., 2006). Although success rates are variable, studies have described huge changes in milk fat composition due to feeding interventions. Diet-induced milk fat depression, for example, can lead to a 40% reduction in milk fat percentage (Bauman & Griinari, 2003), though this might not be a desirable change in relation to cow health. A more favorable effect can be achieved by oil supplementation, which potentially can increase CLA levels up to 300% (Chilliard et al., 2000). Commercial applications of dietary treatments have resulted in special products like the Dutch Campina milk, which has 20% more UFA than traditional milks (in winter milk this would approximate 30 ww% UFA as opposed to 25 ww%). Table 8.1 shows that a similar increase can be obtained by collection of milk from the best 10% of cows.

Results from chapter 3, 4, and 5 indicate considerable differences in milk fat composition between farms. These differences might be caused by diet, but also by soiltype, feed quality, health status and other management factors. To illustrate the differences between herds, herd effects were estimated using models described in chapter 3 of this thesis. Table 8.2 shows the 10% herds with lowest and highest effects on proportion of C16:0 or UFA, for winter and summer milk fat. The results are based on 1,905 winter milk samples when all cows were kept inside 24 hours/

Table 8.2. Extreme herd effect for C16:0 and UFA, based on 1,905 winter milk records and 1,795 summer milk records, from approximately 398 farms.

Trait		10%lowest	average	10%highest
C16:0	Winter	-2.35	32.59	2.22
	Summer	-3.67	29.17	4.21
UFA	Winter	-1.75	25.75	2.25
	Summer	-3.37	29.24	3.66

[1] UFA and C16:0 are given as ww% of total fat fraction

[2] 10% lowest and highest extremes are shown as deviation from phenotypic average.

day, and on 1,795 summer milk samples when about half of the cows had grazing opportunities.

Firstly, there are considerable differences between the extreme groups of farms; in winter samples there is a difference of over 4 ww% in proportion of both C16:0 and UFA between the 10% lowest and highest farms. In summer samples the difference in proportion of C16:0 is approximately 8 ww%, and for UFA 7 ww%. Secondly, herd averages are higher in summer than in winter (result not shown), as was observed for individual cows in Table 8.1. This might be a partial effect of lactation stage, fresh grass availability (Elgersma et al., 2004; Elgersma et al., 2006) or a seasonal effect (Soyeurt et al., 2008).

However, of the 40 farms that have lowest C16:0 proportion in winter, only 8 also have the lowest C16:0 proportion in summer. Likewise, of the 40 farms with highest UFA levels in winter, 12 have also highest UFA levels in summer. This low correlation is shown in Figure 8.1, where the estimated effect for C16:0 of each herd in summer is plotted against the herd effect for C16:0 in winter (see also chapter 5). The correlation is estimated to be 0.20. If the aim is to decrease C16:0 and increase UFA, summer milk has a more favorable milk composition. It might be important to identify the causal factors for the observed changes, as these may offer opportunities to improve milk fat composition in the winter and result in a more constant composition and more constant herd performance throughout the year.

Results from literature show that feeding interventions can lead to large changes in milk fat composition. The results from table 8.2 support this and show considerable differences between herds. Herd was modeled as a random effect, which means that for low numbers of cows per herd

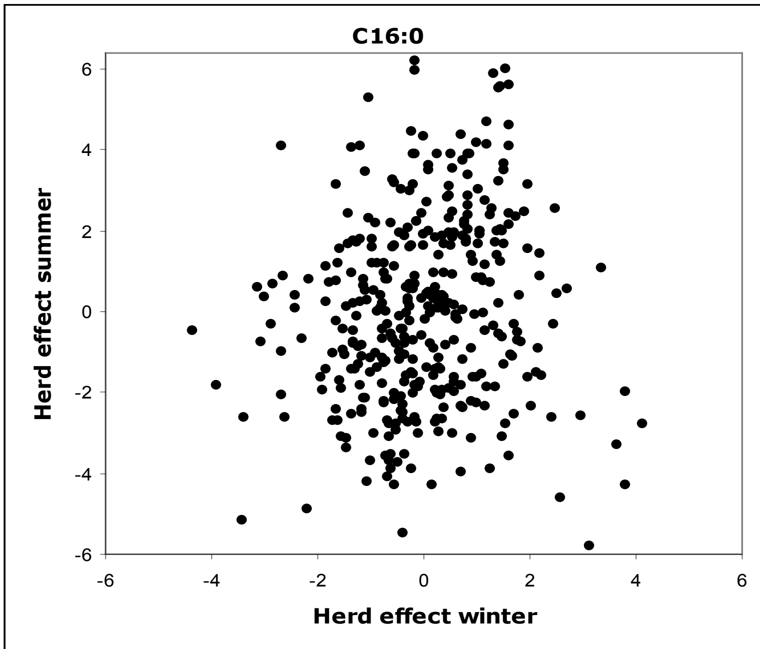


Figure 8.1. Estimated effect of each herd for C16:0 in winter and summer. The herd correlation for C16:0 was estimated at 0.20.

the herd effect will be regressed towards the mean. This implies that possibly larger effects could be observed within the Dutch population. Based on the herd variation estimated from the model, predictions can be made on the size of effects expected to be found in Dutch herds as $i*\sigma_H$, which is the intensity times the standard deviation. In summer, it is expected that the 10% best performing herds for C16:0 have 4.44 ww% less C16:0 than average, whereas the 10% best performing herds for UFA have 4.04 ww% more UFA than average herds. And this estimated variation between herds is only variation measured in this study and might increase with the introduction of feed additives, as no special dietary treatments were practiced at the studied herds.

Table 8.1 and 8.2 show that there are opportunities to change milk fat composition by differentiating between and within herds. As the studied herds did not practice any special (dietary) treatments directed to improve milk fat composition, the potential effect of herd could be much larger. Additional research on management and dietary factors affecting

milk fat composition could lead to management tools to maintain a more favorable milk fat composition in herds throughout the year.

DIFFERENTIATION & GENETIC SELECTION

There are several opportunities to implement genetic selection for milk fat composition. Three scenarios will be described: one based on cow performance, one based on selective use of bulls within the current breeding program, and one based on the development of a new breeding program.

Scenario A: on-farm differentiation based on cow performance

This strategy reflects opportunities of differentiation on farm, where selection is based on own performance of the cows. This strategy would separate milk on-farm to a milk container used for one end-product or a different milk container for another end-product. The group of cows can be split in two production herds within a farm or milk of cows can be separated within the milk robot.

The change in C16:0 or UFA proportion that can be achieved with this strategy depends on three parameters: the proportion of cows that is selected to produce the specialized milk, the repeatability of measurements on which cows are selected, and the phenotypic standard deviation of the trait. Figure 8.2. shows the change in C16:0 or UFA for increasing proportions of selected cows. When the 10% cows with best performance for C16:0 are selected, they produce on average 2.3 ww% less C16:0 than the herd average. Likewise, when the 10% cows with best performance for UFA are selected, they produce on average 2.0 ww% more UFA than the herd average. Differences decrease when larger numbers of cows are selected. Average herd size in the Dutch Milk Genomics Dataset is 82 cows per herd. If specialized milk is collected on only a handful of cows, implementation costs will be relatively high. Selecting the 25% best performing cows will result, depending on whether selection is on C16:0 or UFA, in either a decrease of 1.7 ww% in C16:0 or an increase of 1.5 ww% in UFA.

Results in this scenario are based on fatty acid proportions measured with gas chromatography. For introduction of differentiation on milk fat composition, phenotyping of large numbers of milk samples milk fat composition is required. Gas chromatography is an expensive and time-consuming analysis method, which is unsuitable for large amounts of data.

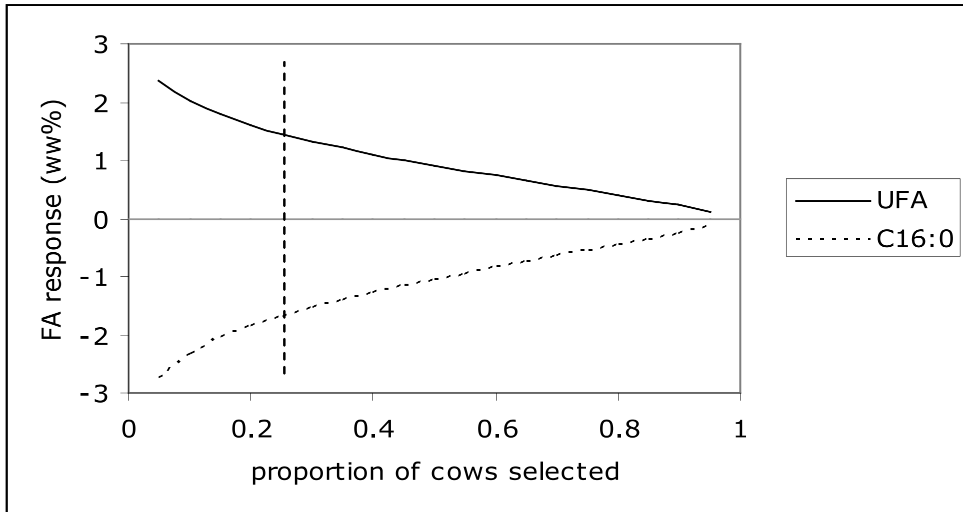


Figure 8.2. Response of C16:0 or UFA for on-farm differentiation, based on cow performance. Based on 3,378 records from winter and summer milk, calculated as intensity*repeatability*σ_P. Repeatability was 0.55 for C16:0 and 0.49 for UFA.

The current operational Milk Production Registration in the Netherlands and many other countries depend on infrared analysis, which is a fast and cheap method to estimate fat, protein and lactose percentage and several other milk production traits.

Some studies have shown a potential to analyze milk fat composition using infrared technology (e.g. Soyeurt et al., 2006). A recent study by Rutten (personal communication 2008), based on the milk samples from this thesis, suggest that mid-infrared patterns may be used to accurately predict proportions of several FA in milk. The correlation between C16:0 measured by gas chromatography and infrared was 0.86, and for UFA 0.87. This implies that it is possible to predict C16:0 and UFA proportion from infrared spectra, which lowers the costs of implementation considerably and can additionally aid farmers in their management decisions. The lower accuracy of infrared measurements will result in a small decrease in response to differentiation: selecting the 25% best performing cows will result, depending on whether selection is on C16:0 or UFA, in either a decrease of 1.5 ww% in C16:0 or an increase of 1.3 ww% in UFA, which is only slightly smaller than the expected effect based on gas chroma-

tography. The accuracy can be increased by more frequent analysis of samples.

This strategy of on-farm differentiation involves separation of milk streams and requires either a milk robot to separately collect the milk or splitting the herd in two production groups. Additionally, two milk containers are needed to store the milk, and costs of the fatty acid measurements. Separation of milk streams is already practiced in the Netherlands for specialized milk as the Campina unsaturated milk, where differentiation is practiced on herd level. Studies by Dooley et al. (2005a, 2005b, 2006) from New Zealand showed that differentiating milk streams is possible when the added milk price compensates farmers for transition costs, and possibly production losses. Dooley et al. (2005a) estimated that separate collection of differentiated milk is a relatively low cost, which increased 4.5 to 22 % from conventional transportation to separate collection of differentiated milks. Demeter et al. (2009), based on a discussion of stakeholders within the Dutch dairy chain, claims that changes within the dairy sector require not only consideration of the entire chain, but also concerted and coordinated actions by all involved stakeholders: breeders, farmers, transporters, dairy plants, and consumers. In summary, this scenario is easy to implement and can result in a considerable reduction in C16:0 or an increase in UFA.

Scenario B: differentiation within bulls; selective use of bulls within the current breeding program

This strategy uses a selective set of bulls to produce specialized milk. Farms that want to improve there over-all c16:0 or UFA level, start using these bulls to improve the herd average. These bulls (and possibly cows) can be selected on estimated breeding values (**EBV**), which are based on own performance (if available) as well as performance of relatives, as estimated by an animal model. The animals with highest EBV will produce the offspring with best performance.

The left part of Figure 8.3 explains the basis of differentiation. As there is no direct selection on the trait of interest (C16:0 or UFA), the assumption is that the population average of the trait does not change over generations. Within the current breeding program, however, it is possible to select the bulls with highest EBV for the trait of interest (grey colour in Figure 8.3) to produce specialized milk. When each generation the best bulls are used, the resulting change in FA proportion will be maintained each generation.

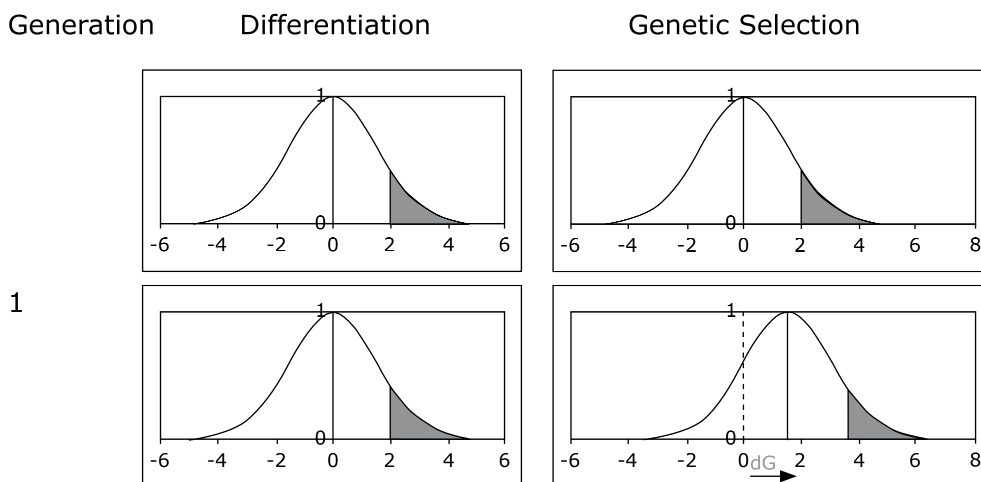


Figure 8.3. Differentiation and genetic selection

As an example, Table 8.3 shows the estimated breeding values of the 10% best young bulls from the Dutch Milk Genomics dataset. For differentiation on protein yield (resembling the current breeding program in The Netherlands), the 10% best bulls have an average EBV of 47.58 kg, which means that their offspring will on average produce 0.5*47.58 is 23.79 kg more protein in one lactation than the population average. When these daughters are subsequently mated to the the best bulls, the second generation will on average produce 0.75*47.58 more kg protein per lactation. The third generation will then reach the maximum difference of 47.58 kg protein, and subsequent generations mated to the best bulls will maintain this level of performance. These same bulls have also positive EBV for C14:0 and UFA, and a negative EBV for C16:0. This result implies that ac-

Table 8.3. Estimated breeding values of 10% best young bulls in the Milk Genomics dataset for protein yield, C14:0. C16:0, and UFA. Based on 3,378 records from 1,689 cows.

Trait	EBV	EBV of related traits			
		Protein yield	C14:0	C16:0	UFA
Protein yield	47.58	X	0.377	-0.302	0.097
C16:0	-1.499	30.5	0.689	X	1.142
UFA	1.726	11.59	0.223	-0.985	X

[1] protein yield is recalculated to resemble 305-day yield.

tually there is a small change in C16:0 and UFA over generations, due to the genetic correlation with protein yield, and that the population average is not exactly constant over generations. Differentiation on C16:0, instead of on protein yield, results in offspring that have on average 1.5 ww% less C16:0. Differentiation on UFA results in offspring that have on average 1.7 ww% more UFA. Offspring performance can be further increased when accuracies of EBV are increased (by testing more daughters per sire), or when differentiation (on EBV) is also done for the cows.

Gibson (1991) advocates this strategy as most promising, as different products require a different optimal milk fat composition. This strategy can be implemented within the current breeding scheme. It requires phenotyping for fatty acid proportions, and estimation (and publication) of breeding values. A possible drawback of the differentiation on EBV within the current breeding program is that there is no genetic improvement over generations in the trait of interest (except for the slight change due to genetic correlations with traits in the current breeding program such as protein yield). Like on-farm differentiation or feeding of special additives, the improved performance will be lost in a single generation when no selective bulls are being used. In summary, selection on bulls can give a minimum response of 1.5 ww% less C16:0, or – when selecting for UFA – 1.7 ww% more UFA. Response can be further increased by an increased accuracy of the EBV, as EBV in this example were based on 20 daugters per sire.

Scenario C: Developing a new breeding program

In scenario C, genetic selection would be based on cow and sire EBV. Each generation, the cows and bulls with best EBV for the trait of interest are chosen as parents to produce the next generation, which will improve the entire population, as is shown in the right side of Figure 8.3. Genetic selection requires a new breeding program where the trait of interest (C16:0 or UFA) is incorporated into the breeding goal. This will lead to genetic selection for the trait and thus a change in population average over generations.

To quantify the size of the changes that can be achieved using this strategy, genetic gains are predicted using the program SelAction (Rutten et al., 2002). Input parameters for SelAction are shown in textbox 8.1, and are based on genetic parameters estimated from a repeatability model with 3,378 records from 1,689 cows. The selection responses (Table 8.4) are calculated for single trait selection on protein yield, C16:0,

Table 8.4. Potential genetic gain of the population with single trait selection, estimated from SelAction.

Trait	Response	Correlated response			
	per year	Protein yield	C14:0	C16:0	UFA
Protein yield	4.88	X	0.070	-0.138	0.033
C16:0	-0.267	2.44	0.124	X	0.116
UFA	0.252	0.61	0.023	-0.124	X

[1] protein yield is recalculated to resemble 305-day yield.

or UFA. Genetic selection on protein yield will result in 4.88 kg more protein per lactation. Correlated responses will slightly increase C14:0 (0.07) and UFA (0.033), and will decrease C16:0 by 0.138 ww% per year. Selection on either C16:0 or UFA will result in a genetic gain of approximately 0.25 ww% per year. When an average generation interval of 6 years is assumed, this will lead to a response to selection of about 1.5 ww% per generation.

Note that the difference with differentiation is that selection of best bulls in the differentiation strategy takes place within a different breeding program, so that the population average for the trait does not change, whereas with genetic selection we select bulls and cows within a breeding program aimed to change the trait, so that bulls in the next generation are better than in the current generation. Selection responses might be improved by including genomic information as data source. Chapter 6 and 7 showed several QTL for milk fat composition. Additional studies with 60.000 SNP will likely result in more genomic information. This genomic selection might improve the EBV and decrease the generation interval. In a few years time, commercial chips may aid the farmer in selecting his best cows based on genomic information instead of on phenotype. Implementation of scenario C requires fatty acid phenotyping, breeding value estimation, and a change of the current breeding program.

CONSEQUENCES OF SELECTION

The above results show that the current selection on protein yield results in minor changes in UFA proportions, a slight increase in C14:0, and a decrease in C16:0. In terms of desired responses, the current index on protein yield has a favorable (reducing) effect on C16:0 proportion. Direct selection on UFA, however, would not only result in a similar reduction in C16:0 as the selection on protein yield, but will also increase UFA levels

with 0.25 ww% each year. Selection on UFA has some consequences. First, there is a negative correlation between UFA and fat percentage and yield (chapter 3). In the current NVI index there is, besides protein yield, also emphasis on fat yield (CRV, 2008). Increased levels of UFA are expected to positively affect cow and human health, but partly contradicts the current selection criteria. Secondly, selection on UFA would result in a small positive correlated response in C14:0, a FA associated with increased LDL-cholesterol.

There is a possible interaction between genetics and other factors. Mulder & Bijma (2006) described genes by environment (GxE) interactions which complicate genetic selection over countries, or even over production environments (organic versus conventional farming) or breeds. This might not be important when differentiating within the current breeding program and within the current Dutch Holstein-Friesian population to produce niche products. When large scale changes in the Dutch dairy sector are expected, however, additional research should study the international context in which these changes occur.

Then there might be an interaction between genetics and cow nutrition. This nutrigenomics may aid in improving milk fat quality by selecting the best cows on the most optimal diet, but at the same time feed effects may be different for different genotypes (GxE). Nutrigenomics may prove an exceptional opportunity to combine herd and genetic effects and optimize the use of variation in milk fat composition to improve dairy product quality.

This thesis has shown large genetic variation in milk fat composition, which can be used to improve milk fat quality. The different scenarios presented in this final chapter show that there are opportunities to implement genetic changes in milk fat composition gradually. First, performance could be improved by differentiating cows on-farm and collect their milk in different containers. Separate collection of several milk streams is already practiced on small scale initiatives in the Netherlands and might be enhanced by a faster analysis methods for milk fat composition, such as infrared technology. Secondly, specialized farms could differentiate within the existing breeding program and use bulls with best EBV on particular fatty acid proportions to further utilize the genetic variation in milk fat composition currently present in the population. Thirdly, it is possible to select the entire population in one or more directions aimed to improve milk fat quality. Scenario A, B, and C could be combined to separately collect milk from the best cows, mate these cows to the best

Textbox 8.1.

Input parameters SelAction

Name	Var(P)	h²
Proteinyield[2]	0.005	0.29
C14:0	0.729	0.55
C16:0	5.79	0.28
UFA	5.485	0.28

[1] Based on summer and winter dataset with 3,378 records
[2] Var(P) for protein yield is based on morning milk samples

Simulated population: 10 sires x 200 dams

Information sources Index

age class	male candidates	male information source	female candidates	female information source
1	0	BLUP	0	BLUP
2	0	BLUP	0	BLUP
3	0	BLUP, HS10	100	BLUP,HS9,OP
4	0	BLUP, HS10	95	BLUP,HS9,OP
5	100	BLUP, HS10, Progeny	90	BLUP,HS9,OP
6	99	BLUP, HS10, Progeny	85	BLUP,HS9,OP
7	98	BLUP, HS10, Progeny	80	BLUP,HS9,OP
8	97	BLUP, HS10, Progeny	75	BLUP,HS9,OP

[1] HS10 = Half sib group with 10 female half sibs; HS9 = Half sib group with 9 female half sibs; OP = Own performance; Progeny = Progeny group with 100 female progeny; BLUP = Best Linear Unbiased Prediction

Phenotypic (below diagonal) and Genetic (above diagonal) correlations

	Proteinyield	C14:0	C16:0	UFA
Proteinyield		0.47	-0.45	0.11
C14:0	0.33		-0.85	0.1
C16:0	-0.07	-0.22		-0.46
UFA	-0.01	-0.31	-0.63	

[1] Based on summer and winter dataset with 3,378 records

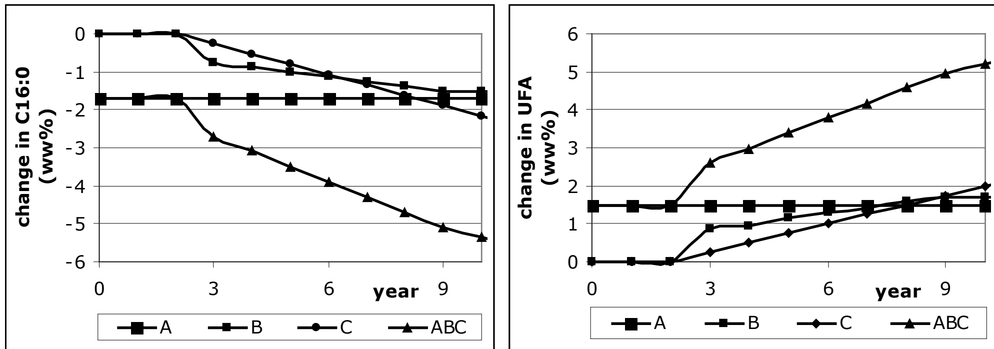


Figure 8.4. Potential changes in C16:0 (left) or UFA (right), when combining scenario A, B, and C.

bulls, while in the meantime improving the entire population. Figure 8.4 shows the potential stacked improvements in either C16:0 or UFA, when combining scenario A, B, and C, based on the Milk Genomics data. Note that the actual improvement will be higher as in subsequent generations best cows (Figure 8.2) and best bulls (Table 8.3) will be better due to the genetic selection from scenario C. And that response to selection can be increased when accuracy of EBV is increased. The new breeding program could gradually evolve in the developing market, which already started by scenario A and B, and thus maximize the genetic potential for changes in milk fat composition. Finally, initiatives outside genetics, such as dietary measures, could be used to maximize cow performances in all scenarios presented.

REFERENCES

Bauman, D.E. and J.M. Griinari. 2003. Nutritional regulation of milk fat synthesis. Annu. Rev. Nutr. 23: 203-227.

Bobe, G., J.A. Minick Bormann, G.L. Lindberg, A.E. Freeman, and D.C. Beitz. 2008. Short communication: Estimates of genetic variation of milk FA in US Holstein cows. J. Dairy Sci. 91: 1209-1213.

Chilliard, Y., A. Ferlay & M. Doreau. 2001. Effect of different types of forages, animal fat or marine oils in cow's diet on milk fat secretion and composition, especially conjugated linoleic acid (CLA) and polyunsaturated FA. Livest. Prod. Sci. 70: 31-48.

Chilliard, Y., A. Ferlay, R.M. Mansbridge & M. Doreau. 2000. Ruminant milk fat plasticity: nutritional control of saturated, polyunsaturated, trans and conjugated FA. Ann. Zootech. 49: 181-205.

Demeter, R.M., M.P.M. Meuwissen, A.G.J.M. Oude Lansink, and J.A.M. van Arendonk. 2009. Scenarios for a future dairy chain in the Netherlands. Submitted.

Dooley, A.E., W.J. Parker, and H.T. Blair. 2005a. Modelling of transport costs and logistics for on-farm milk segregation in New Zealand dairying. Computer and electronics in agriculture 48:75-91.

Dooley, A.E., W.J. Parker, H.T. Blair, and E.M. Hurley. 2005b. Implications of on-farm segregation for valuable milk characteristics. Agricult. Systems 85: 82-97.

Dooley, A.E., W.J. Parker, H.T. Blair, and N. Lopez-Villalobos. 2006. Selection and segregation of herds for a valuable milk trait. Livest. Sci. 102: 60-71.

Elgersma, A., G. Ellen, H. van der Horst, H. Boer, P.R. Dekker, and S. Tamminga. 2004. Quick changes in milk fat composition from cows after transition from fresh grass to a silage diet. Anim. Feed Sci. and Techn. 117: 13-27.

Elgersma, A., S. Tamminga, and G. Ellen. 2006. Modifying milk composition through forage. Anim. Feed Sci. and Techn. 131: 207-225.

Gibson, J. P. 1991. The potential for genetic change in milk fat composition. J. Dairy Sci. 74:3258–3266.

Heck, J.M.L., H.J.F. van Valenberg, J. Dijkstra, A.C.M. van Hooijdonk. 2009. Seasonal variation in the Dutch bovine raw milk composition. Submitted.

Laurenco, M., B. Vlaeminck, M. Bruinenberg, D. Demeyer, V. Fievez. 2005. Milk fatty acid composition and associated rumen lipolysis and fatty

acid hydrogenation when feeding forages from intensively managed or semi-natural grasslands. Anim. Res. 54: 471-484.

Maijala, K. 1995. Current possibilities of breeding to influence milk composition from the viewpoint of human nutrition and health. Page 23 in Proc. NJF/NMR—Seminar No. 252. Milk in Nutrition: Effects of Production and Processing Factors. Turku, Finland. S. Mantere-Alhonen and K. Maijala, University Printing House, Helsinki, Finland.

Maijala, K. 2000. Cow milk and human development and well being. Livest. Prod. Sci. 65: 1-18.

Mulder, H.A., and P. Bijma. 2006. Benefits of cooperation between breeding programs in the presence of genotype by environment interaction. J. Dairy Sci. 89: 1727-1739.

Palmquist, D.L. & A.D. Beaulieau. 1993. Feed and Animal factors influencing milk fat composition. J. Dairy Sci. 76: 1753-1771.

Parodi, P.W. 1997. Cows' milk fat components as potential anticarcinogenic agents. J. Nutr. 127: 1055-1060.

Rutten, M.J.M., P. Bijma, J.A. Woolliams, and J.A.M. van Arendonk. 2002. SelAction: software to predict selection response and rate of inbreeding in livestock breeding programs. J. Heredity 93(6): 456-458.

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

Soyeurt, H., P. Dardenne, F. Dehareng, C. Bastin, and N. Gengler. 2008. Genetic parameters of saturated and monounsaturated fatty acid content and the ratio of saturated to unsaturated FA in bovine milk. J. Dairy Sci. 91: 3611-3626.

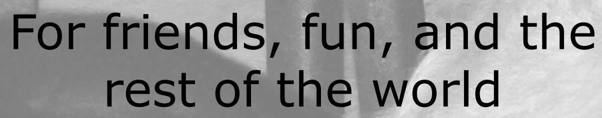
Soyeurt, H., P. Dardenne, F. Dehareng, G. Lognay, D. Veselko, M. Marlier, C. Bertozzi, P. Mayeres, and N. Gengler. 2006. Estimating fatty acid content in cow milk using mid-infrared spectrometry. J. Dairy Sci. 89: 3690-3695.

Van Knegsel, A.T.M, H. van den Brand, J. Dijkstra, W.M. van Straalen, M.J.W. Heetkamp, S. Tamminga, and B. Kemp. 2007a. Dietary energy source in dairy cows in early lactation: energy partitioning and milk composition. J. Dairy Sci. 90: 1467-1476.

Van Knegsel, A.T.M, H. van den Brand, E.A.A Graat, J. Dijkstra, R. Jorritsma, E. Decuypere, S. Tamminga, and B. Kemp. 2007b. Dietary energy source in dairy cows in early lactation: metabolites and metabolic hormones. J. Dairy Sci. 90: 1477-1485.

Walker, G.P., F.R. Dunshea, and P.T. Doyle. 2004. Effects of nutrition and management on the production and composition of milk fat and protein: a review. Australian J. of agricult. Res. 55: 1009-1028.

Williams, C.M. 2000. Dietary FA and human health. Ann. Zootech. 49: 165-180.



For friends, fun, and the
rest of the world

SUMMARY

Bovine milk is widely recognized as an important food containing many nutrients. It is a major contributor to human welfare and an important base for infant foods. The last 40 years milk quality is being increasingly questioned, especially the high amount of saturated FA and relatively low amounts of polyunsaturated FA. More information about milk fat properties and the possibilities to change milk fat composition should become available.This thesis is part of the Dutch Milk Genomics Initiative and aimed to unravel the genetic background of milk fat composition and to identify opportunities to change milk fat composition by genetic selection. Aims were to estimate genetic variance in milk fat composition, to identify other factors contributing to variation in milk fat composition, and to identify QTL that affect milk fat composition. For this purpose, three milk samples and one blood sample (DNA) of each of almost 2,000 first lactation cows were collected in winter, spring and summer 2005. All milk samples were analyzed for milk production traits, and winter milk samples were analyzed for fat composition. Later, also summer milk samples were analyzed for milk fat composition.

In chapter 2 we estimated genetic parameters for milk urea nitrogen (MUN) and evaluated its relationships with milk production traits. Heritability of MUN was low. Phenotypic correlations of MUN with milk production traits were close to zero, and genetic correlations were low to moderate and positive. This suggested that selection for milk production traits tends to increase yield of MUN. Heritabilities for milk production traits were comparable to literature and set the frame-work to which to relate the findings on new traits in later chapters.

In chapter 3 genetic parameters for major milk FA (FA) were estimated and the relationship of major milk FA with milk production traits was studied for winter milkfat composition. Results showed considerable genetic variation for all milk FA, with C4:0 to C16:0 having higher heritabilities (approximately 0.60) than C18 FA (approximately 0.25). High genetic correlations existed within the groups of short- and long-chain FA, which coincide with the origin of the FA and the biological pathways of synthesis. Selection for fat may lead to an increased proportion of C16:0 and a decreased proportion of unsaturated C18 FA.

In chapter 4 other sources of variation in milk fat composition were studied by estimating the effect of lactation stage and energy status throughout lactation on winter milk fat composition. Moderate changes of

0.5 to 1 phenotypic SD were found with lactation stage for most FA. Both lactation stage and energy balance significantly contributed to variation in milk fat composition and altered the activity of different fatty acid pathways, although their effect was smaller than genetic variation or variation due to herd.

In chapter 5 genetic correlations between winter and summer milk fat composition were studied to infer whether there are genetic differences in milk fat composition throughout the year, and to evaluate the effect of season on the estimated genetic parameters. Milk fat composition in summer is characterized by a more unsaturated composition, compared to winter milk samples, and was more variable. Variation due to herd was about 50% higher in summer than in winter, and was likely caused by changes in diet. Heritabilities for milk fat composition were comparable for winter and summer milk samples, and genetic correlations between winter and summer FA proportions were high. This indicated that despite the phenotypic differences, milk fat composition in winter and summer can be considered as the same genetic trait.

Chapters 6 and 7 present the results of a genome-wide scan to identify QTL for fatty acid composition of bovine milk. For short and medium chain FA, we detected 4 significant QTL on BTA6, 14, 19, and 26, and for long chain FA, we detected 3 significant QTL on BTA14, 15, and 16. The QTL explained 3 to 19 % of phenotypic variance in the affected traits. DGAT1 (BTA14) affected both short and medium, and long chain FA, whereas other QTL were specific to either short and medium, or long chain FA. This finding indicates that short and medium chain FA on one hand, and long chain FA on the other hand, undergo distinct processes of synthesis and metabolism. QTL that affect specific FA might increase our understanding of physiologic processes involved in fat synthesis and may aid in locating the underlying genes.

The final chapter discusses the opportunities for genetic selection. The different selection scenarios presented in the final chapter showed that there are opportunities to implement genetic improvement in milk fat composition. First, performance could be improved by differentiating cows on-farm and collect their milk in different tanks. Separate collection of several milk streams is already practiced on small scale initiatives in the Netherlands and might be enhanced by a faster analysis methods for milk fat composition, such as Infra Red technology. Secondly, specialized farms could differentiate within the existing breeding program and use bulls with best EBV on particular fatty acid proportions to further uti-

lize the genetic variation in milk fat composition currently present in the population. Thirdly, it is possible to select the entire population in one or more directions aimed to improve milk fat quality. This separate breeding program(s) could gradually evolve in the developing market, which already started by step 1 and 2. Finally, initiatives outside genetics, such as dietary measures, could be used to maximize cow performances in all scenarios presented.

SAMENVATTING

Melk wordt algemeen gezien as een belangrijke voedselbron met vele nutrienten. Het levert een grote bijdrage aan humaan welzijn en vormt een belangrijke grondstof voor voedsel voor kleine kinderen. De laatste 40 jaar wordt melkkwaliteit steeds vaker ter discussie gesteld, waarbij de nadruk ligt op de scheve verhouding van een relatief hoog aandeel verzadigde vetzuren en een relatief laag aandeel meervoudig onverzadigde vetzuren. Meer informatie dient beschikbaar te komen over melkvet eigenschappen en de mogelijkheden om melkvetsamenstelling te veranderen. Dit proefschrift is onderdeel van het Nederlandse Milk Genomics Initiatief. Doelstellingen van dit proefschrift waren ondermeer meer kennis te verkrijgen over de genetische achtergrond van melkvetsamenstelling en het identificeren van de mogelijkheden om melkvetsamenstelling genetisch, met behulp van fokkerij, te veranderen. Dit wordt bereikt door het schatten van genetische variatie in melkvetsamenstelling, het identificeren van andere factoren die bijdragen aan variatie in melkvetsamenstelling en het identificeren van QTL die melkvetsamenstelling beinvloeden.

Verzameld materiaal omvat drie melkmonsters en een bloedmonster (DNA) van bijna 2000 koeien in eerste lactatie, verzameld in de winter, lente en vroege zomer van 2005. Alle melkmonsters werden geanalyseerd voor melkproductie kenmerken en van de winter monsters werd ook de melkvetsamenstelling bepaald. Later werd ook vetsamenstelling van zomer monsters bepaald.

In hoofdstuk 2 werden genetische parameters geschat voor melk ureum (MUN) en werden relaties van ureum met melkproductie kenmerken geevalueerd. De erfelijkheidsgraad van MUN was laag. Fenotypische correlaties tussen MUN en melkproductie kenmerken waren nul en genetische correlaties waren laag tot gemiddeld en positief. Dit suggereerde dat selectie op melkproductie kenmerken leid tot een verhoogd ureum getal in de melk. Erfelijkheidsgraden voor melkproductie kenmerken waren vergelijkbaar met literatuur en vormden de basis om bevindingen in volgende hoofdstukken aan te relateren.

In hoofdstuk 3 werden genetische parameters voor de belangrijkste melkvetzuren geschat en werd, op basis van winter melkmonsters, de relatie van deze vetzuren met melkproductie kenmerken bestudeerd.

Uit de resultaten bleek dat er aanzienlijke genetische variatie bestond voor alle melkvetzuren, waarbij C4:0 tot C16:0 hogeren erfelijkheidsgraden hadden (0.60) dan C18 vetzuren (0.25). Genetische correlaties waren

hoog binnen de korte keten vetzuren en binnen de lange keten vetzuren, wat overeenkomt met de herkomst en fysiologie van de vetzuren. Selectie op vet kan leiden tot een verhoogd aandeel C16:0 en een verlaagd aandeel C18 vetzuren in melk.

In hoofdstuk 4 werden andere bronnen van variatie in melkvetsamenstelling geanalyseerd door het effect van lactatiestadium en energy status op wintermelkvetsamenstelling te schatten. Vetzuren veranderen in het algemeen 0.5 tot 1 standaard deviatie gedurende lactatie. Zowel lactatiestadium als energy status had een significante bijdrage aan variatie in melkvetsamenstelling en had een effect op de activiteit van verschillende vetzuursynthese systemen, maar beide factoren hadden een kleiner effect op variatie dan genetica en bedrijf.

In hoofdstuk 5 werden genetische correlaties tussen winter en zomer melkvetsamenstelling bestudeerd om te bepalen of er genetische verschillen tussen seizoenen bestaan en om te evalueren wat het effect van seizoen is op geschatte genetische parameters. Melkvetsamenstelling in de zomer wordt gekarakteriseerd door een meer onverzadigde compositie en grotere variatie tussen dieren en bedrijven, vergeleken met wintermelk. Bedrijfsvariatie was ongeveer 50% hoger in de zomer dan in de winter en werd vermoedelijk veroorzaakt door verandering in dieet. Erfelijkheidsgraden voor melkvetsamenstelling waren vergelijkbaar tussen winter en zomer en genetische correlaties tussen winter en zomer vetzuur proporties waren hoog. Dit was een indicatie dat ondanks grote fenotypische verschillen, melkvetsamenstelling in winter en zomer genetisch gezien hetzelfde kenmerk is.

Hoofdstuk 6 en 7 presenteren de resultaten van een genoomwijde scan om QTL te identificeren met een effect op melkvetsamenstelling. Voor korte en medium keten vetzuren werden vier significante QTL gevonden op BTA6, 14, 19 en 26. Voor lange keten vetzuren werden drie significante QTL gevonden op BTA14, 15 en 16. Deze QTL verklaarden 3 tot 19% van de fenotypische variatie in de betreffende kenmerken. DGAT1 (BTA14) had een effect op zowel korte, medium en lange keten vetzuren, maar de andere QTL waren specifiek ofwel op korte en medium keten vetzuren, ofwel op lange keten vetzuren. Dit gaf aan dat korte en medium keten vetzuren aan de ene kant en lange keten vetzuren aan de andere kant, verschillende processen van synthese en metabolisme ondergaan. QTL die specifieke vetzuren beinvloeden kunnen de kennis verhogen van fysiologische processen betrokken bij melkvetsynthese en kunnen bijdragen aan de identificatie van onderliggende genen.

Het laatste hoofdstuk bediscussiert de mogelijkheden voor genetische selectie. De verschillende selectie strategieen die in het laatste hoofdstuk gepresenteerd worden, laten zien dat er mogelijkheden zijn om genetische verbetering voor melkvetsamenstelling te implementeren. Als eerste kan de prestatie worden verbeterd door koeien binnen (en tussen) bedrijven te differentieren en melk gescheiden op te halen. Gescheiden verzameling van verschillende melkstromen wordt in Nederland al op kleine schaal toegepast en kan mogelijk verder gestimuleerd worden wanneer snellere analyse methoden voor melkvetsamenstelling beschikbaar komen, zoals infrarood technieken. Ten tweede kunnen bedrijven met een gespecialiseerd productiedoel differentieren binnen het huidige fokprogramma en allen stieren gebruiken met de hoogste fokwaarden voor bepaalde vetzuren. Dit verhoogt efficient gebruik van de genetische variatie in melkvetsamentelling die op dit moment in de Nederlandse populatie aanwezig is. Ten derde kan de volledige populatie in een of meerdere richtingen geselecteerd worden met als doel het verbeteren ven de melkvet kwaliteit. Dit aparte fokprogramma kan geleidelijk ontstaan in de ontwikkelende markt, zoals die is ingezet met stap 1 en twee. Als laatste kunnen nieuwe initiatieven buiten de genetica, zoals dieet aanpassingen, gebruikt worden om koe-prestatie te maximaliseren.

CURRICULUM VITAE

Wendy Marianne Stoop was born April 22 1982 in Ermelo, the Netherlands. She completed her V.W.O. at the Hendrik Pierson College in Zetten in 2000, for the courses Dutch, English, biology, chemics, physics, mathematics A, mathematics B, and history. She studied Animal Sciences at Wageningen University, where she was the first to obtain an international B.Sc degree in Animal Science. In November 2004 she graduated and received her Master degree in Animal Science and Aquaculture, specializing in Animal Breeding and Genetics and in Ethology. Already in October 2004 she had started her PhD studies at Wageningen University, within the Animal Breeding and Genomics group, which resulted in this thesis. Nowadays Marianne is working as data analyst/ programmer at the Animal Evaluation Unit of CRV Holding, the main cattle breeding organization in the Netherlands. Marianne currently resides in Renkum with her daughter Lily.

LIST OF PUBLICATIONS

Peer-reviewed papers

Stoop, W.M., H. Bovenhuis, and J.A.M. van Arendonk. 2007. Genetic parameters for milk urea nitrogen in relation to milk production traits. J. Dairy Sci. 90: 1981-1986.

Stoop, W.M., J.A.M. van Arendonk, J.M.L. Heck, H.J.F. van Valenberg, and H. Bovenhuis. 2008. Genetic parameters for major milk FA and milk production traits of Dutch Holstein Friesians. J. Dairy Sci. 91: 385-394.

Stoop, W.M., H. Bovenhuis, J.M.L. Heck, and J.A.M. van Arendonk. 2009. Effect of lactation stage and energy status on milk fat composition of Holstein-Friesian cows. J. Dairy Sci. 92: in press.

Stoop, W.M., H. Bovenhuis, and J.A.M. van Arendonk. 2009. Genetic correlations between winter and summer milk fat composition. Concept.

Stoop, W.M., A. Schennink, M.H.P.W. Visker, E. Mullaart, J.A.M. van Arendonk, and H. Bovenhuis. 2009. Genome-wide scan for bovine milk fat composition I. QTL for short and medium chain fatty acids. Submitted.

Schennink, A., W.M. Stoop, M.H.P.W. Visker, J.J. van der Poel, H. Bovenhuis, and J.A.M. van Arendonk. 2009. Genome-wide scan for bovine milk fat composition II. QTL for long chain fatty acids. Submitted.

Schennink, A., W.M. Stoop, M.H.P.W. Visker, J.M.L. Heck, H. Bovenhuis, J.J. van der Poel, H.J.F. van Valenberg, and J.A.M. van Arendonk. 2007. DGAT1 underlies large genetic variation in milk-fat composition of dairy cows. Animal Genetics 38 (5): 467-473.

Abstracts, posters, and conference papers

Stoop, W.M., H. Bovenhuis, and J.A.M. van Arendonk. 2006. Genetic parameters of milk urea in relation to milk production traits. In : 8th World Congress on Genetics Applied to Livestock Production, Belo Horizonte, Brazil, August 13-18, 2006.

Stoop, W.M., A. Schennink, M.H.P.W. Visker, E. Mullaart, J.A.M. van Arendonk, and H. Bovenhuis. 2008. Genome-wide scan for short and medium chain milk fatty acids. XXXI Conference of the International Society for Animal Genetics (ISAG), Amsterdam, July 2008.

Stoop, W.M., J.A.M. van Arendonk, J.M.L. Heck, H.J.F. van Valenberg, and H. Bovenhuis. 2008. Genetic parameters for major milk fatty acids and milk production traits of Dutch Holstein-Friesians. In: Proceedings of the 59th Conference of the European Association for Animal Production (EAAP), Vilnius, Lithuania, 24 - 27 August, 2008.

Miscellaneous

Stoop, W.M., H. Bovenhuis, and J.A.M. van Arendonk. 2006. Genetic and herd effects on milk composition. Milk Genomics and Human Health congress, Brussels, Belgium, 21-23 September, 2006.

Stoop, W.M., A. Schennink, H. Bovenhuis, M.P.H.W. Visker, E. Mullaart, J.J. van der Poel, and J.A.M. van Arendonk. 2008. QTL for Milk Fat Composition: short,medium and long chain fatty acids. Milk Genomics and Human Health congress, Sydney, Australia, 14-16 October, 2008.

W.M. Stoop, J.A.M. van Arendonk, J.M.L. Heck, H.J.F. van Valenberg, H. Bovenhuis. 2008. Genetic Parameters for Major Milk Fatty Acids. Wageningen Institute for Animal Sciences Science Day, Wageningen, The Netherlands, 13 march, 2008.

TRAINING AND SUPERVISION PLAN – WIAS

The Basic Package	
WIAS Introduction Course 2005	
Course on philosophy of science and/or ethics 2005	
Subtotal Basic Package	**3**
Scientific Exposure	
International conferences	
WCGALP, Belo Horizonte, Aug 13-18 2006	
Milk Genomics and Human Health, Brussels, Sep 19-21 2006	
ISAG, Amsterdam, July 20-24 2008	
EAAP, Vilnius Lithuania, August 23-27 2008	
Milk Genomics and Human Health, Sydney, Oct 14-16 2008	
Seminars and workshops	
F&G connection days, Vught, November 2004/2006/2008	
NZO symposium: Zuivel, Ede, December 2004/2005	
Farm Genomics, Wageningen 2004	
WIAS science day, Wageningen, March 2006/2007/2008	
Seminar Biodiversity:..., Wageningen, December 2006	
NZO research middag, Wageningen, March 2006/2007/2008	
Presentations	
Genetic parameters of Milk Urea, WCGALP 2006 (oral)	
Genetic and Herd effects on milk composition, MGHH 2006 (poster)	
Genetic parameters of milk fat composition, EAAP 2008 (oral)	
QTL for milk fat composition:…, MGHH 2008 (poster)	
Genome-wide scan for short and medium chain milk FA, ISAG 2008 (poster)	*scholarship*
Genetic parameters of milk fat, WIAS science day 2008 (oral)	*Best presentation*
Subtotal International Exposure	**16**
In-Depth Studies	
Disciplinary and interdisciplinary courses	
The biological basis for improved management and selection tools, 10-14 oct 2005	
QTL detection and fine mapping in complex pedigrees, 17-21 oct 2005	
Molecular techniques in animal breeding 4-8 apr 2005	
Fortran95 for animal breeders, 11-15 jun 2007	
Linear Models 2-6 Jul 2007	
PhD students' discussion groups	
Quantitative Discussion: Falconer & Mackay: 20 hrs 2004/2005	

Quantitative Discussion: Lynch&Walsh: 20 hrs 2006/2007	
MSc level courses	
Biology, IVLOS, University Utrecht 2008	
Subtotal In-Depth Studies	**9**
Professional Skills Support Courses	
WIAS Course Techniques for writing and presenting a Scientific Paper 2006	
AV organiseren en begeleiden 2006	
WGS course Career Perspectives 2006	
cursus Hoorcollege geven 2008	
Writing and presenting scientific papers, EAAP 2008	
Subtotal Professional Skills Support Courses	**4**
Research Skills Training	
Preparing own PhD research proposal 2005	
Subtotal Research Skills Training	**3**
Didactic Skills Training	
Lecturing	
Animal Breeding and Genetics 2006/2008	
Supervising practicals and excursions	
Inleiding Dierwetenschappen 2005/2006	
Supervising theses	
Linda Roest, Msc minor 2007	
Douwe-Jan Sietsma, Bsc 2008	
Surya Prasad Paudel, Msc major 2008	
Tutorship	
Rianne Memelink 2005	
Subtotal Didactic Skills Training	**10**
Education and Training Total	**45**

ACKNOWLEDGMENTS

I know… Most of you will read this page as one of the first. It is relatively short, it's personal and you just might learn a creative new way of saying 'thank you', since it ususally involves an endless list of superlative forms of these two words.

The other chapters in this book are probably not really of interest to you; depending on your interest in milk genomics, you either already read them before or you never intend going to read them at all.

Did it ever occur to you that regardless of your interest in the topic, this remains the most important page? In the end - literally and figuratively - nothing else matters more than family and friends. So if you read this page as one of the first: well done, you know what really matters in life.

Know that I do not write (actually I do not write it all, I type it) this page for me, I write it for you. Yes you. The fact that you take the effort to read this, likely means you know me, you know my family, friends – in this world or Azeroth -, and colleagues, and very possibly be one of them. And so you have been involved in completing this thesis, in your own unique way.

Thank you.

J.R.R. Tolkien wrote his poem 'The Road goes ever on and on', and compared the road (of life) to a river: … 'He (Bilbo Baggings) often used to say there was only one Road; that it was like a great river: its springs were at every doorstep, and every path was its tributary.'

I have come a long way, where this PhD has proven a constant factor during the last four years. It is an encouraging thought that the path that leads through great challenges is the same that one day will turn towards new zeniths.

Special thanks are for Henk and Anke; for all the discussions, suggestions, and teamwork. For mum and dad; for your never-ending support. Michiel, thanks for your artistic input. Jeroen, thanks for evenings of home cinemas and introducing me into Draco Noir. And for Lily, for all those times I was away working when I rather had been home with you.

Marianne

COLOPHON

This thesis was financially supported by Wageningen University, Dutch Dairy Association NZO and breeding organization CRV, with additional funding from technology foundation STW for molecular work and QTL studies (chapter 6 and 7).

Cover design and chapter photos by Michiel Stoop – Mastarte, Nijmegen.

Cover photo:
Jeltje 272 (Himster Grandprix x Etazon Addison x Etazon Lord Lily).

Chapters 2, 3, 4, 6, and 7 in this thesis are printed with permission of Journal of Dairy Science.