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GENETIC POLYMORPHISMS IN THE *STEAROYL-CoA DESATURASE1 (SCD1)* GENE AND THEIR INFLUENCE ON THE CONJUGATED LINOLEIC ACID (CLA) AND MONOUNSATURATED FATTY ACIDS (MUFA) CONTENT OF MILK FAT OF CANADIAN HOLSTEIN AND JERSEY COWS

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ABSTRACT

Stearoyl-CoA desaturase1 (SCD1) catalyzes the synthesis of conjugated linoleic acid (CLA) and mono-unsaturated fatty acids (MUFA) in the mammary gland of ruminant animals. We hypothesized that single nucleotide polymorphisms (SNPs) in the coding region, 5′ and 3′ untranslted regions (UTRs) of the *SCD1* gene would influence the activity of *SCD1* enzyme and consequently account for some within-breed variations in milk CLA and MUFA. Sequence analysis of the coding region of the *SCD1* gene of Jerseys and Holsteins revealed c.702A→G, c.762T→C and c.878C→T SNPs in exon 5 in both breeds and c.435G→A in exon 3 in Holsteins. The SNPs resulted in: *A* ($G_{435}A_{702}T_{762}C_{878}$), *A1* ($A_{435}A_{702}T_{762}C_{878}$), *B* ($G_{435}G_{702}C_{762}T_{878}$) and *B1* ($A_{435}G_{702}C_{762}T_{878}$) coding variants in Holsteins and only variants *A* and *B* in Jerseys. Only SNP 878C→T resulted in a non-synonymous codon change resulting in p.293Ala and p.293Val protein variants or alleles at the *SCD1* locus. Subsequent association studies found significantly higher C10 index, C12 index and C14 index and consequently higher concentrations of C10:1 and C12:1 in p.293AA cows compared to the p.293VV cows in both breeds. The *SCD1* genotype had no influence on concentrations of C14:1, C16:1, C18:1 and CLA in both breeds.

Sequence analysis of the 5′ and 3′ UTRs revealed no SNPs in the 5′UTR and a total of 14 SNPs in the 3′UTR of both breeds. The SNPs were in complete linkage disequilibrium resulting in 3 haplotypes or regulatory variants: H1 ($G_{1571}G_{1644}C_{1763}C_{2053}A_{2584}A_{3007}C_{3107}G_{3208}T_{3290}G_{3497}G_{3682}A_{4399}C_{4533}G_{4881}$), H2 ($G_{1571}G_{1644}A_{1763}C_{2053}A_{2584}G_{3007}C_{3107}G_{3208}T_{3290}G_{3497}G_{3682}A_{4399}C_{4533}G_{4881}$) and H3 ($T_{1571}C_{1644}A_{1763}T_{2053}G_{2584}G_{3007}T_{3107}A_{3208}C_{3290}A_{3497}A_{3682}T_{4399}T_{4533}A_{4881}$) in Holsteins and only H1 and H3

variants in Jerseys. A subsequent association study involving 862 Holstein cows, found the H1 regulatory variant to be associated with higher C10 and C12 desaturase indices and consequently with higher concentrations of C10:1 and C12:1 compared with the H3 variant. The effects of the H2 variant were intermediate to those of H1 and H3. 3′UTR genotype had no influence on the concentrations of C14:1, C16:1, C18:1 and CLA. The concentrations of C10:1 and C12:1 in milk fat could therefore be due to effects of SNPs in the open reading frame and the 3′UTR regions of the *SCD1* gene. These results indicate that SNPs in the coding and 3′UTR regions of the *SCD1* gene could be used as markers for genetic selection for increased C10:1 and C12:1 contents of milk.

RÉSUMÉ

La Stearoyl-CoA desaturase (*SCD1*) catalyse la synthèse de l'acide linoléique conjuguée (CLA) et des acides gras mono-saturés (MUFA) dans la glande mammaire des animaux ruminants. Nous faisons l'hypothèse que des polymorphismes à nucléotide unique dans la séquence codante, les séquences non traduites 5' et 3' (UTRs) du gène *SCD1* doit influencer l'activité de l'enzyme *SCD1* et être responsable de certaines variations intra géniques dans le CLA et le MUFA du lait.

Une analyse de séquence codante du gène *SCD1* de Jerseys et Holsteins révèle 702A→G, 762T→C et 878C→T SNPs dans l'exon 5 dans les deux races et 435G→A dans l'exon 3 chez les Holsteins. Les SNPs ont été résulté en les variantes codantes A (G435A702T762C878), A1 (A435A702T762C878), B (G435G702C762T878) et B1 (A435G702C762T878) chez les Holsteins et seulement les variantes A et B chez les Jerseys. Seuls SNP 878C→T a résulté en un changement de codon non-synonyme résultant en variantes protéines p.293Ala and p.293Val ou des allèles au locus *SCD1*. Des études d'associations subséquentes ont découvert des index C10, C12 et C14 et par conséquent des concentrations élevées de C10:1 and C12:1 chez des vaches p.293AA comparativement aux vaches p.293VV chez les deux races. Le génotype *SCD1* n'a eu aucune influence sur les concentrations C14:1, C16:1, C18:1 et CLA chez les deux races.

Les analyses de séquence des UTRs 5' et 3' n'ont été révélé aucuns SNP chez les UTR 5' et un total de 15 SNPs chez les UTR 3' des deux races. Les SNPs étaient en déséquilibre complet de liaison résultant en 3 haplotypes ou variantes régulatrices: H1 ($G_{1571}G_{1644}C_{1763}C_{2053}A_{2584}A_{3007}C_{3107}G_{3208}T_{3290}G_{3497}G_{3682}A_{4399}C_{4533}G_{4881}$), H2 ($G_{1571}$ G_{1644} $A_{1763}C_{2053}A_{2584}G_{3007}C_{3107}G_{3208}T_{3290}G_{3497}G_{3682}A_{4399}C_{4533}G_{4881}$) and H3 ($T_{1571}C_{1644}A_{1763}$

$T_{2053}G_{2584}G_{3007}T_{3107}A_{3208}C_{3290}A_{3497}$ $A_{3682}T_{4399}T_{4533}A_{4881}$) chez les Holsteins et seulement les variantes H1 et H3 chez les Jerseys. Une étude postérieure d'association impliquant 862 vaches Holstein, a été révélé la variante régulatrice H1 comme étant associée à des indices désaturés C10 et C12 élevés et par conséquent avec des concentrations élevées de C10:1 et C12:1 comparativement à la variante H3. Les effets de la variante H2 étaient intermédiaires à ceux de H1 et H3. Le génotype 3'UTR n'a eu aucune influence sur les concentrations de C14:1, C16:1, C18:1 et CLA. Les concentrations de C10:1 et C12:1 dans la matière grasse du lait peuvent être aux effets combinés des SNPs à la fois dans le cadre ouvert de lecture et les séquences 3'UTR du gène *SCD1*.

Ces résultats indiquent que les SNPs dans la région codante et la séquence 3'UTR du gène *SCD1* pourraient être utilisés pour la sélection génétique afin d'obtenir une teneur élevée en C10:1 et C12:1 du lait.

DEDICATION

I dedicate this work to the memories of my late grandparents, Maipelo Kgwatalala and Moikwathai J. Kgwatalala who developed my love for Agriculture and always encouraged me to work hard to achieve my goals.

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CONTRIBUTIONS TO KNOWLEDGE

1. Chapter II: The study documented SNPs in the coding region of *stearoyl-CoA desaturase1 (SCD1)* gene in Canadian Holstein and Jersey cows and the resulting sequence variants and protein variants (alleles) at the *SCD1* locus. A novel mutation was found in exon 3 of the *SCD1* gene in Canadian Holsteins.

2. Chapter III: For the first time gene and genotypic frequencies at the *SCD1* locus in Canadian Holsteins were determined. The influences of *SCD1* genotype and stage of lactation on milk fatty acid composition were demonstrated through an association study.

3. Chapter IV: This is the first study to report gene and genotypic frequencies at the *SCD1* locus in Canadian Jersey Cows. This is also the first study to ever report the influence of *SCD1* genotype on the fatty acid composition of milk in Jerseys.

4. Chapter V: The study documented all SNPs in the 3′UTR of the *SCD1* gene and the resulting sequence variants in Canadian Holsteins and Jerseys. Three novel SNPs were found in the 3′UTR of the *SCD1* gene in both breeds and a novel 3′UTR sequence variant was found in Canadian Holsteins. For the first time, the study reported the influence of 3′UTR genotype on the fatty acid composition of milk in Holsteins.

FORMAT OF THESIS

This thesis has been written in the format of manuscripts submitted to scientific journals in accordance with the "Guidelines for Thesis Preparation, C: Manuscripts-based Thesis," which states,

Candidates have the option of including, as part of the thesis, the text of one or more papers submitted, or to be submitted, for publication, or the clearly- duplicated text (not the reprints) of one or more published papers. These texts must conform to the "Guidelines for Thesis Preparation" with respect to the font size, line spacing and margin sizes and must be bound together as an integral part of the thesis.

The thesis must be more than a collection of manuscripts. All components must be integrated into a cohesive unit with a logical progression from one chapter to the next. In order to ensure that the thesis has continuity, connecting texts that provide logical bridges preceding and following each manuscript are mandatory.

The thesis must conform to all other requirements of the "Guidelines for Thesis Preparation" in addition to the manuscripts.

The thesis must include the following:

1. a table of contents;

2. a brief abstract in both English and French;

3. an introduction which clearly states the rational and objectives of the research;

4. a comprehensive review of the literature (in addition to that covered in the introduction to each paper;

5. a final conclusion and summary;

6. a thorough bibliography;

7. Appendix containing an ethics certificate in the case of research involving human or animal subjects, microorganisms, living cells, other biohazards and/or radioactive material.

As manuscripts for publication are frequently very concise documents, where appropriate, additional material must be provided (e.g., in appendices) in sufficient detail to allow a clear and precise judgement to be made of the importance and originality of the research reported in the thesis.

In general, when co-authored papers are included in a thesis the candidate must have made substantial contribution to all papers included in the thesis. In addition, the candidate is required to make an explicit statement in the thesis as to who contributed to such work and to what extent. This statement should appear in single section entitled "Contributions of Authors" as a preface to the thesis. The supervisor must attest to the accuracy of the statement at the doctoral oral defence. Since the task of the examiners is made more difficult in these cases, it is the candidate's interest to clearly specify the responsibilities of all the authors of the co-authored papers.

When previously published copyright material is presented in a thesis, the candidate must include signed waivers from the publishers and submit these to the Graduate and Postdoctoral Studies Office with the final deposition, if not submitted previously. The candidate must also include signed waivers from the co-authors of unpublished manuscripts".

CONTRIBUTIONS OF AUTHORS

Four co-authored manuscripts submitted for publication are included in the thesis.

Authors of Manuscript 1 (Chapter II): P. Kgwatalala, E. M. Ibeagha-Awemu, J. F. Hayes, and X. Zhao.

P. Kgwatalala carried out all experiments and part of data analysis and E. M. Ibeagha-Awemu did the other part of data analysis. J. F. Hayes and X. Zhao assisted P. Kgwatalala in the experimental design. The manuscript was written by P. Kgwatalala and revised by the other three co-authors.

Authors of Manuscript 2 (Chapter III): P. Kgwatalala, E. M. Ibeagha-Awemu, A. F. Mustafa and X. Zhao.

P. Kgwatalala carried out all experiments and part of data analysis and E. M. Ibeagha-Awemu did the other part of data analysis. A. F. Mustafa and X. Zhao assisted P. Kgwatalala in the experimental design. The manuscript was written by P. Kgwatalala and reviewed by the other three co-authors.

Authors of Manuscript 3 (Chapter IV): P. Kgwatalala, E. M. Ibeagha-Awemu, A. F. Mustafa and X. Zhao.

P. Kgwatalala carried out all experiments and part of data analysis and E. M. Ibeagha-Awemu did the other part of data analysis. A. F. Mustafa and X. Zhao assisted P. Kgwatalala in the experimental design. The manuscript was written by P. Kgwatalala and revised by the other three co-authors.

Authors of Manuscript 4 (Chapter V): P. Kgwatalala, E. M. Ibeagha-Awemu, J. F. Hayes, and X. Zhao.

P. Kgwatalala carried out all experiments and part of data analysis and E. M. Ibeagha-Awemu did the other part of data analysis. J. F. Hayes and X. Zhao assisted P.

Kgwatalala in the experimental design. The manuscript was written by P. Kgwatalala and reviewed by the other three co-authors.

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LIST OF ABREVIATIONS

CLA.....conjugated linoleic acid

DGAT.....diacyl glycerol acyl transferase

DHA.....decosahexaenoic acid

DNA.....deoxyribonucleic acid

EPA.....eicosapentaenoic acid

HDL.....high density lipoprotein

LA.....linoleic acid

LDL.....low density lipoprotein

LNA.....linolenic acid

MUFA.....monounsaturated fatty acids

ORF.....open reading frame

PUFA.....polyunsaturated fatty acids

QTL.....quantitative trait locus

RNA.....ribonucleic acid

SCD.....stearoyl-CoA desaturase

SFA.....saturated fatty acids

SNP.....single nucleotide polymorphism

SREBP.....sterol regulatory element binding protein

SSR.....simple sequence repeats

STR.....short tandem repeats

TMR.....total mixed rations

TVA.....transvacennic acid

UTR.....untranslated region

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CHAPTER I. GENERAL INTRODUCTION AND LITERATURE REVIEW

GENERAL INTRODUCTION

Milk is an important dietary source of nutrients, providing energy, high quality protein, and a variety of vitamins and minerals. Bovine milk typically contains 70% saturated fatty acid (SFA), 25% monounsaturated fatty acids (MUFA) and 5% polyunsaturated fatty acids (PUFA) (Grummer, 1991). Results from epidemiologic, clinical and animal studies indicate that high quantities of dietary saturated fatty acids, as is the case with bovine milk, can lead to an increase in serum total cholesterol and low-density lipoprotein (LDL) cholesterol and consequently increase risk for atherosclerosis and coronary heart disease in humans (Kromhout et al., 2002). To the contrary, MUFA and PUFA have been reported to lower both serum total cholesterol levels and LDL cholesterol and therefore lower the risk of coronary heart diseases (Mensink et al., 2003). The cholesterol raising effects of saturated fatty acids in bovine milk are confined to the medium-chain fatty acids; lauric (C12:0), myristic (C14:0) and palmitic (C16:0) acids while the short-chain (C4:0-C8:0) and some medium-chain (C10:0) saturated fatty acids are reported neutral, with C18:0 reported as effective as C18:1 in reducing plasma cholesterol levels (Bonanome and Grundy, 1988). A few saturated fatty acids with some cholesterol raising effects tarnished the image of milk as a complete and well-balanced food until the discovery of omega-3 and omega-6 fatty acids, and conjugated linoleic acid (CLA). CLA is of interest to human health because it may be anticarcinogenic, anti-atherosclerotic, decrease fat accumulation and can modulate the immune response (Dhiman et al., 2005) Increasing the MUFA and CLA content of bovine milk at the expense of saturated fatty acids could be an important selection objective. Although most

of the current research is focused on changing the fatty acid composition of milk through environmental manipulation (feeding), selection for increased MUFA and CLA content of milk can be quite effective due to the moderate heritability of the trait. Changing the fatty acid profile of milk through genetic selection will have permanent and accumulative effects, with improvements in milk composition every generation.

In cattle, the gene with marked influence on the CLA and MUFA content of milk, the *Stearoyl-CoA desaturase1 (SCD1)* gene has already been identified and sequenced DDBJ (Accession No. AB075020). The *SCD1* gene has been mapped to chromosome 26 and codes for the enzyme stearoyl-CoA desaturase1 or delta-9-desaturase that has been associated with *de novo* synthesis of CLA and MUFA from their saturated counterparts in the mammary gland and adipose tissues of ruminants. Single nucleotide polymorphisms have been identified in the *SCD* gene in sheep, pigs and beef cattle. The single nucleotide polymorphisms in beef cattle have been reported to influence MUFA content and melting point of intra-muscular fat of Japanese Black cattle (Taniguchi et al., 2004). There is less information regarding SNPs in *SCD1* gene in dairy cattle and the possible influence of such SNPs in the fatty acid composition of milk fat. The purpose of this study was therefore to identify the polymorphisms in the *SCD1* gene and to determine the contributions of these polymorphisms towards the fatty acid composition of milk fat in Canadian Holstein and Jersey cows. The results from this study could lay down the foundation for possible genetic selection to improve the fatty acid composition or nutritional quality of milk.

REVIEW OF LITERATURE

1. Milk Fat

1.1. Biosynthesis of milk fat

Bovine milk typically contains about 4% milk fat (40g fat/ L of milk) comprised almost entirely (~98%) of triacylglycerol and the balance are monoacylglycerol, free fatty acids, phospholipids, sterols as well as trace amounts of fat soluble vitamins, β carotene and fat soluble flavoring compounds. Milk fat is synthesized in the mammary gland from glycerol and free fatty acids and is known to contain more than 500 individual fatty acids (Hermansen, 1995), although only considerable amounts of 12-15 fatty acids are found in any single fat (Mansbridge and Blake, 1997). The fatty acids found in milk are almost equally derived from either *de novo* synthesis in the mammary gland from circulating acetate and β-hydroxybutyrate or directly from preformed fatty acids in blood lipoproteins derived mainly from digestion and absorption of dietary fat or mobilization from body reserves (Mansbridge and Blake 1997).

De novo fatty acid synthesis in the mammary gland produces the majority of the saturated fatty acids from C4-C14 and half of palmitic acid (C16:0) catalyzed by acetyl Co-A carboxylase and fatty acid synthase (Mansbridge and Blake, 1997). The hydrolysis of triglycerides in LDL and very low density lipoprotein (VLDL) or chylomicrons by lipoprotein lipase supply preformed fatty acids found in milk. The major preformed fatty acids are C16:0, C18:0 and C18:1 (Mansbridge and Blake, 1997). Inside the mammary gland, preformed fatty acids undergo very little further modifications except for extensive desaturation of medium- and long-chain saturated (SFA) by SCD1 (Bickerstaffe and Annison, 1968). Polyunsaturated fatty acid (PUFA) tend to be concentrated in the

phospholipids and cholesterol esters of the HDL (very low triglycerol content) and the uptake of fatty acids from HDL by the mammary gland is very poor which may partially explain low levels of PUFA in bovine milk (Mansbridge and Blake, 1997).

Bovine milk also contains significant amounts of odd- and branched-chain fatty acids (OBCFA) mainly derived from the digestion of microbes leaving the rumen by the host animals. According to Vlaeminck et al. (2006) the main OBCFA in milk are isomers of pentadecanoic acid (C15:0, *iso* C15:0 and *anteiso* C15:0) and heptadecanoic acid (C17:0, iso C17:0, anteiso C17:0) and trace amounts of *iso* C14:0 and *iso* C16:0.

1.2. Fatty acid composition of bovine milk

A list of principal fatty acids found in Holstein and Jersey milk under two different feeding regimens is shown in Table 1.1. On average bovine milk contains 70% saturated fatty acids (SFA), 25% monounsaturated fatty acids (MUFA), and 5% polyunsaturated fatty acids (PUFA) (Grummer, 1991) including CLA. CLA is a collective term for the group of geometric and positional isomers of linoleic acid or octadecadienoate (C18:2) in which the constituent double bonds are separated by a single carbon-carbon bond instead of a methylene group. A total of 24 different CLA isomers have been found in bovine milk and the major isomers are in the following rank order: *cis*-9, *trans*-11 CLA (also called rumenic acid) > *trans*-7, *cis*-9 CLA > *cis*-11, *trans*-13 CLA > *cis*-8, *trans*-10 CLA > *trans*-10, *cis*-12 CLA > other isomers (Ma et al., 1999, Fritsche et al., 1999 and Kramer et al., 1998). CLA will be further discussed later in details.

Table 1.1 Least squares means for fatty acid composition (g/100g of total fatty acids) of Holstein and Jersey cows consuming pasture or a total mixed ration

	Pasture		Total Mixed Ration	
Fatty acid	**Holstein**	**Jersey**	**Holstein**	**Jersey**
C4:0	1.06	1.08	1.05	1.09
C6:0	1.56	1.74	1.47	1.71
C8:0	1.00	1.23	0.92	1.17
C10:0	2.22	2.89	2.00	2.68
C12:0	2.65	3.48	2.34	3.14
C14:0	10.22	11.47	9.43	10.44
C14:1	0.80	0.82	0.59	0.60
C16:0	31.19	31.53	31.67	31.32
C16:1	1.25	1.07	1.12	1.00
C18:0	13.35	13.45	15.36	15.47
C18:1	23.09	19.44	23.28	20.87
C18:2	1.82	1.86	2.49	2.49
CLA	0.72	0.59	0.41	0.32
C18:3	0.71	0.75	0.38	0.37
C20:0	0.13	0.11	0.17	0.17

Adapted from White et al. (2001)

1.2.1 Saturated fatty acids

Saturated fatty acids contain the maximum number of hydrogen atoms that their molecular structure can permit and are characterized by the absence of double bonds in their molecular structure. Saturated fatty acids are the most stable of all classes of fatty acids found in bovine milk and have relatively high melting point. The saturated fatty acids found in bovine milk vary in chain length from 4 to 20 carbon atoms. On average bovine milk fat contains about 7% short-chain saturated fatty acids (C4:0 to C8:0), 15-

20% medium-chain saturated fatty acids (C10:0 to C14:0) and 42-48% long-chain saturated fatty acids (C16:0 and higher). The most abundant saturated fatty acids in bovine milk are C16:0, C14:0 and C18:0. It is now commonly accepted that the cholesterol-raising effects of saturated fatty acids in milk are confined to lauric (C12:0), myristic (C14:0) and palmitic acid (C16:0). C18:0 has been reported as effective as C18:1 in reducing plasma cholesterol levels (Bonanome and Grundy, 1988; Keys et al., 1965). In addition, butyric acid (C4:0) is known to modulate gene function and may play a role in cancer prevention while C8:0 and C10:0 may have antiviral activities and C8:0 in particular has been reported to delay tumor growth (Thormar et al. 1994; German, 1999). Dietary short- and medium-chain fatty acids are predominantly absorbed into the hepatic portal vein and metabolized in the liver and long chain fatty acids, in contrast, are mostly incorporated into lipoprotein lipids by the intestines and secreted into the lymphatic system and these differences in metabolism between short- and medium-chain fatty acids and long chain fatty acids may account for their contrasting effects on plasma cholesterol concentrations in humans (Leveille et al. 1967).

1.2.2. *Cis*- unsaturated fatty acids

Unsaturated fatty acids are characterized by the presence of one (monounsaturated fatty acids, MUFA) or more (polyunsaturated fatty acids, PUFA) double bonds in their molecular structure and generally have a lower melting point than their saturated counterparts. Most of the unsaturated fatty acids in bovine milk are synthesized in the intestines and the mammary gland from their saturated counterparts through the action of SCD. The presence of a double bond in unsaturated fatty acids makes them very unstable and highly susceptible to oxidative stress, which may result in off-flavors or rancidity in milk. Unsaturated fatty acids are further categorized into *cis* and *trans* fatty acids on the

basis of their configuration and *cis*- unsaturated fatty acids are by far the most abundant in milk. Oleic acid (C18:1 *cis*-9) is the single most abundant *cis*-MUFA accounting for 15%-25% of the total fatty acids and more than half of all the unsaturated fatty acids in bovine milk. Other *cis*-MUFA found in bovine milk include C16:1 (1%), C14:1 (1%), C12:1 (>0.5%) and C10:1 (>1%). *Cis*-MUFA are considered beneficial for human health and MUFA-rich diets have been reported to lower both plasma total cholesterol, LDL-cholesterol and triacylglycerol concentrations and lowers the risk for coronary artery disease (Kris-Etherton et al., 1999; Mensink et al., 2003).

PUFA are found at relatively low concentrations in bovine milk fat due to their extensive biohydrogenation in the rumen. PUFA in bovine milk fat are almost exclusively the essential fatty acids linoleic acid (C18:2 omega-6) comprising 1-3% of total milk fatty acids and alpha-linolenic acid (C18:3 omega -3) comprising 0.5 -1.5% of total fatty acids in milk or their derivatives. Linoleic acid can be converted into arachidonic acid (20:4 omega-6) and linolenic acid into eicosapentaenoic acid (EPA) and decosahexaenoic acid (DHA) and further converted into eicosanoids, all of which are found in small but significant quantities in bovine milk (Haug et al., 2007). PUFA have also been reported to participate in signal transduction and modulation of gene expression and together with MUFA help maintain the fluidity of cell membranes and the fluidity of milk (Jump and Clarke, 1999; Bagga et al., 2003).

Both MUFA and PUFA are important components of cellular membranes but are highly susceptible to oxidative stress with free radicals and secondary peroxidation products which may be detrimental to cellular proteins and DNA and may contribute to the incidence of cancer and mitochondrial DNA mutations (Bartsch et al. 2002; Pamplona et al., 2004). Oleic acid has been reported more stable to oxidation than omega-3 and

omega-6 fatty acids and a diet with high MUFA to PUFA ratio has been found to give better protection against cardiovascular diseases than a diet rich in PUFA (De lorgeril et al. 1994). According to Haug et al. (2007), a favourable meal should be rich in oleic acid, have a low ratio between omega-6 and omega-3 fatty acids (1-2:1) and milk fits this description probably better than any other food item.

1.2 3. *Trans*-unsaturated fatty acids

Bovine milk also contains appreciable quantities of *trans* fats formed as a result of incomplete biohydrogenation of unsaturated dietary lipids in the rumen. *Trans* fats have attracted a lot of attention because of the adverse nutritional or health effects associated with industrially produced *trans* fat. The main *trans* fat in bovine milk is C18:1 t-11 or vaccenic acid constituting 2-4% of the total fatty acids content and 30-60% of all the trans fat in bovine milk (Precht and Molktentin, 1996, Roche et al., 2005). Vaccenic acid serves as a precursor for the synthesis of CLA and approximately 90% of the CLA in milk fat is produced endogenously in the mammary gland through the action of SCD on vaccenic acid, furthermore, rodents, pigs and humans can convert dietary vaccenic acid into CLA (Kay et al., 2004; Santora et al., 2000; Glaser et al., 2002; Turpeinen et al., 2002). Industrially produced *trans* fats have been shown to adversely alter the HDL to LDL ratio and increase the incidence of coronary heart disease (Lichtenstein, et al. 2003; Mensink et al. 1990) but is not clear if *trans* fats of animal origin have the same effects. Willet et al. (1993) and Tricon et al. (2006) reported no detrimental effects of *trans* fat of animal origin on most cardiovascular disease risk parameters. The verdict has not been reached on the effects of vaccenic acid on blood lipids and risk of cardiovascular diseases.

1.3. Factors influencing milk fatty acid composition

The fatty acid composition of bovine milk fat is completely different from the fatty acid composition of the diet the animals consume. Fatty acids synthesized by rumen microbes, endogenous fatty acids of adipose origin, de novo synthesized fatty acids in the mammary gland and biohydrogenation of dietary unsaturated fatty acids all serve to modify dietary fatty acids prior to incorporation into milk fat (Grummer et al. 1991). The fatty acid composition of milk fat is the most variable component of milk and is influenced by breed (genetics), stage of lactation and quality and quantity of feed (Grummer 1991; Palmquist et al., 1993).

1.3.1. Genetics

Karijord et al. (1982) reported strong positive correlations (both genotypic and phenotypic) between the proportions of short- and medium-chain fatty acids (C6-C16) and fat percentage and consistently negative correlations between long-chain fatty acids and milk fat percentage. Strong positive correlations (both phenotypic and genotypic) have been found among the proportions of various short-chain fatty acids (C6 to C14:0) and among various unsaturated C18 fatty acids (Soyeurt et al. 2007; Palmquist et al. 1993). The correlations between short-chain and unsaturated C18 fatty acids were however consistently negative in all cases. Fatty acid composition of milk fat can thus be changed by genetic selection for milk fat percentage, and continued selection for increased fat percentage will lead to an increase in the proportion of short- and medium-chain fatty acids and a decrease in the proportion of long-chain fatty acids (Karijord et al. 1982). Selection for decreased fat percentage will have the opposite effect.

Considerable variations in milk fatty acid composition also occur among individuals of a particular breed belonging to the same contemporary group, suggesting that genetic

selection for individual fatty acids or classes of fatty acids is feasible. Soyeurt et al. (2007) have calculated heritability estimates for the relative proportions of some individual fatty acids found in milk fat and reported that heritabilities for FA in milk and fat ranged from 5 to 38%.

1.3.2. Effects of stage of lactation

At the bigining of lactation, dairy cows are normally in negative energy balance which leads to the mobilization of adipose fatty acids and the incorporation of these long-chain fatty acids into milk fat (Belyea and Adams, 1990). The mobilization and incorporation of long-chain fatty acids especially C18:1 into milk fat inhibits *de novo* short chain fatty acid synthesis resulting in lower proportions of short and medium chain fatty acids at initiation of lactation. Thereafter, there is a consistent increase in the proportion of short- and medium-chain fatty acids (C6-C16) with advancing lactation, accompanied by a compensatory decrease in the proportions of all C18 fatty acids, with 90% of peak C6-C16 fatty acids production occurring at around 8 weeks of lactation (Palmquist et al., 1993). The time required for the stabilization of milk fatty acid composition from the initial negative energy balance depends on the amount of fat stores, milk fat yield, extent of the initial negative energy balance and the quantity of fat in the diet.

1.3.3. Nutritional factors

The fatty acid profile of bovine milk is far from optimal and nutrition manipulation through the diet remains the key avenue for modifying the fatty acid profile of bovine milk. The fatty acid composition of bovine milk is however completely different from the fatty acid composition of the diets that the animals consume due to extensive biohydrogenation in the rumen, *de novo* fatty acid synthesis and desaturation of

certain fatty acids in the intestines and mammary glands of ruminants animals (Grummer, 1991). Nutritional strategies for increasing the concentration of desired fatty acids in milk are therefore based on increasing the amount or concentration of the desired fatty acids or their precursors in the diet, reducing the extent of biohydrogenation in the rumen, and/or enhancing the activity of enzymes involved in the conversion of the desired fatty acids from their precursor molecules. According to Mansbridge and Blake (1997), fatty acids present in milk are derived either directly or indirectly from the diet and the main sources of fatty acids in ruminant diet are forages, oils and oil seeds, fish oil and fat supplements.

1.3.3.1. Forage:concentrate ratio

Despite their low oil content, forages may contribute 25-35% of the total fatty acids in bovine milk and are the source of acetate and butyrate, which are the precursors for *de novo* synthesis of milk fat in the mammary gland (Mansbridge and Blake, 1997). Lowering the forage:concentrate ratio of ruminant diets has been reported to reduce the C6:0 to C16:0 fatty acid content of milk fat and increase the proportions of C18:1 and C18:2 (Grummer, 1991), consistent with a reduction in the supply of acetate and butyrate for *de novo* fatty acid synthesis and a compensatory increase in C18:1 and C18:2 from adipose tissue fatty acid mobilization. Latham et al. (1972) related the high C18:2 content of cows fed low forage diets or alternatively high concentrate diets to changes in ruminal pH (low pH) which inhibits its biohydrogenation, allowing it to escape the rumen and be incorporated in milk.

1.3.3.2. Oils and oilseeds

The fatty acid composition of seed oils varies widely but generally contains long chain fatty acids, C18:1, C18:2 and C18:3 in varying proportions. A number of studies

have reported changes in the fatty acid profile of milk fat as a result of feeding various forms of rapeseed, linseed, sunflower seeds, soya beans and evening primrose oil (Murphy et al. 1990; Ashes et al 1992; Dhiman et al. 1991; Choiunard et al. 1992; Stegman et al. 1992; Ali et al. 1991; Hermansen et al. 1995). In general, the inclusion of oils or oilseeds in the diets of dairy cows results in reductions in the levels of *de novo* synthesized fatty acids (C4-C16) and a concomitant increase in the levels of one or more of the long chain fatty acids (C18:0, C18:1, C18:2 or C18:3) (Mansbridge and Blake, 1997). Although the fatty acid profiles change similarly regardless of the form of supplementation (free oils or oilseeds), feeding free oils depresses milk fat yield, while feeding oil as part of whole oilseeds does not alter milk fat yield (Grummer, 1991). Processing of oil seeds generally has no effects on the direction of fatty acid profile change and has minimal effects on the magnitude of the fatty acid changes. Mahamed et al. (1988) reported similar milk fatty acid composition between cows fed raw and roasted soybeans or cottonseeds. Mielke and Schingoethe (1981) obtained similar results between feeding heated and unheated extruded soybeans. Kennelly (1989) however reported more increase in the contents of C18:0 and C18:1 and a more decrease in the content of C6:0-C16:0 fatty acids as a result of feeding ground canola seeds relative to the un-ground seeds.

The most profound changes in milk fatty acid composition and the greatest ability to selectively change the proportions of certain fatty acids in milk have been obtained by feeding protected oils. In ruminant animals, casein and formaldehyde are used to protect oils from biohydrogenation in the rumen and the greater the degree of protection from biohydrogenation, the greater the transfer efficiencies of the fatty acids contained in the oil into milk fat. Feeding protected sunflower or linseed oil has increased the C18:2 or

C18:3 content of milk fat to as high as 35 or 22%, respectively (Macdonald and Scott, 1977). Feeding of protected oils increased efficiency of transfer of C18:2 or C18:3 from <5% when feeding unprotected seeds to 35-45% (Bitman et al. 1975; Plowman et al. 1972).

1.3.3.3. Fish oils

There has been considerable interest in recent years to increase the omega-3 content of bovine milk fat to counter the imbalance in omega-6 to omega-3 intakes in most western diet. Plants unlike animals have the ability to synthesize *de novo* α-linolenic acid (C18:3n-3) , the parent compound of the omega-3 series of essential fatty acids and marine plants in particular, can be elongated and desaturated to yield omega-3 PUFA with C20 and C22 chain lengths (Mansbridge and Blake, 1997). Fish accumulate these long-chain omega-3 PUFA within their fat through the consumption of marine algae and plankton and the C20:5 omega-3 and C22:6 omega-3 contents of fish oils can be as high as 25% of the total fatty acids by weight (Grummer, 1991). Hagemeister et al. (1988) reported an increase of 6.7% by weight of milk fat C20:5 omega-3 plus C22:6 omega-3 following postruminal infusion of 420g of menhaden fish oil representing transfer efficiency from diet to milk of 35-40%. Intravenous infusion of cod liver oil by Storry et al. (1969), however, resulted in no detectable increases in the amounts of C20:5 omega-3 and C22:6 omega-3 fatty acids clearly indicating that those fatty acids were delivered in a form not suitable for mammary gland extraction. Fish oils need to be incorporated into lipoproteins for efficient extraction by the mammary gland and postruminal infusion is necessary to avoid milk fat depression associated with feeding moderate to high levels of fish oils.

1.3.3.4 Fat supplements

Fat supplements are usually given to dairy cows as energy supplements and have been carefully developed to minimize their adverse effects on fiber digestion or microbial activity in the rumen (Mansbridge and Blake, 1997). Development of rumen-inert or bypass fats to minimize digestibility problems associated with feeding unsaturated oils to dairy cows led to commercial development of a variety of bypass fats, including calcium salts of fatty acids and products enriched in saturated fatty acids (Jenkins and McGuire, 2005).

2. Conjugated linoleic acid (CLA)

2.1. Health Benefits of CLA

Conjugated linoleic acid (CLA) is a collective term for the group of geometric and positional isomers of linoleic acid or octadecadienoate (C18:2) in which the constituent double bonds are separated by a single carbon-carbon bond instead of a methylene group. CLA is found mainly in milk fat, tissue fat and to a lesser extent in the egg yolk as well as in the dairy products (Khanal and Olson, 2004). The major isomers in foods are in the following rank order: *cis*-9, *trans*-11 CLA (also called rumenic acid) > *trans*-7, *cis*-9 CLA > *cis*-11, *trans*-13 CLA > *cis*-8, *trans*-10 CLA > *trans*-10, *cis*-12 CLA > other isomers (Ma et al., 1999, Fritsche et al., 1999 and Kramer et al., 1998). The principal and biologically active isomer present in both milk and meat fat is the *cis*-9, *trans*-11 CLA that accounts for 80 to 90% of the total CLA (Chin et al., 1993). The *trans*-10, *cis*-12 isomer is also very active biologically and constitutes less than 5% of the total CLA in ruminant products.

The discovery and subsequent identification of CLA as the active ingredient on the extract from cooked beef that inhibited mutagenesis in mice by Pariza et al. (1979) sparked considerable interest in the potential health benefits of CLA. Since then, the list of the purported benefits of CLA is very impressive. CLA has been shown to inhibit skin papillomas (Belury et al., 1996), fore-stomach neoplasia (Ha et al., 1990), mammary tumors (Ip et al., 1996) and colon aberrant crypt foci (Liew et al., 1995). CLA has also been reported effective in reducing the size and metastasis of transplanted human breast and prostrate cancer cells in severe combined immune deficiency (SCID) mice (Cessano et al., 1998). CLA isomers have also been shown to modulate immune function (Yamasaki et al., 2003) as well as markers of atherosclerosis (Mcleod et al., 2004), diabetes (Belury, 2002) and obesity risk factors (Brown and McIntosh, 2003).

2.2. Biosynthesis of CLA

The CLA found in milk and meat fat of ruminant animals is formed during rumen biohydrogenation of linoleic acid (LA) or is synthesized by the animal's tissues from *trans*-11C18:1 (transvaccenic acid, TVA). Upon consumption by ruminant animals, dietary lipids comprising mainly linoleic (LA) and linolenic (LNA) acids undergo two important transformations in the rumen. The first transformation involves the hydrolysis of the ester linkages catalyzed by microbial lipases to release free fatty acids and the second step is the biohydrogenation of the now free fatty acids by rumen bacteria (*Butyrivibrio fibrisolvens* and others).

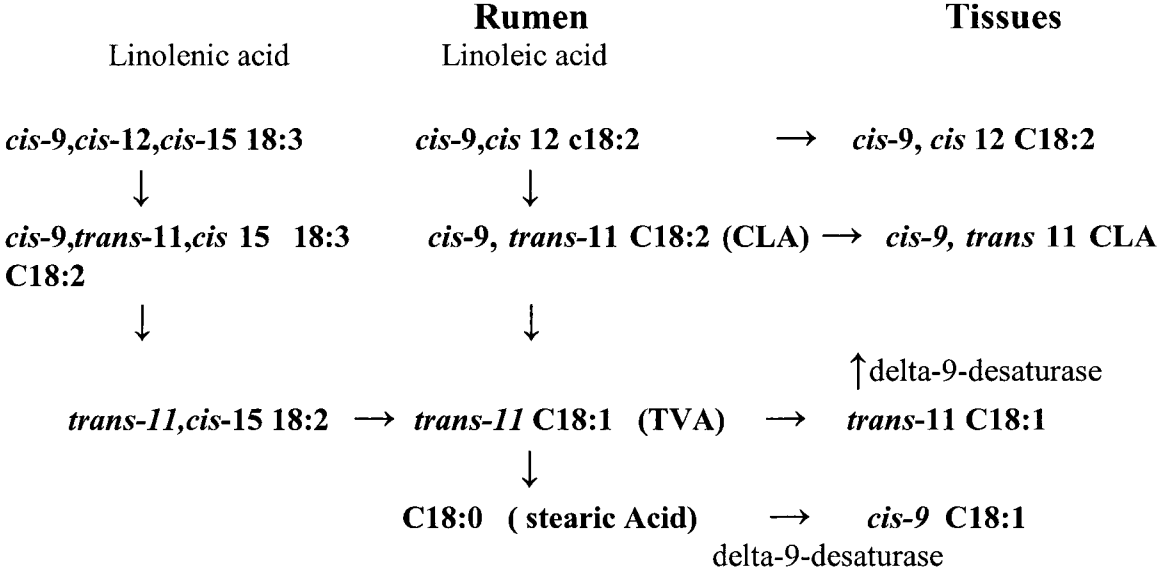
The biohydrogenation of linoleic acid (*cis*-9, *cis*-12 C18:2) begins with the isomerization of the *cis*-12 double bound to form *cis*-9, *trans*-11 CLA and is catalyzed by the enzyme linoleate isomerase. The second reaction is a reduction in which *cis*-9, *trans*-11 CLA is converted to *trans*-11 C18:1 (TVA). The second reduction will convert TVA

into stearic acid. The hydrogenation of TVA into stearic acid occurs very slowly (rate limiting step), leading to the accumulation of TVA in the rumen (Keeney, 1970). Similar to the biohydrogenation of linoleic acid, the biohydrogenation of linolenic (*cis*-9, *cis*-12, *cis*-15 C18:3) begins with an isomerization followed by a sequence of reductions and terminates with the formation of stearic acid (Bauman et al., 2000). TVA is the only common intermediate in the biohydrogenation of both linoleic and linolenic acids.

For quite some time, it was assumed that the escape of *cis*-9, *trans*-11 CLA from the rumen was the sole source of this CLA isomer in the milk until detailed studies across a wide range of diets demonstrated this to be incorrect (Griinari et al., 2000; Corl et al. 2001; Lock and Garnsworthy, 2002; Piperova et al., 2002; Kay et al., 2004). Analysis of milk samples by Jiang et al. (1996) revealed a close linear relationship between *cis*-9, *trans*-11 CLA and TVA and this relationship, which was consistent with precursor-product relationship, led to Griinari et al. (1997) proposing that a portion of the CLA in milk fat was of endogenous origin. Corl et al. (1998) hypothesized endogenous synthesis of *cis-9, trans-11* CLA from TVA by SCD1 and subsequently demonstrated that abomasally infusing 12.5 g/d TVA for 3 d increased the CLA content of milk by 40%, indicating lactating cows' ability to endogenously synthesize CLA. Inhibition of SCD1 activity by abomasal infusion of 10g/d sterculic acid (potent inhibitor of delta-9-desaturase) for 4 consecutive days resulted in a drastic reduction in milk fat content of *cis*-9, *trans*-11 CLA in lactating cows. Corl et al. (2001) reported a 60-65% reduction in *cis*-9, *trans*-11 CLA after abomasally infusing 8.8 g/d sterculic acid for 4 d. Consistent with the inhibition of SCD1, sterculic acid treatment also increased the milk fat ratios for fatty acids pairs affected by SCD1, C14:0/*cis*-9 C14:1, C16:0/*cis*-9 C16:1, and C18:0/*cis*-9 C18:1. In fact endogenous synthesis of *cis*-9, *trans*-11 CLA from TVA via SCD1

represent the predominant source of CLA in both milk fat and tissue fat. The *trans*-7, *cis*-9 CLA in milk fat is also almost exclusively of endogenous origin being synthesized by SCD1 from rumen-derived *trans*-7 18:1 as the precursor (Corl et al., 2002; Piperova et al. 2002). Endogenous synthesis of CLA via SCD1 has been estimated to contribute between 64% and 80% of the total CLA in milk (Griinari et al., 2000; Corl et al., 2001; Lock and Garnsworthy, 2002). Kay et al. (2002) even estimated 100% of CLA to be derived from endogenous synthesis. Details on the biohydrogenation of linoleic and linolenic acids are shown in Figure 1.1

Figure 1.1 Pathways for rumen and endogenous synthesis of cis-9, trans-11 CLA in the dairy cow.



Adapted from Bauman et al. (2003)

Certain dietary and rumen conditions often result in bacterial population shifts and some concomitant changes in patterns of biohydrogenation end products. Changes in the rumen environment induced by high concentrate, low fiber diets often result in a

reduction in rumen pH and alterations in biohydrogenation pathways, resulting in more production of *trans-10,cis-12* CLA and a concomitant increase in *trans-10* C18:1 in the rumen and finally in the milk (Baumen and Griinari, 2003). Putative pathways for the production of *trans*-10 C18:1 instead of trans-11 C18:1 as the predominant isomer in milk fat have been proposed (Griinari and Baumen, 1999) and these involve a specific *cis-9, trans-10* isomerase in rumen bacteria with the formation of *trans-10, cis-12* CLA as the first intermediate in the biohydrogenation of linoleic acid. Further evidence for the existence of such a pathway is provided by an increase in the proportion of *trans-10, cis-*12 CLA isomer in milk fat when animals are fed low fiber diets. Other CLA isomers are believed to be exclusively of rumen origin and formed as intermediates in the bacterial biohydrogenation of polyunsaturated fatty acids by less prominent and unelucidated pathways (Baumen et al., 2003), possibly because they have not been assigned any biological roles as yet.

Although there are as many as 28 possible isomeric forms of CLA (Banni, 2002), by far the most abundant isomer in nature is the *cis-9-trans-11* CLA (c9, t11-isomer). In human diets, CLA is consumed in milk fat and in meats derived from ruminant animals whereby they represent 0.2-2% of the fatty acids, with milk having higher concentrations than meat (Khanal and Olsen, 2004). More than 70% of the CLA in these foods is the *cis-9, trans*-11 isomer (Mcleod et al., 2004).

2.3. Factors affecting the CLA content of ruminant products

The CLA content in fat from ruminant-derived food products will be dependent on the rumen production of both CLA and vaccenic acid and the tissue activity of SCD (Baumen et al., 2000). A host of factors appear to affect the CLA content in milk and

meat from ruminant animals, and large variations in milk and meat CLA have been observed in individual animals fed the same diet and raised under similar environmental conditions (Khanal and Olsen, 2004). The CLA content of milk and meat from ruminant animals is influenced by dietary factors, animal factors and post-harvest (processing) related factors.

2.3.1. Dietary factors

Manipulation of animal diet has been the focus for increasing the CLA content of foods from ruminants and will continue to be the mainstay of animal nutrition research intended for enhancing CLA content in milk and meat (Kay et al. 2004; Khanal et al., 2003b; Watkins et al., 2003). Dietary manipulations to enhance the CLA content in ruminants' milk and meat basically involve supplying linoleic (LA) or linolenic (LNA) acids as substrates for rumen biohydrogenation. The assumption being the linoleic and linolenic acids contained in feeds will be converted either to CLA or transvaccenic (TVA) by the rumen flora and eventually into CLA in the mammary gland or adipocytes by SCD1. Dietary manipulation is therefore related probably more to the fatty acid composition of the feed being used than anything else (Khanal and Olsen, 2004).

2.3.1.1. Effects of fats, oils and oilseeds on CLA content of milk fat

Inclusions of plant oils in ruminant diets result in substantial increases in milk fat concentration of CLA. Plant oils or oil seeds that have a positive influence in the CLA content of milk include soybean, sunflower, safflower, solin, cottonseed, rapeseed, canola, peanut, corn and linseed. However, feeding grain and silage harvested from high-oil varieties corn has minimal effect on the concentration of CLA in milk fat (Baumen et al.,

2000). Oil seeds that are rich in LA are more effective than LNA rich seeds in increasing the milk fat CLA (Kelly et al., 1998b), and there is a clear dose-dependent increase in milk fat CLA with the feeding of such seeds. However feeding soybean and linseed seeds to goats on a low forage diet (30:70 forage to concentrate ratio), did not increase goat milk fat CLA (Chilliard et al., 2003) probably because the low forage diet reduced rumen pH below 6.0, which has negative effect on both CLA and transvaccenic acid concentrations in the rumen (Martin and Jenkins, 2002). Supplementation of LA to cows fed conventional Total Mixed Rations (TMR) appears to have an edge over supplementation with LNA probably because it contributes to both increased CLA and TVA production in the rumen, which ultimately become the substrate for CLA synthesis by delta-9 desaturase in the mammary gland, whereas LNA contributes TVA only during its biohydrogenation (Harfoot and Hazlewood, 1988). Additionally high levels of LA irreversibly inhibit the hydrogenation of TVA into stearic acid in the rumen, and this result in additional substrate for endogenous synthesis of *cis-9, trans-11* CLA (Harfoot et al., 1973a).

Plant oils are not normally included in ruminant diets because they produce inhibitory effects on rumen microbial growth (Jenkins, 1993). Calcium salts of fatty acids are therefore normally fed to ruminants as an alternative to plant oils. When calcium salts of fatty acid are fed to lactating animals, increased concentrations of milk fat CLA and TVA were observed (Chouinard et al., 2001) with effects relatively smaller compared to supplementing free oils.

Another alternative is to feed full-fat seeds. However, studies have demonstrated that feeding raw seeds has no effect on the milk fat concentration of CLA, suggesting that the polyunsaturated fatty acids in the intact seeds are relatively unavailable to rumen

microflora (Baumen et al., 2000). In contrast, substantial increases in milk fat CLA occur when the diet supplement contains full fat seeds that have been processed. Extruding, roasting or micronizing of soybeans resulted in two or three fold increases in milk fat CLA content compared with the control diet containing ground soybeans (Chouinard et al., 2001). It thus appears that processing (grinding, roasting, micronizing, flaking and extruding) makes the fatty acids contained in seeds accessible to rumen microbes.

Fish oil has also been found equally or even more effective than plant oils or oil seeds in increasing milk fat CLA content from cows fed conventional TMR diets (Whitlock et al., 2002; Chouinard et al., 2001). The highest concentration of milk fat CLA (2.2-2.5%) has been achieved with fish oil supplementation included at 2% of the diet DM (Baer et al., 2001; Donavan et al., 2000; Ramaswany et al., 2001). Although the rumen biohydrogenation of the long-chain polyunsaturated fatty acids in fish oil is not well understood (Harfoot and Hazlewood, 1988), neither CLA nor TVA seems to be intermediates. It has been suggested that the polyunsaturated fatty acids from fish oil inhibit the growth of bacteria responsible for rumen biohydrogenation of TVA into stearic acid, leading to more escape of TVA from the rumen and its eventual assembly into CLA by SCD1 in the mammary gland. Franklin et al. (1999) also reported a six- or seven-fold increase in milk fat CLA content when a control diet was supplemented with marine algae (*Schizochytrium sp*). More research is needed to elucidate the mechanisms for rumen biohydrogenation of longer chain polyunsaturated fatty acids from fish oils and other fatty acid sources of marine origin.

2.3.1.2. Pasture

The effects of pasture on milk fat concentration of CLA have been described in a number of studies (Timmen and Patton, 1988; Dhiman et al., 1996; Zegarska et al., 1996) and generally, pasture feeding increases milk fat CLA content compared to feeding a total mixed ration with similar lipid content or conserved forages. The drastic increase in milk fat CLA content after turning cows out to pasture were confirmed later in a series of experiments (Stanton et al., 1997; Kelly et al., 1998b; Dhiman et al., 1999b) and continue to be the mainstay of research for enhancing CLA content in the cow's milk (Kay et al., 2004; Ward et al., 2003). The forage lipid content and composition seemed only to partly explain the observed differences in milk content of CLA. A mature pasture with higher proportions of C14:0 and C16:0 and less LNA is not likely to produce as much milk fat CLA content as does the lush green pasture when grazed by lactating dairy cows (Loor et al., 2002b). Dhiman et al. (1999a) also reported a higher CLA content of milk in cows receiving all of their diet from pasture compared with cows receiving either one-third or two-thirds of their diet from pasture. Diversity in the species of forages available on pasture also increased the milk fat CLA (Collomb et al., 2002).

2.3.1.3. CLA supplements

Milk fat and tissue fat content of CLA can also be increased by dietary supplements of CLA (Loor and Herbein, 1998; Chouinard et al., 1999a). In most studies, the CLA supplements were abomasally infused to protect the supplements from alterations by rumen bacteria (Loor and Herbein, 1998; Chouinard et al., 1999a,b). All studies have established that dietary CLA supplements result in dose-dependent increases in the concentration of CLA in milk fat. In most studies, all the CLA isomers contained in

the CLA supplements were successfully transferred or incorporated into milk fat (Chouinard et al., 1999a,b). The effects of different dietary factors on the CLA content of milk fat are summarized in Table 1.2.

Table 1.2: Dietary factors and their effects on the CLA content of milk and tissue fat

Factors	**Effect on CLA**
Fresh/lush Pasture	Highly Positive
Pasture + Full fat extruded soybean	No effect
Pasture + Soy oil	No effect
Pasture + Fish oil	Positive
Maturity of Pasture	Negative
Diversity of Plant species	Positive
Fresh cut Pasture	Fresh>conserved
High Forage diet	Positive
High Grain diet	Negative
Raw oil seeds	Minimal
Roasted oil seeds/meals	Positive
Extruded oil seeds	Positive, better than roasted
Plant oils	Positive, better than Processed seeds
Fish meal	Positive, efficient than Plant seeds
Fish oil	Positive, efficient than plant oils
Ca salts of fatty acids	Positive
Marine algae	Positive
Rumen pH	>6.0 pH Positive
CLA supplementation	Positive
Trans fats	Positive

Adapted from Khanal and Olsen (2004)

Direct fortification of milk with conjugated linoleic acid through the use of CLA oil by Campbell et al. (2003) resulted in significantly less white colour and more blue colour than typical milk and the presence of a grassy/vegetable oil flavour and a concomitant decrease in milk fat flavour. Fortification of milk with CLA also decreased overall acceptability, overall flavour, and freshness perception of milk.

2.3.2. Animal related factors

Considerable variation in milk fat CLA content has been observed among individuals belonging to the same contemporary group (Khanal and Olsen, 2004) and the variation among individuals would primarily be due to differences in rumen production of CLA and TVA, and the activity of SCD1. Rumen production of TVA and CLA is influenced by the animals' diet and the type and activity levels of rumen microflora responsible for the biohydrogenation of available substrates into CLA and TVA. Since endogenous synthesis of CLA contributes from a minimum of 64% or 80% (Griinari et al., 2000 and Lock and Garnsworthy, 2002) to a maximum of 91% and even 100% (Kay et al., 2004: Kay et al., 2002) of the total CLA found in the milk, it is reasonable to suggest that SCD activity is responsible for at least some of the animal to animal variation in the milk fat CLA of dairy cows. However, Fievez et al. (2003) have shown that changes in CLA content of milk fat depended mainly on TVA supply and to a lesser extent on the activity of SCD1. There are four major fatty acid pairs in milk fat that represent a Product/Substrate ratio for SCD1 viz *cis-9, trans*-11-CLA/TVA, Oleic/Stearic, Palmitoleic/Palmitic and Myristoleic/Myristic and the ratios of these four pairs of fatty acids (Desaturase Index) are regarded as a proxy for the activity of SCD1 (Choi et al., 2000). A consistency in the individual hierarchy in desaturase ratios over time has been

shown when cows are fed the same diet or when they are switched between diets (Peterson et al., 2002), suggesting individual differences between cows in SCD1 activity.

Kelly et al. (1998a) reported a larger variation in milk fat CLA content for cows fed sunflower oil (rich in LA) than either peanut (rich in oleic acid) or linseed oil (rich in LNA). Higher variations in CLA content of milk fat among individuals were also reported with diets such as all pasture or diets with higher forage to concentrate ratio (Jiang et al., 1996) all of which are conducive to higher CLA concentrations in milk fat. Cows in confinement fed TMR had smaller variations in milk fat CLA content compared to cows grazing pastures (White et al., 2001). From all this, Khanal and Olsen (2004) suspected that the variation in the activity of SCD1 was expected only under dietary conditions suitable for higher TVA production leading to greater variations in milk fat CLA content among individual cows.

Contradictory findings have been reported regarding the influence of breed, parity and days into lactation on the CLA content of milk fat. It has been reported that given the same diet, Holsteins produce higher CLA in milk fat than Jerseys or Normandes (White et al., 2001; Lawless et al., 1999). Lal and Narayanan (1984) reported 15% more CLA in milk fat of cows and buffaloes with 7 or higher lactations compared to either 1-3 or 4-6 lactations. Stanton et al. (1997) also reported a positive effect on milk fat CLA content emanating from increased lactation number. To the contrary, Kelsey et al. (2003) observed minor variations in milk fat CLA content between Holstein and Brown Swiss cows fed the same diet, and breed contributed only 1% of the total variation while parity contributed about 10% of the total variation. Animal related factors and their contributions towards the CLA content of milk fat are summarized in Table 1.3.

Table 1.3: Animal related factors and their influence on the CLA content of milk fat

Factor	Effect on CLA
Species	Ruminants > Non- ruminants
Breed	Holstein>Brown Swiss>Normandes>Jersey
Stage of lactation	Minimal
Parity	Minimal
Animal to animal	Positive with higher delta-9 desaturase activity

Adapted from Khanal and Olsen (2004)

2.3.3. Post-harvest related factors

The effects of post-harvest related factors such as processing conditions, storage, cooking, aging, or converting one product to another (milk into cheese) on the CLA content of milk fat is controversial (Khanal and Olsen, 2004). Although it can be argued that CLA content of processed products depend on the CLA content of the original milk or meat, it is likely that the bacterial cultures used for making other dairy products from milk may contain enzymes that can isomerize LA into CLA. Most bacterial species such as *Lactobacillus acidophilus*, *lactobacillus casei* and *lactobacillus debruckii* used for making cheese, yogurt and other fermented milk products have been shown to convert LA into CLA (Alonso et al., 2003; Ogawa et al., 2001) and can thus increase the CLA content of milk products. Lin et al. (1999) observed small differences in CLA levels at various processing stages when raw milk was processed into cheddar cheese aged for 6 months while Dhiman et al. (1999b) noted no increases in CLA content of mozzarella cheese compared to the original milk. Baer et al. (2001) reported no differences in CLA contents of raw milk, cream and butter. Cooking tended to increase the CLA content of sirloin tip

roast (Ma et al., 1999) but had no effect on either cooked or uncooked extra lean ground beef or rib roast.

Compared to dietary and animal related factors, changes in CLA content of milk or tissue fat due to processing are usually minor and of very little significance.

3. DNA polymorphisms

3.1. Types of polymorphisms

Genetic polymorphisms refer to subtle differences or variations in DNA sequences among individuals, groups or populations as a result of genetic mutations. Genetic polymorphisms account for part of the phenotypic differences between individuals such as hair color in humans, coat color in livestock and polled or horned conditions in cattle, but some polymorphisms may result in diseases or an increased risk for diseases in both humans and livestock. There are two kinds of genetic polymorphisms: genetic polymorphisms due to a substitution of DNA bases and genetic polymorphisms due to an insertion or deletion of base pairs. Developments in DNA sequencing have made it easy to look for allelic versions of a gene from different members of a population (or from heterozygous individuals) and alleles whose sequences reveal only single changed nucleotide are called single nucleotide polymorphism or SNPs for short. Single nucleotide polymorphisms are single base changes in the genome that occur at a frequency of 1% or more in the population (Cullis, 2002) and other types of genetic polymorphisms result from insertions or deletions of a section of DNA, which include repeat sequences (mini and micro satellites) and gross genetic losses and rearrangements (Bellaterra, 2003).

Micro-satellites, otherwise known as simple sequence repeats (SSRs) or short tandem repeats (STRs) are short (usually <6 base pairs) tandemly repeated sequences or repeats of multiple copies of the same sequence motif composed of 1-6 base pair long units such as $(GA)_n$, $(GGA)_n$ or $(AGGA)_n$, where n is the number of tandem repeats. Micro-satellites are widely dispersed throughout the genome and the polymorphisms result from the variability in the number of repeats at a given site. Short tandem repeats are widely distributed in the genomes of both prokaryotes and eukaryotes and are used extensively as genetic markers in linkage studies in livestock (Cullis, 2002) for the identification of QTLs.

Hyper-variable mini-satellites are defined as the repetition in tandem of a short sequence motif, usually 6-100 bp spanning 0.5 kb to several kilo bases. The polymorphisms in mini-satellites arise from the differences in their lengths, and are located between genes where they are dispersed unevenly in the genome preferably in telometric locations (Lathrop et al., 1988). For many years, micro-satellites and hyper-variable mini-satellites have been classified as junk DNA because of their occurrence between genes and their seemingly no role in protein synthesis. Although mini-satellites and micro-satellites polymorphisms may not directly cause amino acid substitutions in proteins, they may however play important roles in gene expression through transcriptional and post-transcriptional regulation of gene expression.

Of all the different types of polymorphisms, SNPs are by far the most common and have the most profound effect on the animal's phenotype. SNPs are estimated to occur with a prevalence of one SNP per 1300 bases in the human genome (Lander et al., 2001; Venter et al., 2001) and can be located in the coding and regulatory regions of the gene. SNPs can occur as a result of either transition or transversion of nucleotide bases,

and transition mutations are by far the most prevalent. Single nucleotide polymorphisms in the coding region of a gene may cause amino acid substitutions in the resulting protein (non-synonymous or non conservative substitution) or may have no effect on the amino acid sequence of the resulting protein (synonymous or conservative substitution). Within the coding regions, the SNPs are four–fold lower compared to non-coding regions, and only about half of the SNPs result in non-synonymous codon changes (Nikerson et al., 1998). Generally, more SNPs occur between genes than in the regulatory and coding regions of most genes, so as to avoid interference with some important biochemical processes in the animal's body. The consequences of the SNP on the animal's phenotype will thus depend on the position of the SNP (whether it occurs in the coding, regulatory or non-coding regions of a gene), on whether or not it result in any amino acid substitution and on the types of the amino acid substitutions and on the degree of dominance at the locus containing the SNP. In general, association studies have to be carried out to scientifically establish if alleles of a gene are somehow related to the phenotypic expression of a particular trait in the population.

3.2. Origin of single nucleotide polymorphisms

One fundamental requirement of the genetic material is that it must exhibit a high degree of stability and the accuracy of DNA replication ensures the genetic stability of the cell. During the process of DNA replication, the DNA polymerase ensures high fidelity in complimentary base pairing between the template strand and the newly synthesized strand through its exonuclease editing or proof reading activity (Hartl and Jones, 2001). Errors in incorporation that are occasionally missed by the editing function are corrected by a post-replication process called mismatch repair system. Even with such elaborate and

seemingly error proof systems to guard against modifications in the DNA structure especially during replication, several endogenous and exogenous factors can still induce modifications in the genetic material resulting in SNPs. Endogenous mechanisms responsible for spontaneous mutagenesis include errors occurring during replication, spontaneous alteration of bases, and events related to the insertion and excision of transposable elements (Hartl and Jones, 2001).

The DNA mismatch repair system responsible for crosschecking and correcting errors missed by the DNA polymerase editing function ensures fidelity of nucleotide pairing between the template and the newly synthesized strand by first recognizing the incorrectly base-paired nucleotides within a strand and excising a contiguous nucleotide sequence that includes the nucleotides of the unmatched pair (Snustad and Simmons, 2003). The excision of the DNA segment containing the unmatched nucleotide pair always occurs in the newly synthesized strand and leaves a gap that will be filled by DNA polymerase, this time with correctly matched nucleotide pair that will then be sealed by DNA ligase. The distinction as to which strand to use as the template strand and which one to cut to remove the incorrectly paired bases by the mismatch repair system is based entirely on the degree of methylation between the two strands. The fully methylated parental or original strand is normally used as a template to determine the correct order of bases and the newly synthesized, under-methylated strand will be excised occasionally to ensure fidelity in base pairing (Snustad and Simmons, 2003). At times tautomeric bases form complimentary base pairs with the parental DNA strand and therefore escape the editing function of the DNA polymerase. However, when the bases assume their normal structure, mismatched base pairs result in the DNA molecule. Normally the mismatch repair system is capable of correcting such errors but if the elapsed time is so great that

the daughter strand containing the tautomeric bases has become fully methylated, the mismatch repair system will be unable to distinguish between the parental and daughter strand and SNPs occur (Griffiths et al., 2005). Other sources of spontaneous mutations or SNPs are deamination of cytosine and 5-methyl-cytosine (MeC), a methylated form of cytosine that normally pairs with guanine and constitutes about 5% of the cytosines in the DNA of many organisms (Hartl and Jones, 2001). Deamination of cytosine yields uracil and since uracil pairs with adenine instead of guanine, replication of the molecule containing GU base pair would ultimately lead to substitution of an AT pair for the original GC pair by the process GU→AU→AT in successive rounds of replication. Cells however possess the enzyme uracil glycosylase that specifically removes uracil from DNA so that cytosine to uracil conversion rarely causes SNPs. Deamination of MetC result in 5-methyluracil which is the same as thymine so that GMetC pair becomes a GT pair. Normally the mismatch repair system would correct the occurrence of the unknown GT pair but since MetC is a methylated base and is present in the methylated strand, the mismatch repair system does not recognize the thymine as incorrect (Hartl and Jones, 2001). During successive replications, the direction of correction is normally random, sometimes yielding the correct GC pair and sometimes the incorrect AT pair. Although the MetC sites comprise a small percentage of the genome, they constitute highly mutable sites also known as the hot spots, and their mutations are always GMetC→AT transitions.

Another endogenous mechanism contributing the occurrence of SNP or spontaneous mutagenesis is the occurrence of transposable elements or transposons in the nucleus of most eukaryotic organisms. Transposons are novel pieces of DNA found in the nucleus capable of inserting anywhere in the organism's genome (Hartl and Jones, 2001). The mutations result from the ability of these elements to be inserted into DNA molecules

and to alter the expression of proximal genes. Transposons may get inserted directly within a gene and interrupt the coding sequence of a gene (frame shift mutation). The ability of transposable elements to become inserted into DNA molecules or genomes of any cells including even germ cells have made them very valuable for the transfer of genes between organisms. Genes of interest can be attached to transposable elements and these genetically modified transposons can then be incorporated into the genome of interest with subsequent expression of the genes of interest (Hartl and Jones, 2001). Apart from spontaneous mutations, mutations can also be induced by mutagenic elements in the environment such as chemicals and radiation.

Mutations are abrupt, heritable changes in single genes or small regions of a chromosome that result in different allelic or polymorphic forms of a gene. Different polymorphic forms of a gene do not necessarily code for proteins with different amino acid sequences as the polymorphisms or mutations may occur in the non-coding regions or introns of a gene, and base changes in the nucleotide sequence of a gene need not result in amino acid change because of the redundancy or degeneracy of the genetic code. Even when the mutation or polymorphism results in amino acid substitution, the substitution will not necessarily create a mutant phenotype. For instance the substitution of one amino acid for a second one with the same charge, for example lysine for histidine may in some cases have no effect on either protein structure or phenotype (Hartl and Jones, 2001). Amino acid substitutions that usually result in phenotypic changes include: charged to uncharged and reverse, change of sign of charge, small side chain to bulky side chain, hydrogen bonding to non-hydrogen bonding, and any change to and from proline (which changes the shape of the polypeptide backbone) or cysteine (whose sulfhydryl group may be engaged in a disulphide bond). Most proteins however contain regions that are fairly

tolerant of amino acid substitutions such as those near the amino and carboxyl termini, but any change in the active site of an enzyme will usually decrease enzymatic activity substantially (Hartl and Jones, 2001).

Base substitution mutations may also be classified as transition or transversion mutations depending on the orientation of the pyrimidine and purine bases in the wild type and mutant bases. If one purine is substituted for the other purine and the complimentary pyrimidine for the other pyrimidine, the mutation is called a transition (e.g. AT→ GC or CG → TA, in which purines are underlined) and if the substitution is Purine for pyrimidine and pyrimidine for purine, the mutation is a transversion (e.g. AT→CG or GC→TA, again with the purines underlined).

4. Stearoyl-CoA desaturase 1 (SCD1)

4.1. Characteristics of stearoyl-CoA desaturase 1

Fatty acid desaturases introduce a double bound at a specific location of fatty acids and are conserved across different mammalian species. Three desaturase systems (delta-9-desaturase, delta-5-desaturase and delta-6-desaturase) have been identified in mammalian species. All mammalian desaturases identified so far are membrane-bound desaturases and analysis of their predicted amino acid sequences indicate that they contain two long hydrophobic domains capable of spanning the membrane bilayer twice (Nakamura and Nara, 2004). Mammalian desaturases also contain three regions of conserved His-box motifs that contain eight histidine residues believed to be the catalytic center of the enzymes. All mammalian desaturases are also regulated at the transcriptional level and share a common mechanism of a feedback regulation to maintain products in membrane phospholipids (Nakamura and Nara, 2004).

The SCD system is a multi-enzyme complex that includes in order, NAD(P)H, NADH-cytochrome b_5 reductase, cytochrome b_5 and the terminal delta-9-desaturase or SCD (Ntambi, 1995). The SCD catalyzes the formation of a *cis*-double bond between carbons 9 and 10 of saturated fatty acyl-CoAs acids. The preferred substrates for SCD are palmitoyl- and stearoyl-CoA (palmitic and stearic acids) that are subsequently converted into palmitoleoyl- and oleoyl-CoA, or palmitoleic and oleic acids, respectively. Although preferred substrates for SCD are palmitic and stearic acids, a wide range of saturated and unsaturated acyl-CoA can serve as substrates including trans-11 octadecadienoic acid or TVA, C10:0 and C12:0 (Pollard et al., 1980; Schennink et al., 2008). Desaturation of TVA by SCD results in the formation of *cis*-9, *trans*-11 CLA. In addition to *cis*-9, *trans*-11 CLA, SCD is responsible for the synthesis of other *cis*-9, *trans*-n octadecadienoic acids in milk fat such as *trans*-7, *cis*-9 octadecadienoic acid (Yuracecz et al., 1998) and *cis*-9, *trans*-13 octadecadienoic acid in milk fat (Henninger, 1994). Schennink et al. (2008)io In ruminant animals, SCD is thus responsible for the synthesis of monounsaturated fatty acids from their saturated counterparts as well as the synthesis of CLA in milk fat or tissue fat.

4.2. Expression and regulation of *SCD* genes

Four isoforms of *SCD* have been identified in mice (*SCD*-1, -2, -3, and -4) whereas only one *SCD* that is highly homologous to mouse SCD-1 has been identified in humans, sheep and goats (Miyizaki et al., 2003; Bernard et al., 2001). The mouse *SCD* genes are localized in close proximity on chromosome 19 and code for a transcript of about 4.9 kb (Ntambi and Miyizaki, 2004), and the human *SCD* gene has been mapped on chromosome 10 with a pseudo gene on chromosome 17 (Zhang et al., 1999). The human

genome has only one *SCD* gene but the gene generates two transcripts of 3.9 and 5.2 kb long by alternative usage of the polyadenylation sites probably resulting in two transcripts that differ in stability or translatability. Bovine and caprine *SCD1* genes have been mapped on chromosome 26 and the ovine *SCD* gene on chromosome 22 (Bernard et al., 2001). Northern-blot analysis provides evidence for the presence of a single *SCD1* transcript of approximately 5-kb in bovine, caprine and ovine mammary, intestinal and adipose tissues (Chung et al., 2000; Chang et al., 1992; Bernard et al., 2001). The bovine *SCD*1 transcript is 5331 nucleotides long, contains an open reading frame of 1080 nucleotides coding for 359 amino acids and has an unusually long 3′UTR of 3884 nucleotides (Taniguchi et al., 2004). The amino acid sequence of bovine *SCD*1 demonstrated high homology with that of the goat (93.9% AF325499; Bernard et al., 2001), sheep (93.6%, AJ001048; Ward et al., 1998), human (87.2%, HM_005063; Zhang et al., 1999) and the mouse (80.55%, NM_009127; Ntambi et al., 1988). The second isoform of *stearoyl-CoA desaturase* (*SCD5*) gene has recently been identified in cattle (Lengi and Corl, 2007). SCD5 is expressed mainly in the brain and its contribution to mammary SCD activity is currently unknown (Lengi and Corl, 2007).

The four isoforms of mouse *SCD* gene have been shown to share 85-88% identity at their amino acid sequences but their 5′-flanking regions showed some striking differences resulting in divergent tissue-specific gene expression (Ntambi and Miyizaki, 2004). Under normal dietary conditions, *SCD1* is mainly expressed in the liver and adipose tissue and not in the brain, and *SCD2* is mainly expressed in the brain, less in the adipose tissue and not at all in the liver and *SCD 3* is expressed in the skin (Ntambi, 2004). In growing ruminant animals (sheep and cattle), SCD1 is mainly expressed in the adipose tissue and some SCD1 activity has been reported in the liver and brain of

growing sheep (Chung et al. 2000; Ward et al. 1998; Page et al. 1997). High SCD1 activity has also been reported in the intestinal mucosal cells (Chang et al., 1992) and mammary glands of lactating ruminants which was consistent with the role of SCD1 in *de novo* synthesis of CLA and MUFA found in milk fat of ruminant animals (Ward et al., 1998; Chung et al., 2000).

Many studies have attempted to elucidate the mechanisms regulating the expression of *SCD1* and results demonstrate that *SCD1* mRNA expression and enzyme activity are responsive to many developmental, dietary, hormonal and environmental factors. High-carbohydrate diets, insulin, glucose, fructose, cholesterol, cold temperatures, light, some drugs (fibrates and peroxisome proliferators) and retinoic acid have been shown to induce hepatic *SCD1* expression in mice while PUFA especially of the omega-6 and omega-3 families, CLA, cAMP (or drugs that increase its intracellular levels), tumor necrosis factor-α, thyroid hormone and leptin have the opposite effect of inhibiting *SCD1* expression (Ntambi and Miyizaki, 2004). Studies on the effects of different factors on the expression of *SCD1* in ruminants are limited. Ward et al. (1998) reported tissue specific changes in mRNA abundance of *SCD* in sheep at different physiological states and observed a decrease in mRNA abundance in adipose tissue and an increase in mammary tissue during late pregnancy and lactation. Furthermore, a 15-fold increase in mRNA of *SCD1* (from 151 ±37 mRNA copies per cell to 2198 ± 340 mRNAs copies) was observed after culturing adipose tissue explants from the subcutaneous depot of lactating sheep in medium 199 containing insulin and dexamethasone. A comprehensive list of different dietary, hormonal and environmental factors regulating *SCD1* expression are shown in Table 1.4.

Table 1.4: Regulation of stearoyl-CoA desaturase

Dietary factors	Hormones	Others
▲Glucose	▲Insulin	▲Peroxisome
▲Fructose	▲Growth hormone	▲Temperature
▲ Vitamin A	▲Estrogen	▲Iron
▲Cholesterol	▲Androgen	▲liver-X- receptor agonist
▲Vitamin D	▼Leptin	▲TGF-β
▼PUFA	▼Glucagon	▲light
▲▼ Alcohol	▼Thyroid hormone	▲β-Amyloid
▲▼ CLA	▼▲ dehydroepiandrosterone	▼Thiazolidinediones
		▼Cadmium
		▼TNF-α

▲= increase ▼= decrease Adapted from Ntambi and Miyizaki (2004)

4. 3. Polymorphisms in bovine *SCD1* and their phenotypic consequences

Taniguchi et al. (2004) reported a total of 8 single nucleotide polymorphisms (SNPs) within the cDNA of bovine *SCD1* gene in Japanese Black cattle (Beef Breed) with 3 of the SNPs occurring in the open reading frame (ORF) region and the rest in the 3′Untranslated region (UTR). SNPs in the 3′UTR of the *SCD1* gene were T1905C, C3143T, A3351G, A3537G and A4736G. SNPs in the ORF region comprised G702A, C762G and T878C, and the latter occurred within the catalytic site of the enzyme and was predicted to cause amino acid substitution of valine for alanine in the resulting SCD1 protein or enzyme (Ala 293 Val). Genotyping of the Japanese black at the *SCD1* locus resulted in three distinct genotypes *p.293AA*, *p.293VA* and *p.293VV* and the frequency of the *p.293A* allele was higher (0.59) than that of the *p.293V* allele (0.41). A relatively

higher frequency of the *p.293A* allele has recently been reported in Italian Holsteins (0.57) (Mele et al., 2007), Dutch Holstein Friesians (0.73) (Schennink et al., 2008) Jerseys (0.94), and the Valdostana (0.65) breeds (Moioli et al., 2007). To the contrary, a relatively higher frequency of the *p.293V* allele (0.58) has been reported in the double-muscled Piedmontese breed (Moioli et al., 2007).

An association analysis between different *SCD1* genotypes and fatty acid composition of intramuscular fat in the Japanese Black cattle, revealed a significant influence of the *SCD1* locus on the fatty acid composition and melting point of intramuscular fat (Taniguchi et al., 2004). The *p.293AA* genotype had the highest percentage of MUFA, followed by the *p.293VA* genotype and the *p.293VV* genotype had the least amount of MUFA. The MUFA content of fat influences its melting point and consequently the AA genotype had the lowest melting point of intramuscular fat and the VV genotype the highest. Recent association studies on the influence of *SCD1* genotype on the fatty acid composition and desaturase indices of milk fat are inconsistent. In comparison with the *p.293V* allele, Schennink et al. (2008) reported a significant positive influence of the *p.293A* allele on C10:1, C12:1, C14:1, C18:0 and C18:1 trans-11, and its negative influence on C10:0, C12:0, C14:0, C16:1 and CLA in the Dutch Holstein Friesian heifers. Furthermore, the *p.293A* allele had a positive influence on C10 index (C10I), C12I and C14I but a negative influence on C16I, C18I and CLAI, in comparison with the *p.293V* allele. Moioli et al. (2007) found a positive influence of the *p.293A* allele on C10:1, C14:1, C10I, C14I and no influence on CLA, total MUFA and PUFA in the Piedmontese and Valdostana breeds. The *p.293A* allele was however associated with comparatively lower C16:1 in both breeds and lower C18:1 and C16I in the Valdostana

cows. In comparison with the *p.293V* allele, Mele et al. (2007) found a positive influence of the *p.293A* allele only on C14:1, C14I, C18:1 and total MUFA in Italian Holsteins.

5. Objectives of this research

From the review of literature, it became apparent that there were no published papers regarding polymorphisms in the *SCD1* gene in dairy cattle before 2006. Recent association studies between polymorphisms in the *SCD1* gene and milk fatty acid composition in dairy cattle are based on SNPs reported in the Japanese Black, which is a beef breed. Most of the recent association studies between *SCD1* polymorphisms and milk fatty acid composition also focused mainly on the Holstein breed and ignored other dairy breeds. Furthermore, results from association studies involving Holsteins differed between countries. In view of all these, the objectives of our study were:

1) To determine SNPs in the coding, 5′ and 3′ UTR regions of the *SCD*1 gene in Canadian Holstein and Jersey cows.

2) To investigate the influence of SNPs in the coding region of the *SCD1* gene on the fatty acid composition of milk fat of Canadian Holstein and Jersey cows, with emphasis on MUFA and CLA contents.

3) To investigate the influence of SNPs in the 5′and 3′UTRs of the *SCD1* gene on the fatty acid composition of milk fat of Canadian Holstein and Jersey cows, with emphasis on MUFA and CLA contents.

4) To investigate the influence of stage of lactation on desaturase indices and milk fatty acid composition of Canadian Holsteins and Jerseys.

REFERENCES

Ali T., Kurihara M., Ishida S. 1991. Increase in alpha-linoleic acid in milk fat by feeding the calcium soap of fatty acids prepared from linseed oil. Aim. Feed Sci. Tech. 62:58-62.

Alonso L., Cuesta E. P., Gilliand S. E. 2003. Production of free conjugated linoleic acid by Lactobacillus acidophilus and lactobacillus casei of human intestinal origin. J. Dairy Sci. 86:1941-1946.

Ashes J. R., St Vincent Welch P., Gulati S. K., Scott T. W., Brown G. H., Blackeley S. 1992. Manipulation of the fatty acid composition of milk by feeding protected canola seed. J. Dairy Sci. 75:1090-1096

Baer R. J., Ryali J., Schingoethe D. J., Kasperson K. M., Donavan D. C., Hippen A. R., Franklin S. T. 2001. Composition and properties of milk and butter from cows fed fish oil. J. Dairy Sci. 84:345-353.

Bagga D., Wang L., Farais-Eisner R., Glaspy J. A., Reddy S. T. 2003. Differential effects of prostaglandin derived from omega-6 and omega-3 polyunsaturated fatty acids on COX-2 expression and IL-6 secretion. Proc. Natl. acad. Sci. 100:1751-1756.

Banni S. 2002. Conjugated linoleic acid metabolism. Curr. Opin. Lipidol. 13:261-266.

Bartsch h., Nair J., Owen R. W. 2002. Exocyclic DNA adducts as oxidative stress markers in colon carcinogenesis: potential role of lipid peroxidation, dietary fat and antioxidants, Biol. Chem. 383:915-921.

Baumen D. E., Baumgard L. H., Corl B. A., Griinari J. M. 2000. Biosynthesis of conjugated linoleic acid in ruminants. Proc. Am. Soc. Anim. Sci. 1999.

Baumen D. E., Baumgard L. H., Corl B. A., Griinari J. M. 2000. Biosynthesis of conjugated linoleic acid in ruminants. In (2000) Available at: http://www.asas.org/jas/symposia/proceedings.

Baumgard L. H., Corl B. A., Dwyer D. A., Bauman D. E. 2000. Identification of the conjugated linoleic isomer that inhibits milk fat synthesis. Am. J. Physiol. 278:R179-R184.

Belury M. A. 2002. Dietary conjugated linoleic acid in health: physiological effects and mechanisms of action. Annu. Rev. Nutr. 22:505-531.

Belury M. A., Bird C., Nickel K. P., Wu B. 1996. Inhibition of mouse skin tumor promotion by dietary conjugated linoleate. Nutr. Cancer 26:149-157.

Belyea R. L., adams M. W. 1990. Energy and nitrogen utilization of high versus low producing dairy cows. J. dairy Sci. 73:1023-1030.

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

Bickerstaffe R., Annison E. F. 1968. Desaturation of stearic acid by mammary gland tissue of the lactating goat and sow. J. Biochem. 107:27

Bitman J., Wrenn T. R., Wood D. L., Mustakas G. C., Baker E. C., Wolf W. J. 1975. Effects of feeding formaldehyde treated, full fat soybean flours on milk fat polyunsaturated fatty acids. J. Am. Oil Chem. Soc. 52:415-418.

Bonanome A., Grundy S. M. 1988. Effect of dietary stearic acid on plasma cholesterol and lipoprotein levels. New England J. Med. 318:1244-1248.

Brown M. J., McIntosh M. K. 2003. Conjugated linoleic acid in Humans: Regulation of adiposity and insulin sensitivity. J. Nutr. 133(10):3041-3046.

Campbell W., Drake M. A., Larick D. K. 2003. The impact of fortification with conjugated linoleic acid (CLA) on the quality of fluid milk. J. Dairy Sci. 86:43-51.

Cessano A., Visonneau S., Scimeca J. A., Kritchevsky D., Santoli D. 1998. Opposite effects of linoleic acid and conjugated linoleic acid on human prostrate cancer in SCID mice. Anticancer Res. 18:1429-1434.

Chang J. H., Lunt D. K., Smith S. B. 1992. Fatty acid composition and fatty acid elongation and stearoyl-CoA desaturase activity in tissues of steers fed high oleate sunflower seed. J. Nutr. 122:2074-2080.

Chilliard Y., Ferlay A., Rouel J., Lamberet G. 2003. A review of nutritional and physiological factors affecting goat milk lipids synthesis and lipolysis. J. Dairy Sci. 86:1751-1770.

Chin S.F., Strokson J.M., Pariza M. W. 1993. Conjugated dienoic derivatives of linoleic acid: A new class of anticarcinogens. Food Flavor and Safety pp: 262-271. Amer. Chem. Soc.

Choi Y., Park Y., Pariza M. W., Ntambi J. M. 2000 Regulation of stearoyl-coenzyme A desaturase activity by trans-10, cis -12, isomer of conjugated linoleic acid in HepG2 cells. Biochem. Biophys. Res. Commun. 284:689-693.

Chouinard P. Y., Corneau L., Butler W. R., Chiliard Y., Drackley J. K., Baumen D. E. 2001. Effect of dietary lipid source on conjugated linoleic acid concentrations in milk fat. J. Dairy Sci. 84:680-690.

Chouinard P. Y., Corneau L., Barbano D. M., Metzger L. E., Bauman D. E. 1999a. Conjugated linoleic acids alter milk fatty acid composition and inhibit milk fat secretion in dairy cows. J. Nutr. 129:1579-1584.

Chouinard P. Y., Corneau L., Saebo A., Bauman D. E. 1999b. Milk yield and composition during abomasal infusion of conjugated linoleic acids in dairy cows. J. Dairy Sci. 82:2737-2745.

Chung M., Ha S., Jeong S., Bok J., Cho K., Baik M., Choi Y. 2000. Cloning and characterization of bovine stearoyl-CoA desaturase 1 cDNA from adipose tissues. Biosci. Biotechnol. Biochem. 64(7):1526-1530.

Collomb M., Butikofer U., Sieber R., Jeangros B., Bosset J. O. 2002a. Composition of fatty acids in cow's milk fat produced in the lowlands, mountains and highlands of Switzerland using high-resolution gas chromatography. Int. Dairy J. 12:649-659.

Collomb M., Butikofer U., Sieber R., Jeangros B., Bosset J. O. 2002b. Correlation between fatty acids in cow's milk fat produced in the lowlands, mountains and highlands of Switzerland and botanical composition of fodder. Int. Dairy J. 12:661-666.

Corl B. A., Chouinard P. Y., Baumen D. E., Dwyer D. A., Griinari J. M., Nurmela K. V. 1998. Conjugated linoleic acid in milk fat of dairy cows originates in part by endogenous synthesis from trans-11 octadecenoic acid. J. Dairy.Sci. 81(suppl.):233 (Abstr.).

Corl B. A., Baumgard L. H., Dwyer D. A., Griinari J. M., Phillips B. S., Bauman D. E. 2001. The role of delta-9-desaturase in the production of cis-9, trans-11 CLA. J. Nutri. Biochem. 12:622-630.

Cullis C. A. 2002. The use of DNA polymorphisms in genetic mapping. Gent. Eng. 24:179-179.

De lorgeril M., Renaud S., Mamelle N., Salen P. 1994. Mediterranean alpha-linolenic acid-rich diet in secondary prevention of coronary heart disease. Lancet 343:1454-1459.

Dhiman T. R., Anand G. R., Satter L. D., Pariza M. W. 1999a. Conjugated linoleic acid content of milk from cows fed different diets. J. Dairy Sci. 82:2146-2156.

Dhiman T. R., Helmink E. D., Mcmahon D. J., Fife R. L., Pariza M. W. 1999b. Conjugated linoleic acid content of milk and cheese from cows fed extruded soybeans. J. Dairy Sci. 82: 412-419.

Dhiman T. R., Anand G. R., Satter L. D., Pariza M. 1996. Conjugated linoleic acid content of milk from cows fed different diets. J. Dairy Sci. 79(Suppl. 1):137 (Abstr.).

Dhiman T.R., Nam S., Ure A. 2005. Factors affecting conjugated linoleic acid content in milk and meat. *Crit. Rev. Food Sci. and Nutr*, **45,** 463-482.

Dhiman T. R., van Zanten K., Satter L. D. 1991. Source of dietary fat and its effect on milk fatty composition. J. Dairy Sci. 74(suppl. 1): 249.

Donavan D.C., Schingoethe D. J., Baer R. J., Ryilla J., Hippen A. R., Franklin S. T. 2000. Influence of dietary fish oil on conjugated linoleic acid and other fatty acids in milk fat from lactating dairy cows. J. Dairy Sci. 83:2620-2628.

Fievez V., Vlaeminck B., Dhanoa M. S., Dewhurst R. J. 2003. Use of principal component analysis to investigate the origin of heptadecenoic and conjugated linoleic acids in milk. J. Dairy Sci.86:4047-4053.

Franklin S. T., Martin K. R., Baer R. J., Schingoethe D. J. , Hippen A. R. 1999. Dietary marine algae (Schizochytrium sp.) increases concentrations of conjugated linoleic acid, docsohexaenoic, and trans vaccenic acids in milk of dairy cows. J. Nutr. 129:2048-2054.

Fritsche J., Ricket R., Steinhart H. 1999. Formation, contents, and estimation of daily intake of conjugated linoleic acid isomers and trans-fatty acids in foods. In: Advances in Conjugated Linoleic Acid Research, Vol. 1 (Yurawecz M. P., Mossoba M. M. Kramer J. K. G., Pariza M. W. and Nelson G. J. Eds.) pp.378-396. AOCS Press, Champaign, IL.

German, J. B., L. Monrad, C. J. Dillard, and R. Xu. 1997. Milk fat composition: Targets for alteration of function and nutrition. Pages 35-72 in milk Composition, Production, Biotechnology. R. A. S. Welch, D .J. W. Burns, S. R. Davis, A. I. Popay and C. J. Prosser, eds. CAB International, Wallingford, UK.

Glaser K. R., Wenk C., Scheeder M. R. 2002. Effects of feeding pigs increasing levels of C18:1 trans fatty acidson fatty acid composition of backfat and intramuscular fat as well as backfat firmness. Arch. Tierenahr 56:117-130.

Griffiths A. J. F., Wessler S. R., Lewontin R. C., Gelbart W. M., Suzuki D. T., Miller J. H. 2005. Mutation, repair and recombination. In. Introduction to genetic analysis. 8th Edition. W. H. Freeman and Company, New York, NY. pp. 451-480.

Griinari J. M., Bauman D. E. 1999. Biosynthesis of conjugated linoleic acid and its incorporation into meat and milk in ruminants. In Yurawecz MP., Mossoba M. M., Kramer J. K. G., Pariza M. W., Nelson G. J. (ed) Advances in conjugated linoleic acid research. Vol 1: 180-200. AOCS Press, Champaign, IL.

Griinari J. M., Chouinard P. Y., Baumen D. E. 1997. Trans fatty acid hypothesis of milk fat depression revised. In: Proc. Cornell Nutr. Conf., Ithaca, NY. pp 208-216.

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

Ha Y. L., Storkson J., Pariza M.W. 1990. Inhibition of benzo(a)pyrene induced mouse forestomach neoplasia by conjugated denoic derivatives of linoleic acids acid. Cancer Res. 50:1097-1101

Hagemeister V. H., Prect D., Barth C. A. 1988. Studies on transfer of omega-3 fatty acids into bovine milk fat. Milchwissenschaft 43:153-158.

Harfoot C. G., Hazlewood G. P. 1988. Lipid metabolism in the rumen. In P.N. Hobson (Ed) the Rumen Microbial Ecosystem pp285-322. Elsevier, London.

Harfoot C. G., Noble R. C., Moore JH. 1973. Factors influencing the extent of biohydrogenation of linoleic acid by rumen micro-organisms in vitro. J. Sci. Food Agric. 24:961-970.

Hartl D. L., Jones E. W. 2001. Molecular mechanisms of of mutation and DNA repair. In. Genetics: Analysis of genes and genomes. 5th edition. Sudbury, MA. pp. 264-304.

Haug A., Hallaq H., Leaf A. 1992. Potential antiatherogenic effects of omega-3 fatty acids. In Thrombosis, an update Edited by: Neri Serneri G. G., Gensini G. F., Abbate R., Prisco D. Scientific press, Florence; pp 361-372.

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

Hawke J. C., Taylor M. W. 1983. Influence of nutritional factors on the yield, composition and physical propertiesof milk fat. Page37 in Developments in dairy Chemistry-2, Lipids. P.F. Fox, ed. Applied Science, New York, NY.

Hermansen J. E. 1995. Prediction of milk fatty acid profile in dairy cows fed various fat in relation to human nutrition and health. In *lipids in Animal Nutrition, Seminar of Lipid Forum, Danish Institute of animal Science, Foulum, October 25-26, 1995,* p.3. Scandinavian Forum for Lipid Research and Technology.

Hermansen J. E., Jonsbo F., Anderson J. O., Michaelsen K. F. , Weisbjerg M. R. 1995. On the transfer of gamma-linoleic acid into milk fat and its possible elongation to arachidonic acid by cows. Milchwissenschaft 50:3-6.

Horton J. D., Shimomura I. 1999. Sterol regulatory element-binding proteins: activators of cholesterol and fatty acid biosynthesis. Curr. Opin. Lipidol. 10:143-150.

Ip C., Briggs S. P., Haegele A. D., Thompson H. J., Storkson J., Scimeca J. 1996. The efficacy of conjugated linoleic acid in mammary cancer prevention is independent of the level or type of fat in the diet. Carcinogenesis 17:1045-1050.

Jiang J. L., Bjoerck J. L., Fonden R., Emanuelsen. 1996. Occurrence of conjugated cis-9, trans-11 octadecadienoic acid in bovine milk: effects of feed and dietary regimen. J. Dairy Sci. 79:438-445.

Jump D. B., Clarke S. D. 1999. Regulation of gene expression by dietary fat. Ann. Rev. Nutr. 19:63-90.

Karijord O., Standal N., Syrstad O. 1982. Sources of variation in composition of milk fat. Z. Tierrz. Zuechtungbiol. 99:81.

Kay J. K., Mackle T. R., Auldist M. J., Thompson N. A., Baumen D. E. 2004. Endogenous synthesis of cis-9, trans-11 conjugated linoleic acid in dairy cows fed fresh pasture. J. Dairy Sci. 87:369-378.

Kay J. K., Roche J. R., Thompson N. A., Griinari J. M., Shingfield K. J. 2003. Increasing milk fat cis-9 trans-11 conjugated linoleic acid content in pasture-fed cows. J. Dairy Sci. 86 (Suppl. 1):146 (Abstr.).

Kay J. K., Mackle T. R., Auldist M. J., Thompson N. A., Bauman D. E. 2002. Endogenous synthesis of cis-9, trans-11 conjugated linoleic acid in pasture-fed dairy cows. J. Dairy Sci. 85 (Suppl. 1):176(Abstr.).

Keeney M. 1970. Lipid metabolism in the rumen. In: A.T. Phllipson (Ed) Physiology of Digestion and Metabolism in the Ruminant. Pp 489-503. Oriel Press, Newcastle upon Tyne, U.K.

Kelly M. L., Berry J. R., Dwyer D. A., Griinari J. M., Chouinard M. E., Van Amburgh M. E., Bauman D. E. 1998a. Dietary fatty acid sources affect conjugated linoleic acid concentrations in milk from lactating dairy cows. J. Nutr. 128:881-885.

Kelly M. L., Kolver E. S., Baumen D. E., Van Amburgh M. E., Muller L. D. 1998b. Effect of intake of pasture on concentrations of conjugated linoleic acid in milk of lactating cows. J. Dairy Sci. 81:1630-1636.

Kelsey J. A., Corl B. A., Collier R. J., Bauman D. E. 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.

Kennelly J. J. 1989. Fat supplementation of dairy cow diets: impact on milk quality. Page 27in advances in dairy technology. Vol. 1. J. Martin, ed. Univ. Alberta, Edmonton, AB, Can.

Keys A., Anderson J. T., Grande F. 1965. Serum cholesterol response to changes in the diet. IV.Particular saturated fatty acids in the diet . Metabolism 14:776.

Khanal R. C., Olsen K. C. 2004. Factors affecting conjugated linoleic acid (CLA) content in milk, meat and egg: A review. Pakistan J. Nutri. 3:82-98.

Khanal R. C., Dhiman T. R., McMahon D. J., Boman R. L. 2003. Consumer acceptability characteristics of conjugated linoleic acid (CLA) enriched milk and cheese. J. Dairy Sci. 86 (suppl. 1): 356 (Abstr.).

Kramer J. K.G., Nelson G., Pariza M. W., eds.), pp180-200. AOCS Press, Chmpaign, IL. Griinari J. M., Corl B. A., Lacy S. H., Choiunard P. Y., Nurmela K. V. V., Bauman D. E. 2000. Conjugated linoleic acid is synthesized endogenously in lactating dairy cows by delta-9- desaturase. J. Nutr. 130:2285-2291.

Kramer J. K. G., Sehat N., Dugan M. E. R., Mossoba M. M., Yuracecz M. P., Roach J. A. G., Eulitz K., Aalhus J. L., Schaefer A. L., Ku Y. 1998. Distributions of conjugated linoleic isomers in tissue lipid classes of pigs fed a commercial CLA mixture determined by gas chromatography and silver ion high performance lipid chromatography. Lipids 33:549-558.

Kris-Etherton P. M., Pearson T. A., Wan Y., Hargrove R. L, Moriarty K., Fishell V., Etherton T. D. 1999. High- monounsaturated fatty acid diets lower both plasma cholesterol and triacylglycerol concentrations. Am. J. Clin. Nutr. 70:1009-1015.

Kromhout D., Menotti A., Kestleloot H., Sans S. 2002. Prevention of coronary heart disease by diet and lifestyle: Evidence from prospective cross-cultural, cohort, and interventional studies. Circulation 105:893-898.

Lal D., Narayana K. M. 1984. The effect of lactation number on the polyunsaturated fatty acids and oxidation stability of milk fats. Ind. J. Dairy Sci. 37:225-229.

Lander E. S., et al. 2001. Initial sequencing and analysis of the human genome. Nature 409: 860-921.

Latham M. J., Storry J. E., Sharpe M. E. 1972. Effect of low-roughage diets on the microflora and lipid metabolism in the rumen. Appl. Microbiol. 24:871-877.

Lathrop M., Nakamura Y., O'Cornell P., Leppert M., Woodward S., Lalouel J. M., White R. 1988. A mapped set of genetic markers for human chromosome 9. Genomics 3:361-366.

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

Lengi A. J., Corl B. A. 2007. Identification and characterization of a novel bovine stearoyl- CoA desaturase isform with homology to human SCD5. Lipids, 42:499-508.

Leveille G. A., Pardini R. S., Tillotson J.A. 1967. Influence of medium chain triglycerides on lipid metabolism in the rat. Lipids 2:287-294.

Liev C., Schut H. A. J., Chin S. F., Pariza M. W., Dashwood R. H. 1995. Protection of conjugated linoleic acid against 2-amino-3-methy-limidazol[4,5-f]quinoline-induced colon canrcinogenesis in the F334 rat: a study of inhibitory mechanisms. Carcinogenesis 16:3037-3043.

Lin H., Boylston T. D., Chang M. J., Luedecke L. O., Shultz T. D. 1995. Conjugated linoleic acid content of cheddar-type cheese as affected by processing. J. Food Sci. 64:874-878.

Litchtenstein A. H., Erkkila A. T., Lamarche B., scwab U. S., Jalbert S. M., Ausman L. M. 2003. Influence of hydrogenated fat and butter on CVD risk factors: remnant –like particles, glucose and insulin, blood pressure and C-reactive protein. Atherosclerosis 171:97-107.

Lock A. L., Garnsworthy P. C. 2002. Independent effects of dietary linoleic acid and linolenic fatty acids on the conjugated linoleic acid content of cow's milk. J. Anim. Sci. 74:163-176.

Loor J. J., Herbein J. H., Polan C. E. 2002. trans18:1 and 18:2 isomers in blood plasma and milk fat of grazing cows fed a grain supplement containing solvent-extracted or mechanically extracted soybean meal. J. Dairy Sci. 85:1197-1207.

Loor J. J., Herbein J. H. 1998. Exogenous conjugated linoleic acid isomers reduce bovine milk fat concentration and yield by inhibiting de novo fatty acid synthesis. J. Nutr. 128:2411-2419.

Ma D. L., Wierzbicki A. A., Field C. J., Clandinin M. T. 1999. Conjugated linoleic acid in Canadian dairy and beef product. J. Agri. Food Chem. 47:1956-1960.

Mansbridge R. J., Blake J. S. 1997. Nutritional factors affecting the fatty acid composition of bovine milk. Br. J. Nutr. 78(suppl. 1):S37-S47.

Martin S. A., Jenkins T. C. 2002. Factors affecting conjugated linoleic acid and trans-C18:1 fatty acid production by mixed ruminal bacteria. J. Anim. Sci. 3347-3352.

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

McLeod R. S., LeBlanc A. M., Langille M. A., Mitchell P. L., Currie D. L. 2004. Conjugated linoleic acids, atherosclerosis, and hepatic very-low-density lipoprotein metabolism. Am. J. Clin. Nutr. 79:1169S-1174S.

Mele, M., G. Conte, B. Castiglioni, S. Chessa, N. P. P. Macciotta, A. Serra, A. Buccioni, G. Pagnacco, and P. Secchiari. 2007. Stearoyl-Coenzyme A desaturase gene polymorphism and milk fatty acid composition in Italian Holsteins. J. Dairy Sci. 90:4458-4465.

Mensink R. P., Katan M. B. 1990. Effect of dietary trans fatty acids on high-density and low-density lipoprotein cholesterol levels in healthy subjects. New Engl. J. Med. 323:439-445.

Mensink R. P., Zock P. L., Kester A. D., Katan M. B. 2003. Effects of dietary fatty acids and carbohydrates on the ration 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.

Mielke C. D., Schigoethe D. J. 1981. Heat treated soyabeans for lactating cows. J. Dairy Sci. 64:1579-1585.

Mohamed O. E., Satter L. D., Grummer R. R., Ehle F. R. 1988. Influence of dietary cottonseed and soyabean on milk production and composition. J. Dairy Sci. 71:2677-2688.

Moioli, B., G. Contarini, A. Avalli, G. Catillo, L. Orru, G. De Matteis, G. Masoero, F.Napolitano. 2007. Short communication: Effect of stearoyl- coenzyme A desaturase polymorphism on fatty acid composition of milk. J. Dairy Sci. 90:3553-3558.

Murphy J. J., McNeil G. P., Connolly J. F., Gleeson P. A. 1990. Effect on cow performance and milk fat composition of including full fat soyabeans and rapeseeds in the concentrate mixture for lactating dairy cows. J. Dairy Res.57:295-306.

Nakamura M. T., Nara T. Y. 2004. Structure, function and dietary regulation of Δ6, Δ5, and Δ9 desaturases. Annu. Rev. Nutr. 24:345-376.

Nickerson D. A., Taylor S. L., Weiss K. M., Clark A. G., Hutchinson R. G., Stugard J., Salomaa V., Vertiainen E., Boerwinkle E., Sing F. 1988. DNA sequence diversity in 9.7 Kb region of human lipoprotein lipas gene. Nature Genetics 16: 252-255.

Ntambi J. M. 1995. The regulation of stearoyl-CoA desaturase (SCD). Prog. Lipid Res. 34:139-150.

Ntambi J.M., Bushrow S..A., Kaestner K..H., Christy R..J., Sibley E. 1988. Differentiation-induced gene expression in 3T3-L1 preadipocytes. J. Biol. Chem. 263:17291-17300.

Ntambi J. M., Miyazaki M., Store J. P., Lan H., Kendziorski C. M., Yandell B. S., Song Y., Cohen P., Friedman J. M., Attie A.D. 2002. Loss of stearoyl- CoA desaturase-1 function protects mice against adiposity. Proc. Natl. Acad. Sci. 99:11482-11486.

Ntambi J. M., Miyazaki M. 2004. Regulation of stearoyl-CoA desaturase and role in metabolism. Prog. Lipid Res. 43:91-104.

Ogawa, Matsumura J. K., Kishino S., Omura Y., Shimizu S. 2001. Conjugated linoleic acid accumulation via 10-hydroxy-12-octadecaenoic acid during microaerobic transformation of linoleic acid by lactobacillus acidophilus. Appl. Envirn. Microbiol. 67:1246-1252.

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

Pamplona R., Portero-Otin M., Sanz A., Requena J., Barja G. 2004. Modification of the longevity-related degree of fatty acid unsaturation modulates oxidative damage to proteins and mitochondrial DNA in liver and brain. Exp. Gerontol. 39:725-733.

Pariza M. W., Ashoor S. H., Chu F. S., Lund D. B. 1979. Effects of temperature and time on mutagen formation in pan-fried hamburger. Cancer lett. 7:63-69.

Peterson D. G., Kelsey J. A., Bauman D. E. 2002. Analysis of variation in cis-9, trans-11 conjugated linoleic acid (CLA) in milk fat of dairy cows. J. Dairy Sci. 85:2164-2172.

Piperova L. S., Moallem U., Teter B. B., Sampugna J., Yurawecz M. P, Morehouse K. M., Lutshini D., Erdman R. A. 2004. Changes in milk fat in response to dietary supplementation with calcium salts of trans-18:1 or conjugated linoleic fatty acids in lactating dairy cows. J. Dairy Sci. 87:3836-3844.

Plowman R. D., Bitman J., Gordon C. H., Dryden L. P., Goering H. K., Edmondson L. P, Yoncoskie R. A., Douglas F. W. 1972. Milk fat with increased polyunsaturated fatty acids. J. Dairy Sci. 55:204-207.

Pollard M. R., Gunstone F. D., Morries L. J. 1980. Desaturation of positional and geometric isomers of monoenoic fatty acids by microsomal preparations from rat liver. Lipids 15:306-314.

Precht D., Molktentin J. 1996. rapid analysis of isomersof trans-octadecenoic acid in milk fat. Int. Dairy J. 6:791-809.

Ramaswamy N., Baer R. J., Schingoethe D. J., Hippen A. R., Kasperson K. M., Whitlock L. A. 2001. Composition and flavour of milk and butter from cows fed fish oil, extruded soybeans, or their combination. J. Dairy Sci.84:2144-2151.

Roche J. R., Petch S., Kay J. K. 2005. Manipulating the dietary cation-anion difference via drenching to early-lactation dairy cows grazing pasture. J. Dairy Sci. 88:264-276.

Santora J., Palmquist D., Rorhrig K. L. 2000. Vaccenic acid is desaturated to conjugated linoleic acid in mice. J. Nutr. 130;208-215.

Shennink, 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.

Shimano H. 2001. Sterol regulatory element-binding proteins (SREBPs): transcriptional regulators of lipid synthetic genes. Prog. Lipid Res. 40:439-452.

Singh S., Hawke J. C. 1979. The in vitro lipolysis and biohydrogenation of monogalactosyldiglycerides by whole rumen contents and its fractions. J. Sci. Food Agric. 30:603-612.

Shennink, A., Heck J. M. L, Bovenhuis H. , Visker M. H. P. W. , Van Valenberg H. J. F., van Arendonk J. A. M. 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.

Snustad D. P., Simmons M. J. 2003. Mutation, DNA repair and recombination. In. Principles of genetics. 3rd edition. John Wiley and Sons, New York, NY. pp. 332-362.

Soyeurt, H., P. Dardenne, A. Gillion, 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.

Stanton C., Lawless F., Kjellmer G., Harrington D., Devery R., Connolly J. F., Murphy J. 1997. Dietary influences on bovine milk cis-9, trans-11-conjugated linoleic acid content. J. Food Sci. 62:1083-1086.

Stegman G. A., Casper D. P., Schingoethe D. J., Baer R. J. 1992. Lactational responses of dairy cows fed unsaturated dietary fat and receiving bovine somatotrophin. J. Dairy Sci. 75:1936-1945.

Storry J. E., Hall A. J, Tuckley B., Millard D. 1969. The effects of Intravenous infusions of cod-liver and soya-bean oils on the secretion of milk fat in the cow. Br. J. Nutr. 3:173-180.

Taniguchi M., Utsugi T., Oyama K., Manmen H., Kobayashi M., Tanabe Y., Ogino A., Tsuji S. 2004. Genotype of stearoyl-CoA desaturase is associated with fatty acid composition in Japanese Black Cattle. Mamm. Genome 14:142-148.

Thormar H., Isaacs E. E., Kim K. S., Brown H. R. 1994. Interaction of visna virus and other enveloped viruses by free fatty acidsand monoglycerides. Ann. N. Y. Acad. Sci. 724:465-471.

Timmen H., Patton S. 1988. Milk fat globules: Fatty acid composition, size, and in vivo regulation of fat liquidity. Lipids, 23:685-689.

Turpeinen A. M., Mutanen M., Aro A., Salminen I, Basu S., Palmquist D.L., Griinari J. M. 2002. Bioconversion of vaccenic acid to conjugated linoleic acid in humans. Am. J. Clin. Nutr. 76:504-510.

Tricon S., Burdge G. C., Jones E. L., Russell J. J., El-Khazen S., Moretti E., Hall W. L., Gerry A. B., leake D. S., Grimble R. F., Williams C. M., Calder p. C., Yaqoob P. 2006. Effects of dairy products naturally enriched with cis-9, trans-11 conjugated linoleic acid on blood lipid profile in healthy middle-aged men. Am. J. Clin. Nutr. 83:744-753.

Venter C., et al. 2001.The sequence of the human genome. Science 291:1304-1351.Ward A. T, Wittenberg K. M., Froebe H. M., Przybylski R., Malcolmson L. 2003. Fresh forage and solin supplementation on conjugated linoleic acid levels in plasma and milk. J. Dairy Sci. 86:1742-1750.

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

Ward R. J., Travers M. T., Richards S. E., Vernon R. G., Salter A. M., Buttery P. J., Barber M. C. 1998. Stearoyl-CoA desaturase mRNA is transcribed from a single gene in the ovine genome. Biochem. Biophys. Acta. 1391: 145-156.

Watkins B. A., Feng S., Strom A. K., DeVitt A. A., Yu L., Li Y. 2003. Conjugated linoleic acids alter the fatty acid composition and physical properties of egg yolk and albumen. J. Agri. Food Chem. 51:6870-6876.

White S. L., Bertrand J. A., Wade M. R., Washburn S. P., Green J. T., Jenkins T. C. 2001. Comparison of fatty acid content of milk from Jersey and Holstein cows consuming pasture or a total mixed ration. J. Dairy Sci. 84:2295-2301.

Whitlock L. A., Schingoethe D. J., Hippen A. R., Kalscheur K. F., Baer R. J., Ramaswamy N., Kasperson K. M. 2002. Fish oil and extruded soybeans fed in combination increase conjugated linoleic acids in milk of dairy cows more than when fed separately. J. Dairy Sci. 85: 234-242.

Willet W. C., Stampfer M. J., Manson J. E., Colditz G. A., Speizer F. E., Rosner B. A., Sampson L. A., Hennekens C. H. 1993. Intake of trans fatty acids and risk of coronary heart disease among women. The Lancet 341:581-585.

Yamasaki M., Ikeda A., Oji M., Tanaka Y., Hirao A., Kasi M., Iwata T., Tachibana H., Yamada K. 2003. Modulation of body fat and serum leptin levels by dietary conjugated linoleic acid in Sprague-Dawley rats fed various fat-level diets. Nutrition 19:30-35.

Yurawecz M. P., Roach J. A. G., Sehat N., Mossoba M. M., Kramer J. K. G., Fritsche J., Steinhart H., Ku Y. 1998. A new conjugated linoleic acid isomer, 7-trans, cis-9 octadecadienoic acid, in cow milk, cheese, beef and human milk and adipose tissue. Lipids 33:803-809.

Zhang L.G., Parimoo S., Stenn K., Prouty S. M. 1999. Human stearoyl-CoA desaturase: alternative transcripts generated from a single gene by usage of tandem polyadenylation sites, Biochem. J. 340:255-264

Zergarska Z., Paszczyk B., Borejszo Z. 1996. Trans fatty acids in milk fat. Pol. J. Food Nutr. Sci. 5:89-96.

CHAPTER II. SINGLE NUCLEOTIDE POLYMORPHISMS IN THE OPEN READING FRAME OF THE *STEAROYL-COA DESATURASE 1* GENE AND THE RESULTING GENETIC VARIANTS IN CANADIEN HOLSTEIN AND JERSEY COWS

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Abstract

Stearoyl-CoA desaturase 1 (SCD1) catalyzes the synthesis of conjugated linoleic acid (CLA) and mono-unsaturated fatty acids (MUFA) from their saturated counterparts in the mammary gland and adipose tissue of ruminant animals. We hypothesized that single nucleotide polymorphisms (SNPs) in the *SCD1* gene account for some of the differences in SCD1 activity, and consequently for some of the variations in CLA and MUFA content of milk fat between Holsteins and Jersey cows and within these two breeds. We analyzed the open reading frame of the *SCD1* gene of 44 Holsteins and 48 Jerseys for SNPs by sequencing. Three SNPs: 702A→G, 762T→C and 878C→T were identified in both breeds and a further SNP, 435G→A, was unique to Holsteins. The SNPs characterized 4 different sequence variants or genetic variants in Holsteins: *A* ($G_{435}A_{702}T_{762}C_{878}$), *A1* ($A_{435}A_{702}T_{762}C_{878}$), *B* ($G_{435}G_{702}C_{762}T_{878}$) and *B1* ($A_{435}G_{702}C_{762}T_{878}$), with only variants *A* and *B* in Jerseys. SNP 878C→T resulted in a non-synonymous codon change while the rest resulted in synonymous codon changes giving rise to two protein variants, *A* having alanine and *B* having valine. Allele *A* was the most prevalent in the two breeds. These differences may therefore contribute to existing variations in CLA and fat content between and within Canadian Holstein and Jersey cows.

Keywords *stearoyl-CoA desaturase*1, single nucleotide polymorphisms, dairy cattle

Introduction

Stearoyl-CoA desaturase1 (SCD1) catalyses the introduction of a *cis*-configuration double bond (between carbons 9 and 10) into a variety of Fatty Acyl-CoAs with 12 to 19 carbon atoms (Ntambi, 1999). The desaturase system is a multi-enzyme complex that includes in order NADH-cytochrome b_5 reductase, cytochrome b_5 and the terminal delta-9-desaturase or stearoyl-CoA desaturase (Ntambi, 1995). The terminal Stearoyl-CoA desaturase is the rate-limiting enzyme in the insertion of a double bond and is responsible for the synthesis of mono-unsaturated fatty acids (MUFA) from their saturated counterparts. It has also been reported to play a crucial role in the synthesis of conjugated linoleic acid (CLA) found in milk and tissue fat of ruminant products.

The genes encoding stearoyl-CoA desaturase have been cloned and characterized in a number of mammalian species including rodents, ovine, porcine, bovine and human (Ward *et al.*, 1998; Bernard *et al.*, 2001; Zhang *et al.*, 1999; Miyazaki *et al.*, 2003). Bovine and caprine *SCD1* genes have been mapped on chromosome 26 q 21 and the ovine *SCD1* gene on chromosome 22 q 21 (Bernard *et al.*, 2001). Northern blot analysis provides evidence for the existence of a single *SCD1* transcript of approximately 5-kb with an unusually long 3′UTR in bovine, caprine and ovine mammary gland and adipose tissues (Chung *et al.*, 2000; Bernard *et a*l., 2001). The *SCD1* transcript is approximately the same size in cattle, sheep and goats. The bovine *Stearoyl-CoA desaturase1* gene just like its caprine counterpart, is approximately 17 kb, comprises 6 exons and 5 introns and contains an open reading frame of 1080 nucleotides coding for a protein of 359 amino acids (GenBank Accession Nos. AY241932 and AY241933).

Breed differences in milk fat composition and a two- to three-fold variation in milk fat CLA has been reported among animals on the same diet (Kelsey *et al.*, 2003;

Kelly *et al.*, 1998a, b; Lawless *et al.*, 1999; Peterson *et al.*, 2002). The variation among individuals in CLA would primarily be due to differences in ruminal production of CLA and transvaccenic acid (TVA), and the activity of delta-9-desaturase in the mammary gland. Peterson *et al.* (2002) reported a range of approximately two-fold for the individuals' variation in delta-9-desaturase activity and consistency in the individual hierarchy in desaturase ratios over time when cows were fed the same diet or when they were switched between diets. It has also been reported that given the same diet, Holsteins secrete significantly higher levels of CLA and MUFA (palmitoleic and oleic acids) but lower levels of all the short and medium chain saturated fatty acids in their milk than Jerseys (White *et al.*, 2001; Lawless *et al.*, 1999). White *et al.* (2001) reported CLA levels of 0.41% vs. 0.32% of total fatty acids on total mixed rations (TMR) and 0.72% vs. 0.59% of total fatty acids on pasture in Holsteins and Jerseys, respectively. All these suggest the existence of some genetic influence on *SCD1* activities.

Single nucleotide polymorphisms (SNPs) are by far the most common polymorphisms and have the most profound effect on the animal's phenotype. As for the bovine *SCD1* gene, the only published study on SNPs is from Taniguchi *et al.* (2004) who reported the existence of a single nucleotide polymorphism (SNP) in the ORF of the *SCD1* gene with significant influence on the MUFA content of marbling fat of Japanese Black cattle.

Given all the above information, we hypothesize that SNPs in the *SCD1* gene account for some of the differences in *SCD1* activity, and consequently for some of the variations in CLA and MUFA content of milk fat between and within breeds. With a view to eventually studying associations of polymorphisms in the *SCD1* gene with milk CLA and MUFA, it is necessary to first determine the existence of polymorphisms and their

frequencies at the *SCD1* locus. The objectives of this study were therefore to determine the existence of polymorphisms in Canadian Holstein and Jersey breeds and based on the polymorphisms in the ORF, to determine the alleles and estimate allele frequencies at the *SCD1* locus in the two breeds.

Materials and methods

Experimental animals

Blood or milk samples were collected from 44 randomly selected Holsteins and 48 randomly selected Jerseys enrolled in the Programme d'analyse des troupeaux laitiers du Québec (PATLQ). Holsteins and Jerseys were used because these breeds differ in milk fatty acid composition particularly MUFA and CLA with Holsteins having higher milk CLA and MUFA content than Jerseys.

DNA isolation and primer design

Total genomic DNA was isolated from either blood or milk samples. Isolation of DNA from blood samples was carried out using Nucleospin Blood Mini Kit (Macherey-Nagel Inc, Easton., PA, USA) and following the supplier's protocol. Milk samples (2 ml per sample) were centrifuged at 15000 x *g* for 15 min to remove excess fat prior to following the Nucleospin Blood Kit manufacturer's protocol for DNA isolation. Primers were designed for selective amplification of the six exons of the *SCD1* gene using as reference sequence GenBank Accession No AY241932 and Primer3 web Program (Primer 3 _www.cgi v 0.2). The primers were synthesized by Invitrogen (Invitrogen Canada Inc., Burlington, ON, Canada) (Table 1).

PCR amplification and DNA sequencing

PCR amplifications were performed with a programmable thermal cycler, PTC-100™ (MJ-Research, Inc., Watertown, MA, USA) in a final reaction volume of 50 µl using PCR reagents from Invitrogen. The PCR reaction mix contained 50 ng of genomic DNA, 0.2 mM dNTPs, 1.5 mM (exons 1, 3 and 6) or 2 mM (exons 2 and 4) or 2.25 mM (exon 5) $MgCl_2$, 0.6 µM each primer and 1.5 units *Taq* DNA polymerase. After an initial denaturation step of 94 °C for 3 min, the reaction mix was subjected to the following cycling conditions: 34 cycles of 94 °C for 45 s, 54 °C for 30 s, and 72 °C for 1 min 30 s, plus a final extension step of 72 °C for 10 min for all the six exons. Amplification was confirmed by running the PCR products on 2 % agarose gels and visualizing under uv rays. Forty µl of each PCR product was sent to the McGill University/Genome Quebec Innovation Centre for purification and sequencing using the Big Dye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA) and the thermocycler GeneAmp PCR System 9700 (Applied Biosystems, Foster City, CA, USA). Sequences were generated with both the forward and reverse primers and read on a 3730xl DNA analyzer (Applied Biosystems, Foster City, CA, USA).

Sequence analysis

Chromatographs generated from sequencing were processed using Chromas version 1.45 (http://www.technelysium.com.au/chromas14x.html) to verify the sequences in the six exons of the *SCD1* gene. Both forward and reverse primer sequences were then aligned using the ClustalW multiple sequence alignment program (http://www.ebi.ac.uk/clusatlw/) to determine the presence of genetic polymorphisms.

Statistical analysis

Different nucleotide combinations at the polymorphic sites for all the individuals sequenced were used to determine the different sequence variants or genetic variants at the *SCD1* locus. Allele and genotypic frequencies, observed and expected homozygotes and heterozygotes at the *SCD* locus as well as tests for Hardy-Weinberg Equilibrium were performed using GENEPOP Web version 3.4 program (http://wbiomed.curtin.edu.au/genepop/) (Raymond and Rousset, 2001). Observed and expected number of homozygotes and heterozygotes were computed using the algorithm of Levene (1949). To obtain the exact p-value of Hardy-Weinberg Equilibrium, the Markov Chain method (Guo and Thompson, 1992) with a dememorization number of 1000, 100 batches and 1000 iterations per batch was used. The p-value returned by this method is calculated as the sum of the probabilities of all tables and its standard error.

Results and discussion

SNPs in the ORF of the SCD1 gene and resulting variants at the SCD1 locus

SNPs in the ORF of the *SCD1* gene in Canadian Holsteins and Jerseys are shown in Table 2. A total of 4 and 3 SNPs were found in the ORF of the *SCD1* gene in Canadian Holsteins and Jerseys, respectively. Based on GenBank No. AY241933, the SNPs were found at 702 (A to G), 762 (T to C) and 878 (C to T) nucleotide positions of the *SCD1* gene in both Holsteins and Jerseys and an additional SNP at 435 (G to A) was unique to Holsteins. All the reported SNPs led to transitional exchanges (A/G or C/T) and three of them (435G→A, 702A→G and 762T→C) were synonyms leading to no amino acid change in the resulting protein. High ratios of transitional over transversional (A/C or T/G)

exchanges ranging from 1.7 to 4 were also observed in the study of Human and mammalian SNPs (Vignal *et al.*, 2002). Synonymous mutations in the ORF regions of most genes are common in order to introduce genetic variability and at the same time maintain the integrity and functionality of the resulting proteins (enzymes) and minimize disturbances to important physiological processes catalyzed by the enzymes. The SNP at nucleotide 878 (C→T) was however predicted to cause amino acid substitution in the resulting SCD1 protein, namely alanine to valine. Multiple comparisons of the SCD1 amino acid sequences from different mammalian species show a highly conserved region in the two-third carboxyl-terminal part of the enzyme suggesting that this may contain the catalytic site (Bernard *et al.*, 2001; Shanklin *et al.*, 1994), and the occurrence of a non-synonymous SNP (878C→T) outside this region and only synonymous SNPs within this region are consistent with this hypothesis. SNPs 702A→G, 762T→C and 878C→T are consistent with those found in the Japanese Black (beef breed) cattle (Tanigushi *et al.*, 2004). GenBank, Accession No. 241933 also reported the existence of the three SNPs in *Bos taurus* species. The SNP 435G→A found only in Holsteins has never been reported before and to the best of our knowledge, this is the first report of the existence of such an SNP in dairy cattle.

The SNPs in the ORF of the *SCD1* gene characterize four different genetic variants that results in two protein variants as shown in Table 3. Sequence alignment shows our samples to have similar nucleotide sequences with the reference sequence AY241933 except at polymorphic sites where they have the sequences shown in table III, according to the polymorphic sites 435, 702, 762 and 878 respectively. Two (*A* and *B*) and four (*A*, *A1*, *B*, *B1*) different genetic variants were found in the Jersey and Holstein

breeds, respectively. The two genetic variants (*A* and *B*) found in both Jersey and Holstein breeds have been previously described (Tanigushi *et al.*, 2004; GenBank Accession No. 241933) and are distinguishable by the bases at 702, 762 and 878 positions (ATC vs. GCT, respectively) while the other two variants (*A1* and *B1*) found only in Holsteins are being described for the first time in this paper. These silent variants (*A1* and *B1*) are the direct result of the SNP 435G→A. Furthermore, the SNP 435G→A was not previously described within *A* and *B* or *V* (as named by Taniguchi *et al.*, 2004).

The *A* and *B* genetic variants found in both breeds have been predicted to result in two different protein variants, *A* and *B* (or *V* as named by Tanigushi *et al.*, 2004), containing amino acids alanine and valine, respectively, due to the SNP 878C→T. The *A1* and *B1* genetic variants in Holsteins are also predicted to result in the same *A* and *B* protein variants respectively, since the nucleotide substitutions did not result in amino acid changes. Holsteins thus have two different genetic variants, *A* and *A1* or *B* and *B1*, coding for the same protein variant due to the degeneracy of the genetic code.

Gene and genotypic frequencies at the SCD1 locus

The estimated frequencies of the identified alleles at the *SCD1* locus in both Holsteins and Jerseys are shown in Table 4. The *A* variant is by far the most frequent in Canadian Jerseys and Holsteins (0.948 and 0.784, respectively), followed by the *B* variant (0.136 and 0.052 in Holsteins and Jersey, respectively). The *A1* and *B1* variants were not identified in the Jersey breed and are therefore assumed to be non-existence in this particular breed of dairy cattle. The *A1* and *B1* variants occur at very low frequencies in the Holsteins. While the actual frequencies of the different genetic variants in the whole

population of Canadian Holsteins and Jerseys might differ from those reported here due to our small sample size, the order of frequency of the different variants is most likely to remain unchanged. Taniguchi *et al.* (2004) reported gene frequencies of 0.59 and 0.41 for the *A* and *B* (or *V* as named in their paper) variants, respectively, in the Japanese Black cattle in a total population of 1003 animals, suggesting that the *A* variant may indeed be the wild allele. The relatively lower frequencies of the *A1* and *B1* variants in Canadian Holstein cattle and their total absence in Jerseys and Japanese Black also suggest that these may be relatively new variants resulting from recent mutations.

The four different genetic variants at the *SCD1* locus are expected to pair up within individuals resulting in an array of genotypes shown in Table 5. Table 5 also shows the observed and the expected genotypic frequencies at the *SCD1* locus assuming Hardy-Weinberg Equilibrium. With only two genetic variants (*A* and *B*) at the *SCD1* locus, only three genotypes (*AA, AB and BB*) were expected in Jerseys while all the 10 genotypes shown in Table 5 were expected in Holsteins. The absence of some genotypes in both breeds could simply be due to the small number of individuals sequenced and genotyped in the current study.

Despite the small sample sizes for both breeds, observed and expected genotypic frequencies under Hardy-Weinberg equilibrium were in complete agreement (see Table 6 for P-values) and the combined Chi-Square probability is 0.994, suggesting random mating and no intentional selection at the *SCD1* locus in Canadian Holsteins and Jerseys.

Mating decisions have the potential to alter both allele and genotypic frequencies and inbreeding in particular can increase homozygosity with a concomitant decrease in heterozygosity. Table 6 shows the observed and expected levels of both homozygosity and heterozygosity in Canadian Holsteins and Jerseys. These levels were in complete

agreement with Hardy-Weinberg equilibrium suggesting a lack of intensive selection effect for milk traits on this locus in Canadian Holsteins and Jerseys.

Our results have demonstrated nucleotide differences between the *SCD1* genes of the Canadian Holstein and Jersey breeds, which may account for the differences observed between the breeds in the levels of CLA and MUFA in their milks (White *et al.*, 2001). Also, within breed differences in these parameters earlier observed by Peterson *et al.* (2002) in Holsteins may be explained by our observations in the Canadian Holsteins.

Considering that CLA has potential health benefits and has been shown to exhibit anti-adipogenic, anti-diabetogenic, anti-carcinogenic and anti-atherosclerotic properties (Belury, 2002; Parodi, 1997) and altered *SCD1* gene activity is thought to be associated with various diseases such as cancer, obesity and aging (Ntambi, 1999), it is important that the responsive variants be identified. We are currently therefore developing a strategy for rapidly genotyping animals at the *SCD1* locus to more accurately estimate gene and genotypic frequencies in a much larger population and more importantly, to relate the alleles and genotypes to milk fat composition traits, particularly MUFA and CLA content.

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References

Belury M. A. 2002. Dietary Conjugated Linoleic Acid in Health: physiological effects and mechanisms of action. Annual Rev. Nutr. 22:505-531.

Bernard L., Leroux C., Hayes H., Gautier M., Chilliard Y., Martin P. 2001. Characterization of caprine stearoyl-CoA desaturase gene and its mRNA showing an

unusually long 3'UTR sequence arising from a single exon. Gene 281:53-61.

Chung M., Ha S., Jeong S., Bok J., Cho K., Baik M., Choi Y. 2000. Cloning and characterization of bovine stearoyl-CoA desaturase 1 cDNA from adipose tissues. Bioscience Biotechnology and Biochemistry 64:1526-1530.

Guo S. W., Thompson E. A. 1992. Performing the exact test fo Hardy-Weinberg proportions for multiple alleles. Biometrics 48: 361-372.

Kelly M. L., Berry J. R., Dwyer D. A., Griinari J. M., Chouinard M. E., Van Amburgh .E., Bauman D. E. 1998a. Dietary fatty acid sources affect conjugated linoleic acid concentrations in milk from lactating dairy cows. J. Nutr. 128:881-885.

Kelly M. L., Kolver E. S., Baumen D. E., Van Amburgh M. E., Muller L. D. 1998b. Effect of intake of pasture on concentrations of conjugated linoleic acid in milk of lactating cows. Journal of Dairy Science 81:1630-1636.

Kelsey JA, Corl B.A., Collier R. J., Bauman D.E. 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.

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

Levene H. 1949. On a matching problem in genetics. Annals Mathematics Statistics 20: 91-94.

Miyazaki, M., M. J. Jacobson, W. C. Man, P. Cohen, E. Asilmaz, J. M. Friedman, and J.M. Ntambi. 2003. Identification and characterization of murine SCD4, a novel heart-specific stearoyl-CoA desaturase isoform regulated by leptin and dietary factors. J. Biol. Chem. 278:33904–33911.

Ntambi J. M. 1995. The regulation of stearoyl-CoA desaturse (SCD). Prog. Lipid Res. 34: 139-150.

Ntambi J. M.1999. Regulation of stearoyl-CoA desaturase by polyunsaturated fatty acids and cholesterol. J. Lipid Res. 40:1549-1558.

Ntambi J. M., Bushrow S. A., Kaestner K. H., Christy R. J., Sibley E. 1988. Differentiation-induced gene expression in 3T3-L1 preadipocytes. J. Biol. Chem. 263:17291-17300.

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

Peterson D. G., Kelsey J. A., Bauman D. E. 2002. Analysis of variation in cis-9, trans-11 conjugated linoleic acid (CLA) in milk fat of dairy cows. J. Dairy Sci. 85:2164-2172.

Raymond M, Rousset F. 2001. Population genetics software and ecumenism. J. Heredity 86:248-249.

Shanklin J., Whittle E., Fox B. G. 1994. Eight histidine residues are catalytically essential in membrane-associated iron enzyme, stearoyl- CoA desaturase, and are conserved in alkane hydroxylase and xylene monooxygenase. Biochem. 33:12787-12794.

Taniguchi M., Utsugi T., Oyama K., Manmen H., Kobayashi M., Tanabe Y., Ogino A., Tsuji S. 2004. Genotype of stearoyl-CoA desaturase is associated with fatty acid composition in Japanese Black Cattle. Mamm. Genome 14:142-148.

Vignal A., Milan D., SanCristobal M., Eggen A. 2002. A review on SNP and other type of molecular markers and their use in animal genetics. Genet. Select. Evol. 34:275-305.

Ward R. J., Travers M. T., Richards S. E., Vernon R. G., Salter A. M., Buttery P. J., Barber M. C. 1998. Stearoyl-CoA desaturase mRNA is transcribed from a single gene in the ovine genome. Biochemica et Biophysica Acta 1391: 145-156.

White S. L., Bertrand J. A., Wade M. R., Washburn S. P., Green J. T., Jenkins T. C. 2001. Comparison of fatty acid content of milk from Jersey and Holstein cows consuming pasture or a total mixed ration. J. Dairy Sc. 84:2295-22301.

Zhang L., Ge L., Parimoo S., Stenn K., Prouty S. M. 1999. Human stearoyl-CoA desaturase: alternative transcripts generated from a single gene by usage of tandem poladenylation sites. Biochemical J. 340: 255-264.

Table 1. Primers used for selective amplification of the six exons in the *SCD1* gene

Exon	Primer sequence
1	Forward: 5'GTT GGC AAC GAA TAA AAG AGG 3'
	Reverse: 5'CGC GGT GAT CTC AAC TCT TC 3'
2	Forward: 5'GGA CCG GGT CTA TGC CTA TC 3'
	Reverse: 5'CCA TCC AGC CTC TCAGGA C 3'
3	Forward: 5'GGA GTT CAG GGG AAA GCA G 3'
	Reverse: 5'CCA CAA GGA GAG AGA GGA AAG TG 3'
4	Forward: 5'GGC AAC TCC ATG ACT TCT CC 3'
	Reverse: 5'CAT GAC CGT CCT AGG TCA AC 3'
5	Forward: 5'CCC ATT CGC TCT TGT TCT GT 3'
	Reverse: 5'CGT GGT CTT GCT GTG GAC T 3'
6	Forward: 5'GCC TCT GAG GGG ATC TAT TTG 3'
	Reverse: 5'AGG CAG AGT TGT TGG CTT TC 3'

Table 2. Single nucleotide polymorphisms, their locations in the ORF of the *SCD1* gene and effects on amino acids identity in Canadian Holsteins and Jerseys

Breed	**Exon and nt position***	**Type**	**Change**	**Amino acid identity**
Holstein	Exon 3, 435	Transition	g→a	Same gc**g****→gc**a** (alanine→alanine)
Holstein/Jersey	Exon 5, 702	Transition	a→g	Same cc**a**→cc**g** (proline→proline)
Holstein/Jersey	Exon 5, 762	Transition	t→c	Same ta**t**→ta**c** (tyrosine→tyrosine)
Holstein/Jersey	Exon 5, 878	Transition	c→t	Altered gcg→gtg (**alanine****→**valine**)

*Nucleotide (nt) position is based on GenBank No. AY241933

** Altered nucleotides and amino acids are shown in bold

Table 3. DNA sequence and protein variants and their characteristic SNPs at the SCD1 locus in Canadian Holsteins and Jerseys

Breed	**Allele notation**	**Characteristic SNPs**	**Protein variant**
Holstein/Jersey	*A*	$G_{435}A_{702}T_{762}C_{878}$	**A**
Holstein	*A1*	$A_{435}A_{702}T_{762}C_{878}$	**A**
Holstein/Jersey	*B*	$G_{435}G_{702}C_{762}T_{878}$	**B**
Holstein	*B1*	$A_{435}G_{702}C_{762}T_{878}$	**B**

Table 4. Alleles and their frequencies at the *SCD1* locus in Canadian Holsteins and Jerseys

Allele	**Holstein**		**Jersey**	
	No. of genes	**Frequency**	**No. of genes**	**Frequency**
A	69	0.784	91	0.948
A1	4	0.046	0	0
B	12	0.136	5	0.052
B1	3	0.034	0	0

Table 5. Observed and expected genotypic frequencies at the *SCD1* locus in Canadian Holsteins and Jerseys

Genotypes	Holstein		Jersey	
	Observed No.	Expected No.	Observed No.	Expected No.
A A	25	26.966	43	43.105
A1A	4	3.172	0	0
A1A1	0	0.069	0	0
BA	12	9.517	5	4.789
BA1	0	0.552	0	0
BB	0	0.759	0	0.105
B1A	3	2.379	0	0
B1A1	0	0.138	0	0
B1B	0	0.414	0	0
B1B1	0	0.034	0	0

Table 6. Observed and expected number of homozygotes and heterozygotes and test for Hardy-Weinberg equilibrium (P-value) at the *SCD1* locus in Canadian Holsteins and Jerseys

Breed	No. of genes	Observed homozygotes	Expected homozygotes	Observed heterozygotes	Expected heterozygotes	P-value
Holstein	88	25	27.828	19	16.172	0.896
Jersey	96	43	43.211	5	4.790	1.000

CONNECTING STATEMENT I

In chapter II, the presence of 4 and 3 SNPs in the coding region of bovine *stearoyl-CoA desaturase* 1 gene was established through sequencing in Holsteins and Jerseys, respectively. The SNPs were in total linkage disequilibrium resulting in two protein variants (p. Ala293Val or *A293V*) or alleles at the *SCD1* locus due to the non-synonymous codon change emanating from SNP 878C→T. We hypothesized differences in the functionality of the resulting enzymes at the *SCD1* locus (allozymes) that could explain in part the variations in CLA and MUFA among cows belonging to the same contemporary group.

In chapter III, we developed a method for quickly genotyping the animals at the *SCD1* locus based on Restriction fragment polymorphism (RFLP) to distinguish between p.293A and p.293V alleles and determined the fatty acid content of 862 Holstein cows to establish an association between the genotype at the *SCD1* locus and the concentrations of individual fatty acids in milk and/ or desaturase indices (proxy for SCD1 activity). The influences of stage of lactation on the concentrations of individual fatty acids were also characterized.

CHAPTER III. *STEAROYL-COA DESATURASE* 1 GENOTYPE AND STAGE OF LACTATION INFLUENCE MILK FATTY ACID COMPOSITION OF CANADIAN HOLSTEIN COWS

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Abstract

Single nucleotide polymorphisms in the coding region of bovine *stearoyl-CoA desaturase1* gene have been predicted to results in *p.293A* and *p.293V* alleles at the *stearoyl-CoA desaturase1* locus. The objectives of this study were to evaluate the extent to which genotypes at the *stearoyl-CoA desaturase1* locus and stage of lactation influence milk fatty acid composition in Canadian Holstein cows. Cows with the *p.293AA* genotype had higher C10 index, C12 index and C14 index and higher concentrations of C10:1 and C12:1 compared to the *p.293AV* or *p.293VV* cows. Cows had higher C18 index and total index, and lower C10 index, C12 index, C14 index and CLA index during early lactation compared to the subsequent lactation stages. Early lactation was also characterized by higher concentrations of C18:1 *cis*-9, C18:1 *trans*-11, C18:2, monounsaturated fatty acids and total polyunsaturated fatty acids, and lower concentrations of C10:0, C10:1, C12:0, C12:1, C14:0, C14:1, C16:0 and total saturated fatty acids compared to the subsequent lactation stages. Neither the *stearoyl-CoA desaturase1* genotype nor the stage of lactation had an influence on conjugated linoleic acid concentrations in milk.

Keywords Canadian Holstein, *stearoyl-CoA desaturase1* genotype, fatty acid composition, lactation stage

Introduction

The fatty acid composition of bovine milk influences its physical and organoleptic properties (Chilliard et al., 2000) and also presents some potential health benefits and/or health risks to consumers (Williams, 2000). Bovine milk fat typically contains 70% saturated fatty acids (SFA), 25% monounsaturated fatty acids (MUFA) and 5% polyunsaturated fatty acids (PUFA) (Grummer, 1991). Saturated fatty acids, in particular C12:0, C14:0 and C16:0 in bovine milk have been reported to increase plasma total and low density lipoprotein (LDL) cholesterol concentrations, considered important biomarkers for cardiovascular diseases (Mattson & Grundy, 1985). PUFA and MUFA have the opposite effect of reducing plasma total and LDL cholesterol concentrations, and increasing the MUFA concentrations of bovine milk at the expense of SFA remains an important selection objective (Williams, 2000).

Current strategies for changing the composition of bovine milk towards increased MUFA and other functional components such as conjugated linoleic acid (CLA) are mainly based on nutritional manipulation of the animals' diet. Genetic selection for increased MUFA and CLA content of milk is however possible in view of the recent moderate heritability estimates of the concentrations of individual MUFA and desaturase indices in dairy cattle (Soyeurt et al., 2007; Schennink et al., 2008). In comparison with nutritional manipulation, genetic selection has the advantage of permanent and accumulative improvements in milk composition every generation.

In cattle, stearoyl-CoA desaturase1 (SCD1) is responsible for the synthesis of MUFA and CLA from precursor molecules in the mammary gland. Three SNPs in total linkage disequilibrium, of which one non-synonymous SNP resulted in a *p.A293V* polymorphism, have been identified in cattle (Taniguchi et al., 2004; Kgwatalala et al.,

2007). An association analysis between the *p.A293V* polymorphism and the fatty acid composition of intramuscular fat in Japanese Black cattle revealed a significant influence of the *p.A293V* polymorphism on fatty acid composition (Taniguchi et al., 2004). Recent association studies on the influence of *p.A293V* polymorphism on the milk fatty acid composition and desaturase indices in Holstein cattle are however inconsistent or differed between countries (Schennink et al., 2008; Mele et al., 2007). Furthermore, the influence of stage of lactation on desaturase indices remains unknown, while literature on the influence of stage of lactation on milk fatty acid composition is limited (Mele et al., 2007; Kay et al., 2005; Auldist et al., 1998; Karijord et al., 1982)

On the basis of the inconsistency of the effect of the *SCD1 p.A293V* polymorphism on milk fatty acid composition of Holsteins and the limited information on the influence of stage of lactation on milk fatty acid composition and desaturase indices, the objectives of our study were to investigate the effects of *SCD1 p.A293V* polymorphism and stage of lactation on milk fatty acid composition and desaturase indices in Canadian Holstein cows.

Materials and Methods

Experimental animals

Eight hundred and sixty two Canadian Holstein cows representing 17 different herds in Southern Quebec were used in this study. All animals were enrolled in Quebec Dairy Production Centre of Expertise (VALACTA) program (www.valacta.com). A single composite milk sample comprising the morning and evening milk was collected for each cow for both fatty acid determination and DNA extraction. The cows used in the study ranged from first to fifth parity of which 290, 223, 159 and 190 cows were of first, second,

third and forth parity or greater, respectively. The cows ranged from 21 to 350 days in milk (DIM) and 199, 261, 402 cows were in early (<100 DIM), mid (100-200 DIM) and late (>200 DIM) lactation stages, respectively.

Fatty acids determination

Lipid extraction was performed according to Hara and Radin (1978) and milk fatty acids were derivatized to methyl esters according to Christie (1982) with modifications as described by Chouinard et al. (1999). Fatty acid methyl esters were analyzed (split inlet 100:1) by gas chromatography (Varian, CP 3900 GC, Walnut Creek, CA, USA) equipped with Supelco-100 m column (100 m x 0.25 mm x 0.2 μm film thickness) and flame ionization detector. Oven temperature was programmed from 60°C to 165°C at 3°C per min and held for 10 min, followed by an increase to 220°C at 5°C per minute and held for 28 minutes. Total running time was 89 minutes and the injector and detector temperatures were maintained at 250°C and 255°C, respectively. The GC calculated peak areas for individual fatty acids automatically and the standard fatty acid mixture comprising 36 known individual fatty acids (Nu Check Prep, Inc) was used to provide reference retention times for the identification of fatty acids in the milk samples. Heptadecanoic acid (C17:0; Nu Check Prep Inc.) was used as the internal standard.

Desaturase indices are used as a proxy for SCD1 activity and individual desaturase indices were calculated according to Schennink et al. (2008) and the total desaturase index (TI) was calculated according to Mele et al. (2007).

DNA isolation and determination of SCD1 genotypes by RFLP

Somatic cells were obtained from the 1 ml sediment following centrifugation of 13 ml of milk at 21000 *g* at 4°C for 30 min. Somatic cells were washed three times with 1X PBS and genomic DNA samples were isolated by proteinase K digestion followed by three

phenol-chloroform extractions. The primer pair, forward - 5′ CCC ATT CGC TCT TGT TCT GT-3′) and reverse- 5′ CGT GGT CTT GCT GTG GAC T-3′, designed based on GenBank Accession No. AY241932 and previously used by Kgwatalala et al. (2007), was used to generate a 400 bp amplicon that contained 3 SNPs in linkage disequilibrium in exon 5 of the *SCD1* gene. The PCR mixture contained 50 ng of genomic DNA, 0.6 μM of each primer, 0.2 mM dNTP mixture, 2.25 mM $MgCl_2$ and 1.0 unit of *Taq* DNA polymerase in a final reaction volume of 25 μl. The PCRs were performed in a programmable thermal cycler, PTC-100^{TM} (MJ-Research, Inc., Watertown, MA, USA) with the following protocol: 94°C for 3 min; followed by 34 cycles of 94°C for 45 s, 54 °C for 30 s and 72°C for 1 min; with a final extension step of 72°C for 10 min.

The genotypes at the *SCD1* locus were determined by restriction digestion of the PCR products with *Nco*I (New England BioLabs Inc., Pickering, ON, Canada) based on *c.A702G* polymorphism which is in complete linkage disequilibrium with *c.C878T* (*p.Ala293Val or p.A293V*) polymorphism (Kgwatalala et al., 2007). The *p.293AA* genotype was digested into two fragments of 200 bp each. In contrast, the PCR product for *p.293VV* genotype remained undigested (400 bp) and the heterozygote had both the 200 bp and 400 bp fragments. The fragments were separated by electrophoresis through a 2% agarose gel stained with ethidium bromide and visualized under uv rays with FX Phosphoimager (BioRad Laboratories INC, Hercules, CA, USA).

Statistical analysis

Least squares means were determined by using a mixed model procedure of SAS version 9.2.1 (Littell et al. 2006). The model included fixed effects of herd (17 herds), parity (first, second, third, fourth or greater), *SCD1* genotype (*p.293AA, p.293AV* and *p.293VV*) and stage of lactation (< 100 DIM [early], 100-200 DIM [mid], >200 DIM [late]), and the

random effect of the sire (336 sires with 1-36 progeny per sire). Means were separated using pairwise t-tests with Scheffe's adjustment and determined to be different at $P \leq 0.05$.

Results

Genotypes at the SCD1 locus

The *SCD1 p.293A* and *p.293V* alleles were present at a frequency of 69.3% and 30.7%, respectively. Among the 862 cows genotyped, 46.8% had the *p.293AA* genotype, 45.0 % were heterozygous *p.293AV* and 8.2% were homozygous for the *p.293VV* genotype. The observed genotypic frequencies were consistent with those expected from Hardy-Weinberg's law, suggesting random mating and lack of new mutations at the *SCD1* locus.

Association of SCD1 genotype with milk fatty acid composition

Effects of *SCD1* genotype on desaturase indices and the concentrations of individual fatty acids are in Table 1. Desaturase indices were generally lower for C10 index (C10I), C12 index (C12I), C14 index (C14I) and C16 index (C16I) and comparatively higher for C18 index (C18I) and CLA index (CLAI). The *SCD1 p.A293V* polymorphism had a significant influence only on C10I, C12I, and C14I. The C10I, 12I and C14I were highest in the *p.293AA* genotype, intermediate in the *p.293AV* genotype and lowest in the *p.293VV* genotype. Significantly higher concentrations of C10:1 and C12:1 were observed in the *p.293AA* genotype compared to the *p.293AV* and *p.293VV* genotypes, but the same was not true for the concentration of C14:1 in milk fat. The *SCD1* genotype had no influence on C16 index (C16I), C18 index (C18I), CLA index (CLAI) and Total index (TI), as well as on the concentrations of C4:0-C8:0 and C14-C18 fatty acids including conjugated linoleic acid (CLA). The *SCD1* genotype had no significant effect on the concentrations of total saturated fatty acids (SFA), MUFA and polyunsaturated fatty acids (PUFA), and on fat yield and fat %, protein yield and protein%, total milk yield, somatic

cell count, lactose %, milk urea nitrogen and 305 d fat yield, protein yield and milk yield (see Appendix 2).

Stage of lactation had a significant influence on several desaturase indices and concentrations of several fatty acids in milk fat of Canadian Holstein cows (Table 2). Lower C10I, C12I, C14I and CLAI and lower concentrations of C10:0, C10:1, C12:0, C12:1, C14:0, C14:1, C16:0 and total SFA were found during early lactation compared to either mid lactation or late lactation. There was no significant difference in C12I, C14I and the concentrations of C10:1, C12:0, C12:1, C14:0 and C14:1 in milk fat between mid lactation and late lactation. Early lactation was however associated with significantly higher C18I and higher concentrations of C18:1 *cis*-9, C18:1 *trans*-11, C18:2, MUFA and PUFA in milk fat compared to either mid lactation or late lactation. Stage of lactation had no significant influence on C16I and milk fat concentrations of C4:0, C8:0, C18:0 and CLA. The animals' parity did not influence the fatty acid composition of milk.

Discussion

The higher frequency of the *p.293A* allele compared to the *p.293V* allele (69.3% vs. 30.7%) at the *SCD1* locus confirms previously reported frequencies of 83% and 17%, respectively, in a limited sample of 44 Canadian Holstein cows (Kgwatalala et al., 2007). Mele et al. (2007) also reported a higher frequency of the *p.293A* allele relative to the *p.293V* allele (57% vs. 43%) in Italian Holstein cows, and a similar trend (73% vs. 27%) was reported in the Dutch Holstein Friesian heifers (Schennink et al., 2008).

The desaturase indices obtained in this study are very comparable to those of Schennink et al. (2008). The significant positive influence of the *p.293A* allele compared with the *p.293V* allele on C10I, C12I and C14I and milk fat concentrations of C10:1 and C12:1 found in this study is consistent with Schennink et al. (2008) who found similar

results in the Dutch Holstein Friesen heifers. Schennink et al. (2008) also reported a significant negative effect of the *p.293A* allele on C16I, C18I and CLAI, and milk fat concentrations of C16:1 and CLA while we found no significant differences between the two alleles for all those parameters. In agreement with our results, Moioli et al. (2007) also reported a significant positive effect of the *p.293A* allele on C10I and C14I and on milk fat concentrations of C10:1 and C14:1 in Piedmontese and Valdostana cows, and no significant effect on CLA, MUFA and PUFA in either breed. The significant influence of *SCD1* genotype on C10I, C12I, C14I and on C10:1 and C12:1 might be related to the origin of fatty acids in bovine milk. All fatty acids with 12 carbons or less, most of C14:0 and about 50% of C16:0 are synthesized endogenously in the mammary gland from acetate and butyrate produced in the rumen, and a minor fraction of C14:0, 50% of C16:0 and all C18 fatty acids are extracted from arterial blood (Enjalbert et al., 1998). The *SCD1 p.293A* allele thus seems to have a significant positive influence only on the desaturation of 100% endogenously-synthesized fatty acids (C10:0 and C12:0) and consequently on the concentrations of their respective MUFA in milk but no significant effect on the desaturation of fatty acids derived mostly or exclusively from blood lipids. The significant positive effect of *p.293A* allele on C14I and lack of influence on C14:1 might be due to the small fraction of C14:1 extracted from blood lipids. Lower C10I, C12I, C14I and C16I compared to C18I and CLAI are consistent with low SCD1 substrate preference and consequently low SCD1 activity with fatty acids shorter than 18-carbon chain length (Chilliard et al., 2000). The lack of influence of *SCD1* genotype on C18I might be related to the low heritability of C18I which was estimated at 3% (Soyeurt et al., 2008) implying that the production of C18:0 and C18:1 *cis*-9 in bovine milk are more influenced by the environment (feeding) than by genetic factors. Higher C18I and a

higher concentration of C18:1 in milk could also be due to selective uptake of stearic acid (C18:0) by the mammary gland and its preferential transport to the endoplasmic reticulum membrane for desaturation by fatty acid binding protein-3 (FABP3), which has high affinity for stearic acid (Whetstone et al. 1986; Hanhoff et al. 2002). Furthermore, higher indices for C18I and CLAI could be due to some contribution of intestinal SCD1 activity on rumen-derived precursors before their extraction from the blood and further desaturation in the mammary gland.

Results on the influence of stage of lactation on the fatty acid composition of milk obtained in this study are fairly consistent with those of Mele et al. (2007). Mele et al. (2007) reported significantly lower C14:0 and C14:1 and higher C18:1 and MUFA during early lactation relative to either mid lactation or late lactation which was also the case in the current investigation. In addition, we report significantly lower C10:0, C10:1, C12:0, C12:1, C16:0, C18:3 and SFA, and significantly higher C18:1 *cis*-9, C18: 1 *trans*-11, C18:2 and PUFA during early lactation compared to either mid lactation or late lactation in Canadian Holstein cows. Auldist et al. (1998) also reported significantly higher milk MUFA and significantly lower SFA during early lactation compared to either mid or late lactation stages in New Zealand Holsteins. Similarly, Kay et al. (2005) reported significantly lower concentrations of C10:0, C12:0, C16:0 and a higher concentration of C18:1 *cis*-9 during early lactation (week 1 of lactation) compared to mid lactation (week 16 of lactation) in Holsteins. The concentrations of most fatty acids (C10:1, C12:0, C14:0, C14:1, C18:0, C18:1 trans-11, cis-9, trans-11 CLA, C18:2 and C18:3) were not significantly different between mid lactation and late lactation, consistent with the report by Auldist et al. (1998) who reported that the effect of stage of lactation on fatty acid profiles was mostly due to differences in early lactation milk. Stage of lactation had no

influence on concentrations of C16:1, C18:0 and CLA as reported by Mele et al. (2007) in Italian Holstein cows. We also report significantly lower C10I, C12I and C14I, and higher C18I, CLAI and TI during early lactation relative to either mid lactation or late lactation. Changes in the fatty acid profile during the entire lactation could be related to the energy balance or status of the cows. During early lactation, dairy cows might be in a negative energy balance leading to the mobilization of adipose tissue fatty acids (mainly palmitoleic acid, oleic acid and other long chain fatty acids) and their eventual secretion into milk, hence the higher concentrations of C18:1 *cis*-9 (oleic acid) , C18:1 *trans*-11 and C18:2 during early lactation. Oleic acid is the preferred substrate for mammary SCD1 and increased availability of oleic acid during early lactation resulted in higher C18I during early lactation compared to mid lactation. C18:1 *cis*-9 and C18:1 *trans*-11 have been shown to depress the activity of enzymes responsible for mammary synthesis of saturated fatty acids in the MAC-T cell line (Jayan & Herbein, 2000) and the increased concentrations of these fatty acids during early lactation might therefore explain relatively lower concentrations of *de novo* synthesized C10:0, C12:0, C14:0 and C16:0 during early lactation compared to the subsequent lactation stages. Drackley et al. (2007) also reported a linear increase in the concentration of C18:1 *cis*-9 and a concomitant linear decrease in the concentrations of C12:0, C14:0 and C16:0 with increasing abomasal infusions of high oleic (C18:1 *cis*-9) sunflower fatty acids in Holstein cows. A limited supply of some of the substrates for SCD1 (C10:0, C12:0 and C14:0) might explain lower C10I, C12I and C14I and lower concentrations of C10:1, C12:1 and C14:1 in milk fat during early lactation compared to the subsequent lactation stages. Attainment of a positive energy balance during mid lactation is expected to reverse the inhibitory effects of C18:1 fatty acid on *de novo* fatty acid synthesis, hence lead to an increase in the concentrations of

C10:0, C10:1, C12:0, C12:1, C14:0, C14:1 and C10I, C12I and C14I during mid lactation compared to early lactation.

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References

Auldist M. J., Walsh B.J., Thomson N.A. 1998. Seasonal and lactation influences on bovine milk composition in New Zealand. J. Dairy Res. 65:401–411.

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

Chouinard P. Y., Corneau L., Saebo A., Bauman D. E. 1999. Milk yield and composition during abomasal infusion of conjugated linoleic acids in dairy cows. J. Dairy Sci. 82: 2737-2745.

Christie W. W. 1982. A simple procedure for rapid transmethylation of glycerolipids and cholesterol esters. J. Lipid Res. 23:1072-1075.

Drackley J. K., Overton T. R., Ortiz-Gonzalez G., Beaulieu A. D., Barbano D. M., Lynch J. M., Perkins E. G. 2007. Responses to increasing amounts of high-oleic sunflower fatty acids infused into the abomasums of lactating dairy cows. J. Dairy Sci. 90: 5165-5175.

Enjalbert F., Nicot M., Bayourthe C., Moncoulon R. 1998. Duodenal infusions of palmitic, stearic or oleic acids differently affect mammary gland metabolism of fatty acids in lactating dairy cows. J. Nutr. 128: 1525-1532.

Glascock R.F., Welch V.A. 1974. Contribution of fatty acids of three low density serum lipoproteins to bovine milk fat. J. Dairy Sci. 57:1364-1370.

Hanhoff T., Lucke C., Spener F. 2002. Insights into binding of fatty acids by fatty acid binding proteins. Mol. Cell Biochem. 239:45-54.

Hara A., Radin N. S. 1978. Lipid extraction of tissues with low toxicity solvent. Analyt. Biochem. 90: 420-426.

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

Karijord O., Standal N., Syrstad O. 1982. Sources of variation in composition of milk fat. Z. Tier.t Ziicht. Biol. 99: 81-93.

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

Kgwatalala P. M., Ibeagha-Awemu E. M., Hayes J. F., Zhao X. 2007. Single nucleotide polymorphisms in the open reading frame of the stearoyl-CoA deasturase gene and the resulting genetic variants in Canadian Holstein and Jersey cows. DNA Sequence 18: 357-362.

Littell R. C., Milliken G. A., Stroup W. W. Wolfinger R. D., Schabenberger O. 2006 SAS® for Mixed Models. 2nd Edition, Cary, NC: SAS Institute.

Mattson F. H., Grundy S. M. 1985. Comparison of effects of dietary saturated, monounsaturated, and polyunsaturated fatty acids on plasma lipids and lipoproteins in man. J. Lipid Res. 26: 194-202.

Mele M., Conte G., Castiglioni B., Chessa S., Macciotta N. P. P., Serra A., Buccioni A., Pagnacco G., Secchiari P. 2007. Stearoyl-Coenzyme A desaturase gene polymorphism and milk fatty acid composition in Italian Holsteins. J. Dairy Sci. 90: 4458-4465.

Moioli B., Contarini G., Avalli A., Catillo G., Orru L., De Matteis G., Masoero G., Napolitano F. 2007. Short Communication : Effect of Stearoyl-Coenzyme A desaturase polymorphisms on fatty acid composition of milk. J. Dairy Sci. 90: 3553-3558.

Schennink A., Heck J. M. L., Bovenhuis H., Visker M. H. P. W., Van Valenberg H. J. F., van Arendonk J. A. M. 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.

Soyeurt H., Dehareng F., Mayeres P., Bertozzi C., Gengler N. 2008. Variation in delta-9-desaturase activity in dairy cattle. J. Dairy Sci. 91:3211-3224.

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

Taniguchi M., Utsugi T., Oyama K., Manmen H., Kobayashi M., Tanabe Y., Ogino A., Tsuji S. 2004. Genotype of stearoyl-CoA desaturase is associated with fatty acid composition in Japanese Black Cattle. Mamm. Genome 14: 142-148.

Whetstone H. D., Hurley W. L., Davis C. L. 1986. Identification and characterization of fatty acid binding protein in bovine mammary gland. Comp. Biochem Physiol. **85:** 687-692.

Williams C. 2000. Dietary fatty acids and human health. Ann. Zootech. 49: 165-180.

Table 1. Effect of *SCD1* genotype on milk fatty acid composition.[1]

Fatty acid	AA n=403	AV n=388	VV n=71
C4: 0	3.68 ± 0.04	3.68 ± 0.04	3.72 ± 0.09
C6:0	2.56 ± 0.02	2.26 ± 0.03	2.63 ± 0.06
C8:0	0.12 ± 0.003	0.13 ± 0.003	0.12 ± 0.006
C10:0	5.57 ± 0.05	5.62 ± 0.06	5.85 ± 0.13
C10:1	0.63^{a} ± 0.01	0.61^{b} ± 0.01	0.56^{b} ± 0.02
C12:0	5.34 ± 0.05	5.31 ± 0.06	5.54 ± 0.13
C12:1	0.19^{a} ± 0.003	0.18^{b} ± 0.003	0.15^{c} ± 0.006
C14:0	14.53 ± 0.09	14.61 ± 0.09	14.82 ± 0.22
C14:1	1.19 ± 0.01	1.15 ± 0.01	1.14 ± 0.03
C16:0	27.52 ± 0.15	27.43 ± 0.15	26.88 ± 0.35
C16:1	1.29 ± 0.01	1.31 ± 0.01	1.34 ± 0.03
C18:0	8.50 ± 0.14	8.75 ± 0.14	8.63 ± 0.33
C18:1 cis-9	19.06 ± 0.23	18.81 ± 0.23	18.86 ± 0.54
C18:1 trans-11	1.14 ± 0.02	1.14± 0.02	1.16 ± 0.05
CLA[2]	0.28 ± 0.005	0.27 ± 0.005	0.28 ± 0.012
C18:2	1.84 ± 0.03	1.88 ± 0.03	1.91 ± 0.07
C18:3	0.40 ± 0.01	0.41 ± 0.01	0.41 ± 0.02
SFA[3]	67.83 ± 0.23	68.07 ± 0.23	68.19 ± 0.53
MUFA[4]	22.36 ± 0.22	22.05 ± 0.22	22.04 ± 0.52
PUFA[5]	3.66 ± 0.05	3.71 ± 0.05	3.76 ± 0.11
C10 Index	10.38^{a} ± 0.08	9.85^{b} ± 0.09	8.97^{c} ± 0.20
C12 Index	3.48^{a} ± 0.05	3.30^{b} ± 0.05	2.62^{c} ± 0.12
C14 index	7.67^{a} ± 0.08	7.37^{b} ± 0.08	7.20^{b} ± 0.18
C16 Index	4.51 ± 0.05	4.60 ± 0.05	4.74 ± 0.11
C18 Index	69.15 ± 0.48	68.37 ± 0.49	68.52 ± 1.13
CLA Index	22.63 ± 0.44	21.98 ± 0.45	21.89 ± 1.04
Total Index	26.45 ± 0.24	26.13 ± 0.25	26.14 ± 0.57

[1]Values are expressed as LSM ± SE. Fatty acid content expressed as g/100g of total fatty acids

[2]Conjugated linoleic acid

[3]Total saturated fatty acids

[4]Total monounsaturated fatty acids

[5]Total polyunsaturated fatty acids

Table 2. Effect of stage of lactation on milk fatty acid composition.[1]

Fatty acid	Early[6] n=199	Middle[7] n =261	Late[8] n=402
C4:0	3.72 ± 0.06	3.73 ± 0.05	3.62 ± 0.04
C6:0	2.58^{ab} ± 0.04	2.64^{a} ± 0.03	2.53^{b} ± 0.03
C8:0	0.12 ± 0.004	0.13 ± 0.004	0.12 ± 0.003
C10:0	5.44^{a} ± 0.09	5.77^{b} ± 0.08	5.53^{a} ± 0.07
C10:1	0.53^{a} ± 0.01	0.64^{b} ± 0.01	0.64^{b} ± 0.01
C12:0	5.10^{a} ± 0.08	5.61^{b} ± 0.07	5.48^{b} ± 0.06
C12:1	0.15^{a} ± 0.004	0.18^{b} ± 0.004	0.19^{b} ± 0.003
C14:0	13.85^{a} ± 0.14	15.15^{b} ± 0.13	14.96^{b} ± 0.11
C14:1	1.04^{a} ± 0.02	1.20^{b} ± 0.02	1.23^{b} ± 0.02
C16:0	26.96^{a} ± 0.22	27.89^{b} ± 0.20	26.98^{a} ± 0.17
C16:1	1.33 ± 0.02	1.33 ± 0.02	1.28 ± 0.02
C18:0	8.94 ± 0.21	8.56 ± 0.19	8.83 ± 0.16
C18:1 cis-9	20.87^{a} ± 0.34	17.05^{b} ± 0.31	18.80^{c} ± 0.27
C18:1 trans-11	1.22^{a} ± 0.03	1.11^{b} ± 0.03	1.12^{b} ± 0.02
CLA[2]	0.28 ± 0.01	0.27 ± 0.01	0.28 ± 0.01
C18:2 *cis*-9, *cis*-12	1.98^{a} ± 0.04	1.84^{b} ± 0.04	1.81^{b} ± 0.03
C18:3 cis-9, cis-12, cis-15	0.39^{a} ± 0.01	0.42^{ab} ± 0.01	0.43^{b} ± 0.01
SFA[3]	66.75^{a} ± 0.34	69.61^{b} ± 0.31	67.73^{c} ± 0.26
MUFA[4]	23.91^{a} ± 0.33	20.40^{b} ± 0.30	22.14^{c} ± 0.26
PUFA[5]	3.86^{a} ± 0.07	3.64^{b} ± 0.06	3.64^{b} ± 0.05
C10- Index	8.92^{a} ± 0.13	9.92^{b} ± 0.12	10.33^{c} ± 0.10
C12 Index	2.85^{a} ± 0.08	3.22^{b} ± 0.07	3.34^{b} ± 0.06
C14 index	7.08^{a} ± 0.11	7.44^{b} ± 0.10	7.71^{b} ± 0.09
C16 Index	4.72 ± 0.07	4.57 ± 0.06	4.57 ± 0.05
C18 Index	69.96^{a} ± 0.73	66.66^{b} ± 0.66	69.43^{a} ± 0.56
CLA Index	20.87^{a} ± 0.66	22.40^{ab} ± 0.60	23.23^{b} ± 0.51
Total Index	28.14^{a} ± 0.36	24.26^{b} ± 0.33	26.31^{c} ± 0.28

[1]Values are expressed as LSM ± SE. Fatty acid content expressed as g/100 g of total fatty acids

[2]Conjugated linoleic acid= *cis*-9, *trans*-11 CLA

[3]Total saturated fatty acids= C4:0+C6:0+C8:0+C10:0+C12:0+C14:0+C16:0+C18:0

[4]Total monounsaturated fatty acids= C10:1+C12:1+C14:1+C16:1+C18:1 *cis*-9+C18:1 *trans*-9

[5]Total polyunsaturated fatty acids = CLA+C18:2 cis-9, cis-12 + C18:3 cis-9, cis-12, cis-15

[6]Early: <100 DIM

[7]Mid: 100-200 DIM

[8]Late: >200

CONNECTING STATEMENT II

In chapter II, the influence of *SCD1* genotype on C10 desaturase index, C12 desaturase and C14 desaturase index, and consequently on the concentrations of C10:1 and C12:1 in milk fat of Holsteins was established. In comparison with the p.293*V* allele, the p.293*A* allele was associated with significantly higher C10 desaturase index, C12 desaturase index and C14 desaturase index and consequently with higher concentrations of C10:1 and C12:1. The Holstein breed exhibit the highest phenotype in terms of milk production and the Jersey breed the highest phenotype in terms of the fat content of milk. Association studies involving *SCD1* genotype and individual fatty acid content in dairy cows, to date, have been biased towards the Holstein breed.

In chapter IV, an association between genotype at the *SCD1* locus and the concentrations of individual fatty acids and/or desaturase indices were established in the Jersey breed. The effects of *SCD1* genotype on traditional milk composition traits (test day milk yield, test day fat yield and fat %, test day protein yield and protein %, lactose %, milk urea nitrogen) and 305 d total milk, fat and protein yields were also investigated. The influences of stage of lactation on the concentration of individual fatty acids in Jersey milk were also characterized.

CHAPTER IV. INFLUENCE OF *STEAROYL-COA DESATURASE1* (*SCD1*) GENOTYPE AND STAGE OF LACTATION ON FATTY ACID COMPOSITION OF CANADIAN JERSEY COWS

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Abstract

Bovine milk contains high proportions of saturated fatty acids (SFA) because of extensive biohydrogenation of dietary fatty acids in the rumen. Stearoyl-CoA desaturase1 (SCD1) catalyzes the conversion of C10-C18 SFA into their monounsaturated (MUFA) counterparts in the mammary gland of ruminant animals; and two alleles (*A* and *V*) have previously been identified at the *SCD1* locus. Genotypes at this locus were identified and and fatty acid contents of milk was measured for 525 Canadian Jersey cows. Association analysis indicated that allele *A* is positively associated with higher C10 (C10I), C12 (12I) and C14 (C14I) indices and, consequently, with higher contents of C10:1 and C12:1, but not C14:1, relative to allele *V*. Allele *A* was also positively associated with increased 305-d milk and protein yields. Allele *A* however had no influence on C16 (C16I), C18 (C18I), or conjugated linoleic acid (CLA) (CLAI) indices in comparison with the *V* allele. Stage of lactation had an influence on desaturase indices and consequently on the MUFA contents of milk fat. The indices C10I, C12I, C14I and CLAI increased from early to mid lactation as did their respective MUFA. Genetic selection for increased unsaturation of the hypercholesterolemic fatty acids in milk fat is feasible and may be accompanied by increased lactation milk and protein yields.

Key Words: *Stearoyl- CoA desaturase1*, desaturase index, fatty acid, Jersey

Introduction

Milk fat composition is one of the factors influencing the technological and nutritional quality of dairy products (Chilliard et al., 2003). Apart from their quantitative contribution to the amount of dietary energy, different fatty acids in dairy and dairy products are potentially involved as positive or negative predisposing factors for the health of human consumers (Parodi, 1999: Williams, 2000). Bovine milk typically contains 70% saturated fatty acid (SFA), 25% monounsaturated fatty acids (MUFA) and 5% polyunsaturated fatty acids (PUFA) (Grummer, 1991). Results from epidemiologic, clinical and animal studies indicate that high quantities of dietary saturated fatty acids, as is the case with bovine milk, can lead to an increase in blood cholesterol and, consequently, an increased risk of atherosclerosis and coronary heart disease in humans (German et al., 1997; Kromhout et al., 2002). To the contrary, MUFA and PUFA have been reported to decrease both serum total cholesterol and low-density lipoprotein (LDL) cholesterol levels and therefore reduce the risk of coronary heart diseases (Kris-Etherton et al., 1999; Mensink et al., 2003). It is now commonly accepted that the cholesterol-raising effects of saturated fatty acids in bovine milk are confined to the medium-chain fatty acids; lauric (C12:0), myristic (C14:0) and palmitic (C16:0) acids, while the short-chain (C4:0-C8:0) and some medium-chain (C10:0) saturated fatty acids are reported to be neutral, with C18:0 reportedly as effective as C18:1 in reducing plasma cholesterol levels (Bonanome and Grundy, 1988).

A more favorable combination of bovine milk lipids to human health would be around 30% SFA, 60% MUFA and 10% PUFA (Pascal, 1996; Hayes and Khosla, 1992); so the current composition is far from optimal. Manipulating animal nutrition is currently the method of choice for modifying the fatty acid composition of milk fat toward the ideal

composition. But even the most extreme modification of the diet by itself will not be sufficient to achieve the desired milk fat composition (Grummer, 1991). The applications of current knowledge of cattle genetics and controlled nutrition of dairy cows have been suggested to be prevailing methods for improving milk fat composition (Goddard, 2001). The application of genetic knowledge for the optimization of milk fat composition is possible on the basis of genetic variability and heritability of milk fat composition both between breeds and within breeds (Soyeurt et al., 2006; Stoop et al., 2008).

In ruminant animals, one of the key enzymes having significant influence on milk fatty acid composition is stearoyl-CoA desaturase1 (SCD1). *Stearoyl Co-A desaturase 1* catalyses the conversion of saturated fatty acids with 10-18 carbon atoms into their monounsaturated counterparts and is also accredited with the synthesis of most of the conjugated linoleic acid (CLA) in the mammary gland of ruminant animals. Conjugated linoleic acid is of interest to human health because it may be anticarcinogenic, anti-atherosclerotic, decrease fat accumulation and can modulate the immune response (Pariza et al., 1999; MacDonald, 2000). Therefore, an alternative way to study the nutritional quality of milk fat is to analyze the variations in SCD1 activity (Soyeurt et al., 2006). The SCD1 activity, also known as the desaturase index can be estimated by calculating the product to substrate ratio for the different fatty acids that SCD1 catalyses and a higher index translates to increased desirable MUFA and a concomitant reduction in the undesirable SFA in milk (Reh et al., 2004). Peterson et al. (2002) reported individual animal differences in SCD1 activity independent of dietary influences, suggesting possible within-breed selection for increased desaturase activity. Schennink et al. (2008) also reported heritabilities for different SCD indices in the range of 0.23 to 0.46 indicating substantial genetic variation for milk fat unsaturation.

Three single nucleotide polymorphisms (SNPs) (A702G, T762C and C878T) in total linkage disequilibrium have been identified in the fifth exon of the bovine *SCD1* gene and the C878T SNP results in two protein variants, A (293Ala) and B or V (293Val) (Kgwatalala et al., 2007; Taniguchi et al., 2004). The amino acid substitution (Ala293Val) lies within the catalytic site of the enzyme. Taniguchi et al. (2004) reported significant associations between Ala293Val *SCD1* genotypes and the fatty acid composition of intramuscular fat in the Japanese black cattle while Schennink et al. (2008) reported significant associations between different *SCD1* genotypes and individual fatty acid contents and various SCD1 indices in Dutch Holstein Friesian heifers.

On the basis of the role of the *SCD1* gene in fatty acid unsaturation and the differences in milk fat percentage between Holsteins and Jerseys, the objectives of our study were to investigate the effects of the *SCD1 Ala293Val* polymorphism and stage of lactation on individual fatty acids contents, various desaturation indices and other milk composition traits in Canadian Jersey cows.

Materials and Methods

Experimental Animals

The analysis was carried out on 525 first to fifth parity Canadian Jersey cows from 17 herds in southern Quebec. The cows were from 21 to 350 DIM and were sired by 160 bulls (1-35 progeny per bull). Cows were enrolled in the VALACTA Program (www.valacta.com) that routinely analyzes milk samples from participating dairy herds in Quebec. A single composite milk sample comprising the morning and evening milk was collected for each cow for milk fatty acid determination and DNA extraction. Samples from participating herds were collected between January and October 2007.

DNA Isolation from Milk

Thirteen ml of raw milk samples were centrifuged at 21,000 g at 4° C for 30 min. The supernatant, including the fat layer, was aspirated and discarded, except for the bottom 1 ml. Five hundred µl of the sediment, which contained mostly somatic cells and casein, was then transferred into a clean 2 ml tube and washed three times with 800 µl of 1X phosphate buffered saline solution. Each wash was followed by centrifugation at 15,000 g for 5 min and removal of 800 µl of the supernatant. After the final centrifugation, 400 µl of sediment was resuspended in 500 µl of the extraction buffer (pH 8.0; 10 mM Tris, 150 mM NaCl, 2 mM EDTA, and 1% SDS) and 25 µl of Proteinase K (Invitrogen Canada Inc., Burlington, ON, Canada). The samples were incubated overnight at 65°C and were left to cool at room temperature. Seven hundred µl of phenol-chloroform (1:1 ratio) mixture (Invitrogen Canada Inc., Burlington, ON, Canada) were added to the lysate before centrifugation at 13,400 g for 15 min. The phenol-chloroform and centrifugation steps above were repeated two more times on the clear aqueous supernatant obtained after centrifugation. The final clear aqueous supernatant obtained after the last centrifugation was then used to precipitate the DNA by addition of 800 µl of 100% ethanol kept at -20°C, followed by centrifugation at 13,400 g for 5 min. The resulting DNA pellet was then washed 3 times with 70% ethanol followed by a final DNA elution step in tris ethylenediaminetetraacetic acid (TE) buffer.

SCD1 Genotyping with PCR –RFLP Method

The Ala293Val polymorphism in exon 5 of the *SCD1* gene was genotyped in 525 cows by the method of PCR-RFLP. Primers were designed based on GenBank No. AY241932 and used to amplify a 400 bp fragment, including exon 5. The PCR

amplifications were performed with a programmable thermal cycler, PTC-100™ (MJ-Research, Inc., Watertown, MA, USA) in a final reaction volume of 25 µl using PCR reagents from Invitrogen. The PCR reaction mix contained 50 ng of genomic DNA, 0.2 mM dNTPs, 2.25 mM $MgCl_2$, 0.6 µM each primer and 1.5 units *Taq* DNA polymerase. Primer sequences were: forward Primer, 5′-CCC ATT CGC TCT TGT TCT GT- 3′; reverse primer, 5′-CGT GGT CTT GCT GTG GAC T- 3′. After an initial denaturation step of 94 °C for 3 min, the reaction mix was subjected to the following cycling conditions: 34 cycles of 94 °C for 45 s, 54 °C for 30 s, and 72 °C for 1 min 30 s, plus a final extension step of 72 °C for 10 min. Amplification was confirmed by running the PCR products on 2 % agarose gels and visualizing under uv rays after staining with ethidium bromide.

The PCR products were digested at 37°C overnight with 10 units of *Nco*I nuclease (New England BioLabs Inc., Pickering, ON, Canada) based on A702G polymorphism which is in complete linkage equilibrium with C878T (Ala293Val) polymorphism, resulting in two 200bp fragments for the AA genotype, 400 bp and 200 bp fragments for the AV genotype and the undigested 400 bp fragments for the VV genotype. The digested products were separated on 2% agarose gels stained with ethidium bromide and visualized and scanned in FX Phosphoimager (Bio-Rad laboratories Inc., Hercules, CA, USA).

Milk Composition

Test day milk fat, protein and lactose contents were determined on fresh milk samples using MilkoScan FT 6000 Series mid-range infrared Fourier Transform Infra Red (FTIR) based spectrometers, and the somatic cell counts were determined by means

of Fossomatic flow cytometric cell counter at VALACTA (Montreal, QC). Test day milk fat and protein yields were determined by multiplying the respective percentages with the total test day milk production. Production parameters for the entire lactation (305 d total milk production, 305 d milk fat yield and milk protein yield) were provided by VALACTA.

Determination of Fatty Acids

Lipid extraction was performed according to Hara and Radin (1978) and milk fatty acids were transesterified with sodium methoxide according to the method of Christie (1982) with modifications as described by Chouinard et al. (1999). The C17:0 methyl ester (Nu Chek Prep, Inc., Elysian, MN) was used as the internal standard. Fatty acid methyl esters in hexane were then injected (split inlet 100:1) into a gas chromatograph (Varian, CP 3900 GC, Walnut Creek, CA, USA) equipped with a flame ionization detector. Separation of fatty acid methyl esters was performed with Supelco 100 m fused silica capillary column (100 m x 0.25 mm x 0.2 μm film thickness). Helium was used as carrier gas at a constant flow of 1 mL/min. Oven temperature was programmed from60°C to 165°C at 3°C per min and held for 10 min followed by an increase to 220°C at 5°C per minute and held for 28 minutes. Injector and detector were set at 250°C and 255°C, respectively. The GC calculated peak areas for individual fatty acids automatically and each peak was identified and quantified by comparison with pure methyl ester standards (Nu Chek Prep, Inc., Elysian, MN, USA).

Desaturase Indices

Desaturase indices were determined by calculating the ratios of cis-9 unsaturated to cis-9 unsaturated + saturated for specific fatty acid pairs and multiplied by 100 (Kelsey et al., 2003). The following indices were calculated: C10 index (C10I), C12 index

(C12I), C14 index (C14I), C16 index (C16I), C18 index (C18I), CLA index (CLAI). The total desaturase index (TI) was calculated according to Mele et al. (2007):

TI= (C10:1 + C12:1 + *cis*-9 C14:1 + *cis*-9 C16:1 + *cis*-9 C18:1 + *cis*-9, *trans*-11 CLA) / (C10:1 + C12:1 + *cis*-9 C14:1 + *cis*-9 C16:1 + *cis*-9 C18:1 + *cis*-9, *trans*-11 CLA + C10:0 + C12:0 + C14:0 + C16:0 + C18:0 + *trans*-11 C18:1) x 100

Statistical Analysis

Data were analyzed with SAS version 9.2.1 (SAS Institute, Cary, NC) using PROC MIXED (Littell et al., 2006) and the following model:

$$Y_{ijklm} = \mu + HERD_i + PARITY_j + DIM_k + SCD_l + SIRE_m + \varepsilon_{ijklm}$$

where Y_{ijklm}= dependent variable or measurable milk composition trait; μ = overall mean; $HERD_i$ = fixed effect of the i^{th} herd (17 herds); $PARITY_j$ = fixed effect of the j^{th} parity (first, second, third, fourth or greater): DIM_k = fixed effect of the k^{th} days in milk interval(3 intervals; <100 = early lactation, 100-200 = mid lactation, >200= late lactation); SCD_l = fixed effect of the l^{th} SCD1 genotype (AA, AV, VV); $Sire_m$ = random effect of the m^{th} sire (160 sires); and ε_{jklm} = random residual effect. All Interactions among various fixed variables were included in the initial model and were eventually dropped from the final model because they were not significant.

Results on the *SCD1* genotype effects are presented as Least Squares means ± SE and mean separation were by paired t-tests with Scheffe's adjustment. Differences between means were declared significantly different at $P \leq 0.05$.

Results and Discussion

Effects of SCD Genotypes on Fatty Acid Profiles and Desaturase Indices

A total of 525 Canadian Jersey cows were genotyped at the *SCD1* locus and the genotypic frequencies were 0.686, 0.244 and 0.070 for the AA, AV and VV genotypes,

respectively. The frequency of the *A* allele was 0.808 and that of *V* allele was 0.192. Genotypic frequencies were in Hardy-Weinberg equilibrium clearly indicating random mating with respect to this locus in Canadian Jersey cows. The relatively high frequency of the *A*-allele confirms an earlier report by Kgwatalala et al. (2007) on a small sample involving 48 Jersey cows.

Moioli et al. (2007) calculated allele frequencies at the SCD1 locus of 3 different Italian breeds and also reported a relatively high frequency of the *A* allele in Italian Jersey cows (0.94 vs. 0.06 for the *V* allele). A higher frequency of the *A* allele relative to the *V* allele has also been reported in the Valdostana (0.65) but not in the Piedmontese (0.42) which had a relatively high frequency (0.58) of the *V* allele (Moioli et al., 2007).

The influence of different *SCD1* genotypes on the milk fatty acid profile of Canadian Jerseys is presented in Table 1. The *SCD1* genotype had a significant influence on C10:1 and C12:1 but had no significant influence on the proportion of short-chain (C4:0 – C8:0) and long-chain (C18) fatty acids in milk fat of Canadian Jersey cows. In comparison with the *V* allele, the *A* allele was associated with significantly higher proportions of C10:1 and C12:1 and numerically lower, but non-significant, proportions of the saturated counterparts of the same fatty acids (C10:0, C12:0). There were however no significant differences between the homozygous AA and heterozygous AV cows in the proportions of C10:1 and C12:1 fatty acid in milk fat. The difference in the proportions of C14:1 between homozygous AA and homozygous VV approached significance (P=0.054). In line with our findings, Schennink et al. (2008) reported a significant positive effect of the *SCD1 A* allele on the relative proportions of C10:1, C12:1, and C14:1 in a population of 1933 Dutch Holstein Friesian heifers. Schennink et al. (2008) also reported a significant negative influence of the *A* allele on the proportions of C10:0, C12:0 and

C14:0 and our results indicate a similar trend, although the differences between the two alleles were not significant in our study (P=0.221 for C10:0; P=0.278 for C12:0; P=0.754 for C14:0). Mele et al. (2007) reported a significant positive effect of the *A* allele only in the proportion of C14:1 fatty acid in a group of 297 Italian Holstein Friesian cows, while Moioli et al. (2007) reported a significant positive effect of the *A* allele on C10:1 and C14:1 in Italian Valdostana and Piedmontese cows. In this study, the *SCD1* genotype had no significant influence on the proportions of C16:1, C18:1, CLA, total saturated fatty acids, MUFA and PUFA in Canadian Jersey cows. Our results were different from those reported for Holstein cows. Schennink et al. (2008) reported a significant positive effect of the *V* allele on C16:1, C18:1 and CLA contents of milk fat of Dutch Holstein Friesian heifers. Furthermore, Mele et al. (2007) reported a significant positive effect of the *A* allele on the total MUFA content of milk fat of Italian Holstein Friesian.

The effects of different *SCD1* genotypes on different fatty acid desaturase indices are in Table 2. Lower desaturase indices (2.881 - 11.188) were found for medium-chain fatty acids (C10-C16) and comparatively higher desaturase indices were found for long-chain fatty acids (C18). The *SCD1* genotype had a significant effect on C10I, C12I and C14I but no significant effect on C16I, C18I, CLAI and total index. The homozygous AA genotype had the highest C10I, C12I and C14I and the homozygous VV genotypes the lowest. There were significant differences in C10I, C12I and C14I between the homozygous AA genotype and the homozygous VV genotype but the differences were not significant between the homozygous AA genotype and the heterozygote. Relative to the *V* allele, the *A* allele was thus associated with significantly higher C10I, C12I and C14I and similar C16I, C18I and CLAI. Our results are consistent with Moioli et al. (2007) who reported a favorable effect of the *A* allele on C10I and C14I and no

significant effects on other desaturase indices in Italian Valdostana and Piedmontese cows. Schennink et al. (2008) also found a significant positive effect of the *A* allele on C10I, C12I and C14I and a negative effect on C16I, C18I and CLAI, although in our case, the differences in C16I, C18I and CLAI between the *A* and *V* alleles were not significant. Mele et al. (2007) found a favorable effect of the *A* allele on C14I and no effect on other indices in the Italian Holstein Friesian cows.

Differences on the effects of *SCD1* genotypes on the fatty acid profiles and desaturase indices between medium-chain fatty acids (C10-C16) and long-chain fatty acids (C18) might be explained in part by the origin of milk fatty acids. The *SCD1 A* allele was found to have a significant positive effect on some of the medium-chain MUFA (C10:1, C12:1 and C14:1) and on some of the medium-chain desaturase indices (C10I, C12I and C14I) and not on the long-chain fatty acid profile and desaturase index. It is commonly accepted that nearly all of the C4 to C14 fatty acids in milk and approximately half of the C16:0 in milk are synthesized *de novo* and the other half of C16:0 and nearly all of the long-chain fatty acids are derived from blood lipoproteins (Baumen and Davis, 1974). The *A* allele thus seems to have a positive effect only in the conversion of exclusively *de novo* synthesized medium–chain saturated fatty acids into their unsaturated counterparts in the mammary gland of ruminant animals. The favorable effect of the *A* allele on medium-chain fatty acid unsaturation and desaturase indices is particularly interesting because saturated medium-chain fatty acid contribute most to the hypercholesterolemic effects on milk consumers (Grummer, 1991). Chilliard et al. (2000) noted a very low SCD1 activity with fatty acids shorter than 18-carbon length that is consistent with our relatively lower desaturase indices for medium-chain fatty acids (C10I-C16I) and higher desaturase indices for long-chain fatty acids. Blood lipoproteins,

which supply most of the long-chain fatty acids found in milk, derive fatty acids from the animal's diet or mobilization of adipose tissue fatty acids (Grummer, 1991). Intestinal SCD1 activity and adipose tissue SCD1 activity convert some of the dietary and adipose tissue saturated fatty acids into MUFA which may then be incorporated into blood lipoproteins and eventually milk, leading to high desaturase indices observed for long-chain fatty acids. The high desaturase indices for C18I and CLAI could thus be a culmination of intestinal SCD1 activity, adipose tissue SCD1 activity and mammary gland SCD1 activity.

Effects of SCD1 Genotype on Routinely Recorded Milk Composition Traits

We also investigated the effect of Ala293Val polymorphism on traditionally recorded milk composition traits in Canadian Jersey cows for possible use of this polymorphism as a marker in selection programs, and the results are shown in Table 3. All the traits or parameters in Table 3 are test day measures except for 305 d milk yield, 305 d fat yield, and 305 d protein yield which cover the entire 305 d lactation period. The *SCD1* genotype had no significant effects on all routinely recorded test day measures (milk yield, fat % and yield, protein % and yield, somatic cell count (SCC), milk urea nitrogen (MUN) and lactose %), but had an effect on 305 d total milk yield and 305 d total protein yield. The *A* allele was associated with higher 305 d protein and milk yields. Consistent with our results, Moioli et al. (2007) reported no significant effect of *SCD1* genotype on test-day fat and protein %, fat and protein yields and milk yield.

Several independent studies have reported QTL for protein yield (Plante et al. 2001; Boichard et al., 2003) and milk yield (Boichard et al., 2003; Jiang et al., 2005) on the same chromosome where *SCD1* gene has been mapped (BTA 26) and in close proximity to the *SCD1* gene. Causal mutations for these QTL effects might be in linkage

disequilibrium with *SCD1* Ala293Val polymorphism resulting in significant effect of *SCD1* genotype on 305 d milk and protein yields.

Effects of Stage of Lactation on Fatty Acid Profiles and Desaturase Indices

We tested the effects of parity and stage of lactation on desaturase indices and milk fatty acid composition of Canadian Jersey cows and found no significant effects of parity on desaturase indices and the contents of individual fatty acids in milk fat (results not shown). The influence of stage of lactation on the fatty acid profile of milk fat of Canadian Jersey cows is shown in Table 4. Stage of lactation significantly affected the proportions of short-chain fatty acids (C4:0 and C6:0), medium-chain fatty acids (C10:1, C12:0, C12:1, C14:0, C14:1 and C16:0) and long-chain fatty acid (C18:1 and CLA), but had no effects on C8:0; C10:0, C16:1 and C18:0 contents. Higher contents of C4:0, C6:0 and C18:1 were observed in early lactation relative to other lactation stages and the opposite was true for the contents of most of the medium-chain fatty acids (C10:1 to C16:0), which increased from early to mid lactation and remained constant until late lactation. Animals in late lactation produced milk fat with a significantly higher CLA content than earlier lactation stages. The saturated fatty acid content increased from early to middle lactation and declined to early lactation proportions during late lactation while the opposite trends were observed for MUFA. The PUFA contents remained relatively constant throughout the whole lactation. Mele et al. (2007) also reported significantly lower contents of C14:0, C14:1 and higher contents of C18:1 and MUFA in early lactation relative to other lactation stages in a group of 297 Italian Holstein Friesian cows, but found no significant effect of stage of lactation on the content of C16:0. Consistent with our results, stage of lactation did not affect the contents of C16:1 and C18:0 in the Italian study and contrary to our findings had no significant effect on the CLA content.

Kelsey et al. (2003) also found no significant effect of stage of lactation on the CLA content of Holstein and Brown Swiss cows. Auldist et al. (1998), however, reported an increase in the CLA content of milk fat from 7.9 mg/g of fatty acids to 9.7 mg/g of fatty acids from early to late lactation. A negative correlation has been reported between the proportions of *de novo* synthesized fatty acids (C6-C12) and the proportions of C18 fatty acids in milk fat of dairy cattle (Palmquist, 1993; Stoop et al., 2008). During early lactation, cows may be in a negative energy balance resulting in the mobilization of fatty acids including C18:1 from the adipose tissue and inhibition of de novo fatty acid synthesis which might explain higher C18:1 and lower contents of C10:1 to C16:0 during early lactation relative to the subsequent lactation stages. Consistent with our findings, Palmquist et al. (1993) reported an increase in the proportion of short- and medium-chain fatty acids (C6-C16:0) with advancing lactation, accompanied by a compensatory decrease in the proportions of all C18 fatty acids. Oleic acid (C18:1) has a direct inhibitory effect on acetyl-CoA carboxylase and the increased availability of C18:1 either from the diet or from adipose tissue mobilization in early lactation, might decrease the percentage of *de novo* synthesized medium-chain fatty acids (C8:0-C14:0) in milk fat (Barber et al., 1997; Natalli et al., 2007). Removal of the inhibitory effect of C18:1 fatty acid with advancing lactation therefore results in increased contents of medium-chain fatty acids in milk fat.

Stage of lactation had a significant effect on all desaturase indices except C16 index (Table 5). Early lactation was associated with significantly lower C10I, C12I, C14I and CLAI than the subsequent lactation stages and the desaturase indices, which are an indirect measure of SCD1 activity, and were consistent with the contents of respective MUFA at different stages of lactation. Literature on the effect of stage of lactation on

different desaturase indices is very limited and contrary to our findings, Kelsey et al. (2003) reported very little effect of stage of lactation on CLAI in 113 Holstein and 106 Brown Swiss cows.

The changing patterns for SCD1 gene activities (estimated from desaturase indices) and its substrates saturated fatty acids were similar throughout the entire lactation, suggesting that substrate availability may be one of the limiting factors for the enzyme activity. Lower SCD1 activity during early lactation relative to the subsequent stages as reflected by most of the indices is also likely to be related to mobilization of fatty acids from adipose tissue. Delbecchi et al. (2001) hypothesized that mammary *SCD1* gene expression may be regulated by the different C18:1/C18:0 ratios brought to the mammary gland by blood lipids. Linoleic acid (C18:2) and C18:3 as well as other long-chain PUFA are known inhibitors of SCD1 (Ntambi, 1999) but in our study, their proportions remained relatively constant throughout the entire lactation, eliminating their possible influence on SCD1 activity throughout lactation.

Conclusions

We have demonstrated that the *A* allele of the *SCD1* gene positively influenced the unsaturation of C10:0 into C10:1, C12:0 into C12:1 and C14:0 into C14:1. However, the *A* allele had no significant influence on the unsaturation of C16:0 into C16:1 and C18:0 into C18:1 in Canadian Jersey cows. This study suggests that the p.A293V polymorphism could be used as a marker for genetic selection for increased unsaturation of C10:0, C12:0 and C14:0 in Canadian Jersey cows. Increased unsaturation of C10:0, C12:0 and C14:0 will be beneficial for human health but the impacts of such increased unsaturation on other traits will have to be evaluated. Stage of lactation had a significant influence on the fatty acid composition of milk fat and the contents of most medium-

chain fatty acids (C10-C16) and CLA increased from early to mid lactation and remained constant through to late lactation.

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References

Auldist, M. J., B. J. Walsh, and N. A. Thomson. 1998. Seasonal and lactation influences on bovine milk composition in New Zealand. J. Dairy Res. 65:401–411.

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.

Baumen, D. E., and C. L. Davis. 1974. Biosynthesis of milk fat. Page 31 in Lactation, a comprehensive treatise. Vol 2. B. L. Larson and v. R. Smith, ed. Academic Press, New York, NY.

Boichard, D., C. Grohs, F. Bourgeois, F. Cerqueira, R. Faugeras, A. Neau, R. Rupp, Y. Amigues, M. Y. Boscher, and H. Leveziel. 2003. Detection of genes influencing economic traits in three French dairy cattle breeds. Genet. Sel. Evol. 35:77-101.

Bonanome, A., and S. M. Grundy. 1988. Effect of dietary stearic acid on plasma cholesterol and lipoprotein levels. New England J. Med. 318:1244.

Chilliard, Y., A. Ferlay, J. Rouel, and G. Lamberet. 2003. A review of nutritional and physiological factors affecting goat milk lipid synthesis and lipolysis. J. Dairy Sci. 86:1751-1770.

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

Chouinard, P. Y., L. Corneau, A. Saebo, and D. E. Bauman. 1999. Milk yield and composition during abomasal infusion of conjugated linoleic acids in dairy cows. J. Dairy Sci. 82:2737-2745.

Christie, W.W. 1982. A simple procedure for rapid transmethylation of glycerolipids and cholesterol esters. J. Lipid Res. 23:1072-1075.

Delbecchi, L., C. E. Ahnadi, J. J. Kennelly, and P. Lacasse. 2001. Milk fatty acid composition and mammary lipid metabolism in Holstein cows fed protected or unprotected canola seeds. J. Dairy Sci. 84:1375-1381.

German, J. B., L. Monrad, C. J. Dillard, and R. Xu. 1997. Milk fat composition: Targets for alteration of function and nutrition. Pages 35-72 in milk Composition, Production, Biotechnology. R. A. S. Welch, D .J. W. Burns, S. R. Davis, A. I. Popay and C. J. Prosser, eds. CAB International, Wallingford, UK.

Goddard, M. 2001. Genetics to improve milk quality. Aust. J. Dairy Technol. 56:166-170.

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

Hara, A., and N. S. Radin. 1978. Lipid extraction of tissues with low toxicity solvent. Anal. Biochem, 90:420-426.

Hayes, K. C., and D. R. Khosla. 1992. Dietary fatty acid thresholds and cholesterolemia. FASEB J. 6:2600-2607.

Jiang, Z., S. De, M. D. Garcia, K. B. Griffin, X. L. Wu, Q. Xiao, J. J. Michal, B. S. Sharma, and G. B. Jansen. 2005. An independent confirmation of a quantitative trait locus for milk yield and composition traits on bovine chromosome 26. J. Anim. Breed. Genet. 122:281-284.

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.

Kgwatalala, P. M., E. M. Ibeagha-Awemu, J. F. Hayes, and X. Zhao. 2007. Single nucleotide polymorphisms in the open reading frame of the stearoyl-CoA desaturase gene and the resulting genetic variants in Canadian Holstein and Jersey cows. DNA Sequence. 18: 357-362.

Kris-Etherton, P. M., T. A. Pearson, Y. Wan, R. L. Hargrove, K. Moriarty, V. Fishell, and T. D. Etherton. 1999. High- monounsaturated fatty acid diets lower both plasma cholesterol and triacylglycerol concentrations. Am. J. Clin. Nutr. 70:1009-1015.

Kromhout, D., A. Menotti, H. Kestleloot, and S. Sans. 2002. Prevention of coronary heart disease by diet and lifestyle: Evidence from prospective cross-cultural, cohort, and interventional studies. Circulation 105:893-898.

Littell, R. C., G. A. Milliken, W. W. Stroup, R. D. Wolfinger, O. Schabenberger. 2006.SAS® for Mixed Models, 2nd Edition, Cary, NC: SAS Institute.

MacDonald, H. B. 2000. Conjugated linoleic acid and disease prevention: A review of current knowledge. J. Am. Coll. Nutr. 19:111S-118S.

Mele, M., G. Conte, B. Castiglioni, S. Chessa, N. P. P. Macciotta, A. Serra, A. Buccioni, G. Pagnacco, and P. Secchiari. 2007. Stearoyl-Coenzyme A desaturase gene polymorphism and milk fatty acid composition in Italian Holsteins. J. Dairy Sci. 90:4458-4465.

Mensink, R. P., P. L. Zock, A. D. Kester, and M. B. Katan. 2003. Effects of dietary fatty acids and carbohydrates on the ration 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.

Moioli, B., G. Contarini, A. Avalli, G. Catillo, L. Orru, G. De Matteis, G. Masoero, and F. Napolitano. 2007. Short communication: Effect of stearoyl- coenzyme A desaturase polymorphism on fatty acid composition of milk. J. Dairy Sci. 90:3553-3558.

Natali, F., L. Siculella, S. Salvati, and G. V. Gnoni. 2007. Oleic acid is a potent inhibitor of fatty acid and cholesterol synthesis in C6 glioma cells. J. Lipid Res. 48:1966-1975.

Ntambi, J. M. 1999. Regulation of stearoyl-CoA desaturase by polyunsaturated fatty acids. J. Lipid Res. 40:1549-1558.

Pariza, M. W., Y. Park, and M. E. Cook. 1999. Conjugated linoleic acid and the control of cancer and obesity. Toxicology. Sci. 52(Suppl.): 107-110.

Peterson, D. G., J.A. Kelsey, and D.E. Bauman. 2002. Analysis of variation in cis-9, trans-11 Conjugated linoleic acid (CLA) in milk fat of dairy cows. J. Dairy Sci. 85:2164-2172.

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

Plante, Y., J. P. Gibson, J. Nadesalingam, H. Mehrabani-Yeganeh, S. Lefebvre, G. Andervoort, and G.B. Jansen. 2001. Detection of quantitative trait loci affecting milk production traits on 10 chromosomes in Holstein cattle. J. Dairy Sci. 84: 1516-1524.

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.

Parodi, P. W. 1999. Conjugated linoleic acid and other anticarcinogenic agents of bovine milk fat. J. Dairy Sci. 82 :1339-1349.

Pascal, G. 1996. Les apports quotidiens recommandes en lipids et en acides gras. OCL 3 :205-210.

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.

Soyeurt, H., P. Dardenne, A. Gillion, 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.

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. J. Dairy Sci. 91:385-394.

Taniguchi, M., T. Utsugi, K. Oyama, H. Manmen, 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 14:142-148.

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

List of Tables

Table 1. Effect of *stearoyl-CoA desaturase1 (SCD1)* genotype on milk fatty acid profile (g/100g of total fatty acids) of Canadian Jersey cows

Parameter	AA	AV	VV	Overall p-values
C4: 0	4.28 ± 0.04	4.28 ± 0.06	4.18 ± 0.10	0.6151
C6:0	3.08 ± 0.02	3.08 ± 0.04	3.07 ± 0.06	0.5529
C8:0	0.133 ± 0.002	0.131 ± 0.003	0.132 ± 0.006	0.8931
C10:0	6.60 ± 0.06	6.78 ± 0.09	6.88 ± 0.16	0.0589
C10:1	0.69^{a} ± 0.01	0.67^{a} ± 0.01	0.61^{b} ± 0.02	0.0002
C12:0	6.21 ± 0.08	6.34 ± 0.10	6.45 ± 0.16	0.1021
C12:1	0.17^{a} ± 0.003	0.16^{a} ± 0.005	0.14^{b} ± 0.008	0.0005
C14:0	15.27 ± 0.10	15.49 ± 0.15	15.47 ± 0.26	0.3092
C14:1	1.06 ± 0.02	1.06 ± 0.03	0.97 ± 0.04	0.0484
C16:0	26.03 ± 0.15	25.96 ± 0.24	26.54 ± 0.44	0.4918
C16:1	1.20 ± 0.01	1.21 ± 0.02	1.23 ± 0.04	0.8237
C18:0	8.20 ± 0.18	8.05 ± 0.27	7.94 ± 0.48	0.8031
C18:1 *cis*-9	17.43 ± 0.16	17.34 ± 0.26	17.56 ± 0.48	0.9025
C18:1 *trans*-11	0.82 ± 0.02	0.78 ± 0.02	0.74 ± 0.04	0.1073
CLA	0.24 ± 0.004	0.23 ± 0.007	0.24 ± 0.012	0.2621
C18:2	1.40 ± 0.02	1.38 ± 0.04	1.32 ± 0.07	0.4726
C18:3	0.47 ± 0.01	0.44 ± 0.02	0.44 ± 0.03	0.3396
SFA	69.80 ± 0.20	70.13 ± 0.32	70.69 ± 0.58	0.2725
MUFA	20.55 ± 0.16	20.43 ± 0.26	20.52 ± 0.47	0.9221
PUFA	2.92 ± 0.04	2.83 ± 0.06	2.74 ± 0.11	0.1650

[a–b]Means within a row with different superscripts differ significantly ($P \leq 0.05$)

[1]SFA= Saturated fatty acids

[2]MUFA= Monounsaturated fatty acids

[3]PUFA= Polyunsaturated fatty acids

Table 2. Effect of *stearoyl-CoA desaturase1 (SCD1)* genotype on SCD1 indices in Canadian Jersey cows

Parameter	AA	AV	VV	Overall p- values
C10 Index[1]	$11.19^{a} \pm 0.11$	$10.68^{a} \pm 0.17$	$9.50^{b} \pm 0.32$	<0.0001
C12 Index[1]	$2.88^{a} \pm 0.05$	$2.72^{a} \pm 0.07$	$2.29^{b} \pm 0.13$	<0.0001
C14 index[1]	$8.14^{a} \pm 0.17$	$8.06^{ab} \pm 0.22$	$7.29^{b} \pm 0.34$	0.0331
C16 Index[1]	5.88 ± 0.07	5.89 ± 0.12	5.95 ± 0.21	0.9487
C18 Index[1]	70.51 ± 0.43	70.94 ± 0.65	71.62 ± 1.15	0.5803
CLA Index[2]	26.43 ± 0.41	26.06 ± 0.66	28.78 ± 1.20	0.1242
Total Index[3]	24.80 ± 0.18	24.63 ± 0.29	24.54 ± 0.53	0.8106

[a–c]Means within a row with different superscripts differ significantly ($P \leq 0.05$)

[1]Indices are calculated according to the following example: C16 index = *cis*-9 C16:1/ (*cis*-9 C16:1 + C16:0) x 100

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

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

Table 3. Effects of *stearoyl-CoA desaturase1 (SCD1)* genotype on routinely recorded lactation parameters in Canadian Jersey cows

Parameter	AA	AV	VV	Overall p-values
Milk Yield (kg)	18.89 ± 0.31	18.76 ± 0.43	18.16 ± 0.76	0.6640
Fat yield (kg)	0.92 ± 0.01	0.94 ± 0.01	0.90 ± 0.04	0.4739
Fat %	5.01 ± 0.05	5.16 ± 0.06	5.10 ± 0.11	0.0615
Protein Yield (kg)	0.72 ± 0.01	0.72 ± 0.01	0.70 ± 0.03	0.7525
Protein %	3.87 ± 0.02	3.93 ± 0.03	3.93 ± 0.05	0.0824
SCC ($x10^3$)	283.29 ± 34.28	233.18 ± 34.92	220.99 ± 80.34	0.1485
MUN	12.36 ± 0.27	12.89 ± 0.43	12.10± 0.75	0.4652
Lactose %	4.41 ± 0.06	4.40 ± 0.06	4.42 ± 0.08	0.8824
305d Milk Yield (kg)	5875.98^{a} ± 111.86	5617.57^{ab} ± 137.10	5340.00^{b} ± 219.81	0.0064
305d Fat yield (kg)	280.24 ± 3.13	271.90 ± 4.81	258.09 ± 9.45	0.0537
305d Protein yield (kg)	222.56^{a} ± 2.92	213.98^{ab} ± 4.02	204.32^{b} ± 7.32	0.0120

[1]MUN= Milk urea nitrogen

[a, b]Means within a row with different superscripts differ significantly ($P \leq 0.05$)

Table 4. Effect of stage of lactation on the fatty acid profile (g/100g of total fatty acids) of Canadian Jersey cows

Parameter	Early[1]	Middle[2]	Late[3]	Overall p-values
C4: 0	$4.40^{a} \pm 0.07$	$4.18^{b} \pm 0.06$	$4.16^{b} \pm 0.06$	0.0007
C6:0	$3.13^{a} \pm 0.04$	$3.04^{ab} \pm 0.04$	$3.02^{b} \pm 0.03$	0.0325
C8:0	0.136 ± 0.004	0.131 ± 0.003	0.128 ± 0.003	0.1170
C10:0	6.72 ± 0.01	6.76 ± 0.09	6.69 ± 0.09	0.2079
C10:1	$0.58^{a} \pm 0.01$	$0.68^{b} \pm 0.01$	$0.70^{b} \pm 0.01$	<0.0001
C12:0	$6.10^{a} \pm 0.11$	$6.52^{b} \pm 0.10$	$6.40^{b} \pm 0.10$	0.0002
C12:1	$0.14^{a} \pm 0.01$	$0.16^{b} \pm 0.01$	$0.17^{b} \pm 0.01$	<0.0001
C14:0	$14.89^{a} \pm 0.16$	$15.68^{b} \pm 0.15$	$15.66^{b} \pm 0.14$	<0.0001
C14:1	$0.94^{a} \pm 0.03$	$1.05^{b} \pm 0.03$	$1.10^{b} \pm 0.03$	<0.0001
C16:0	$25.58^{a} \pm 0.27$	$26.97^{b} \pm 0.25$	$25.98^{a} \pm 0.22$	<0.0001
C16:1	1.21 ± 0.03	1.22 ± 0.02	1.21 ± 0.02	0.8514
C18:0	8.37 ± 0.30	8.10 ± 0.28	7.72 ± 0.25	0.0888
C18:1 *cis*-9	$18.68^{a} \pm 0.29$	$16.17^{b} \pm 0.27$	$17.49^{c} \pm 0.24$	<0.0001
C18:1 *trans*-11	0.81 ± 0.03	0.76 ± 0.02	0.78 ± 0.02	0.3257
CLA	$0.22^{a} \pm 0.01$	$0.23^{ab} \pm 0.01$	$0.25^{b} \pm 0.01$	0.0038
C18:2	1.43 ± 0.04	1.33 ± 0.04	1.35 ± 0.03	0.0868
C18:3	0.44 ± 0.02	0.45 ± 0.02	0.47 ± 0.01	0.3456
SFA	$69.32^{a} \pm 0.35$	$71.54^{b} \pm 0.33$	$69.76^{a} \pm 0.29$	<0.0001
MUFA	$21.56^{a} \pm 0.28$	$19.28^{b} \pm 0.26$	$20.66^{c} \pm 0.24$	<0.0001
PUFA	2.89 ± 0.07	2.77 ± 0.06	2.84 ± 0.06	0.2813

[a–c]Means within a row with different superscripts differ ($P \leq 0.05$)

[1]SFA= Saturated fatty acids

[2]MUFA= Monounsaturated fatty acids;

[3]PUFA= Polyunsaturated fatty acids

<100 DIM = [1]Early; 100-200 DIM = [2]Middle; >200 DIM = [3]Late

Table 5. Effect of stage of lactation on stearoyl-CoA desaturase1 (SCD1) indices in Canadian Jersey cows

Parameter	Early[1]	Middle[2]	Late[3]	Overall p-values
C10- Index	$9.29^{a} \pm 0.19$	$10.80^{b} \pm 0.18$	$11.20^{b} \pm 0.16$	<0.0001
C12 Index	$2.38^{a} \pm 0.08$	$2.70^{b} \pm 0.07$	$2.81^{b} \pm 0.07$	<0.0001
C14 index	$7.38^{a} \pm 0.23$	$7.83^{ab} \pm 0.22$	$8.29^{b} \pm 0.21$	<0.0001
C16 Index	6.01 ± 0.13	5.82 ± 0.12	5.90 ± 0.11	0.4373
C18 Index	$71.34^{ab} \pm 0.71$	$69.67^{a} \pm 0.67$	$72.06^{b} \pm 0.61$	0.0036
CLA Index	$25.59^{a} \pm 0.73$	$27.72^{b} \pm 0.67$	$27.96^{b} \pm 0.61$	0.0066
Total Index	$25.89^{a} \pm 0.32$	$23.17^{b} \pm 0.29$	$24.90^{c} \pm 0.27$	<0.0001

[a–c]Means within a row with different superscripts differ ($P \leq 0.05$)

<100 DIM = [1]Early; 100-200 DIM = [2]Middle; >200 DIM = [3]Late

CONNECTING STATEMENT III

In chapter II and chapter III, the influence of *SCD1* genotype on C10 desaturase index, C12 desaturase index and C14 desaturase index, and consequently on the concentrations of C10:1 and C12:1 in both Holsteins and Jerseys were established. The *SCD1 p.293A* allele was associated with significantly higher C10 desaturase index, C12 desaturase index and C14 desaturase index and consequently higher concentrations of C10:1 and C12:1 compared with the *SCD1 p.293V* allele. Although the general tendency is to investigate the influence of coding sequence variants or SNPs in the coding regions on performance in various economic traits, SNPs in the 5′and 3′UTRs of some genes may have a significant influence on performance in traits of economic importance in livestock. In chapter IV, SNPs in the 3′UTR of the *SCD1* gene and the resulting haplotypes or regulatory variants in Holsteins and Jerseys were established through sequencing. A method for quickly genotyping the animals at the 3′UTR of the *SCD1* gene based on RFLP was developed, and associations between 3′UTR genotype and the concentrations of individual fatty acids and/or individual desaturase indices in Canadian Holsteins established.

CHAPTER V. *STEAROYL-COA DESATURASE1 (SCD1)* 3′UTR SNPs AND THEIR INFLUENCE ON MILK FATTY ACID COMPOSITION OF CANADIAN HOLSTEIN COWS

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Abstract

Stearoyl-CoA desaturase1 (SCD1) catalyzes the synthesis of conjugated linoleic acid (CLA) and mono-unsaturated fatty acids (MUFA) in the mammary gland of ruminant animals. Considerable variations in CLA and MUFA have been reported among animals of the same contemporary group. We hypothesized that single nucleotide polymorphisms (SNPs) in the 5′ and 3′ untranslated regions (UTRs) of the *SCD1* gene would influence the production of SCD1 enzyme and consequently its activity in the mammary gland, which may account for some of the observed within breed variations in CLA and MUFA. The 5′ and 3′ UTRs of the *SCD1* gene of 46 Holsteins and 35 Jerseys were analyzed for SNPs by sequencing. No SNPs were identified in the 5' UTR while fourteen SNPs were identified in the 3′ UTR region. Further analysis revealed 3 haplotype structures or regulatory variants in Holsteins: named H1, H2 and H3 and only H1 and H3 in Jerseys. An IRES motif was found in the H1 variant. A subsequent association study involving the milk fatty acid profiles of 862 Holstein cows found the H1 regulatory variant to be associated with higher C10 and C12 desaturase indices and consequently with higher contents of C10:1 and C12:1 relative to the H3 variant. The effects of the H2 variant were intermediate to those of H1 and H3. SNPs in the 3′UTR of the *SCD1* gene could therefore explain some of the within breed variations in MUFA content of milk fat.

Introduction

Bovine *stearoyl-CoA desaturase 1 (SCD1)* gene codes for an important lipogenic enzyme stearoyl-CoA desaturase1 (SCD1) that catalyzes the biosynthesis of monounsaturated fatty acids (MUFA) from their saturated counterparts as well as the synthesis of conjugated linoleic acid (CLA) in the mammary gland and adipose tissues. SCD1 catalyzes the addition of a double bond (between carbons 9 and 10) in saturated fatty acids with 10-18 carbon atoms. CLA (*cis* 9, *trans* 11 CLA and cis *10*, *trans* 12 CLA) has received wide attention in recent years because of its potential in protecting against cancer, atherogenesis and diabetes mainly in animal models and cell lines (Dhiman et al., 2005). The second isoform of *stearoyl-CoA desaturase* (*SCD5*) gene has recently been identified in cattle (Lengi and Corl, 2007). SCD5 is expressed mainly in the brain and its contribution to mammary SCD activity is currently unknown (Lengi and Corl, 2007).

Higher CLA and MUFA contents have been reported in milk fat of Holstein than of Jersey cows when grazed on pasture or fed diets containing conserved forages and grains (White et al., 2002; Lawless et al., 1999). Kelly et al. (1998) reported a three-fold variation in CLA content of milk among individual cows of the same breed, fed the same diet, at a similar stage of lactation and producing milk with similar fat content. These differences could be due to differences in rumen production of CLA and transvaccenic acid (TVA), desaturase activity in the mammary gland and/or other unknown factors (Dhiman et al., 2005). Peterson et al. (2002) reported a range of approximately two-fold for individuals' variation in desaturase indices (used as proxy for SCD1 activity) when cows were fed the same diet or when switched between diets suggesting that individual differences in SCD1 activity could in part account for some of the observed variation in CLA among individuals on the same diet.

It has been observed in recent years that polymorphisms in the underlying genes or in the regulatory regions of the underlying genes account for some of the observed phenotypic differences among individuals. Taniguchi et al. (2004) reported the existence of SNPs in the open reading frame (ORF) region of the *SCD1* gene with significant effects on the MUFA content of marbling fat in Japanese black cattle. Schennink et al. (2008) reported a significant influence of *c.C878T (p.A293V)* SNP in the ORF region of the S*CD1* gene on desaturase indices and consequently on the contents of various MUFA in milk fat of Dutch Holstein Friesian heifers. Keating et al. (2005) analyzed the promoter region of the *SCD1* gene in several breeds of cattle including Irish Friesian, Montbeliarde, Norwegian Red, Dutch Friesian, Charolois, Limousin and Kerry, and reported no SNPs. The 3'UTRs of most genes have remained largely neglected as potential sources of regulatory variants that may have a significant influence on the animal's phenotype. Current research results however indicate that the 3'UTRs are actively involved in post-transcriptional regulation of gene expression including modulation of the transport of mRNAs out of the nucleus and of translation efficiency, subcellular localization and stability (Mignone et al., 2002; Chabanon et al., 2004). Within the 3'UTRs of some mRNAs, the AU-rich element (ARE) motifs are known to be associated with mRNA degradation and therefore suppress gene expression at translational level (Wang et al., 2006). Conne et al. (2000) described the 3′UTR of mRNAs as a molecular 'hotspot' for pathology or abnormal phenotypes. To date, diseases that have been associated with SNPs in the 3′UTRs of the underlying genes include hereditary thrombophilia (mutation in the thrombin gene), urolithiasis (mutation in the urokinase gene) and hypertension (mutation in the C-type natriuretic peptide gene) (Mazunder et al. 2003). The 5′UTR of messenger RNA contain nucleotide sequences required for the assembly of the translational

machinery (Conne, et al., 2000), and SNPs in the 5′UTR might therefore affect the assembly of the translational machinery and hence translation of the constituent mRNA.

We therefore hypothesize that existence of SNPs in the 5' and 3′UTRs of the *SCD1* gene may result in different regulatory variants with effects on SCD1 activity, and consequently account for some of the variations in CLA and MUFA contents of milk fat between and within breeds. Therefore, the objectives of our study were to (1) determine SNPs in the 5' and 3'UTRs of the *SCD1* gene and the resulting regulatory variants in Canadian Holstein and Jersey cows and (2) investigate the influence of the regulatory variants on desaturase indices and fatty acid composition of Canadian Holstein cows.

Materials and Methods

Phase 1- Sequencing and haplotype structure determination

Experimental Animals

Blood or milk samples were collected from a random selection of 46 Holstein and 35 Jersey cows enrolled in the Quebec Dairy Production Centre of Expertise (VALACTA) program (http://www.valacta.com). VALACTA routinely analyzes milk from member dairy herds in Quebec. The animals used in this investigation were previously used by Kgwatalala et al. (2007).

DNA isolation and primer design

Total genomic DNA was isolated from milk samples using Nucleospin Blood Mini Kit (Macherey-Nagel Inc, Easton., PA) following the supplier's protocol, with slight modifications. Milk samples (2 ml per sample) were centrifuged at 15000 x g for 15 min to remove excess fat prior to following manufacturer's protocol for DNA isolation. Primers were designed for selective amplification of the 5′ and 3′ UTRs of the *SCD1* gene using the reference sequence GenBank No. AY241932 and the Primer3 web

Program (Primer 3 _www.cgi v 0.2). Five primer pairs were used to selectively amplify 5 overlapping fragments that covered the whole length of the unusually long 3′ UTR of the *SCD1* gene. The primers were synthesized by Invitrogen Canada Inc. (Burlington, Ontario) and are shown in Table 1.

PCR Amplification and DNA Sequencing

PCR amplifications were performed on a programmable Thermal Cycler, PTC-100 TM (MJ, Research, Inc., Watertown, MA 02172, USA) in a final reaction volume of 50 µl using PCR reagents and primers from Invitrogen. Each PCR reaction contained 1 X PCR buffer, 0.2 mM dNTPs mix, 0.6 µM of each primer, 1.5-2.25 mM magnesium chloride (Table 1), 1 unit Taq DNA polymerase and 50 ng of DNA template. After an initial denaturation step of 94 °C for 3 min, the reaction mixes were subjected to the following PCR conditions: 34 cycles of 94 °C for 45 s, 54 °C to 56 °C (Table 1) for 30 s, and 72 °C for 1 min 30 s, plus a final extension step of 72 °C for 10 min. Amplification was confirmed by running the PCR products on 1% agarose gels and visualization with uv rays after staining with ethidium bromide. Amplified PCR products were bidirectionally sequenced using both the forward and reverse primers by McGill University/Genome Quebec Innovation Centre (https://genomequebec.mcgill.ca/).

Sequence Analysis

Chromatographs generated from sequencing were processed using Chromas version 1.45 (http://www.technelysium.com.au/chromas14x.html). Both forward and reverse primer sequences were aligned using ClustalW (www.ebi.ac.uk/clusatlw/) multiple sequence alignment program to verify sequence identities and to determine the presence of genetic polymorphisms as compared to the reference sequence. SNPs were also confirmed by the presence of heterozygotes and homozygotes in the chromatographs

of some individuals at the identified polymorphic sites. SNP frequencies were estimated with GENEPOP program (Raymond and Donnely, 2001) while haplotypes and their frequencies were determined with the program PHASE V2.1.1 (Donnelly, 2003, Smith and Donnelly, 2001). Identification of functional motifs in the different 3'UTR regulatory variants of the bovine *SCD1* gene and the 3'UTR of other mammalian *SCD* sequences in the GenBank [caprine (GenBank Accession No. 325499), porcine (GenBank Accession No. AY487829), human (GenBank Accession No AF097514), murine *SCD1* (GenBank Accession No. NM009127), murine *SCD2* (GenBank Accession No. NM009128), and murine *SCD3* (GenBank Accession No. NM 024450)] was performed using a web-based UTRscan program (Pesole and Liuni, 1999).

Phase 2- Association study

Experimental animals and DNA isolation from milk

The analysis was carried out on 862 Canadian Holstein cows of first to fifth parity from 17 different herds in Southern Quebec. There were 290, 223, 159 and 190 cows of first, second, third and ≥fourth parity, respectively. The cows were between 21 and 350 days in milk (DIM) and were all enrolled in VALACTA program. A single composite milk sample comprising the day's morning and evening milk was collected for each cow for both DNA isolation and fatty acid determination. Milk samples were collected from January to October, 2007.

DNA was isolated from this larger sample size using the phenol-chloroform method as described by Lipkin et al. (1993) with slight modifications. Essentially, 13 ml of raw milk samples were centrifuged at 21000 g at 4°C for 30 min and about 500 µl of

sediment was washed three times with 800 µl of 1X PBS. After the final wash, 400 µl of sediment was resuspended in 500 µl of the extraction buffer (10 mM Tris, 150 mM NaCl, 2 mM EDTA, and 1% SDS; pH 8.0) and 25 µl of proteinase K (Invitrogen). The samples were incubated overnight at 65°C and DNA extracted with equal volumes (350 µl) of buffered saturated phenol and chloroform. The phenol-chloroform extraction was repeated three times and DNA was precipitated with 100% ethanol kept at -20 °C. The resulting DNA pellet was washed 3 times with 70% ethanol, air-dried and resuspended in TE buffer.

SNP genotyping with PCR –RFLP method

Genotyping of animals at the 3′UTR was based on the observed exclusive association of H1 and H2 variants with the open reading frame allele *p.293A* and the H3 variant with the *p.293V or p.293B* allele. Animals were therefore genotyped by discriminating first between ORF *p.293AA* and *p.293AV* genotypes followed by discrimination between H1 and H2 regulatory variants. The animal's genotype in the ORF region was determined by restriction digestion of a 400 bp PCR fragment of exon 5 with *Nco*I endonuclease (New England BioLabs Inc., Pickering, ON, Canada). The 400 bp PCR fragment was generated using PCR conditions similar to those of 5′ UTR described above and the primers designed based on GenBank No. AY241932: forward Primer, 5′-CCC ATT CGC TCT TGT TCT GT- 3′; reverse primer, 5′-CGT GGT CTT GCT GTG GAC T- 3′. Digestion of the PCR product with *Nco*I nuclease is based on c.A702G polymorphism which is in complete linkage disequilibrium with c.C878T (p.A293V) polymorphism, resulting in two 200 bp fragments for the *p.293AA* genotype and, 400 bp and 200 bp fragments for the *p.293AV* genotype and the undigested 400 bp fragments for the *p.293VV* genotype (data not shown).

After the initial genotyping at the open reading frame, *p.293AA* and *p.293AV* animals were genotyped as distinct groups to distinguish between H1 and H2 haplotypes or regulatory variants. The distinction was accomplished by PCR amplification of the 3′ UTR1 region as described in Table 1 using conditions described above. Restriction digestion of the resulting 1000 bp PCR product with *Mfe*I endonuclease (New England BioLabs Inc.) was based on g.C1763A polymorphism resulting in different sized fragments (Figures 1 and 2). The g.C1763A mutation distinguishes between H1 and H2 regulatory variants within the ORF genotypes.

Determination of fatty acid profiles

Lipid extraction was performed according to Hara and Radin (1978) and milk fatty acids were transesterified with sodium methoxide according to the method of Christie et al. (1982) with modifications as described by Chouinard et al. (1999). Fatty acid methyl esters in hexane were then injected (split inlet 100:1) into a gas chromatograph (Varian, CP 3900 GC, Walnut Creek, CA, USA) equipped with a flame ionization detector. Separation of fatty acid methyl esters was performed with Supelco-100 m fused silica capillary column (100 m x 0.25 mm x 0.2 μm film thickness). Helium was used as carrier gas at a constant flow of 1 mL/min. Oven temperature was programmed from 60°C to 165°C at 3°C per min and held for 10 min followed by an increase to 220°C at 5°C per minute and held for 28 min. Injector and detector were set at 250°C and 255°C, respectively. The GC calculated peak areas for individual fatty acids automatically. Heptadecanoic acid (C17:0; Nu Check Prep, Inc., Elysian, MN, USA) was used as the internal standard and a standard fatty acid mixture comprising 36 known individual fatty acids (Nu Check Prep, Inc) was used for the provision of standard

retention times. Fatty acids in milk fat were identified by comparison with retention times of fatty acids in the standard fatty acid mixture.

Desaturase Indices

Individual desaturase indices were determined by calculating the ratios of cis-9 unsaturated to cis-9 unsaturated + saturated for specific fatty acid pairs, multiplied by 100 as described by Kelsey et al. (2003) and includes indices for C10, C12, C14, C16, C18 and total desaturase index (TI) was calculated according to Mele et al. (2007). CLA index (CLAI) was determined by calculating the ratio of *cis-9, trans-11* CLA to *cis-9, trans-11* CLA + *trans-11*, C18:1.

Statistical Analysis.

Data were analyzed with the following proc mixed procedure of SAS (SAS, 1999).

$Y_{ijklm} = \mu + HERD_i + PARITY_j + DIM_k + Ge_l + Sire_m + \varepsilon_{ijklm}$

Where Y_{ijkl}= dependent variable (C14:1 content %, C16 index etc); μ = overall mean; $HERD_i$ = fixed effect of the i^{th} herd (17 heads); $PARITY_j$ = fixed effect of the j^{th} parity (First, second, third, fourth or greater): DIM_k = fixed effect of the k^{th} days in milking interval [<100DIM (early lactation), 100-200DIM (Mid lactation), >200DIM (late lactation)]; Ge_l = fixed effect of the l^{th} UTR genotype (H1H1, H1H2, H1H3, H2H2, H2H3, H3H3); $Sire_m$= random effect of the m^{th} sire (336 sires with 1-36 progeny per sire) and ε_{ijklm} = random residual effect. Interactions among various fixed variables were included in the initial model and were eventually dropped from the final model because all interactions were not significant.

Results on the *SCD1* genotype effects were presented as least square means ± SE and mean separation were by paired t-tests with Scheffe's adjustment. Differences between means were declared significant at $P \leq 0.05$.

Results

SNPs in the 3′ UTR region of the SCD1 gene

The 3′UTR of the *SCD1* gene was highly polymorphic while no SNPs were found within the 5' UTR in both Holsteins and Jerseys. SNPs in the 3′UTR region of the *SCD1* gene and their nucleotide positions are shown in Table 2. A total of 14 SNPs were found in the 3′UTR of the *SCD1* gene in both Holsteins and Jerseys. Sequence analysis showed that particular SNPs in the 3′UTR always occurred together (linkage disequilibrium) and this was confirmed by the program PHASE, thus giving a total of three characteristic haplotypes/regulatory variants, here named H1, H2 and H3, and shown in Table 3. The different 3′UTR haplotypes or regulatory variants have the same nucleotide sequences with the reference sequence (GenBank Accession No. AY241933) except at polymorphic sites where they have sequences shown in Table 3. Our analysis of the different 3′UTR regulatory variants and the previously described ORF sequence variants (Kgwatalala et al. 2007) revealed that H1 and H2 variants were always linked to the open reading frame region p.293*A* allele and H3 variant to the p.293*B* or p.293*V* allele.

All the 3 regulatory variants were present in the Holstein breed while the H2 regulatory variant was absent in the Jersey breed. The estimated frequencies of the 3'UTR regulatory variants in both Holsteins and Jerseys are shown in Table 3. The H1 haplotype is by far the most frequent in Canadian Holsteins and Jerseys (0.772 and 0.943, respectively), followed by the H3 haplotype (0.174 vs. 0.057, respectively). The H2 haplotype was absent in the Jersey breed and existed at a low frequency in the Holstein breed (0.054).

Regulatory motifs in the 3'UTR of the SCD1 gene

Analysis of different regulatory variants for functional motifs using UTRscan revealed the presence and/or absence of motifs shown in Table 4. All the 3 regulatory variants contained the Erythroid 15-lipoxygenase differentiation control element (15-LOX DICE), K-Box and the Brd-Box motifs while the internal ribosome entry site (IRES) motif was unique to the H1 regulatory variant.

Effects of 3′UTR genotypes on milk fatty acid composition of Canadian Holsteins

The frequencies of different 3′UTR genotypes in Canadian Holsteins as well as the frequencies of different regulatory variants (gene frequencies) in a sample of 862 Holsteins are shown in Table 5. The H1H1 genotype was by far the most frequent and the H2H2 genotype the least frequent. The gene frequencies were however not in Hardy-Weinberg equilibrium possibly due to selective breeding (non-random mating) of dairy animals through the use of artificial insemination and a few elite bulls.

The influences of different regulatory variants on various desaturase indices and on the fatty acid composition of Canadian Holsteins are shown in Tables 6 and 7, respectively. The *SCD1* indices are used as a proxy for stearoyl-CoA desaturase1 activity with regard to the unsaturation of particular saturated fatty acids into their monounsaturated ones in the mammary gland of ruminant animals. The genotype at the 3′UTR had significant influence on the C10 index (C10I) and C12 index (C12I) but had no influence on C14 index (C14I), C16 index (C16I), C18 index (C18I), CLA index (CLAI) and total desaturase index (TI). The H1 regulatory variant was associated with significantly higher C10I and C12I and the opposite was true for the H3 variant. The effects of the H2 variant on different desaturase indices were intermediate to those of H1 and H3 effects. The higher C10I and C12I translated to the higher contents of C10:1 and

C12:1 in the H1H1 genotype of Canadian Holstein cows (Table 7). Apart from those two MUFA, the animal's genotype at the 3´UTR had no significant influence on the contents of other fatty acids listed in Table 7 including conjugated linoleic acid (CLA).

Discussion

SNPs in the 3'UTR and the resulting regulatory variants

The total absence of SNPs in the 5'UTR and the high density of SNPs in the 3'UTR of the *SCD1* gene is not surprising given the relatively smaller size of the 5'UTR as compared to the 3'UTR (144 nucleotides vs. 3874 nucleotides in cattle). The 3'UTRs of most genes can also tolerate SNPs and other types of polymorphisms because they are not under the same rigid structural constraints as promoter, 5'UTRs or coding sequences which are obliged to accommodate transcriptional or translational machinery (Conne et al., 2000) and therefore require very high fidelity in the primary sequence of DNA and mRNA. The existence of SNPs; g.G1571T, G1644C, C1763A, T2053C, A2584G, T2668C, A3007G, C3107T, G3208A, T3290C, G3497A and C4533T in the 3'UTR of the *SCD1* gene in the *Bos taurus* species was recorded in GenBank Accession Nos. 241932 and 241933. Taniguchi et al. (2004) also reported the existence of SNPs; T2053C, T3290C, G3497A and 2 other SNPs not identified in the current study, in the 3'UTR of the *SCD1* gene in the Japanese Black cattle (Beef Breed). SNPs G3682A, A4399T and G4881A found in both Holsteins and Jerseys have never been reported before and to the best of our knowledge, this is the first report on the existence of such SNPs in dairy cattle.

GenBank Accession No. AY241933 and Taniguchi et al. (2004) implied the presence of only two different haplotypes or regulatory variants (H1 and H3 with minor differences due to SNPs reported in the two studies) linked to ORF *p.293A* and *p.293B* or *p.293V* (according to Taniguchi et al., 2004) variants, respectively, while we report here

an additional haplotype (H2) in Canadian Holstein cows. The H2 haplotype is being described for the first time and is different from the H1 haplotype because of SNPs g.C1763A and A3007G. The relatively low frequency of the H2 variant in Holsteins and its total absence in Jersey cows suggests that this variant may be relatively new, resulting from very recent mutations occurring in the H1 variant.

Regulatory motifs in the 3'UTR of the SCD1 gene

All the 3 regulatory variants contained the 15-LOX DICE, the K- box and the Brd-box motifs. The 15-LOX DICE motif is recognized by specific regulatory proteins, heterogeneous nuclear ribonucleoproteins K (hn RNP K) and heterogeneous nuclear ribonucleoproteins E1 (hn RNP E1), resulting in inhibition of translation of the 15-LOX mRNA (Ostareck-Lederer et al., 1994). The K-Box and the Brd-Box motifs have been reported in the 3'UTR of many Notch pathway genes in drosophila including Helix-loop-helix repressor family and the bearded family and mediate negative post-transcriptional regulation mainly effected by decreased transcript levels, transcript stability and translational efficiency (Lai et al., 1998). miRNAs likely form duplexes with the K-box and Brd-box and repress translation of the constituent mRNAs. The internal ribosome entry site (IRES) structural motif found in the H1 variant mediates internal mRNA ribosome binding translational mechanism, an alternative to the conventional 5'-cap dependent ribosome scanning mechanism, and may thus enhance translation of its constituent mRNA (Le and Maizel, 1997). The presence of IRES motif in the H1 regulatory variant may thus enhance translation of the constituent mRNA while its absence in the other two regulatory variants (because of the SNP) may lower the rate of translation of the constituent mRNAs which may ultimately affect the protein turnover or quantity of the SCD1 enzyme produced.

No functional motifs were identified in the 5' UTR of the *SCD1* gene in cattle, sheep, goats and mice, and our analysis of the 3'UTRs of different mammalian species using the UTRscan program revealed the presence of 15-LOX DICE motif in caprine, porcine, human and murine (*SCD1*, *2* and *3* isoforms) *SCD* genes. Ren et al. (2004) reported the presence of the 15-LOX DICE motif in the 3'UTR of the porcine *SCD* gene and Bernard et al. (2001) reported the existence of 2 copies of the same motif in caprine SCD cDNA. Most studies, however, report the presence of the IRES motif only in the 5'UTRs of some genes but we report for the first time its presence also in the 3'UTRs of the bovine, porcine, caprine and murine *SCD* genes

Effects of 3'UTR genotypes on milk fatty acid composition of Canadian Holsteins

SCD1 is known to convert saturated fatty acids with 10-18 carbon atoms into their monounsaturated counterparts in the mammary glands of ruminant animals. Lower indices for C10I, C12I, C14I and C16I, and relatively higher indices for C18I, CLAI and TI are consistent with Chilliard et al. (2000) who reported lower stearoyl- CoA desaturase activity with fatty acids shorter than 18-carbon chain length. Enoch et al. (1976) also reported substrate specificity for SCD1 with preference for longer chain fatty acids.

Significantly higher C10I, C12I, C10:1 and C12:1 in the H1 regulatory variant compared to the H3 regulatory variant is consistent with significantly higher C10I, C12I, C14I, C10:1 and C12:1 associated with the coding region p.293*A* allele relative to the p.293*V* allele found in this group of Holsteins and in Canadian Jerseys (525 cows from 25 herds) in other studies (Kgwatalala et al. unpublished results). Schennink et al. (2008) also reported a positive effect of the open reading frame p.293*A* allele of the *SCD1* gene on C10I, C12I, C14I and on concentrations of C10:1, C12:1 and 14:1 in milk fat of Dutch Holstein Friesian heifers. Moioli et al. (2007) reported a positive effect of p.293*A* allele

relative to the p.293*V* allele on C10I, C14I, C10:1 and C14:1 in the Piedmontese and Valdostona breeds and Mele et al. (2007) reported a positive effect of the p.293*A* allele on C14:I and C14:1 in Italian Holsteins. We have shown the p.293*A* allele to be in linkage disequilibrium with either the H1 or H2 regulatory variant and the H3 to be in linkage disequilibrium with the p.293*V* allele and the *SCD1* effects reported in the above studies could therefore be due to polymorphisms either in the coding region or in the 3′UTR region of the *SCD1* gene or combined effects of both. Further studies are needed to determine the relative contributions of the different coding region alleles and the 3′UTR regulatory variants towards SCD1 activity. SNPs in the 3′UTR of the *SCD1* gene are however of practical significance for use as markers for genetic selection for increased concentrations of C10:1, C12:1 and C14:1 in milk.

3′UTRs of most genes have been shown to contain motifs that regulate translation of the constituent mRNA and differences in C10I, C12I, C10:1 and C12:1 between the H1 and H3 regulatory variants reported in this study may be indicative of a possible role of the SNPs in the 3′UTR in the expression of the *SCD1* gene. Higher C10I and C12I associated with the H1 variant may be due to enhanced expression or production of the SCD1 enzyme which eventually translates to increased unsaturation of C10:0 and C12:0 into C10:1 and C12:1, respectively, in the mammary glands of Canadian Holstein cattle. SNPs associated with the H3 variant may have the opposite effect and possibly result in decreased production of the SCD1 enzyme and reduced unsaturation of C10:0 and C12:0 into their monounsaturated fatty acids counterparts, hence their relatively low contents in milk fat.

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References

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

Chabanon H., Mickleburgh I., Hesketh J. 2004. Zipcodes and postage stamps: mRNA localization signals and their trans-acting binding proteins. Brief Funct Genomic Proteomic, 3: 240-256.

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

Chouinard P.Y., Corneau L., Saebo A., D. E. Bauman D.E. 1999. Milk yield and composition during abomasal infusion of conjugated linoleic acids in dairy cows. J. Dairy Sci. 82: 2737-2745.

Christie W.W. 1982. A simple procedure for rapid transmethylation of glycerolipids and cholesterol esters. J. Lipid Res. 23:1072-1075.

Conne B., Stutz A., Vassali J.D. 2000. The 3′ untranslated region of messenger RNA : A molecular 'hotspot' for pathology? Nat. Med. 6:637-641.

Hara A., Radin N.S. 1978. Lipid extraction of tissues with low toxicity solvent. Anal. Biochem. 90:420-426.

Dhiman T.R., Nam S., Ure A. 2005. Factors affecting conjugated linoleic acid content in milk and meat. Crit. Rev. Food Sci. and Nutr. 45:463-482.

Enoch H.G., Catala A., Strittmatter P. 1976. Mechanisms of rat liver microsomal stearoyl-CoA-desaturase. Studies of the substrate specificity, enzyme-substrate interactions, and the function of lipid. J. Biol. Chem. 251: 5095-5103.

Keating A.F., Stanton C., Murphy J.J., Smith T.J., Ross R.P., Cairns M.T. 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.

Kelly M.L., Berry J.R., Dwyer D.A., Griinari J.M., Chouinard M.E., Van Amburgh, M.E., Bauman D.E. 1998. Dietary fatty acid sources affect conjugated linoleic acid concentrations in milk from lactating dairy cows. J. Nutr. 128: 881-885.

Kelsey J.A., Corl B.A., Collier R.J., Baumen D.E. 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.

Kgwatalala P.M., Ibeagha-Awemu E.M., Hayes J.F., Zhao X. 2007. Single nucleotide polymorphismsin the open reading frame of the stearoyl-CoA deasturase gene and the resulting genetic variants in Canadian Holstein and Jersey cows. DNA Sequence, 18:357-362.

Lai E. C., Burks C., Poakony J. W. 1998. The K-Box: A conseved 3′UTR sequence motif negatively regulates accumulation of enhancer of split complex transcripts. Development, 125:4077-4088.

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

Le S.Y., Maizel J.V. Jr. 1997. A common RNA structural motif involved in the internal initiation of translation of cellular mRNAs. Nucleic Acids Res. 25:362-369.

Lengi A. J., Corl B. A. 2007. Identification and characterization of a novel bovine stearoyl- CoA desaturase isform with homology to human SCD5. Lipids, 42:499-508.

Lipkin E., Shalom A., Khatib H., Soller M., FriedmanA. 1993. Milk as a source of deoxyribonucleic acidand as a substrate for the polymerase chain reaction. J. Dairy Sci. 76:2025-2032.

Mazumder D., Seshadri V., Fox L.P. 2003. Translational control by the 3′UTR: the ends specify the means. Trends in Biochem Sci. 28:91-98.

Mele M., G. Conte, B. Castiglioni, S. Chessa, N. P. P. Macciotta, A. Serra, A. Buccioni, G. Pagnacco, and P. Secchiari. 2007. Stearoyl-Coenzyme A desaturase gene polymorphism and milk fatty acid composition in Italian Holsteins. J. Dairy Sci. 90: 4458-4465.

Mignone F., Gissi C., Liuni S., Pesole G. 2002. Untranslated regions of mRNAs. Genome Biology, 3: reviews 0004.1-0004.10.

Moioli B., Contarini G., Avalli A., Catillo G., Orru L., De Matteis G., Masoero G., Napolitano F. 2007. Short communication: Effect of stearoyl-coenzyme A desaturase polymorphism on fatty acid composition of milk. J. Dairy Sci. 90:3553-3558.

Ostareck-Lederer A., Ostareck D.H., Standart N., Thiele B.J. 1994. Translation of 15-lipooxygenase mRNA is inhibited by a protein that binds to a repeat sequence in the 3′ untranslated region. Embry J. 13:1476-1481.

Pesole G., Liuni S. (1999) Internet resources for functional analysis of 5′and 3′UTRs of eukaryotic mRNAs. TIG, 15:378.

Peterson D.G., Kelsey J.A., Baumen D.E. 2002. Analysis of variation in cis-9, trans-11 conjugated linoleic acid (CLA) in milk fat of dairy cows. J. Dairy Sci. 85:2164-2172.

Raymond M., Rousset F. 2001. GENEPOP: Population genetics software and ecumenism. J. Hered. 86:248-249, http://wbiomed.curtin.edu.au/genepop/

Ren J., Knorr C., Huang L., Brenig B. 2004. Isolation and molecular characterization of the porcine stearoyl-CoA desaturase gene. Gene, 340:19-30.

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

Taniguchi M., Utsugi T., Oyama K., Manmen H., Kobayashi M., Tanabe Y., Ogino A., Tsuji S. 2004. Genotype of stearoyl-CoA desaturase is associated with fatty acid composition in Japanese Black Cattle. Mamm. Genome, 14:142-148.

Wang G., Yang P., Xie H. 2006. Gene variants in noncoding regions and their possible consequencies. Pharmacogenomics, 7:203-209.

White S.L., Bertrand J.A., Wade M.R., Washburn S.P., Green J.T., Jenkins T.C. 2002. Comparison of fatty acid content of milk from Jersey and Holstein cows consuming pasture or a total mixed ration. J. Dairy Sci. 85:95-104.

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Figure 1. Distinguishing between 3'UTR regulatory variants in individuals of AA open reading frame genotype by RFLP analysis. PCR products were digested with *Mfe*I endonuclease and the distinction between H1and H2 regulatory variants is based on g.C1763A polymorphism. Lane 1= 1KB ladder, lanes 2 and 6= H2H2, Lanes 3 and 4= H1H1 and lanes 5 and 6= H1H2 ,

1 2 3 4 5 6

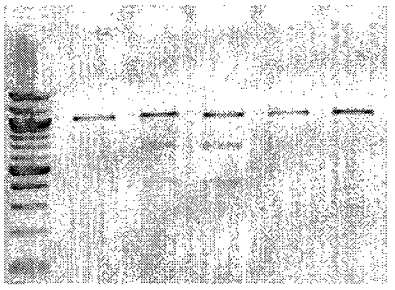

Figure 2. Distinguishing between 3'UTR regulatory variants in individuals of AV open reading frame genotype. PCR products were digested with *Mfe*I endonuclease and the distinction between H1 and H2 regulatory variants is based on g.C1763A polymorphism. Lane 1 = 1 KB Ladder, Lanes 2, 5 and 6= H2H3 and lanes 3 and 4=H1H3

Table 1. Primers used for amplification of the 5′ and 3′ untranslated regions of bovine *SCD1* gene

Region	Primers	Sequence (5' to 3')	$MgCl_2$ concentration	Annealing temperature (°C)
5′UTR	F_{1763}	GTTGGCAACGAATAAAAGAGG	1.5 mM	54
	R_{2146}	CGCGGTGATCTCAACTCTTC		
3′UTR1	F_{13130}	CTCTGGCTTATGACCGGAAG	1.75 mM	56
	R_{14127}	CCTGAGCTGCTGGAATCAG		
3′UTR2	F_{13837}	TCCTGCTGTGATCAGACACC	1.75 mM	56
	R_{14796}	GTGGACAAAGATGGCAGAGC		
3′UTR3	F_{14437}	CTGCTGGACAGGAGATGGAG	2.25 mM	55
	R_{15432}	TCAGCAAAGTTCATGGCATC		
3′UTR4	F_{15219}	GGATAAGCTGCCTACCCTGA	1.75 mM	55
	R_{16208}	GCAAAGGGGCTCCAAAGAG		
3′UTR5	F_{16098}	CTTGCTTTGTGGGTATGCTG	1.75 mM	55
	R_{17085}	GGCAATCAGATTCACTTTTATT ATTG		

* Subscripts ensuing F (forward) and R (reverse) are annealing positions of the primers based on GanBank No. AY241932

Table 2. Single nucleotide polymorphisms and their positions in the 3'UTR of the *SCD1* gene in Canadian Holsteins and Jerseys

Nucleotide position	Nucleotide change
1571	G→T
1644	G→C
1763	C→A
2053	C→T
2584	A→G
3007	A→G
3107	C→T
3208	G→A
3290	T→C
3497	G→A
3682	G→A
4399	A→T
4533	C→T
4881	G→A

*Nucleotide position is based on GenBank No. AY241933

Table 3. Different haplotypes and their frequencies at the 3'UTR of the *SCD1* gene in Canadian Holsteins and Jerseys

Variant	Characteristic SNPs	Holstein		Jersey	
		No.	Freq	No.	Freq
H1	$G_{1571}G_{1644}C_{1763}C_{2053}A_{2584}A_{3007}C_{3107}G_{3208}T_{3290}G_{3497}$ $G_{3682}A_{4399}C_{4533}G_{4881}$	71	0.772	66	0.943
H2	$G_{1571}G_{1644}A_{1763}C_{2053}A_{2584}G_{3007}C_{3107}G_{3208}T_{3290}G_{3497}$ $G_{3682}A_{4399}C_{4533}G_{4881}$	5	0.054	0	0
H3	$T_{1571}C_{1644}A_{1763}T_{2053}G_{2584}G_{3007}T_{3107}A_{3208}C_{3290}A_{3497}$ $A_{3682}T_{4399}T_{4533}A_{4881}$	16	0.174	4	0.057

Table 4. Regulatory sequences or motifs found in different regulatory variants at the 3′ UTR of the *SCD1* gene in Canadian Holsteins and Jerseys

Regulatory sequence	Position	Regulatory variants		
		H1	H2	H3
15-Lox DICE	3357-3373	Present	Present	Present
IRES	4528-4620	Present	Absent	Absent
K-Box	1848-1855	Present	Present	Present
Brd- Box	1628-1634	Present	Present	Present

Table 5. Regulatory variant frequencies at the 3′ untranslated region of the *SCD1* gene in Holsteins

	Genotypic frequencies						Gene frequencies		
	H1H1	H1H2	H2H2	H1H3	H2H3	H3H3	H1	H2	H3
Observed	393	10	2	361	25	71			
Relative	0.456	0.012	0.002	0.419	0.029	0.082	0.671	0.023	0.306

Table 6. Effects of 3′UTR genotype on *SCD* 1 indices in Canadian Holsteins

Indices	H1H1	H1H2	H1H3	H2H2	H2H3	H3H3
*C10I	$10.38^{a} \pm 0.09$	$9.89^{abc} \pm 0.52$	$9.85^{b} \pm 0.09$	$9.82^{abc} \pm 1.16$	$10.07^{abc} \pm 0.33$	$8.98^{c} \pm 0.20$
C12I	$3.50^{a} \pm 0.05$	$3.35^{ab} \pm 0.32$	$3.28^{a} \pm 0.06$	$3.04^{ab} \pm 0.72$	$3.35^{ab} \pm 0.21$	$2.62^{b} \pm 0.12$
C14I	7.67 ± 0.08	7.94 ± 0.47	7.36 ± 0.08	7.27 ± 1.04	7.28 ± 0.30	7.20 ± 0.18
C16I	4.50 ± 0.05	4.65 ± 0.29	4.62 ± 0.05	4.40 ± 0.64	4.50 ± 0.18	4.74 ± 0.11
C18I	69.08 ± 0.49	68.17 ± 2.99	68.33 ± 0.51	78.51 ± 6.64	69.78 ± 1.90	68.51 ± 1.13
CLAI	22.69 ± 0.45	18.12 ± 2.73	22.04 ± 0.46	22.08 ± 6.07	21.55 ± 1.74	21.88 ± 1.04
TI	26.40 ± 0.25	27.12 ± 1.50	26.14 ± 0.25	30.24 ± 3.33	26.17 ± 0.96	26.13 ± 0.57

*C10I is C10 index

[a–c]Means within a row with different superscripts differ significantly ($P < 0.05$)

Table 7. Effects of 3′ UTR genotypes on the contents of selected fatty acids (g/100g of total fatty acids) in milk fat of Canadian Holstein cows

Parameter	H1H1	H1H2	H1H3	H2H2	H2H3	H3H3
C10:0	5.57 ± 0.06	5.04 ± 0.34	5.62 ± 0.06	5.51 ± 0.75	5.69 ± 0.21	5.85 ± 0.13
C10:1	0.63^{a} ± 0.01	0.56^{ab} ± 0.04	0.61^{ab} ± 0.01	0.55^{ab} ± 0.10	0.62^{ab} ± 0.03	0.56^{b} ± 0.02
C12:0	5.35 ± 0.05	5.01 ± 0.33	5.30 ± 0.06	5.18 ± 0.74	5.41 ± 0.21	5.54 ± 0.13
C12:1	0.19^{a} ± 0.003	0.17^{abc} ± 0.017	0.18^{b} ± 0.003	0.16^{abc} ± 0.380	0.18^{abc} ± 0.011	0.15^{c} ± 0.006
C14:0	14.53 ± 0.09	14.25 ± 0.57	14.60 ± 0.10	14.15 ± 1.27	14.88 ± 0.36	14.82 ± 0.22
C14:1	1.19 ± 0.01	1.23 ± 0.08	1.15 ± 0.01	1.07 ± 0.17	1.15 ± 0.05	1.14 ± 0.03
C16:0	27.53 ± 0.15	27.21 ± 0.92	27.42 ± 0.16	27.03 ± 2.05	27.46 ± 0.59	26.88 ± 0.35
C16:1	1.29 ± 0.01	1.31 ± 0.08	1.31 ± 0.01	1.20 ± 0.18	1.28 ± 0.05	1.34 ± 0.03
C18:0	8.51 ± 0.14	9.51 ± 0.86	8.75 ± 0.15	7.09 ± 1.92	8.54 ± 0.55	8.63 ± 0.33
C18:1 *cis*-9	19.01 ± 0.23	19.74 ± 1.42	18.81 ± 0.24	22.90 ± 3.16	18.99 ± 0.91	18.85 ± 0.54
C18:1 *trans*-11	1.14 ± 0.02	1.29 ± 0.12	1.13 ± 0.02	1.31 ± 0.27	1.17 ± 0.08	1.16 ± 0.05
Cis-9,*trans*-11 CLA	0.28 ± 0.01	0.28 ± 0.03	0.27 ± 0.01	0.31 ± 0.07	0.28 ± 0.02	0.28± 0.01

[a–c] Means within a row with different superscripts differ ($P < 0.05$)

CHAPTER VI. CONCLUSIONS AND GENERAL DISCUSSION

Bovine milk typically contains 70% saturated fatty acid (SFA), 25% monounsaturated fatty acids (MUFA) and 5% polyunsaturated fatty acids (PUFA) (Grummer, 1991). Saturated fatty acids, in particular C12:0, C14:0 and C16:0 in bovine milk have been reported to increase plasma total and low density lipoprotein (LDL) cholesterol concentrations, considered important biomarkers for cardiovascular diseases (Kromhout et al., 2000; Mattson & Grundy, 1985). To the contrary, MUFA and PUFA have been reported to lower both serum total cholesterol levels and LDL cholesterol and therefore lower the risk of coronary heart diseases (Kris-Etherton et al., 1999; Mensink et al., 2003). A more favourable combination of bovine milk lipids to human health would be around 30% SFA, 60% MUFA and 10% PUFA and the current composition is far from optimal (Soyeurt et al. 2006). Current strategies for changing the composition of bovine milk towards increased MUFA and other functional components such as conjugated linoleic acid (CLA) are mainly based on nutritional manipulation of the diet but even the most extreme modification of the diet on its own will not be sufficient to achieve the desired milk fat composition (Grummer, 1991). Genetic selection for increased MUFA and CLA contents of milk is however possible on the basis of the recent moderate heritability estimates of the concentrations of individual MUFA and desaturase indices in dairy cattle (Soyeurt et al. 2007; Schennink et al. 2008). According to Dekkers and van de Werf (2007) successful genetic selection is however dependent on advances in: (1) Gene mapping - to identify the genes as well as the polymorphisms within the identified genes; (2) Marker genotyping - genotyping of individuals within the population for a large

number of markers for both QTL detection and routine MAS application; (3) QTL detection - detection and associations of identified genes and markers with economic traits; (4) Gene-assisted selection (GAS) and Marker-assisted selection (MAS) - breeding strategies and use of genetic information in selection and mating programs. In cattle, *stearoyl-CoA desaturase1 (SCD1)* is considered a strong candidate gene influencing milk fatty acid composition because of its role in fatty acid unsaturation. In this study, identification of SNPs in the coding region and 3′UTR of the *SCD1* gene and their association with the fatty acid composition of Canadian Holsteins and Jerseys, were carried out to lay down the foundation for possible GAS or MAS to improve the fatty acid composition of milk.

Sequence analysis of the coding region of the *SCD1* gene revealed SNPs: 702A→G, 762T→C and 878C→T in exon 5 in both Holsteins and Jerseys and a further SNP, 435G→A in exon 3 was found only in Holsteins. The SNPs characterized 4 different coding sequence variants in Holsteins: *A* ($G_{435}A_{702}T_{762}C_{878}$), *A1* ($A_{435}A_{702}T_{762}C_{878}$), *B* ($G_{435}G_{702}C_{762}T_{878}$) and *B1* ($A_{435}G_{702}C_{762}T_{878}$) and only variants *A* and *B* in Jerseys. SNP 878C→T resulted in a non-synonymous codon change while the rest resulted in synonymous codon changes giving rise to two protein variants or alleles at the *SCD1* locus, *A* having alanine and *V* having valine (Ala293Val or A293V). Allele *A* was more prevalent in the two breeds. The three SNPs in exon 5 are consistent with those found in the Japanese Black (beef breed) cattle (Taniguchi *et al.*, 2004) and GenBank Accession No. 241933. The SNP 435G→A found only in Holsteins is a novel mutation and to the best of our knowledge, this is the first report of the existence of such an SNP in dairy cattle.

After the identification of *p.293A* and *p.293V* alleles at the *SCD1* locus, we proceeded to evaluate the extent to which the genotype at the *SCD1* locus and stage of lactation influenced desaturase indices (proxy for SCD1 activity) and milk fatty acid composition of both Holsteins and Jerseys. In comparison with the p.293VV genotype, the p.293AA genotype had significantly higher C10 index (C10I), C12 index (C12I) and C14 index (C14I) which translated into significantly higher concentrations of C10:1 and C12:1 in milk of both Holsteins and Jerseys. The differences in C10I, C12I and C14I and the concentrations of C10:1 and C12:1 in milk between p.293AA and p.293AV individuals were not significant in Jerseys but were significantly different in Holsteins. The *SCD1* genotype had no significant influence on C16 index (C16I), C18 index (C18I), CLA index (CLAI) and total index (TI) and consequently no significant influence on the concentrations of C16:1, C18:1 and CLA in both breeds. The *SCD1* genotype also had no significant influence on the concentrations of C4:0, C6:0, C8:0, C10:0, C18:2, C18:3, total SFA, total MUFA and total PUFA in both breeds.

A comparison between the fatty acid profile of Canadian Holstein cows (862 cows from 17 herds) and Canadian Jersey cows (525 cows from 17 herds) revealed significantly higher concentrations of C10:1, C12:1, C14:1, C16:1, C18:1 cis-9, C18:1 trans-11, CLA, C18:2, MUFA, PUFA and all SCD1 indices, and significantly lower concentrations of C4:0, C6:0, C10:0, C12:0, C16:0, C18:0, C18:3 and SFA in Holstein cows than in Jersey cows (see chapters III and IV). Significantly higher desaturase indices resulted in higher individual and consequently higher total MUFA content in milk of Holsteins compared to that of the Jerseys.

Literature on the influence of parity and stage of lactation on the fatty acid composition of bovine milk is limited, and our experimental design afforded us the opportunity to investigate the two variables. Parity had no influence on the milk fatty acid composition in both breeds. In comparison with the subsequent lactation stages, early lactation was associated with significantly lower C10I, C12I, C14I and CLAI and significantly higher C18I and TI which translated to lower concentrations C10:1, C12:1, 14:1 and consequently total MUFA, and higher C18:1 *cis*-9 concentrations in milk fat in both breeds. In comparison with either mid lactation or late lactation, early lactation was also characterized by higher concentrations of C18:1 *trans*-11, C18:2 and total PUFA in Holsteins and there was no apparent effect of stage of lactation in all those fatty acids in Jerseys. Stage of lactation had no influence on the CLA content of milk in Holsteins but the CLA content was lower during early lactation compared to the subsequent lactation stages in Jerseys. Stage of lactation had no influence on C8:0, C16:1 and C18:0 contents in both breeds. Results on the influence of stage of lactation on the fatty acid composition of both Canadian Holstein and Jersey milk obtained in this study are fairly consistent with those of Mele et al. (2007). Mele et al. (2007) reported significantly lower C14:0 and C14:1 and higher C18:1 and MUFA during early lactation relative to either mid lactation or late lactation which was also the case in the current investigation. Stage of lactation had no influence on concentrations of C16:1 and C18:0 in both breeds as reported by Mele et al. (2007) in Italian Holstein cows. The lack of influence of stage of lactation on CLA content of milk in Holsteins is consistent with Kelsey et al. (2003) who reported no significant effect of stage of lactation on the CLA content of Holstein and Brown Swiss cows.

An increase in the CLA content with advancing lactation in Jerseys is consistent with Auldist et al. (1998), who reported an increase in the CLA content of milk fat from 7.9 mg/g of fatty acids to 9.7 mg/g of fatty acids from early to late lactation.

Changes in the fatty acid profile during the entire lactation could be related to the energy balance or status of the cows. During early lactation, dairy cows might be in a negative energy balance leading to the mobilization of adipose tissue fatty acids (mainly palmitoleic acid and oleic acid) and their eventual secretion into milk, hence the higher concentrations of C18:1 *cis*-9 (oleic acid) during early lactation in both breeds. C18:1 *cis*-9 has been shown to depress the activity of enzymes responsible for mammary synthesis of saturated fatty acids in the MacT cell line (Jayan & Herbein 2000) and the increased concentration of C18:1 *cis*-9 during early lactation might therefore explain relatively lower concentrations of *de novo* synthesized C10:0, C12:0, C14:0 and C16:0 during early lactation compared to the subsequent lactation stages. Drackley et al. (2007) also reported a linear increase in the concentration of C18:1 *cis*-9 and a concomitant linear decrease in the concentrations of C12:0, C14:0 and C16:0 with increasing abomasal infusions of high oleic (C18:1 cis-9) sunflower fatty acids in Holstein cows. A limited supply of some of the substrates for SCD1 (C10:0, C12:0 and C14:0) might explain lower C10I, C12I and C14I and consequently lower concentrations of C10:1, C12:1 and C14:1 in milk fat during early lactation compared to the subsequent lactation stages. Attainment of a positive energy balance is expected to reverse the inhibitory effects of C18:1 on *de novo* fatty acid synthesis, hence an increase in the concentrations of C10:0, C12:0 and C14:0, and C10I, C12I

and C14I and consequently C10:1, C12:1 and C14:1 during mid lactation compared to early lactation.

It has been observed in recent years that polymorphisms in the regulatory regions of the underlying genes account for some of the observed phenotypic differences among individuals. The 3'UTRs of most genes have remained largely neglected as potential sources of regulatory variants that may have a significant influence on the animal's phenotype. Current research results however indicate that the 3'UTRs are actively involved in post-transcriptional regulation of gene expression including modulation of the transport of mRNAs out of the nucleus and of translation efficiency, subcellular localization and stability (Mignone et al., 2002; Chabanone et al., 2004). We therefore also investigated the 3′UTR of the *SCD1* gene for a possible role in the concentrations of individual fatty acids in milk.

Sequence analysis of the 3′UTR of the *SCD1* gene identified SNPS: 1571G→T, 1644 G→C, 1763 C→A, 2053 C→T, 2584 A→G, 3007 A→G, 3107 C→T, 3208 G→A, 3290 T→C, 3497 G→A, 3682 G→A, 4399 A→T, 4533 C→T and 4881 G→A in both Holsteins and Jerseys. SNPs G3682A, A4399T and G4881A have never been reported before (Taniguchi et al., 2004; GenBank Accession No. AY241933) and to the best of our knowledge, this is the first report on the existence of such SNPs in dairy cattle. The SNPs were in complete linkage disequilibrium resulting in 3 haplotypes or regulatory variants that comprised in part: H1 ($G_{1571}G_{1644}C_{1763}C_{2053}$ A_{2584} A_{3007} C_{3107} $G_{3208}T_{3290}$ G_{3497} $G_{3682}A_{4399}C_{4533}G_{4881}$), H2 ($G_{1571}G_{1644}A_{1763}C_{2053}A_{2584}$ $G_{3007}C_{3107}$ $G_{3208}T_{3290}$ $G_{3497}G_{3682}$ A_{4399} $C_{4533}G_{4881}$) and H3 ($T_{1571}C_{1644}A_{1763}T_{2053}G_{2584}G_{3007}T_{3107}A_{3208}C_{3290}A_{3497}A_{3682}T_{4399}T_{4533}$ A_{4881}) in Holsteins

and only H1 and H3 variants in Jerseys. The H1 and H2 regulatory variants were always in linkage disequilibrium with the coding region *p.293A* allele and the H3 variant was in linkage disequilibrium with the *p.293V* allele. The H1 regulatory variant was the most frequent in both breeds and the H2 variant the least frequent in Holsteins. GenBank Accession No. AY241933 and Taniguchi et al. (2004) implied the presence of only two different haplotypes or regulatory variants (H1 and H3 with minor differences due to SNPs reported in the two studies) linked to open reading frame (ORF) p.293*A* and p.293*V* alleles, respectively, while we report here an additional regulatory variant (H2) in Holstein cows. All the 3 regulatory variants contained the Erythroid 15-lipoxygenase differentiation control element (15-LOX DICE), K-Box and the Brd-Box motifs while the internal ribosome entry site (IRES) motif was found only in the H1 regulatory variant. The IRES motif, normally found in the 5′UTRs of some genes, is known to mediate internal mRNA ribosome binding translational mechanism, an alternative to the conventional 5'-cap dependent ribosome scanning mechanism, and may thus enhance translation of its constituent mRNA (Le and Maizel, 1997). A search of literature places the IRES motif only in the 5′UTR of some genes but we report for the first time its presence also in the 3'UTR of the bovine *SCD1* gene.

Association analysis between the different 3′UTR regulatory variants and milk fatty acid composition was done only in Holstein because with the H1 and H3 regulatory variants in complete linkage disequilibrium with coding region *p.293A* and *p.293V* alleles, respectively, the association analysis in Jerseys would yield the same results as those reported for the coding region genotypes. The genotype at the 3′UTR

of the *SCD1* gene had significant influence on the C10I and C12I and consequently on the concentrations of C10:1 and C12:1 but had no significant influence on C14I, C16I, C18I, CLAI, TI and on the resulting concentrations of C14:1, C16:1, C18:1 and CLA in Holstein cows. The H1 regulatory variant was associated with significantly high C10I and C12I and the resulting C10:1 and C12:1 while the opposite was true for the H3 variant. The effects of the H2 variant on different desaturase indices were intermediate to those of H1 and H3 effects. Apart from the concentrations of C10:1 and C12:1, the genotype at the 3′UTR of the *SCD1* gene had no significant influence on the concentrations of all other fatty acids contained in milk.

Differences in C10I, C12I and the resulting concentrations of C10:1 and C12:1 between the H1 and H3 regulatory variants may suggest a possible role of the SNPs in the 3′UTR in the expression of the *SCD1* gene. Higher C10I and C12I associated with the H1 variant may suggests enhanced expression or production of the SCD1 enzyme which eventually translates to increased unsaturation of C10:0 and C12:0 into C10:1 and C12:1, respectively, in the mammary glands of Holstein cows. SNPs associated with the H3 variant may have the opposite effect and possibly result in decreased production of the SCD1 enzyme and reduced unsaturation of C10:0 and C12:0 into their monounsaturated fatty acids counterparts, hence their relatively low contents in milk fat. We have shown a significant positive influence of the open reading frame *A* allele of the *SCD1* gene on C10I, C12I and consequently on C10:1 and C12:1, respectively, in both Holsteins and Jerseys and the opposite effects of the *V* allele of the *SCD1* gene. We have also shown the *A* allele to be in linkage disequilibrium with either the H1 or H2 regulatory variant and the H3 to be in linkage

disequilibrium with the *V* allele. The concentrations of C10:1 and C12:1 in milk fat could therefore be due to the effects of SNPs in the open reading frame or 3′UTR region of the *SCD1* gene. SNPs in the coding and 3' UTR regions of the bovine *SCD1* gene could therefore be used as markers for genetic selection for increased concentrations of C10:1 and C2:1 in milk.

References

Auldist M. J., Walsh B. J., Thomson N. A. 1998. Seasonal and lactation influences on bovine milk composition in New Zealand. J. Dairy Res. 65:401–411.

Chabanon H., Mickleburgh I., Hesketh J. 2004. Zipcodes and postage stamps: mRNA localization signals and their trans-acting binding proteins. Brief Funct Genomic Proteomic 3: 240-256.

Dekkers J. C. M., van de Werf J. H. J. 2007. Strategeies, limitations and opportunities for marker- assisted selection in livestock. In: Guimarães E., Ruane J., Scherf B., Sonnino A., Dargie J. (eds.) Marker-Assisted Selection: Current statuts and future perspectives in crops, livestock, forestry and fish. Food and Agricultural Organization, Rome, p. 167-184,

Drackley J. K., Overton T. R., Ortiz-Gonzalez G., Beaulieu A. D., Barbano D. M., Lynch J. M., Perkins E. G. 2007. Responses to increasing amounts of high-oleic sunflower fatty acids infused into the abomasums of lactating dairy cows. J. Dairy Sci. 90: 5165-5175.

Enjalbert F., Nicot M., Bayourthe C., Moncoulon R. 1998. Duodenal infusions of palmitic, stearic or oleic acids differently affect mammary gland metabolism of fatty acids in lactating dairy cows. J. Nutr. 128: 1525-1532.

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

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

Kelsey J. A., Corl B. A., Collier R. J., Bauman D. E. 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.

Kris-Etherton, P. M., T. A. Pearson, Y. Wan, R. L. Hargrove, K. Moriarty, V. Fishell, and T. D. Etherton. 1999. High- monounsaturated fatty acid diets lower both plasma cholesterol and triacylglycerol concentrations. Am. J. Clin. Nutr. 70:1009-1015.

Kromhout, D., A. Menotti, H. Kestleloot, and S. Sans. 2002. Prevention of coronary heart disease by diet and lifestyle: Evidence from prospective cross-cultural, cohort, and interventional studies. Circulation 105:893-898.

Le S.Y., Maizel J.V. Jr. 1997. A common RNA structural motif involved in the internal initiation of translation of cellular mRNAs. Nucleic Acids Res. 25: 362-369.

Mensink, R. P., P.L. Zock, A. D. Kester, and M. B. Katan. 2003. Effects of dietary fatty acids and carbohydrates on the ration 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.

Mattson F. H., Grundy S. M. 1985. Comparison of effects of dietary saturated, monounsaturated, and polyunsaturated fatty acids on plasma lipids and lipoproteins in man. J. Lipid Res. 26: 194-202.

Mele M., Conte G., Castiglioni B., Chessa S., Macciotta N. P. P., Serra A., Buccioni A., Pagnacco G., Secchiari P. 2007. Stearoyl-Coenzyme A desaturase gene polymorphism and milk fatty acid composition in Italian Holsteins. J. Dairy Sci. 90: 4458-4465.

Mignone F., Gissi C., Liuni S., Pesole G. 2002. Untranslated regions of mRNAs. Genome Biol. 3: 0004.1-0004.10.

Moioli B., Contarini G., Avalli A., Catillo G., Orru L., De Matteis G., Masoero G., Napolitano F. 2007. Short Communication : Effect of Stearoyl-Coenzyme A desaturase polymorphisms on fatty acid composition of milk. J. Dairy Sci. 90: 3553-3558.

Schennink A., Heck J. M. L., Bovenhuis H., Visker M. H. P. W., Van Valenberg H. J. F., van Arendonk J. A. M. 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.

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

Taniguchi M., Utsugi T., Oyama K., Manmen H., Kobayashi M., Tanabe Y., Ogino A., Tsuji S. 2004. Genotype of stearoyl-CoA desaturase is associated with fatty acid composition in Japanese Black Cattle. *Mamm. Genome* 14: 142-148.

APPENDIX 1

Appendix 2: Effects of stearoyl-CoA desaturase1 (SCD1) genotype on routinely recorded lactation parameters in Canadian Holstein cows.

Parameter	**AA**	**AB**	**BB**
Milk Yield (kg)	31.68 ± 0.89	31.66 ± 0.35	31.67 ± 0.36
Fat Yield (kg)	1.19 ± 0.025	1.18 ± 0.02	1.22 ± 0.03
Fat %	3.81 ± 0.03	3.81 ± 0.03	3.76 ± 0.07
Protein Yield (kg)	1.02 ± 0.01	1.02 ± 0.01	1.03 ± 0.03
Protein %	3.28 ± 0.02	3.27 ± 0.02	3.21 ± 0.04
SCC (x 10^3)	383.29 ± 34.28	333.18 ± 34.92	220.99 ± 80.34
MUN	10.27 ± 0.21	10.48 ± 0.21	10.13 ± 0.46
Lactose %	4.50 ± 0.02	4.52 ± 0.02	4.51 ± 0.05
Lactation Period	371.43 ± 3.84	361.81 ± 3.99	361.50 ± 9.02
305d Milk Yield (kg)	9822.35 ± 71.81	9714.57 ± 74.59	9803.95 ± 168.59
305d Fat yield (kg)	377.30 ± 3.00	370.25 ± 3.12	376.37 ± 7.04
305d Protein Yield (kg)	318.99 ± 2.14	315.81 ± 2.23	317.44 ± 5.03