Genetic Analysis of Groups of Mid-Infrared Predicted Fatty Acids in Milk

by

Saranya Gunasegaram Narayana

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ABSTRACT

GENETIC ANALYSIS OF GROUPS OF MID-INFRARED PREDICTED FATTY ACIDS IN MILK

Saranya G. Narayana   Advisors:

University of Guelph   Dr. Filippo Miglior

                                                       Dr. Flavio S. Schenkel

This thesis investigated the phenotypic and genetic variation of five groups of mid-infrared (MIR) predicted fatty acids; short-chain (4 to 10 carbons), medium-chain (12 to 16 carbons), long-chain (17 to 22 carbons), saturated, and unsaturated fatty acids. The predicted fatty acid values (g/dL of milk) were log transformed in order to improve normality. The data set included 49,127 test-day records from 10,029 first lactation Holstein cows in 810 herds. The total number of animals in the pedigree was 76,074. The random regression animal test-day model included: days in milk, herd-test date, and season-age of calving (polynomial regression) as fixed effects, and herd-year of calving, animal and permanent environment effects as random polynomial regressions, and random residual effect. Third and fourth degree of Legendre polynomial were estimated for fixed regression of season-age of calving effect and for the three random regressions, respectively. The average daily heritability estimates were 0.24, 0.32, 0.23, 0.33, and 0.21 for short-chain, medium-chain, long-chain, saturated, and unsaturated fatty acids, respectively. Estimated average daily genetic correlations were positive among all fatty acid groups and ranged from moderate to high (0.63-0.96). This study demonstrates the existence of genetic variation in milk fatty acid profile predicted by MIR spectra. Therefore, milk fatty acid composition can be improved through genetic selection.

Keywords: milk fatty acid, mid-infrared, random regression model, heritability

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TABLE OF CONTENTS

GENETIC ANALYSIS OF GROUPS OF MID-INFRARED PREDICTED FATTY ACIDS IN MILK

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1

1.1. General Introduction

Cattle milk and its products are important components of human diet. Milk is an extremely complex liquid comprising many systems (Jensen et al., 1991). It is composed of nearly 10,000 diverse biomolecules (Jelen, 2007). Fat content in cattle milk varies from 3% to 6%, but typical milk contains fat between the ranges of 3.5% to 4.7% and fat in bovine milk is available in mini globules as an oil-in-water emulsion (MacGibbon and Taylor, 2006). Bovine milk fat provides 75% of total human intake of fat from ruminant animals (Demeyer et al., 1995). However, dairy products supply only 15% of the total fat presented in the human diet and 25-35% of the total saturated fatty acids (O'Donnell, 1993). Fatty acids are important components of milk fat comprising of 90% of its weight (Samková et al., 2012). Milk fat contains around 400-500 fatty acids which makes it the one of the most variable and complex natural fats (Jensen et al., 1991; Barłowska et al., 2009). Investigating the milk lipids is more of interest since 1970s, due to the fact that payments is based on fat content, nutritional aspects, and non-nutritional roles (Jensen et al.,1991). Generally, 70% of the fatty acids in milk are saturated, 25% are mono-unsaturated, 2.3% are poly-unsaturated and 2.7% are *trans* fatty acids. (Grummer, 1991; Jensen, 2002; Månsson, 2008). Approximately, only 15 fatty acids are present at or above 1% of concentration in milk fat (major fatty acids), whereas about 40 are present less than 0.01% of concentration (MacGibbon and Taylor, 2006). Fatty acids can be grouped into different categories based on carbon chain length; short-chain, medium-chain and long-chain, and saturation; saturated and unsaturated (Walstra and Jeness, 1984). Milk fatty acids are produced in two ways; *de novo* synthesis and derived from blood circulation. Bauman and Griinari (2003) reported that short-chain (4 to 8 carbons) and medium-chain (10 to 14 carbons) are produced from *de novo* synthesis, whereas long-chain (>16 carbons) are derived from the uptake of circulating lipids. However, C16:0 carbon originates from both sources. On weight basis of total milk fatty acids, *de novo* synthesis accounts for 40% while fatty acids derived from blood lipids accounts for 60% (Chilliard et al., 2000).

While some fatty acids are beneficial to human health some have negative effects. Many animal model studies have reported anti-atherosclerotic effects of Conjugated Linoleic Acid (CLA) by decreasing the level of total cholesterol, low-density lipoprotein cholesterol (LDL-C),

blood pressure and increasing high-density lipoprotein cholesterol (HDL-C) (Kritchevsky et al., 2004; McLeod et al., 2004; Bhattacharya et al., 2006). CLA was also found to have a cancer preventive effect (Bhattacharya et al., 2006; Flowers and Thompson, 2009; Hsu et al., 2010). Moreover, CLA also lowers hypertension, atherosclerosis and diabetes and also stimulates immune function (Mills et al., 2011). Omega fatty acids are more of interest due to its positive effects on human health. They are essential for growth and development. Further, they also prevent cardio vascular disease, inflammatory diseases and neurological disorders (Yashodhara et al., 2009). On the other hand studies have shown that some of the saturated fatty acids are associated with negative health effects. For instance human intervention studies showed that lauric (C12:0), myristic (C14:0) and palmitic (C16:0) acids significantly elevate the low-density lipoprotein cholesterol whereas, stearic acid (C18:0) has no effect on serum lipid (Denke and Grundy, 1992; Zock et al., 1994; Temme et al., 1996; Mensink et al., 2003). Siri-Tarino et al. (2010) reported that replacing saturated fatty acids with polyunsaturated fatty acids reduced the cardio vascular disease by 42%. Thus, the effect of saturated fatty acids on cardio vascular disease depends on replacement nutrients such as polyunsaturated fatty acids, monounsaturated fatty acids and carbohydrates. However, despite well-established evidence, recent studies have shown that compared to carbohydrates, saturated fatty acids increased total cholesterol, low-density lipoprotein cholesterol but also increased high-density lipoprotein cholesterol which resulted in no significant effects on total cholesterol /high-density lipoprotein cholesterol ratio (Mensink et al., 2003; Micha and Mozaffarian, 2010). Hence, association of saturated fatty acids with human health is still a debate and further studies are needed in this area.

Due to the negative health image, consumption of milk and dairy products has decreased (Samková et al., 2012). This situation has created a need for researchers to investigate ways to alter milk fat composition. Aside from processing milk fat can be modified mainly in two ways; diet and genetic (Palmquist et al., 1993; DePeters et al., 1995). Many studies reported change of milk fat composition by change in diet (Chilliard et al., 2000; Poulsen et al., 2012; Shingfield et al., 2013). But fewer studies have proven existence of genetic variability in fatty acid profile using gas chromatography method and mid-infrared (MIR) spectra prediction (Stoop et al., 2008; Soyeurt et al., 2007a; Bastin et al., 2011; Gion et al., 2011; Lopez-Villalobos et al., 2014).

Studies on genetic variation of milk fat composition reported varying results. This is due to different factors such as: number of samples, definition of traits, unit of expression of the trait, method used to measure the fatty acid content, and model used (Bastin et al., 2011). Generally, fatty acids in milk have been reported to have low to moderate heritability. Fatty acids produced from *de novo* synthesis (short chain and medium chain) seem to be more under genetic control than those fatty acids derived from blood lipids (long chain) (Renner and Kosmack 1974a; Bobe et al., 2008; Garnsworthy et al., 2010). Previous studies also estimated higher heritability for saturated fatty acids than unsaturated fatty acids (Soyeurt et al. 2007a; Bobe et al., 2008; Bastin et al., 2011; Gion et al. 2011). Among unsaturated fatty acids, heritability of polyunsaturated fatty acids was higher than monounsaturated fatty acids (Bobe et al., 2008; Garnsworthy et al., 2010; Bastin et al., 2011). Some studies observed that heritability decreased with increase of chain length (Renner and Kosmack 1974a; Bastin et al., 2011). However, Soyeurt et al. (2007a) could not find any association between chain length and heritability. Renner and Kosmack (1974a) reported higher heritabilities for fatty acids in milk than in milk fat. Conversely, Soyeurt et al. (2008a) found that heritability of monounsaturated fatty acid was lower in milk (0.14) than in milk fat (0.27). In terms of genetic correlations, some studies reported positive correlations among fatty acids which mirrored the similarity in origin (Karijord et al., 1982; Bastin et al., 2011; Gion et al., 2011). Few studies estimated a positive correlation of milk yield with most of the saturated fatty acids and negative correlation with unsaturated fatty acids (Karijord et al., 1982; Bastin et al., 2011). With regard to milk fat, a number of studies observed positive genetic correlations with short chain, medium chain and saturated fatty acids, and negative genetic correlations with long chain and unsaturated fatty acids (Renner and Kosmack, 1974a; Stoop et al., 2008; Bastin et al., 2011; Karijord et al., 1982; Mele et al., 2009). Thus, these studies provide evidence that milk fatty acid profile is partially controlled by genetics.

In terms of measuring fatty acid content, MIR spectra technology is more efficient than gas chromatography method. Gas chromatography method requires skilled labor, more time and it is expensive (Bastin et al. 2011). Hence, analyzing a high number of samples using gas chromatography is not possible. On the other hand MIR predictions can generate large datasets potentially resulting in increased reliability of genetic parameter estimates (Soyeurt et al., 2006a).

Yet, gas chromatography method is used as reference for MIR predictions. Moreover, MIR spectra technology is used routinely in Canadian DHI laboratories to measure milk fat and protein. Thus, using existing resources to analyze genetic variation in milk fatty acid profile is cheaper and more efficient. This is the first study to explore genetic variation of fatty acid groups predicted by MIR spectra in milk of Canadian Holstein cattle.

1.2. Research Objectives

The main objective of this study was to investigate the genetic variation of five predicted fatty acid groups (short-chain, medium-chain, long-chain, saturated, and unsaturated fatty acids) in milk from Canadian Holstein cattle using MIR spectra technology. The specific objectives of this research were:

1. To explore the phenotypic variation of five groups of fatty acids
2. To find the suitable Legendre polynomials for the random regression model
3. To estimate the genetic parameters, heritabilities and correlations for fatty acid groups.

1.3 Thesis structure

This thesis will investigate the phenotypic and genetic variation of predicted five groups of fatty acids. Chapter 2 reviews the different fatty acid groups, their bio synthesis, effect on human health, and current and past research on genetic variation of fatty acids. The phenotypic and genetic variations are examined in chapter 3. General discussion, implication, future studies and conclusions are presented in chapter 4.

CHAPTER 2

LITERATURE REVIEW

2.1. Dairy Industry in Canada

Canadian dairy industry has a great impact on Canadian agriculture and agri-food economy. Among the Canadian Agriculture sectors, dairy sector ranks third following grains and oilseeds, and red meats, with net farm receipts of $ 6.07 billion in 2014 (CDIC, 2016). Furthermore, in 2011, dairy products constituted 14.6% of total food and beverages (CDIC, 2016). According to CDC (2016), total Canadian milk production in 2015 was 81.76 million hl. On average cows recorded in official milk recording programs (above 75%) produced 9,893 kg of milk per lactation (305 days) with an average content of 3.92% fat and 3.22% protein (CDIC, 2016). In addition, total Canadian dairy market represents 36% of fluid market and 64% of industrial market (CDC, 2016). In 2013, Canadian dairy industry contributed 1.1% to the Canadian GDP by generating $18.9 billion (Dairy Farmers of Canada, 2015). Canadian dairy sector operates under a supply management system involving regulation of domestic production, pricing and dairy product imports (CDC, 2016). Figure 2.1 shows total Canadian milk production at the farm from 2006 to 2015. According to CDIC (2016) imports and exports of dairy products in 2014 increased by 19.6% and 7.2% respectively.

High concentrations of dairy farms can be found in Ontario and Quebec (82%), followed by Western provinces (12.6%), and Atlantic Provinces (5.4%) in 2015 (CDC, 2016). In 2014, the total number of dairy farms in Canada was 11,962 (CDIC, 2016) with an average of 73 cows per farm (CDC, 2016). Moreover, Canadian dairy herd composition is made up of 7 breeds; Holstein (94%), Brown Swiss, Jersey, Guernsey, Ayrshire, Milking shorthorn, and Canadienne (CDC, 2016).

2.2. Dairy Genetics and Genomic Evaluation in Canada

Canada is at the lead of new and pioneering research into dairy genetics (CDIC, 2016). The Canadian Record of Performance (R.O.P.) program was established in late 1905, which now contains records of approximately 70% of Canadian dairy herds (AAFC, 2015). Canadian Dairy Network (CDN) is responsible for the calculation and publication of all dairy genetic evaluations

in Canada (Van Doormaal, 2007), whereas the data collection is mainly done by DHI services (Van Doormaal, 2008). Genetic evaluation focuses on selection of superior bulls and cows for breeding. Canadian Dairy Network provides genetic evaluation for 7 breeds: Holstein, Ayrshire, Jersey, Brown Swiss, Guernsey, Canadienne, and Milking Shorthorn on functional, production and conformation traits. Canadian Dairy Network also publishes genetic evaluations for foreign bulls as provided by the International Bull Evaluation Service (Interbull) using a methodology called Multi-Trait Across Country *Evaluations* (MACE) (Schaeffer et al., 1993; Van Doormaal, 2007). Genetic selection decisions were made based mainly on Lifetime Profit Index (LPI) which was introduced in 1991 (AAFC, 2015) until August 2015, with focus on "balance breeding" (Van Doormaal, 2007). Van Doormaal in 2008 stated the LPI formula in simple terms as follows;

LPI= Production+ Durability+ Health and Fertility

Canada was the first country to introduce the most sophisticated methodology in the world, called Canadian test day model for genetic evaluation in 1999 (AAFC, 2015). Modified Canadian test day model with Legendre polynomials of order four (5 coefficients) for both fixed and random effects instead of Wilmink curve was used since 2003 (Kistemaker, 2003). Recently, a new selection index, Pro$, was introduced by Canadian Dairy Network in August 2015 for Holstein and Jersey breeds in addition to the LPI formula. This index is relevant to producers who get all of their farm revenues from milk sales. Pro$ of a sire directly gives the average difference in the profit of their daughters expected to earn over 6 years (Van Doormaal and Beavers, 2015).

Genomic evaluation system together with quantitative genetics theory have a great potential in improving selection of superior animals for breeding (Van Doormaal, 2008; Sullivan, 2009). Major advantages of genomics over other breed improvement systems include high degree of accuracy in selection at early age, generation interval reduction, and a balance approach between genetic gain and inbreeding (Van Doormaal, 2008). Moreover, young animals could be ranked in a much better way against each other than parent averages using genomic information (Schenkel et al., 2009). In Canada genomic evaluations are provided by CDN. Genomic information together with traditional pedigree and progeny performance data was officially released in 2009 (Van Doormaal et al., 2009). Variants or mutation with frequency of at least 1% within a whole genome sequence is called Single Nucleotide Polymorphisms (SNP) (Van Doormaal, 2008). High density

50K panel which contains nearly 45,000 SNP is used for genomic evaluation to estimate Direction Genomic Values (DGVs) (Sullivan, 2009). Genomic evaluations are routinely estimated for over 60 different traits (CDIC, 2016).The genomic evaluation published by CDN contains DGVs on trait by trait basis combined with traditional genetic evaluation according to their reliabilities (CDC, 2016).

2.3. Milk Consumption Trend in Canada

Milk consumption in Canada has been decreasing over the years. Per capita milk consumption has fallen from 90.3 liters in 1995 to 73.3 Liters in 2014 (Figure 2.2) (CDIC, 2016). According to CDC (2016), Canadians are switching to lower fat milk from higher fat milk. Increasing public concerns regarding health issues of milk and milk products, are one of the reasons for reduction in consumption of milk. This is because milk fat contains high amount of saturated fatty acids (70%) (Grummer, 1991). Some of the saturated fatty acids have negative effects on human health; heart diseases, weight gain, and obesity (Parodi, 2009; Bauman and Lock, 2010). In human diet, dairy products account for 15-25% of fat and 25-35% of saturated fatty acids intake (Debry, 2001). Academy of Nutrition and Dietetics suggested that dietary fat should account for 20-35% of the energy and recommended a reduction in saturated and *trans* fatty acids and an increase in n-3 polyunsaturated fatty acids (Vannice and Rasmussen, 2014).

2.4. Bovine Milk Composition

Milk is a complex food composed of nearly 10,000 diverse biomolecules (Jelen, 2007). Milk is an important resource of lipids, proteins, carbohydrates, vitamins and minerals (Fox et al., 2015). Nutrient components of typical whole milk are shown in Table 2.1. Typical whole milk has 4% fat, however, on a dry basis, fat content increases up to 27% where most of it is saturated (65%) (Jenkins and McGuire, 2006). Components of milk meet the needs of a growing calf through changes in their proportion over the lactation (Haug et al., 2007). Milk composition regulates its nutritional quality, properties, and its value as raw material for food products (Walstra and Jeness, 1984). The finest nutritional quality of milk depends on fat quality, fat soluble vitamins and n-3 fatty acids, and conjugated linoleic acid (CLA) (Markiewicz-Kęszycka et al., 2013).

2.4.1. Bovine Milk Fat

Lipids are the esters of fatty acids and similar or derived components that are soluble in nonpolar organic solvents and insoluble, or nearly so, in aqueous liquids (Walstra and Jeness,1984). Fat content in cattle milk varies from 3% to 6%, but typical milk contains fat between the range of 3.5% to 4.7% (MacGibbon and Taylor, 2006). Milk fat content tends to decrease over the lactation (Bitman and Wood, 1990). Milk fat contains around 400-500 fatty acids which makes it the one of the most variable and complex natural fats. Investigating the milk lipids is of increasing interest since 1970s, due to the fact that payment is based on fat content, nutritional aspects, and non-nutritional roles (Jensen et al., 1991; Barłowska et al., 2009). Studies have shown that milk fat comprises 70% saturated and 30% unsaturated fatty acids which includes 25% of monounsaturated and 5% polyunsaturated fatty acids (Grummer et al., 1991; Bilal et al., 2014). Bovine milk fat provides 75% of total human intake of fat from ruminant animals (Demeyer et al., 1995). However, dairy products supplies only 15% of the total fat presented in the human diet and 25-35% of the total saturated fatty acids (O'Donnell, 1993).

The most concentrated lipid in milk fat is triacylglycerol which accounts for 96%-98% of the total lipid content and is relatively constant over the lactation (Walstra and Jeness, 1984; Bitman and Wood,1990). The structure of triglyceride molecule is shown in Figure 2.3. The triacylglycerol varies according to type and amount of fatty acid (4 to 24 carbons) present (Jensen, 2002). Triacylglycerides also affect the hydrophobicity, density and melting properties of milk fat. Figure 2.3 shows the triacylglycerol structure. Other minor lipids in milk fat are phospholipids (0.8%), sterols (0.3%) and free fatty acids (0.1%). Cholesterol is the major lipid in sterols accounting for 95% (MacGibbon and Taylor, 2006). Table 2.2 shows the main lipid classes in bovine milk.

The fat in bovine milk is available in mini globules as an oil-in-water emulsion (MacGibbon and Taylor, 2006). Mammary epithelial cells produce the milk fat globules and fuse them into the alveolar lumen. During this fusion process the globules are enveloped by a portion of the cell membrane, which is called milk fat globule membrane (MFGM) (Walstra and Jeness, 1984; Keenan and Dylewski, 1985; Jensen et al., 1991). The fat globule core mainly consists of triacylglycerol (Keenan and Dylewski, 1994). The membrane is 2% of the total milk fat globule

mass which ranges from 0.1 to 10 μm in diameter and contains mainly polar lipids, proteins and many enzymes. The number of globules per ml of milk is 10^{10} with surface area of 700 cm^2/ml of milk (Walstra and Jeness, 1984). Milk fat globule size increases with increase in fat due to the limitation of milk fat globule membrane production (Wiking et al., 2004).

Milk fat is involved in a number of functions. The primary purpose of milk fat is to supply energy to the new born calf (MacGibbon and Taylor, 2006). Milk fat provides nearly 3.7 KJ g^{-1} of energy and nutrients such as essential fatty acids and bioactive lipids; triacylglycerides, diacylglycerides, saturated, polyunsaturated, and phospholipids and also fat-soluble vitamins to the consumer (Walstra and Jeness, 1984; German and Dillard, 2006). It is also responsible for nutritive, physical, manufacturing, and organoleptic properties of milk and milk products (Jensen et al., 1991; MacGibbon and Taylor, 2006).

2.4.2. Bovine Milk Fatty Acids

In current years comprehensive studies of fine milk fat composition have become more of interest due to several factors. The main key reason is to fulfill consumer demands with quality milk and milk products. Milk fat composition affects its nutritional, industrial and sensory qualities, as well as has an impact on economic value of milk (Bastin et al., 2011). Moreover, milk fatty acid profile reflects the energy status of cow (Stoop et al., 2009b) and in vivo methane output in ruminants (Chilliard et al., 2009).

Bovine milk fat consists of a wide range of fatty acids differing in carbon chain length and saturation. Fatty acids are main components of triacylglycerol, diacylglycerol, monoacylglycerol, phospholipids, and sterol esters. Fatty acids are made up of carbon, hydrogen, and oxygen. These elements are arranged in a linear carbon chain skeleton of different length with a carboxyl group at the end (Chow et al., 2007). Approximately 15 fatty acids are present at or above 1% of concentration in milk fat (major fatty acids) (Table 2.3), whereas about 40 are present less than 0.01% of concentration (Table 2.4) (MacGibbon and Taylor, 2006). Fatty acids are the major milk component accounting for 90% of its weight (Samková et al., 2012).

According to Walstra and Jeness (1984), fatty acids can be classified into different groups depending on the following variables;

9

1. Chain length

   Fatty acids occur as even numbers (predominant), as well as odd numbers of carbons. Depending on chain length fatty acids could be classified into three different groups; long-chain, medium-chain, and short-chain. Different studies have given different definitions for these 3 groups based on the purpose of the research (MacGibbon and Taylor, 2006; FAO, 2010; Bastin et al., 2011).

2. Number of double bonds /saturation

   Based on saturation fatty acids can be divided mainly into two groups; saturated (no double bonds) and unsaturated (one or more double bonds). Unsaturated fatty acids can be further grouped into two; monounsaturated (one double bond) and polyunsaturated (more than one double bonds).

3. Position of double bond

   Depending on position of the double bond many isomers can occur. The position of the double bond should be specified by counting from the carboxyl group.

4. Geometric isomers

   Each double bond can be either *cis* or *trans*
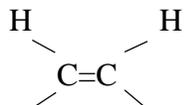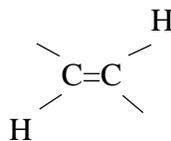
   cis   *trans*

5. Branching

   Branched fatty acids can be either iso acids or anteiso. Very small quantity of multi branched and cyclic fatty acids also exists.

6. Keto and Hydroxy Groups

   Keto group have $\overset{\overset{O}{\|}}{C}$ at various position and hydroxy group have -CHOH-.

7. 1-Glycerol Ethers

   These category contains group of $-C-O-CH_2-R$.

   R- Radical group containing any number of carbon and hydrogen atom attached to the rest of the molecule

2.4.2.1. Saturated Fatty Acids

Saturated fatty acids account for 70-75% of total fat in a typical bovine milk. Significant amount of saturated fatty acids are unbranched and have carbon chain length of 4 to 18. Major saturated fatty acids, such as palmitic (C16:0), stearic (C18:0) and myristic acids (C14:0) account for 27.9%, 12.2%, 11.1%, respectively (MacGibbon and Taylor, 2006). Similarly, a study done by Bastin et al. (2011) using MIR spectrometry also showed a high amount of saturated fatty acids in milk. A research done in Canada by Bilal et al. (2014) found 60-65% of saturated fatty acids with high proportions of C16:0, C14:0 and C18:0. Short-chain saturated fatty acids (C4:0 to C10:0) are present at 10.9% with butyric acid (C4:0) and caproic acid (C6:0) about 4.4% and 2.4% of the total fatty acids. Medium-chain (C12:0 to C16:0) and long-chain (C17:0 to C22:0) saturated fatty acids account for approximately 46% and 12% of the total fatty acids, respectively (Månsson, 2008).

2.4.2.2. Unsaturated Fatty Acids

The total amount of unsaturated fatty acids in bovine milk is 30%. Moreover, monounsaturated fatty acids account for 25%, while polyunsaturated and transunsaturated fatty acids account for 2.3% and 2.7%, respectively (Månsson, 2008). Unsaturated fatty acids are synthesized by the introduction of *cis* double bond between carbon 9 and 10, in a carbon chain length of 10 to 18 saturated fatty acids. This desaturation is catalyzed by the iron containing stearoyl-CoA desaturase enzyme (SCD) (Ntambi, 1995). Due to the biohydrogenation of dietary unsaturated fatty acid that takes place in the rumen, the amount of unsaturated fatty acid absorbed is very minimal, thus their incorporation into milk is low (Sauer et al., 1998).

2.4.2.2.1. Monounsaturated Fatty Acids

Most common monounsaturated fatty acids have carbon chain length of 16 to 22 with double bonds of *cis* configuration occurring at different positions. During industrial process, *trans* isomers can be produced which have the melting point greater than *cis* (Rustan and Drevon, 2005). The *cis*-monoenoic fatty acid accounts for 18 to 24% of bovine milk fat (MacGibbon and Taylor, 2006). Oleic acid (C18:1-9c) is the highest concentrated *cis* monounsaturated fatty acid with 22.8% by weight of total fatty acids (Månsson, 2008) and also accounts for 80% of total unsaturated fatty acids (Bilal et al., 2014). Monounsaturated fatty acid C18:1-11c is present at 0.5% while other

18:1-*cis* are found in small trace amount. Other *cis* monounsaturated fatty acids found in significant quantities are C14:1 (about 1.0%), C16:1 (about1.5%) (MacGibbon and Taylor, 2006) and vaccenic acid (C18:1-11t) (1.5%-5%) which is a precursor of CLA in human (Markiewicz-Kęszycka, et al., 2013).

2.4.2.2.2. Polyunsaturated Fatty Acids

Most of the polyunsaturated fatty acids are long chain fatty acids. If the first double bond is found between 3rd and 4th carbon atom from the omega carbon, it is called omega-3 fatty acids. Whereas, if the first double bond is found between 6th and 7th carbon atom from the omega carbon, it is called omega-6 fatty acids. Fatty acids of omega-3 and omega-6 families are essential nutrients because they cannot be synthesized in humans due to the lack of $\Delta12$- and $\Delta15$-desaturase enzyme which facilitates the *de novo* synthesis from stearic acid (Goyens et al., 2006) and these two groups of fatty acids cannot be interconverted (Rustan and Drevon, 2005). Linoleic acid (LA) (C18:2) and α-linolenic acid (ALA) (C18:3) are the main omega-6 and omega-3 polyunsaturated fatty acids accounting for 1.6% and 0.7% of total weight of fatty acids, respectively (Månsson, 2008). α-linolenic acid (C18:3) is higher in milk from pasture-fed cows than barn-fed cows (MacGibbon and Taylor, 2006). Ratio between omega-6 and omega-3 fatty acids was 2.3:1 in Swedish cow's milk (Månsson, 2008). During the metabolism of α-linolenic acid eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are produced. Likewise arachidonic is produced from metabolism of linoleic acid (Rustan and Drevon, 2005). In human, up to 8% of α-linolenic acid in phospholipid was reported to be converted to eicosapentaenoic acid. However, only 8% of α-linolenic acid was incorporated into phospholipids (Goyens et al., 2006). Polyunsaturated fatty acids are microbial dehydrogenated in the rumen (Markiewicz-Kęszycka et al., 2013).

2.4.2.2.3. Trans Fatty Acids

Trans fatty acids account for 2-4% of milk (Bauman and Lock, 2010). Precht and Molkentin (2000) found that C18:1-*trans* (vaccenic acid) and C18:2-*trans* account for 3.67% and 1.12%, respectively, under normal feeding conditions. Other *trans*-polyunsaturated fatty acids found in milk fat are CLA (minor *trans* fatty acids). Approximately 20% of average daily intake of total *trans* fatty acid of human diet come from ruminant derived food while the rest come from

industrial sources (Bauman and Lock, 2010). Hulshof et al. (1999) found that in some European countries *trans* fatty acids, derived from butter and from milk fats and dairy products including cheese, account for 3 to 50% and 14% to 50%, respectively. *Trans* fatty acids are produced in the rumen by bacteria during the hydrogenation of polyunsaturated fatty acids. During the metabolism double bonds of fatty acids are used as acceptors for hydrogen due to lack of oxygen. This leads to the production of *trans* fatty acids. These *trans* fatty acids are of increasing interest due to their negative health effects (Mensink and Katan, 1993). *Trans* fatty acids usually have a high melting point, which makes it more preferable for semi-solid cooking fats and margarines (Mensink and Katan, 1993).

2.4.2.2.4. Conjugated Linoleic Acids (CLA)

In contrast to other *trans* fatty acids CLA has attracted much more attention due its positive health effects. A group of position and geometric isomers of linoleic acids (*c*9, *c*12-octadecadienoic acid) are called CLA. They are characterized by conjugated double bonds at carbon 9 and 11 or 10 and 12 separated by one single bond. Approximately total CLA ranges between 0.71%-1.06 % (Kargar et al., 2012). Among the 28 CLA isomers rumenic acid (C18:2 *cis*-9, *trans*-11) is the principal isomer (Markiewicz-Kęszycka, et al., 2013), which accounts for 80-90% of total CLA (Parodi, 1977). Rumenic acid is synthesized through the desaturation of vaccenic acid in the mammary gland (Månsson, 2008). CLA has both *cis* or *trans* configuration or both located along the carbon chain (O'Quinn et al., 2000). Diagram of linoleic and conjugated linoleic acids is shown in Figure 2.4.

2.4.3. Categorization of Fatty Acids Based on Chain-Length

Fatty acids have been defined in different ways based on chain length in different studies (Hou, 2005; Månsson, 2008; MacGibbon and Taylor, 2006; Kargar et al., 2012), which leads to different ranges of contents in fatty acid. MacGibbon and Taylor (2006) stated that short-chain (4 to 6 carbons), medium-chain (8 to 12 carbons) and long-chain (14 to 18 carbons) fatty acids account for 6.4%, 8.7% and 85.3%, respectively. Contrarily, Kargar et al. (2012) estimated short-chain (4 to 12 carbons), medium-chain (14 to 16 carbons) and long-chain (18 to 22 carbons) in the ranges of 13.08%-17.86%, 43.17%-48.33% and 34.23%-42.52% respectively. Stoop et al. (2009a)

grouped the fatty acids based on their effect on human health; short-chain (4 to12 carbons)-neutral, medium-chain (14 to 16 carbons)-negative and long-chain (carbon18 family)-positive. They found that short-chain, medium-chain and long-chain accounted for 15%, 44% and 29.5% of total fat. Bastin et al. (2011) found a high amount of medium-chain fatty acids (12 to16 carbons) than other two short-chain (4 to 10 carbons) and long-chain fatty acids (17 to 22 carbons) fatty acids using MIR spectrometry. Categorization of fatty acids based on chain length and saturation following MacGibbon and Taylor (2006) is shown in Figure 2.5.

2.4.4. Functions of Fatty Acids

Milk fatty acid composition has a great influence on both milk's physical and nutritional qualities. Short-chain and medium-chain fatty acids lower the melting point of triacylglycerol thus enhance the liquidity of milk fat in cow's body temperature (MacGibbon and Taylor, 2006). Milk fatty acids also affect the hardness of butter, crystallization and fractionation of milk fat. Organoleptic properties of milk are also affected by free short-chain fatty acids and oxidative changes in fatty acids (Chilliard, 2001). Moreover, both free and as part of complex lipid fatty acids are involved in numerous functions in the body. Fatty acids act as metabolic fuel components of all membranes and as gene regulators. Polyunsaturated fatty acids are the precursors of metabolites (Rustan and Drevon, 2005).

2.5 Bovine Milk Synthesis

Milk fatty acids synthesis in ruminants take place in two ways; *de novo* synthesis in mammary epithelial cells and derived from blood circulation (Dils, 1986; Neville and Picciano, 1997; Bauman and Griinari, 2003; Bauman and Davis, 2013). In ruminants, half of the fatty acids (molar percentage) are derived from *de novo* synthesis (Bauman and Griinari, 2003). However, by weight of milk fatty acids, *de novo* synthesis accounts for 40% while fatty acids derived from blood lipids account for 60% (Chilliard et al., 2000). Milk fat synthesis and secretion based on Chilliard et al. (2000) is shown in Figure 2.6.

2.5.1. *De Novo* Synthesis

Short chain fatty acids (4 to 8 carbons) and medium-chain fatty acids (10 to 16 carbons) are produced from *de novo* synthesis, whereas long-chain fatty acids (>16 carbons) are derived from the uptake of circulating lipids. However, 16 carbon fatty acids originate from both sources. During the *de novo* synthesis ruminants utilize acetate produced by the fermentation of carbohydrate in rumen as the main carbon source (Bauman and Griinari, 2003). In addition, half of the first 4 carbons of fatty acids produced during *de novo* synthesis are derived from β-hydroxybutyrate which accounts for 15% of carbon content (Chilliard et al., 2000). β-hydroxybutyrate are produced from absorbed butyrate by the rumen epithelium. *De novo* synthesis involves two main enzymes; acetyl CoA carboxylase (ACC) and fatty acid synthase (FAS). First, to activate the process acetate is converted to acetyl-CoA (ACC) by acyl-CoA synthetase short-chain family (ACSS) (Ha and Kim, 1994). Then Malnoyl-CoA is formed from acetate by acetyl CoA carboxylase. Fatty acid synthase involves in the formation of malnoyl CoA either from acetyl CoA or butyryl CoA, which arise from acetate or 3- hydroxybutyrate metabolism, respectively. Fatty acid synthase also is involved in thioesterase activity which produces C14:0 and mostly C16:0 through chain-termination production. Fatty acid synthase show a transacylase with both loading and releasing activity for acyl chains from 2 to 12 carbon chain length (Chilliard et al., 2000). Enzyme involved in the biosynthesis of milk are found in the high speed supernatant (cytosol) of mammary tissue and in other lipogenic tissues, such as adipose and liver (Ganguly, 1960). *De novo* synthesis in liver and adipose tissues produce mainly palmitate (Bauman and Davis, 2013). Most of the fatty acid produced from *de novo* synthesis are saturated due to low activity of delta-9 desaturase enzyme on fatty acids shorter than 18 carbon chain length. However small promotion of C14:0 and C16:0 are desaturated to C14:1 and C16:1 (Chilliard et al., 2000). Conversion of C16:0 to C18:0 is not possible by chain elongation in the lactating mammary gland (Sauer et al., 1998). Milk fat synthesis and secretion in ruminants based on Rustan and Drevon. (2005) is showed in Figure 1.7.

2.5.2. Preformed Fatty Acids

The fatty acids derived from the circulating lipids are directly incorporated into the milk. Most of the preformed fatty acids are long-chain and unsaturated fatty acids. The circulating lipids

in the blood are produced from two sources; lipoproteins and nonesterified fatty acids (NEFA) derived from digestive tract during the absorption of lipids and from mobilization of body fat reserves (Bauman and Davis, 2013; Barber et al., 1997). The main source of fatty acids derived from blood circulation lipids is dietary and microbial fatty acids in the intestine. Fatty acids derived from body fat mobilization and lipolysis accounts for < 10% of fatty acids in milk. However, Bauman and Griinari (2003) reported that, when cows are in negative energy balance, fatty acids produced from body fat reserve mobilization increase in direct proportion of energy deficit and it can vary from 5-20% of milk fatty acids in well fed cows.

2.5.3. Desaturase Activity

Delta-9 desaturase enzyme (SCD) introduces a *cis* double bond between carbons 9 and 10 thus producing unsaturated fatty acids, especially monounsaturated fatty acids and conjugated linoleic acid isomers (CLA) only found in ruminants. Delta-9 desaturase enzyme produced by fully differentiated mammary secretory cells converts stearic acid (C18:0) to oleic acid (C18:1 cis-9) (Kinsella, 1972). Nearly 40% of absorbed stearic acid is desaturated and produced more than 50% of oleic acid in milk fat (Bickerstaffe et al., 1974, Enjalbert et al., 1998). Further, in milk 50-56% of palmitoleic (C16:1 *cis*-9) and 90 % of myristoleic (C14:1 *cis*-9) originated from palmitic (16:0), myristic (14:0) by the activity of delta-9 destaurse enzyme. More than 60% of rumenic acid (C18:2 cis-9 trans-11) formed by the desaturation from vaccenic acid (C18:1 *trans* 11) which is produced in the rumen during biohydrogenation and absorbed into the intestine. Rumenic acid (C18:2 *cis*-9 trans-11) is a major isomers of CLA (Griinari and Bauman, 1999; Bernard et al., 2013).

Delta-5 desaturase and delta-6 desaturase enzymes are involved in the production of omega-3 and omega-6 fatty acids which are essential fatty acids (Simopoulos, 1991). Omega-3 fatty acids account for 0.5% of total fatty acids and the main omega-3 fatty acid is α-Linolenic acid (ALA). α-Linolenic acid is converted to very long chain fatty acids; eicosapentaenoic (EPA) and docosahexaenoic (DHA) through many desaturation activities (Bauman and Lock, 2010). Biosynthesis of omega-3 and omega-6 is showed in Figure 2.7. Delta-9 desaturase enzyme is also involved in the production of CLA. It is produced through two sources: ruminal biohydrogenation and from C18:1 *trans*-11: another intermediate in the biohydrogenation of unsaturated fatty acids. Synthesis of CLA based on Bauman et al., (2000) is shown in Figure 2.8.

2.6. Effect of Fatty Acids on Human Health

2.6.1. Saturated Fatty Acids and Cardio Vascular Disease (CVD)

Heart disease and stroke statistics report (2016) stated that in 2013 overall death rate caused by cardio vascular disease (CVD) was 222.9 per 100,000 Americans (Mozaffarian et al., 2015). Based on 2013 data report, 1 American dies every 40 seconds due to cardio vascular disease. Cardio vascular disease is caused by high blood cholesterol, hypertension, diabetes, obesity and an atherogenic diet (Huth and park, 2012). A pillar of international dietary recommendations to reduce the risk of cardiovascular disease is the reduction of saturated fatty acids consumption (Bauman and Lock, 2010; Micha and Mozaffarian, 2010). In 2010 "Expert consultation on fats and fatty acids in human nutrition" recommended saturated fatty acid dietary intake as 10% of total energy intake (FAO, 2010).

Milk and dairy products are the major sources of saturated fatty acids in human diet (Kliem and Shingfiled, 2016). As milk fat has 60 to 70% saturated fatty acids (Grummer et al., 1991; Bilal et al., 2014), milk is associated with increase of serum cholesterol levels and therefore, considered as a risk factor for coronary heart disease (CHD) (Parodi, 2009) thus affects the consumption of milk and dairy products. Saturated fatty acid from dairy products accounts for 20-30% of total dietary intake (Bauman and Lock, 2010). These reasons have led to reduction of saturated fatty acid proportion in milk without changing consumer eating habits (Kliem and Shingfiled, 2016). However, not all saturated fatty acid equally affect hypercholesterolemic effect, the links between saturated fatty acid, cholesterol and coronary heart disease are more complex and each fatty acid should be evaluated individually with respect to the risk of coronary heart disease (Parodi, 2009).

It is a generally accepted statement that saturated fatty acids increase total cholesterol (TC) and low density lipoprotein-cholesterol (LDL-C) in humans (Kliem and Shingfiled, 2016). A review was done by Hooper et al. (2015) based on 15 randomized controlled trials. The study showed that reducing dietary saturated fatty acids reduced the cardiovascular events by 17%. However, despite well-established evidence, recent studies have shown that compared to carbohydrate, saturated fatty acids increased total cholesterol, and low density lipoprotein-cholesterol, but also increased high density lipoprotein-cholesterol (HDL-C), which resulted in no significant effects on total cholesterol / high density lipoprotein-cholesterol ratio (TC/HDL-C)

(Mensink et al., 2003; Micha and Mozaffarian, 2010). Contrarily, meta-analysis of 21 cohort studies showed that there is no association of saturated fatty acids with coronary heart disease, stroke or cardio vascular disease. However, likely there was not sufficient data available for the study to arrive at a conclusion of association of saturated fatty acids with cardio vascular disease (Siri-Tarino et al., 2010).

There is convincing evidence from clinical trials, controlled metabolic intervention and cohort studies stating that: when saturated fatty acids are replaced by *cis* polyunsaturated fatty acids, they reduce the low density lipoprotein-cholesterol as well as total cholesterol / high density lipoprotein-cholesterol ratio rather than replacing saturated fatty acids with *cis* monounsaturated fatty acids, which reduces the coronary heart disease. Contrarily, when saturated fatty acids is replaced with *trans* fatty acids, they reduce the high density lipoprotein-cholesterol and total cholesterol / high density lipoprotein-cholesterol ratio, which increases the cardio vascular disease (Mensink et al., 2003; Jakobsen et al., 2009; Micha and Mozaffarian, 2010). Siri-Tarino et al. (2010) reported that replacing saturated fatty acids with polyunsaturated fatty acid reduced the cardio vascular disease by 42%. Thus, the effect of saturated fatty acids on cardio vascular disease depends on replacement nutrients such as polyunsaturated fatty acids, monounsaturated fatty acids and carbohydrate. Therefore, it is important to consider the replacement nutrient with regard to development of policies that prioritize the reduction of consumption of saturated fatty acids. The most common replacement is carbohydrate (Micha and Mozaffarian, 2010). Some reports stated that the effect of saturated fatty acids from milk and cheese on cardio vascular disease was less compared with saturated fatty acids derived from other foods (de Oliveira Otto et al., 2012; Siri-Tarino et al., 2015). However, the underlying relationship between the intake of saturated fatty acids and cardio vascular disease risk still remains controversial (Huth and Park, 2012). Hooper et al. (2012) conducted a review based on human intervention studies, which examined the effect of saturated fatty acid on cardio vascular disease with reduced or modified dietary fat. It revealed that modified dietary fat had less effect on cardio vascular disease than reduced total dietary fat. Similarly, a review done by Livingstone et al. (2012) reported that modified milk and dairy products reduced total and low density lipoprotein-cholesterol. Therefore, research so far shows that modified milk and dairy products are more beneficial than typical milk and dairy products,

however, further research need to be conducted to study complexity of association of fatty acids with cardio vascular disease.

The effect of saturated fatty acids on plasma lipoprotein cholesterol fractions varies depending on the chain length. For instance human intervention studies shows that lauric acid (C12:0), myristic (C14:0) and palmitic acid (C16:0) significantly elevate the low density lipoprotein-cholesterol, whereas stearic acid (C18:0) has no effect (Denke and Grundy, 1992; Temme et al., 1996; Mensink et al., 2003). This leads to a general consensus that excessive consumption of medium chain saturated fatty acids is a risk factor for cardio vascular diseases (Kliem and Shingfiled, 2016). However, a meta-analysis of 60 controlled studies done by Mensink et al. (2003) showed that all three saturated fatty acids also increase the high density lipoprotein-cholesterol and raising effects increase with lower chain length. Overall, palmitic and myristic acids have no significant effect on total cholesterol / high density lipoprotein-cholesterol ratio, but the ratio is significantly reduced by lauric acid while reduction by stearic acid is not significant. Authors concluded that replacing myristic, palmitic and stearic acids with carbohydrate has little benefits, while replacing lauric acids with carbohydrate would have potential harm. Likewise, as cited by Parodi, 2009, short-chain fatty acids C4:0 to C10:0 and a portion of C12:0 have no serum cholesterol-raising effect. Therefore, milk fat composition should be modified in terms of proportion of C12:0, C14:0 and C16:0 rather than the total saturated fatty acids per serving (Kliem and Shingfiled, 2016).

2.6.2. Unsaturated Fatty Acids

2.6.2.1. Trans Fatty Acids

The average intake of *trans* fatty acids in United States population was about 2.6% of daily calories of which 20% is derived from ruminants (Bauman and Lock, 2010). Vaccenic acid (C18:1 11t) is a main *trans* fatty acids in milk (Brouwer et al., 2013). Human intervention studies have demonstrated that *trans* fatty acids are associated with cardio vascular diseases. They increase low density lipoprotein-cholesterol and decrease high density lipoprotein-cholesterol and increase total cholesterol / high density lipoprotein-cholesterol ratio (Mensink et al., 1990; Mensink et al., 2003; Wang et al., 2014). Due to association of *trans* fatty acids with cardio vascular diseases "Expert consultation on fats and fatty acids in human nutrition" recommended intake <1% of total energy

intake (FAO, 2010). Oomen et al. (2001) reported that consumption of *trans* fatty acids around 2% of total energy intake would increase cardio vascular diseases by 25%. Zock et al. (1992) reported that Vaccenic acid (C18:1 11t) has antherogenic effect than palmitic C16:0 which increased both low density lipoprotein-cholesterol and high density lipoprotein-cholesterol. However, some studies have shown no association of *trans* fatty acids from animals with cardio vascular diseases (Willett et al., 1993). Tricon et al. (2006) found that Vaccenic acid and 11 t CLA in milk did not have any effect on cardio vascular diseases. Thus, association of *trans* fatty acids with cardio vascular diseases need to be further researched. In addition, high consumption of *trans* fatty acids increase body weight, visceral adiposity, insulin resistance, unfavorably affect lipid level in blood, activate systematic inflammation and induce endothelial dysfunction (Dorfman et al., 2009; Micha and Mozaffarian, 2009). Studies have also shown that *trans* fatty acids are associated with breast and colorectal cancers (Slattery et al., 2001; Chajès et al., 2008). Chavarro et al. (2014) reported that *trans* fatty acids are associated with low quality semen in men.

2.6.2.2 Conjugated Linoleic Acid (CLA)

A group of polyunsaturated fatty acids are called CLA which accounts for 30mg/g of fat. Twenty-eight CLA isomeres have been identified. However only two of the isomers (cis-9, *trans* 11 and *trans*-10, cis12) are positively associated with human health (Micinski et al., 2012). Main sources of CLA are milk and dairy products. Many animal model studies have reported anti-atherosclerotic effect of CLA by decreasing the level of total cholesterol, low density lipoprotein-cholesterol, blood pressure and increasing high density lipoprotein-cholesterol (Kritchevsky et al., 2004; McLeod et al., 2004; Bhattacharya et al., 2006). CLA has also been found to have cancer preventive effect (Bhattacharya et al., 2006; Flowers and Thompson, 2009; Hsu et al., 2010). Moreover, CLA also lowers hypertension, atherosclerosis and diabetes and stimulates the immune function (Mills et al., 2011).

2.6.2.3. Essential Fatty Acids (omega-6, omega-3)

Omega fatty acids (6, 3) are more of interest due to positive effect on human health. They are essential for growth and development. Further, they also prevent cardio vascular diseases, inflammatory diseases and neurological disorders (Yashodhara et al., 2009). Omega-6 fatty acid

cannot be converted to omega-3 in humans due to the lack of omega-desaturase enzyme. Linoleic Acid (LA) and α-Linoleic Acid (ALA) and their long-chain derivative are essential components in animal cell membranes. For good health and normal development balance of these two fatty acids are important. Western diet lack omega-3, and the ratio of omega-6/ omega-3 is 15-20/1, which should be around 1/1 (Simopoulos, 2002).

2.7. Possibility of Altering Milk Fatty Acid Composition

The idea of modifying milk fat composition started in 1980s. During the Wisconsin milk marketing board meeting held in 1988, it was decided that ideal milk in terms of human health should consist of less than 10% polyunsaturated fatty acids including omega-3 and omega-6 and up to 8% saturated fatty acids and the rest should be monounsaturated fatty acids (O'Donell, 1989). Moreover, as cited by Soyeurt et al. (2006a), Pascal (1996) estimated that favorable milk lipid to human health should contain 30% of saturated fatty acids and Hayes and Khosla (1992) found that it should have 60% monounsaturated and 10% polyunsaturated fatty acids. Contrary, a typical milk contains 70% saturated, 25% monounsaturated and 5% poly unsaturated fatty acids, which is far from optimal in terms of human health (Grummer et al., 1991; Bilal et al., 2014). Furthermore, studies have shown that increased unsaturated and short-chain fatty acids improves softness of milk fat, the spreadability and adhesiveness of butter, especially increasing the content of C18:1 and C18:2 and reducing the content of fatty acids with carbon of C8:0 to C14:0. (MacGibbon and McLennan, 1987; Bobe et al., 2007). However, Palmquist et al. (1993) stated that increase of C18:2 above 20% would create off flavors in milk and thus affect the sensory quality of butter. Thus, the reasons discussed above reflect the need and opportunity to modify milk fat composition, especially with regards to human health and to cater specific dairy products. Yet, the opinion of optimal fatty acids may vary among nutritionists and dairy processors. Currently, there is no selection program for fatty acids in Canada. However, as dairy processors' interest in producing differentiated products is increasing, it creates the need to select dairy cattle based on different milk fat composition. Therefore, in order to develop a breeding program to modify milk fatty acid profile there should be genetic variation and genetic parameters for fatty acids should be estimated. Moreover, there should also be a selection mechanism and sufficient economic incentives (Shingfield et al., 2013).

21

Multiple factors impact the final milk fat composition. These factors can be classified into 3 main groups: animal, feed and environment (Palmquist et al., 1993; Jensen, 2002). Animal factors include breed, stage of lactation, diet, parity, cow individuality and milk and fat yield (Samková et al., 2012). Some studies have investigated the effect of environment such as season and heat stress on milk fat composition (Karijord et al., 1982; Lock and Garnsworthy 2003; Mele, 2009; Wang et al., 2010). Comparing to animal and environmental factors affecting fatty acid composition, an abundance of studies is available on effect of feed on milk fatty acids composition (Bauman et al., 2001; Chilliard et al., 2001). Milk fat can be modified mainly via feeding and genetic selection (Palmquist et al., 1993; DePeters et al., 1995). Modification by feeding is a temporary way. As long as a cow is fed a diet containing modified fat content, they will produce milk with favorable fat composition. Conversely, genetic modification is more permanent, where animals will be selected based on genetic merit for milk fat composition and produce progeny with enhance milk composition. Beaulieu and Palmquist (1995) observed difference in milk fat composition between Jersey and Holstein, thus reflecting the effect of genetics in different breeds. However, studies are scarce with regards to within-breed genetic variation (Gibson, 1991). Similarly, studies have shown that half of variation of milk fat percentage is due to genetic variation (Fuerst and Solkner, 1994; Ikonen et al., 2004).

2.7.1. Genetic Variation of Fatty Acids

There has been a limited number of studies exploring the genetic variability of fatty acids. Most of the research focused on estimating the basic genetic parameters such as heritabilities of fatty acids and/or correlations among fatty acids. The results vary across different studies and this might be due to different number of samples, model, method used to measure fatty acids, units used for the expression of the traits and definition of each trait (Bastin et al., 2011). In order to select for improved fatty acid profile, factors such as genetic variation as well as interdependence between different fatty acids and other traits should be taken into account. To estimate the genetic parameters accurately, a large number of records is required (Soyeurt et al., 2007a) and it should be collected in an efficient and affordable way. Gas chromatography, which is generally used to measure fatty acid content may not be the best option in this case. It requires significant amount of more time and is labor intensive, thus reducing the possible number of cows and milk samples

used for the analysis. Recent studies have shown the use of MIR spectra as an alternative solution (Soyeurt et al., 2006a; Rutten et al., 2009; Soyeurt et al., 2011; Lopez-Villalobos et al., 2014)).

2.7.1.1. Mid-Infrared Spectroscopy

Mid-infrared is one of the region on electromagnetic radiation, which ranges between 2,500 to 25,000 nm. When a sample is hit by MIR radiation the bonds of the molecules starts to vibrate which take in part of the supplied energy. Thus, a sample's chemical composition can be determined based on the supplied and absorbed energy, as well as using spectra mathematical pretreatments (De Marchi et al., 2014). In the recent years, MIR spectroscopy has been considered as a potential tool for collecting data at a population level for phenotypic and genetic analyses of, for instance, milk related traits (De Marchi et al., 2014). Soyeurt et al. (2006a) first developed a calibration equation to predict fatty acid concentration using MIR spectra. .The advantages of using MIR spectra are many. The MIR spectra are already available in milk laboratories during routine milk testing and they can predict a large number of traits, in an affordable and efficient way (Arnould and Soyeurt, 2009).

These genetic studies on fatty acids could be categorized into two groups based on the method used to measure the fatty acids: gas chromatography and MIR spectroscopy.

2.7.1.2. Fatty acid Analysis Using Gas Chromatography Method

A study conducted by Edwards et al. (1973) was one of the first studies to estimate the heritability for fatty acids (mol%) using 50 winter milk samples. They found value ranging from 0.64-0.98 which were overvalued (Arnould and Soyeurt, 2009). Renner and Kosmack (1974a) as cited by Karijord et al. (1982) and Soyeurt et al. (2008a) estimated heritabilities for three groups of fatty acids in milk and in milk fat percentage using a sire model based on 2,082 samples. They found higher heritabilities for fatty acids groups in milk than in fat percentage. The heritabilities for short-chain (4 to 8 carbons) and medium-chain (10 to 16 carbons) and long-chain (C18 family) in milk were 0.26, 0.25 and 0.02, respectively, and in milk fat percentage were 0.26, 0.06, and 0.04, respectively. The heritability for short-chain fatty acids was similar in both milk and in milk fat percentage. These estimates shows that heritability of fatty acids in milk is greater than in milk fat percentage. C18 family had the lowest heritability in both milk and in milk fat percentage as

most of them are derived from blood lipids. This showed that heritability decreased with the increase of chain length. One of the first studies to examine the correlation among fatty acids was done by Renner and Kosmack (1974b). They estimated correlations between milk yield, fat content, protein content, short-chain, medium-chain and C18 family fatty acids. They found a positive correlation of 0.83 between short-chain fatty acids and milk yield, while the other two groups showed negative correlation. Short-chain and medium-chain fatty acids groups were positively correlated to milk fat. However, a negative correlation was observed between C18 family in fat and milk fat content.

Karijord et al. (1982) also used a sire model to estimate the genetic variance of fatty acids based on 7,000 milk samples. Heritability in fat (g/100g of fat) for 14 fatty acids analyzed ranged between 0.06-0.26. If the fatty acids were grouped into three groups such as short-chain (6 to10 carbons), medium-chain (12 to 17 carbons) and long-chain (>18), the heritabilities would be 0.11 - 0.16, 0.07 -0.26 and 0.06-0.15 respectively. This shows that medium-chain has higher heritability than other two groups. They also estimated the correlation between milk yield, fat %, and protein % and among individual fatty acids. Most of the saturated fatty acids had positive correlation with milk yield between the ranges of 0.08-0.24 except for C15:0, and C16:0 which had negative correlations of 0.58 and 0.14 respectively. C18:1 and C18:3 had negative correlation with milk yield, however C18:2 had a positive correlation. C18 family had a negative correlation with fat %. Most saturated and unsaturated fatty acids were positively correlated among themselves, while a negative correlation was found between saturated and unsaturated fatty acids.

Stoop et al. (2008) estimated heritabilities and correlations for individual and groups of fatty acids using linear animal model. Milk samples were collected from 1,918 Holstein first lactation cows from 398 herds. Fatty acids concentrations were determined using gas chromatography. Heritability for individual fatty acids (weight as proportion of total fat weight) ranged from 0.22 to 0.71. Heritabilities estimated for fatty acids groups were 0.67, 0.16, 0.26 for short-chain (6 to12 carbons), medium-chain (14 to 16 carbons), and long-chain (18 unsaturated fatty acid family), respectively. These estimated values were higher compared to Renner and Kosmack (1974a) and Karijord et al. (1982). Grouping of fatty acids was based on their effect on human health. Authors also found negative genetic correlation of -0.30 and -0.62 for medium-chain and short-chain with long-chain fatty acids, respectively. A negative genetic correlation of -

0.43 was found between short-chain and medium- chain fatty acids. Genetic correlation between fat percentage and fatty acids groups were 0.14, 0.65 and -0.72 for short-chain, medium-chain and long-chain fatty acids, respectively, demonstrating that an increase in fat percentage would be associated with an increase short-chain and medium-chain while reducing the long-chain fatty acids content in milk fat.

Using a single trait mixed linear animal model Bobe et al. (2008) estimated the heritability and repeatability for milk fatty acids. For the study 592 milk samples from 233 cows, daughters of 53 sire were used. A constant residual variance was assumed over the lactation. Fatty acid concentrations were expressed in g/L and wt % (individual fatty acid weight/total weight of fatty acids in milk). Heritability of saturated, monounsaturated and polyunsaturated fatty acids were 0.27, 0.09 and 0.25 respectively in milk (g/L). Authors found higher heritability of 0.28 for *de novo* synthesized fatty acids (6 to 14 carbons), showing that fatty acids synthesized by *de novo* synthesis are more under genetic control than those derived from blood lipids. Estimated heritabilities were in line with those values estimated by Renner and Kosmack (1974a). Heritabilities for fatty acids expressed as a proportion of total fatty acids (wt %) were small to moderate compared to those in milk (g/L).

Heritability and genetic correlations were estimated by Mele et al. (2009) using a linear multiple trait animal model based on 900 Holstein from 34 herds. Contrary to Stoop et al. (2008), they found lower heritabilities of 0.07, 0.03 and 0.08 for saturated fatty acids (C14:0, C16:0 and C18:0, respectively) and higher heritabilities of 0.19, 0.14 and 0.17 for unsaturated fatty acids (C14:1 cis-9, C16:1 cis-9 and C18:1 cis-9, respectively) in milk fat. High negative genetic correlation of -0.84 and -0.89 were found between C14:0 and C16:0 with mono unsaturated fatty acids. A positive correlation of 0.06 was found between C18:0 and mono unsaturated fatty acids. These results were similar to Karijord et al. (1982), Stoop et al. (2009a) and Soyeurt et al. (2007a). Fat percentage showed a high positive correlation with C16:0 (0.74) and low positive correlation with monounsaturated fatty acids (0.01). C14:0 and CLA (C18:1 trans-11 cis -9) showed negative correlation of -0.40 and -0.55 with fat percentage. These results showed that increase in fat percentage would increase C16:0 and reduce CLA.

25

Garnsworthy et al. (2010) estimated the heritability of certain major fatty acids (g/100 of total fatty acids) and desaturase index (g/100g). Fatty acids were determined using gas chromatography, methylation and rapid lipid extraction methods. Milk samples were collected from 2,408 Holstein cows of 597 sires from 325 farms. Heritability estimates for C6:0, C8:0, C12:0, C14:1 cis 9 and C18:1 cis 9 were 0.27, 0.27, 0.13, 0.28, and 0.12, respectively. They found significant heritability of 0.14 and 0.15 for saturated fatty acids and *de novo* fatty acids (6 to 14 carbons) groups. However Bobe et al. (2008) found heritability of 0.05 and 0.08 for saturated and *de novo* fatty acids (6 to 14 carbons) groups, which were not significant.

Bilal et al. (2014) estimated heritabilities and genetic correlations using amixed linear multivariate animal model based on 2,573 Holstein cows from 46 herds. Fatty acid content was determined using gas chromatography and expressed as g/100g of total fatty acids. Estimates of heritability for saturated (4 to 24 carbons), monounsaturated (14 to 18 carbons) and polyunsaturated (18 to 22 carbons) fatty acids were 0.20, 0.21 and 0.15, respectively. For individual saturated fatty acids, heritabilities ranged from 0.03 to 0.34 (C10:0). Similar to Stoop et al. (2009a), authors found high correlations of 0.74 among C12:0 and C14:0 reflecting the similar origin of those two fatty acids. A moderate negative correlation of -0.42 was estimated between C14:0 and C16:0. Saturated fatty acids had negative genetic correlation with monounsaturated (-0.99) and polyunsaturated fatty acids (-0.71). However, a moderate positive correlation of 0.60 was observed between monounsaturated and polyunsaturated fatty acids.

2.7.1.3 Fatty Acid Analysis Using MIR Spectroscopy

In the past 10 years there has been an increasing interest in using MIR spectrometry to predict fatty acids, as it is more efficient than gas chromatography. Studies have proven that some fatty acids could be predicted accurately using MIR spectrometry (Soyeurt et al., 2006a; Rutten et al., 2009; Soyeurt et al., 2011; Lopez-Villalobos et al., 2014) and also these data could be used to estimate genetic parameters of milk fatty acids (Soyeurt et al., 2007a; Bastin et al., 2011; Lopez-Villalobos et al., 2014).

Soyeurt et al. (2006a) developed first calibration equations for predicting fatty acids using mid- infrared spectrometry. Results showed that developed calibration equations for C10:0, C12:0,

C14:0, C16:0, C16:1*cis*-9, C18:1, and saturated and monounsaturated fatty acids in milk could be used for predicting fatty acids. Soyeurt et al. (2007a) estimated heritabilities and genetic correlations from 7,700 milk samples in milk and milk fat using MIR spectroscopy method. They used 40,007 test day records on 2,047 animals from 25 herds composed of 7 breeds with 1 to 3 lactations. They used calibration equation developed by Soyeurt et al. (2006a) to predict the fatty acids (g/100g of milk). Fatty acids content was also converted to g/100g of fat using density of milk (1.03 g/cm^3) and using MIR predicted fat percentage. A multi-trait mixed repeatability test-day model was used to estimate the genetic parameters, assuming constant genetic variation throughout the lactation. Estimated results showed that heritability of saturated fatty acids (0.36) was greater than monounsaturated fatty acids (0.15) in milk (g/100g of milk). Moderate heritability in milk was estimated for medium-chain fatty acids (C12:0-C16:0) ranging between 0.29-0.38, which were in line with Renner and Kosmack (1974a). Within C18 family, C18:1 had the lowest heritability (0.05) in milk, while C18:0 and C18:2 had moderate heritability of 0.30 and 0.20, respectively. This showed no relationship between chain length and heritability. Results also showed that permanent environmental variance within the lactation was greater than across lactation in milk. Monounsaturated fatty acids were more variable than saturated fatty acids within lactation. Authors also estimated the heritability in milk fat (g/100g of fat), which showed that saturated fatty acids had lower heritability (0.14) in fat than in milk (g/100g of milk). However the heritability of monounsaturated fatty acids (0.24) was greater in fat than in milk. They also observed more variability in the contents of saturated fatty acids and monounsaturated fatty acids within lactation, due to effect of seasonal changes. Moreover, based on the residual variance estimate, results showed that the model used was more suitable to analyze fatty acids in milk fat than in milk. With regards to genetic correlation in milk, Soyeurt et al. (2007a) observed negative correlation between milk and all fatty acids. This is due to the effect of dilution of higher level of milk yeild. They also found high correlation between saturated fatty acids and fat percentage than with unsaturated fatty acids. Similarly, high correlation was observed between monounsaturated and unsaturated fatty acids. From the results it is obvious that fatty acids from the same origin were highly correlated to each other. Genetic correlation in milk fat (g/100g fat) showed that selection for fat percentage would increase most of the saturated and decrease monounsaturated and C18:2 fatty acids. However, not all of saturated fatty acids showed similar changes to increase of fat percentage. The genetic correlation between fat percentage and C12:0, C14:0, C16:0 and

C18:0 were 0.55, -0.06, 0.60 and 0.84, respectively. The overall results from Soyeurt et al. (2007a) shows that there is a genetic variance present in milk fatty acids content.

Using a multi-trait random regression test-day model Soyeurt et al. (2007b) estimated heritability for saturated:unsaturated ratio which reflects the hardness of butter. They found an average daily heritability of 0.11 and lactation heritability of 0.22 for saturated:unsaturated fatty acid ratio. A negative genetic correlation of -0.71 was found between milk and saturated:unsaturated fatty acid ratio. This shows that part of hardness of butter is influenced by genetics.

Soyeurt et al. (2008a) estimated the heritability and genetic correlation of saturated and unsaturated fatty acids using multi trait test day random regression model based on 100,799 test day records from 11,626 first parity Holstein cows (Holstein composition > 84%). The calibration equations used for the saturated and unsaturated fatty acids (g/dL of milk) had RPD (ratio of performance deviation) of 5.78 and 3.65 respectively and R^2cv (coefficient of determination of the cross-validation) of 0.97 and 0.93, respectively. Authors found higher heritability of 0.42 for saturated fatty acids in milk (g/100g of milk) than in milk fat (g/100g of fat) which was 0.24. However, heritability of monounsaturated fatty acids was lower in milk (0.14) than in milk fat (0.27). They also observed higher heritability for both saturated fatty acids and monounsaturated fatty acid at the beginning and at the end of lactation in milk fat. Higher values at the beginning of lactation could be due to negative energy balance of cow, which involves the mobilization of fat from adipose tissues. Higher value at the later part of lactation could be associated with the persistency of lactation. They also found heritability of 0.27 for saturated: unsaturated fatty acid ratio. With regarding to genetic correlation authors observed a negative correlation between milk yield and the studied milk components such as: FAT%, PRO%, saturated, monounsaturated fatty acids in milk and saturated:unsaturated fatty acid ratio. The genetic correlation between saturated and monounsaturated fatty acids in milk was low at the beginning of lactation and increased until the mid-lactation and then reduced. However the genetic correlations between saturated and monounsaturated fatty acids were negative and stable throughout the lactation.

Soyeurt et al. (2008b) found heritability of 0.17 for monounsaturated fatty acids (g/100g of fat) using multi-trait mixed model using 52,950 test day records from 3,217 cows from 7 breeds.

This value was lower than the value estimated by Soyeurt et al. (2007a). Similar to previous results authors found high heritability of 0.20, 0.22 and 0.17 for monounsaturated fatty acids; C14:1 *cis* -9, C16:1 *cis* -9 and C18:1 *cis* respectively in milk fat than the saturated fatty acids such as C14:0, C16:0 and C18:0. Authors estimated heritabilities of 0.20, 0.20 and 0.03 for the following fatty acids ratios: C14:1 *cis* -9/ C14:0, C16:1 *cis* -9/ C16:0 and C18:1 *cis/* C18:0.

Arnould and Soyeurt (2009) published a paper summarizing the heritability of fatty acids in milk from the previously presented studies (Ewards et al., 1973, Karijord et al., 1982, Soyeurt et al., 2007a, Soyeurt et al 2008b, Bobe et al., 2008 and Stoop et al., 2008), thus showing the evidence for existence of genetic variability among fatty acids. The heritability ranged from 0.00 to 0.98. This wide range of heritability estimates is due to reasons such as; the number of samples, the statistical model, the method used and the unit used to express the content of fatty acids.

Using a single-trait random regression animal test day model Bastin et al. (2011) estimated heritability and genetic correlation for individual and groups of fatty acids: short-chain (4 to 10 carbons), medium-chain (12 to 16 carbons), long-chain (17 to 22 carbons), saturated, unsaturated, polyunsaturated, and monounsaturated fatty acids. The estimates were based on 130,285 test day records from 26,166 cows in 531 herds. The residual variances were assumed to be constant and independent over the lactation. Estimated heritabilities for saturated, unsaturated, monounsaturated and polyunsaturated were 0.426, 0.223, 0.212 and 0.298, respectively. Heritabilities estimated for groups based on chain length were 0.438, 0.434 and 0.198 for short-chain, medium-chain and long-chain fatty acids, respectively. Similar to Renner and Kosmack (1974), the heritability decreased with increase in chain length. Heritabilities values estimated were higher than the estimates of Soyeurt et al. (2007a), Karijord et al. (1982), Bobe et al. (2008), and Mele et al. (2009). This might be due to reasons such as; unit used to express content of fatty acids, model, methodology used to estimate the fatty acids content, and also depend on the accuracy of calibration equations used. This study also showed that short-chain and medium-chain fatty acids are more controlled by genetics more than the long-chain fatty acids reflecting the different of origin of fatty acids. Short-chain and medium-chain fatty acids are produced by *de novo* synthesis which involves two main enzymes such as acetyl-coenzyme A carboxylase and fatty acid synthetase, which are more under genetic control. However long-chain fatty acids are derived from blood lipids, which depends more on diet and are less under genetic control. The

genetic correlations estimated in Bastin et al. (2011) were approximated using daily breeding values (EBVd). Authors found negative average daily genetic correlation between milk yield and individual and groups of fatty acids ranging between -0.27 to -0.43, reflecting a dilution effect. However average daily genetic correlation did not vary strongly, indicating selection for improved milk yield would have on average equal effect on all fatty acids content in milk over the lactation, which was similar to results from Kay et al. (2005) and Bobe et al. (2007). These estimated values were higher than the values estimated by Soyeurt et al. (2007a). They also estimated positive genetic correlation between fat percentage and individual and groups of fatty acids varying between 0.67-0.97. This showed that increase in fat percentage would increase all fatty acids content in milk. However, increase of saturated, short-chain and medium-chain fatty acids would be higher when selecting for more fat content than the increase of long-chain, mono unsaturated and polyunsaturated. Authors also found average daily genetic correlation in the range of 0.18-0.60 for protein percentage and individual and groups of fatty acids. Genetic correlations among individual fatty acids were all positive and high correlation were found between fatty acids of the same groups. Similar to Soyeurt et al. (2007a) the genetic correlation reflected the common origin of fatty acids: *de novo* synthesis or deriving from circulating blood lipids.

Gion et al. (2011) estimated the heritabilities and genetic correlations for fatty acids using MIR was done for three French dairy breeds using single-trait mixed animal model. They found higher heritability for saturated than unsaturated fatty acids in milk. Also the heritabilities were higher when fatty acids content were expressed in g/100g of milk than in g/100g of fat. They estimated positive correlation among saturated as well as among unsaturated fatty acids. Genetic correlations between saturated and unsaturated fatty acids were positive in milk and negative in fat.

Lopez-Villalobos et al. (2014) estimated the heritability of fatty acids from 26,769 milk samples of 7,385 Holstein, Jersey and first cross of Holstein and Jersey cows, from 78 herds. Fatty acids were predicted by MIR spectroscopy and expressed in unit of percentage of total fatty acids. Heritability of individual fatty acids ranged from 0.14-0.45. Estimated heritabilities for short-chain (4:0, 6:0, 8:0), medium-chain (10:0, 10:1, 12:0, 12:1), long-chain (14:0 to 22:0), saturated, unsaturated, and polyunsaturated fatty acids were 0.39, 0.30, 0.50, 0.46, 0.48 and 0.42, respectively. These heritability values were greater than those values estimated by Soyeurt et al.

(2007a); Bobe et al. (2008); Mele et al. (2009); Bastin et al. (2011); Krag et al . (2013). However, the estimates were similar to results of Stoop et al. (2008). A summary of heritability estimates for MIR predicted fatty acids are provided in Table 2.5.

Studies previously discussed above show the existence of genetic variance among fatty acids in milk. However, comparing the results is difficult due to various reasons such as: different model, unit used to express fatty acid content, methodology used to measure fatty acid content and amount of data used in the analysis (Arnould and Soyeurt, 2009). Generally, studies shows that heritability of fatty acids is greater when fatty acids content is expressed in milk (g /100g of milk and g/ L of milk) than in milk fat or as proportion of total weight (g/100g fat and %wt) (Arnould and Soyeurt, 2009; Gion et al., 2011; Soyeurt et al., 2007a; and Bobe et al., 2008). Also studies show that fatty acids produced from *de novo* synthesis are under more genetic control than those derived from blood lipids. Most of fatty acids have negative correlations with milk yield. Saturated fatty acids have positive correlations with fat percentage, whereas unsaturated fatty acids have a negative correlation.

2.8 Conclusions

As consumer awareness regarding healthy diets is increasing, there is a need for the Canadian dairy industry to prepare itself for future needs. Due to the negative image of milk to be associated with cardiovascular diseases, consumption of milk has reduced over the past years. Therefore, improving milk fat composition would be a better solution to cater consumer needs. Altering milk fat composition via feeding has been explored in depth. However, modification through genetic selection needs to be investigated further. This literature review examined the Canadian dairy industry, different fatty acids, bio synthesis, effect of fatty acids on human health, and genetic variability of the milk fatty acids, providing evidence for the presence of genetic variation in milk fatty acid composition. Hence, altering milk fat composition genetically seems possible.

2.9 Tables

Table 2.1 Nutrient composition of whole milk (Source- USDA National Nutrient Database for Standard Reference 28 Software v.2.3.2 (2015))

Components	Unit	
Water	g	88.13
Energy	kcal	61
Protein	g	3.15
Total lipid (fat)	g	3.25
Ash	g	0.67
Carbohydrate, by difference	g	4.8
Fatty acids		
Total saturated	% of total	64.93
Total monounsaturated	% of total	28.27
Total polyunsaturated	% of total	6.78

Table 2.2. Main lipid classes in bovine milk (adapted from Walstra and Jeness, 1984)

Lipid Classes	Amount (%,w/w)
Triacylglycerols	98.3
Diacylglycerols	0.3
Monoacylglycerols	0.03
Free fatty acids	0.1
Phospholipids	0.8
Sterols	0.3
Carotenoids	trace
Fat-soluble vitamins	trace
Flavor compounds	trace

Table 2.3. Major fatty acids in bovine milk fat (adapted from MacGibbon and Taylor, 2006)

Common Name		Composition		
		Typical		Range
		%(w/w)	mol%	%(w/w)
4:0	Butyric	3.9	10.1	3.1-4.4
6:0	Caproic	2.5	4.9	1.8-2.7
8:0	Caprylic	1.5	2.4	1.0-1.7
10:0	Capric	3.2	4.3	2.2-3.8
12:0	Lauric	3.6	4.1	2.6-4.2
14:0	Myristic	11.1	11.1	9.1-11.9
14:1	Myristoleic	0.8	0.8	0.5-1.1
15:0	-	1.2	1.1	0.9-1.4
16:0	Palmitic	27.9	24.9	23.6-31.4
16:1	Palmitoleic	1.5	1.4	1.4-2.0
18:0	Stearic	12.2	9.8	10.4-14.6
18:1 cis	Oleic	17.2	13.9	14.9-22.0
18:1 trans	-	3.9	3.2	
18:2	Linoleic	1.4	1.1	1.2-1.7
18:2 conj	Conjugated Linoleic Acid	1.1	0.9	0.8-1.5
18:3	α Linolenic	1	0.8	0.9-1.2
	Minor acids	6	5.1	4.8-7.5

Table 2.4. Minor fatty acids in bovine milk fat (adapted from MacGibbon and Taylor, 2006)

(i=iso a=anteiso)

Composition (% w/w, of total fatty acids (FA))							
Saturated				Unsaturated			
Straight-chain		Branched-chain		Monounsaturated		Polyunsaturated	
FA	% (w/w)	FA	% (w/w)	FA	% (w/w)	FA	% (w/w)
11:0	0.20	13:0i	0.03	10:01	0.15	20:2	0.07
13:00	0.19	14:0a	0.02	12:01	0.06	20:3	0.10
17:00	0.60	15:0i	0.40	13:01	0.03	20:4	0.14
19:00	0.15	15:0a	0.44	17:01	0.36	20:5	0.09
20:00	0.35	16:0i	0.40	19:01	0.16	22:2	0.04
21:00	0.04	17:0i	0.50	20:01	0.32	22:3	0.07
22:00	0.20	17:0a	0.52	21:01	0.04	22:4	0.03
23:00	0.12	18:0i	0.16	22:01	0.06	22:5	0.04
24:0	0.14	19:0i	0.10			22:6	0.01
25:0	0.03						
26:0	0.06						

Table 2.5. Heritability estimates of MIR predicted fatty acids

Trait	Soyeurt et al., 2007a		Soyeurt et al., 2008a		Soyeurt et al. 2008b	Bastin et al., 2011	Gion et al., 2011		Lopez-Villalobos et al., 2014
Number of test-day records/ milk samples	40,007		100,799		52,950	227,313	101,858		26,769
unit	g/100g of milk	g/100g of fat	g/100g of milk	g/100g of fat	g/100g of fat	g/dL of milk	g/100g of milk	g/100g of fat	% of the total FA
Short-chain						0.44 (4 to 10 carbons)			0.39 (4 to 8 carbons)
Medium-chain						0.43 (12 to 16 carbons)			0.3 (10 to 12 carbons)
Long-chain						0.2 (17 to 22 carbons)			0.5 (14 to 22 carbons)
Saturated	0.36	0.14	0.42	0.24		0.43	0.28	0.16	0.46
Monounsaturated	0.15	0.24	0.15	0.27		0.21	0.15	0.15	
Polyunsaturated						0.30	0.22	0.2	0.42
Unsaturated						0.22	0.14	0.13	0.48
C4:0						0.35		0.31	0.38
C6:0						0.44			0.32
C8:0						0.44			0.29
C10:0						0.43			0.17
C12:0	0.29	0.09				0.43			0.16
C14:0	0.31	0.19			0.15	0.44			0.19
C14:1 cis-9					0.2		0.32	0.29	0.27
C16:0	0.38	0.2			0.15	0.41	0.28	0.21	0.29
C16:01 cis-9					0.22				0.3
C18:0	0.30	0.28			0.16	0.23			0.26
C18:1 cis					0.17	0.18			0.22
C18:1 cis-9						0.18	0.11	0.17	
C18:1 cis-9, cis-12	0.20	0.15							
C18:1 cis-9 trans-11							0.11	0.14	0.27

36

2.10 Figures

Figure 2.1. Total Canadian milk production at the farm, 2006-2015 (CDIC, 2016)
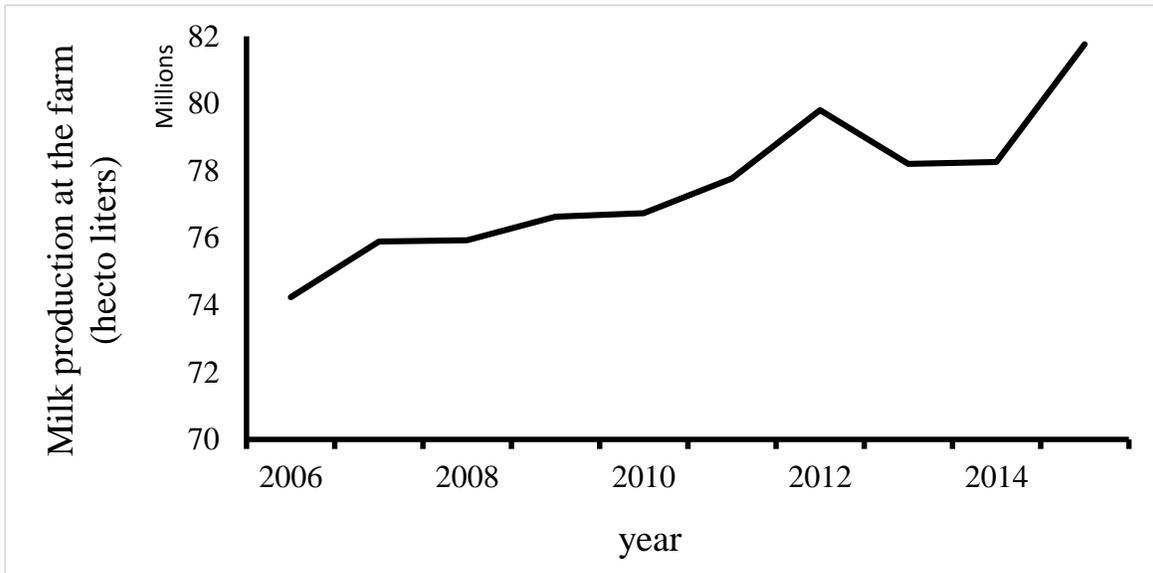
Figure 2.2. Per capita consumption of milk (Liters) in Canada- (CDIC, 2015).

Figure 2.3. Triacylglycerol molecule (Adapted from Taylor and MacGibbon, 2011)
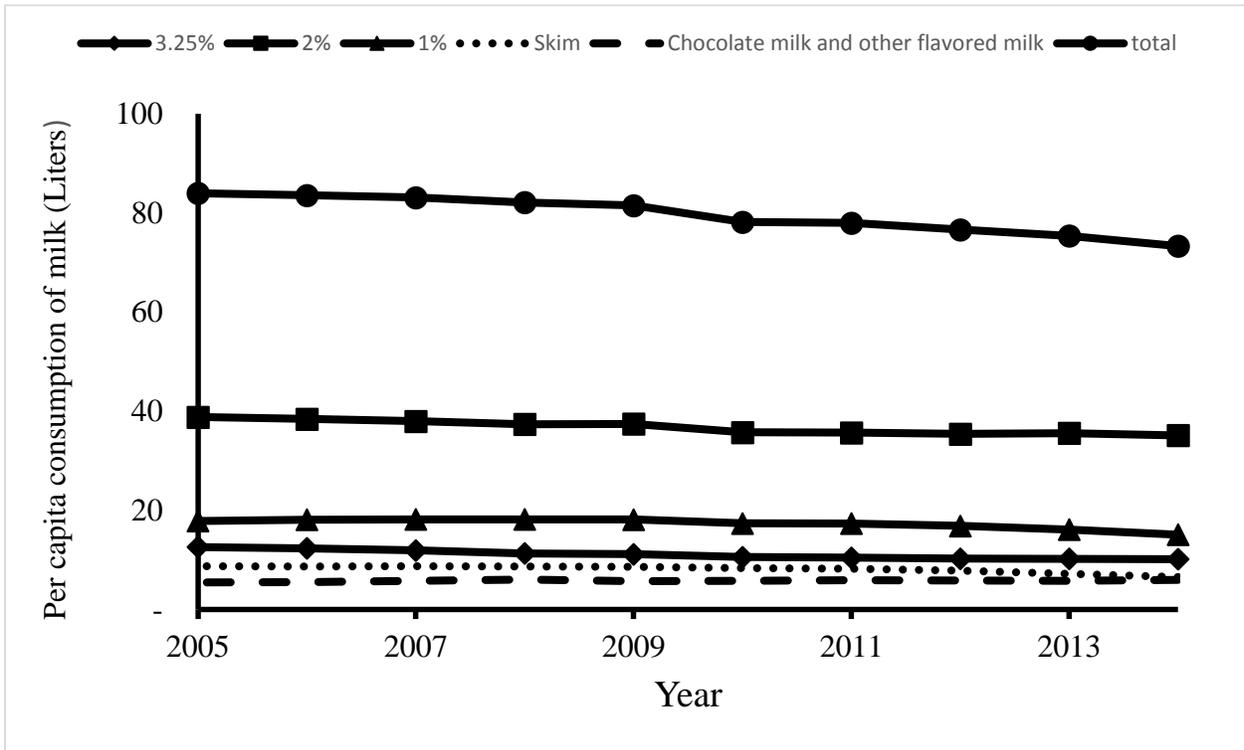
Figure 2.4. Diagram of linoleic and conjugated linoleic acids (Adapted from O'Quinn et al., 2000)
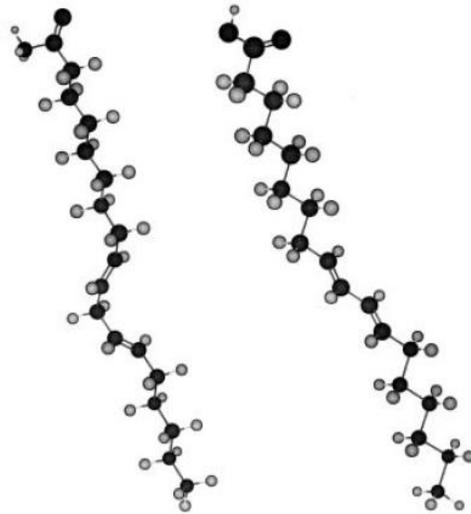
Linoleic Acid   Conjugated Linoleic Acid

(*cis* 9, *cis* 12)   (*cis* 9, *cis* 11)

Figure 2.5. Categorization of fatty acids based on chain length and saturation (Content of fatty acids adapted from MacGibbon and Taylor, 2006) [Short-chain (4 to 10 carbon), medium-chain fatty acids (12 to16 carbon) and long-chain fatty acids (17 to 22 carbon)]
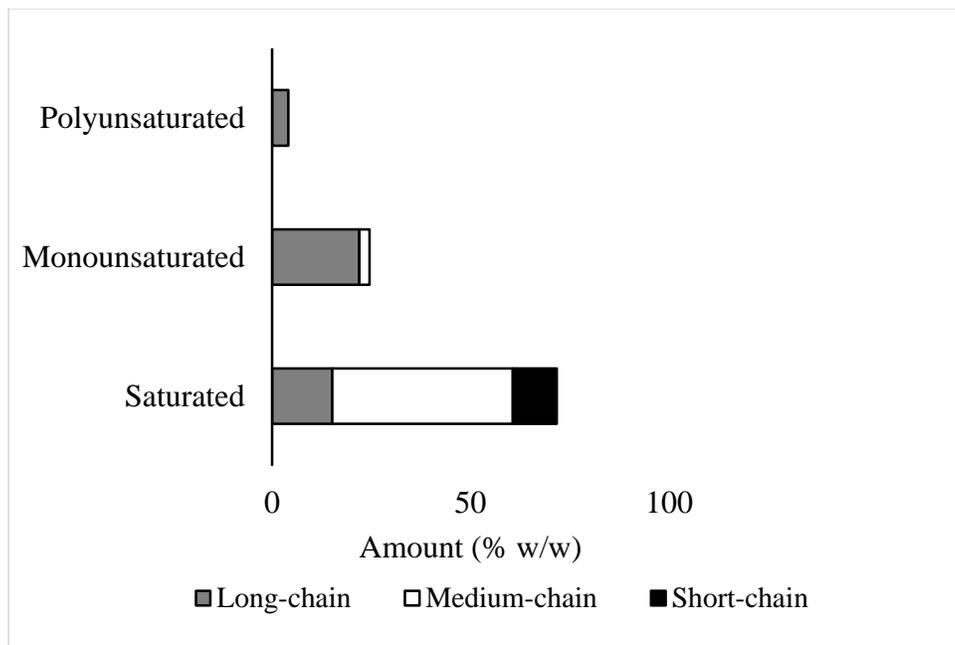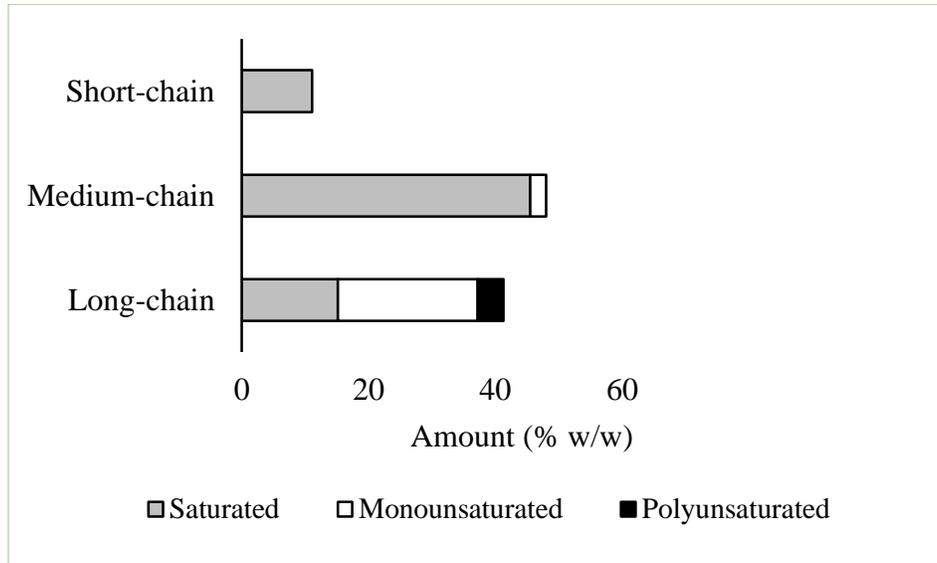
Figure 2.6. Milk fat synthesis and secretion (Adapted from Chilliard et al., 2000)

Abbreviation used: ACC- Acetyl-CoA carboxylase, CM-Chylomicron, DES= Delta-9 desaturase, FA-Fatty acids, FAS-Fatty acid synthase, Glut 1-Glucose transporter 1, LPL-Lipo protein lipase, MFG- Milk fat globule, TG-Triglycerides, VDL-Very low density lipoprotein
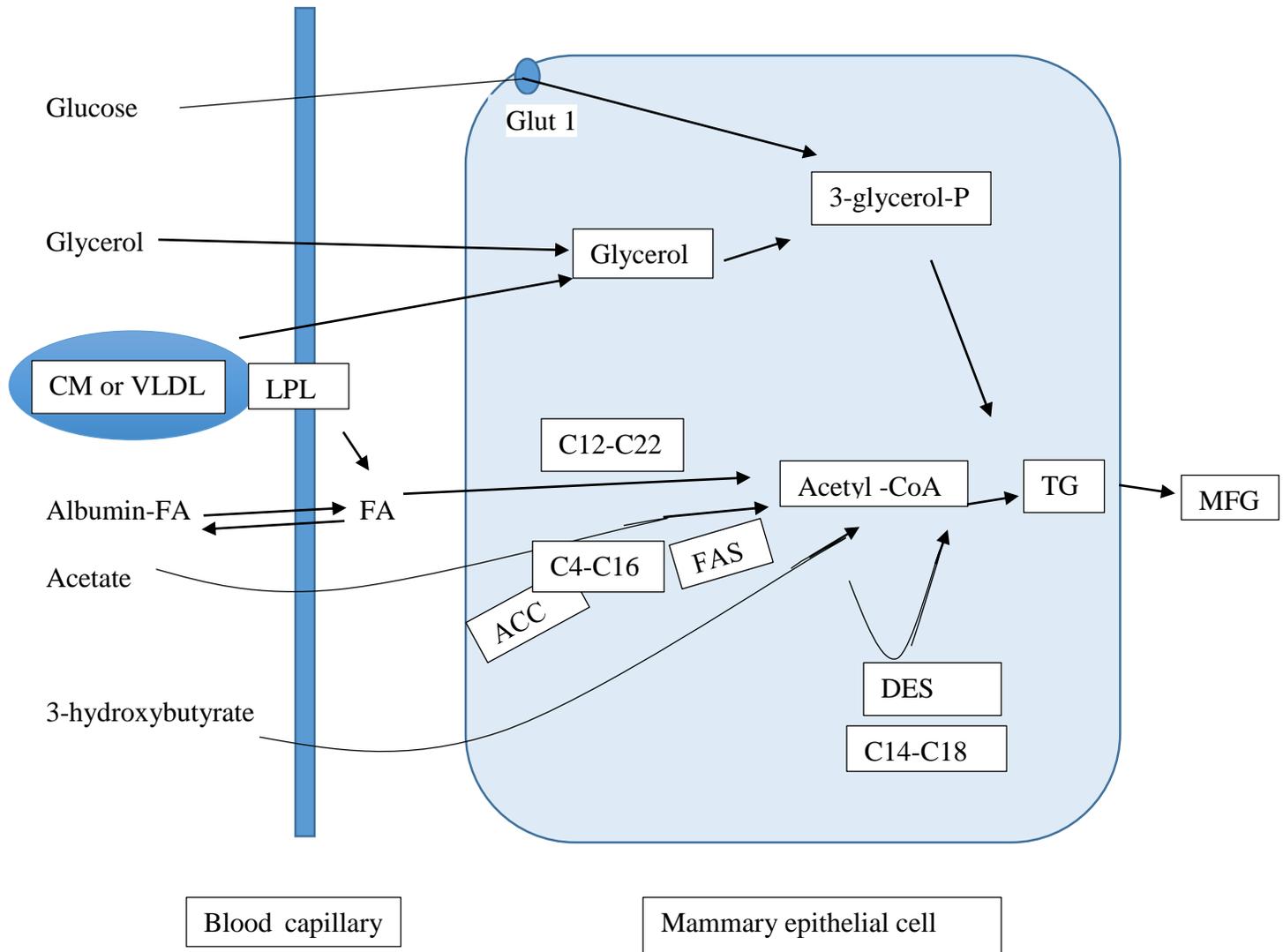
Figure 2.7 Synthesis of w-3 and w6 polyunsaturated fatty acids (adapted from Rustan and Drevon, 2005)

Omega-6 fatty acids	Enzyme	Omega-3 fatty acids
Linoleic 18:2 (LA)		α-Linoleic 18:3 (ALA)
↓	Δ^6 –destaurase	↓
ϒ-Linolei 18:3		Octadectatraenoic 18:4
↓	elongase	↓
Dihomo- ϒ-linnolenic 20:3		Eicosatetraenoic 20:4
↓	Δ^5 -destaurase	↓
Arachidonic 20:4		Eicosapentaenoic 20:5
↓	elongase	↓
Adrenic 22:4		Docosapentaenoic 22:5
↓	elongase	↓
Teracosatetraenoic 24:4		Teracosapentaenoic 24:5
↓	Δ^6 -destaurase	↓
Teracosateraenoic 24:5		Tetracosahexaenoic 24:6
↓	β-oxidation	↓
Dcosapentaenoic 22:5		Dcosahexaenoic 22:6

Figure 2.8 Synthesis of conjugated linoleic acid through rumaen biohydrogenation and Δ^9 desturase activity in tissues (adapted from Bauman et al., 2000)

Rumen

Tissues

Dietary fat e.g. Linoleic acid

C18:2 cis-9, cis12

C18:2 cis-9, trans-11 (CLA)

C18:1trans-11(Vaccenic acid)

C18:0 (Stearic acid)

C18:2 cis-9, cis-12

C18:2 cis-9, trans-11 (CLA)

Δ^9 desturase

C18:1 trans-11

C18:0   C18:1 cis-9

Δ^9 desturase
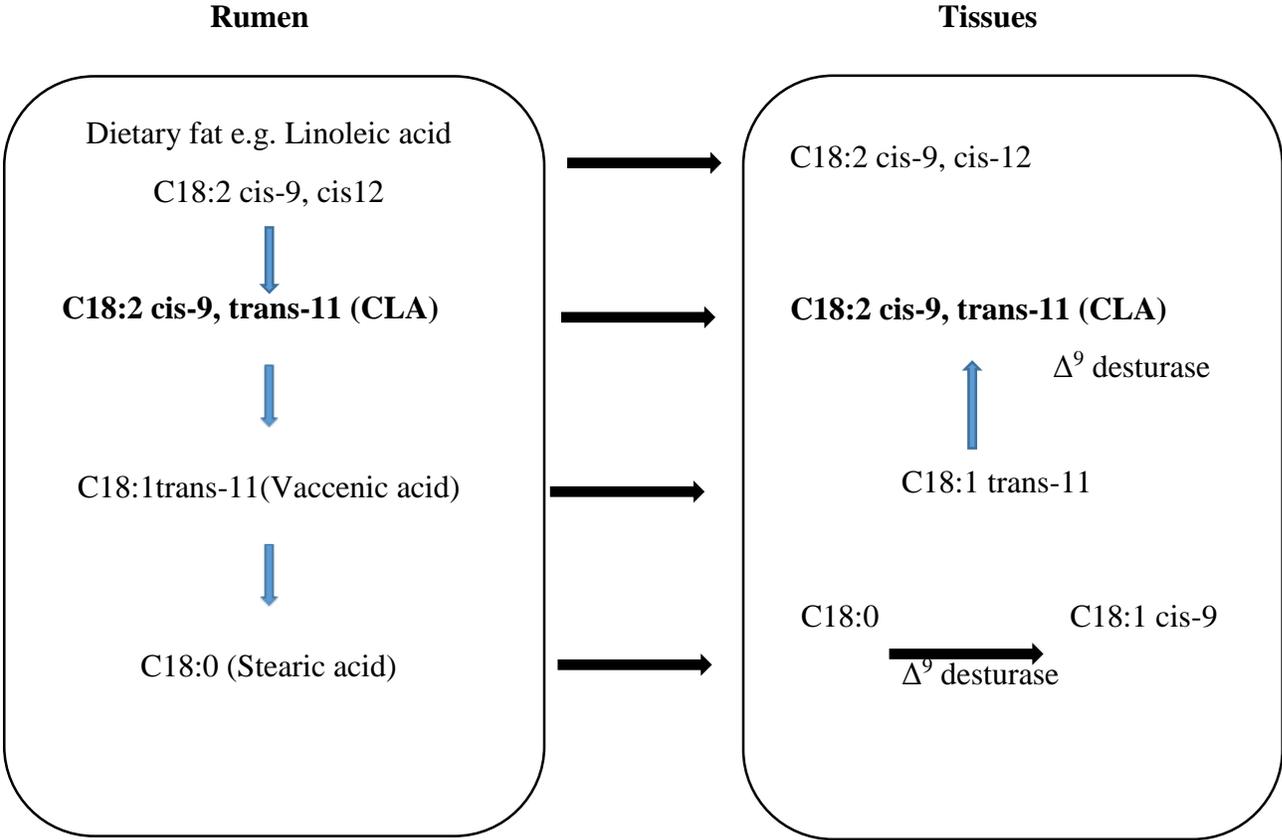
CHAPTER 3
GENETIC ANALYSIS OF GROUPS OF MID-INFRARED PREDICTED FATTY ACIDS IN MILK

3.1. Abstract

The objective of this study was to investigate genetic variability of MIR predicted fatty acids groups in Canadian Holstein cattle. Genetic parameters were estimated for five groups of fatty acids: short-chain (4 to 10 carbons), medium-chain (12 to 16 carbons), long-chain (17 to 22 carbons), saturated, and unsaturated fatty acids. The data set included 49,127 test-day records from 10,029 first lactation Holstein cows in 810 herds. The total number of animals in the pedigree was 76,074. The random regression animal test-day model included: days in milk, herd- test date, and age-season of calving (polynomial regression) as fixed effects, herd-year of calving, animal additive genetic effect, and permanent environment effects as random polynomial regressions, and random residual effect. The significance of the fixed effects and the best degree of the fixed Legendre polynomial regressions for age-season effect (3rd degree) were determined using AI-REML. Bayesian methods via Gibbs sampling were then used for fitting models with different degree of random regressions, assuming the best degree for fixed polynomial regression for age-season effect, and the same increasing degree for all random effects (from intercept only to the 4th degree). Fourth degree random regressions yielded the best fitting based on the Deviance Information Criterion (DIC). No polynomials with degree higher than 4 were fitted due to low number of cows with more than 5 fatty acid measurements and the cubic shape of the phenotypic distribution of the fatty acid groups. The estimate of average daily heritability over the lactation for the medium-chain fatty acid group (0.32) was higher than for the short-chain (0.24) and long-chain (0.23) fatty acid groups. The average daily heritability for the saturated fatty acid group (0.33) was greater than for the unsaturated fatty acid group (0.21). Estimated average daily genetic correlations were positive among all fatty acid groups and ranged from moderate to high (0.63-0.96). They illustrated similarities and differences in their origin and the makeup of the groupings based on chain length and saturation. These results provide evidence for the existence of genetic variation in MIR predicted fatty acids groups, and the possibility of improving milk fatty acid profile through genetic selection in Canadian dairy cattle.

Keywords: milk fatty acid, mid-infrared, random regression model, heritability

45

3.2 Introduction

Milk contains essential nutrients needed for young mammals as well as for humans. The main components of milk are water, lipids, proteins, carbohydrates, salts and vitamins (Fox et al., 2015). Milk fat contains approximately 400 different fatty acids (Jensen et al., 1991). Almost 70% of the fatty acids in milk are saturated, 25% are monounsaturated, 2.3% are poly-unsaturated, and 2.7% are *trans* fatty acids. (Månsson, 2008). Milk fat composition reflects the metabolism and environment of the cow, and affects the nutritional, technological and sensory quality, and economic value of milk and milk products (Bastin et al., 2011). In nutritional terms, while some fatty acids are beneficial to human health (CLA and omega fatty acids), some may have negative effects. Human intervention studies have shown that lauric (C12:0), myristic (C14:0) and palmitic acid (C16:0) significantly elevate the low-density lipoprotein cholesterol, whereas stearic acid (C18:0) had no effect on serum lipid (Denke and Grundy, 1992; Zock et al., 1994; Temme et al., 1996; Mensink et al., 2003). Despite well-established evidence, the association of saturated fatty acids with human health is still controversial. Increasing concern about health and diet has created a need for researchers to investigate ways to alter milk fat composition. In addition to food processing milk fat can be modified mainly through feeding and genetics (Palmquist et al., 1993; DePeters et al., 1995). Many studies have reported changes in milk fat composition by feeding different diets and supplementations (Grummer, 1991; Chilliard et al., 2001). However, only a small number of studies have estimated the genetic variability in the fatty acid profile of milk measured using gas chromatography (GC) (Renner and Kosmack, 1974a; Karijord et al., 1982; Stoop et al., 2008). There have also been a limited number of studies reporting genetic variation of milk fatty acids predicted using MIR spectral technology (Soyeurt et al., 2008b; Bastin et al., 2011; Gion et al., 2011; Lopez-Villalobos et al., 2014). The number of samples was generally limited when GC was used for the quantification of fat composition. Infrared prediction of fatty acid contents generates large datasets potentially resulting in increases in the accuracy of genetic parameters estimates (Soyeurt et al., 2006a). Moreover, MIR spectroscopy is less expensive and requires less labor.

Soyeurt et al. (2006a) first developed calibration equations for fatty acids using MIR spectrometry. Results indicated that the developed calibration equations for C10:0, C12:0, C14:0, C16:0, C16:1 *cis*-9, C18:1, and saturated and monounsaturated fatty acids in milk could be used

with high accuracy. Following these results, several studies have investigated the genetic variability of MIR predicted individual fatty acids, as well as groups of fatty acids (Soyeurt et al., 2007a; Soyeurt et al., 2007b; Soyeurt et al., 2008a; Soyeurt et al., 2008b; Gion et al., 2011; Bastin et al., 2011; Lopez-Villalobos et al., 2014). Generally, these studies revealed that *de novo* synthesized fatty acids are under more genetic control than preformed fatty acids (fatty acids synthesized from dietary lipids and body fat reserves). Additionally, saturated fatty acids are more heritable than unsaturated fatty acids. Soyeurt et al. (2007a) reported high genetic correlation among fatty acids of similar origin, and therefore, suggested developing a selection index by incorporating groups of fatty acids of similar origin rather than distinct individual fatty acids.

Milk MIR spectroscopy is the standard method for Canadian DHI laboratories to quantify fat and protein contents for payment and animal performance recording purposes. To take further advantage of this already existing practice and acquired spectral data, Fleming (2016) developed calibration equations to predict fatty acid composition from the MIR spectra of milk for the Canadian dairy industry. Investigating fatty acid profile in depth using the large number of MIR predicted fatty acid phenotypes would provide useful information to perform selection and genetic improvement of the milk fatty acid profile efficiently. Moreover, phenotypic information on the fatty acid profile could potentially be used for herd management, including feeding, health and reproduction.

The objectives of this thesis were to investigate the phenotypic and genetic variation of the following five groups of MIR predicted fatty acids: short-chain, medium-chain, long-chain, saturated, and unsaturated fatty acids.

3.3 Material and Methods
3.3.1 Data

Milk MIR spectra obtained during routine milk recording from Canwest DHI (Ontario, Canada) and Valacta (Quebec, Canada) laboratories were collected and stored in a database. Milk samples were analyzed by one of the two labs using a MilkoScan FT6000 spectrometer (Foss, Hillerød, Denmark). Milk spectral records were standardized across the two instruments and across time by Bonfatti et al. (2016). Fatty acid data used in this study were predicted by applying the

calibration equations developed by Fleming (2016). The development of the calibration models and model performances were previously described by Fleming (2016). Fatty acids were classified into five groups based on the length of the carbon chain and by saturation; short-chain (4 to 10 carbons), medium-chain (12 to 16 carbons), long-chain (17 to 22 carbons), saturated (no double bond) and unsaturated (one or more double bonds). Definitions of the milk fatty acid groups are given in Table 3.1.

The coefficient of determination of the cross-validation (R^2_{cv}) and ratio of performance deviation (RPD) of the used calibration equations (Fleming, 2016) are shown in Table 3.2. Not all fatty acids were predicted with the same accuracy from the MIR spectra. As shown in Table 3.2, the R^2_{cv} for the short-chain fatty acid group was 70.69%, which was lower than that for the other fatty acid groups. The lower predictability for the short-chain fatty acid group may have an effect on the estimation of genetic parameters. The predicted fatty acid values were expressed in grams per decilter (g/dL) of milk and log transformed in order to improve normality.

3.3.2 Data Editing

The initial data set of predicted fatty acids had 2,053,396 test-day records from 569,260 Holstein cows in 6,785 herds, collected from January 2013 to July 2015. Only test-day records between 5 and 305 days in milk (DIM) were considered for the genetic analysis (1,499,607 records from 488,047 cows in 6,759 herds). Not all test-day milk samples from a given cow have spectral data saved. Only a small proportion of the FOSS lines (2 out of 12) currently output milk spectral records, and which samples go through these lines is largely random. For this reason, only herds with more than 70 cows were chosen in order to have enough repeated test-day fatty acid records to effectively model the shape of lactation curve using a random regression model (1,100,810 records from 338,431 cows in 2,858 herds). Further, only first lactation Holstein cows with an age at calving between 19 and 43 months were considered for the analysis (390,068 records from 174,545 cows in 2,858 herds). Cows were required to have the first test-day within the first 50 DIM, and records were deleted for a given herd × test-day if fewer than 4 records were available. Moreover, cows were expected to have fatty acid records for at least 4 test-days. The last three criteria were applied in a loop sequence until a constant number of test-day fatty acid records were achieved. The final data set included 49,127 records from 10,029 Holstein cows in 810 herds.

Descriptive statistics of the data set after editing are shown in Table 3.2. An animal pedigree file was generated by tracing back the pedigrees of sires and dams as far as possible and contained a total of 76,074 animals.

3.3.3 Models

The following general random regression animal test-day model was used for all the analysis:

$$\mathbf{y} = \mathbf{X}\boldsymbol{\beta} + \mathbf{Z}_{hy}\mathbf{hy} + \mathbf{Z}_a\mathbf{a} + \mathbf{Z}_p\mathbf{p} + \mathbf{e}$$

Where:

$\mathbf{y}$ is a vector of observations (fatty acid content $\ln(g/dL$ of milk)) ;

$\boldsymbol{\beta}$ is a vector of systematic effects of herd-test date, days in milk (defined as single day classes) and fixed Legendre polynomial coefficients for age-season of calving (4 seasons: Jan-March, April-June, July-Sept, and Oct-Dec);

$\mathbf{hy}$ is a vector of random Legendre polynomial coefficients for herd-year of calving effect;

$\mathbf{a}$ is a vector of random Legendre polynomial coefficients for animal additive genetic effect;

$\mathbf{p}$ is a vector of random Legendre polynomial coefficients for permanent environmental effect;

$\mathbf{e}$ is a vector of random residuals.

$\mathbf{X}$, $\mathbf{Z}_{hy}$, $\mathbf{Z}_a$, and $\mathbf{Z}_p$ are the corresponding incidence matrices.

The expectations and (co)variance structure for the random effects, all assumed normally distributed, were:

$$E \begin{bmatrix} y \\ hy \\ p \\ a \\ e \end{bmatrix} = \begin{bmatrix} X\beta \\ 0 \\ 0 \\ 0 \\ 0 \end{bmatrix}$$

and

$$
\mathrm{V}
\begin{bmatrix}
hy \\
p \\
a \\
e
\end{bmatrix}
=
\begin{bmatrix}
H & O & O & O \\
O & P & O & O \\
O & O & G & O \\
O & O & O & E
\end{bmatrix}
$$

Where;

$$\mathbf{H} = \mathbf{I} \otimes \mathbf{H_0}, \quad \mathbf{P} = \mathbf{I} \otimes \mathbf{P_0}, \quad \mathbf{G} = \mathbf{A} \otimes \mathbf{G_0},$$

$\mathbf{I}$ is an identity matrix, $\mathbf{A}$ is the additive relationship matrix, and $\mathbf{H_0}$, $\mathbf{P_0}$, and $\mathbf{G_0}$ are (co)variance matrices for herd-year of calving, permanent environment, and additive genetic regression coefficients, respectively. $\mathbf{E}$ is a block-diagonal residual (co)variance matrix, assumed heterogeneous across 20 classes of 15 DIM and $\otimes$ is the Kronecker product.

3.3.4 Selection of Suitable Legendre Polynomial and Model Comparisons

Single-trait models were used to determine the significance of fixed effects and the best degree of the fixed Legendre polynomial regression for age-season of calving effect using ASReml (Gilmour et al., 2009). Bayesian methods via Gibbs sampling were then used to find best degree of the Legendre polynomial for the random regressions using single-trait models. Deviance Information Criterion (DIC) values for models with different degree of random regressions were estimated by keeping the best degree for fixed regression for age-season of calving constant. In addition, the same increasing degree for all three random regressions were assumed (Table 3.3). Five single-trait models were fitted using Bayesian methods via Gibbs sampling to estimate posterior means of covariance components, assuming heterogeneous residual variance in 20 DIM classes of equally spaced intervals of 15. The five single-trait models were as follows:

M_0- model with Legendre polynomial of degree 0 (intercept)
M_1- model with Legendre polynomial of degree 1 (2 coefficients)
M_2- model with Legendre polynomial of degree 2 (3 coefficients)
M_3- model with Legendre polynomial of degree 3 (4 coefficients)
M_4- model with Legendre polynomial of degree 4 (5 coefficients)

Regression curves were modeled using Legendre polynomial as in Jamrozik et al. (2002) and were as follows:

L0 = 1.0,

L1 = $3^{0.5}$x,

L2 = $5^{0.5}$($1.5x^2 - 0.5$),

L3 = $7^{0.5}$($2.5x^3 - 1.5x$),

L4 = $9^{0.5}$($35x^4 - 30x^2 + 3$)/8,

where; x = 2(t−5)/300 − 1, and x is a standardized (in interval from −1 to +1) time.

The best single-trait model for three random effects was selected based on the Deviance Information Criterion (**DIC**). The smaller the DIC value, the better the model fit (Spiegelhalter et al., 2002). Posterior means and standard deviations of (co)variance components were estimated using 70,000 samples after 30,000 burn-in iterations. Prior values were set to 0.01 for variances and 0 for co-variances.

3.3.5 Estimation of Genetic Parameters using Multiple Trait Model

A five-trait random regression animal test-day model was used to estimate the genetic parameters. The best degree of Legendre polynomial from the above univariate analysis for both fixed and random regressions were used for the multi-trait analysis. The Bayesian methods via Gibbs sampling were based on posterior means of parameters estimated using 270,000 samples after a burn-in of 30,000 samples assuming heterogeneous residual variance in 15 DIM intervals. Prior values were set to 0.01 for variances and 0 for co-variances. Flat prior distributions were assumed for all fixed effects. All random effects were assumed to have a non-zero covariance between traits.

Daily heritability was defined as:

$h^2 =$ _____ Additive genetic variance _____

{herd-year of calving + additive genetic+ permanent environmental+ residual} variances and was calculated for 5-305 days.

Average daily heritability was estimated by the sum of all the daily heritability divided by 300. Additive genetic correlations were calculated as:

51

$$r_g = \frac{Cov\ (t_1, t_2)}{\sqrt{v1 * v2}}$$

Where;

Cov is the additive genetic covariance between trait 1 and trait 2

$v1$ and $v2$ are the additive genetic variance for trait 1 and trait 2, respectively

Average correlation between traits was estimated by the sum of daily genetic correlation (5-305 DIM) divided by 300.

Average daily herd-year of calving, permanent environment, residual and phenotypic correlations were estimated using their corresponding variances and covariances.

3.4 Results and Discussion

3.4.1 Descriptive Statistics

On average, the proportions of short-chain, medium-chain, and long-chain fatty acid groups were 15%, 47.85% and 37.15%, respectively. These fatty acid percentages were comparable to those found by Bastin et al. (2011) who examined the same groupings (8.42%, 51.47% and 40.12% for short-chain, medium-chain, and long-chain fatty acids, respectively). The percentages of the various fatty acid groups might differ in other studies as a result of how the fatty acids groups were defined. The medium-chain fatty acid group represented the bulk of the milk fatty acids in the current study because included in this group are two major milk fatty acids, C14:0 and C16:0. Månsson (2008) reported C14:0 and C16:0 account for approximately 11% and 30% of total weight of fatty acids, respectively. Similarly, Stoop et al. (2008) observed a high percentage for the 14 to 16 carbon group of fatty acids (44.24% of total fat). In the current study, saturated and unsaturated fatty acid groups represented 64.15% and 35.85% of the total fatty acids, respectively. Due to the biohydrogenation of dietary unsaturated fatty acids that takes place in the rumen, the amount of unsaturated fatty acids absorbed is minimal. Thus, their incorporation into milk is lower than that for saturated fatty acids (Sauer et al., 1998). This is in agreement with other studies (Grummer, 1991; Jensen, 2002, Månsson, 2008).

Figure 3.1 shows the phenotypic variation of fatty acid groups across the lactation. Higher amounts of saturated fatty acids were observed across the entire lactation compared to unsaturated

fatty acids in the present study. This is in accordance with the overall lactation estimates reported by Grummer et al. (1991). Long-chain fatty acid contents followed the same trend as unsaturated fatty acids, as most of the long-chain fatty acids are unsaturated fatty acids. Likewise, short-chain and medium-chain fatty acids followed the similar trend as saturated fatty acids. This is because most of the fatty acids in the short-chain as well as medium-chain fatty acid groups, are saturated fatty acids (Table 3.1). Short-chain and medium-chain show similar trends as both groups of fatty acids are produced from *de novo* synthesis, while long-chain fatty acids are derived from blood lipids. However, C16:0 is produced from both *de novo* synthesis and blood lipids (Bauman and Griinari, 2003).

The effect of stage of lactation in milk fatty acid composition has been reported by many studies (Karijord et al., 1982; Kay et al., 2005; Garnsworthy et al., 2006; Soyeurt et al., 2008a; Mele et al., 2009; Bastin et al., 2011). In the current study, content of fatty acid groups were more variable during the initial part of lactation compared to mid and late lactation (Figure 3.1). In early lactation, long-chain fatty acid contents decreased more rapidly than short-chain and medium-chain fatty acids. In the same way, unsaturated fatty acids decreased more in early lactation than saturated fatty acids. This result is in accordance with Bastin et al. (2011), who found high variation for the monounsaturated fatty acid group as well as monounsaturated individual fatty acids (e.g., C18:1, C18:1cis and C18:1cis-9) compared to saturated fatty acids for four classes of DIM (1-20, 21-40,41-60, and 61-80) as a proportion of their concentration at class 81-100 DIM. They also observed greater variation in long-chain than medium-chain and short-chain fatty acid groups in early lactation. Soyeurt et al. (2008a) also observed a similar pattern of saturated and monounsaturated fatty acid content over the lactation. The distribution of fatty acid contents over the lactation could be explained by the energy status of cow.

An increased content of long-chain and unsaturated fatty acids observed at the very beginning of the lactation is likely due to the negative energy balance status of the cow. As mentioned by Garnsworthy et al (2006), the fatty acid profile of milk is the output of complex interactions between feed intake, diet composition, body fat mobilization, liver metabolism, rumen fermentation, mammary absorption, and synthesis of fatty acids. Thus, when cows are in negative energy balance during early lactation, feed intake is low, which leads to the mobilization of body

fat (Garnsworthy and Topps, 1982; Palmquist et al., 1993). In adipose tissue, the main fatty acids forming the stored triacylglycerides are C16:0, C18:0 and C18:1 cis -9. The mobilization of body fat causes direct incorporation of these fatty acids into the milk in early lactation (Christie, 1981; Chilliard et al., 2000). However, studies have reported higher contents of C18 family fatty acids than C16:0 in milk, during high lipolysis in early lactation (Barber et al., 1997; Bastin et al., 2011). Bauman and Griinari (2003) reported that when cows are in negative energy balance, fatty acids produced from body fat reserve mobilization increases in direct proportion to the energy state.

 As the lactation progresses, cows start to stabilize their energy balance and body fat mobilization decreases. Therefore, incorporation of these fatty acids into milk lowers and becomes constant. This results in a decrease in long-chain fatty acid contents after the initial stage of lactation. Lower quantities of *de novo* synthesized fatty acids (short-chain and medium-chain fatty acids) in the initial stage of lactation may be in part caused by a high content of long-chain fatty acid. The long-chain fatty acids inhibit the mammary lipogenic enzyme, acetyl-coenzyme A carboxylase, which catalyzes the synthesis of malonyl-CoA, a catabolic intermediate in fatty acid synthesis (Palmquist et al., 1993; Bastin et al., 2011). In mid and late lactation, fatty acid group contents become more constant as the cows attain a positive energy balance and the inhibitory effect of long-chain on *de novo* synthesized fatty acids is reversed back. Similarly, Garnsworthy et al. (2006) and Kgwatalala et al. (2009) found no differences in the fatty acid profile in mid and late lactation. Phenotypic variation of fatty acid groups may therefore be a useful indicator of the energy status of cows in early lactation.

3.4.2 Suitable Legendre Polynomials

Wald F-statistics showed 3rd degree Legendre polynomial (cubic) was significant for the fixed age-season of calving effect. The most suitable Legendre polynomial for the random effects was chosen based on the DIC values of the 5 alternate models that had the same degree of Legendre polynomial for age-season of calving (3rd degree), and included different degrees of Legendre polynomial (from 0 (intercept only) to 4 (quartic)) for the random effects. The DIC values decreased with increase of degree of Legendre polynomials (Table 3.3). Fourth degree random regressions yielded the best fit based on the lowest DIC value (Table 3.3). No polynomials with

degree higher than 4 were fitted due to the low number of cows with more than 5 fatty acid measurements and the cubic shape of the phenotypic distribution of the fatty acid groups.

3.4.3 Heritability

Average daily heritabilities for fatty acids from the multiple-trait analysis are shown in Table 3.4. Average daily heritabilities were 0.24 for short-chain, 0.32 for medium-chain, 0.23 for long-chain, 0.33 for saturated, and 0.21 for unsaturated fatty acids. These estimates were lower than the heritability estimates reported by Bastin et al. (2011) for short-chain, medium-chain and saturated, and unsaturated fatty acid groups (0.44, 0.43 and 0.43, respectively). But similar for long-chain (0.20) and unsaturated (0.22) fatty acids. The greatest difference was for short-chain fatty acids, where the estimate from Bastin et al. (2011) was much higher than the heritability estimated in the current study. The lower heritability estimated in the current study might be due to the lower accuracy of the calibration equation ($R^2cv = 70.69\%$) as reported by Fleming (2016). Previous studies have reported that heritability tends to decrease with an increase in carbon chain length of milk fatty acids (Renner and Kosmack, 1974a; Bastin et al., 2011). However, in the present study, the heritability estimated for the short-chain fatty acid group was low.

Previous studies have shown that, in general, fatty acids produced from *de novo* synthesis (short-chain and medium-chain) are under more genetic control than preformed fatty acids (long-chain) (Renner and Kosmack, 1974a; Karijord et al., 1982; Bastin et al., 2011). This was observed in the present study, where medium-chain fatty acids had a higher average daily heritability than long-chain fatty acids. However, the heritabilities estimated for short-chain and long-chain fatty acids were similar. Again, the lower than anticipated heritability for the short-chain fatty acid group may be due to the lower prediction accuracy. *De novo* synthesis involves metabolic enzymes, such as acetyl-coenzyme A carboxylase and fatty acid synthetase (Chilliard, 2000; Bauman and Griinari, 2003), which are under genetic control. Contrarily, preformed fatty acids are taken up by the mammary gland from circulating blood lipids. The circulating lipids in the blood are produced from two sources; lipoproteins and nonesterified fatty acids derived from the digestive tract during the absorption of lipids, and from the mobilization of body fat reserves (Bauman and Davis, 2013; Barber et al., 1997). This may be one of the reasons for the lower heritability estimates for preformed fatty acids. However, the heritability of 0.23 for long-chain

fatty acids indicates that the process of their incorporation into milk is partially under genetic control.

A higher heritability was found for the saturated fatty acid group than for the unsaturated fatty acid group. This is due to the fact that most of the *de novo* synthesized fatty acids are saturated because of the low activity of the delta-9 desaturase enzyme on fatty acids shorter than 18 carbons in length (Chilliard, 2000). However, a small proportion of C14:0 and C16:0 are desaturated to C14:1 and C16:1 (Chilliard, 2000). Similarly, most unsaturated fatty acids are preformed fatty acids derived from blood lipids.

Daily heritabilities for short-chain, medium-chain and long-chain and saturated and unsaturated fatty acid groups are shown in Figure 3.2. Posterior standard deviations for average and daily heritabilities ranged from 0.000650 to 0.001930 and 0.000076 to 0.000628, respectively. Heritabilities for all fatty acid groups were lower in the beginning of lactation until approximately 45 DIM. In mid lactation, heritabilities tended to increase, followed by a decrease for short-chain, medium-chain, and saturated fatty acids while long-chain and unsaturated fatty acid group slightly increased. Lower heritabilities in early lactation may be due to high residual variance estimates found in the beginning of lactation compared to mid and late lactation in this study (Figure 3.4). The variances across lactation of genetic, herd-year of calving and permanent environmental effects estimated in the current study are shown in the Appendix. Variation of heritabilities in early lactation may be due to negative energy balance, lipolysis of adipose tissue and inhibition of *de novo* synthesis by long-chain fatty acids (Soyeurt et al., 2008a). As the cows reach positive energy balance, heritability increased. Variation of heritability among fatty acid group in late lactation may be due to less number of test-day records in late lactation. A similar pattern was observed by Soyeurt et al. (2008a) for monounsaturated fatty acid group in milk. Even though, high heritabilities during mid lactation were reported by Bastin et al. (2011), the variation of daily heritabilities in early lactation was not clearly visible.

3.4.4 Correlations

Averaged daily genetic correlations among fatty acids were estimated from the multivariate analysis (Table 3.4). All genetic correlations were positive among fatty acid groups and ranged

56

from moderate to high (0.63-0.96). Posterior standard deviations for average daily genetic correlations ranged from 0.000000 to 0.001000. The estimated genetic correlations between the fatty acid groups illustrate similarities and differences in their origin and the makeup of the groupings based on chain length and saturation. A high genetic correlation of 0.86 was found between short-chain and medium-chain fatty acids, which indicates their similar origin. The lower genetic correlation estimated between long-chain fatty acids and short-chain (0.70) and medium-chain fatty acids (0.73), is due to their different origins. As expected, high genetic correlations were found for the saturated fatty acid group with short-chain and medium-chain fatty acid groups. Saturated fatty acids consist of nearly 80% short-chain and medium-chain fatty acids (Månsson, 2008). A genetic correlation of 0.86 was estimated between long-chain and unsaturated fatty acids, thus suggesting that the unsaturated fatty acids are mostly comprised of long-chain fatty acids. These estimated values were slightly different from estimates of Bastin et al. (2011), even though both studies used similar groupings of fatty acids. The estimated genetic correlations in the present study were higher than those reported by Bastin et al. (2011) except for genetic correlation between short-chain and medium chain, unsaturated and long-chain, short-chain and saturated, and medium-chain and saturated fatty acid groups. This might be due to differences in the number of samples, model, population, and units (log transformed) used. Also, Bastin et al. (2011) approximated daily genetic correlations among traits from correlations among daily breeding values, not using a multivariate model like the present study used. As presented by Calo et al. (1973), correlations between breeding values do not fully reflect the genetic relationships between traits and they might be underestimated. Figure 3.3 shows genetic correlations among the different fatty acid groups over the lactation. Most of the daily genetic correlations were low in early and late lactation while high during mid-lactation. Genetic correlations between saturated and unsaturated fatty acids over the lactation found in the present study (Figure 3.3d), were similar to those reported between saturated and monounsaturated fatty acids by Soyuert et al. (2008a). Bastin et al. (2011) described the genetic correlations over the lactation between different individual saturated fatty acids and C18:1 cis-9, and the genetic correlations were generally low in early lactation and constant during mid and late lactation. Correlations for effects of herd-year of calving and permanent environment are shown in Table 3.5. Residual correlations are shown in Table 3.6. Comparatively herd-year of calving correlations were the lowest, ranging from 0.15 to 0.48. Posterior standard deviations ranged from 0.001000 to 0.005000.

Positive and significant average daily phenotypic correlations were observed among five fatty acid groups in the current study ranging from 0.32 to 0.80 (Table 3.4). Similar to the other correlations, phenotypic correlations reflected the similarity in origin of fatty acids. Soyeurt et al. (2007a) found high phenotypic correlations among saturated fatty acid group with C12:0, C14:0, C16:0 and C18:0. Similarly, high phenotypic correlations were observed between monounsaturated fatty acid group with C18:1 and C18:2. However, Soyeurt et al. (2006b; 2007a) reported different phenotypic correlations between saturated and monounsaturated fatty acid groups (0.70 and 0.49, respectively). This may be due to different number of test-day records and units used in the studies. Bilal et al. (2014) reported a wide range (-0.01 to 0.83) of phenotypic correlations for individual fatty acids, which were measured using GC method. Soyeurt et al. (2008a) observed phenotypic correlations over the lactation between saturated and monounsaturated fatty acid groups. They were lower in initial lactation and increased during mid and late lactation.

3.4.5 Residual Variance

Residual variance over the lactation is shown in Figure 3.4. Except for short-chain fatty acid group, higher residual variances were observed for all other fatty acid groups in the early lactation. In mid and late lactation residual variances decreased gradually for medium-chain, long-chain, saturated and unsaturated fatty acid groups. The residual variance of short-chain remained constant over the mid and late lactation. This may be due the effect of lower predictability of short-chain using MIR spectra (Fleming, 2016). Heterogeneity and lower value of residual variance (0.00048 to 0.00556) was observed in Figure 3.4, thus justifying the choice of separating residual variances in 15 DIM intervals.

3.5 Implications

The main advantage of MIR spectroscopy is, it offers an opportunity to use a large number of records for genetic analysis, and thus, may provide more accuracy for genetic analyses. For efficient genetic improvement three factors are required: genetic variation, selection mechanism, and economic incentive (Gibson, 1991). This study found moderate heritabilities and moderate to high genetic correlations for groups of milk fatty acids. This provides evidence of genetic variation

in these groups and the possibility for modification of fatty acid composition through genetic selection. However, defining the ideal milk fat composition and developing an appropriate selection index with proper weightings for these groups of fatty acids still needs to be completed. The fatty acid proportion that is beneficial in human health may not be favorable for dairy food processing. For example, a higher proportion of unsaturated fatty acids and lower amounts of saturated fatty acids are preferred in order to reduce the cardiovascular diseases (Bauman and Lock, 2010; Micha and Mozaffarian, 2010). However, higher unsaturated fatty acid lowers the melting point of butter and impairs the whipping properties of butter, eventually making it liquid at room temperature. Also within dairy products, a fatty acid profile good for one product might be detrimental to another. Hence, the ideal fatty acid profile may need to be defined in multiple ways in order to cater to specific products or niche markets. Defining the milk fatty acid profile to tailor to each specific product may or may not be economical (Gibson, 1991). Thus, defining the correct milk fat composition is difficult and more research needs to be done in the future to address these aforementioned issues.

Furthermore, studies have reported negative genetic correlations among proportions of unsaturated fatty acids and fat and protein contents (Karijord et al., 1982; Soyeurt et al., 2007a; Stoop et al., 2008). Milk payment is positively associated with fat and protein contents, and therefore, increasing the amount of unsaturated fatty acids in milk could affect farmers negatively. This could lead to the development of new payment systems (Arnould and Soyeurt, 2009). Information on milk fatty acids could also be used for other purposes, such as feed and herd management, or as indicators for health and reproductive traits. However, this would require further research.

3.6 Conclusions

The present work provides evidence for phenotypic and genetic variation in the contents of fatty acids over lactation stages. The phenotypic variation reflects the association of the milk fatty acid profile with the energy status of the cow, suggesting that the fatty acid profile could be used as an indicator of the energy status of a cow. Moderate heritabilities were estimated for the five fatty acids groups. The observed differences in heritabilities showed that *de novo* synthesized fatty acids are more genetically regulated than the preformed fatty acids. Genetic correlations

among all fatty acid groups were positive and ranged from moderate to high. Estimated genetic correlations coincided with the biological pathways of fatty acid synthesis. Therefore, developing a selection index by incorporating groups of fatty acids from similar origins may be beneficial. This research provides evidence for the existence of genetic variation for milk fatty acid groups, thus changing milk fatty acid composition through genetics may be possible. Moreover, the work done presently is an initial step toward the potential future implementation of genetic evaluation for milk fatty acid contents in the Canadian dairy industry. However, additional research is required to establish how changing the milk fatty acid composition could affect the cow (production, reproductive, and health traits), the processing milk industry and milk for human consumption, and to define proper selection goals for milk fat composition.

3.7 Tables

Table 3.1. Definition of milk fatty acid groups.

Fatty acid group	Content
Short-chain	C4:0, C6:0, C8:0, C10:0
Medium-chain	C12:0, C13:0, C14:0, C14:1,C15:0, C16:0, C16:1
Long-chain	C17:0, C18:0, C17:1, C18:1n9t, C18:1n9c, C18:02n6t, C18:02n6c, C18:3 n3, C22:6n3,CLA
Saturated	C4:0, C6:0, C8:0, C10:0, C11:0, C12:0, C13:0, C14:0, C15:0, C16:0, C17:0, C18:0
Unsaturated	C14:1, C16:1, C17:1, C18:1n9t, C18:1n9c, C18:02n6t, C18:02n6c, C18:3 n3, C22:6n3,CLA

Table 3.2. The coefficient of determination of the cross-validation (R^2_{cv}), ratio of performance deviation (RPD) and descriptive statistics of analyzed data (N = 49,127)

Fatty acid group	R^2_{CV}[*]	RPD[*]	Mean	SD	Minimum	Maximum
Short-chain	70.69	1.85	0.356	0.057	0.054	0.635
Medium-chain	89.17	3.04	1.136	0.127	0.505	1.80
Long-chain	80.17	2.25	0.882	0.124	0.397	1.828
Saturated	93.37	3.88	1.349	0.125	0.644	1.989
Unsaturated	82.11	2.36	0.754	0.108	0.319	1.629

*From Fleming (2016)

Table 3.3 Deviance Information Criterion (DIC) values for models with different degree of Legendre polynomial

Degree	Short-chain	Medium-chain	Long-chain	Saturated	unsaturated
0	-183684	-117679	-116279	-112570	-128700
1	-188726	-122920	-119040	-117232	-131850
2	-192046	-126512	-121401	-120678	-134615
3	-195563	-130590	-124171	-124418	-137591
4	-199075	-134939	-127481	-128708	-141296

Table 3.4. Averaged daily heritability (on diagonal), averaged daily genetic correlation (above diagonal) and average daily phenotypic correlation (below diagonal) for fatty acid groups in first lactation Holstein cows

Fatty acid	Short-chain	Medium-chain	Long-chain	Saturated	Unsaturated
Short-chain	0.24	0.86	0.70	0.89	0.63
Medium- chain	0.58	0.32	0.73	0.96	0.68
Long-chain	0.38	0.45	0.23	0.77	0.86
Saturated	0.62	0.80	0.52	0.33	0.70
Unsaturated	0.32	0.42	0.64	0.45	0.21

Table 3.5. Average daily permanent environmental correlations (above the diagonal) and average daily herd-year of calving effect correlations (below the diagonal) for fatty acid groups in first lactation Holstein cows

Fatty acid	Short- chain	Medium- chain	Long- chain	Saturated	Unsaturated
Short-chain	1	0.81	0.60	0.85	0.51
Medium-chain	0.24	1	0.63	0.95	0.58
Long-chain	0.10	0.15	1	0.69	0.83
Saturated	0.28	0.48	0.22	1	0.60
Unsaturated	0.07	0.14	0.34	0.16	1

Table 3.6. Average daily residual correlations (above the diagonal) for fatty acid groups in first lactation Holstein cows

Fatty acid	Medium- chain	Long-chain	Saturated	Unsaturated
Short-chain	0.67	0.40	0.69	0.36
Medium-chain		0.43	0.84	0.43
Long-chain			0.56	0.83
Saturated				0.52

3.8 Figures

Figure 3.1. Content of short-chain, medium-chain, long-chain, saturated and unsaturated fatty acid groups across lactation of first lactation Holstein cows
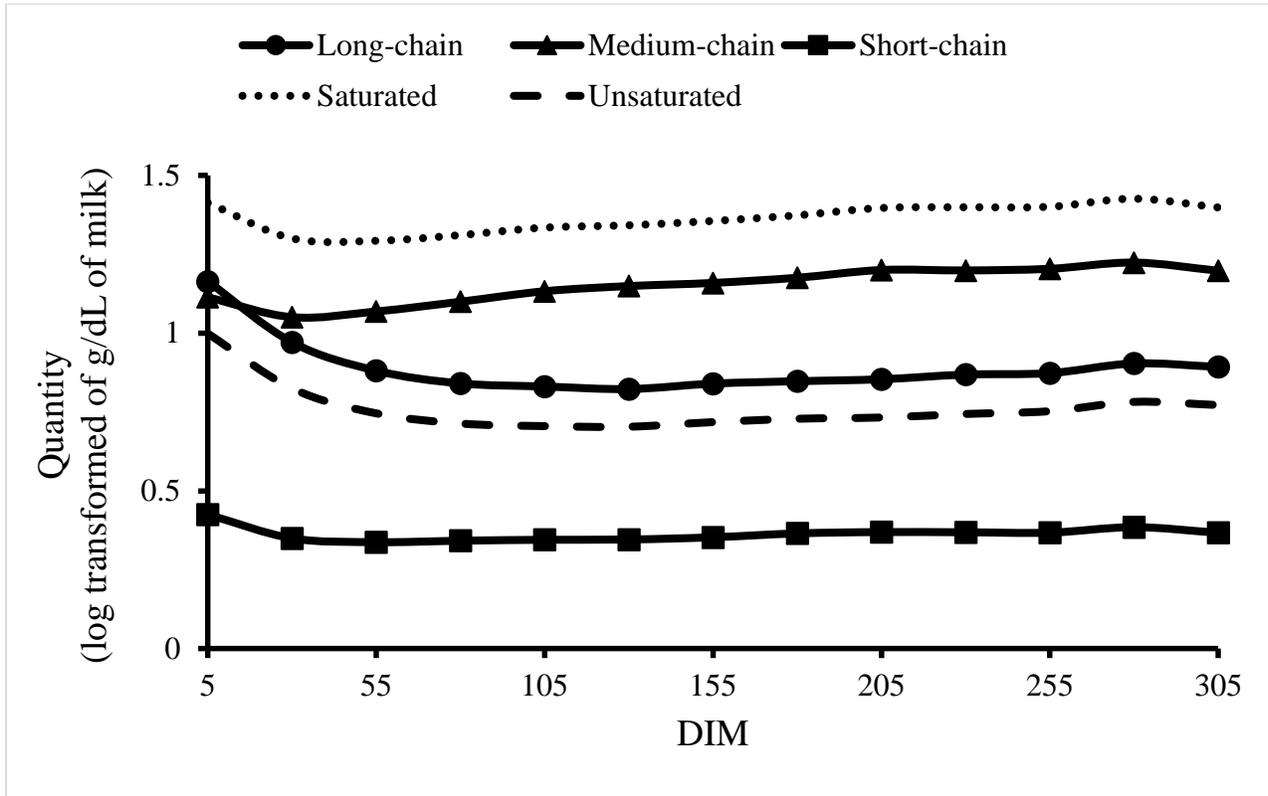
Figure 3.2. Daily heritabilities of short-chain, medium-chain, long-chain, saturated and unsaturated fatty acid groups in first lactation Holstein cows
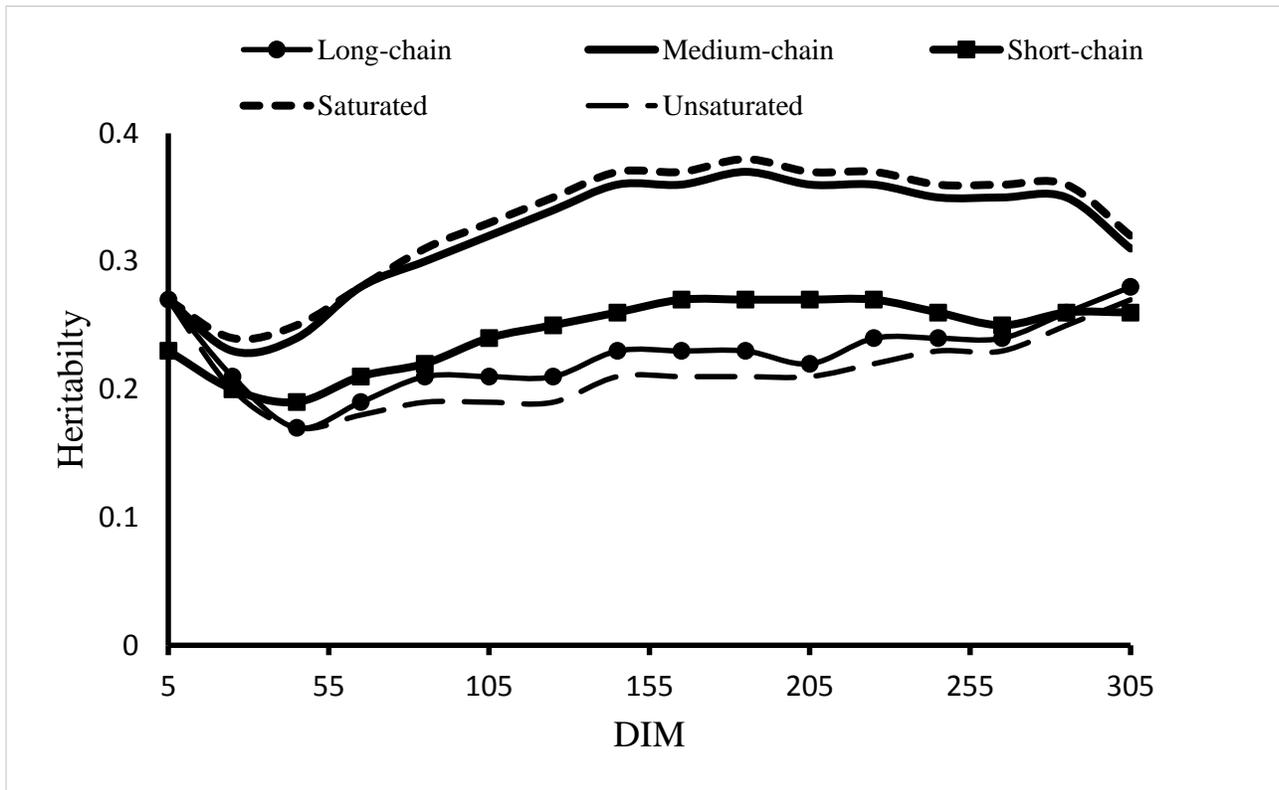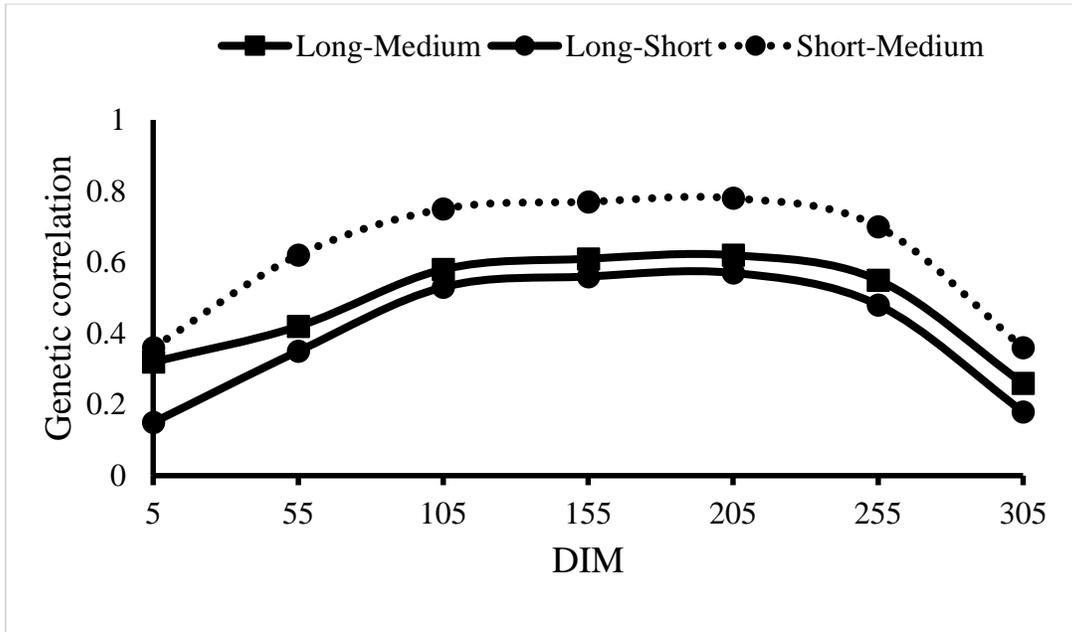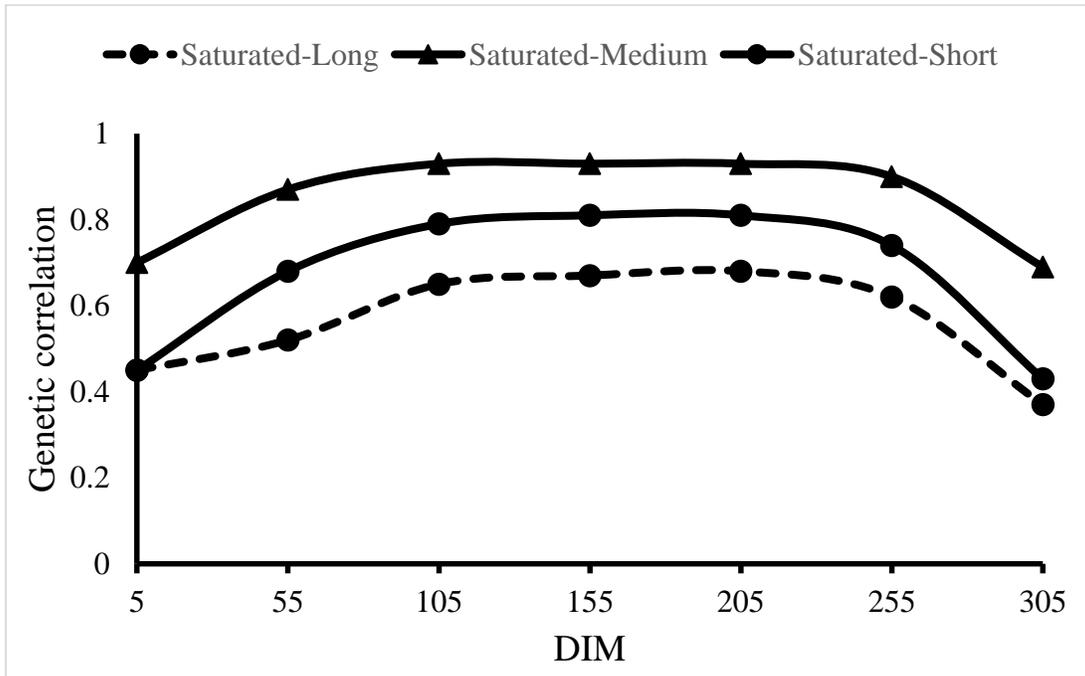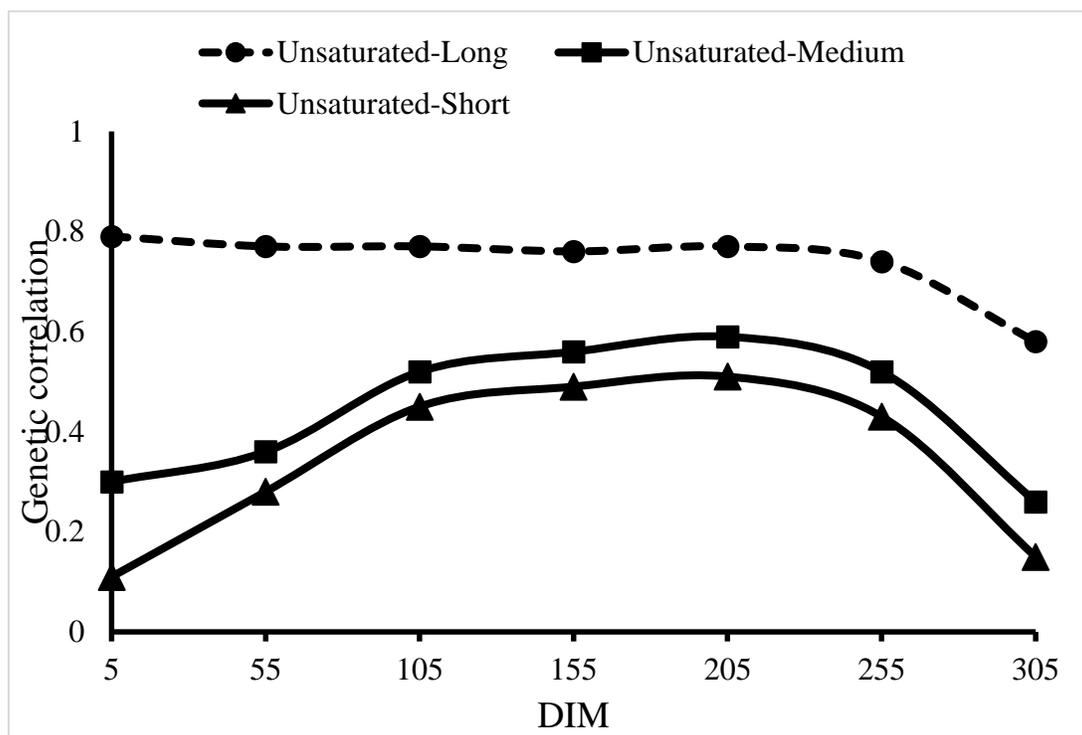
Figure 3.3. Daily genetic correlations among; short-chain, medium-chain, long-chain fatty acid groups (a), saturated fatty acid with short-chain, medium-chain, long-chain fatty acid groups (b), unsaturated fatty acid with short-chain, medium-chain, long-chain fatty acid groups (c) and saturated and unsaturated fatty acid groups (d) in first lactation Holstein cows
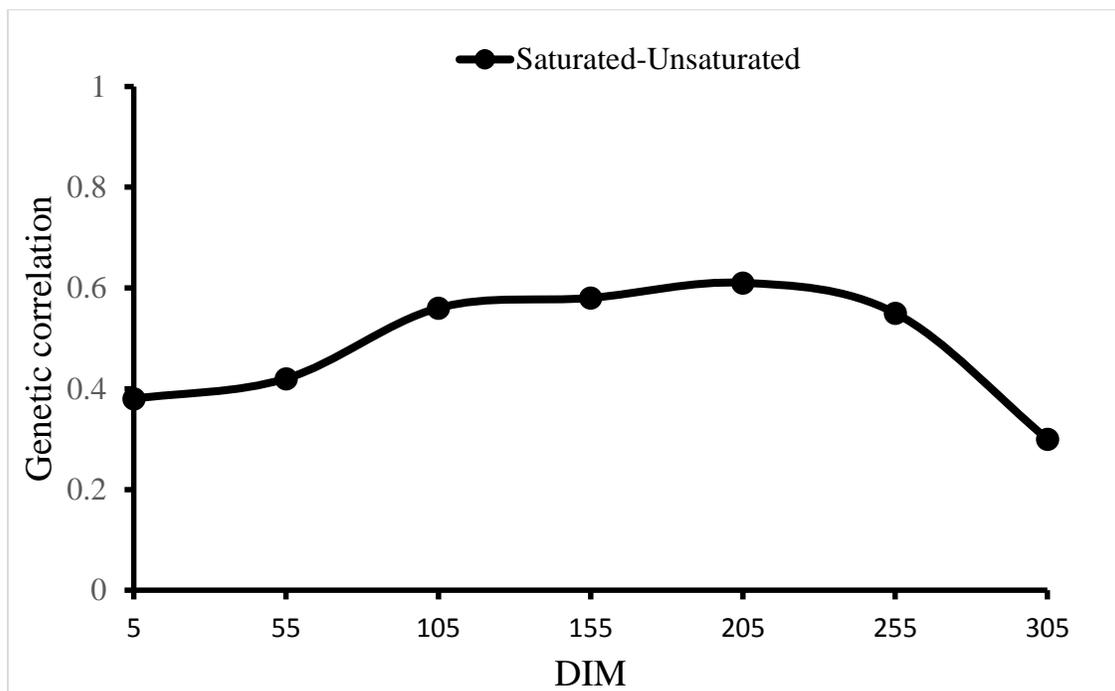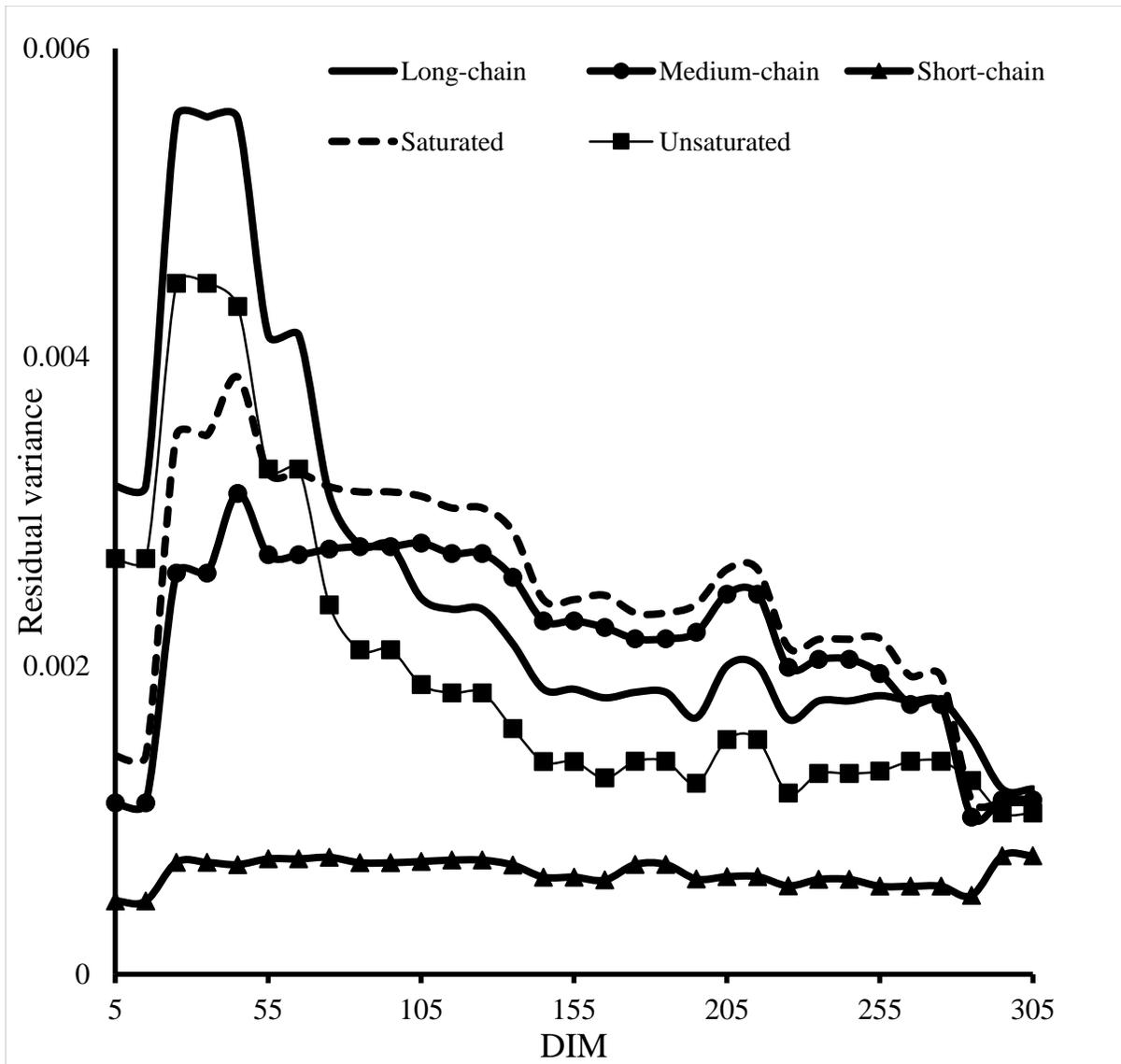
(a)

(b)

(c)

(d)

Figure 3.4. Residual variances over the lactation for long-chain, medium-chain, short-chain, saturated, and unsaturated fatty acid groups in first lactation Holstein cows

3.9. Appendix

Figure A1. Genetic variances over the lactation for long-chain, medium-chain, short-chain, saturated and unsaturated fatty acid groups in first lactation Holstein cows
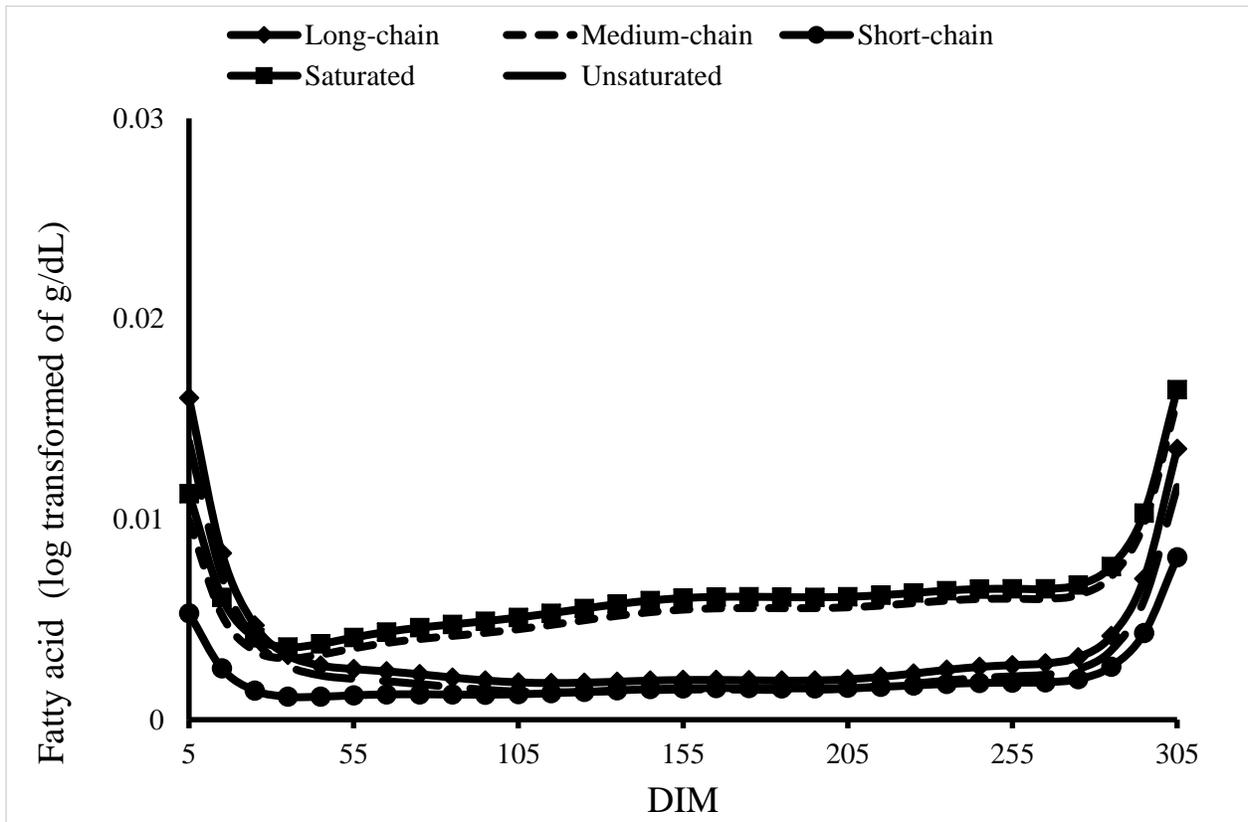
Figure A2. Herd-year of calving variances over the lactation for long-chain, medium-chain, short-chain, saturated, and unsaturated fatty acid groups in first lactation Holstein cows
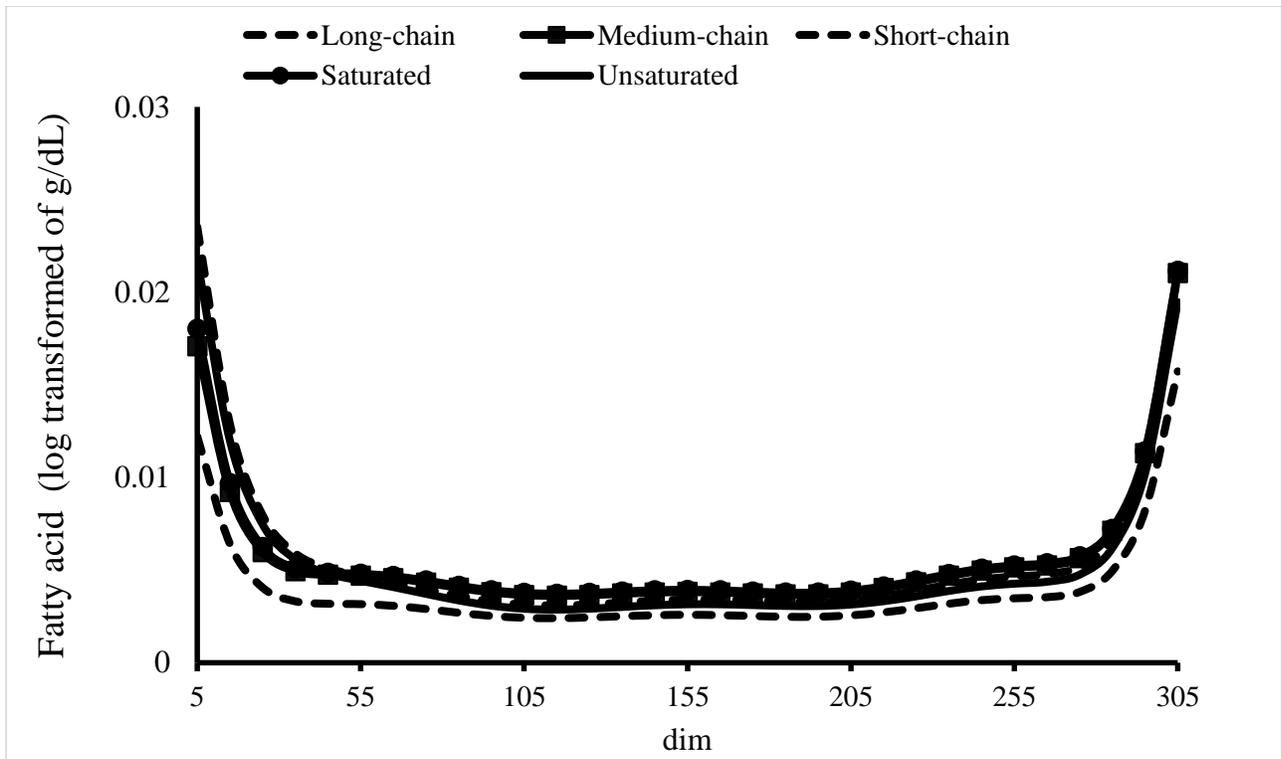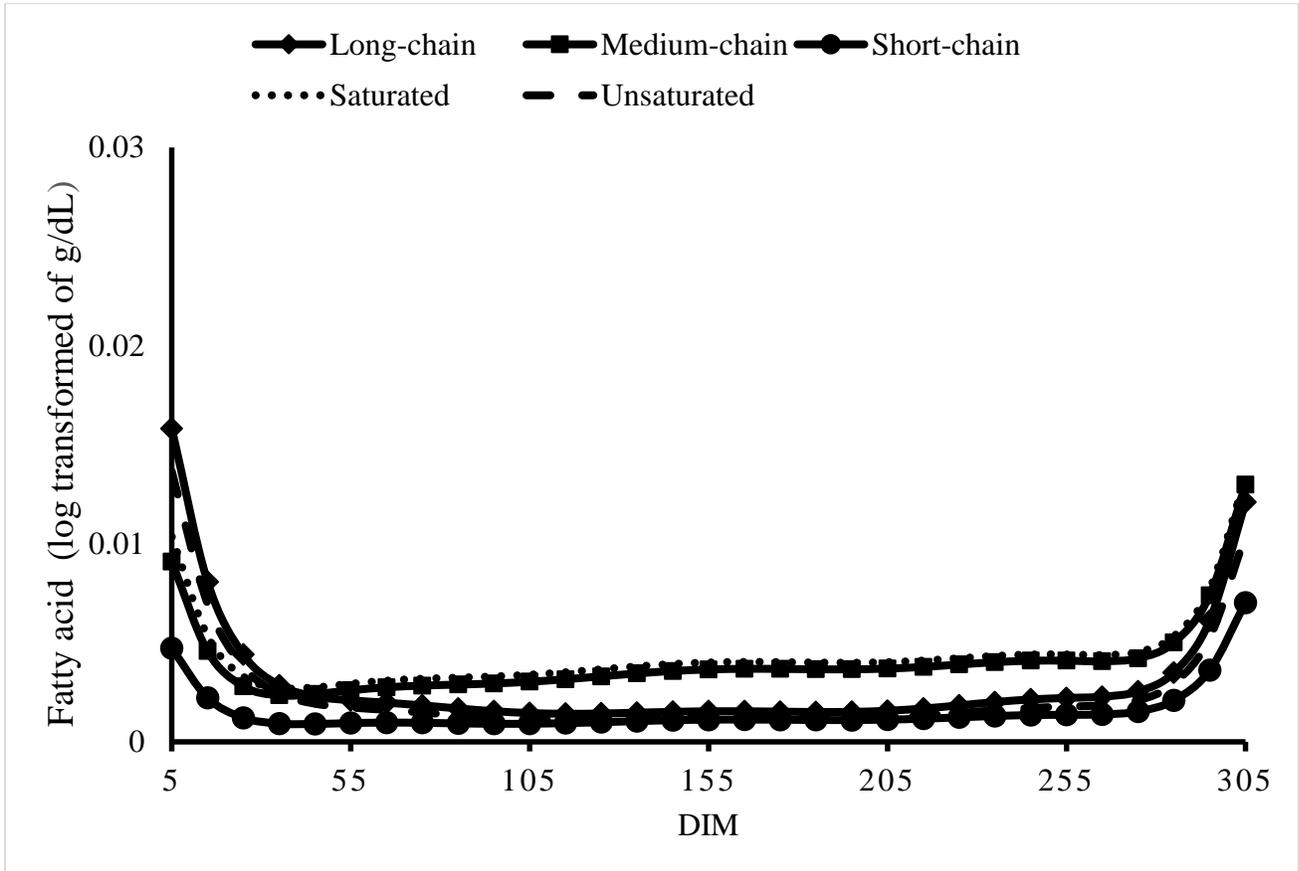
Figure A3. Permanent environmental variances over the lactation for long-chain, medium-chain, short-chain, saturated and unsaturated fatty acids in in first lactation Holstein cows

CHAPTER 4

GENERAL DISCUSSION AND CONCLUSIONS

4.0. General Discussion

The present study explored the genetic variation of five MIR predicted fatty acid groups in milk of Canadian Holstein cattle. Investigating genetic variability of milk fatty acid composition is essential in order to modify milk fatty acid composition in a favorable way through genetic selection. Basic genetic parameters such as heritabilities and genetic correlations are essential to select cows with favorable genetic merit for milk fatty acid composition. In accordance with other studies, the current study found higher heritability for *de novo* synthesized fatty acids (short-chain and medium-chain fatty acids) than the blood derived fatty acids (long-chain). As discussed in chapter 3, the small difference between short-chain and long-chain was likely due to the low prediction accuracy of the short-chain fatty acid group. Moreover, the present study also estimated higher heritability for saturated fatty acids than unsaturated fatty acids. In terms of genetic correlations, this study again confirmed that fatty acids from similar origin are more correlated to each other than the fatty acids synthesized from different sources. Thus, the present study provides evidence for presence of genetic variability among fatty acids, which is the initial step in improving milk fatty acid profile genetically.

In the current study a multiple trait random regression model was used to estimate the genetic variance components, which was similar to the Canadian test-day model. Random regression coefficients allow the shape of lactation curve to differ for each animal. A random regression model gives two sets of regressions on days in milk for an individual cow, i.e. fixed and random regressions. The general shape of lactation curve for cows belonging to same sub classes (e.g. age and season of calving in this thesis) is given by fixed regressions. On the other hand, random regressions give each cow to have different shape of lactation curve due to additive genetic, herd-year of calving and permanent environment effects, which describes the deviation from the fixed regressions (Jamrozik et al., 1997). Thus, a random regression model enables to model the shape of lactation with a restricted number of parameters (Pool et al., 2000). In the present study, random regression for each herd-year of calving subclass was considered to account

75

for management effects on the shape of lactation curves of cows within the herd (Jamrozik et al., 1997).

Orthogonal Legendre polynomials were used for analyzing patterns of genetic variation. Orthogonal Legendre polynomials are more suitable as higher orders are estimable due to better convergence (Pool et al., 2000). Furthermore, orthogonal Legendre polynomials reduce the correlations among estimated coefficients (Schaeffer, 2004). The order of Legendre polynomials is important as the genetic parameters tend to differ with the order (Misztal et al., 2000). In the current study different order of Legendre polynomials for fixed and random effects were investigated. No polynomials with degree higher than 4 were fitted due to the low number of cows with more than 5 fatty acid test day measurements and the cubic shape of the phenotypic distribution of the fatty acid groups.

This is the first study in Canada using MIR predicted fatty acid groups to explore the genetic variation. The studies done by Bilal et al. (2012, 2014) investigated the genetic variation of milk fatty acids in Canadian Holsteins using gas chromatography to measure the fatty acids. The gas chromatography method is expensive, more time consuming and requires skilled staff. Thus, previous studies used limited number of samples on limited number of cows. As an alternative method Soyeurt et al. (2006a) introduced the calibration equation for predicting milk fatty acid using MIR technology. The use of infrared predictions generates large amount of data, thus may yield to better genetic parameters estimates. Moreover, MIR spectra technology is routinely used in Canadian DHI laboratories to measure milk fat and protein. Therefore, utilizing existing MIR spectra is less expensive and requires less labor.

However, some limitations exist in the current study. One of the main limitations of this study is that not all test-day milk samples from a given cow have spectral data saved. Only a small proportion of the FOSS lines currently output milk spectral records in Canada, and which samples go through these lines is largely random. Therefore, only herds with more than 70 cows were chosen to have enough repeated test-day spectral records in order to model the shape of lactation curve using a random regression model. The other constraint is the prediction accuracy of fatty acid groups. Fitting statistics of calibration equations were shown in Table 3.2 of chapter 3. Short-chain fatty acids had the lowest R^2cv and RPD. An RPD measure greater than 2 is desired for good

calibration equation (Karoui et al., 2006). Moreover, some of the test-day records had missing information, which were deleted during the data edits.

4.1 Selection Scope, Implications and Future Studies

Desired direction of selection for milk fatty acid profile is controversial. Because the proportion of preferred fatty acids in terms of human health perspective may not be suitable for industrial standpoint (Gibson, 1991). Generally, milk contains 70% saturated fatty acids, 25% mono unsaturated fatty acids and 5% poly unsaturated fatty acids. For human health, Pascal (1996) estimated that favorable milk lipid should contain 30% of saturated fatty acids and Hayes and Khosla (1992) found that it should have 60% monounsaturated fatty acids and 10% polyunsaturated fatty acids. Thus current milk fat composition is far from optimal. A high amount of unsaturated fatty acid is preferred because of its positive association on human health. It decreases cholesterol level, coronary heart diseases, diabetes, cancer, atherosclerotic and obesity (Williams, 2000; Kritchevsky et al., 2004; McLeod et al., 2004; Bhattacharya et al., 2006; Hsu et al., 2010). Increased proportion of unsaturated /long-chain fatty acid reduces the melting point of fat and improves the spreadability of butter. However, a very high proportion of unsaturated fatty acids lower the melting point of butter more and impairs the whipping properties of butter and eventually making it liquid at the room temperature (Gibson, 1991). Similarly, a very high amount of unsaturated fatty acids reduce the shelf-life of other dairy products such as cheese by increasing probability of oxidation (Chilliard et al., 2001).

With regard to saturated fatty acids their association with cardiovascular disease is still questionable. A pillar of international dietary recommendations to reduce the risk of cardiovascular disease is the reduction of saturated fatty acid consumption (Bauman and Lock, 2010). However, despite well-established evidence, recent studies have shown that compared to carbohydrates, saturated fatty acids increased total cholesterol, low density lipoprotein-cholesterol, but also they increased high density lipoprotein-cholesterol, which resulted in no significant effects on total cholesterol and high density lipoprotein-cholesterol ratio (Mensink et al., 2003; Micha and Mozaffarian, 2010). Concerning individual saturated fatty acids; lauric (C12:0), myristic (C14:0) and palmitic acid (C16:0) significantly elevate the low density lipoprotein cholesterol, whereas stearic acid (C18:0) has no effect on it (Denke and Grundy, 1992; Zock et al., 1994; Temme et al.,

1996; Mensink et al., 2003). Thus, defining a general milk fat composition is difficult as it will not work for all at all times. Therefore, milk fat composition needs to be designed to cater different needs: human health and industry. However, defining milk fat composition to cater to specific needs to be economical is doubtful. Therefore urgent research is needed to address these issues. However, Stoop et al. (2009a) stated that in Netherlands low fat %, C14:0 and C16:0 and higher proportion of unsaturated fatty acids are preferred. Moreover, commercially the Dutch Campina milk is produced with 20% more unsaturated fatty acids (Stoop et al., 2009a).

Genomic information of fatty acid composition would provide insight into the multifaceted networks of genes underlying variation in fatty acid synthesis. To our knowledge there has been no studies concerning the genomic information on MIR predicted fatty acids in Canadian Holstein milk. Therefore, additional research is required to study this potential application. Also possible interaction between genetic and other factors such as management system (organic versus conventional) and nutritional factors need to be studied in order to implement selection program for fatty acids. Furthermore, consequences of changing milk fat composition need to be considered, especially on other production, reproductive and health traits of cow. It would be also interesting to study milk fatty acid profile of other cattle breeds and different species using MIR technology. However, phenotypic and genetic information on fatty acid composition could be used for other purposes such as feed and herd management and indicator for health and reproductive traits. But this would require further research with related traits.

4.2. General Conclusions

The present study provides evidence for the presence of genetic variation in milk fatty acid composition of Canadian Holstein cattle. The estimated heritabilities were moderate and higher values were observed for *de novo* synthesized fatty acids. Estimated average daily genetic correlations were positive among all fatty acid groups and ranged from moderate to high and reflected similarity in origin of fatty acids. Moreover, the phenotypic variation reflects the association of fatty acid profile with the energy status of cow, thus suggesting fatty acid profile could be used as an indicator of energy status of a cow.

REFERENCES

Agriculture and Agri-Food Canada (AAFC). 2015. Canadian Dairy Genetics. Accessed Dec. 20, 2015.http://www5.agr.gc.ca/resources/prod/Internet-Internet/MISB-DGSIM/CB-MC/PDF/4663-eng.pdf

Arnould, V. R., and H. Soyeurt. 2009. Genetic variability of milk fatty acids. J. Appl. Sci. 50:29-39.

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

Barłowska, J. 2009. Nutritional and pro-health properties of milk fat. Medycyna Wet. 65:171-174.

Bastin, C., N. Gengler, and H. Soyeurt. 2011. Phenotypic and genetic variability of production traits and milk fatty acid contents across days in milk for Walloon Holstein first-parity cows. J. Dairy Sci. 94:4152–4163.

Bauman, D. E., L. H. Baumgard, B. A. Corl, and D. J. Griinari. 2000. Biosynthesis of conjugated linoleic acid in ruminants. J. Anim. Sci.77:1-15.

Bauman, D. E., and C. L. Davis. 2013. Biosynthesis of milk fat. Lactation: a comprehensive treatise2: 31-75.

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

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

Bauman, D. E., and A. L. Lock. 2010. Milk fatty acid composition: Challenges and opportunities related to human health. Proc. 26th World Buiatrics Congr. 278–289.

Beaulieu, A. D., and D. L. Palmquist.1995. Differential effects of high fat diets on fatty acid composition in milk of Jersey and Holstein cows. J. Dairy Sci. 78:1336-1344.

Bernard. L., C. Leroux, and Y. Chilliard. 2013. Expression and nutritional regulation of stearoyl-CoA desaturase genes in the ruminant mammary gland: Relationship with milk fatty acid composition J.M. Ntambi (Ed.), Stearoyl-CoA Desaturase Genes in Lipid Metabolism, Springer Science + Business Media, New York, NY.161–193.

Bhattacharya, A., J. Banu, M. Rahman, J. Causey, and G. Fernandes. 2006. Biological effects of conjugated linoleic acids in health and disease. J. Nutr. biochem, 17: 789-810.

Bickerstaffe, R.., E. F. Annison and J. L. Linzell. 1974. The metabolism of glucose, acetate, lipids and amino acids in lactating dairy cows. J. Agric. Sci.82: 71-85.

Bilal, G., R. I. Cue, A. F. Mustafa, and J. F. Hayes. 2012. Short communication: Estimates of heritabilities and genetic correlations among milk fatty acid unsaturation indices in Canadian Holsteins. J. Dairy Sci. 95:7367-7371.

Bilal, G., R. I. Cue, A. F. Mustafa, and J. F. Hayes. 2014. Short communication: Genetic parameters of individual fatty acids in milk of Canadian Holsteins. J. Dairy Sci. 97:1150–1156.

Bitman, I., and D. L. Wood. 1990. Changes in milk: fat phospholipids during lactation. J. Dairy Sci. 73: 1208.

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

Bobe, G., G. L. Lindberg, A. E. Freeman, and D.C. Beitz. 2007. Short communication: Composition of milk protein and milk fatty acids is stable for cows differing in genetic merit for milk production. J. Dairy Sci.90:3955–3960.

Bonfatti, V., A. Fleming, A. Koeck, and F. Miglior. 2016. Standardization of milk infrared spectra for the retroactive application of calibration models. J. Dairy Sci. (submitted).

Brouwer, I. A., A. J. Wanders, and M. B. Katan. 2013. Trans fatty acids and cardiovascular health: research completed and quest. Eur. J. Clin. Nutr .67:541-547.

Calo, L. L., R. E. McDowell, L. D. VanVleck, and P. D. Miller. 1973. Genetic aspects of beef production among Holstein-Friesians pedigree selected for milk production. J. Anim. Sci. 37:676–682.

Canadian Dairy Commission (CDC). 2016. The industry. Accessed Jun. 23, 2016. http://www.cdc-ccl.gc.ca/CDC/index-eng.php?id=3796.

Canadian Dairy Information Center (CDIC).2016. Canada's dairy industry at glance. Accessed Jun. 23, 2016. http://www.dairyinfo.gc.ca/index_e.php?s1=cdi-ilc&s2=aag-ail

Chajès, V., A. C. Thiébaut, M. Rotival, E.Gauthier, V. Maillard, M. C. Boutron-Ruault, V. Joulin, G. M. Lenoir and F. Clavel-Chapelon. 2008. Association between serum trans-monounsaturated fatty acids and breast cancer risk in the E3N-EPIC Study. Am. J. epidemiol. 167: 1312-1320.

Chavarro, J. E., L. Mínguez-Alarcón, J. Mendiola, A. Cutillas-Tolín, J. J. López-Espín, and A. M. Torres-Cantero. 2014. Trans fatty acid intake is inversely related to total sperm count in young healthy men. Hum. Reprod. 29:429-440.

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

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

Chilliard, Y., C. Martin, J. Rouel, and M. Doreau. 2009. Milk fatty acids in dairy cows fed whole crude linseed, extruded linseed, or linseed oil, and their relationship with methane output. J. Dairy Sci. 92:5199–5211.

Chow, C. K. 2007. Fatty acids in foods and their health implications. 3rd ed .CRC Press.

Christie, W. W. 1981. The composition, structure and function of lipids in the tissues of ruminant animals. Pages 95–191 in Lipid Metabolism in Ruminant Animals. W. W. Christie, ed. Pergamon Press, Oxford, UK.

Dairy farmers of Canada. 2015.The economic impacts of the Canadian dairy industry in 2013.Final report.

de Oliveira Otto, M. C., D. Mozaffarian, D. Kromhout, A. G. Bertoni, C. T. Sibley, D. R.Jacobs, and J. A. Nettleton. 2012. Dietary intake of saturated fat by food source and incident cardiovascular disease: the Multi-Ethnic Study of Atherosclerosis. Am. J. Clin. Nutr.96: 397-404.

Debry, G. 2001. Milk, nutrition and health. Edition TEC and DAC. France: Lavoisier, 566 p.

De Marchi, M., V. Toffanin, M. Cassandro, and M. Penasa. 2014. Invited review: Mid-infrared spectroscopy as phenotyping tool for milk traits. J. Dairy Sci. 97: 1171-1186.

Demeyer D., C. Van Nevel, and L. Fiems. 1995. Nutritional engineering of beef fat composition: motive, target and approach, in: Lundström K., I. Hansson, and E. Wiklund (Eds.). Composition of meat in relation to processing, nutritional and sensory quality, ECCEAMST Utrecht.15–36.

Denke, M. A., and S. M. Grundy.1992. Comparison of effects of lauric acid and palmitic acid on plasma lipids and lipoproteins. Am. J. Clin. Nutr.56:895-898.

DePeters, E. J., J. F. Medrano, and B. A. Reed. 1995. Fatty acid composition of milk fat from three breeds of dairy cattle. Can. J. Anim. Sci.75:267-269.

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

Dorfman, S. E., D. Laurent, J. S. Gounarides , X. Li, T. L. Mullarkey, E. C. Rocheford, F. Sari-Sarraf, E. A. Hirsch, T. E. Hughes, and S. R. Commerford. 2009. Metabolic Implications of Dietary *Trans*-fatty Acids. Obesity. 17:1200-1207.

Edwards, R. A., J. W. B. King, and I. M. Yousef.1973. A note on the genetic variation in the fatty acid composition of cow milk. Anim. Prod.16: 307-310.

Enjalbert, F., M. C Nicot, C. Bayourthe, and R. Moncoulon. 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.

Fleming, A. 2016. Phenotypic and genetic variation of milk fat components incorporating mid-infrared technology. PhD Thesis. University of Guelph., Canada

Flowers, M., and P. A. Thompson. 2009. t10C12 conjugated linoleic acid suppresses HER2 protein and enhances apoptosis in SKBr3 breast cancer cells: possible role of COX2. PLoS One, 4: e5342.

Food and Agriculture Organization of the United Nations (FAO). 2010. Fats and fatty acids in human nutrition. Report of an expert consultation.91.

Fox, P. F., Uniacke-Lowe. T, McSweeney. P.L.H, and J. A. O'Mahony. 2015. Dairy chemistry and Biochemistry. 2nd ed. Springer international publishing.

Fuerst, C., and J. Sölkner. 1994. Additive and nonadditive genetic variances for milk yield, fertility, and lifetime performance traits of dairy cattle. J. Dairy Sci. 77:1114-1125.

Ganguly, J. 1960. Studies on the mechanism of fatty acid synthesis. Biochim. Biophys. Acta. 40.110–118.

Garnsworthy, P. C., and J. H. Topps. 1982. The effect of body condition score at calving on their food intake and performance when given complete diets. Anim. Prod. 35:113–119.

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

Garnsworthy, P. C., S. Feng, A. L. Lock, and M. D. Royal. 2010. Short communication: Heritability of milk fatty acid composition and stearoyl-CoA desaturase indices in dairy cows. J. Dairy Sci.93*: 1743-1748.

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

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

Gilmour, A. R., B. J. Gogel, B. R. Cullis, R. Thompson and D. Butler. 2009. ASReml user guide release 3.0. VSN International Ltd, Hemel Hempstead, UK.

Gion, A., H. Larroque, M. Brochard, F. Lahalle, D. Boichard. 2011. Genetic parameter estimation for milk fatty acids in three French dairy cattle breeds. Interbull Bulletin.44.

Goyens, P. L., M. E. Spilker, P. L. Zock, M. B.Katan, and R. P. Mensink. 2006. Conversion of α-linolenic acid in humans is influenced by the absolute amounts of α-linolenic acid and linoleic acid in the diet and not by their ratio. Am. J. Clin. Nutr. 84: 44-53.

Griinari J.M and D.E Bauman. 1999. Biosynthesis of conjugated linoleic acid and its incorporation into meat and milk in ruminants, in: Yurawecz M.P., M.M. Mossoba, J.K.G. Kramer, M.W. Pariza, and G.J. Nelson (Eds.), Advances in Conjugated Linoleic Acid Research, Vol. 1, Champaign, Illinois: AOCS Press.180–200.

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

Ha, J. and K. Kim. 1994. Inhibition of fatty acid synthesis by expression of an acetyl-CoA carboxylase-specific ribozyme gene. Proc. Natl. Acad. Sci. 91:9951-9955.

Haug, A., A. T Høstmark, and O. M. Harstad. 2007. Bovine milk in human nutrition–A review. Lipids in Health and Dis. 6:25-40.

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

Hooper, L., C. D. Summerbell, R. Thompson, D. Sills, F. G. Roberts, H. J. Moore, and G. D. Smith. 2012. Reduced or modified dietary fat for preventing cardiovascular disease. Cochrane Database Syst Rev. *5*.

Hooper, L., N. Martin, A. Abdelhamid, and G. D.Smith. 2015. Reduction in saturated fat intake for cardiovascular disease. Cochrane Database Syst Rev.6.

Hou, C. T. 2005. Handbook of industrial biocatalysis. CRC press.

Hsu, Y. C., X. Meng, L. Ou, and M. M. Ip. 2010. Activation of the AMP-activated protein kinase–p38 MAP kinase pathway mediates apoptosis induced by conjugated linoleic acid in p53-mutant mouse mammary tumor cells. Cell. Sig. 22:590-599.

Hulshof, K. F., M. A. van Erp-Baart , M. Anttolainen, W. Becker, S. M. Church , C. Couet, E. Hermann-Kunz, H. Kesteloot, T. Leth, I. Martins, O. Moreiras, J. Moschandreas, L. Pizzoferrato, A.H. Rimestad, H. Thorgeirsdottir, J. M. van Amelsvoort, A. Aro, A.G. Kafatos, D. Lanzmann-Petithory and G. van Poppel. 1999. Intake of fatty acids in Western Europe with emphasis on *trans* fatty acids: the TRANSFAIR Study. Eur. J. Clin. Nutr. 53:143-157.

Huth, P. J., and K. M. Park. 2012. Influence of dairy product and milk fat consumption on cardiovascular disease risk: a review of the evidence. Adv Nutr: An International Review Journal, 3: 266-285.

Ikonen, T., S. Morri, A. M. Tyrisevä, O. Ruottinen, and M. Ojala. 2004. Genetic and phenotypic correlations between milk coagulation properties, milk production traits, somatic cell count, casein content, and pH of milk. J. Dairy Sci. 87:458-467.

Jakobsen, M. U., E. J. O'Reilly, B. L. Heitmann, M. A. Pereira, K. Bälter, G. E. Fraser, U. Goldbourt, G. Hallmans, P. Knekt, S. Liu, P. Pietinen, D. Spiegelman, J. Stevens, J. Virtamo, W. C Willett, and A. Ascherio. 2009. Major types of dietary fat and risk of coronary heart disease: a pooled analysis of 11 cohort studies. Am. J. Clin. Nutr 89: 1425-1432.

Jamrozik, J., L. R. Schaeffer, and J. C. M. Dekkers.1997. Genetic evaluation of dairy cattle using test day yields and random regression model. J. Dairy Sci.80:1217-1226.

Jamrozik, J., L. R. Schaeffer, and K. A. Weigel. 2002. Estimates of genetic parameters for single- and multiple-country test-day models. J. Dairy Sci. 85:3131-3141.

Jelen, P. 2007. Innovative uses of milk in human nutrition and health. Proceedings of the 35th Biennal Session of ICAR, Kuopio, Finland. EAAP publication 121. EAAP, Rome, Italy.

Jenkins T.C., and M.A. McGuire. 2006. Major advances in nutrition: Impact on milk composition. J. Dairy Sci., 89:1302–1310.

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

Jensen, R. G., Ferris. A. M, and Lammi-Keefe. C. J. 1991. The Composition of Milk Fat. J Dairy Sci. 74: 3228-3243.

Kargar, S., G. R. Ghorbani, M. Alikhani, M. Khorvash, L.Rashidi, and D. J. Schingoethe. 2012. Lactational performance and milk fatty acid profile of Holstein cows in response to dietary fat supplements and forage: Concentrate ratio. Livest. Sci. 150:274-283.

Karijord, Ø., N. Standal, , and O. Syrstad. 1982. Sources of variation in composition of milk fat. Zeitschrift für Tierzüchtung und Züchtungsbiologie, 99: 81-93.

Karoui, R., A. M. Mouazen, E. Dufour, L. Pillonel, D. Picque, J. De Baerdemaeker, and J.-O. Bosset. 2006. Application of the MIR for the determination of some chemical parameters in European Emmental cheeses produced during summer. Eur. Food Res. Technol. 222:165–170.

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

Keenan, T. W., and D. P. Dylewski. 1985. Aspects of intracellular transit of serum and lipid phases of milk. J. Dairy Sci. 68:1025.

Keenan, T. W., and D. P. Dylewski. 1994. Intracellular origin of milk lipid globules and the nature and structure of the milk lipid globule membrane. Pages 89–130 in Advanced Dairy Chemistry-2-Lipids. P. F. Fox, ed. Chapman and Hall, London, United Kingdom.

Kgwatalala, P. M., E. M. Ibeagha-Awemu, A. F Mustafa, and X. Zhao. 2009. Stearoyl-CoA desaturase 1 genotype and stage of lactation influences milk fatty acid composition of Canadian Holstein cows. Anim. Genet. 40: 609-615.

Kinsella, J.E. 1972. Stearyl CoA as a precursor of oleic acid and glycerolipids in mammary microsomes from lactating bovine: possible regulatory step in milk triglyceride synthesis, Lipids 7:349–355.

Kistemaker, G.J. 2003 .The Canadian test model using Legendre polynomial. Accessed Dec. 20, 2015. https://www.cdn.ca/articles.php.

Kliem, K. E., and K. J. Shingfield. 2016. Manipulation of milk fatty acid composition in lactating cows: Opportunities and challenges. Eur. J. Lipid. Sci. Tec.

Krag, K., N. A. Poulsen, M. K. Larsen, L. B. Larsen, L. L. Janss, and B. Buitenhuis.2013. Genetic parameters for milk fatty acids in Danish Holstein cattle based on SNP markers using a Bayesian approach. BMC genet. 14: 1.

Kritchevsky, D., S. A. Tepper, S. Wright, S. K. Czarnecki, , T. A. Wilson, and R. J. Nicolosi.2004. Conjugated linoleic acid isomer effects in atherosclerosis: growth and regression of lesions. *Lipids*, *39*:611-616.

Livingstone, K. M., J. A. Lovegrove, and D. I. Givens. 2012. The impact of substituting SFA in dairy products with MUFA or PUFA on CVD risk: evidence from human intervention studies. Nutr. Res. Rev 25:193-206.

Lock, A. L., and P. C. Garnsworthy. 2003. Seasonal variation in milk conjugated linoleic acid and Δ 9-desaturase activity in dairy cows. Livest. Prod. Sci.79:47-59.

Lopez-Villalobos, N., R. J. Spelman, J. Melis, S. R. Davis, S. D. Berry, K. Lehnert, S. E. Holroyda, A. K. H. MacGibbon, and R. G. Snell. 2014. Estimation of genetic and crossbreeding parameters of fatty acid concentrations in milk fat predicted by mid-infrared spectroscopy in New Zealand dairy cattle. J. Dairy Res.81:340-349.

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

MacGibbon, A. K. H., and McLennan, W. D. 1987. Hardness of new-zealand patted butter-seasonal and regional variations. N.Z. J. Dairy Sci and technol.22:143-156.

Månsson, H. L. 2008. Fatty acids in bovine milk fat. Food Nutr. Res. 52

Markiewicz- Kęszycka, M., G. Czyĺak-Runowska, P. LipiĚska, and J. Wójtowski. 2013. Fatty acid profile of milk—A review. Bull Vet Inst Pulawy. 57:135–139.

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

Mele, M., R. Dal Zotto, M. Cassandro, G. Conte, A. Serra, A. Buccioni, G. Bittante and P. Secchiari. 2009. Genetic parameters for conjugated linoleic acid, selected milk fatty acids, and milk fatty acid unsaturation of Italian Holstein-Friesian cows. J. Dairy Sci.92:392-400.

Mensink, R. P., and 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., and M. B. Katan. 1993. *Trans* monounsaturated fatty acids in nutrition and their impact on serum lipoprotein levels in man. Prog. in Lipid Res. 32:111-122.

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

Micha, R., and D. Mozaffarian. 2009. *Trans* fatty acids: effects on metabolic syndrome, heart disease and diabetes. Nutr. Rev. Endocrinol, 5: 335-344.

Micha, R., and D. Mozaffarian.2010. Saturated fat and cardiometabolic risk factors, coronary heart disease, stroke, and diabetes: a fresh look at the evidence. Lipids, 45: 893-905.

Miciński, J., G. Zwierzchowski, I. M. Kowalski, J. Szarek, B. Pierożyński, and J. Raistenskis, 2012. The effects of bovine milk fat on human health. Pol. Ann. Med. 19: 170-175.

Mills, S., R. P. Ross, C. Hill, G. F. Fitzgerald, and C. Stanton. 2011. Milk intelligence: Mining milk for bioactive substances associated with human health. Int. Dairy J.21:377-401.

Misztal, I., T. Strabel, J. Jamrozik, E. A. Mäntysaari, and T. H. E. Meuwissen.2000. Strategies for estimating the parameters needed for different test day models. J. Dairy Sci. 83:1125–1134.

Mozaffarian, D., E. J. Benjamin, A. S. Go, D. K. Arnett, M. J. Blaha, M. Cushman, S.R. Das, S. de Ferranti, J.P. Després, H.J Fullerton and V. J. Howard. 2015. Heart Disease and Stroke Statistics—2016 Update. Circulation. pp. CIR-0000000000000350.

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

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

O'Donnell J.A. 1993. Future of milk fat modification by production or processing: integration of nutrition, food science, and animal science, J. Dairy Sci. 76:1797–1801.

O'Donnell, J. A. 1989. Milk fat technologies and markets: a summary of the Wisconsin Milk Marketing Board 1988 Milk Fat Roundtable. J. Dairy Sci.72: 3109-3115.

Oomen, C. M., M. C. Ocké, E. J. Feskens, M. A. J. van Erp-Baart, F. J. Kok, and D. Kromhout. 2001. Association between trans fatty acid intake and 10-year risk of coronary heart disease in the Zutphen Elderly Study: a prospective population-based study. The Lancet, 357: 746-751.

O'Quinn, P. R., J. L. Nelssen, R. D. Goodband, and M. D. Tokach. 2000. Conjugated linoleic acid. Anim. Health Res. Rev. 1: 35-46.

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.

Parodi, P. W. 1977. Conjugated octadecadienoic acids of milk fat. J. Dairy Sci.60: 1550-1553.

Parodi, P. W. 2009. Has the association between saturated fatty acids, serum cholesterol and coronary heart disease been over emphasized? Int. Dairy J. 19: 345-361.

Pascal, G. 1996. Les apports quotidiens recommande´s en lipides eten acides gras. OCL 3:205–210.

Pool, M. H., L. L. G. Janss, and T. H. E. Meuwissen.2000. Genetic parameters of Legendre polynomials for first parity lactation curves J. Dairy Sci. 83: 2640-2649.

Poulsen, N. A., F. Gustavsson, M. Glantz, M. Paulsson, L. B. Larsen, and M. K. Larsen.2012. The influence of feed and herd on fatty acid composition in 3 dairy breeds (Danish Holstein, Danish Jersey, and Swedish Red). J. Dairy Sci. 95:6362-6371.

Precht, D., and J. Molkentin. 2000. Frequency distributions of conjugated linoleic acid and *trans* fatty acid contents in European bovine milk fats. Milchwissenschaft. 55:687-691.

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

Renner, E., and Kosmack, U. 1974b. Genetic aspects concerning fatty acid composition of milk fat. 3: Genetic correlations with percentage of butter fat and amount of fat produced. Zuechtungskunde. 46:257–264

Rustan, A. C., and C. A. Drevon. 2005. Fatty acids: structures and properties. *eLS*.

Rutten, M. J. M., H. Bovenhuis, K. A. Hettinga, H. J. F. van Valenberg, and J. A. M. Van Arendonk.2009. Predicting bovine milk fat composition using infrared spectroscopy based on milk samples collected in winter and summer. J. Dairy Sci. 92:6202-6209.

Samková, E., J. Spicka, M. Pesek, T. Pelikanova, and O. Hanus. 2012. Animal factors affecting fatty acid composition of cow milk fat: A review. S. Afr. J. Anim. Sci. 42: 83-100.

Sauer F.D., V. Fellner, R. Kinsman, J.K.G. Kramer, H.A. Jackson, A.J. Lee, and S. Chen. 1998. Methane output and lactation response in Holstein cattle with monensin or unsaturated fat added to the diet, J. Anim. Sci. 76:906–914.

Schaeffer, L. R. 2004. Application of random regression models in animal breeding. Livest. Prod. Sci, 86:35-45.

Schaeffer, L. R., A. Robinson, J. Chesnais, H. Wilmink, G. Wiggans, P. Rozzi, H. DeBoer, and M. Hunt. 1993. Multi-trait, across country evaluation of dairy sires. Interbull Bulletin 8.

Schenkel, F., M .Sargolzaei, G. Kistemaker, G. Jansen, P. Sullivan, B. Van Doormaal ,P. Van Raden, G. Wiggans . 2009. Reliability of genomic evaluation of Holstein cattle in Canada. Pages 51-58 in Proc. Interbull Int. Workshop, Bulletin No. 39. Interbull, Uppsala, Sweden

Shingfield, K. J., M. Bonnet, and N. D. Scollan. 2013. Recent developments in altering the fatty acid composition of ruminant-derived foods. Anim.7: 132-162**.**

Simopoulos, A. P. 1991. Omega-3 fatty acids in health and disease and in growth and development. Am. J. Clin. Nutr. 54:438-463.

Simopoulos. A. P. 2002 The importance of the ratio of omega-6/omega-3 essential fatty acids. Biomed. Pharmacother. 56:365–379.

Siri-Tarino, P. W., Q. Sun, F. B. Hu, and R. M. Krauss. 2010. Meta-analysis of prospective cohort studies evaluating the association of saturated fat with cardiovascular disease. Am. J. Clin. Nutr. 27725.

Siri-Tarino, P. W., S. Chiu, N. Bergeron, and R. M. Krauss. 2015. Saturated fats versus polyunsaturated fats versus carbohydrates for cardiovascular disease prevention and treatment. Annu. Rev. Nutr.*35*: 517-543.

Slattery, M. L., J. Benson, K. N. Ma, D. Schaffer, and J. D. Potter.2001. Trans-fatty acids and colon cancer. Nutr and cancer. 39:170-175.

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

Soyeurt, H., F. Dehareng, N.Gengler, S. McParland, E. P. B. D. Wall, D. P. Berry , M. Coffey and P. Dardenne. 2011. Mid-infrared prediction of bovine milk fatty acids across multiple breeds, production systems, and countries. J. Dairy Sci. 94: 1657-1667.

Soyeurt, H., F. Dehareng, C. Bertozzi, and Gengler, N. 2007b. Genetic parameters of butter hardness estimated by test-day model. Interbull Bulletin, 37:49.

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

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

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

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

Spiegelhalter, D. J., N. G. Best, B. P. Carlin and A. Van Der Linde. 2002. Bayesian measures of model complexity and fit. J. R. Statist. Soc. B.64:583-639.

Stoop, W. M. 2009a. Genetic variation in bovine milk fat composition. PhD thesis, Wageningen University, the Netherlands.

Stoop, W. M., H. Bovenhuis, J. M. L. Heck, and J. A. M. van Arendonk. 2009b. Effect of lactation stage and energy status on milk fat composition of Holstein-Friesian cows. J. Dairy Sci. 92:1469–1478.

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.

Sullivan, P. 2009.Options for combining direct genomic and progeny-test results. Accessed Dec. 20, 2015. https://www.cdn.ca/articles.php.

Temme, E. H., R. P. Mensink, and G. Hornstra. 1996. Comparison of the effects of diets enriched in lauric, palmitic, or oleic acids on serum lipids and lipoproteins in healthy women and men. Am. J. Clin. Nutr .63: 897-903.

Tricon, S., G. C. Burdge , E. L. Jones, J. J. Russell, S. El-Khazen, E. Moretti, W. L. Hall, A. B. Gerry, D. S. Leake, R. F. Grimble, C. M. Williams, P. C. Calder , P. Yaqoob and C. M. Williams. 2006. Effects of dairy products naturally enriched with cis-9, trans-11

conjugated linoleic acid on the blood lipid profile in healthy middle-aged men. Am. J. Clin. Nutr. 83:744-753

Van Doormaal, B., and L.Beavers. 2015. How do I use Pro$ for cows and heifers? Accessed Dec. 20, 2015. https://www.cdn.ca/articles.php.

Van Doormaal, B. 2007. Genetic evaluation of dairy cattle in Canada. Accessed Dec. 20, 2015. https://www.cdn.ca/articles.php.

Van Doormaal, B. 2008. Trends in the Canadian dairy cattle improvement industry. Accessed Dec. 20, 2015. https://www.cdn.ca/articles.php.

Vannice, G., and H. Rasmussen. 2014. Position of the academy of nutrition and dietetics: dietary fatty acids for healthy adults. J Acad Nutr Diet.114: 136-153.

Walstra, P., and R. Jenness. 1984. Dairy Chemistry and Physics. John Wiley and Sons, New York, NY.

Wang, J. P., D.P. Bu, J.Q. Wang, X.K. Huo, T.J. Guo, H.Y. Wei, L.Y. Zhou, R.R. Rastani, L.H. Baumgard, and F.D. Li. 2010. Effect of saturated fatty acid supplementation on production and metabolism indices in heat-stressed mid-lactation dairy cows. J. Dairy Sci. 93:4121-4127.

Wang, Q., F. Imamura, R. N. Lemaitre, E. B. Rimm, M. Wang, I. B. King, X. Song, D. Siscovick and D. Mozaffarian. 2014. Plasma phospholipid *trans*-fatty acids levels, cardiovascular diseases, and total mortality: the cardiovascular health study. J. Am. Heart. Assoc.3: e000914.

Wiking, L., J. Stagsted, L. Bjo¨rck, and J. H. Nielsen. 2004. Milk fat globule size is affected by fat production in dairy cows. Int. Dairy J. 14:909–913.

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

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

Yashodhara, B. M., S. Umakanth , J. M. Pappachan , S. K. Bhat, R. Kamath, and B. H. Choo. 2009. Omega-3 fatty acids: a comprehensive review of their role in health and disease. Postgrad. Med. J, 85: 84-90.

Zock, P. L., J. H. De Vries, and M. B. Katan. 1994. Impact of myristic acid versus palmitic acid on serum lipid and lipoprotein levels in healthy women and men. Arterioscler Thromb Vasc Biol. 14:567-575.

Zock, P. L., and M. B. Katan.1992. Hydrogenation alternatives: effects of *trans* fatty acids and stearic acid versus linoleic acid on serum lipids and lipoproteins in humans. J. Lipid Res. 33: 399-410.