GENETIC INFLUENCE OF HOST ANIMAL ON FATTY ACID COMPOSITION IN BEEF CATTLE TISSUES

by

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A thesis submitted in partial fulfillment of the requirements for the degree of

Doctor of Philosophy

in

Animal Science

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ABSTRACT

Beef fatty acid (FA) composition has emerged as a trait of economic importance as consumers have become more aware of the relationship between diet and health. As a result, they are paying more attention on the health impact of the food they consume including the type of dietary fat. Clinical studies have shown that the type of dietary fat has a more profound impact on human health than the amount of fat in the diet. In animal studies, phenotypic variations of fatty acid contents in animal tissues are commonly observed.

The objectives of this study were: (1) to estimate heritabilities, genetic and phenotypic correlation for FAs in the brisket adipose tissue, and heritabilities for FAs in the subcutaneous adipose tissue (SQ) and longissimus lumborum (LL) muscle in beef cattle, (2): to estimate phenotypic and genetic correlations between FAs in the LL muscle with 6 carcass and 13 meat quality traits of economic importance, (3): to identify single nucleotide polymorphism markers (SNP) associated with FA composition in order to help in designing effective genetic selection tool / genotype based management program for selection of beef with healthier FA profile to meet consumers' demands.

Heritability for 15 individual and 10 groups of FAs having a concentration greater than 0.5% was estimated in the brisket adipose of 223 Angus based crossbred steers, and for over 80 individual and groups of FAs in the LL muscle and SQ adipose tissue of 1366 crossbred steers and heifers using a univariate animal model. Across the three tissues, heritability ranged from 0 for 7c-17:1 in the LL and 7c-17:1, 12t-18:1, 6t, 8t-18:2, 9t, 11t-18:2, n-6/n-3 in the SQ to 0.68 ± 0.1 for

9c-16:1 in the LL nuscle tissue. The result suggested the presence of host animal genetic effects on some FAs, including harmful 14:0 (0.61 ±0.13)and 16:0 (0.54 ±0.1), beneficial MUFA (0.44±0.09), and health index (0.54±0.1) in the muscle. The results also showed that non-genetic factors played a major role in determining the concentration of many other FAs in the muscle including 9c, 11t-18:2 conjugated linoleic acid (CLA, 0.16 ±0.07) and vaccenic acid (18:1, trans-11, 0.24 ±0.08), which have been shown to benefit humam health. Phenotypic correlation between FAs in the brisket adipose tissue did not indicate significant antagonistic relationships between harmful and healthy FAs. The relationship revealed that reducing the concentration of harmful 16:0 would yield a correlated response of increased concentration of beneficial 9c-18:1, 11t-18:1, 9c,11t-18:2, and 18:3n3. However, in the LL muscle, genetic correlations revealed antagonistic relationships between monounsaturated FA (MUFA) and hot carcass weight (HCW) (-0.4±0.13) and between 18:3n3, 22:3n6, and total n-3 with marbling (MARB) (-0.82±0.11,-0.60±0.17, -0.84±0.11). For FAs and meat quality, unfavourable genetic relationships existed between 11t-18:1 with WBSF_29d, shear force on 26-day aged steaks and flavor (0.49±0.22, -0.37±0.32). There was also a moderate to strong antagonistic genetic relationship between beneficial polyunsaturated FAs, 9c, 11t-18:2, 18:3n3, 20:5n3, 22:6n3 and total omega 3 with meat quality traits particularly flavor, tenderness and juiciness.

A two step bayesian analysis approach was used to evaluate the association of each of 15 individual and 10 grouped FAs for associations with 947 polymorphic SNP markers in 556 growth- and fat metabolism- related genes. The markers were

developed and genotyped on 223 commercial crossbred beef steers that had FA profiles measured in brisket adipose tissue. The analyses identified 24 SNPs in 22 genes involved in various cellular processes were significantly associated with 8 FAs at a genome-wise threshold of $P<0.05$. Phenotypic variance explained by significant SNPs at genome-wide threshold for each of the 8 traits ranged from 0.0001% for MUFA with a heritability of 0.06±0.10 to 19.61% for cis-13-octadecenoic acid (13c-18:1) with a heritability of 0.43±0.1. The results show that FA concentrations in brisket adipose tissue of beef cattle are influenced by multiple genes, with different functional roles in the cell: several having small effects

The results of the study will not only help us gain more insight into the genetic influence of host animals on FA composition in beef cattle tissues but also provide genetic parameters and DNA markers for more effective genetic evaluation and selection as well as DNA marker assisted diet management to improve FAs profiles in beef cattle.

PREFACE

Research conducted for this thesis was led by Dr. Changxi Li who was involved in every part of this project.

I was responsible for profiling and quantifying all conjugated linoleic acids in the *longissimus lumborum* muscle and subcutaneous adipose tissue samples using high performance liquid chromatography (HPLC). I carried out quality checks on all the phenotype and genotype data, analysed the data using statistical models, interpretated results from data analysis, and composed the manuscripts and the thesis including the literature review and general conclusions.

For a version of Chapter 2 that has been published in the Meat Science Journal (Ekine-Dzivenu C., Chen L., Vinsky M., Aldai N., Dugan M.E.R, McAllister T.A., Wang Z., Li C., 2014. Estimates of genetic parameters for fatty acids in brisket adipose tissue of Canadian commercial crossbred beef steers. Meat Science 2014, 96(4):1517-1526), I was responsible for data quality checks, data analysis, interpretation of results from data analysis, and manuscript composition. Michael Vinsky was involved in data compilation, genotyping and contributed to manuscript edits. Liuhong Chen was involved in writing computer programs for data analysis, helped with statistical data analysis, and contributed to manuscript edits. Noelia Aldai, Mike Dugan, Tim McAllister, Erasmus Okine and Zhiquan Wang contributed to manuscript edits. Changxi Li was the lead investigator and was involved in every part of the study.

For a version of Chapter 3 that has been submitted to the Journal of Animal Breeding and Genetics for publication (JABG-15-0006), I was responsible for profiling and quantifying the conjugated linoleic acids in the *longissimus lumborum* muscle and subcutaneous adipose tissue samples using high performance liquid chromatography (HPLC). I carried out the data quality checks, data analysis, interpretation of results from data analysis, and manuscript composition. Shurong Xiong was involved in profiling and quantifying fatty acids in the *longissimus lumborum* muscle and subcutaneous adipose tissue samples using Gas Chromatography (GC). Michael Vinsky was involved in profiling and quantifying fatty acids in the *longissimus lumborum* muscle and subcutaneous adipose tissues using the GC and HPLC, pedigree compilation, and contributed to manuscript edits. Liuhong Chen was involved in with statistical data analysis and manuscript editing. Changxi Li was the lead investigator and was involved throughout every part of the study.

For I am the LORD your God
who takes hold of your right hand
and says to you, Do not fear;
I will help you.

Isaiah 41:13
New International Version (NIV)

ACKNOWLEDGEMENT

I would like to express my special appreciation and thanks to my supervisor Dr. Changxi Li, you have been a tremendous mentor to me. I would like to thank you for allowing me to grow as a research scientist and for encouraging my research. Your advice has been priceless.

I would also like to thank my supervisory committee members, Dr. Erasmus Okine and Dr. Zhiquan Wang for serving as my committee members, for your brilliant comments and suggestions that helped fine tune this research. Thanks Dr. Okine for cheering me on and being such an encourager. It gave me strength.

I would especially like to thank Liuhong Chen, Mike Vinsky and Shurong Xiong for their help with analyzing the samples and data used in this project. Thank you Liuhong for your prompt response to all the many questions I had and for being nice.

Special thanks to my all colleagues at the Livestock Gentec. All of you have incented me to strive towards this goal. Thank you to my Mclauren Church family. I rode on the wheels of your prayers.

I would also like to thank my parents, in-laws and siblings. They were always supporting me and encouraging me. Thank you mum for helping me take care of Dzesi while I returned back to school. Thanks “sister” for your constant prayers. They sustained me.

I would never have been able to finish my dissertation without support from my husband and son. Words cannot express how grateful I am for all of the sacrifices that you made on my behalf. I love you guys!

At the end I would like express my appreciation to my God for the GRACE to accomplish this task.

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LIST OF ABBREVIATIONS

Ag-HPLC	Silver ion High Performance Liquid Chromatography
Ag-TLC	Silver-ion Thin-Layer Chromatography
AHA	American Health Association
AI	Atherogenic Index
BCFA	Branched Chain Fatty Acid
CLA	Conjugated Linoleic Acid
DHA	Docosahexaenoic Acid
DNA	Deoxyribonucleic Acids
EPA	Eicosapentaenoic Acid
FA	Fatty Acid
FAME	Fatty Acid Methyl Esters
GC	Gas Chromatography
GWAS	Genome Wide Associaition Study
HI	Health Index
LDL	Low Density Lipoprotein
mRNA	Messenger Ribonucleic Acid
MUFA	Monounsaturated Fatty Acid
n-3	Omega 3
n-6	Omega 6
NR	Not Reported
P/S	Polyunsaturated /Saturated fatty acid ratio
PHVO	Partially Hydrogenated Vegetable Oils
PUFA	Polyunsaturated Fatty Acid

QTL	Quantitative Trait Loci
SFA	Saturated Fatty Acid
SNP	Single Nucleotide Polymorphisms
TAG	Triacylglycerol
VA	Vaccenic Acid
HCW	Hot Carcass Weight
BFAT	Back fat thickness
REA	Rib eye area
CMAR	Carcass Marbling
LMY	Lean Meat Yield
YG	Yield Grade
WBSF	Warner Bratzler Shear Force
BFI	Beef Flavor Intensity
OF	Off Flavor
OT	Overall Tenderness
SJ	Sustained Juiceness

CHAPTER 1

A LITERATURE REVIEW OF THE GENETIC INFLUENCE OF HOST ANIMAL ON THE FATTY ACID COMPOSITION IN BEEF CATTLE

1.1 Introduction

The saturated fat content in meat is the reason most consumers label meat as unhealthy (Jakobsen, 1999). Indeed, the mantra for healthy eating in many quarters is eating diets low in fat because excessive dietary fat intake has been associated with various health conditions ranging from cardiovascular diseases to obesity and some forms of cancer in humans (Gormley et al., 1987; Lin et al., 2004; Uemoto et al., 2010). However, with a growing body of evidence from epidemiological studies, metabolic studies and clinical trials, it has become increasing clear and is now being stressed that the type of fat in a diet (diet fatty acid composition) matters more than the amount of fat in managing cardiovascular health (Hu et al., 2001; Woodside and Kromhout, 2005). For instance, in terms of reducing the risk of cardiovascular diseases in middle aged men, total monounsaturated fatty acid (MUFA) and polyunsaturated fatty acid (PUFA) consumed was more important than the total fat consumed (Laaksonen et al., 2005). In infants, it was shown that ingesting more PUFA and less saturated fatty acid (SFA) not only reduced total cholesterol but more importantly reduced low density lipoprotein (LDL) also known as bad cholesterol early in life (Ohlund

et al., 2008). With increasing demand for healthier food options, improving the fatty acid profile of beef, by reducing the SFA content, and increasing the concentration of beneficial polyunsaturated fatty acids (PUFA) , particularly the omega 3's (n-3), and conjugated linoleic acid (CLA), is important. Moreover, the profile of fat also impacts on the flavor and tenderness of beef. As a result, the fatty acid composition in beef is fast evolving to becoming a trait of economic importance, and meeting this demand should come into focus in the beef industry (Mir et al., 2004) so as to maintain the sustainability of beef production.

1.2 Lipids, Fats and Fatty Acids

Lipids are group of chemical compounds which have diverse biological functions but share a common characteristic of being insoluble in water. Lipids can be broadly classified as storage lipids (fats and oils) or membrane lipids (phospholipids, glycoplipids, archebacterial lipids). Fats and oils also called neutral lipids are the principal form in which energy is stored almost universally in living organisms where they also serve as insulation against cold temperature (Lehninger et al., 2005). Storage lipids (neutral lipids or triacylglycerol) make up about 80% of the mass of an adipocyte while membrane lipids make up 5 – 10% of the dry mass of adipocyte cells. Triacylglycerol, are the simplest lipids derived from fatty acids and are made up of three fatty acids each in an ester linkage with a single glycerol (fig.1).

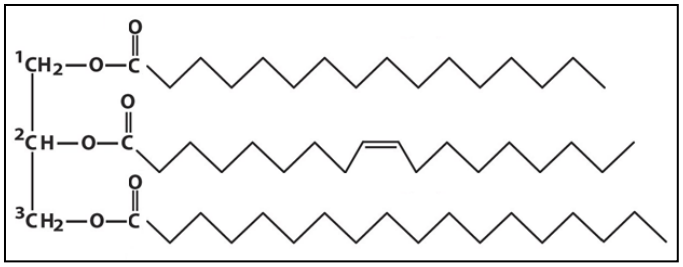

Fig 1. Structure of a triacylglycerol

Fatty acids in the three positions can be the same (simple) or different (mixed). However, most naturally occurring triacylglycerols (TAGs) are mixed, containing two or more fatty acids at the different positions. Fats in animal products are a combination of simple and mixed triacylglycerol with fatty acids having different lengths and degree of saturation (Lehninger et al., 2005; McDonald, 1988).

Natural waxes are esters of very long chain fatty acids (C14-C36) and very long chain alcohols (C16-C30) thus, they have a firm consistency and very high melting point. These attributes give them repellant and protective functions (Lehninger et al., 2005; McDonald, 1988). Waxes secreted by birds and animals repel water from the wool and feathers while some leaves have thick layer of waxes which protects then against parasites and keeps the leaves from excessively losing water through transpiration (McDonald, 1988). Planktons which are free floating microorganism found at the bottom of the food chain of marine animals store their energy in the form of waxes (Lehninger et al., 2005).

Fatty acids are carboxylic acids which have hydrocarbon chains ranging from 4-36 carbons. They can be broadly classified into saturated and unsaturated fatty acid. Saturated fatty (SFA) acids have no double bonds in their carbon chain while unsaturated fatty acids have at least one double bond in their carbon chain, and are further divided into monounsaturated (MUFA) or polyunsaturated

(PUFA) with PUFA having more than one double bond. Conjugated Linoleic acids (CLA) are a special class of PUFA. They are called conjugated linoleic acid because they have alternating single and double bonds and are not separated by a methylene group like the PUFAs (fig 2). They are positional isomers of linoleic acid (cis-9, cis-12 18:2). Eight of these positional isomers are possible but the most predominant isomer is the cis-9, trans11 isomer otherwise called rumenic acid. This class of fatty acids are originally found in meat and milk from ruminant animals because they are synthesized by rumen microflora (Bauman et al., 2003). Most naturally occurring unsaturated fatty acid is in cis configuration (McDonald, 1988) but some trans fatty acids are produced in the rumen of ruminant animals through the process of biohydrogenation and are deposited in the meat. Trans fats are also artificially produced during the partial hydrogenation of vegetable oils (PHVO) and are found mainly in processed foods. There is a distinction between these trans fats. One is naturally occurring and the other is man-made. The relationship between trans fats and cardiovascular diseases is specifically related to the man-made ones, and structural differences (position of the double bond) between them has been proposed as the possible reason for the difference in health effects (Belury, 2002; Lock et al., 2004; Willett et al., 1993). A major trans fat occurring in beef, vaccenic acid (18:1, trans-11) is a precursor to cis-9, trans-11 18:2, a major conjugated linoleic acid (CLA) isomer which has sparked a lot of research interest due to its purported anticarcinogenic, anti-atherosclerotic (Corl et al., 2003) and anti-diabetic effect (Rainer and Heiss, 2004). It has also been suggested that CLAs enhance the immune system, enhance bone

mineralization (Belury, 1995; Chin et al., 1994; Dugan et al., 1997; Pariza et al., 1999) and might cause a re-apportioning of nutrients such that more lean is deposited in place of fat (anti-obesity). Recent studies with animal models showed that, vaccenic acid (trans-11 18:1), may also have a number of potential health benefits (Bassett et al., 2010; Wang et al., 2008).

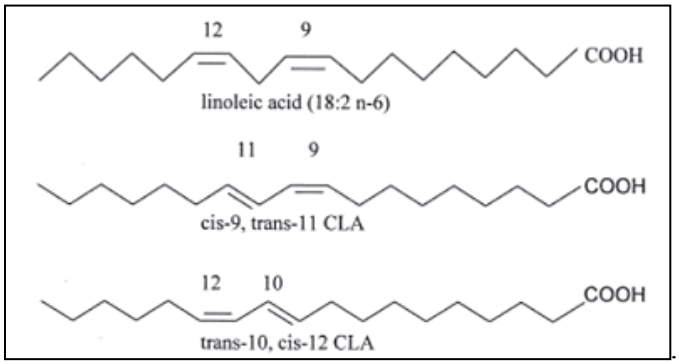

Fig 2. Difference between polyunsaturated fatty acid and conjugated linoleic acid

Physical properties of fatty acids are determined by the length and the degree of unsaturation of the carbon chain. The least soluble molecules have longer chains and fewer double bonds (Lehninger et al., 2005; McDonald, 1988).The degree of packing of the fatty acid molecules also influences their physical properties. In saturated fatty acids, the molecules pack together tightly in a crystalline form, thus they are solid at room temperature whereas unsaturated fatty acids are oily liquids because the cis double bond introduces a kink in the carbon chain which makes it impossible to pack tightly (fig 3). Because unsaturated fatty acids are not tightly packed, less energy is required to break the van der waal forces between

them making them have a lower melting point than saturated fatty acids of the same length (Lehninger et al., 2005) .

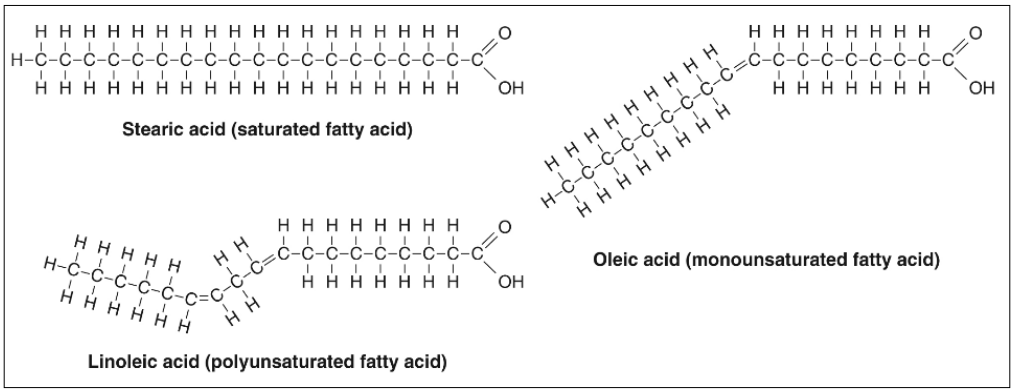

Fig 3. Packing of saturated fatty acid and unsaturated fatty acids

In general, there are 5 types of membrane lipids all with polar head groups and non polar tail groups namely, glycerophospholipids which are the most abundant membrane lipids, have their hydrophobic (water fearing) region comprising two fatty acid molecules attached on the first and second carbon of the glycerol backbone and the hydrophilic (water loving) region is either a polar group attached to the third carbon through a phosphodiester linkage (phospholipids) or a simple or complex sugar attached directly to the third carbon (galactolipids); Sphingolipids where the fatty acid joined to sphingosine (long chain aliphatic amino alcohol) backbone is hydrophobic and polar head group attached either by a phosphodiester linkage or a glycosidic linkage; Archebacterial which have very long branched hydrocarbon chain linked to glycerol at both ends by ether linkages and the hydrophilic unit is either a phosphate or sugar residue linked to glycerol at each end of the molecule; and sterols with hydrophobic groups consisting four

fused hydrocarbon rings, three with 6 carbon and one with 5 and a hydrocarbon side on carbon 17 and hydrophilic side, a hydroxyl group on carbon 3 (Lehninger et al., 2005).

Cholesterol is the main sterol in animal tissues. Phospholipids and sterols serve as structural lipids in cell membranes where they act as barriers to the passage of molecules and ions. Sterols are also precursor to hormones. One end of membrane lipids are hydrophobic while the other end is hydrophilic, in other words, they are amphipathic. These lipids pack in to sheets called membrane bilayers because of the hydrophobic interactions of the lipids among each other and the hydrophilic interaction with water (Lehninger et al., 2005)

There are other kinds of lipids which are present in small amounts but play crucial roles as enzymes cofactors (vitamin K), hormones (estrogen, testosterone), intracellular messengers (prostaglandins) and emulsifying agents in the digestive tract (bile) (Lehninger et al., 2008).

1.3 Fatty Acids and Health

In recent years, a lot of attention has been directed towards the fatty acid profile of beef because its high saturated fatty acid content has been associated with cardiovascular diseases, obesity and some cancers (Gormley et al., 1987; Raes et al., 2001). In addition, there has been reports of the benefits of vaccenic acid (11t-18:1) and CLA to human health. The nutritional quality of dietary fat is mostly assessed by looking at indices like the ratio of omega 6 PUFA to omega 3 PUFA (n-6/n-3), PUFA to SFA ratio (P/S), atherogenic index (AI) and the health index

(HI). The recommended P/S ratio is 0.4 - 0.7 (Ulbricht and Southgate, 1991; Webb and O'Neill, 2008) but this ratio is said to be flawed because it consideres all SFA as hypercholestrolaemic which is not the case (Ulbright and Sought, 1991). It has been proposed that this ratio be replaced with the index of atherogenicity (AI) which considers only the hypercholesterolaemic saturated fatty acid so diets that have the most atherogenic effect have the highest index (Ulbricht and Southgate, 1991). Zhang et al. (2008) inverted this index and redefined it as a health index (HI) so that healthier diets have higher indices. Presently, the focus has shifted to the type of PUFA. The precursor molecule to the n-6 and n-3 class of PUFA is linoleic acid (cis-9, cis-12, 18:2) and alpha linolenic acid (cis-9, cis-12, cis-15, 18:3). Important as they are, linoleic acid and alpha linolenic acid cannot be synthesized by the animal de novo and are therefore required to obtain it in the diet from plant materials thus they are called essential fatty acids. Linoleic acid goes through a series of elongation and desaturation steps to form arachidonic acid which is precursor to eicosanoids (prostaglandins-PG2, thromboxanes and leukotrienes) (Webb and O'Neill, 2008; Wood et al., 2008) which function as short range messengers affecting tissues near cells that produce them (Lehninger et al., 2005). Some of the important fatty acid of the n-3 class include eicosapentaenoic acid (EPA) 20:5(n-3) and docosahexaenoic acid (DHA) 22:5(n-3). EPA and DHA are commonly found in fish oils (Smith, 2008). EPA is the precursor of the PG3 series of prostaglandins (Christie, 2014) which have anti-inflamatory effects. There is evidence to suggest that an increased level of DHA is associated with the development of improved

cognitive and behavioral function in infants and the elderly and EPA has been linked with alleviating the symptoms of neurological disorders such as schizophrenia (Christie, 2014). Pathways for the formation of these two fatty acids are shown in Fig4.

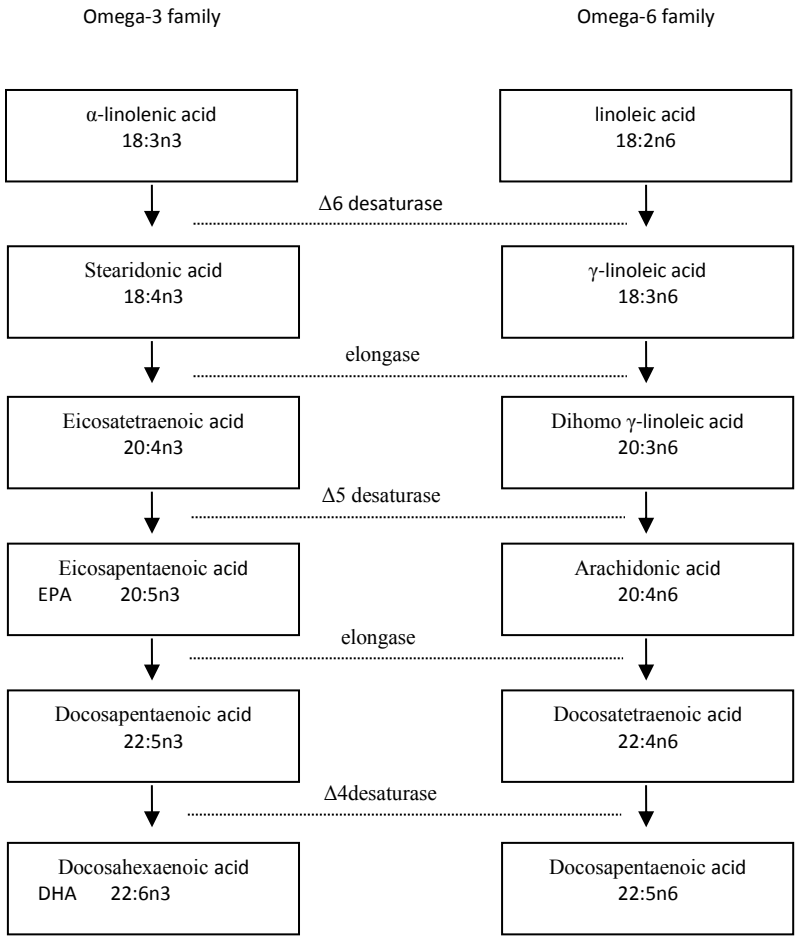

Fig 4. Pathway for the formation of Omega 3 and Omega 6 family of fatty acids

The rate of the conversion of alpha linolenic acid (18:3n-3) to longer chain metabolites is very slow in humans (Christie) and it is suggested that a higher proportion should come from the diet. The same enzymes are involved in the

series of elongation and desaturation reaction for both the n-3 and n-6 and the excess of one can suppress the conversion of the other (Mohrhauer and Holman, 1963). As a matter of fact, increase in the use of vegetable oils rich in linoleic acid in western countries over the past 30 years and a reduction in the consumption of fish and vegetables has resulted in diets with n-6:n-3 ratios as high as 20:1 in sharp contrast with a ratio of 2:1 in historical times (Christie, 2014; Webb and O'Neill, 2008). A high ratio of n-3 to n-6 is predisposes one to cardiovascular disease and cancer (Webb and O'Neill, 2008) and there is a call to cut down this ratio with medical experts recommending a ratio of less than 4 (Webb and O'Neill, 2008). EPA, DHA are potential antiarrythmic agent, i.e. they correct abnormal cardiac rhythms they improve vascular endothelial function, lower blood pressure and lower serum triacyglycerol (Wijendran and Hayes, 2004).

Relatively small amounts of these FA are needed in the diet to meet nutritional requirements. Nutritional guidelines/recommendations designed by the American Heart Association (AHA) to protect against the risk of these diseases suggest consumption of less ≤ 30% of total daily caloric intake as fat with 8-10% of as SFA, ≈10% as PUFA, ≈15% as MUFA, ≤ 300mg as cholesterol (Krauss et al., 1996). Trans fats should be limited to less than 1% of total energy.

1.4 Fatty Acid and Meat Quality

The degree of beef fatty acid unsaturation influences its shelf life and eating qualities (Webb and O'Neill, 2008). The palatability of beef is influenced by the quantity of oleic, 9c-18:1 acid, due to the low melting point of unsaturated fatty

acid and their fluidity at room temperature, very much unlike saturated fatty acids which are solids at room temperature (Smith et al., 2006; Westerling and Hedrick, 1979). Oleic acid is also the most abundant fatty acid in beef and it's precursor, stearic acid, determines the degree of hardness of fat in beef (Smith et al., 1998). Fatty acids with many double bonds are easily oxidized (Elmore and Mottram, 2009) leading to change in meat color, reduced shelf life, a loss of flavor and nutritional value of beef due to rancidity. Vitamin C and Vitamin E (α-tocopherol) have the ability to prevent oxidation of lipids because they have antioxidant properties (Elmore and Mottram, 2009; Li and Liu, 2012). Pasture contains a substantial amount of vitamin E which acts as a natural antioxidant, and protects lipids from oxidation. In an experiment where animals were fed only grain, grain supplemented with vitamin E, pasture and pasture supplemented with vitamin E, the authors (Descalzo et al., 2005) found a clear distinction between grain fed and pasture fed cattle, and concluded that, regardless of supplementation of grain with vitamin E, pasture fed animals had higher levels of vitamin E which is enough to prevent oxidation of lipid in fresh cut beef .

1.5 Origin of Fatty Acids in Beef Tissues

Fatty acids found in beef tissues is either from the animal's diet which has undergone modification in the rumen by rumen microbes, is synthesized by rumen microbes, or is a product of de novo synthesis by the animal. More than 5% dry matter inclusion rate of lipid in ruminant diets have negative effects on appetite, palatability, cellulolytic activity, shelf life of concentrate feed, and poor

consistency of feed in high and low temperatures (Church, 1988; Dukes and Reece, 2004).

Microbial modification of dietary fatty acids through biohydrogenation has been described as a mechanism of rumen microbes to detoxify the toxic effect of unsaturated fatty acids on them (Harfoot and Hazlewood, 1997; Maia et al., 2010). Alternative suggestions as to the role of biohydrogenation in both the rumen environment and in the physiology of the residing bacteria include serving as a means of disposing reducing power (hydrogen sink) because of the lack of oxygen in the rumen (Lennarz, 1966), which limits metabolic options (Church, 1988; Harfoot and Hazlewood, 1997).

Rumen bacteria involved in the biohydrogenation pathway have been grouped into two classes, A and B depending on their metabolic pathway (Kemp et al., 1984) and both are needed to achieve total hydrogenation of PUFA. Generally group A bacteria hydrogenate PUFA to trans 18:1 and group B converts trans18:1 to stearic acid (Bauman et al., 2003). The process involves the isomerizaion of cis 12 double bond to trans 11 (yielding a conjugated fatty acid), a reduction of cis 9 double bond and finally, hydrogenation of the trans 11 double bond to stearic acid.

A number of factors affect the extent of biohydrogenation in the rumen, mainly the concentration of fat in the diet, and composition of the diet (Leat, 1977). One theory explaining the inhibitory effect of lipids on rumen microbes is the coating of microbes by lipids, denying them access to attach to feed particles thereby preventing hydrolysis and metabolism. Other theories have attributed it to

antimicrobial effect of lipids on microbial membrane function thereby altering membrane function, modification of ruminal population associated with fiber digestion and reduction in the availability of Ca needed for microbial function by lipids forming soaps (Jenkins, 1993).

In addition to modifying dietary fatty acids, microbes also synthesize odd chain, iso and anteiso branched chain fatty acid which is deposited in beef tissues. Iso and anteiso-methyl branched chains have the branched point one and two carbon from the penultimate carbon (Christie, 2012). Precursors for synthesizing these lipids originate from endogenous (de novo synthesis) and exogenous sources particularly uptake of dietary fatty acids, mainly PUFA (Bauman et al., 2003; Jenkins, 1993). De novo synthesis yields mainly 16:0 and 18:0 (Jenkins, 1993). Substrates for de novo synthesis of straight odd chain fatty acid are propionic and valeric acid, for straight even chain fatty acid, butyric and caproic and for the branched chain fatty acid isovalerate, isobutyrate and 2- methylbutyrate are primers (Church, 1988; Hobson and Stewart, 1997; Jenkins, 1993). These branched chain precursors are generated from the metabolism of amino acids (Christie, 2012; Hobson and Stewart, 1997). Many microbial lipids are in the trans configuration while most plant unsaturated fatty acids are in the cis form and so beef contains fatty acids in both forms. PUFAs are not commonly synthesized by rumen bacteria, those that exist are as a result of uptake of already formed ones /dietary ones (Jenkins, 1993). Microbes do not store triacylglycerol, and fatty acids present are mostly in membrane phospholipid or as free fatty acids (Church, 1988). As some of these microbes flow out of the rumen and go through intestinal

digestion, PUFAs are released for the animal's use as this is most likely the main source of PUFAs in animal tissues (Dukes and Reece, 2004).

Like other species, ruminants also synthesize fatty acids de novo and this takes place much more in the adipose tissue than in the liver as found in non-ruminants.

Endogenous fatty acids synthesis occurs in the cytosol of fat cells from acetyl Co-A derived from acetate produced during ruminal fermentation. The first step is the conversion of acetyl Co-A to malonyl Co-A in the presence of acetyl Co-A carboxylase. This is the rate limiting step in the synthesis of fatty acids. Subsequent reactions elongate the chain by addition of 2 carbon units (acetyl units) donated by malonyl Co-A (Hillgartner et al., 1995). The synthesis of fatty acid is catalyzed by the fatty acid synthase enzyme complex which is a multifunctional enzyme composed of two identical polypeptide chains each consisting of seven distinct enzyme activities which are necessary for the elongation of fatty acids (Smith, 1994). Even though shorter chain fatty acids can be released, palmitic acid is usually the end product of de novo fatty acid synthesis in animal tissues (Drackley, 2000). This fatty acid constitutes 20-30% of the total fatty acid in the adipose tissue (Rule, 1995). Biosynthesis of fatty acid in ruminants is also influenced by nutrition, metabolites and hormones (Dukes and Reece, 2004)

High insulin concentrations activate both acetyl CoA carboxylase and fatty acid synthase gene, promoting fat storage, as well as citrate and isocitrate which signal increased availability for storage in the form of fat (Drackley, 2000) while glucagon and growth hormones inhibit the activity of these enzymes (Drackley,

2000; Dukes and Reece, 2004). Fatty acid synthesis is reduced with the consumption of diets high in fat and it increases greatly with diets high in carbohydrates (Dukes and Reece, 2004)

Accessory enzymes are involved in further elongation and desaturation of fatty acids and although elongation by addition of acetyl units (two carbons atoms) by malonyl CoA can take place in the mitochondria, the action of fatty acid elongase in the endoplasmic reticulum of the cell seems to predominate (Drackley, 2000; Dukes and Reece, 2004; Lehninger et al., 2005; McDonald, 1988). Oleic acid (18:1), the most abundant fatty acid found in animal tissues is derived from the elongation of palmitic acid (16:0) to stearic acid (18:0) in a reaction catalysed by a fatty acid elongase and a desaturation of stearic acid (18:0) to oleic acid (18:1) by a stearoyl Co-A desaturase enzyme (Drackley, 2000; Lehninger et al., 2005). It inserts a double bond nine carbons from the carboxyl end of the fatty acid molecule (Drackley, 2000). The omega 9 family of fatty acids originate from oleic acid (Dukes and Reece, 2004). Delta 5 desaturase and Delta 6 desaturase are responsible for further desaturation in longer chain fatty acids (De Smet et al., 2004). The desaturase enzyme system found in animals cannot introduce a double bond beyond the 9^{th} carbon from the carboxyl end of the fatty acid chain (Dukes and Reece, 2004).

Fatty acids ingested and synthesized by the body are either incorporated into membranes if the animal is growing, or they are incorporated into triacylglycerol and stored if the animal is not growing and has enough food supply (Lehninger et al., 2005). Most fatty acids found in the body are in esterified form. The initial

step of triacylglycerol biosynthesis involves the transfer of two fatty acyl chains to glycerol 3 phosphate derived from dihydroxyacetone phosphate, an intermediate in the glycolytic pathway, to form diacylglycerol 3 phosphate (phosphatidic acid). Phosphatidic acid can then be converted to a triacylglycerol or a phospholipid. To yield triacylglycerol, phosphatidic acid is hydrolysed by phosphatidic acid phosphatase to yield 1,2 diacylglycerol which reacts with another fatty acyl group to form triacylglycerols (Dukes and Reece, 2004; Lehninger et al., 2005). Unsaturated fatty acids usually occupy the 2^{nd} position on the glycerol backbone (Dukes and Reece, 2004) and removal of fatty acids occupying this position on the glycerol backbone is harder than those at the other positions (1 and 3) (McDonald, 1988) .

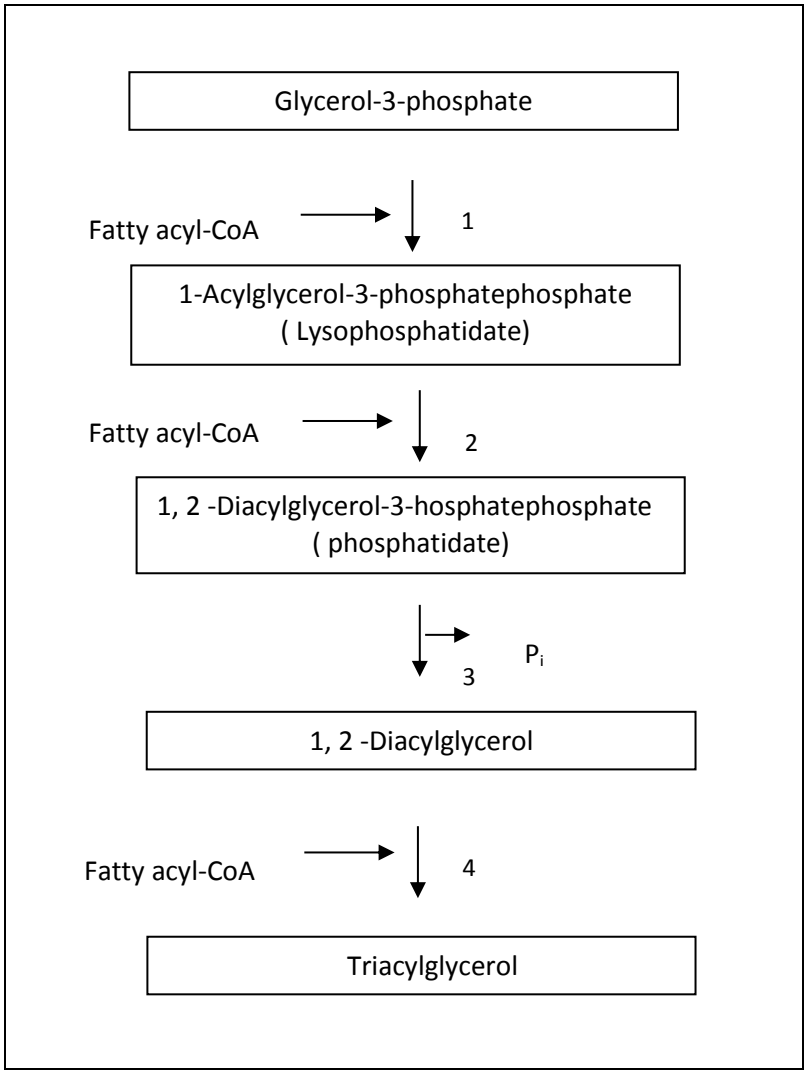

Fig 5. Formation of triacylglycerol.
Enzymes involved are: 1. glycerophosphate acyltransferase (GPAT) 2. lysophosphatidate acyltransferase (LPAAT) 3. phosphatidate phosphohydrolase (PAP) 4. diacylglycerol acyltransferase (DGAT) and Pi, inorganic phosphate. Adapted from Rule (1995).

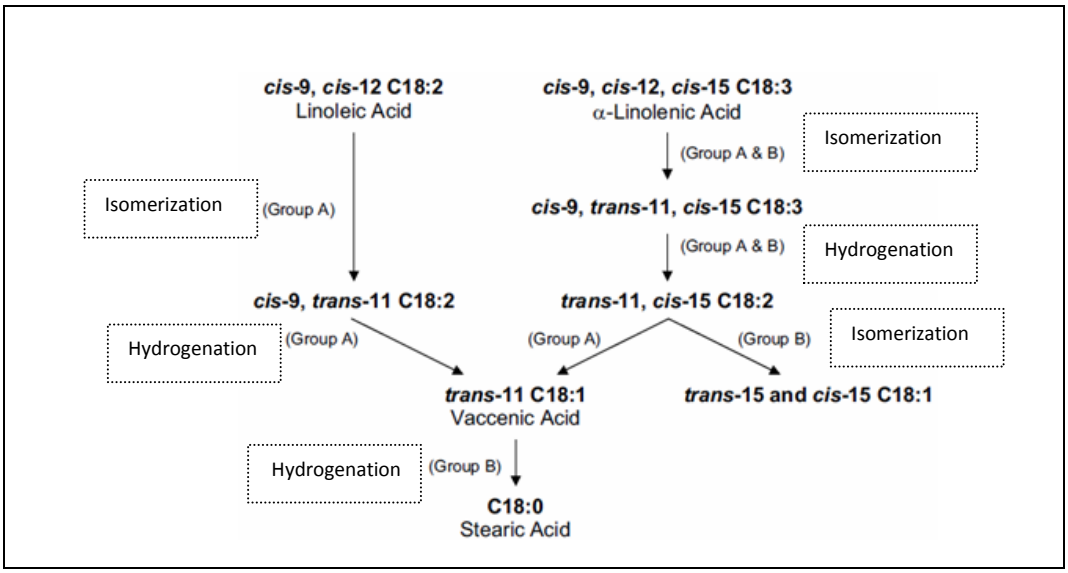

Fig 6. Biohydrogenation of fatty acids in the rumen.

Adapted from Bauman 2003

1.6 Factors Influencing Beef Fatty Acid Composition

Animal Diet:

Change in the rumen environment due to feeding grain can alter the profile of fat deposited by the animal by lowering the pH, causing a reduction in the bacterial population responsible for biohydrogenation and lead to deposition of lower SFA (van de Vossenberg and Joblin, 2003; Wood et al., 1999). Even though a high forage diet increases n-3 content, it also increases the concentration of SFA (Church, 1988; Smith et al., 2009). Feeding diets supplemented with antibiotics may depress microbial activity and increase amount of MUFA deposited in tissues (Church, 1988). Smith et al. (2009) showed that stearic acid was lower and oleic acid higher in 12 month old steers fed a corn based diet than steers that

grazed pasture for 4 months. Expression of the Stearoyl Co-A Desaturase gene, SCD gene was not detected in the pasture fed cattle but was detected in the corn fed steers. Forage is high in omega 3 and it has been reported that omega 3 depresses the expression of the SCD gene in animal tissues which might explain the lower ratio of MUFA/PUFA in the pasture fed cattle (Waters et al., 2009). Daniel et al. (2004) fed concentrate and forage to sheep and reported that there was greater stearoyl CoA to acetyl CoA carboxylase mRNA ratio in the adipose of sheep fed concentrate than the forage fed sheep. Hauseman et al. (2009) suggest elevated levels of trans-10,cis-12 associated with pasture feeding might be responsible for the depression in SCD activity (Chung et al., 2006). Chung et al. (2006) also found that trans-10 cis-12 depressed lipid filling of adipocytes, suggesting that production practices that encourage the accumulation of tran-10 cis-12 would lead to beef with less lipid, more saturated fatty acid and less 9c,11t 18:2 (Smith et al., 2009). Due to the significant effects of diets on the fatty acid composition in beef tissues, designed diet supplements have been a very attractive method to alter the fatty acid composition in beef (Scollan et al., 2014). However, the diet supplement approach sometimes involves the use of formaldehyde which is not permitted in the feed of meat producing animals (Scollan et al., 2006; Scollan et al., 2014).

Animal tissue type:

Oka et al. 2002 compared fatty acid composition of subcutaneous, intermuscular, intramuscular and perinephric fat among steers and showed that fatty acid

composition differed depending on the depot of fat in animal tissues. Subcutaneous adipose tissue had a higher percentage of 14:1, 16:1, 18:1, MUFA, 18:1/18:0 ratio, and lower percentages of 18:0 and SFA than other sites while perinephric fat had lower percentages of 14:1, 16:1, 18:1, 18:2 , 18:1/18:0 ratio, MUFA and PUFA and higher percentages of 18:0 and SFA. This finding is in agreement with established knowledge that MUFA percentages are higher for tissues near the body surface. Fat depots in the different regions of an animal have functions according to the need of the region which may dictate the composition of the fat and amount of different fatty acids deposited in them.

Animal Age:

In their study to determine the effect of breed and age on carcass quality, fatness and fatty acid composition, Ugarković et al. (2013) found no significant effect of age at slaughter on the fatty acid profile of total lipid in the subcutaneous adipose and longissimus muscle of Simmental, Hereford and Chairolais breeds. However, Smith et al. (2009) reported that older cattle have higher MUFA:SFA due to elevated expression and activity of the SCD gene. Malau-Aduli et al.(1997) and (1998) showed that as age increased, concentration of MUFA increased and SFA decreased in the triacylglycerol and phospholipid fraction of both Limousin and Jersey cattle. They reported a general increase in desaturase enzyme activity as animals aged. The effect of age on the fatty acid profile in beef cattle was also confirmed in the study of Huerta-Leidenz et al. (1996) and Chung et al. (2006).

Breed, sex differences and host genetics:

Beef fatty acid composition differs by breed which may be due to the segregation of major genes like the doubled muscle gene in Belgian blue cattle for leanness caused by a mutuation in the myostatin gene (De Smet et al., 2004). Japanese Black (Wagyu) and Korean Hanwoo Cattle which share similar ancestry (Jung, 2003) have a genetic disposition for producing lipids with a higher MUFA concentration than other breeds (Zembayashi et al., 1995). These breeds are known to have extensive marbling and less external fat compared to other breeds as well as a higher MUFA to SFA ratio (May et al., 1993; Mir et al., 2000). The higher MUFA content in the Wagyu cattle reflects an elevated delta 9 desaturase activity (Sturdivant et al., 1992). In comparison to Holstein cattle, Taniguchi et al. (2004) showed that Japanese Black cattle had higher SCD expression levels which may lead to higher MUFA content. Also, Taniguchi et al. (2004) found a mutation in the SCD gene that contributed to higher MUFA in the Japanese Black cattle. Differences have also been reported in the MUFA content of Brahman and Hereford steers raised under similar production conditions and also in the muscle and adipose tissue lipid content of Limousin and Jersey (Huertaleidenz et al., 1993; HuertaLeidenz et al., 1996; Malau-Aduli et al., 1998) suggesting a genetic basis for differences in fatty acid profile among beef breeds.

Generally higher MUFA/SFA ratio has been reported for heifers when compared to steers (Calles et al., 2000; Zembayashi et al., 1995). Heifers have more 18:1 and MUFA than steers (Zembayashi et al., 1995) which might be due to hormonal differences between steers and heifers. Malau-Aduli et al. (1998) associates this

difference in 18:1 concentration with the difference in fatness between steers and heifers and the fact that heifers mature faster physiologically. Zembayashi et al. (1995) showed that fatty acid composition was related to fatness with fatter animals having higher proportions of MUFA. Between steers and heifers fed a forage-based diet, SFA content of total lipids was higher in steers compared to bulls (Eichhorn et al., 1985) and this was attributed to higher fat content in the steers. Gillis et al. (1973) reported higher concentration of linoleic acid in bulls compared to steers suggesting the effect of sex hormones on the enzymes involved in fatty acid metabolism (De Smet et al., 2004).

In addition to breed and sex effects, significant variation of fatty acid content was observed among beef steers of the same breed fed a typical western Canadian finishing diet (Basarab et al., 2007), indicating the influence of the difference of genotypes among animals, i.e. host genes had an effect on the fatty acid composition and that is supported by chromosomal regions or quantitative trait loci (QTL) that were identified to be associated with fatty acid composition in beef cattle (Abe et al., 2008; Alexander et al., 2007; Gutierrez-Gil et al., 2010; Morris et al., 2010; Morris et al., 2007). In parallel to the QTL scan for fatty acids, SNP markers of several genes including SCD (Stearoyl-CoA Desaturase (Delta-9-Desaturase)), FASN (Fatty Acid Synthase), SREBP-1 (Sterol Regulatory Element Binding Transcription Factor 1), FABP4 (Fatty Acid Binding Protein 4, Adipocyte), LXR-ALPHA (Liver X Nuclear Receptor Alpha) also known as NR1H3 (Nuclear Receptor Subfamily 1, Group H, Member 3), ACACA (Acetyl-CoA Carboxylase Alpha), LEP (Leptin), PPARG (Peroxisome Proliferative

Activated Receptor Gamma), THRSP (Thyroid Hormone Responsive Spot 14 Protein), PPARGC1A (Peroxisome Proliferator-Activated Receptor-Gamma Coactivator 1 Alpha), have been reported to have associations with fatty acid composition in beef cattle populations (Abe et al., 2009; Barton et al., 2010; Dunner et al., 2013; Han et al., 2013; Hoashi et al., 2007; Hoashi et al., 2008; La et al., 2013; Li et al., 2012; Li et al., 2010; Ohsaki et al., 2009; Orru et al., 2011; Sevane et al., 2013; Taniguchi et al., 2004a; Zhang et al., 2008; Zhang et al., 2010). A Genome scan of a higher density single nucleotide polymorphisms (SNP) panel of the Illumina BovineSNP50 BeadChip was also carried out by Uemoto et al. (2010) for oleic acid (cis-9 18:1) in the intramuscular fat of Japanese Black cattle, and 32 SNPs were significantly associated with oleic acid. Ishii et al. (2013) performed a genome wide association study (GWAS) for 9 individual and groups of fatty acids in the trapezius muscle of Japanese Black cattle using the Illumina BovineSNP50v2 beadchip and 35 SNPs were significantly associated with various fatty acids. Saatchi et al. 2013, using the 50K SNP panel explained up to 57% of the genetic variance among fatty acids. The associations between DNA variants of animals and their fatty acid composition further indicate the effects of host genes on the fatty acid profiles.

1.7 Heritability of Fatty Acid Concentration in Beef Cattle Tissues

Estimates of heritability of the different individual and groups of FAs in beef cattle are outlined in Table 1-4. These studies have reported a considerable amount of genetic variation for some FAs in beef, with heritability, quantifying

the additive effect of host animal genes on the FA concentrations, ranging from very low (0.00) to very high (0.86) depending on the origin of the FA (Ekine-Dzivenu et al. 2014; Inoue et al., 2011; Kelly et al., 2013; Malau-Aduli et al., 2000; Nogi et al., 2011; Pitchford et al., 2002; Saatchi et al., 2013; Tait et al., 2007; Yokota et al., 2012), indicating genetic influence of host animal on FA composition in beef tissues. Improvement of the FA profile of beef cattle is traditionally carried out by manipulating non-genetic factors primarily through supplements in designed diets (Dugan et al., 2010; Gillis et al., 2004; Mir et al., 2004). However, the genetic influence of host animal genes on the FA composition in beef tissues may offer another opportunity to further enhance the content of beneficial FAs, perpetually and accumulatively, by selecting and breeding genetically superior cattle or by managing cattle based on their genetic potential.

1.8 Phenotypic and Genetic Correlation among FAs and between FAs and Carcass Quality

Genetic selection of cattle for some beneficial FAs may affect the concentrations of other beneficial FAs, as well as carcass and and meat quality. Phenotypic and genetic correlations between FAs and between FAs, carcass traits and meat quality will help design optimal multiple-trait selection index. A wide range of phenotypic and genetic correlation between FAs (0 to 1) was reported by Inoue et al. (2011) in the Musculus trapezius muscle of Japanese Black cattle and they suggested no strong evidence of antagonism on other traits existed to prevent the improvement of beef FA. Genetic correlations between FA and carcass trait was

reported by Tait et al. (2008) in the longissimus muscle of Angus sired cattle in the range of -0.98 to 0.83. Moderate antagonism between percentage kidney, pelvic and heart fat with 9c-18:1 (0.36) and between marbling with 14:0 were observed (0.31). Nogi et al. (2011) reported a range of genetic correlations of -0.28 to 0.39 between FA and carcass merit traits in the longissimus muscle of Japanese Black cattle and concluded that FA and carcass merit traits could be simultaneously improved since there was no severe antagonism between the traits. Garmyn et al. (2011) reported moderate negative phenotypic correlations between marbling and 18:2n-6, 20:4, total PUFA and total n-6 (-0.40, -0.46, -0.38, -0.48), respectively, in the longissimus muscle of Angus sired cattle.

1.9 Phenotypic and Genetic Correlation among FAs and between FAs and Meat Quality

For FAs and meat quality traits, Sevane et al. (2014) reported low phenotypic correlations (0.01 to 0.27 in magnitude) between relative proportions of 18:2n6, n-3, n-6, n-6/n-3 with flavor and juiciness in longissimus thoracis muscle of 15 breeds of European cattle. Garmyn et al. (2011) also reported low phenotypic correlations between FAs and overall tenderness, overall juiciness and beef flavor (0 to -0.22). In Hereford steers, Dryden et al (1970) reported phenotypic correlations between FAs with tenderness, juiciness and flavor in the range of 0 to 0.87 in three beef tissues. In particular, 18:2n6 had moderate to high negative relationship with tenderness, juiciness and flavor (-0.36, -0.74, -0.32 respectively) in the longissimus muscle. In the semimembranosus muscle, phenotypic correlation estimates ranged from 0.01 to 0.71 with positive unfavourable

relationship between 14:0 with tenderness and juiciness (0.35, 0.45), and a negative relationship between 18:2n6 and juiciness (-0.34). In the triceps brachii, phenotypic correlation between FAs and sensory traits ranged from 0.01 to 0.87 with 18:2n6 and tenderness having a negative relationship of -0.37. Oleic acid, 9c-18:1 was highly correlated with flavor in the the longissimus muscle and had low correlation with flavor in the semimembranosus and triceps brachii (0.66, 0.13, 0.19). Phenotypic correlation estimates of Melton et al. (1982) for FAs and flavor score ranged from 0.04 to -0.51 in the neutral and polar lipid fractions of ground beef of Hereford and predominantly Angus breeds. They found a low positive correlation (0.29) between oleic acid and flavor in neutral lipid but high negative correlation between 18:3n3 and flavor in both neutral lipid and polar lipid fraction (-0.51, -0.41). In the longissimus muscle of Hereford steers and heifers, Westerling (1979) reported correlations between FAs with flavor, juiciness and tenderness in the range of 0 to 0.67, 0 to 0.41, 0 to -0.36 respectively. Oleic acid, 9c-18:1 had a high positive correlation of 0.67 with flavor, while 18:2n6 had a high negative correlation of -0.63 with flavor. O'Quinn et al. (2012) reported phenotypic correlations in the range -0.05 to -0.65 between FAs in the beef strip loins of Angus, Holstein and American Wagyu cattle and overall flavor desirability. Total trans 18:1 and 18:3n3 had negative correlations (-0.37, -0.65) with overall flavor desirability but a positive correlation (0.49) between 9c-18:1 with flavor. In the longissimus, semimembranosus and triceps brachii muscle obtained from Korean Hanwoo and Australian Angus beef cattle, Cho et al. (2005) reported a phenotypic correlation of 0.017 to -0.34 for fatty

acids with with tenderness, juiciness and flavor. Total MUFA had a negative association with tenderness juiciness and flavor, while the sensory traits were all positively associated with total SFA.

Althrough fatty acid composition has emerged as an economically important trait, reports on genetic variations, heritability, genetic correlations between FAs with carcass and meat quality traits are still in the early stage and are limited to certain type of fatty acids and a few breeds/populations. In addition, identification of gene variants associated with FA composition in beef cattle has been limited to a few candidate genes.

1.10 Research Hypothesis and Objectives

Our hypotheses are: (1) there are genetic variations of fatty acid contents in beef tissues due to genetic variation of host animals; (2). Genetic correlations of fatty acid contents in beef tissues with carcass and meat quality exist and there is a potential to improve the fatty acid profile of beef cattle without severe antagonism on traits of economic importance like meat and carcass quality traits; (3). Genetic markers of host animals are associated with beef fatty acid profile, which can be identified and used for potential marker assisted selection or genomic prediction to select animals with healthier fatty acid profile. These three hypothesese are tested by conducting the following three studies (i.e. objectives of this study) :

1. To estimate genetic parameters (heritabilities, genetic and phenotypic correlations) for fatty acids in beef tissues to assess the potential for

genetic improvements of fatty acids and evaluate the relationship between fatty acids in the tissue.

2. To estimate genetic and phenotypic correlations between fatty acids in beef tissues with carcass and meat quality trait of economic importance, which would help in exploring relationships between fatty acids with carcass and meat quality traits of economic importance.

3. To carry out a candidate gene association study using single nucleotide polymorphism markers (SNPs) of multiple genes involved in fat metabolism to identify SNP markers singificnaly associated with fatty acid contents in animal tissues..

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Table 1-1. Tissue, breed, ranges of fatty acid means and heritability reported in literature and references. (NR=Not Reported)

Fatty Acids	Tissue	Breed	Range of Mean	Range of Heritability	Reference
10:0	Longissimus muscle Intermuscular fat (L.*dorsi*) Subcutaneous adipose, Kidney fat Omental fat Brisket adipose Pars costalis diaphragmatis	Angus sired Jersey Mixed breed	0.02 - 0.05±0.049	0.11	Saatchi et al. (2013) Jiang et al. (2013) Shah (2006)
12:0	Longissimus muscle Intermuscular fat (L.*dorsi*) Subcutaneous adipose, Kidney fat Omental fat Pars costalis diaphragmatis	Angus sired Jersey Mixed breed	0.016 - 0.062±0.055	0.08	Saatchi et al. (2013) Jiang et al. (2013) Shah (2006)
13:0	Longissimus muscle	Angus sired	0.005±0.01	0.23	Saatchi et al. 2013
14:0	Brisket adipose, Subcutaneous adipose, Intramuscular adipose, Musculus trapezius, Longissimus dorsi, Intermuscular Fat (M. *serratus)* Intermuscular fat (M. *transversalis)* Kidney fat Intermuscular fat (L.*dorsi*) Omental fat Pars costalis diaphragmatic	Angus sired Crossbred Multiple breeds, Japanese Black, Belgian Blue Jersey Mixed breed	2.42 - 4.2±0.8	0.17±0.12 - 0.82±0.1	Saatchi et al. (2013) Ekine-Dzivenu et al. (2013) Kelly et al. (2013) Yokota et al. (2012) Nogi et al. (2011) Inoue et al. (2011) Tait et al. (2007) Pitchford et al. (2002) Webb et al. (1998) Jiang et al. (2013) Shah et al. (2006)

Fatty Acids	Tissue	Breed	Range of Mean	Range of Heritability	Reference
16:0	Intramuscular adipose, longissimus muscle, Musculus trapezius, Intramuscular adipose tissue, Intermuscular Fat (M. serratus) Intermuscular fat (M. transversalis) Kidney fat Intermuscular fat (L.*dorsi*) Subcutaneous adipose, Omental Fat Pars costalis diaphragmatis Brisket adipose	Angus sired, Crossbred, Multiple breeds, Japanese Black Angus sired Multi-breed, Belgium Blue Hereford Jersey Limousine, Devon, Wagyu	23.27-30.20±0.4	0.05±0.12 - 0.65±0.09	Saatchi et al. (2013) Ekine-Dzivenu et al. (2013) Kelly et al. (2013) Yokota et al. (2012) Nogi et al. (2011) Inoue et al. (2011) Tait et al. (2007) Pitchford et al. (2002) Malau-Aduli et al. (2000) Webb et al. (1998) Jiang et al. (2013) Shah et al.(2006)
17:0	Brisket adipose, Subcutaneous adipose, Longissimus muscle, Intermuscular fat (L.*dorsi*) Kidney fat Omental fat Pars costalis diaphragmatis Brisket adipose	Angus sired, Crossbred, Multiple breeds, Japanese Black, Angus sired, Jersey	0.593±0.33 - 1.74	0.13±0.08 - 0.35	Saatchi et al. (2013) Ekine-Dzivenu et al. (2013) Kelly et al. (2013) Nogi et al. (2011) Tait et al. (2007) Jiang et al. (2013) Shah et al. (2006)
18:0	Brisket adipose Subcutaneous adipose Intramuscular adipose, Musculus trapezius, Longissimus dorsi Intramuscular adipose tissue Intermuscular fat (M. *serratus)* Intermuscular fat (M. *transversalis)* Kidney Fat Intermuscular fat (L.*dorsi*) Omental Fat Pars costalis diaphragmatis	Angus sired Crossbred, Multiple breeds, Japanese black, Multi-breed, Belgium Blue Hereford Jersey Limousine Devon Wagyu Mixed breed	6.6±1.2 - 30.82±4.15	0.12±0.11- 0.71±0.1	Saatchi et al. (2013) Ekine-Dzivenu et al. (2013) Kelly et al. (2013) Yokota et al. (2012) Nogi et al. (2011) Inoue et al.(2011) Tait et al. (2007) Pitchford et al. (2002) Malau-Aduli et al. (2000) Webb et al. (1998) Jiang et al. (2013) Shah et al.(2006)

Fatty Acids	Tissue	Breed	Range of Mean	Range of Heritability	Reference
19:0	Subcutaneous adipose	Multiple breeds	0.59±0.24	0.22±0.09	Kelly et al. (2013)
20:0	longissimus muscle Intermuscular fat (L.*dorsi*) Subcutaneous adipose Kidney fat Omental Fat Pars costalis diaphragmatis Brisket adipose	Angus sired, Japanese black Angus sired Jersey Mixed breed	0.02±0.04 - 0.18	0±0.01 - 0.11	Saatchi et al. (2013) Nogi et al. (2011) Tait et al. (2007) Jiang et al. (2013) Shah et al.(2006)
22:0	Longissimus dorsi	Angus sired	0.02±0.06 - 0.11±0.152	0±0.044 - 0.09	Saatchi et al. (2013) Tait et al. (2007)
23:0	Longissimus dorsi	Angus sired	0.069±0.17	0.11	Saatchi et al. (2013)
24:0	Longissimus dorsi	Angus sired	0.143±0.37 - 0.02±0.05	0±0.05 - 0.51	Saatchi et al. 2013 Tait et al. 2007
SFA	Subcutaneous adipose Intramuscular adipose Longissimus dorsi Intermuscular Fat (M. *serratus)* Intermuscular fat (M. *transversalis)* Kidney fat Pars costalis diaphragmatis Brisket adipose Intramuscular (L. *thoracis*) Intermuscular	Multi-breed, Angus Belgium Blue Hereford Jersey Limousine Devon Wagyu Japanese Black Mixed Austriana de los valles Austriana de la montana	32.7±2.7 - 56.76±3.80,	0.07±0.11 - 0.66±0.09	Saatchi et al. (2013) Ekine-Dzivenu et al. (2013) Kelly et al. (2013) Yokota et al. (2012) Nogi et al. (2011) Pitchford et al. (2002) Malau-Aduli et al. (2000) Webb et al. (1998) Shah et al.(2006) Aldai et al. (2007)
iso14:0	NR	NR	NR	NR	NR
iso15:0	NR	NR	NR	NR	NR
ai15:0	NR	NR	NR	NR	NR

Fatty Acids	Tissue	Breed	Range of Mean	Range of Heritability	Reference
iso16:0	NR	NR	NR	NR	NR
iso17:0	NR	NR	NR	NR	NR
ai17:0	NR	NR	NR	NR	NR
iso18:0	NR	NR	NR	NR	NR
BFA	Intramuscular (L.*thoracis*) Intermuscular Subcutaneous	Austriana de los valles Austriana de la montana	0.62 - 1.20	NR	Aldai 2007
SFA+BFA	NR	NR	NR	NR	NR
9c-14:1	Subcutaneous adipose Intramuscular adipose longissimus muscle Musculus trapezius Intermuscular fat (L.*dorsi*) Kidney fat Omental Fat Pars costalis diaphragmatis Brisket adipose	Angus sired Crossbred, Multiple breeds Japanese Black Jersey Mixed breed	0.565±0.20 - 2.40	0.13±0.08 - 0.86±0.1	Saatchi et al. (2013) Ekine-Dzivenu et al. (2013) Kelly et al. (2013) Yokota et al. (2012) Nogi et al. (2011) Inoue et al. (2011) Tait et al. (2007) Jiang et al. (2013) Shah et al.(2006)
9c-15:1	NR	NR	NR	NR	NR
7c-16:1	NR	NR	NR	NR	NR
9c-16:1	Brisket adipose Subcutaneous adipose Intramuscular adipose Musculus trapezius Longissimus dorsi Intramuscular adipose tissue Intermuscular fat (M. *serratus)* Intermuscular fat (M. *transversalis)* Kidney Fat Intermuscular fat (L.*dorsi*) Omental Fat Pars costalis diaphragmatis	Angus sired Crossbred, Multiple breeds, Japanese black, Angus sired, Multi-breed, Belgium Blue Hereford Jersey Limousine Devon Wagyu Mixed breed	3.478±0.71 - 9.0 ±1.4	0.02±0.09 - 0.76±0.09	Saatchi et al. (2013) Ekine-Dzivenu et al. (2013) Kelly et al. (2013) Yokota et al. (2012) Nogi et al. (2011) Inoue et al. (2011) Tait et al. (2007) Pitchford et al. (2002) Malau-Aduli et al. (2000) Webb et al. (1998) Jiang et al. (2013) Shah et al.(2006)

Fatty Acids	Tissue	Breed	Range of Mean	Range of Heritability	Reference
11t-16:1	NR	NR	NR	NR	NR
12c-16:1	NR	NR	NR	NR	NR
7c-17:1	NR	NR	NR	NR	NR
9c-17:1	Subcutaneous adipose Intramuscular adipose longissimus muscle Pars costalis diaphragmatis Brisket adipose	Angus sired Crossbred, Multiple breeds, Japanese Black Mixed breed	0.93±0.22 - 1.85	0.04±0.10 - 0.25	Saatchi et al. (2013) Ekine-Dzivenu et al. (2013) Nogi et al. (2011) Shah et al.(2006) Kelly et al. (2013)
9c-18:1	Brisket adipose Subcutaneous adipose Intramuscular adipose, Musculus trapezius, Longissimus dorsi Intramuscular adipose tissue Intermuscular fat (M. *serratus)* Intermuscular fat (M. *transversalis)* Kidney Fat Intermuscular fat (L.*dorsi*) Omental Fat Pars costalis diaphragmatis	Angus sired Crossbred, Multiple breeds, Japanese black, Angus sired, Multi-breed, Belgium Blue Hereford Jersey Limousine Devon Wagyu Mixed breed	38.6±2.79 - 56.2±2.7	0.09±0.07 0.78±0.09	Saatchi et al. (2013) Ekine-Dzivenu et al. (2013) Kelly et al. (2013) Yokota et al. (2012) Nogi et al. (2011) Inoue et al.(2011) Tait et al. (2007) Pitchford et al. (2002) Malau-Aduli et al. (2000) Webb et al. (1998) Jiang et al. (2013) Shah et al.(2006
11c-18:1	Subcutaneous adipose Longissimus muscle Intermuscular fat (L.*dorsi*) Kidney fat Omental Fat Pars costalis diaphragmatis Brisket adipose	Angus sired Crossbred, Multiple breeds, Jersey Mixed breed	0.1 ±0.11 - 2.47±0.37	0.04±0.11 - 0.21±0.09	Saatchi et al. (2013) Ekine-Dzivenu et al. (2013) Shah et al.(2006) Kelly et al. (2013) Jiang et al. (2013)
12c-18:1	Longissimus muscle	Angus sired	0.26 ±0.162	0.26	Saatchi et al. (2013)

Fatty Acids	Tissue	Breed	Range of Mean	Range of Heritability	Reference
13c-18:1	Longissimus muscle Brisket adipose	Angus sired Crossbred,	0.10±0.10 - 0.75±0.21	0.06 - 0.43±0.10	Saatchi et al. (2013) Ekine-Dzivenu et al. (2013)
14c-18:1	NR	NR	NR	NR	NR
15c-18:1	NR	NR	NR	NR	NR
9c-20:1	Longissimus muscle Pars costalis diaphragmatis Brisket adipose	Angus sired Mixed breed	0.09±0.11 – 0.29	0 - 0.12	Saatchi et al. (2013) Shah et al.(2006)
11c-20:1	NR	NR	NR	NR	NR
9c-22:1	Longissimus muscle	Angus sired	0.01±0.06	0.09	Saatchi et al. (2013)
6t/8t-18:1	Longissimus muscle	Angus sired	0.13±0.19*	0.09	Saatchi et al. (2013)
9t-18:1					
10t-18:1	Longissimus muscle Brisket adipose	Angus sired Crossbred	0.82±0.5 - 3.60 ±1.38*	0.19±0.12 - 0.4	Saatchi et al. (2013) Ekine-Dzivenu et al. (2013)
11t-18:1	Subcutaneous adipose Longissimus muscle Intermuscular fat (L.*dorsi*) Kidney fat Omental Fat Pars costalis diaphragmatis Brisket adipose	Angus sired Crossbred, Multiple breeds, Jersey Mixed breed	0.54±0.16 - 3.36	0.11±0.11 - 0.12±0.08	Ekine-Dzivenu et al. (2013) Shah et al.(2006) Kelly et al. (2013) Jiang et al. (2013)
12t-18:1	Longissimus muscle	Angus sired	0.063±0.128	0.14	Saatchi et al. (2013)
15t-18:1	Longissimus muscle	Angus sired	1.037±0.506	0.14	Saatchi et al. (2013)
16t-18:1	NR	NR	NR	NR	NR
sumtrans18:1	Brisket adipose Intramuscular (L.*thoracis*) Intermuscular (L.*thoracis*) Subcutaneous	Austriana de los valles Austriana de la montana Crossbred	2.30±0.6 - 10.70±0.28	0.11±0.11	Ekine-Dzivenu et al. (2013) Aldai et al. (2007)

Fatty Acids	Tissue	Breed	Range of Mean	Range of Heritability	Reference
MUFA	Brisket adipose Subcutaneous adipose Intramuscular adipose, Musculus trapezius, Longissimus dorsi Intramuscular adipose tissue Intermuscular fat (M. *serratus)* Intermuscular fat (M. *transversalis)* Kidney Fat Intermuscular fat (L.*dorsi*) Omental Fat Pars costalis diaphragmatis Intramuscular (L.*thoracis*) Intermuscular(L.*thoracis*)	Angus sired Crossbred, Multiple breeds, Japanese black, Angus sired, Multi-breed, Belgium Blue Hereford Jersey Limousine Devon Wagyu Mixed breed Austriana de los valles Austriana de la montana	33.31±0.30 - 65.2±2.7	0.06±0.10 - 0.68±0.09	Aldai et al. (2007) Saatchi et al. (2013) Ekine-Dzivenu et al. (2013) Kelly et al. (2013) Yokota et al. (2012) Nogi et al. (2011) Inoue et al.(2011) Tait et al. (2007) Pitchford et al. (2002) Malau-Aduli et al. (2000) Webb et al. (1998) Jiang et al. (2013) Shah et al.(2006
9c,13t/8t,12c-18:2	NR	NR	NR	NR	NR
9c,15c-18:2	NR	NR	NR	NR	NR
8t,13c-18:2	NR	NR	NR	NR	NR
11t,15c-18:2	NR	NR	NR	NR	NR
6t,8t-18:2	NR	NR	NR	NR	NR
7t,9c-18:2	NR	NR	NR	NR	NR
7t,9t-18:2	NR	NR	NR	NR	NR
8t,10c-18:2	NR	NR	NR	NR	NR
8t,10t-18:2	NR	NR	NR	NR	NR
9c,11t/9t,11c -18:2	Subcutaneous adipose Longissimus muscle Intermuscular fat (L.*dorsi*) Kidney fat Omental Fat Pars costalis diaphragmatis Brisket adipose	Angus sired Multiple breeds, Jersey Mixed breed	0.13 ±0.13 - 0.70	0.11 - 0.24±0.09	Saatchi et al. (2013) Shah et al.(2006) Kelly et al. (2013) Jiang et al. (2013)

Fatty Acids	Tissue	Breed	Range of Mean	Range of Heritability	Reference
9t,11t-18:2	NR	NR	NR	NR	NR
10t,12c-18:2	Pars costalis diaphragmatis Brisket adipose	Mixed breed	0.01	NR	Shah 2006
10t,12t-18:2	Longissimus muscle	Angus sired	0.051±0.071	0.12	Saatchi
11t,13c/11c,13t -18:2	NR	NR	NR	NR	NR
11t,13t-18:2	NR	NR	NR	NR	NR
12t,14c/12c,14t -18:2	NR	NR	NR	NR	NR
12t,14t-18:2	NR	NR	NR	NR	NR
sumCLA	Brisket adipose Intramuscular (L.*thoracis*) Intermuscular (L.*thoracis*) Subcutaneous	Austriana de los valles Austriana de la montana Crossbred	0.22±0.01 - 0.59±0.11	0.06±0.10	Ekine-Dzivenu et al. (2013) Aldai et al. (2007)
18:2n-6	Intermuscular Fat (M. *serratus)* Intermuscular fat (M. *transversalis)* Kidney Fat Longissimus muscle Intermuscular fat (L.*dorsi*) Subcutaneous adipose, Kidney fat Omental Fat Pars costalis diaphragmatis Brisket adipose	Angus sired Crossbred Multiple breeds Japanese Black Multi-breed Belgium Blue Jersey Mixed breed	1±0.43 - 7.02±2.99	0.06±0.07 - 0.58±0.09	Saatchi et al. (2013) Ekine-Dzivenu et al. (2013) Kelly et al. (2013) Yokota et al. (2012) Nogi et al. (2011) Inoue et al.(2011) Tait et al. (2007) Webb et al. (1998) Jiang et al. (2013) Shah et al.(2006)
18:3n-3	Intermuscular Fat (M. *serratus)* Intermuscular fat (M. *transversalis)* Kidney Fat Longissimus muscle Intermuscular fat (L.*dorsi*) Subcutaneous adipose, Kidney fat Omental Fat pars costalis diaphragmatis Brisket adipose	Belgian Blue Jersey Mixed breed	0.13±0.16 - 0.79±0.20	0±0.01- 0.14	Saatchi et al. (2013) Kelly et al. (2013) Nogi et al. (2011) Tait et al. (2007) Webb et al. (1998) Jiang et al. (2013) Shah et al.(2006)

Fatty Acids	Tissue	Breed	Range of Mean	Range of Heritability	Reference
18:3n-6	Longissimus muscle Intermuscular fat (L.*dorsi*) subcutaneous adipose, Kidney fat Omental Fat	Angus Sired Jersey	0.014±0.03 - 0.05±0.07	0.03±0.06 - 0.08	Saatchi et al. (2013) Tait et al. (2007) Jiang et al. (2013)
20:2n-6	Longissimus muscle Intermuscular fat (L.*dorsi*) subcutaneous adipose, Kidney fat Omental Fat	Angus Sired Jersey	0.04±0.05* - 0.07±0.06	0±0.06 - 0.07*	Saatchi et al. (2013) Tait et al. (2007) Jiang et al. (2013)
20:3n-3	Longissimus muscle	Angus sired	0.02±0.09 - 2.03±0.98*	0.06 - 0.26±0.10*	Saatchi et al. (2013) Tait et al. (2007)
20:3n-6	Longissimus muscle Intermuscular fat (L.*dorsi*) Subcutaneous adipose, Kidney fat Omental Fat	Angus Sired Jersey	0.12 ±0.15 - 0.45±0.21	0.11- 0.22±0.10	Saatchi et al. (2013) Tait et al. (2007) Jiang et al. (2013)
20:3n-9	NR	NR	NR	NR	NR
20:4n-6	Longissimus muscle Intermuscular fat (L.*dorsi*) subcutaneous adipose, Kidney fat Omental Fat pars costalis diaphragmatis Brisket adipose	Angus Sired Jersey Mixedbreed	0.77±0.39* - 0.99	0.01- 0.14*	Saatchi et al. (2013) Jiang et al. (2013) Shah et al.(2006)
20:5n-3	Longissimus muscle Intermuscular fat (L.*dorsi*) subcutaneous adipose, Kidney fat Omental Fat	Angus Sired Jersey	0.04 - 0.18±0.17*	0.2* - 0.07±0.068*	Saatchi et al. (2013) Tait et al. (2007) Jiang et al. (2013)

Fatty Acids	Tissue	Breed	Range of Mean	Range of Heritability	Reference
22:4n-6	Longissimus muscle Intermuscular fat (L.*dorsi*) subcutaneous adipose, Kidney fat Omental Fat	Angus Sired Jersey	0.06 ±0.14* - 0.28±0.15	0.16* - 0.19±0.088	Saatchi et al. (2013) Tait et al. (2007) Jiang et al. (2013)
22:5n-3	Longissimus muscle Intermuscular fat (L.*dorsi*) subcutaneous adipose, Kidney fat Omental Fat pars costalis diaphragmatis Brisket adipose	Angus Sired Jersey Mixed breed	0.13±0.16* - 0.5±0.25	0.01- 0.16±0.09	Saatchi et al. (2013) Tait et al. (2007) Jiang et al. (2013) Shah (2006)
22:6n-3	Longissimus muscle Intermuscular fat (L.*dorsi*) subcutaneous adipose, Kidney fat Omental Fat	Angus Sired Jersey	0.08±0.16 - 0.1±0.15	0±0.04 - 0.24*	Saatchi et al. (2013) Tait et al. (2007) Jiang et al. (2013)
PUFA	Intermuscular Fat (M. serratus) Intermuscular fat (M. transversalis) Kidney fat Longissimus muscle Intermuscular fat (L.*dorsi*) subcutaneous adipose Omental Fat pars costalis diaphragmatic Intramuscular (L.*thoracis*) Intermuscular(L.*thoracis*) Brisket adipose	Angus sired Crossbred Belgian Blue Multiple breed Japanese Black Belgium Blue Jersey Mixed breed Austriana de los valles Austriana de la montana	1.26±0.38 - 19.76±0.33	0.05±0.08 - 0.47±0.08	Saatchi et al. (2013) Ekine-Dzivenu et al. (2013) Kelly et al. (2013) Nogi et al. (2011) Webb et al. (1998) Jiang et al. (2013) Shah et al.(2006) Aldai et al. (2007)

Fatty Acids	Tissue	Breed	Range of Mean	Range of Heritability	Reference
n-3	Longissimus muscle Intramuscular (L.*thoracis*) Intermuscular(L.*thoracis*) Subcutaneous	Angus sired Austriana de los valles Austriana de la montana	0.34±0.02 - 0.54±0.54	0.28	Saatchi et al. (2013) Aldai et al. (2007)
n-6	Longissimus muscle Intermuscular fat (L.*dorsi*) subcutaneous adipose, Kidney fat Omental Fat Intramuscular (L.*thoracis*) Intermuscular(L.*thoracis*) Brisket adipose	Angus sired, Crossbred Jersey Austriana de los valles Austriana de la montana	1.46±0.22 - 17.86±0.32	0.16±0.13 - 0.19	Saatchi et al. (2013) Ekine-Dzivenu et al. (2013) Jiang et al. (2013) Aldai et al. (2007)
n-6/n-3	Longissimus muscle Intermuscular fat (L.*dorsi*) subcutaneous adipose, Kidney fat Omental Fat Intramuscular (L.*thoracis*) Intermuscular(L.*thoracis*) Brisket adipose	Crossbred Jersey Austriana de los valles Austriana de la montana	7.99±1.21 - 12.68	0.03±0.10	Ekine-Dzivenu et al. (2013) Jiang et al. (2013) Aldai et al. (2007)
P/S	Longissimus muscle Intermuscular fat (L.*dorsi*) subcutaneous adipose, Kidney fat Omental Fat Intramuscular (L.*thoracis*) Intermuscular (L.*thoracis*) Brisket adipose	Angus sired, Crossbred Jersey Austriana de los valles Austriana de la montana	0.06±0.02 - 12.6±4.285	0.21 - 0.47±0.08	Saatchi et al. (2013) Ekine-Dzivenu et al. (2013) Jiang et al. (2013) Aldai et al. (2007)
P/(S+B)	NR	NR	NR	NR	NR
AI	Longissimus muscle	Angus sired,	68.73±8.86 - 0.67±0.08	0.58 - 0.52±0.144	Saatchi et al. (2013) Tait et al. (2007)
Health Index	Brisket adipose	Crossbred	1.49±0.23	0.16±0.12	Ekine-Dzivenu et al. (2013)

CHAPTER 2

ESTIMATES OF GENETIC PARAMETERS FOR FATTY ACIDS IN BRISKET ADIPOSE TISSUE OF CANADIAN COMMERCIAL CROSSBRED BEEF STEERS[1]

2.1 Introduction

It has been widely recognized that the type of dietary fatty acid (FA) has a more profound impact on human health than the amount of fat (Hu et al., 2001; Woodside & Kromhout, 2005). Both fat content and the FA profile of beef products are associated with its taste and flavor (Melton et al., 1982; Smith et al., 2006; Westerling & Hedrick, 1979). Therefore, the FA composition in beef cuts plays a role in determining the healthfulness and eating quality of beef.

Like many other quantitative traits in beef cattle, the composition of FAs in tissues is influenced by both genetic and non-genetic factors and their interactions (Aldai et al., 2010; De Smet et al., 2004; Malau-Aduli et al., 2000; Wood et al., 2008). Traditionally, improvement in the FA profile of beef cattle is primarily focused on the manipulation of non-genetic factors mainly through supplements in designed diets (Dugan et al., 2010; Gillis et al., 2004; Mir et al., 2004). However, the genetic influence of host animal genes on the FA composition in beef tissues may offer another opportunity to further enhance the content of

[1] A version of this chapter has been published. Ekine-Dzivenu et al., (2014) Meat Science, http://dx.doi.org/10.1016/j.meatsci.2013.10.011

beneficial FAs, perpetually and accumulatively, by selecting and breeding genetically superior cattle. Therefore, estimation of heritability and genetic correlations will facilitate the design of effective genetic evaluation and selection programs and/or genetic based diet management to improve the composition of FA profiles in beef cattle.

Several studies have been conducted to estimate the heritability and genetic correlations for FAs in beef cattle. Malau-Aduli et al. (2000) and Pitchford et al., (2002) reported a range of heritability estimates from 0.02 to 0.30 for 14:0, 16:0, 18:0, 9c-16:1, 9c- 18:1, total saturated (SFA), monounsaturated (MUFA), and polyunsaturated (PUFA) FAs in the subcutaneous fat of British crossbred beef cattle. Tait et al. (2007) estimated the heritability for 24 FAs in Longissimus dorsi samples of Angus-sired bulls and steers, and the estimates of heritability ranged from 0.00 to 0.49. Recently, Inoue et al., (2011), Nogi et al, (2011) and Yokota et al. (2012) analyzed the FA composition of trapezius and longissimus dorsi muscles of Japanese black cattle and their estimates of heritability ranged from 0.00 to 0.86. A wide range of genetic correlations, from near 0 to 1 has been reported for a few FAs by Inoue et al. (2011). However, in comparison to other beef carcass and meat quality traits, reports of heritability and genetic correlations for FAs in beef cattle are few (Pitchford et al., 2002) and the estimates of the genetic parameters are not consistent across studies. Therefore, the objective of this study was to estimate the heritability and phenotypic and genetic correlations of 25 major individuals and groups of FAs in the brisket adipose tissue of a Canadian commercial crossbred steer population.

2.2 Materials and Methods

2.2.1 Animals and Management

Two hundred and twenty-three Angus and Charolais based Canadian commercial crossbred steers, which originated from Deseret Ranches near Lethbridge, Alberta, Canada, were used in this study. The steers were part of a study that examined the impact of nonionophore antibiotics on feedlot cattle production (Aldai et al., 2008), and were cared for according to the guidelines set by the Canadian Council of Animal Care (CCAC, 1993). Feeding management, diets, and nonionophore antibiotic treatments were described previously (Aldai et al., 2008). Briefly, steers had similar body weight (198±20 kg) and were randomly assigned to 24 feedlot pens. A barley silage-based grower diet, which consisted of 53.9% barley silage, 37.1% barley, 6.8% supplement, and 2.2% antibiotic premix was fed for 80 days. The steers were subsequently adapted from the silage based grower diet to a grain-based finishing diet using 4 transition diets over a 21-day period. The grain-based finishing diet consisted of 81.1% barley, 9.1% barley silage, 7.5% supplement, and 2.3% antibiotic premix and was fed for 120 days. The steers were randomly assigned to 1 of 5 nonionophore antibiotic treatments, and antibiotic was administered throughout the feeding period and withdrawn 21 days before slaughter. The effect of nonionophore antibiotic treatments on the FA composition was also reported by Aldai et al. (2008).

2.2.2 Animal Tissue Collection and Fatty Acid Analyses

The animals used in this study were slaughtered at 580±34 kg and samples of brisket adipose tissue were collected within 48 h post mortem from each steer, placed in plastic bags, frozen on dry ice and stored at−80°C. Details of FA analyses have been described previously (Aldai et al., 2008). Briefly, brisket adipose tissue samples were freeze-dried and directly methylated with sodium methoxide. The fatty acid methyl esters (FAME) were analyzed by gas chromatography (GC) and silver-ion high performance liquid chromatography (Ag-HPLC) using the methods outlined by Cruz-Hernandez et al. (2004). However, the trans18:1 isomers were further separated using two complementary GC temperature programs instead of a preparatory silver-ion thin-layer chromatography (Ag-TLC) separation combined with GC analyses at 120 °C (Kramer et al., 2008).

The concentrations of FAs were expressed as a percentage of total FAME quantified. Eighty-five fatty acids were quantified and 25 FAs (15 individuals and 10 groups including ratios of FAs) with a concentration greater than 0.5% were selected and analyzed in this study. The 15 individual FAs included 5 SFAs (14:0, 15:0, 16:0, 17:0 and 18:0), 8 MUFAs (9c-14:1, 9c-16:1, 9c-17:1, 9c-18:1, 10t-18:1, 11c-18:1, 11t-18:1, 13c-18:1), 1 branched-chain fatty acid (BCFA), 17:0 ai (anteiso); and 1 PUFA (18:2n−6) while the 10 groups of fatty acids were SFA, MUFA, PUFA, BCFA, SFA + BCFA, sumCLA (conjugated linoleic acid), sum trans18:1, n−6, n−6/n−3 ratio, and Health Index, which were calculated by summing the appropriate components as described in Tables 2-1 and 2-2. The Health Index (HI) (Zhang, Knight, Reecy, & Beitz, 2008), a modification on the

atherogenicity index proposed by Ulbricht & Southgate (1991), was computed as: HI = (Total MUFA+Total PUFA) / (4×14:0+16:0).

2.2.3 DNA Marker Genotyping and Construction of Genomic Relationship Matrix

DNA was extracted from the adipose tissue using the phenol/chloroform/isoamyl alcohol method as described by Sambrook and Russel (2001). The steers were genotyped for 1536 single nucleotide polymorphism (SNP) markers using an Illumina Goldengate Assay. SNPs that had a minor allele frequency less than 5% and/or a genotype missing rate larger than 5% were excluded from analyses. After evaluating the quality of the genotypes, 961 polymorphic SNP markers were selected for further analyses. As the pedigrees of the animals were not available, a genomic relationship matrix was constructed using the SNP genotypes based on the proportion of total loci shared by two individuals (Hayes, Visscher, & Goddard, 2009; VanRaden, 2008), which was defined as: $\mathbf{G} = \mathbf{MM}'/\Sigma 2p_iq_i$, where **M** is an n ×m matrix of the number of animals (n) and number of marker loci (m), and it specifies the marker genotype coefficient at each locus. The SNP marker genotype coefficients for each locus (the ith column in the matrix) were defined as (0-2pi) for genotype AA, (1-2pi) for genotype AB and (2-2pi) for genotype BB; where pi was the frequency of allele A of the SNP, and qi was the frequency of allele B. In $\mathbf{MM}'$ the number of alleles shared by relatives was reported on the off-

diagonals and an individual's relationship with itself was reported on the diagonals.

2.2.4 Estimation of Heritability and Phenotypic and Genetic Correlations

Phenotypic and genetic variances and covariances of FAs were estimated using an animal model implemented in ASReml 3.0 (Gilmour et al., 2009). The animal model included fixed effects of contemporary groups of combinations of antimicrobial (nonionophore) treatment by feedlot pen, random effects of additive polygenic effects with the genomic relationship matrix defined above, and residual effects. A preliminary univariate animal model was fitted for each FA to obtain initial values of variances for subsequent REML bivariate analyses. Pairwise bivariate analyses were performed for each combination of FAs to estimate the variance and covariance components, which were used to calculate the phenotypic and genetic correlations as well as the heritability as implemented in ASReml 3.0. The standard error (SE) for heritability was calculated as in ASReml 3.0 and the standard error for a genetic correlation coefficient between FAs was computed as described by Falconer and Mackay (1996).

$$SE_{(rA)} = \frac{1 - r_A^2}{\sqrt{2}} \sqrt{\frac{SE(h_x^2)SE(h_y^2)}{h_x^2 h_y^2}}$$

2.3 Results and Discussion

2.3.1 Variability of Fatty Acids and Heritability

The descriptive statistics and heritability estimates are presented in Table 2-1 for 15 individuals and 10 groups of FAs with a concentration greater than 0.5% of total FAME. These FAs in total accounted for approximately 95.18% of total FAME in the brisket adipose tissue. The most abundant individual FA in the brisket adipose tissue was 9c-18:1 (40.13%), followed by 16:0 (25.56%), 18:0 (8.92%) and 9c-16:1 (5.60%). Trans-11 octadecenoic (11t-18:1), also known as vaccenic acid, accounted for the lowest proportion of total FAME (0.54%) among the 15 individual FAs analyzed. Among the 10 groups of FAs as a percentage of total FAME, SFAs averaged 40.29%, MUFAs averaged 55.41% and PUFAs averaged 2.81%. Conjugated linoleic acids (CLAs) accounted for only 0.59% of total FAME. In general, the relative proportions of individual FAs 18:0, 9c-18:1, 16:0, 9c-16:1, 14:0, and 18:2n−6, and groups of FAs SFA and MUFA in the beef fat aligned with the reports by other researchers (Pitchford et al., 2002; Wood et al., 2008) in subcutaneous fat of finishing cattle. As reported by others, the concentration of individual and groups of FAs varied considerably, with a CV from 5.3% for MUFA to 61.0% for 10t-18:1. Heritability estimates are presented in Table 2-1 for the 25 FAs. In general, most of the heritability estimates were below 0.20 except for 15:0, 9c-14:1, and 13c-18:1. These three FAs had heritability estimates of 0.31, 0.51, and 0.43, respectively.

Adipose tissue plays an important role in the regulation of energy balance through fat storage and lipolysis to provide the appropriate “fuel” in the form of FAs to

meet the animal's energy requirements (Chilliard, 1993; Laliotis et al., 2010). Lipid metabolism is a complex process, which includes lipolysis of dietary fat and biohydrogenation in the rumen, de novo synthesis of FAs by microbes from carbohydrate precursors, uptake and transport of FAs by host animals, de novo synthesis in host animal tissues, elongation and desaturation of FAs in animal organ and tissues, FA esterification and triglyceride hydrolysis, and FA oxidation or metabolism to other products (Bauchart, 1993; Chilliard, 1993; Jenkins, 1993; Laliotis et al., 2010). The FA composition in adipose tissue is believed to be influenced by multiple factors that are involved in the above complex processes of lipid metabolism. The wide range of estimates of heritability for different FAs in this study reflects, to some extent, the different origins of FAs, and is thus indicative of various additive genetic proportions that host animal genes contribute to the phenotypic variation of the FAs in the adipose tissue. In ruminants, dietary lipids are subjected to lipolysis and the fatty acids to biohydrogenation, a process which converts unsaturated dietary FAs with 18 carbons to a final product, stearic acid 18:0 (Harfoot & Hazlewood, 1997). Stearic acid, 18:0, in adipose tissue can also be produced by elongation from shorter chained SFAs as a result of de novo synthesis by the host animal (Drackley, 2000; Mapiye et al., 2012). In this study, the heritability estimates for the 5 SFAs ranged from 0.05 for 16:0 to 0.31 for 15:0 with other fatty acids ranging from 0.12 to 0.17. In general, the estimates of heritability for the SFAs in this study are lower than those reported by Nogi et al. (2011), Inoue et al. (2011) and Yokota et al. (2012) for 14:0 (0.49 to 0.82), 16:0 (0.40 to 0.65) and 18:0 (0.55 to 0.71) in

Longissimus dorsi samples of Japanese Black cattle whereas heritability estimates for 14:0 and 18:0 are similar to those reported by Malau-Aduli et al. (2000) and Pitchford et al. (2002) for subcutaneous adipose tissue of crossbred beef cattle. Pitchford et al. (2002) reported a heritability estimate of 0.21 for 16:0, higher than our estimate of 0.05. Estimates of heritability for 15:0 and 17:0 were reported by Tait et al. (2007) in Longissimus dorsi samples of Angus beef cattle, and were lower (0.10) and similar, respectively, to our study. Even though the estimates of heritability were not consistent across studies, the genetic variation of SFAs indicates the influence of host animal genes on the concentration of different SFAs in animal tissues, which is supported by several host gene DNA marker association studies. Zhang et al. (2008) identified significant associations between SNPs of the fatty acid synthase gene (FASN) for 14:0, 15:0 and 16:0 of fat in the muscle of American Angus bulls. FASN gene is one of the major genes involved in the FA biosynthetic pathway (Corazzin et al., 2013), and the same FASN gene SNP also showed significant effects on the content of 14:0, 15:0 and 18:0 in brisket adipose tissue of the Canadian crossbred beef steer population (Li et al., 2012). However, the relatively low estimates of heritability also suggest a strong environmental contribution to the variation of SFAs in beef tissue.

Monounsaturated FAs in cattle tissues originate from the desaturation of SFAs in the tissue and partly from the uptake of intermediates including 10t-18:1, 11c-18:1, 11t-18:1, and 13c-18:1 that are generated mainly from microbial lipolysis of dietary FAs and incomplete biohydrogenation of PUFA in the rumen (Harfoot & Hazlewood, 1997). In this study, of the 9 MUFAs examined, myristoleic acid (9c-

14:1) had the highest heritability of 0.51. Similarly, Inoue et al. (2011) observed that the estimate of heritability for 9c-14:1 was the highest (0.86) among the 8 individuals and groups of FAs they analyzed. Myristoleic acid is predominantly produced from its precursor 14:0 through desaturation (Mele et al., 2009; Rioux et al., 2011) suggesting that the amount of 9c-14:1 in beef tissue is more influenced by host genetics than other MUFA's. Indeed, a number of other studies have also shown that genes directly associated with the desaturation of FA's have a significant influence on the 9c-14:1 content of beef tissues (Barton et al., 2010; Li et al., 2012; Narukami et al., 2011; Taniguchi et al., 2004).

Heritability for other 9c-MUFAs including 9c-16:1, 9c-17:1 and 9c-18:1 was in a low range of 0.04 for 9c-17:1 to 0.13 for both the 9c-16:1 and 9c-18:1. Low heritability for 9c-16:1 (0.02 to 0.16) and 9c-18:1 (0.09 to 0.17) was also reported by Malau-Aduli et al. (2000) and Pitchford et al. (2002) for subcutaneous adipose tissue of crossbred beef populations. However, much higher estimates of heritability for 9c-16:1 (0.66 to 0.76) and 9c-18:1 (0.42 to 0.78) were observed by Nogi et al. (2011), Inoue et al. (2011) and Yokota et al. (2012) in Japanese black cattle. This suggests that the activity of Δ9 desaturase differs among beef breeds, a possibility supported by the higher expression of the stearoyl-CoA desaturase (SCD) gene in Japanese black cattle as compared to other breeds (Ohsaki et al., 2007; Smith, Gill, Lunt, & Brooks, 2009; Taniguchi et al., 2004).

Of the intermediates 10t-18:1, 11c-18:1, 11t-18:1 and 13c-18:1 examined, 13c-18:1 showed a higher heritability of 0.43, followed by 10t-18:1 (0.19), 11t-18:1

(0.11) and 11c-18:1 (0.04). A higher estimate of heritability for 13c C18:1 indicates a stronger effect of host genes. Indeed, previous studies have identified that SNPs of the SCD gene have significant associations with 13c-18:1 in brisket adipose tissue of Canadian commercial steers as well as in Spanish breeds (Li et al., 2010; Li et al., 2012). Also a SNP of the FA desaturase 1 gene (FADS1) was found to be significantly associated with the concentration of 13c-18:1 (Han et al., 2013).

Vaccenic acid (11t-18:1) is a naturally occurring trans fat found in red meat and dairy products, and has reported beneficial health effects on human health (Bauman et al., 2004; Lock et al., 2004). However, its heritability estimate is low (0.11). Low heritability was also estimated for 10t-18:1 (0.19). It has been proposed that 10t-18:1 and 11t-18:1 are formed by two distinct rumen bacteria populations in the metabolic pathways of PUFA (Aldai et al., 2008; Kramer et al., 2004). The relative low heritability estimates for 10t-18:1 and 11t-18:1 confirm stronger effects of the rumen and other environmental factors on their concentrations in adipose tissue.

17:0ai is the only individual BCFA that had a concentration of greater than 0.5% in the adipose tissue, and it had a low heritability (0.05) similar to that (0.14) reported by Nogi et al. (2011) in the *longissimus* muscle of Japanese Black cattle. The low heritability estimate for the BCFAs likely reflects the fact that it is primarily synthesized by rumen microorganisms (Drackley, 2000). The main function of BCFAs is to maintain fluidity in the cell membranes of microbes as an

alternative to double-bond FAs (Christie, 2012), and therefore its presence in the tissue is likely influenced by the rumen environment. Linoleic acid (18:2n−6) is one of the individual PUFA that was analyzed in this study and had an estimated heritability of 0.17, which is similar to the estimates of 0.14 to 0.23 reported by Tait et al. (2007) and Yokota et al. (2012) in the muscle fat of Angus and Japanese Black cattle. The low heritability of 18:2n−6 reflects the fact that it cannot be synthesized by the animal and that it arises primarily from dietary sources, and rumen microbes may have stronger effects on the concentration as they convert most PUFA to 18:0 through biohydrogenation. However, Nogi et al. (2011) and Inoue et al. (2011) reported a heritability of 0.34 to 0.58 in the intramuscular fat of muscle of Japanese Black cattle, suggesting that the host genetic influence on 18:2n−6 varies among beef breeds or among tissues when comparing brisket versus intramuscular fat.

The heritability estimates for 10 groups of FAs ranged from 0.03 for n−6/n−3 and BCFA to 0.16 for n−6 and HI. Low estimates of heritability for PUFA (0.05), MUFA (0.17 to 0.20) and SFA (0.27 to 0.30) were also reported by Malau-Aduli et al. (2000) and Pitchford et al. (2002). However, other studies reported that heritability estimates for these groups of FAs were higher with 0.47 for PUFA, 0.35 to 0.66 for SFA and 0.35 to 0.68 for MUFA in Japanese Black cattle (Inoue et al., 2011; Nogi et al., 2011; Yokota et al., 2012). Higher estimates of heritability for major individual components of these three FA groups e.g. 14:0 (0.49–0.82), 16:0 (0.40 to 0.65), 18:0 (0.55 to 0.71), 9c-14:1 (0.60 to 0.86), 9c-18:1 (0.42–0.78), and 18:2n−6 (0.14 to 0.58) were also reported in these

populations, suggesting that these individual FAs are under stronger host genetic influences in Japanese Black cattle.

Estimating heritability of FAs is still at its early stage and we report the first heritability estimates of individual FAs 9c-17:1, 10t-18:1, 11c-18:1, 11t-18:1, and 13c-18:1 and groups and ratios of FAs including n−6, n−6/n−3, BCFA, SFA + BCFA, sum trans18:1, total CLA and HI. Estimates of heritability for the major FAs are different across studies, in particular, when breeds or animal tissues are different, which may suggest difference in the genetic control of fatty acids in different tissues or genetic differences of beef cattle breeds or populations, and therefore the genetic control of host genes on FAs in the animal tissues may be different. However, other factors such as sample sizes and the statistical models used may also contribute to the difference of heritability estimates across studies.

2.3.2 Phenotypic and Genetic Correlations between Fatty Acids

Phenotypic and genetic correlations among individuals and groups of FAs are shown in Table 2-2. Both the phenotypic and genetic correlations between FAs ranged from low (close to 0) to high (near 1) depending on the pairs of FAs under investigation. For the 5 SFAs analyzed, strong positive phenotypic and genetic correlations among 14:0, 15:0 and 16:0 were observed with the phenotypic correlation coefficients ranging from 0.53 to 0.78 and genetic correlation coefficient from 0.71 to 1.0. However, the relationship between the longer chain

SFAs 17:0 and 18:0 was weaker, with a phenotypic and genetic correlation coefficient of 0.24 and −0.25, respectively (Table 2-2). Both longer chain SFAs 17:0 and 18:0 had low genetic and phenotypic correlations, from −0.02 to 0.23, with shorter chain SFAs 14:0. However, longer chain FA 17:0 had an increased genetic correlation with 15:0 and 16:0 (0.71 and 0.66) whereas the genetic correlations of longer chain FA 18:0 with 15:0 and 16:0 were moderate to high but negative (−0.44 and −0.79). Phenotypically, 17:0 was also positively correlated with 15:0 (0.55), possibly because 15:0 is a precursor of 17:0 but its phenotypic correlation with 16:0 was near zero while 18:0 had low phenotypic correlations with both the 15:0 (− 0.19) and 16:0 (0.02). Inoue et al. (2011) also reported high genetic correlations between 14:0 and 16:0 (0.70), but lower genetic correlations of 14:0 and 16:0 with longer chain SFA 18:0 (<0.28). The positive genetic relationships among 14:0, 15:0, and 16:0 may indicate their similar origins of de novo synthesis from carbohydrate, amino acids and volatile FA precursors in animal tissues and organs (Mapiye et al., 2012; Palmquist, 2006). The high and positive phenotypic correlations among 14:0, 15:0 and 16:0 also suggest that environmental conditions had similar effects on the FAs. The high and positive genetic correlation of 17:0 with 15:0 and 16:0 suggests that 17:0 may also be primarily produced by the same de novo synthesis that is regulated by the same host genes and/or host genes in close linkage.

Stearic acid (18:0) can also be derived from shorter chain SFAs through elongation in animal tissues (Drackley, 2000; Lehninger, Nelson, & Cox, 2008; Mapiye et al., 2012). The high but negative genetic correlations of 18:0 with 15:0

and 16:0 suggest that host animal genes that regulate its elongation may have a close links with the genes affecting de novo synthesis of 15:0 and 16:0, and the elongation may lead to a reduction of concentration of 15:0 and 16:0 in adipose tissue. The high and positive phenotypic correlation of 15:0 and 17:0 implies that environmental conditions may have a similar effect on both FAs. The low phenotypic correlations of 18:0 with other SFAs suggest that the environmental conditions influencing 18:0 content in adipose tissue differ from other SFAs owing to the ability of 18:0 to originate from the biohydrogenation of 18:2n−6 and 18:3n−3 by rumen microorganisms.

Saturated FAs can be converted to MUFAs in the adipose tissue of the host animal. SCD gene plays a rate-limiting role in the synthesis of unsaturated FAs by inserting a cis (c)-double bond in the delta 9 position of SFAs. Kim and Ntambi (1999) proposed that palmitic (16:0) and stearic (18:0) acids were the preferred substrates for SCD, being converted to palmitoleic (9c-16:1) and oleic (9c-18:1) acid, respectively. In brisket adipose tissue, 9c-18:1 was the most abundant among MUFA, followed by 9c-16:1. However, these two MUFAs were negatively correlated with genetic and phenotypic correlation coefficients of − 0.97 and − 0.35, respectively. The negative correlation between 9c-16:1 and 9c-18:1 is likely attributable to the high and negative genetic correlation between their precursors 16:0 and 18:0 (− 0.79). 9c-16:1 showed a high and positive genetic correlation with its precursor 16:0 (0.88) but, 16:0 had a negative genetic correlation with 18:0 (− 0.79). These high genetic correlations suggest the linkage of host animal genes or pleiotropic effects of host animal genes on the production

of 16:0, 18:0 and 9c-16:1 through de novo FA synthesis, elongation and desaturation of FAs. The reduced genetic correlations of 9c-18:1 with 16:0 and 18:0 may suggest that host animal genes influencing 9c-18:1 are less likely associated with host genes that involved in the production of 16:0 and 18:0.

Myristoleic acid (9c-14:1) is also a major MUFA and originates from 14:0 through desaturation of 14:0. In this study 9c-14:1 was poorly genetically correlated with 14:0 (0.12), even though the phenotypic correlation was highly positive (0.57). 9c-14:1 had a moderate and positive genetic correlation with 15:0 and 16:0 (0.50, 0.40). Another c9-MUFA, 9c-17:1, was moderately genetically correlated with 9c-14:1 and 9c-16:1 (0.39, 0.50), but its genetic correlation with 9c-18:1 was high and negative (− 0.75). 9c-17:1 was also found to be positively genetically correlated with 14:0 (0.84), 16:0 (0.96), and 17:0 (0.60) but negatively correlated (− 0.53) with 18:0. These correlations were in line with the observation that 18:0 was negatively genetically correlated with 14:0 (− 0.17), 16:0 (− 0.79) and 17:0 (− 0.25), indicating that the concentration of 9c-MUFA in the adipose tissue was influenced by both the genes involved in the synthesis of their substrates as well as those coding for enzymes involved in desaturation. In Japanese Black cattle (Inoue et al. 2011), the genetic correlation of 9c-14:1 with 14:0 was higher (0.51) but the genetic correlation of 9c-14:1 with 16:0 was lower (0.22), suggesting that genetic activities of host animal genes are different in different breeds.

In this study, intermediates of PUFA hydrogenation and rumen lipolysis include 10t-18:1, 11c-18:1, 11t-18:1, and 13c-18:1. These fatty acids, when saturated produce C18:0 (Harfoot & Hazlewood, 1997; Jenkins, 1993). The genetic correlations among the intermediates were at a low range (− 0.25 to 0.22) except for the genetic correlation between 10t-18:1 and 11t-18:1, which showed a strong negative correlation of −0.74. This observation supports the suggestion that two distinct rumen bacteria favor the production of either 10t-18:1 or 11t-18:1 (Aldai et al., 2008; Bauman, Perfield, De Veth, & Lock 2003; Kramer et al., 2004) resulting in a reduction in the concentration of the other. Of the 4 intermediates, 11t-18:1 had a high and positive genetic correlation with 18:0 (0.70) whereas 13c-18:1 was negatively correlated with 18:0 (−0.87) but the genetic correlation coefficients of 10t-18:1 and 11c-18:1 with 18:0 were lower (− 0.33, 0.16), which might indicate other origins of 18:0 in the beef tissue in addition to the origin of 18:0 as an end product of rumen lipolysis and biohydrogenation of PUFA.

Vaccenic acid (11t-18:1), a FA proposed to be beneficial to human health, had negative genetic correlations with the two most harmful saturated FA, 14:0 (−0.36) and 16:0 (−0.84), and a positive genetic correlation with CLA (0.57). Even though 11t-18:1 had a positive genetic correlation with 18:0 (0.7), genetic improvement of 11t-18:1 would possibly lead to a much healthier FA profile because 18:0 is considered to be neutral in terms of its impact on human health (Haumann, 1998).

Linoleic acid (18:2n−6) and 17:0ai are two individual FAs in PUFA and branched FAs, respectively, with the two FAs having a positive genetic correlation close to 1 and a moderate phenotypic correlation of 0.34. In general, these two FAs had similar genetic correlations with other FAs. 17:0ai and 18:2n−6, were both genetically positively correlated with 17:0 (0.45, 0.93) and 18:0 (0.76, 0.68) but had a negative genetic correlation with 14:0 (−0.73, −0.35) and 16:0 (−0.84, −0.88). These correlations reflect to some extent the genetic correlations among the SFAs and also suggest the linkage and/or pleiotropic effects of host animal's genes on the concentration of 17:0ai, 18:2n−6 and the concentrations of SFAs 14:0, 16:0, 17:0 and 18:0 in the beef tissue. Similarly, 17:0ai and 18:2n−6 were both genetically correlated with MUFA 9c-17:1 (0.94, 0.95) but negatively correlated with 9c-14:1 (−0.87, −0.57). However, 18:2n−6 and 17:0ai showed different genetic correlations in terms of scale with 9c-18:1 (0.14 vs. 0.91) and 9c-16:1 (−0.51 vs. 0.15). The genetic correlations of 17:0ai and 18:2n−6 with intermediates 10t-18:1, 11c-18:1, 11t-18:1, and 13c-18:1 were generally moderate (−0.56 to 0.42) except for the genetic correlations of 17:0ai with 11c-18:1 (0.82) and 18:2n−6 with 10t-18:1 (0.97), both were high and positive. As 17:0ai is primarily produced by rumen microorganisms (Kaneda, 1991), the concentrations of 17:0ai, 18:2n−6 and the intermediates in the adipose tissue are likely dependent on the concentrations of those FAs in rumen, as well as processes of host uptake and deposition of the FAs in the adipose tissue. The higher genetic correlation of 17:0ai and 18:2n−6 with the MUFAs and intermediates suggests a stronger co-

effect of host animal genes on the production and storage of the FAs in beef tissue.

The 10 groups of FAs investigated in this study were calculated based on individual FAs, and therefore they, in general, showed stronger phenotypic and genetic correlations with their major component FAs or precursor FA. For example, Sumtrans18:1 had a high and positive phenotypic and genetic correlation with 10t-18:1 (0.89 and 1.0) due to the fact that 10t-18:1 is a major FA of sumtrans18:1 FA in the calculation of the total amount. Total CLA had a positive genetic correlation with 11t-18:1 (0.59), suggesting that 11t-18:1 is a major precursor of CLA. Both 9c,11t-18:2 as the major CLA isomer and its precursor 11t-18:1 have been shown to have a number of potential health benefits including reducing the risk of various forms of cancer, atherosclerosis, diabetes, and having anti-obesity effects (Belury, 2002; Corl et al., 2003; De Smet et al., 2004; Park et al., 1997). The moderately high and positive genetic correlation between CLA and 11t-18:1 suggests a genetic effect of host animal genes in endogenous conversion from 11t-18:1 via delta 9 desaturase activity to 9c,11t-18:2 (Bauman et al., 2003). With the positive genetic correlation, genetic improvement of 11t-18:1 will lead to an increase of CLA in adipose tissue. In addition, CLA showed high and negative genetic correlations with the two most harmful SFAs, 14:0 (−0.74) and 16:0 (−0.65), as well as total SFA (−0.70), but was genetically positively correlated with MUFA (0.7) and PUFA (0.37), indicating that there will be no antagonistic effects in genetically increasing the content of CLA, MUFA and PUFA or decreasing 14:0 and C16:0 in beef cattle.

Although CLA had low phenotypic correlations with MUFAs 9c-14:1, 9c-16:1, 9c-17:1 and 9c-18:1 (−0.01 to 0.26), it showed high and positive genetic correlations with 9c-14:1 (0.55) and 9c-17:1 (0.91), but a negative correlation with 9c-16:1 (−0.54), suggesting a genetic co-regulation of these MUFAs and CLA production. Genetically increasing the concentration of 9c-14:1 and 9c-17:1 will lead to an increase of CLA in the beef tissue. However, CLA had a low genetic correlation with 9c-18:1 (0.19), a FA that confers flavor, juiciness and tenderness on beef, indicating that genetically improvement of CLA will not result in correlated responses in the concentration of 9c-18:1 in the beef tissue.

Furthermore, CLA had a positive genetic and phenotypic correlation with total MUFA (0.7, 0.2), PUFA (0.37, 0.61) and a negative genetic and phenotypic correlation with SFA (−0.7,−0.29). These results imply that selection for increased CLA is expected to also increase other beneficial individuals and groups of FA while reducing individuals and groups of non-beneficial FAs.

SFA and SFA + BCFA had the highest phenotypic and genetic correlation to each other and the correlations are near 1. This is not surprising since the proportion of BCFA in the sum of SFA + BCFA is very low. SFA and SFA+BCFA had negative genetic correlations with MUFAs with total CLA being the highest in scale (−0.70, −0.73), followed by 9c-18:1 (−0.69, −0.68). However, SFA and SFA+BCFA showed moderate to high positive genetic correlation with 9c

16:1 (0.75, 0.77), 9c-17:1 (0.76, 0.83) and 11c-18:1 (0.35, 0.40), likely due to the fact that 9c-16:1, 9c-17:1 and 11c-18:1 are highly negatively correlated with 9c-18:1. SFA and SFA+BCFA were phenotypically and genetically negatively correlated with MUFA (−0.99, −0.99), which is expected as these two groups of FAs are negatively correlated by definition. Therefore, MUFA showed similar magnitude of correlations with other FAs as SFA and SFA+BCFA but in an opposite direction.

Polyunsaturated FAs, however, had a moderate negative genetic correlation with SFA (−0.41) and SFA + BCFA (−0.38) as well as positive but low genetic correlation with MUFA (0.2), and therefore, showed a different pattern of correlations with other FAs in comparison to SFA, SFA+BCFA and MUFA. Phenotypically and genetically, PUFAs were highly and positively correlated with a major individual PUFA 18:2n−6 (0.84 and 0.87). The phenotypic correlation with other individual FAs is in general low to moderate (−0.31 to 0.45). Although dietary PUFAs including 18:2n−6 are subjected to microbial hydrogenation processes and are converted to intermediates such as 10t-18:1, 11c-18:1, 11t-18:1, 13c-18:1 and finally to 18:0 (Harfoot & Hazlewood, 1997), other PUFA such as 20:3n−9 can originate from the elongation and desaturation of oleic acid 9c-18:1 when n−6 and n−3 PUFAs are limited in the diet (Holman, 1981).

Phenotypically and genetically, 18:2n−6, n−6 and PUFA are highly positively correlated with 10t-18:1 (0.45−0.63, 0.86−0.97), reflecting that 18:2n−6 is a

precursor of 10t-18:1 and suggesting that the same host genes or linked genes influence the uptake and storage of 18:2n−6 and 10t-18:1, and therefore their concentrations in the beef tissue. However, 18:2n−6 and PUFA had weak phenotypic correlation with 11c-18:1, 11t-18:1, and 13c-18:1 (−0.14 to 0.23) and the genetic correlation of PUFA and 11t-18:1 was also weak (−0.26), indicating that genetic improvement of either one will not significantly affect the content of another FA in the beef tissue. Phenotypically and genetically, n−6 fatty acids showed a high and positive correlation with their major component 18:2n−6 (0.98, 1). As a result, n−6 showed a very similar pattern of phenotypic and genetic correlations with other FAs in comparison to 18:2n−6. However, n−6 had lower phenotypic and genetic correlations with the n−6/n−3 ratio (0.26, 0.49), likely due to different genetic and environment influences on n−6 and n−3. Therefore, n−6/n−3 had a reduced phenotypic and genetic correlation with 18:2n−6 (0.25, 0.44) and had a different pattern of correlations in terms of sign and magnitude with some FAs in comparison to n−6 and 18:2n−6. In contrast to n−6 and 18:2n−6, n−6/n−3 was genetically positively correlated with harmful saturated FAs 14:0 (0.42), 16:0 (0.90), although their phenotypic correlations were negative and low (−0.36, −0.18). It has been shown that delta 5 and delta 6 desaturase enzymes have a preference in metabolizing the n−3 PUFA over the n−6 PUFA when they are in a ratio of 1:1–4 (n−3:n−6) (Patterson, Wall, Fitzgerald, Ross, & Stanton, 2012).

Health Index was calculated directly from MUFA + PUFA and 14:0 + 16:0, and there were high and negative phenotypic and genetic correlations with its component fatty acid 14:0 (− 0.91, − 0.97), C16:0 (− 0.93, −.85), the two most harmful SFA, and a high and positive phenotypic and genetic correlation with MUFA (0.90, 0.96), and the major MUFA, 9c-18:1 (0.91, 0.81). The phenotypic and genetic correlations of HI with PUFA decreased to 0.23 and 0.48, respectively, as PUFA had a relatively low concentration or weighting factor in the calculation of HI. Health Index was also positively genetically correlated with other beneficial FAs such as total CLA (0.68) and 11t-18:1 (0.32), indicating that a genetic response would be positive when selection is in favor for either of the beneficial FAs.

2.4 Conclusion

In conclusion, results from this study have shown that there is a considerable genetic variation among FAs in beef cattle and hence indicate a potential for genetically improving beef FA profiles through genetic selection and breeding programs. FAs with higher estimates of heritability such as 9c-14:1 indicates a stronger additive genetic effect from host animal genes. However, most of the FAs had low heritability estimates, suggesting stronger environment effects including rumen conditions and/or stronger non-additive genetic effects, e.g. interactions of host animal genes. In ruminants, rumen conditions play an important role in microbial lipolysis and hydrogenation processes as well as de novo synthesis of some FAs. As such, host animal genes may also interact with

genes of microorganisms in the rumen in the production of FAs in addition to the direct effect of the host genes on the concentrations of FAs in the beef tissues. Future investigations on how host animal genes shape the genetic regulation of microbes in the lipolysis, hydrogenation, and de novo synthesis of FAs under different diets would help improve understanding of lipid metabolism in ruminants and it may also provide a means to improve FA profiles in animal tissues by manipulating the interactions of genes between host animal and rumen microbes. Although it may be more feasible to improve the contents of FA with low heritability through manipulating the environment and non-additive genetic factors, genetic improvement by genetic evaluation and selections of superior cattle is still possible with the DNA marker-assisted selection or genome selection for which the genetic merit of FAs for a potential parent can be evaluated through including the DNA marker genotype information or can be predicted solely based on the DNA markers. However, for FAs with low heritability, a larger number of phenotype data needs to be collected and genotyped on high density DNA markers in order to establish a reliable relationship between the DNA markers and the phenotype before effective genome selection can be implemented.

Higher genetic correlations among FAs observed in this study suggest a common origin of the FAs and thus similar biochemical pathways involved in their production as well as the influence of common host animal genes or genes in linkage. Practically, high genetic correlations between two FAs indicate that a correlated genetic response can be achieved when the genetic improvement is made on either one. In general, there was no evidence of antagonism between

groups of beneficial fatty acids, thus suggesting that they can be simultaneously improved. However, genetic correlations do not always correspond to their phenotypic correlations as observed in many pairs of FAs in this study. A high phenotypic correlation between two FAs when their genetic correlation is low suggests a stronger common environment or non-additive gene effect on the two FAs whereas a low phenotypic correlation between two highly genetically correlated FAs indicates the strong different effects of the environments or non-additive effects on the FAs. It is important to point out that the estimates of heritability and correlation coefficients reported in this study as well as in other studies are still rudimentary. With the relatively small sample size used in this study as well as in other studies, relatively larger SEs were observed in the estimates, and the estimation of the heritability and correlation coefficients may also be biased. Future investigations with larger sample sizes in various tissues of different breeds or populations will lead to more accurate estimates of genetic parameters and thus will further enhance our understating of host animal genetic influence on the FA profiles in animal tissues.

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Table 2-1. Descriptive statistics and heritability estimates for 25 individual and groups of fatty acids in brisket adipose tissue of Canadian commercial crossbred beef steers.

Trait[a]	Mean±SD	Min	Max	CV%	Additive genetic variance	Residual variance	Heritability
Saturated							
14:0	3.55±0.65	1.80	5.36	18.3	0.0743	0.3542	0.17±0.12
15:0	0.62±0.11	0.31	0.91	17.7	0.0037	0.0082	0.31±0.12
16:0	25.56±1.86	20.40	30.94	7.3	0.1604	3.2995	0.05±0.12
17:0	1.40±0.23	0.93	2.33	16.4	0.0091	0.0451	0.17±0.11
18:0	8.92±1.5	5.46	13.94	16.8	0.2607	1.9849	0.12±0.11
Monounsaturated							
9c-14:1	1.48±0.51	0.49	3.41	35.5	0.1263	0.1224	0.51±0.11
9c-16:1	5.60±1.11	3.12	8.97	19.8	0.1580	1.0667	0.13±0.11
9c-17:1	1.49±0.25	0.98	2.35	16.8	0.0027	0.0594	0.04±0.10
9c-18:1	40.13±2.89	32.22	48.87	7.2	1.0631	7.3327	0.13±0.12
10t-18:1	0.82±0.5	0.15	3.37	61.0	0.0481	0.2067	0.19±0.12
11c-18:1	2.47±0.37	1.60	3.57	15.0	0.0061	0.1309	0.04±0.11
11t-18:1	0.54±0.16	0.19	1.23	29.6	0.0029	0.0232	0.11±0.11
13c-18:1	0.75±0.21	0.27	1.49	28.0	0.0166	0.0217	0.43±0.10
Polyunsaturated							
18:2n-6	1.26±0.21	0.79	2.02	16.7	0.0069	0.0347	0.17±0.13
Branched fatty acid							
17:0 ai	0.59±0.07	0.35	0.82	11.9	0.0002	0.0045	0.05±0.11
Group fatty acids							
Sum trans18:1	2.30±0.6	0.39	1.13	26.1	0.0393	0.3267	0.11±0.11
SumCLA	0.59±0.11	0.39	1.13	18.6	0.0008	0.0114	0.06±0.10
SFA	40.29±2.94	32.79	49.69	7.3	0.5830	8.0718	0.07±0.11
MUFA	55.41±2.96	46.54	62.68	5.3	0.5476	8.2173	0.06±0.10
PUFA	2.81±0.33	2.00	3.82	11.7	0.0127	0.0943	0.12±0.12
BCFA	1.49±0.21	0.79	2.43	14.1	0.0013	0.0422	0.03±0.10
SFA+BCFA	41.79±3.04	34.34	51.31	7.3	0.5785	8.6247	0.06±0.11
n-6	1.46±0.22	0.92	2.24	17.5	0.0074	0.0404	0.16±0.13
n-6/n-3	7.99±1.21	4.36	11.34	15.1	0.0462	1.4077	0.03±0.10
Health Index	1.49±0.23	0.95	2.28	15.4	0.0086	0.0450	0.16±0.12

[a] The concentrations of fatty acids were expressed as a percentage of total fatty acid methyl esters (FAME) quantified. Only fatty acids with a concentration greater than 0.5% of total FAME are presented. c=cis, t=trans. Sum trans18:1 = 6t/8t-18:1 + 9t-18:1 + 10t-18:1 + 11t-18:1 + 12t-18:1 + 13t/14t-18:1 + 15t-18:1 +16t-18:1. SumCLA (sum of conjugated linoleic acid) = 8t,10c-18:2 + 9c,11t-18:2 + 7t,9c-18:2 + 9t,11c-18:2 + 10t,12c-18:2 + 11c,13t-18:2 + 11t,13c-18:2 +12t,14c-18:2+12c,14t-18:2+ 9c,11c-18:2 + 10c,12c-18:2 +6t,8t-18:2+ 9t,11t-18:2 + 11t,13t-18:2 + 12t,14t-18:2 + 10t,12t-18:2 + 8t,10t-18:2 + 7t,9t-18:2. SFA (sum of saturated fatty acid) = 10:0 + 12:0 + 13:0 + 14:0 + 15:0 + 16:0 + 17:0 + 18:0 + 20:0 + 23:0. MUFA (sum of monounsaturated fatty acid)= 9c-14:1 + 9c-15:1 + 7c-16:1 + 9c-16:1 + 9c-17:1 + 6t/7t/8t-18:1 + 9t-18:1 + 10t-18:1 +11t-18:1 + 12t-18:1 + 13t/14t-18:1 + 15t-18:1 + 16t-18:1 + 9c-18:1 + 11c-18:1 + 12c-18:1 + 13c-18:1 + 14c-18:1 + 16c-18:1 + 9c-20:1 + 11c-20:1. PUFA (sum of polyunsaturated fatty acid) = 18:2n-6 + 18:3n-6 + 18:3n-3 + 20:2n-6 + 20:3n-9 + 20:3n-6 + 20:4n-6 + 22:4n-6 + 22:5n-3. BCFA (sum of branched-chain fatty acid) = iso-14:0 + iso-15:0 + anteiso-15:0 + iso-16:0 + iso-17:0 + anteiso-17:0 + iso-18:0. SFA+BCFA: sum of saturated and branched chain fatty acids. n-6 (sum of omega 6 fatty acids) = 18:2n-6 + 18:3n-6 + 20:2n-6 + 20:3n-6 +20:4n-6 + 22:4n-6. n-3 (sum of omega 3 fatty acids) = 18:3n-3 + 22:5n-3. n-6/n-3: ratio between n-6 and n-3 PUFA. Health Index: (total MUFA + total PUFA) / (4 x 14:0 + 16:0)

Table 2-2. Estimates of phenotypic correlations ±SE (above diagonal) and genetic correlation ±SE (below diagonal) between 25 individual and groups of fatty acids in beef brisket adipose tissue Canadian commercial crossbred beef steers.

Trait[a]	14:0	15:0	16:0	17:0	18:0	9c-14:1	9c-16:1	9c-17:1	9c-18:1	10t-18:1	11c-18:1	11t-18:1
14:0		0.6±0.04	0.78±0.03	-0.12±0.07	-0.02±0.07	0.57±0.05	0.36±0.06	-0.32±0.06	-0.91±0.01	-0.08±0.07	-0.44±0.05	0.25±0.06
15:0	0.71±0.17		0.53±0.05	0.55±0.05	-0.19±0.07	0.39±0.06	0.15±0.07	0.42±0.06	-0.69±0.04	0.33±0.06	-0.27±0.06	-0.09±0.07
16:0	0.85±0.3	1±0.0001		0±0.07	0.02±0.07	0.4±0.06	0.2±0.06	-0.35±0.06	-0.83±0.02	-0.12±0.07	-0.59±0.04	0.15±0.07
17:0	0.23±0.46	0.71±0.18	0.66±0.45		0.24±0.06	-0.37±0.06	-0.55±0.05	0.57±0.04	-0.01±0.07	0.56±0.05	-0.29±0.06	-0.26±0.06
18:0	-0.17±0.5	-0.44±0.32	-0.79±0.36	-0.25±0.54		-0.61±0.04	-0.73±0.03	-0.44±0.05	0±0.07	-0.08±0.07	-0.61±0.04	0.5±0.05
9c-14:1	0.12±0.37	0.5±0.15	0.4±0.37	0.16±0.3	-0.99±0.01		0.77±0.03	-0.04±0.07	-0.57±0.05	-0.17±0.07	0.1±0.07	-0.02±0.07
9c-16:1	0.92±0.09	0.45±0.34	0.88±0.24	-0.39±0.49	0.18±0.89	0.43±0.3		0.1±0.07	-0.35±0.06	-0.23±0.06	0.42±0.05	-0.14±0.07
9c-17:1	0.84±0.27	0.8±0.19	0.96±0.08	0.6±0.57	-0.53±0.72	0.39±0.48	0.5±0.83		0.25±0.06	0.39±0.06	0.43±0.05	-0.56±0.05
9c-18:1	-0.74±0.27	-0.96±0.03	-0.62±0.74	-0.79±0.2	0.35±0.54	-0.43±0.28	-0.97±0.04	-0.75±0.28		-0.06±0.07	0.47±0.05	-0.28±0.06
10t-18:1	-0.2±0.44	0.36±0.3	-0.18±0.85	0.94±0.05	-0.33±0.45	-0.32±0.24	-0.1±0.56	0.86±0.15	-0.09±0.55		0±0.07	-0.39±0.06
11c-18:1	0.45±0.7	-0.62±0.36	0.31±1.08	-1±0.001	0.16±0.92	-0.27±0.49	0.99±0.02	-0.58±0.69	-0.97±0.09	-0.04±0.89		-0.44±0.05
11t-18:1	-0.36±0.49	0.2±0.4	-0.84±0.24	0.42±0.44	0.7±0.32	-0.31±0.3	-0.11±0.67	0.63±0.51	0.57±0.46	-0.74±0.24	0.22±0.88	
13c-18:1	-0.1±0.28	0.13±0.21	0.18±0.39	-0.09±0.29	-0.87±0.12	0.99±0.01	0.1±0.4	-0.02±0.49	-0.18±0.28	-0.07±0.27	-0.18±0.45	-0.25±0.3
18:2n-6	-0.35±0.42	0.31±0.34	-0.88±0.17	0.93±0.07	0.68±0.32	-0.57±0.21	-0.51±0.45	0.95±0.07	0.14±0.58	0.97±0.04	0.42±0.74	-0.49±0.41
17:0ai	-0.73±0.31	-0.48±0.47	-0.84±0.24	0.45±0.7	0.76±0.36	-0.87±0.13	0.15±1.03	0.94±0.16	0.91±0.14	0.17±0.82	0.82±0.31	0.33±0.91
Sum trans18:1	-0.44±0.43	0.6±0.29	-0.76±0.39	0.94±0.06	-0.02±0.64	-0.59±0.22	-0.24±0.69	0.89±0.11	0.25±0.63	1±0.001	0.06±1.06	-0.58±0.44
SumCLA	-0.74±0.41	0.51±0.42	-0.65±0.82	0.65±0.46	-0.39±0.69	0.55±0.31	-0.54±0.78	0.91±0.22	0.19±0.87	-0.24±0.68	-0.06±1.31	0.59±0.57
SFA	0.9±0.13	0.71±0.23	0.63±0.75	0.37±0.56	0.13±0.74	-0.31±0.43	0.75±0.34	0.76±0.51	-0.69±0.42	-0.21±0.61	0.35±1.39	-0.11±0.78
MUFA	-0.94±0.09	-0.78±0.18	-0.47±0.99	-0.54±0.45	-0.35±0.65	0.51±0.45	-1.0±0.0.01	-0.54±0.69	0.62±0.49	0.05±0.69	-0.56±1.14	0.1±0.8
PUFA	-0.55±0.4	0.48±0.32	-0.94±0.11	0.93±0.07	0.16±0.59	-0.22±0.31	-0.8±0.29	0.98±0.02	0.1±0.69	0.86±0.14	0.08±1.16	-0.26±0.54
BFA	-0.44±0.78	0.26±0.77	-0.44±0.83	0.85±0.28	0.82±0.31	-0.57±0.32	0.61±0.83	0.31±0.98	0.25±1.14	0.24±1.03	0.78±0.48	0.58±0.85
SFA+BFA	0.99±0.02	0.71±0.23	0.57±0.84	0.4±0.55	0.21±0.75	-0.42±0.44	0.77±0.33	0.83±0.39	-0.68±0.43	-0.21±0.62	0.4±1.33	-0.08±0.8
n-6	-0.21±0.48	0.21±0.37	-0.89±0.16	0.74±0.22	0.97±0.03	-0.72±0.17	-0.43±0.49	0.91±0.12	0.05±0.62	0.97±0.03	0.43±0.78	-0.47±0.43
n-6/n-3	0.42±0.8	-0.41±0.55	0.9±0.35	-0.51±0.52	-0.39±0.84	-0.23±0.49	0.73±0.55	-0.71±0.83	-0.84±0.41	0.27±0.8	0.97±0.08	-0.57±0.67
Health Index	-0.97±0.04	-0.75±0.15	-0.85±0.36	-0.36±0.41	0.03±0.52	-0.22±0.3	-0.76±0.25	-0.79±0.37	0.81±0.22	0.11±0.48	-0.26±0.87	0.32±0.51

Table 2-2 cont'd. Estimates of phenotypic correlations ±SE (above diagonal) and genetic correlation ±SE (below diagonal) between 25 individual and groups of fatty acids in beef brisket adipose tissue Canadian commercial crossbred beef steers.

Trait[a]	13c-18:1	18:2n-6	17:0ai	Sum trans18:1	SumCLA	SFA	MUFA	PUFA	BFA	SFA+BFA	n-6	n-6/n-3	Health_index
14:0	-0.11±0.07	-0.05±0.07	0.16±0.07	0.02±0.07	-0.01±0.07	0.73±0.03	-0.74±0.03	-0.1±0.07	0.38±0.06	0.74±0.03	-0.09±0.07	-0.36±0.06	-0.91±0.01
15:0	0.01±0.07	0.11±0.07	0.09±0.07	0.31±0.06	-0.12±0.07	0.46±0.05	-0.48±0.05	0.04±0.07	0.2±0.06	0.46±0.05	0.04±0.07	-0.1±0.07	-0.59±0.04
16:0	-0.24±0.06	-0.15±0.07	0.08±0.07	-0.07±0.07	-0.27±0.06	0.84±0.02	-0.81±0.02	-0.31±0.06	0.22±0.06	0.83±0.02	-0.2±0.06	-0.18±0.06	-0.93±0.01
17:0	-0.24±0.06	0.18±0.07	-0.05±0.07	0.47±0.05	-0.36±0.06	0.2±0.06	-0.19±0.06	-0.01±0.07	-0.11±0.07	0.18±0.06	0.12±0.07	0.24±0.06	-0.03±0.07
18:0	-0.74±0.03	0.02±0.07	0.36±0.06	0.16±0.07	-0.18±0.06	0.53±0.05	-0.53±0.05	-0.18±0.07	0.37±0.06	0.54±0.05	0±0.07	-0.05±0.07	-0.17±0.07
9c-14:1	0.63±0.04	-0.11±0.07	-0.08±0.07	-0.21±0.06	0.25±0.06	0.04±0.07	-0.05±0.07	0.06±0.07	0.04±0.07	0.05±0.07	-0.12±0.07	-0.24±0.06	-0.4±0.06
9c-16:1	0.59±0.04	-0.15±0.07	-0.25±0.06	-0.35±0.06	0.26±0.06	-0.2±0.06	0.2±0.06	0.04±0.07	-0.12±0.07	-0.2±0.06	-0.13±0.07	-0.16±0.07	-0.16±0.07
9c-17:1	0.4±0.06	0.08±0.07	-0.34±0.06	0.15±0.07	-0.11±0.07	-0.46±0.05	0.48±0.05	0.11±0.07	-0.41±0.06	-0.48±0.05	0.08±0.07	0.28±0.06	0.39±0.06
9c-18:1	0.12±0.07	-0.1±0.07	-0.25±0.06	-0.18±0.07	-0.01±0.07	-0.76±0.03	0.79±0.03	-0.03±0.07	-0.44±0.05	-0.77±0.03	-0.05±0.07	0.3±0.06	0.91±0.01
10t-18:1	-0.13±0.07	0.63±0.04	0.07±0.07	0.89±0.01	-0.15±0.07	-0.08±0.07	0.03±0.07	0.45±0.05	-0.02±0.07	-0.08±0.07	0.59±0.04	0.29±0.06	0.09±0.07
11c-18:1	0.6±0.04	-0.02±0.07	-0.35±0.06	-0.18±0.06	0.2±0.06	-0.82±0.02	0.82±0.02	0.19±0.06	-0.41±0.06	-0.82±0.02	0.03±0.07	0.11±0.07	0.64±0.04
11t-18:1	-0.35±0.06	0.03±0.07	0.57±0.04	0.03±0.07	0.57±0.05	0.38±0.06	-0.45±0.05	0.23±0.06	0.7±0.03	0.42±0.06	0.02±0.07	-0.51±0.05	-0.28±0.06
13c-18:1		-0.14±0.07	-0.39±0.06	-0.33±0.06	0.27±0.06	-0.57±0.04	0.58±0.04	0.11±0.07	-0.39±0.06	-0.58±0.04	-0.13±0.07	0.02±0.07	0.3±0.06
18:2n-6	-0.13±0.29		0.34±0.06	0.7±0.03	0.13±0.07	-0.07±0.07	-0.04±0.07	0.84±0.02	0.21±0.06	-0.05±0.07	0.98±0.01	0.25±0.06	0.07±0.07
17:0ai	-0.56±0.52	1±0.001		0.37±0.06	0.28±0.06	0.27±0.06	-0.37±0.06	0.36±0.06	0.9±0.01	0.32±0.06	0.33±0.06	-0.17±0.06	-0.21±0.06
Sum trans18:1	-0.17±0.34	0.9±0.14	0.23±0.96		0.05±0.07	0.09±0.07	-0.18±0.06	0.57±0.04	0.32±0.06	0.11±0.07	0.66±0.04	0.11±0.07	-0.02±0.07
SumCLA	0.44±0.4	-0.25±0.79	-0.29±1.2	-0.04±0.91		-0.29±0.06	0.2±0.06	0.61±0.04	0.39±0.06	-0.26±0.06	0.14±0.07	-0.51±0.05	0.19±0.06
SFA	-0.4±0.39	-0.07±0.7	-0.6±0.63	-0.33±0.69	-0.7±0.52		-0.99±0.01	-0.31±0.06	0.41±0.06	1±0.001	-0.13±0.07	-0.2±0.06	-0.91±0.01
MUFA	0.48±0.44	-0.18±0.75	0.47±0.8	0.15±0.81	0.7±0.55	-0.99±0.03		0.17±0.06	-0.52±0.05	-0.99±0.01	0.02±0.07	0.24±0.06	0.9±0.01
PUFA	0.07±0.33	0.87±0.15	0.97±0.05	0.91±0.13	0.37±0.61	-0.41±0.64	0.2±0.82		0.32±0.06	-0.28±0.06	0.85±0.02	-0.09±0.07	0.23±0.06
BFA	-0.81±0.3	0.73±0.39	0.62±1.03	0.74±0.68	-0.63±1.14	-0.02±1.43	-0.17±1.45	0.81±0.39		0.47±0.05	0.19±0.06	-0.43±0.05	-0.38±0.06
SFA+BFA	-0.46±0.41	0±0.72	-0.57±0.68	-0.31±0.71	-0.73±0.5	1±0.001	-0.99±0.02	-0.38±0.67	0.03±1.46		-0.11±0.07	-0.23±0.06	-0.9±0.01
n-6	-0.26±0.31	1±0.01	0.81±0.23	0.97±0.05	-0.46±0.7	0.2±0.75	-0.53±0.64	0.8±0.25	0.77±0.36	0.29±0.75		0.26±0.06	0.12±0.07
n-6/n-3	0.01±0.52	0.44±0.71	0.44±1.13	-0.33±1.16	-0.08±1.47	0.08±1.27	-0.37±1.35	0.14±1.04	0.69±0.93	0.11±1.28	0.49±0.69		0.26±0.06
Health Index	0.05±0.27	0.28±0.49	0.87±0.17	0.36±0.51	0.68±0.44	-0.99±0.01	0.96±0.05	0.48±0.45	0.43±0.85	-0.99±0.01	0.15±0.55	-0.28±0.87	

a The concentrations of fatty acids were expressed as a percentage of total fatty acid methyl esters (FAME) quantified. Only fatty acids with a concentration greater than 0.5% of total FAME are presented. c=cis, t=trans. Sum trans18:1 = 6t/8t-18:1 + 9t-18:1 + 10t-18:1 + 11t-18:1 + 12t-18:1 + 13t/14t-18:1 + 15t-18:1 +16t-18:1. SumCLA (sum of conjugated linoleic acid) = 8t,10c-18:2 + 9c,11t-18:2 + 7t,9c-18:2 + 9t,11c-18:2 + 10t,12c-18:2 + 11c,13t-18:2 + 11t,13c-18:2 +12t,14c-18:2+12c,14t-18:2+ 9c,11c-18:2 + 10c,12c-18:2 +6t,8t-18:2+ 9t,11t-18:2 + 11t,13t-18:2 + 12t,14t-18:2 + 10t,12t-18:2 + 8t,10t-18:2 + 7t,9t-18:2. SFA(sum of saturated fatty acid) = 10:0 + 12:0 + 13:0 + 14:0 + 15:0 + 16:0 + 17:0 + 18:0 + 20:0 + 23:0. MUFA (sum of monounsaturated fatty acid)= 9c-14:1 + 9c-15:1 + 7c-16:1 + 9c-16:1 + 9c-17:1 + 6t/7t/8t-18:1 + 9t-18:1 + 10t-18:1 +11t-18:1 + 12t-18:1 + 13t/14t-18:1 + 15t-18:1 + 16t-18:1 + 9c-18:1 + 11c-18:1 + 12c-18:1 + 13c-18:1 + 14c-18:1 + 16c-18:1 + 9c-20:1 + 11c-20:1. PUFA (sum of polyunsaturated fatty acid) = 18:2n-6 + 18:3n-6 + 18:3n-3 + 20:2n-6 + 20:3n-9 + 20:3n-6 + 20:4n-6 + 22:4n-6 + 22:5n-3. BCFA (sum of branched-chain fatty acid)= iso-14:0 + iso-15:0 + anteiso-15:0 + iso-16:0 + iso-17:0 + anteiso-17:0 + iso-18:0. SFA+BCFA: sum of saturated and branched chain fatty acids. n-6 (sum of mega 6 fatty acids) = 18:2n-6 + 18:3n-6 + 20:2n-6 + 20:3n-6 +20:4n-6 + 22:4n-6. n-3(sum of omega 3 fatty acids) = 18:3n-3 + 22:5n-3. n-6/n-3: ratio between n-6 and n-3 PUFA. Health Index: (total MUFA + total PUFA) / (4 x 14:0 + 16:0).

CHAPTER 3

VARIATION AND HERITABILITY ESTIMATES FOR FATTY ACID COMPOSITION IN SUBCUTANEOUS ADIPOSE AND LONGISSIMUS LUMBORUM MUSCLE OF BEEF CATTLE[2]

3.1 Introduction

Beef can be an excellent source of protein in the human diet and it also contains many essential vitamins and minerals (Biesalski, 2005; Scollan et al., 2006b). However, it is well documented that atherosclerosis and other cardiovascular diseases, cancers and diabetes in humans are correlated with excessive dietary fat intake associated with consumption of red meat including beef (Micha et al., 2010; Pan et al., 2011; Pan et al., 2012). However, clinical studies have showed that the type of dietary fat (or the fatty acid composition) actually has a more profound impact on human health than the amount of fat in the diet (Hu et al., 2001; Woodside and Kromhout, 2005) and the risk of cardiovascular diseases can be moderately reduced by decreasing intake of saturated fatty acids (SFA) and replacing it by a combination of polyunsaturated fatty acids (PUFA) and monounsaturated fatty acids (MUFA) (Michas et al., 2014; Scollan et al., 2006a). Moreover, some preliminary studies in human clinical trials have also shown that some trans fatty acids that are produced naturally by ruminant animals, including

[2] A version of this manuscript has been submitted to BMC Genetics

trans-11 18:1 (vaccenic acid) and cis-9, trans-11 isomer of conjugated linoleic acid (CLA), have a number of potential health benefits (Bassett et al., 2010; Corl et al., 2003; Rainer and Heiss, 2004; Scollan et al., 2006a; Wang et al., 2008). Therefore, increasing the content of beneficial fatty acids and/or reducing the concentration of harmful fatty acids in beef will benefit human health and thus will add values to beef products by enhancing the healthfulness of beef.

It has been a common approach to feed animals with designed diet supplements to improve contents of beneficial fatty acids in beef. For instance, increased PUFA content in beef can be achieved through dietary supplements designed to bypass biohydrogenation of unsaturated FAs by rumen microbes (Demeyer and Doreau, 1999). However some of the approaches often involves the use of formaldehyde which is not permitted in the feed of meat producing animals by some food regulatory authorities, including those in the United States (Chow, 1999; Scollan et al., 2006a; Scollan et al., 2014). In addition, it has the potential of increasing production feeding costs and some of the designed diet supplements have an adverse effect on meat quality in terms of flavor and shelf life of beef (Richardson et al., 2004; Scollan et al., 2004; Wood et al., 2003).

Many studies have reported substantial phenotypic variations of fatty acid content in animal tissues among beef steers fed the same diet (Basarab et al., 2007b; De Smet et al., 2004). This variability offers a great potential to further improve fatty acid profiles by capitalizing on the natural genetic differences among animals through genetic selection. However, our understanding on host genetic control of

variations in fatty acid composition in beef tissues is still rudimentary. To date, reports on estimates of heritability of fatty acid concentration in beef tissues have been limited to certain types of fatty acids of a few beef breeds/populations (Ekine-Dzivenu et al., 2014; Inoue et al., 2011; Kelly et al., 2013; Malau-Aduli et al., 2000; Nogi et al., 2011; Pitchford et al., 2002; Saatchi et al., 2013; Tait et al., 2007; Yokota et al., 2012), and the heritability estimates for certain fatty acids were not consistent across studies. The objective of this study was to estimate heritability of over 80 individual and grouped fatty acids in subcutaneous adipose tissue and longissimus lumborum muscle of Canadian crossbred beef cattle.

3.2 Materials and Methods

3.3 Animals and Management

All animals used in this study were cared for according to the guidelines set out by the Canadian Council of Animal Care (1993). A total of 1366 spring-born heifers and steers from four crossbred beef cattle herds comprising three commercial cow-calf herds in Alberta, Canada and one experimental beef cattle herd at Agriculture and Agri-food Canada (AAFC) Lacombe Research Centre were used in this study. The description of animal populations, the breeding and the animal management were described in previous reports. Briefly, the population consisted of 6 Aberdeen Angus crossbred (ANAN), 93 Charolais–Red Angus crossbred (CHAR), 120 Hereford–Angus crossbred (HEAN), 209 Hereford-Angus-Gelbvieh crossbred (HEANGV) crossbred and 938 Hereford ×

Black Angus × Red Angus × Limousin crossbred animals (TXX). After weaning in each year, calves were assigned to one of four production systems of combinations of calf fed and yearling fed with usage and no-usage of growth implants, i.e. (1) growth implant, calf fed; (2) no growth implant, calf fed; (3) growth implant, yearling fed; (4) no growth implant, yearling fed. Yearling fed animals were backgrounded before entering the feedlot while calf fed animals entered the feedlot immediately after weaning. The calf fed animals were fed a high forage diet in the feedlot for a 27-42 day period of dietary adjustment and were then finished on a high grain diet of 81.4% rolled barley grain and 1.8% protein supplement premix, 8.9% barley silage, and 7.9% grass silage on dry matter basis for 76-112days days. Calf-fed cattle were implanted with 200 mg progesterone (Synovex -S) and 20 mg estradiol benzoate at weaning and subsequently re-implanted with 120 mg trenbolone acetate and with 24 mg estradiol (Revalor-S) 90-100 days before slaughter. Yearling finished cattle were implanted with 200 mg progesterone (Synovex-S) at weaning, and this was re-implanted subsequently at 83, 154 and 240 days after weaning with 200 mg progesterone (Synovex-S) and with 24 mg estradiol Revalor-S 90 days before slaughter. For yearling fed animals, they rotationally grazed alfalfa (*Medicago sativa* L)/meadow bromegrass (*Bromus riparius* Rehm) fall pasture for 52 days. When the snow prevented grazing, they were then placed on a grower diet comprising on a dry matter basis 43.1% barley silage, 41.1% grass hay, 15.8% rolled barley: oat grain mix (60:40) for 192 days and then returned to pasture (summer pasture) for 90 days after which they entered a feedlot. In the feedlot,

they were allowed a 21-23 day adjustment period to adapt to the high grain diet before finishing on a high concentrate diet with 79% barley, and 21% barley silage for 86 days.

3.3.1 Animal Tissue Collection

All animals were targeted to be slaughtered at a constant back fat thickness of 9-10 mm at the 12^{th} and 13^{th} rib. This corresponded to 11-14 months for the calf fed and 19-23 months of age for the year fed cattle. The animals were sent to the abattoir for slaughter at 1-2 week interval in a batch of 14 consisting of 7 implanted and 7 non-implanted cattle. At slaughter, the animals ranged from 330 to 691days old, averaging 474 days (SD=67). After early morning grading (48hr post-mortem), the left striploin (i.e. longissimus lumborum (LL) muscle) of each animals was removed, vacuum packed and then chilled at 2°C. The striploin samples were transported to a lab where approximately 10 grams of LL muscle of striploin of each animal was sub-sampled and about 5 grams of subcutaneous adipose (SQ) tissue was also sub-sampled from the side of the striploin. The LL muscles and SQ adipose tissues were vacuum packed and frozen at -80 C for subsequent fatty acid analyses.

3.3.2 Fatty Acid Analyses

Lipid extraction: The procedure for lipid extraction from the LL muscle tissue was based on the Folch method (Folch et al., 1957) as outlined by Cruz-Hernandez et al. (2004). In brief, the LL muscle samples were freeze dried for at least 3 days to remove all traces of water. Approximately 1g of tissue was cut from each sample while frozen, ground into powder and weighed in a glass test tube. To each tube 20 mL chloroform / Methanol ($CHCl_3$ / MeOH) mixture (2:1, v/v.) was added, inverted several times, and then incubated in the dark for 1 hour. The tubes were centrifuged at 2000 rpm for 5 minutes, then 15mL was removed into a new tube, and 3mL of 0.9% saline was added. All tubes were then mixed by inversion and spun at 2000 rpm for 5 minutes. From this, the entire top layer and interface was discarded to remove any possible contamination, and 8 mL of the bottom layer was placed into a new pre-weighed tube. These samples were then put in a dry bath at 40°C and dried under a constant stream of nitrogen gas. The tube was then weighted and the total lipid per sample was calculated. Subsequently, each dried lipid sample was dissolved in enough $CHCl_3$ to give 5 mg / 50µl [0.1mg/µl] of lipid. For the SQ adipose tissues, lipid was extracted based on the procedures in Dugan et al. (2007) and Cruz-Hernandez et al. (2004). In brief, adipose tissue samples were freeze dried to remove all water and kept at -80°C. Approximately 0.1g adipose tissue was cut on dry ice from each frozen sample and weighed in a pre-weighed glass test tube. $CHCl_3$ was added to give a

concentration of 1mg / 10µl of fat and the samples were incubated at room temperature for 1 hour to extract all lipid from the tissue.

Derivatization of fatty acids: From each lipid sample 250 µL was removed and added to a test tube containing 2 mL of 0.5 N Sodium Methoxide. Each tube was carefully mixed and incubated at 50°C for 15 minutes. After cooling to room temperature, 0.1 mL water, 3mL hexane, and 0.5 mL of 5.0 mg/mL methyl-C23 as an internal standard was added to each sample. These fatty acid methyl esters (FAME) were then mixed vigorously by hand for 30 seconds and centrifuged at 2000 rpm for 2 minutes. The top layer was then carefully removed and added to a 2 mL vial containing 30 mg anhydrous sodium sulfate to remove all traces of water, and subsequently 600 µl of the solution was transferred to a gas chromatography (GC) vial containing 400 µl hexane for GC and silver-ion high performance liquid chromatography (Ag+HPLC) analysis.

Fatty acid quantification: Separation and quantification of derivatized fatty acids was achieved using two complementary GC programs as outlined in Kramer et al. (2008) and by Ag+HPLC as described in Cruz-Hernandez et al. (2004). To summarize, the first GC program was 45°C for 5 minutes, 13°C/minute to 175°C for 27 minutes, followed by 4°C/minute to 215°C for 35 minutes. The second GC program was 45°C for 4 minutes, 13°C/minute to 150°C for 47 minutes followed by 4°C/minute to 215°C for 30 minutes. Both programs were run with a split ratio of 19:1 and hydrogen as a carrier gas at a flow rate of 1-1.3mL/minute. FAMEs were then identified using Nu-Check standard 463 (Nu-Check Prep Inc. (Elysian,

MN, USA). Analysis of conjugated linoleic acid (CLA) was conducted using in series three ChromSpher 5 Lipids (250 x 4.6 mm id; 5 μm particle size) analytical, silver ion-impregnated columns (Varian Inc.). The mobile phase was hexane containing 0.1% acetonitrile and 0.5% diethyl ether at a flow rate of 1.0 mL/minute. The mobile phase was made fresh daily and mixed continuously using a magnetic stir plate. The UV detector was set for an absorbance of 233nm as the optimal wavelength for detecting all CLA isomers in a single run which lasted 80 minutes per sample. The CLA isomers were identified using standard no. UC-59M from Nu-Chek Prep Inc. as it contained all four positional CLA isomers. Each individual fatty acid component was quantified as a percentage of total FAME. Concentrations of grouped fatty acids were obtained by summing up the percentages of individual fatty acids within the fatty acid group (Table 3-1). In addition, a Health Index (Zhang et al, 2008), a modification of the atherogenicity index proposed by Ulbricht and Southgate (1991), was computed as HI = (total MUFA + total PUFA) / (4 x C14:0 + C16:0).

3.3.3 Statistical Analysis

A univariate animal model analysis was conducted to estimate variance components for each trait. The univariate animal model can be written as follows:

$$\mathbf{Y}=\mathbf{Xb}+\mathbf{Za}+\mathbf{Wc}+\mathbf{e},$$

Where **Y** is the vector of phenotypic observations, i.e., contents of the fatty acid for individual animals. **b** is the vector of fixed effects including breed types

(ANAN, CHAR, , HEAN, HEANGV, TXX), production systems (4 combinations of calf fed and yearling fed with two growth implant usages: yes or no), gender, a fixed linear covariate effect of slaughter age, diet energy content, and number of days between slaughter and fatty acid extraction. For analyzing fatty acids in the LL tissue, marbling score (intramuscular fat content) was included as an additional fixed linear covariate. **a** is the vector of animals' additive genetic effects, **c** is a vector of random contemporary group effects (combinations of feedlot test locations, feedlot pens and feedlot test years), $\boldsymbol{e}$ is a vector of random residual effects, **X**, **Z** and **W** are known design matrices relating the phenotypic values to the fixed effects, random additive genetic effects and random contemporary group effects, respectively. The random effects **a**, **c** and **e** were assumed to follow a normal distribution with means equal to 0, which leads to E(**Y**) = **Xb**. The variance–covariance matrix for the random effects is described as below:

$$\text{var}\begin{bmatrix}\boldsymbol{a}\\ \boldsymbol{c}\\ \boldsymbol{e}\end{bmatrix}=\begin{bmatrix}\boldsymbol{A}\sigma_a^2 & 0 & 0\\ 0 & \boldsymbol{I}_{n_c}\sigma_c^2 & 0\\ 0 & 0 & \boldsymbol{I}_{n_e}\sigma_e^2\end{bmatrix}$$

where **A** is the additive genetic relationship matrix constructed based on pedigree of one generation, σ_a^2 is the additive genetic variance, $\mathbf{I}_{\boldsymbol{n_c}}$ is the identity matrix with dimension $n_c x\, n_c$, where n_c is the number of random contemporary groups and σ^2_c is variance of random contemporary group effect. $\mathbf{I}_{\mathbf{n_e}}$ is an $n_e x\, n_e$ identity matrix where n_e is the number of animals with records and σ_e^2 is the

residual variance. Covariance between **a** and **c**, **a** and **e**, **e** and **c** were considered 0. The variances for each trait were estimated by restricted maximum likelihood as implemented in the ASReml version 3.0 software package (Gilmour et al., 2009) and were used to calculate heritability and its standard error (SE) for each trait. Heritability (h^2) was calculated as: $h^2 = \sigma_a^2/\sigma_p^2$, where phenotypic variance for each trait was computed as $\sigma_p^2 = \sigma_a^2 + \sigma_c^2 + \sigma_e^2$.

3.4 Results and Discussion

3.4.1 Variation of Fatty Acid Concentrations in Beef Cattle Subcutaneous Adipose (SQ) and Longissimus Lumborum (LL) Muscle Tissues

In total, 81and 83 individual and grouped fatty acids (FA) were analyzed and quantified in the SQ adipose and LL muscle tissues, respectively, for each of the 1366 animals. The descriptive statistics are reported in Table 3-1. In the SQ tissue, the concentrations of individual FAs ranged from 0.0023% (18:3n-6) to 37.92% (9c-18:1) and from 0.0017% (8t,10t-18:2) to 36.68% (9c-18:1) in the LL tissue. The most abundant individual FA in both tissues is 9c-18:1 (oleic acid), comprising 37.9% of total individual fatty acids in SQ and 36.7% in LL muscle followed by 16:0 (25.09% SQ, 24.61% LL) and 18:0 (10.54% SQ, 12.41% LL). The relatively higher concentrations of the three individual fatty acids over other fatty acids are consistent with the fatty acid profile reported in other studies for subcutaneous adipose tissue (Pitchford et al., 2002, Kelly et al., 2013), various muscle tissues (Tait et al., 2007, Inoue et al., 2011, Nogi et al., 2011, Yokota et

al., 2012, Saatchi et al., 2013), and brisket adipose tissue of beef cattle (Ekine-Dzivenu et al., 2014). Gamma linoleic acid (18:3n6) in SQ and conjugated linoleic acid (CLA) isomer, 8t,10t-18:2, in LL had the least concentration of approximately 0.002% of all individual FAs quantified in the tissue. In general, contents of most individual fatty acids are comparable between the two tissues (Table 3-1). However, the substantial differences in concentrations of some PUFA were observed between the tissues ($p<0.001$). Eicosapentaenoic acid (EPA, 20:5n3), and docosahexaenoic acid (DHA, 22:6n3) are two FAs that were not detectable in the SQ tissue, most probably due to very low concentrations, while in the LL muscle the contents of 20:5n3 and 22:6n3 were 0.0291% and 0.046%, respectively. Amounts of 18:3n6, 8t,10t-18:2, 20:5n3 and 22:6n3 in beef tissues are only reported in a few studies with equally low concentrations. Saatchi et al. 2013 reported a concentration of 0.014% for 18:3n6 in longissimus muscle of Angus sired beef cattle and Li et al. (2014) reported a concentration of 0.002% for 8t,10t-18:2 in brisket adipose tissue of Canadian crossbred beef cattle. Similarly, in the study carried out by Warren et al. (2008) using a population of Aberdeen Angus and Holstein–Friesian cattle fed grass silage or concentrate diet, EPA (20:5n3) and DHA(22:6n3) were only detected in the phospholipid fraction of lipids of the longissimus muscle with concentrations ranging from 0.39% - 4.15% for 20:5n3 and 0.07% -1.13% for 22:6n3 but not in the neutral lipid fraction (adipose) of the muscle. Scollan et al. (2001) detected no 20 or 22 carbon PUFA in the subcutaneous adipose tissue of 32 Charolais steers fed different sources of long chain PUFA. It is suggested that these FAs are conserved in the phospholipid

fraction of muscle lipids where they are not likely to be used for energy production because of their metabolic roles (Wood et al., 2008b). However, Jiang et al. (2013) detected and quantified DHA (0.05%) in the subcutaneous fat of Jersey steers but not EPA. Individual PUFAs 18:2n-6, 18:3n-3, 20:2n-6, 20:3n-6, 20:3n-9, 20:4n-6, 20:5n-3, 22:4n-6, 22:5n-3 were also quantified in this study and alpha Linoleic acid, 18:2n6 was the most represented in both tissues (1.8761% in SQ and 4.3867% LL). All individual PUFAs as well as their precursor fatty acids were more abundant in the LL muscle (Table 3-1). While there was not so much difference between the quantity of 18:3n3 in both tissues (0.2113% vs. 0.2969%), the concentration of 18:2n6 in the LL muscle was, however, more than 2 folds higher compared with its amount in the SQ tissue (4.3867% vs. 1.8761%). This agrees with what has been observed about the preferential deposition of 18:2n6 in the muscle and the near equal partitioning of 18:3n3 into muscle and adipose (De Smet et al., 2004; Wood et al., 2008a). In muscle, phospholipids are important components of cell membranes and are rich in PUFA because it ensures the fluidity of cell membranes necessary for cell functionality and shape changes that come with cell growth (Nelson et al., 2008). Higher contents of PUFA, n-6 and n-3 in the muscle relative to intermuscular and subcutaneous adipose tissues was also reported by Aldai et al. (2007) in Asturiana de la Montana and Asturiana de los Valles cattle breeds with and without the double muscling (mh) allele, while reports from Jiang et al. (2013) also showed a higher concentration of total PUFA, total n-6, 18:2n6, 20:3n6, 20:4n6, 20:5n3 in the muscle, with no significant difference between quantities of 18:3n3, 18:3n6, 20:2n6, 22:4n6, 22:5n3, 22:6n3

in both the muscle and intermuscular, subcutaneous, kidney and omental fat tissues.

Trans fatty acids result from the isomerization and biohydrogenation of PUFAs in the diet of the animal by rumen microorganisms (Bauman et al., 2000). Of the trans fatty acids, 10t-18:1 (trans-10 octadecenoic acid) has been referred to as the most common trans 18 isomer accounting for up to 60-80% of total trans FAs in milk and meat (Bauman et al., 2004a; Craig-Schmidt, 1998; Emken, 1995). In the present study, 10t-18:1 was the most abundant individual monounsaturated trans fatty acids accounting for about 65% of the total trans FAs in the SQ tissue and about 62% in the LL tissue while trans vaccenic acid 11t-18:1 only accounted for about 12% (SQ) and 14% (LL) of the total trans FA. This may be a reflective of the ruminal bacterial population and biohydrogenation pathway due to decreased rumen pH resulting from feeding high concentrate diets (Bauman et al., 2003; Griinari and Bauman, 1999). Indeed, Dugan et al. (2007) also reports a large difference between the proportion of 10t-18:1 isomer and 11t-18:1 isomer (2.13% vs, 0.77% of total lipid) in the subcutaneous adipose tissue of beef cattle fed a typical western Canadian feedlot diet consisting more than 70% barley grain.

CLA isomers are proposed to originate during ruminal biohydrogenation of linoleic acid and are endogenously synthesized from its substrate trans vaccenic acid (11t-18:1) through the action of the SCD enzyme in inserting a double bond at the delta 9 position of the trans vaccenic acid molecule (Griinari and Bauman, 1999) . Elevated gene expression and enzymatic activity of the Stearoyl CoA desaturase gene (SCD) has been reported in SQ adipose tissue of cattle in

comparison to the muscle (Cameron et al., 1994; Chang et al., 1992; Smith, 1995), and the predominant gene expression in adipose tissues might contribute to higher concentrations in the SQ tissue for all CLA isomers quantified in this study in comparison to their contents in the LL muscle tissue (Table 3-1).

Individual fatty acids are summed up into their respective groups of fatty acids with MUFA being the most abundant in both tissues (52.9% in SQ and 48.6% in LL), followed by SFA (41.60% in SQ and 42.42% in LL). In general the concentrations of grouped fatty acids between the two tissues, when they are compared, follow a similar trend of the major individual fatty acids within the fatty acid group. As a result, the LL muscle contained a higher total PUFA with an average concentration of 6.70% in comparison to 2.29% in SQ. The concentration of total CLA in the SQ tissue almost doubled the concentration in the LL tissue (0.70% vs. 0.40%).

In general, the relative concentrations of individual and group FAs quantified in this study were comparable or within the ranges of their relative concentrations observed in other studies for the respective tissues (Saatchi et al., 2013, Tait et al., 2007, Yokota et al., 2012, Nogi et al., 2011, Kelly et al., 2013, Inoue et al., 2011, Pitchford et al., 2002, Ekine-Dzivenu et al., 2014) However, for 11t- 18:1, its concentration of 0.5455% (SQ) and 0.4405% (LL) (Table 3-1), is much lower than a concentration of 3.33% in the subcutaneous fat of a population of multiple breeds of cattle in Australia (Kelly et al. 2013), which may also be attributable to other factors including breed type, diet, and animal management.

Phenotypic variations were observed for all fatty acids in both the SQ and LL tissues with coefficient of variation (CV) (calculated based on the mean and SD in Table 3-1) ranging from 6.8% (MUFA) to 268.5% (18:3n-6) with an average 43.76% in SQ and from 5.5% (MUFA) to 199.8% (6t,8t-18:2) with an average CV of 33.42% for FAs in the LL muscle tissue. Relatively larger values of CV were also observed for fatty acids with low concentrations and were also reported by Saatchi et al. (2013), which is likely due to increased sensitivity of measurement to random variations in the quantification process for fatty acids with lower concentrations.

3.4.2 Heritability Estimates of Fatty Acids in Beef Cattle Subcutaneous Adipose (SQ) and Longissimus Lumborum (LL) Muscle Tissues

Additive genetic variances and estimates of heritability for the FAs quantified are presented in Table 3-1 for the SQ and LL muscle tissues. Heritability estimates of the 81 individual and grouped fatty acids in the SQ ranged from 0 (7c-17:1, 12t-18:1, 6t,8t-18:2, 9t,11t-18:2, n-6/n-3,) to 0.64 ± 0.11 (12:0) with 34 fatty acids having heritability estimates below 0.20 (Table 3-1). Heritability estimates of the 83 FAs in the LL muscle were from 0 (7c-17:1) to 0.68 ± 0.1 (9c-16:1) with heritability estimates of 45 FAs below 0.20 (Table 3-1). The heritability estimates for the same FAs in both tissues are generally consistent with Person correlation and Spearman rank-order correlation coefficients of 0.595 ($P<0.001$) and 0.596 ($P<0.001$), respectively.

Fatty acid composition in animal tissues of ruminants are believed to be influenced by multiple factors that are involved mainly in lipolysis and biohydrogenation of dietary fat by rumen microbes, de novo synthesis of FAs by microbes in the rumen, absorption and transport of FAs by the host animal, de novo synthesis, elongation and desaturation of FAs in the host animal's tissues, esterification of FA to triacylglycerol (TAG), oxidation or FA or metabolism to other products (Chilliard, 1993; Jenkins, 1994; Jenkins, 1993; Laliotis et al., 2010). The wide range of heritability estimates for FAs in this study reflects, to some extent, different origins of the FAs and thus also reflects the direct genetic controls of the host animal's genes on variations of fatty acid concentrations in these tissues.

Heritability for the concentration of 12 SFAs was estimated for both the SQ and LL tissues in this study. In general, medium length even-numbered SFAs 10:0, 12:0, 14:0, 16:0 18:0 were more heritable (0.28±0.09 to 0.64±0.11for SQ and 0.28±0.09 to 0.67±0.11 for LL) than medium length odd-numbered SFAs 13:0, 15:0, 17:0, 19:0 (0.07±0.04 to 0.43±0.14 for SQ and 0.13±0.06 to 0.31±0.12 in LL) and long chain saturated fatty acids 20:0, 22:0, and 24:0 (0.03±0.04 to 0.23±0.08 for SQ and 0.05±0.03 to 0.21±0.07 in LL). Palmitic acid, 16:0 had a relatively lower heritability estimate (0.28±0.09) among the medium length even-numbered SFA while 17:0 had a relatively higher estimate of heritability (0.43±0.14) among medium length odd-numbered SFAs in the SQ tissue. In the LL tissue, C18:0 showed a relatively lower heritability estimate (0.28±0.09) among the even-carbon numbered medium chain SFA while 17:0 had a relatively

higher estimate of heritability (0.31±0.12) among odd-carbon numbered medium chain SFA. In general, the moderate to high heritability estimates of 14:0 (0.5±0.16 in SQ, 0.61±0.13 in LL), 16:0 (0.28±0.09 in SQ, 0.54±0.1 in LL) and 18: 0 (0.43±0.1 in SQ, 0.28±0.09 in LL) are consistent with those reported by Nogi et al. (2011), Inoue et al. (2011) and Yokota et al. (2012) for 14:0 (0.49±0.08 to 0.82±01), 16:0 (0.40±0.01 to 0.65±0.09) and 18:0 (0.55±0.11 to 0.71±0.01) in intramuscular fat of longissimus dorsi of Japanese Black cattle and by Saatchi et al. (2013) (0.57, 0.51 and 0.52, respectively) in the muscle of Angus-sired calves (fat percentage based), and Kelly et al. (2013) for 14:0 (0.55±0.12), 16:0 (0.43±0.11), 18:0 (0.44±0.11) in subcutaneous adipose tissue of a multiple breed Australian cattle population, indicating a strong direct influence of host genes on the concentrations of these fatty acids in the animal tissues. However, lower estimates were reported by Ekine-Dzivenu et al (2014) for 14:0 (0.17±0.12), 16:0 (0.05±0.12) and 18:0 (0.12±0.11) in brisket adipose tissue of crossbred beef cattle, Malau-Aduli et al. (2002) for 16:0 (0.13±0.08) and 18:0 (0.12±0.08)) in the intramuscular fat of crossbred animals and Pitchford et al. (2002) for 14:0 (0.18), 16:0 (0.21), and 18:0 (0.14) in the subcutaneous adipose tissue of crossbred beef cattle. For the medium and long chain odd-numbered SFAs, the lower estimated heritability for 13:0, 15:0, 19:0 and long chain SFA 20:0, 22:0, and 24:0 are in agreement with the heritability reported by Nogi et al. (2011) for 17:0 (0.21±0.06), 20:0 (0.00±0.01), Kelly et al. (2013) for 19:0 (0.22±0.09) and Saatchi et al (2013) for 13:0 (0.23), 15:0 (0.11), 17:0 (0.35), 20:0 (0.11), 22:0 (0.09) except for 24:0 (0.51). Moderate to moderately high

heritability estimates for medium length odd-numbered SFAs 17:0 were also observed by Kelly et al. (2013) in subcutaneous adipose tissue of a multiple breed Australian cattle population (0.28±0.10) and Saatchi et al (2013) in the muscle of Angus-sired calves (0.35).

In ruminants, acetate and propionate are utilized to synthesize medium length even and odd-numbered FAs, respectively (Drackley, 2000; Vernon, 2005; Vernon and Flint, 1988) with chain termination and elongation mechanisms giving rise to different length of fatty acids (Drackley, 2000). Adipose tissue is a major tissue where de novo fatty acids synthesis takes place (Beitz and Nizzi, 1997; Vernon, 2005), and host genes such as acetyl-CoA carboxylase alpha (ACACA), fatty acid synthase gene (FASN) and stearoyl-CoA desaturase (SCD) involved in lipid metabolism have been shown to have effects on contents of 14:0, 15:0 and 16:0 in beef muscle and adipose tissues (Abe et al., 2009; Li et al., 2012; Matsumoto et al., 2012; Zhang et al., 2010). However, SFAs in animal tissues may also originate from end products of complete biohydrogenation of dietary fat as well as de novo synthesis by rumen microbes (Jenkins, 1993). The relatively lower estimate of heritability for most odd-numbered SFAs suggest a non-host direct gene effect, likely the effect of microbes in the rumen due to the greater synthesis of these FAs by rumen bacteria (Drackley, 2000). However, Kelly et al. (2013) reported a higher estimate of heritability for odd-numbered SFA 15:0 (0.43±0.11) in subcutaneous fat adipose tissue of a multiple breed Australian cattle population. The inconsistent estimates for heritabilities of these

fatty acids may also be attributed to different populations/tissues investigated, various sample sizes and statistical models used.

Estimates of heritability for individual branched chain FAs were generally low in both tissues with magnitude ranging from 0.07±0.04 for iso17:0 in the LL muscle to 0.30±0.09 for iso16:0 in SQ tissue, except for ai15:0 that had an estimate of 0.50±0.11 in the SQ. Ekine-Dzivenu et al (2014) also reported a low estimate of heritability 0.05±0.11 for ai17:0 in brisket adipose of crossbred beef cattle. The major function of branched chain fatty acid is to maintain fluidity in the cell membranes in both animals and bacteria as an alternative to double-bond FAs (Christie, 2012). In ruminants, rumen bacteria play a greater role in the synthesis of branched chain fatty acids (Drackley, 2000), therefore the rumen environment likely has a larger influence on the concentrations of branched chain fatty acids in animal tissues, which is also reflected by the lower values of heritability estimates.

Of the twenty-three individual monounsaturated FAs analyzed in the study, 9c-14:1, 9c-16:1, 12c-16:1, 13c-18:1, 11c-20:1 had consistently higher estimates of heritability in both the tissues, ranging from 0.34±0.09 (12c-16:1) to 0.51±0.12 (9c-16:1) in SQ and from 0.45±0.09 (12c-16:1) to 0.68±0.11 (9c-16:1) in the LL muscle tissue. 9c-18:1 also showed a higher estimate of heritability of 0.42±0.09 in the LL tissue but it had a lower estimate of 0.17 ± 0.07 in the SQ tissue. Higher estimates of heritability for 9c-14:1 (0.60±0.09 to 0.86±0.1), 9c-16:1 (0.66±0.1 to 0.76±0.09) and 9c-18:1 (0.42±0.1 to 0.78±0.09) were observed by Nogi et al.

(2011), Inoue et al. (2011) and Yokota et al. (2011) in intramuscular adipose tissue of Japanese black cattle. Kelly et al. (2013) and Saatchi et al. (2013) also reported moderate to high estimates of 9c-14:1 (0.50 and 0.51±0.12), 9c-16:1 (0.49 and 0.38±0.11), 9c-18:1 (0.55 and 0.56±0.12) respectively in subcutaneous fat of multiple breed Australian beef cattle and muscle of Angus cattle, indicating strong effects of host delta 9 desaturase enzyme genes in determining the concentrations of the these FAs in animal tissues (Garnsworthy et al., 2010; Smith, 1995). However, low heritability for 9c-16:1 (0.02 to 0.16) and 9c-18:1 (0.09 to 0.17) were reported by Malau-Aduli et al. (2000) and Pitchford et al. (2002). Ekine-Dzivenu et al (2014) also obtained low heritability estimates for 9c-16:1 (0.13±0.11) and 9c-18:1 (0.13±0.12) in brisket adipose tissue of a crossbred beef population.

Estimates of heritability for most biohydrogenation intermediates were low (<0.30) in both tissues (Table 3-1), indicating a greater influence of rumen microbes on their concentration in animal tissues. Vaccenic acid (11t-18:1), a naturally occurring trans fat found in ruminant red meat and dairy products, has reported beneficial effects on human health (Bauman et al., 2004b; Lock et al., 2005). It is proposed that 11t-18:1 is formed by rumen bacteria during the biohydrogentation of PUFAs (Bauman et al., 2004b; Dugan et al., 2011). In this study heritability estimate is low in both tissues (0.16±0.07 in SQ and 0.24±0.08 in LL). Low heritability of 11t-18:1 has also been reported in subcutaneous fat of multiple Australian beef breeds (0.12±0.08) and in brisket adipose tissue of

Canadian crossbred beef cattle steers (0.11±0.08) (Ekine-Dzivenu et al (2014), suggesting stronger effects of the rumen and other environmental factors on their concentration in the adipose and muscle tissues. However, intermediate 13c-18:1 showed a higher estimate of heritability in both tissues (0.37±0.09 in SQ and 0.51±0.09 in LL). A higher estimate of heritability of for 13c-18:1 (0.43±0.10) was also reported in brisket adipose tissue of a crossbred beef population (Ekine-Dzivenu et al. 2014). Previous studies have also found significant association of SNPs in genes SCD and fatty acid desaturase 1 gene (FADS1) with concentrations of 13c-18:1 in brisket adipose tissue of Canadian commercial steers (Han et al., 2013; Li et al., 2012) as well as significant associations of SCD SNPs on 13c-18:1 in Spanish beef breeds (Li et al., 2010), supporting a stronger effect of these host genes on the concentrations of this fatty acids in beef tissues.

Heritability estimates for individual PUFA ranged from 0 for 6t,8t-18:2 and 9t,11t-18:2 to 0.43±0.1 for 18:2n6 and 18:3n3 in SQ and from 0.01±0.03 for 6t,8t-18:2 to 0.37±0.10 for 8t,10c-18:2 in the LL tissue with 17 of 26 individual PUFAs in the SQ and 20 of 26 individual PUFA in LL being low heritable (<0.20). Of the PUFA analyzed, most CLA isomers had low heritability estimates <0.02 except for 7t,9c-18:2 (0.29±0.10), 9c-11t/9c,11c-18:2 (0.24±0.08), 10t,12c-18:2(0.25±0.09) in the SQ tissue and 8t,10c-18:2 (0.37±0.10), 10t,13t-18:2 (0.23±0.07) in the LL tissue. Low heritability estimates of CLA were also reported in brisket adipose tissue of beef cattle by Li et al, (2014) with 14 of the 17 CLA isomers showing heritability estimates smaller than 0.20. The most

abundant CLA isomer 9t-11t:18:2 is also lowly heritable with an estimate of 0.24±0.08 in SQ and 0.16±0.07 in the LL tissue. Kelly et al. (2013) and Saatchi et al. (2013) also reported low estimates of heritability (0.11 to 0.24±0.09) for this major CLA isomer, 9c,11t-18:2 in beef tissues (Kelly et al., 2013; Saatchi et al., 2013), suggesting a stronger non-host direct genetic effects, most likely the influence of rumen metabolism, on contents of CLA isomers in animal tissues. Aside from being a product of partial PUFA biohydrogenation in the rumen, major CLA isomer 9c,11t-18:2 also originates from the action of Δ9-desaturase on 11t- 18:1(Griinari and Bauman, 1999). The relatively low estimates of heritability of 11t- 18:1 in both the tissues (0.16±0.07 and 0.24±0.08) (Table 3-1) are in line with the low estimates of heritability for major CLA isomer 9c,11t-18:2. However, CLA isomer 8t,10c-18:2 showed a moderately high estimate of heritability (0.37±0.10) in the LL tissue. Moderately high estimates of CLA isomers have also been reported for 11c,13t-18:2 (0.37±0.12) and 12c,14t-18:2 (0.45±0.11) in the brisket adipose tissue of Canadian crossbred beef cattle (Li et al. 2014). Inter-conversion of CLA isomers and presence of previously uncharacterized desaturases (Rioux et al. 2013) may have some influences on the contents of the CLA isomers in the animal tissues (Schneider et al. 2012). However, the relatively small sample size used in the previous study (Li et al. 2014) and the high sensitivity of CLA isomers to quantification random variations due to their low concentrations may bias the heritability estimates. For other individual PUFA, Saatchi et al. (2013) reported low estimates of heritability for 18:3n-3, 18:3n-8, 20:n-3, 20:3n-6 (0.04-0.14) in muscle of Angus cattle, which

are in agreement with our low estimates of PUFA in the LL muscle tissue. The low heritability of PUFA likely reflects stronger influence of the rumen environment on its concentration as most PUFA are subject to be converted to C18:0 through biohydrogenation in the rumen. However, we observed that 20:2n-6, 18:2n-6 and 18:3n-3 had a moderate to high estimate (0.31±0.08 to 0.43±0.1) in the SQ tissue. Inoue et al. (2011) and Nogi et al. (2011) also reported a heritability of 0.34±0.08 to 0.58±0.09 for PUFA 18:2 in the intramuscular fat of muscle of Japanese Black cattle, suggesting a role for host genes in the content of PUFAs in animal fat tissues.

Heritability for group FAs were 0.24±0.08 (BCFA) to 0.42±0.09 (PUFA) in the adipose tissue and from 0.13±0.06 (BCFA) to 0.46±0.1 (SFA and SFA+BCFA) in the LL muscle. Heritability estimates for fatty acid ratios, ranged from 0 (n6/n3) to 0.47±0.01 (P/S, P/S+B) and from 0.17 ± 0.07 (P/S) to 0.54±0.01 (HI) in the SQ and LL muscle tissues, respectively. The magnitude of heritability for grouped fatty acids largely reflects the heritability estimate of major individual fatty acids within the group. In SQ tissue, SFA, SFA+BFA, MUFA, PUFA, Sumtrans 18:1, n-3. n-6, P/S, P/(S+B) and Health Index (HI) had moderate to moderately high estimates of heritability (0.32±0.09 to 0.47±0.1) while heritability estimates for BFA and sumCLA and n6/n3 were lower (0 to 0.30±0.08). In the LL muscle tissue, SFA, SFA+BFA, MUFA, n-6/n-3, and HI had moderately high estimates of heritability (0.44±0.09 to 0.54±0.1) while BFA, sumCLA, Sumtrans 18:1, PUFA, n-3, n-6, P/S, P/(S+B) had lower estimates of heritability (0.13±0.06 to

0.26±0.08). It is noticed that the estimate of heritability for n6/n3 is 0.46±0.1 in the LL tissue although both n-3 and n-6 had relatively low estimates of heritability (0.16±0.07 and 0.20±0.07). However, Saatchi et al. (2013) reported a lower heritability for n-6/n-3 in the muscle of Angus sired cattle (0.12). A low heritability estimate for n-6/n-3 (0.03±0.10) was also reported by Ekine-Dzivenu et al. (2014) in the brisket adipose tissue, which was similar to our heritability estimate of zero in the SQ tissue. In comparison to a low estimate of P/S ratio (total PUFA/total SFA) in the LL tissue (0.16±0.06), the P/S ratio in SQ was moderately heritable (0.47 ±0.10), likely due to a moderately high estimate of heritability for PUFA in SQ (0.42±0.09). Nogi et al. (2007) reported a high estimate of heritability for P/S ratio (0.47±0.08) in intramuscular fat of Japanese cattle but Saatchi et al. (2013) reported a similarly low estimate (0.21) in the muscle of Angus-sired beef cattle population. Nutritional experts recommend the PS ratio be greater than 0.4 and the n-6/n-3 ratio be less than 4 (Wood et al., 2003), as a lower ratio of P/S and higher n6/n3 ratio are associated with cardiovascular diseases and cancers (Patterson et al., 2012; Simopoulos, 2006; Simopoulos, 2008). The relative high heritability estimates of n-6/n-3 in the LL muscle tissue may indicate an opportunity to reduce the n-6/n-3 ratio through genetic selection.

Health index (Zhang et al., 2008) is a modification of the atherogenic index (AI) defined as a ratio of the sum of total MUFA and PUFA to the sum of 16:0 and four times 14:0 content. 14:0 is considered to have four times the potential of

raising serum cholesterol compared to 16:0 (Hegsted et al., 1965). Healthier dietary components have a higher HI index. Estimates of heritability for HI in both tissues were moderately high (0.38±0.11 and 0.54±0.1). These estimates of heritability were comparable to that of Saatchi et al. (2013) and Tait et al. (2007) for Atherogenic Index (AI) in longissimus dorsi muscle (0.58, 0.52±0.14). However, Ekine-Dzivenu et al. (2014) reported a lower estimate of heritability (0.16±0.12) for HI in the brisket adipose tissue.

Fatty acids are complex traits and it is relatively difficult/expensive to quantify them in animal tissues. Therefore, estimating heritability of FAs is still at its early stage in comparison to many other economically important traits in beef cattle, and heritability estimates for some fatty acids were generally not consistent across studies. The discrepancy of heritability estimates in magnitude across different studies may also be attributable to differences in breeds/populations, tissue types, sample sizes and the statistical models used. It is also noted that the heritability estimates only quantify additive genetic effects of the host genes on fatty acid concentrations in the tissues. As a result, fatty acids with low heritability estimates might also be influenced by host genes through dominant or epistatic effects of the genes involved and/or through interactions of the host genes with genes of the rumen microbes or with the rumen environments. More studies will help gain better insight into host genetic control on contents of various fatty acids in beef tissues and will lead to better estimates of genetic parameters for fatty acid traits in beef cattle.

3.4 Conclusion

There are wide ranges of heritability estimates as well as genetic variations for fatty acid contents in both the SQ and LL muscle tissues of beef cattle, which likely reflect various origins of the fatty acids analyzed in this study. In general, medium length even-numbered SFAs were more heritable than medium and long chain odd-numbered SFAs in both the SQ and LL muscle tissues with 12:0 and 14:0 being highly heritable in both the issues. Individual MUFA 9c-14:1, 9c-16:1 had higher estimates of heritability of in both the tissues, indicating a strong host genetic direct effect on the concentrations of these fatty acids in the animal tissues. However, CLA precursor trans vaccenic acid (11t-18:1) and most CLA isomers including the most isomer 9t-11t:18:2 as well as most PUFA were low heritable in both tissues, suggesting strong influence of non-direct host genetic effect, most likely the rumen microorganisms and/or the ruminal environments, on the concentrations of the fatty acids in the animal tissues.

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Table 3-1. Mean, standard deviation (SD), range, estimates of additive genetic variance and heritability ± Standard error (SE) for fatty acids quantified in subcutaneous adipose tissue and longissimus lumborum muscle of beef cattle

Fatty Acids*	subcutaneous adipose tissue				longissimus lumborum muscle			
	Mean (SD)	Range	additive genetic variance	h^2±SE	Mean (SD)	Range	additive genetic variance	h^2±SE
10:00	0.0508 (0.0138)	0.0145 - 0.1238	0.000084	0.54 ± 0.09	0.0558 (0.0116)	0.0236 - 0.1104	0.000073	0.43 ±0.11
12:00	0.0705 (0.017)	0.029 - 0.1392	0.000196	0.64 ± 0.11	0.0722 (0.0149)	0.0252 - 0.1329	0.000163	0.67 ±0.11
13:00	0.0283 (0.0103)	0 - 0.0753	0.000029	0.28 ± 0.08	0.0269 (0.0091)	0 - 0.0739	0.000012	0.13 ±0.06
14:00	3.2036 (0.5992)	0.8044 - 6.5868	0.256701	0.5 ± 0.16	2.8035 (0.4857)	0.826 - 4.7912	0.152569	0.61 ±0.13
15:00	0.6423 (0.1668)	0.2642 - 1.4939	0.010928	0.25 ± 0.12	0.5024 (0.1112)	0.2158 - 1.0217	0.003762	0.22 ±0.1
16:00	25.092 (2.5941)	15.111 - 47.635	1.784560	0.28 ± 0.09	24.6068 (2.0556)	15.3686 - 32.7135	1.45072	0.54 ±0.1
17:00	1.7088 (0.4451)	0.7336 - 3.6467	0.067626	0.43 ± 0.14	1.5484 (0.3288)	0.7624 - 2.9748	0.029425	0.31 ±0.12
18:00	10.545 (1.9563)	4.532 - 18.6466	1.689360	0.43 ± 0.1	12.4062 (1.4167)	8.5218 - 17.9958	0.597542	0.28 ±0.09
19:00	0.108 (0.0323)	0.0203 - 0.2566	0.000096	0.07 ± 0.04	0.0902 (0.0286)	0.034 - 0.1895	0.000148	0.15 ±0.08
20:00	0.0815 (0.0187)	0.026 - 0.1619	0.000080	0.2 ± 0.07	0.0892 (0.016)	0.0377 - 0.1487	0.000056	0.18 ±0.07
22:00	0.0327 (0.0092)	0 - 0.1074	0.000003	0.03 ± 0.04	0.0686 (0.0205)	0.0058 - 0.1955	0.000041	0.05 ±0.03
24:00	0.0345 (0.0151)	0 - 0.1546	0.000064	0.23 ± 0.08	0.1511 (0.0702)	0.0214 - 0.5136	0.000762	0.21 ±0.07
SFA	41.598 (3.4694)	28.195 - 72.944	5.252040	0.39 ± 0.1	42.4213 (2.695)	31.0342 - 52.5723	2.71353	0.46 ±0.1
iso14:0	0.0313 (0.0115)	0.0111 - 0.1347	0.000020	0.17 ± 0.07	0.027 (0.0077)	0 - 0.0753	0.000006	0.11 ±0.06
iso15:0	0.1088 (0.0258)	0.0107 - 0.283	0.000109	0.22 ± 0.07	0.0818 (0.0153)	0.044 - 0.1593	0.000059	0.28 ±0.08
ai15:0	0.1803 (0.0476)	0.0159 - 0.409	0.001136	0.5 ± 0.11	0.14 (0.0283)	0.0205 - 0.2702	0.000294	0.28 ±0.1
iso16:0	0.177 (0.0411)	0.0795 - 0.415	0.000532	0.3 ± 0.09	0.1399 (0.0271)	0.0676 - 0.275	0.00018	0.2 ±0.08
iso17:0	0.3817 (0.0622)	0.1886 - 0.7749	0.001024	0.25 ± 0.08	0.3451 (0.0624)	0.2004 - 0.6624	0.000339	0.07 ±0.04
ai17:0	0.6718 (0.0946)	0.1405 - 1.2052	0.001646	0.16 ± 0.06	0.4893 (0.0757)	0.1085 - 0.8814	0.001542	0.18 ±0.08
iso18:0	0.1631 (0.0366)	0.0062 - 0.3202	0.000379	0.29 ± 0.09	0.1328 (0.0284)	0.0555 - 0.2611	0.000207	0.24 ±0.09
BFA	1.7139 (0.2607)	1.0097 - 3.295	0.018686	0.24 ± 0.08	1.3561 (0.2033)	0.768 - 2.5281	0.008174	0.13 ±0.06
SFA+BFA	43.312 (3.5606)	29.502 - 75.802	5.421110	0.38 ± 0.1	43.7774 (2.6805)	32.1366 - 53.9354	2.71062	0.46 ±0.1
9c-14:1	1.0459 (0.39)	0.125 - 3.3092	0.055500	0.41 ± 0.1	0.6401 (0.1835)	0.1299 - 1.6569	0.015941	0.52 ±0.1
9c-15:1	0.0341 (0.012)	0.0105 - 0.1862	0.000040	0.22 ± 0.08	0.0256 (0.0085)	0.002 - 0.0595	0.000002	0.03 ±0.03

Fatty Acids*	subcutaneous adipose tissue				longissimus lumborum muscle			
	Mean (SD)	Range	additive	h^2±SE	Mean (SD)	Range	additive	h^2±SE
7c-16:1	0.1395 (0.0249)	0.0828 - 0.3308	0.000162	0.27 ± 0.08	0.1364 (0.0185)	0.0743 - 0.2171	0.000088	0.2 ±0.08
9c-16:1	4.247 (1.0957)	1.2855 - 10.2681	0.618134	0.51 ± 0.12	3.4079 (0.5635)	1.5941 - 5.6582	0.186882	0.68 ±0.11
11t-16:1	0.047 (0.0116)	0.0064 - 0.091	0.000020	0.11 ± 0.05	0.042 (0.0118)	0.0056 - 0.0932	0.000006	0.03 ±0.03
12c-16:1	0.2386 (0.0898)	0.0161 - 0.8396	0.002365	0.34 ± 0.09	0.1681 (0.0442)	0.0475 - 0.3863	0.000807	0.45 ±0.09
7c-17:1	0.0229 (0.0094)	0 - 0.0702	0.000000	0	0.0253 (0.0131)	0 - 0.0807	0	0
9c-17:1	1.3774 (0.34)	0.5975 - 2.8457	0.025849	0.18 ± 0.09	1.1914 (0.2977)	0.0209 - 2.7239	0.013796	0.16 ±0.07
9c-18:1	37.917 (4.343)	37.917 - 51.601	3.938750	0.17 ± 0.07	36.6794 (2.9969)	20.3734 - 53.4145	3.18631	0.42 ±0.09
11c-18:1	1.9604 (1.7357)	0 - 29.033	0.085595	0.03 ± 0.04	1.8359 (0.2439)	1.0023 - 2.5227	0.011174	0.21 ±0.09
12c-18:1	0.2604 (0.0791)	0.0764 - 0.5603	0.000524	0.07 ± 0.04	0.2266 (0.0709)	0.0684 - 0.4501	0.000533	0.11 ±0.05
13c-18:1	0.4869 (0.1594)	0.1402 - 1.6239	0.007287	0.37 ± 0.09	0.3957 (0.0888)	0.1444 - 0.7663	0.003574	0.51 ±0.09
14c-18:1	0.0533 (0.0105)	0.0255 - 0.0959	0.000012	0.09 ± 0.05	0.0476 (0.0089)	0.0168 - 0.0833	0.000011	0.17 ±0.06
15c-18:1	0.2484 (0.0596)	0.0201 - 0.5081	0.000926	0.26 ± 0.07	0.204 (0.0444)	0.0852 - 0.4019	0.000508	0.24 ±0.08
9c-20:1	0.1068 (0.0188)	0 - 0.2607	0.000069	0.19 ± 0.07	0.0903 (0.0128)	0.0549 - 0.1591	0.000026	0.11 ±0.05
11c-20:1	0.2695 (0.0751)	0 - 0.8547	0.001572	0.36 ± 0.09	0.1975 (0.0351)	0.0856 - 0.4821	0.000696	0.53 ±0.11
6t/8t-18:1	0.2751 (0.121)	0.0658 - 1.3494	0.002984	0.26 ± 0.07	0.1927 (0.0847)	0.0388 - 0.718	0.000888	0.13 ±0.05
9t-18:1	0.291 (0.0939)	0.1055 - 1	0.001423	0.21 ± 0.07	0.2322 (0.0667)	0.0954 - 0.6012	0.000559	0.13 ±0.05
10t-18:1	2.908 (1.6847)	0.2021 - 11.0885	0.830434	0.3 ± 0.1	2.0278 (1.119)	0.1804 - 8.0507	0.27801	0.24 ±0.09
11t-18:1	0.5455 (0.2339)	0.1199 - 2.3296	0.011219	0.16 ± 0.07	0.4405 (0.1642)	0.1397 - 1.2134	0.006475	0.24 ±0.08
12t-18:1	0.184 (0.1717)	0 - 3.5503	0.000000	0	0.1365 (0.029)	0.0458 - 0.2963	0.000071	0.09 ±0.05
15t-18:1	0.1689 (0.1788)	0 - 2.2305	0.002351	0.08 ± 0.05	0.1296 (0.0786)	0 - 0.8428	0.000119	0.02 ±0.04
16t-18:1	0.1134 (0.0369)	0.0269 - 0.2717	0.000191	0.08 ± 0.05	0.0924 (0.0263)	0.0215 - 0.1795	0.000152	0.17 ±0.07
sumtrans18:1	4.4859 (1.6872)	1.5443 - 14.8333	0.848921	0.32 ± 0.09	3.2516 (1.1313)	1.2459 - 9.1328	0.306133	0.26 ±0.08
MUFA	52.941 (3.583)	16.276 - 68.55	5.057950	0.35 ± 0.09	48.5654 (2.6907)	30.5368 - 62.6315	2.52459	0.44 ±0.09
9c,13t/8t,12c-	0.2424 (0.0452)	0.1033 - 0.4639	0.000574	0.32 ± 0.1	0.1647 (0.0285)	0.0848 - 0.266	0.000157	0.21 ±0.08
9c,15c-18:2	0.1842 (0.0443)	0.0235 - 0.4426	0.000497	0.25 ± 0.08	0.1779 (0.0355)	0.0128 - 0.3036	0.000315	0.23 ±0.09
8t,13c-18:2	0.1646 (0.0432)	0.02 - 0.3876	0.000346	0.16 ± 0.07	0.1211 (0.025)	0.0184 - 0.2157	0.000034	0.05 ±0.04
11t,15c-18:2	0.1617 (0.1002)	0.017 - 0.7725	0.003211	0.33 ± 0.09	0.1224 (0.0699)	0.0154 - 0.5504	0.001541	0.34 ±0.09
6t,8t-18:2	0.0024 (0.0031)	0 - 0.028	0.000000	0	0.0019 (0.0037)	0 - 0.0994	1.22E-05	0.01 ±0.03
7t,9c-18:2	0.1077 (0.0828)	0 - 0.7696	0.001325	0.29 ± 0.1	0.0602 (0.06)	0 - 2.0869	0.000145	0.04 ±0.04
7t,9t-18:2	0.0069 (0.005)	0 - 0.0396	7.5E-06	0.03 ± 0.03	0.0041 (0.0026)	0 - 0.017	0.000001	0.06 ±0.04
8t,10c-18:2	0.0118 (0.0101)	0 - 0.07	0.000002	0.11 ± 0.05	0.0079 (0.0028)	0 - 0.0317	0.000003	0.37 ±0.1
8t,10t-18:2	0.0026 (0.0023)	0 - 0.0201	2.12E-06	0.12 ± 0.06	0.0017 (0.0015)	0 - 0.0182	1.84E-06	0.04 ±0.04

Fatty Acids*	subcutaneous adipose tissue				longissimus lumborum muscle			
	Mean (SD)	Range	additive	h^2±SE	Mean (SD)	Range	additive	h^2±SE
9c,11t-18:2**	0.4709 (0.3576)	0 - 2.1543	0.003197	0.24 ± 0.08	0.2573 (0.0619)	0.0984 - 0.5592	0.00098	0.16 ±0.07
9t,11t-18:2	0.0135 (0.0102)	0 - 0.1519	0.000000	0	0.0092 (0.0032)	0 - 0.0353	0.000001	0.13 ±0.06
10t,12c-18:2	0.025 (0.0119)	0 - 0.0955	0.000033	0.25 ± 0.09	0.018 (0.0099)	0 - 0.0647	0.000003	0.04 ±0.03
10t,12t-18:2	0.0096(0.0053)	0 - 0.0998	1.88E-05	0.02 ± 0.04	0.0061 (0.0023)	0 - 0.0177	0.000001	0.13 ±0.06
11t,13c/11c,13t -	0.0235 (0.024)	0 - 0.1933	0.000019	0.18 ± 0.07	0.0118 (0.0054)	0 - 0.0494	0.000002	0.09 ±0.05
11t,13t-18:2	0.0083 (0.0061)	0 - 0.0541	0.000003	0.17 ± 0.07	0.006 (0.0019)	0 - 0.015	0.000001	0.23 ±0.07
12t,14c/12c,14t -	0.0121 (0.0099)	0 - 0.1051	0.000003	0.05 ± 0.03	0.0068 (0.0029)	0 - 0.0383	0.000002	0.19 ±0.07
12t,14t-18:2	0.0088 (0.0113)	0 - 0.0734	0.000007	0.04 ± 0.02	0.0061 (0.0081)	0 - 0.0591	0.000003	0.03 ±0.02
sumCLA	0.7043 (0.4931)	0.2335 - 3.5626	0.008102	0.3 ± 0.08	0.395 (0.0803)	0.1783 - 0.8006	0.001315	0.17 ±0.07
18:2n-6	1.8761 (0.5868)	0.7607 - 4.5082	0.085310	0.43 ± 0.1	4.3867 (1.6124)	1.3942 - 16.3132	0.28197	0.22 ±0.07
18:3n-3	0.2113 (0.0545)	0 - 0.5687	0.001122	0.43 ± 0.1	0.2969 (0.0818)	0.125 - 0.7315	0.00098	0.22 ±0.07
18:3n-6	0.0023 (0.0063)	0 - 0.0813	0.000001	0.04 ± 0.04	0.043 (0.016)	0 - 0.1449	0.000033	0.11 ±0.05
20:2n-6	0.0377 (0.0132)	0 - 0.114	0.000035	0.31 ± 0.08	0.0682 (0.0219)	0.017 - 0.18	0.000026	0.07 ±0.05
20:3n-6	0.0591 (0.0166)	0.0224 - 0.129	0.000036	0.09 ± 0.05	0.2919 (0.0979)	0.0897 - 0.921	0.001464	0.21 ±0.07
20:3n-9	0.0166 (0.0161)	0 - 0.1484	0.000031	0.14 ± 0.06	0.0659 (0.025)	0.0073 - 0.2138	0.000087	0.14 ±0.06
20:4n-6	0.0399 (0.0123)	0 - 0.1246	0.000025	0.16 ± 0.06	0.9996 (0.4122)	0.2398 - 3.731	0.015575	0.15 ±0.06
20:5n-3	ND	ND	ND	ND	0.0291 (0.0086)	0 - 0.0732	0.000003	0.03 ±0.04
22:4n-6	0.0305 (0.0113)	0 - 0.0909	0.000021	0.15 ± 0.06	0.1361 (0.0448)	0.0412 - 0.4496	0.000298	0.13 ±0.06
22:5n-3	0.0166 (0.0096)	0 - 0.0511	0.000006	0.12 ± 0.05	0.3319 (0.1255)	0.0905 - 0.9482	0.002142	0.15 ±0.06
22:6n-3	ND	ND	ND	ND	0.046 (0.0234)	0 - 0.253	0.000073	0.15 ±0.06
PUFA	2.2902 (0.6273)	1.0051 - 5.2125	0.104775	0.42 ± 0.09	6.6953 (2.231)	2.2715 - 22.7526	0.487321	0.18 ±0.07
n-3	0.2278 (0.0535)	0.017 - 0.5687	0.001034	0.39 ± 0.09	0.7039 (0.2079)	0.2744 - 1.6389	0.005603	0.16 ±0.07
n-6	2.0457 (0.6015)	0.8656 - 4.6909	0.087198	0.42 ± 0.09	5.9255 (2.107)	1.9763 - 21.3471	0.444558	0.2 ±0.07
n-6/n-3	9.2625 (5.0778)	3.9705 - 167.4736	0.000004	0 ± 0	8.6283 (2.526)	2.9992 - 16.6226	1.04493	0.46 ±0.11
P/S	0.0555 (0.0161)	0.0247 - 0.1155	0.000074	0.47 ± 0.1	0.1599 (0.0583)	0.0508 - 0.51	0.000376	0.17 ±0.07
P/(S+B)	0.0532 (0.0153)	0.0238 - 0.1083	0.000066	0.47 ± 0.1	0.1548 (0.056)	0.0497 - 0.4926	0.000346	0.18 ±0.07
Health Index	1.4875 (0.265)	0.2751 - 3.4901	0.029236	0.38 ± 0.11	1.5655 (0.2317)	0.9931 - 3.5777	0.022769	0.54 ±0.1

*The concentrations of FAs (FAs) were expressed as a percentage of FA methyl esters (FAME) quantified.

**also included 9t-11c-18:2.,ND: Not detected, c=cis, t=*trans*. SFASFA+BCFA: sum of saturated and branched chain FAs; Sum *trans*18:1: sum of *trans*-18:1; MUFA: sum of all cis and all *trans* mono-unsaturated FAs.; SumCLA: sum of conjugated linoleic acids; PUFA: sum of polyunsaturated FAs; n-6/n-3: ratio between n-6 and n-3 PUFA; Sum *trans*18:1 = 6t/8t-18:1 + 9t-18:1 + 10t-18:1 + 11t-18:1 + 12t-18:1 + 15t-18:1 +16t-18:1 ; SumCLA = 8t,10c-18:2 + 9c,11t-18:2 + 7t,9c-18:2 + 9t,11c-18:2 + 10t,12c-18:2 + 11c,13t-18:2 + 11t,13c-18:2 +12t,14c-18:2+12c,14t-18:2 +6t,8t-18:2+ 9t,11t-18:2 + 11t,13t-18:2 + 12t,14t-18:2 + 10t,12t-18:2 + 8t,10t-18:2 + 7t,9t-18:2+ 9c,13t/8t,12c-18:2 + 9c,15c-18:2 + 8t,13c-18:2 + 11t, 15c-18:2 ;SFA = 10:0 + 12:0 + 13:0 + 14:0 + 15:0 + 16:0 + 17:0 + 18:0 + 19:0 + 20:0 + 22:0 + 24:0; MUFA = 9c-14:1 + 9c-15:1 + 7c-16:1 +11t-16:1+ 12c-16:1+ 9c-16:1 + 7c:17:1 + 9c-17:1 + 6t/8t-18:1 + 9t-18:1 + 10t-18:1 +11t-18:1 + 12t-18:1 + 15t-18:1 + 16t-18:1 + 9c-18:1 + 11c-18:1 + 12c-18:1 + 13c-18:1 + 14c-18:1 + 15c-18:1 + 9c-20:1 + 11c-20:1 ; PUFA = 18:2n-6 + 18:3n-6 + 18:3n-3 + 20:2n-6 + 20:3n-9 + 20:3n-6 + 20:4n-6 + 22:4n-6 + 22:5n-3+ 22:6n-3.BCFA iso-14:0 + iso-15:0 + anteiso-15:0 + iso-16:0 + iso-17:0 + anteiso-17:0 + iso-18:0 ; n-6 PUFA = 18:2n-6 + 18:3n-6 + 20:2n-6 + 20:3n-6 +20:4n-6 + 22:4n-6 ; n-3 PUFA = 18:3n-3 + 22:5n-3; Health Index: (total MUFA + total PUFA) / (4 x 14:0 +16:0)

CHAPTER 4

PHENOTYPIC AND GENETIC CORRELATIONS OF FATTY ACID CONCENTRATION IN THE LONGISSIMUS LUMBORUM MUSCLE WITH CARCASS MERIT TRAITS OF BEEF CATTLE[3]

4.1 Introduction

Enhancing beef fatty acid profile by increasing the concentration of beneficial fatty acids while reducing the concentration of unhealthy ones will positively influence the consumption of beef which is often perceived as a fatty protein source (Garmyn et al., 2011; Wood et al., 2004). In addition to designed diet supplement to enhance contents of beneficial fatty acids, genetic selection and breeding of beef cattle provides a great potential to capitalize on genetic variation of fatty acids among animals to reduce the concentration of unhealthy fatty acids and increase beneficial ones (Ekine-Dzivenu et al., 2014; Inoue et al., 2011; Kelly et al., 2013; Malau-Aduli et al., 2000; Nogi et al., 2011; Pitchford et al., 2002; Saatchi et al., 2013; Tait et al., 2007; Yokota et al., 2012). Genetic selection and breeding is perpetual and accumulative, therefore the relationship of FA concentrations in beef with carcass merit traits traditionally included in beef breeding programs needs to be evaluated for the design of optimal multiple trait selection indices to ensure that carcass merit traits are not compromised when

[3] Manuscript in preparation

enhancing fatty acid composition in beef. Nogi et al. (2011) reported genetic correlations in the range of -0.28 to 0.39 between FA and carcass merit traits in the longissimus muscle of a population of Japanese Black steers and heifers. They concluded that FA traits and carcass merit traits could be improved simultaneously because severe genetic antagonism was not observed. In the study of Tait et al. (2008) in the longissimus dorsi of Angus sired bulls and steers, genetic correlations were in the range of -0.98 to 0.83 and they found moderate antagonistic association between harmful myristic acid (14:0) with marbling (0.31) and between beneficial oleic acid (9c-18:1) with percentage of kidney, pelvic, and heart fat (0.36) amongst others. However, these reports only focused on a few beef breeds and correlations of many other fatty acids with carcass merit traits remain unknown (Inoue et al., 2011; Nogi et al., 2011; Pitchford et al., 2002; Tait et al., 2008). Therefore, the objective of this study was to estimate the phenotypic and genetic correlation of 83 fatty acids in longissimus lumborum muscle tissue of 1366 crossbred animals with 6 carcass merit traits to understand how genetic improvement of fatty acid concentrations in beef may affect carcass merit traits.

4.2 Materials and Methods

4.2.1 Animals and Management

The animals used in this study were cared for according to the Canadian Council of Animal Care 1993 guidelines (Olfert et al. 1993). The animal population and management were described previously (Basarab et al., 2011; Basarab et al.,

2007; López-Campos et al., 2013; McKeown et al., 2013). Briefly, spring born crossbred heifers and steers (n=1366) from the Lacombe research center, Lacombe and three commercial cow-calf herds located in Alberta were used in this study. The beef calves were produced from multi-sire mating groups. The population consisted of 6 Aberdeen Angus crossbred (ANAN), 93 Charolais–Red Angus crossbred (CHAR), 120 Hereford–Angus crossbred (HEAN), 209 Hereford-Angus-Gelbvieh crossbred (HEANGV) and 934 Hereford × Black Angus × Red Angus × Limousin crossbred (TXX).

After weaning at an average age of 182 days, calves were randomly assigned to one of four combination of production systems and growth implants based on breed cross, birth date, calf weight and dam age i.e. (1) Growth implant, calf fed (2) No growth Implant, calf fed (3) Growth implant, yearling fed (4) No growth implant, yearling fed. Calf fed animals entered the feedlot immediately after weaning while yearling fed animals were backgrounded before entering the feed lot (to build frame especially in the small to medium framed animals). Over a 27-42 day period, the calf fed steers were adjusted from a high forage diet to a high grain diet and then were finished on a high grain diet (on a dry matter basis) with 81.4% barley grain and protein supplement premix, 8.9 % barley silage, and 7.9 % grass silage for 76-112days days. Some of the calf-fed cattle (n=1198) were implanted with 200 mg progesterone (Synovex -S) and 20 mg estradiol benzoate at weaning and re-implanted with 120 mg trenbolone acetate and 24 mg estradiol (Revalor-S) 90-100 days before slaughter. The yearling fed animals rotationally grazed for 52 days fall pasture, alfalfa (*Medicago sativa L*) /meadow bromegrass

(*Bromus riparius* Rehm). When the snow prevented grazing, a grower diet comprising 43.1% barley silage, 41.1% grass hay, 15.8% rolled barley:oat grain mix (60:40) (on a dry matter basis) was fed for 192 days after which they returned to pasture (summer pasture) for 90 days before entering a feedlot. The yearling steers were allowed a 21-23 day adjustment period before finishing on a high concentrate diet composed of 79% barley grain, and 21% barley silage (on a dry matter basis) for 86 days. Some of the yearling finished cattle (n=56) were implanted with Synovex-S at weaning. Subsequently the yearling finished cattle were re-implanted 83, 154 and 240 days after weaning. They were finally implanted with Revalor-S 90 days before slaughter.

4.2.2 Collection of Carcass Data and Tissue Sample

All animals were targeted to be slaughtered at a constant back fat thickness of 9-10mm over the right longissimus thoracis muscle between the 12^{th} and 13^{th} rib as determined by ultrasound using an Aloka 500V diagnostic real time ultrasound machine with a 17cm 3.5Mhz linear array transducer (Overseas Monitor Corporation Ltd., Richmond BC). This corresponded to 11-14 months of age for the calf fed and 19-23 months of age for the year fed cattle. The animals were sent to the abattoir for slaughter at 1-2 week interval in a batch of 14 consisting of 7 implanted and 7 non-implanted cattle. Carcass were assessed according to the Canadian beef carcass grading system (AgricultureCanada, 1992) by trained personnel after which carcass number and slaughter data were recorded. Following slaughter, (24 h post mortem), each carcass was split, and the left and

right sides weighed and summed up to obtain hot carcass weight (HCW), and then chilled at 2^{o}C for 48 h. The left side of the carcasses sides were ribbed at the grading site between the 12th and 13th ribs and assessed for back fat thickness (BFAT), longissimus thoracic area (rib eye area (REA), marbling score (MARB), quality grade (QG), yield grade (YG) and lean meat yield (LMY) measurements. Carcass marbling was measured as the flecks of fat deposits interspersed between the muscle fibers of the longissimus thoracis at the grading site on the left side of the carcass and it was classified into 4 groups (100-399 = trace marbling or less; 400-499 = slight marbling; 500-799 = small to moderate marbling; 800-1199 = slightly abundant or more marbling). Quality grades were A, AA, AAA, and Prime reflecting the amount of intramuscular fat. Yield grade was classified as YG1>59%, YG2= 54 to 59%, and YG3 <54% based on the estimated proportion of lean meat derived from primal cuts, grade fat thickness, rib eye area and percent of kidney, pelvic and heart fat as YG%= (2.5 + (2.5 × BFAT, mm)) + (0.2 × %KPH) + (0.0038 × HCW, kg) – (0.32 × REA, cm2) where, KPH is assumed to be 2.5 (McKeown et al., 2013). Lean meat yield (LMY) is an estimate of the saleable meat and it was calculated as LMY% = 57.96 + (0.202 × REA, cm^2) – (0.027 × HCW, kg) – (0.703 × AFAT, mm) as described by Basarab et al. (2003). After being chilled at 2^{o}C for 24h to 48h longissimus lumborum muscle (meat) was sampled from the 12th rib, vacuum packed in plastic bags, frozen on dry ice and stored at -80^{o}C for subsequent fatty acid analysis.

4.2.3 Fatty Acid Analysis

Fatty acid analyses in the longissimus lumborum muscle were described previously in Chapter 3. Briefly, muscle was thawed and ground and lipid extracted using chloroform methanol (2:1, v/v) solvent which was directly methylated with sodium methoxide to fatty acid methyl esters (FAME). Then it was analyzed by gas chromatography (GC) and silver-ion high performance liquid chromatography (Ag-HPLC) using the methods outlined by (Cruz-Hernandez et al., 2004). A total of 83 individual and groups of fatty acids were quantified in the longissimus lumborum muscle and were expressed as percentages of total fatty acids detected. Group fatty acids were calculated by summing the appropriate components and the health index (HI) (Zhang et al., 2008), a modified version of the atherogenicity index (AI) proposed by Ulbricht and Southgate (1991) was calculated as:

$$\mathrm{HI} = (\text{Total MUFA} + \text{Total PUFA})/(4 \times 14{:}0 + 16{:}0).$$

4.2.4 Statistical Analysis

Preliminary analyses using a simple linear regression model were carried out to adjust each of the 83 fatty acid concentrations in the longissimus lumborum muscle for marbling scores to take into account differences in FA composition with increase in intramuscular fat, after which a pair-wise bivariate animal model was fitted to estimate phenotypic and genetic variance and covariance components for each of the adjusted 83 fatty acid concentrations against 6 carcass

merit using pair-wise bivariate animal model as implemented in ASReml3 (Gilmour et al., 2009).

The bivariate model can be written as follows:

$$\begin{bmatrix} \boldsymbol{y}_1 \\ \boldsymbol{y}_2 \end{bmatrix} = \begin{bmatrix} \boldsymbol{X}_1 & 0 \\ 0 & \boldsymbol{X}_2 \end{bmatrix} \begin{bmatrix} \boldsymbol{b}_1 \\ \boldsymbol{b}_2 \end{bmatrix} + \begin{bmatrix} \boldsymbol{Z}_1 & 0 \\ 0 & \boldsymbol{Z}_2 \end{bmatrix} \begin{bmatrix} \boldsymbol{a}_1 \\ \boldsymbol{a}_2 \end{bmatrix} + \begin{bmatrix} \boldsymbol{W}_1 & 0 \\ 0 & \boldsymbol{W}_2 \end{bmatrix} \begin{bmatrix} \boldsymbol{c}_1 \\ \boldsymbol{c}_2 \end{bmatrix} + \begin{bmatrix} \boldsymbol{e}_1 \\ \boldsymbol{e}_2 \end{bmatrix},$$

where $\boldsymbol{y_1}$ and $\boldsymbol{y_2}$ are vectors of phenotypic values (concentrations of fatty acid adjusted for marbling scores) and phenotypic values of carcass merit trait, respectively. $\mathbf{b_1}$ and $\mathbf{b_2}$ are vectors of fixed effects for trait 1 and trait 2, respectively, which included fixed effects of breed type (HEANGV, TXX, CHAR, ANAN, HEAN), production system (calf fed, yearling fed), growth implant use (yes, no), and gender. Diet (diet energy content), number of days between slaughter and fatty acid extraction for fatty acid traits, and slaughter age were included as fixed linear covariates. Random effects include $\boldsymbol{a_1}$ *and* $\boldsymbol{a_2}$, vectors of random additive genetic effects, $\mathbf{c_1}$ and $\mathbf{c_2}$, vectors of random contemporary group effects (combinations of feedlot test locations, feedlot pens and feedlot test years), $\boldsymbol{e_1}$ and $\mathbf{e_2}$ are vectors of random residual effects; **X**, **Z** and **W** are known design matrices relating the phenotypic values to the fixed, random additive and random contemporary group effects, respectively. For ***a***, ***c*** and ***e***, multivariate normal distributions were assumed with means equal to zero, leading to $E(\mathbf{y}) = \boldsymbol{X}\mathbf{b}$, and the variance-covariance matrix for the random effects can be described as:

$$var\begin{bmatrix} \boldsymbol{a_1} \\ \boldsymbol{a_2} \\ \boldsymbol{c_1} \\ \boldsymbol{c_2} \\ \boldsymbol{e_1} \\ \boldsymbol{e_2} \end{bmatrix} = \begin{bmatrix} \boldsymbol{A}\sigma^2_{a_1} & \boldsymbol{A}\sigma_{a_1a_2} & \boldsymbol{0} & \boldsymbol{0} & \boldsymbol{0} & \boldsymbol{0} \\ \boldsymbol{A}\sigma_{a_1a_2} & \boldsymbol{A}\sigma^2_{a_2} & \boldsymbol{0} & \boldsymbol{0} & \boldsymbol{0} & \boldsymbol{0} \\ \boldsymbol{0} & \boldsymbol{0} & \boldsymbol{I_{n_c}}\sigma^2_{c_1} & \boldsymbol{I_{n_c}}\sigma_{c_1c_2} & \boldsymbol{0} & \boldsymbol{0} \\ \boldsymbol{0} & \boldsymbol{0} & \boldsymbol{I_{n_c}}\sigma_{c_1c_2} & \boldsymbol{I_{n_c}}\sigma^2_{c_2} & \boldsymbol{0} & \boldsymbol{0} \\ \boldsymbol{0} & \boldsymbol{0} & \boldsymbol{0} & \boldsymbol{0} & \boldsymbol{I_{n_e}}\sigma^2_{e_1} & \boldsymbol{I_{n_e}}\sigma_{e_1e_2} \\ \boldsymbol{0} & \boldsymbol{0} & \boldsymbol{0} & \boldsymbol{0} & \boldsymbol{I_{n_e}}\sigma_{e_1e_2} & \boldsymbol{I_{n_e}}\sigma^2_{e_2} \end{bmatrix},$$

where $\sigma^2_{a_1}$ and $\sigma^2_{a_2}$ are the additive genetic variance for trait 1 and trait 2, respectively, and $\sigma_{a_1a_2}$ is the additive genetic covariance between the two traits; $\boldsymbol{A}$ is the additive genetic relationship matrix constructed from the pedigree which was traced back one generation; $\sigma^2_{c_1}$ and $\sigma^2_{c_2}$ are the variance of contemporary group effects for trait 1 and trait 2 respectively and $\sigma_{c_1c_2}$ is the covariance between the two traits due to the same contemporary groups. The covariance between different contemporary group effects were assumed to be zero; $\boldsymbol{I_{n_c}}$ is the identity matrix with dimension $n_c \times n_c$, where n_c is the number of random contemporary groups; $\sigma^2_{e_1}$ and $\sigma^2_{e_2}$ are the residual variance for trait 1 and trait 2, respectively, and $\sigma_{e_1e_2}$ is the residual covariance between the two traits; $\boldsymbol{I_{n_e}}$ is the identity matrix with dimension $n_e \times n_e$, where n_e is the number of animals with records. Initial values of variances were obtained by a preliminary univariate animal model analysis for subsequent REML bivariate analyses. Pairwise bivariate analyses were conducted for each combination of the fatty acid and carcass merit traits, and variance and covariance components were estimated by restricted

maximum likelihood (REML) as implemented in the ASReml v3.0 software package (VSN International Ltd., Hemel Hempstead, UK; Gilmour et al., 2009). Phenotypic variance and covariance were calculated as $\sigma_p^2 = \sigma_a^2 + \sigma_c^2 + \sigma_e^2$ and $\sigma_{p_1 p_2} = \sigma_{a_1 a_2} + \sigma_{c_1 c_2} + \sigma_{e_1 e_2}$, respectively. The phenotypic and genetic correlations were then estimated as $r_p = \sigma_{p_1 p_2} / \sqrt{\sigma_{p_1}^2 \sigma_{p_2}^2}$ and $r_a = \sigma_{a_1 a_2} / \sqrt{\sigma_{a_1}^2 \sigma_{a_2}^2}$, respectively, and their SE were approximated as described in Falconer and Mackay (1996). Heritability estimates for carcass merit traits were the average of the estimates of the corresponding pairwise bivariate analysis.

4.3 Results and Discussion

Descriptive statistics and heritability for 6 carcass merit traits are presented in Table 4-1. The 6 carcass quality traits are hot carcass weight (HCW), average back fat thickness (BFAT), rib eye area (REA), carcass marbling (MARB), lean meat yield (LMY), and YG (yield grade). The mean, range, SD and heritability estimates of the 83 fatty acids in the LL muscle have been previously reported (Chapter 2).

Table 4-2 lists phenotypic and genetic correlations of 24 major FAs (14 individual and 10 grouped FAs including an index) that had an average concentration greater than 0.5%. The 14 individual FAs include 5 saturated fatty acids (SFA) (14:0, 15:0, 16:0, 17:0 and 18:0), 6 monounsaturated fatty acids (MUFA) (9c-14:1, 9c-16:1, 9c-17:1, 9c-18:1, 10t-18:1, 11c-18:1), 1 branched-chain fatty acid (BCFA),

ai17:0 (anteiso), 2 polyunsaturated fatty acid (PUFA) (18:2n-6, 20:4n6) and 10 groups of fatty acids calculated by summing up proportions of the relevant components as previously described (SFA, MUFA, PUFA, BCFA, SFA+BCFA, sumtrans18:1, n-3, n-6, n-6/n-3 ratio and Health Index (HI)). The sum of concentrations of the major 14 individual fatty acids accounted for 94% (93.53%) of the total concentrations of 83 fatty acids in LL muscle. Phenotypic and genetic correlations of other minor fatty acids with the 6 carcass merit traits are presented in Appendix S4-1. However, in this chapter, we focus on the results of phenotypic and genetic correlation of the 6 carcass merit traits with the 24 major FAs in this chapter.

4.3.1 Variability in Carcass Quality Traits

There was considerable variation across all carcass quality traits with YG and BFAT being the most variable while LMY was the least variable with a CV of 39.7% and 7.2%, respectively. The average and range of the 6 carcass merit traits are in the ranges for Canadian beef cattle populations reported in other studies (Fernandes et al., 2002; Mao et al., 2013; Miar et al., 2013; Nkrumah et al., 2007). The estimates of heritability for the 6 carcass traits were moderate to moderately high, ranging from 0.34±0.08 for REA to 0.52±0.09 for HCW. The moderate to moderately high heritability estimates for carcass merit traits in this population indicates the presence of sufficient additive genetic variance for improving carcass merit traits through selection and breeding. The heritability estimate for

HCW (0.52±0.09) reported in this study is similar to the estimate reported by Nogi et al. (2011) (0.61±0.09) and higher than the estimate of 0.3 reported by Fernandes et al. (2002) in a population of crossbred steers and heifers, 0.33±0.14 reported by Nkrumah et al. (2007) in a population of animals produced from crosses of Angus, Charolais, or Alberta Hybrid Bulls, 0.37±0.08 reported by Chen et al. (2014) in a purebreed Angus population and 0.4±0.12 reported by Mair et al. (2013) in a population of crossbred steers. Lower estimates of 0.23±0.08 and 0.29±0.06 were reported by Mao et al. (2013) in purebred Angus and Chairolais steers and Tait et al. (2008) reported a heritability of 0.15 in Japanese heifers and steers. For BFAT, our estimate was moderate, 0.43±0.09, and in agreement with estimates of Chen et al. (2014), Nkrumah et al. (2007), Mao et al. (2013) for the Charolais population and Nogi et al. (2011) (0.34±0.07 to 0.51±0.15). Mao et al. (2013), however, reported a low heritability of 0.17±0.11 in the Angus population, same as 0.17 reported by Fernandes et al. (2002) in crossbred steers and heifers while Mair et al. (2014) reported an estimate of 0.22±0.1. Except for Mair et al. (2014), where heritability for LMY was 0.28±0.11, other reported estimates were greater than 0.3, (Chen et al., 2014; Fernandes et al., 2002; Mao et al., 2013; Nkrumah et al., 2007). Heritability estimates for marbling score varied most across the different beef cattle populations, ranging from 0.26±0.07 to 0.74±0.14. In line with our moderate estimate of 0.44±0.1, Tait et al. (2008), Fernandes et al. (2002), Mao et al. (2013) for the Angus population, Mair et al. (2014), and Nkrumah (2007), also reported moderate estimates in the range of 0.35 to 0.51±0.08. Moderately high to high heritability of 0.51±0.08 and

0.74±0.14 were reported by Nogi et al. (2011) and Mao et al. (2013) for the Charolais population respectively. Chen et al. (2014) however reported a low heritability of 0.26±0.7. Moderate heritability estimate for REA (0.34±0.08) reported in this study is within the range of moderate heritability reported by Chen et al. (2014), Fernandes et al, (2002), Nkrumah et al. (2007), Nogi et al. (2011), and Mao et al. (2013) for the Angus cattle population (0.34±0.08 to 0.49±0.14). Mao et al. (2013) reported a higher estimate of 0.64±0.15 for the Chairolais population while Tait et al. (2008) and Mair et al. (2014) reported lower estimates of (0.24±0.1 to 0.28). For YG, our estimate of 0.37±0.09 was lower than that reported by Nogi et al. (2011) and Nkrumah et al. (2007) 0.55±0.09 and 0.58±0.18 respectively. Heritability estimates for all of the carcass traits varied across studies and this could be due to differences in the way the trait was measured or estimated, differences in the methods of parameter estimation, effects fitted/accounted for in the statistical model, number of observations, breed, sex and management of the animals. The wide range of heritability estimates for marbling could partly be related to subjectivity in assessing the trait.

4.3.2 Phenotypic and genetic relationships of fatty acids with carcass quality traits

Phenotypic correlations between individual SFA 14:0, 15:0, 16:0, 17:0 and 18:0 with all carcass quality traits were generally low (<0.27) except for a moderate estimate between 16:0 and 17:0 with MARB (-0.3±0.06 and 0.36±0.08). The genetic correlations of these saturated FAs with carcass merit traits also tended to

be weak (< 0.19) apart from the moderate to moderately strong positive relationship of 16:0 and 18:0 with HCW (0.38±0.12, and 0.42±0.15) and a moderately negative favorable relationship of 16:0 with MARB (-0.31±0.13). These results suggest that breeding and selection to reduce concentration of individual SFA, including 14:0 would have a small effect on carcass merit traits in general except for a tendency of reduction in 16:0 to increase marbling score in the carcass but reduce hot carcass weight along with 18:0. Palmitic acid, 16:0 is considered as one of the harmful SFAs to human health. The moderate negative phenotypic and genetic correlations of 16:0 with marbling and positive genetic correlation with HCW suggests that muscle with more marbling actually tends to have a smaller concentration of 16:0 whereas leaner heavier animals tend to have a high concentration of 16:0. Inoue et al. (2011) also reported a favorable genetic relationship between 16:0 and MARB (-0.31±0.12) in the Musculus trapezius muscle of Japenese black cattle and Nogi et al. (2011) found a weak favorable negative genetic correlation for 16:0 with MARB (-0.16) and no association of 16:0 with HCW (0) in the Longissimus dorsi muscle of Japanese Black cattle in addition to a weak genetic relationship (0 to -0.27) between 14:0, 17:0 and 18:0 with comparable carcass traits in this study. However, Tait et al. (2008) reported a weak positive genetic correlation for 16:0 and MARB (0.26) in the Longissimus dorsi muscle of Angus sired beef cattle. They also found weak genetic correlations (0 to 0.27) for 14:0, 16:0 and 18:0 with carcass merit traits with a few exceptions. 14:0 had a moderate antagonistic relationship with MARB (0.32) and

18:0 had a moderate to strong negative relationship with BFAT (-0.54), REA (-0.50) and MARB (-0.45).

Phenotypic correlations between individual MUFAs with all carcass merit traits were mostly below 0.21, including phenotypic correlations of oleic acid (9c-18:1), the most abundant MUFA in beef associated with beef palatability (Smith et al., 2006), with all carcass merit traits (0.1±0.1 to 0.18±0.6). The exception was a moderate phenotypic correlation of 9c-17:1, 10t-18:1, 11c-18:1 with MARB (0.35±0.09, 0.30±0.08, and 0.31±0.08). Genetic correlations of individual MUFAs with all carcass merit traits ranged from 0 to -0.49±0.14. Fatty acid 11c-18:1 and HCW had a moderately strong negative genetic correlation of -0.49± 0.14, suggesting heavier cattle tend to have a smaller amount of 11c-18:1 in the LL muscle. Moderate to moderately high genetic correlations were also found between 9c-16:1 and BFAT (-0.44±0.13), 9c-17:1, 11c-18:1, 10t-18:1 and MARB (0.35±0.09, 0.31±0.08, 0.3±0.08) and 9c-16:1 with LMY (0.33±0.14) and with YG (-0.33±0.13). The moderate to moderately high positive genetic correlations between 9c-17:1, 11c-18:1, 10t-18:1 with marbling score suggests that selection to increase the content of these MUFAs will tend to improve marbling score. Selection to increase 11c-18:1 would lead to decreased HCW whereas animals with less back fat and more LMY and less YG (lower values indicate better yield grade) would have higher amounts of 9c-16:1 in the LL muscle. For the most abundant MUFA 9c-18:1, Nogi et al. (2011) and Tait et al (2011) reported weak genetic relationship for 9c-18:1 and HCW, BFAT, REA (-0.01, 0.17, 0.12 and -0.14, 0.18, 0.01 respectively) which is consistent with our report. Also in line with

our result, Nogi et al. (2011) reported a weak genetic correlation between 9c-18:1 and MARB (0.19) in the longissimus dorsi of Japanese Black Cattle. However, Tait et al et al. (2011) found a very strong favourable genetic correlation between 9c-18:1 and MARB (0.83) in the longissimus dorsi of Angus sired bulls and steers, and Inoue et al (2011) reported a moderate correlation of 0.40±0.11 with MARB in the Musculus trapezius of Japanese Black steers. Tait et al. (2011) obtained a moderate negative genetic correlation between 9c-14:1 and HCW (-0.42), a moderate positive genetic correlation between 9c-16:1 and MARB (0.51), and Inoue et al. (2011) reported a moderate negative genetic correlation between 9c-14:1 and MARB (-0.42±0.05).

Estimates of phenotypic correlations between major individual polyunsaturated fatty acids 18:2n6 (alpha linoleic acid) and its metabolite, 20:4n6 (Arachidonic acid) with carcass merit traits was low, ranging from 0.01±0.12 to 0.18±0.08 in magnitude, indicating that, phenotypically, the content of PUFA in LL muscle are not correlated with the carcass traits after the adjusting for marbling scores. The genetic correlations between 18:2n6 with the entire carcass merit trait were low (-0.30±0.22 to 0.22±0.20). Low genetic correlations (≤0.30) between 18:2n6 with HCW, BFAT, MARB was reported by Nogi et al. (2011) and Inoue et al. (2011), and they also reported low genetic correlation between 18:2n6 with MARB (0.05±0.16) in the Musculus trapezius of Japanese Black steers. Tait et al. (2008) found a low genetic correlation between 18:2n6 and BFAT (-0.17) but a moderate to very high genetic correlation for 18:2n6 with HCW (0.43) and MARB (-0.93)

in the longissimus dorsi of Angus sired cattle. Genetic correlations of 20:4n6 with carcass merit traits ranged from 0.06±0.2 for HCW to -0.43±0.21 for MARB.

With the clamor to reduce the high ratio of n-6 PUFA to n-3 PUFA typical of diets in the developed world (Daley et al., 2010; Wood et al., 2003), our results suggests that beef production practices that reduce the concentration of 18:2n6, which is a major n-6 PUFA component, would not significantly alter carcass merit and also breeding for increased marbling score would yield a correlated response of reduced 20:4n6. Increased amounts/consumption of n-6 FAs in western diets cause larger quantities of metabolic products of 18:2n6, particularly arachidonic acid (20:4n6) to be formed in larger quantities than those from 18:3n3 especially eicosapentaenoic acid (EPA, 20:5n3). Because metabolic products from arachidonic acid are biologically active even in small quantities, when they are formed in large amounts, they contribute to the formation of thrombi (blood clot) and atheroma (atherosclerosis – hardening of the arteries) (Simopoulos, 1999a, 2003, 1999b, 2006) which is the root cause of a number of cardiovascular diseases.

Branch chained fatty acid ai17:0 showed low phenotypic correlations with carcass merit traits (-0.28±0.10 to 0.18±0.11). However, its genetic correlations with HCW, BFAT, and YG are moderately high and negative (-0.37±0.15 to -0.45±0.13). This suggests that breeding for reduced back fat thickness might tend to increase the concentration of ai17:0 while increasing HCW and YG through selection and breeding would tend to decrease the concentration of ai17:0

Phenotypic correlation estimates for group SFA and SFA+BFA with the carcass merit traits are similarly low, ranging from 0.01±0.06 to -0.21±0.07 and -0.01±0.06 to -0.2±0.07 respectively, while the genetic correlation estimates ranged from 0 to 0.44±0.12 and -0.02±0.14 to 0.42±0.12 respectively. Genetic correlation estimates of SFA and SFA+BFA with BFAT, REA, LMY, YG were less than 0.3. However, SFA and SFA+BFA had a moderate to moderately high positive genetic correlation with HCW (0.44±0.12 and 0.33±0.15), indicating that selection to decrease total SFA in the LL muscle would tend to result in an unfavorable reduction of HCW. In line with our result, Nogi et al. reported low genetic correlation between SFA with REA, BFAT, and MARB (-0.21, -0.24, -0.25) in the longissimus dorsi of Japanese Black steers and heifers. In contrast however, they reported low genetic correlation between SFA and HCW (-0.04).

For group MUFA, phenotypic and genetic correlation was from -0.01±0.16 to 0.15±0.04 and -0.01±0.06 to -0.40±0.13 respectively with the carcass merit traits. Moderate genetic correlations of MUFA with HCW and MARB (-0.4±0.13, 0.3±0.15) indicates that genetic improvement of total MUFA would be associated with a slight favorable increase in carcass marbling score but an unfavorable decrease in HCW. Nogi et al. (2011) and Inoue et al. (2011) found a similar pattern of correlation for MUFA and MARB (0.23, 0.28±0.12) in the longissimus dorsi and Musculus trapezius muscle of Japanese Black cattle. In contrast however, Nogi et al. (2011) reported very weak negative genetic correlations of -0.02 for MUFA and HCW. Pitchford et al. (2002) also reported a weak phenotypic and genetic correlation for MUFA and HCW (0.04, -0.10).

Phenotypic correlations ranged from 0.1±0.11 for BCFA and MARB to -0.31±0.08 for BCFA and BFAT while genetic correlations were from -0.04±0.18 to -0.42±0.17. BCFA had moderate genetic correlation with LMY, HCW, BFAT and YG (0.3±0.18, -0.31±0.15, -0.4±0.16, -0.42±0.17). The negative genetic and phenotypic correlation of BFAT with BCFA suggests that reducing BFAT in cattle would increase BCFA concentration in the LL muscle. Increasing LMY and improving YG would also tend to result in more BCFA while increasing HCW would tend to cause a reduction in BCFA.

Total PUFA had low phenotypic correlation with all carcass merit traits (-0.02±0.13 to 0.18±0.08) and low genetic correlations ranging from -0.07±0.19 to -0.29±0.23 with the relationship of PUFA and MARB having the largest estimate of -0.29±0.23, indicating that selection for increasing total PUFA content in LL muscle would lead to a slight reduction of marbling scores. In contrast, the results of Nogi et al. (2011) showed that PUFA had the weakest relationship with MARB (0.02) amongst all carcass traits evaluated in the longissimus dorsi of Japanese Black steers and heifers.

Phenotypic correlations between n-3, n-6 and n-6/n-3 ratio with carcass merit traits ranged from 0 to 0.49±0.06. There was moderate negative and positive phenotypic correlations between n-3 and n-6/n-3 ratio with MARB (-0.33±0.09 and 0.49±0.06) suggesting that muscles with more n-3 would tend to have a lower marbling score. In other words, leaner muscle tissues would have more n-3. This result also suggests that the higher the n6/n-3 ratio (unfavorable) in the LL

muscle, the more the marbling score. Genetic correlations between these FA groups and ratio with carcass merit traits ranged from low, 0.02±0.15 to very high, -0.84±0.11. There was moderately high to high positive and negative genetic correlations for n-3 with HCW and MARB (0.56±0.13, -0.84±0.11) and a moderate to high positive and negative genetic correlation between n-6/n-3 with MARB and HCW (0.49±0.11, -0.69±0.1). This indicates that selection for heavier carcass would result in increased amounts of n-3 and a lower n-6/n-3 ratio, which is favorable. However, selection to increase marbling score would tend to result in lower concentration of n-3 in the muscle and a higher n-6/n-3 ratio.

Health index is a measure of the healthfulness of fatty acids in an animal tissue. Phenotypic correlations of HI with carcass quality traits were generally weak, ranging from -0.02±0.08 for REA to 0.15±0.17 for MARB and genetic correlations were also low, ranging from 0.05±0.14 for YG to -0.25±0.12 for HCW. Tait et al. (2011), reported a similar range (0.11 to 0.25) in the same direction for Atherogenic index (AI), inverse of the health index reported here with HCW, BFAT, REA, MARB in the longissimus dorsi of Angus sired bulls and steers. The low genetic and phenotypic correlations of all carcass merit traits with HI is likely due to the generally low phenotypic and genetic correlations of the component traits, 14:0, 16:0, MUFA and PUFA with carcass merit traits observed in this study. With the exception of a moderate negative correlation for 16:0 and MARB (-0.3±0.06), all other phenotypic correlations of component traits with carcass merit traits were low (<0.2) and apart from moderate genetic correlations of 16:0 and MUFA with MARB and HCW (0.31±0.13, 0.3±0.15 and

0.38±0.12, 0.4±0.13 respectively), all other genetic correlations of component traits with carcass quality were low (<0.3). This suggests that improving beef fatty acid profile using the health index is not expected to have negative effects on carcass quality traits. However, although FA ratios showed low to moderate heritability in both tissues, selection based on ratios are not optimal and might not result in the actual change desired since different combinations of beneficial MUFA and PUFA in the numerator and non-beneficial 14:0 and 16:0 FAs in the denominator would likely yield similar ratios. A selection index with appropriate weights for healthy and unhealthy FAs would be a better tool for selection.

For each pair of FA and carcass merit trait, differences in estimates in the literature may reflect differences in several factors including sample size, breed, sex, herd management, how the trait was measured or estimated, method of data analysis and effects fitted in the statistical model. This implies that the magnitude and direction of correlated response to selection would differ across populations.

Phenotypic and genetic correlations of 59 minor FA quantified in the LL muscle with carcass merit traits are reported in the supplementary Table (see Appendix). Phenotypic correlations ranged from 0 for 9c-15:1 and YG, iso16:0 and MARB, 15t-8:1 and 8t, 10t-18:2 with BFAT to -0.39±0.09 for 11t-8:1 and MARB. Moderate phenotypic correlations were observed for 7c-16:1, 12t,14t-18:2, iso16:0 and ai15:0 with BFAT (-0.31±0.08 , 0.32±0.12, -0.35±0.09, -0.36± 0.08), 16t-18:1, 18:3n3, 10t,12c-18:2, 10t,12t-18:2, 11t-18:1, with MARB (-0.31±0.1, -0.31±0.08, 0.37±0.08, 0.38±0.06, -0.39±0.09), 12t,14t-18:2 with HCW

(0.34±0.12) and 19:0 with REA (0.31±0.14). Genetic correlations ranged from 0 for 10t, 12c-18:2 and 12t, 14c/12c,14t-18:2 with HCW, 9c,15c-18:2 and 6t/8t-18:1 with BFAT, 12:0 and YG, 11t-16:1 and REA to -0.82±0.11 for 18:3n3 and MARB. There were several moderate to high genetic correlations between the various minor FAs and the 6 carcass quality traits considered in this study. The largest estimates (≥0.6) were between 12t-18:1, 9c-15:1, and 8t,13c-18:2 with BFAT (-0.61±0.18, -0.63±0.3, -0.64±0.25), 22:6n3, 6t,8t-18:2, 24:0, 11t-16:1, 22:5n3, 11t,13c/11c,13t-18:2, 18:3n3 with MARB (-0.6±0.17, 0.61±0.83, -0.62±0.15, 0.7±0.32, -0.72±0.13, -0.76±0.14, -0.82±0.11) and 15t-18:1 with HCW (0.82±1.0).

It is interesting to note the relationship of some minor FAs like vaccenic 11t-18t:1 and its metabolite 9c,11t-18:2, a major CLA isomer with carcass merit traits owing to their potential beneficial effect on human health (Bassett et al., 2010; Corl et al., 2003; Rainer and Heiss, 2004; Wang et al., 2008). Phenotypic correlations of 11t-18:1 with carcass merit traits ranged from (0.1±0.01 to -0.39±0.09). The strongest phenotypic correlation was between 11t-18:1 and MARB (-0.39±0.09). The negative phenotypic correlation of 11t-18:1 with MARB suggests that management practices, like finishing cattle with high grain diet that leads to increased marbling in the carcass will result in less 11t:18:1. Indeed, Dugan et al. (2007) showed that beef cattle fed a 73% based barley diet had more 10t-18:1 compared to 11t-18:1 in the subcutaneous adipose tissue of beef cattle. In this study, 10t-18:1 accounted for 62% of total trans fatty acids

quantified and it had a moderate positive correlation of 0.3±0.08 with MARB. This is particularly not favorable considering that 10t-18:1 was associated with increasing the risk of atherosclerosis in rabbits fed butters differing in trans18:1 and CLA contents (Bauchart et al., 2007).

Genetic correlations for 11t-18:1 ranged from 0.01±0.1 (BFAT) to -0.26±0.15 (HCW) and for 10t-18:1, it ranged from -0.03±0.19 (LMY) to -0.26±0.15 (HCW). This suggests modifying carcass merit traits through selection would not be expected to result in substantial change for these fatty acids.

Phenotypic and genetic correlations of CLA 9c, 11t/9t, 11c-18:2 varied between -0.05±0.1 (BFAT) to -0.22±0.13 (REA) and between -0.02±0.18 (REA) to -0.2±0.18 (LMY) respectively. In addition, SumCLA had low phenotypic (-0.02±0.07 (LMY) to -0.16±0.12 (REA)) and genetic (-0.04 ±0.2 (MARB) to 0.26±0.17(HCW)) correlations with all carcass merit traits considered in this study indicating that selection to improve carcass merit traits will not be associated with substantial changes in 9c,11t-18:2 or SumCLA.

Alpha linoleic acid (18:3n3) and its metabolites eicosapentanoic acid (EPA 20:5n3), and docosahexaenoic acid (DHA 22:6n3) are important to human health/nutrition as they have been shown to have multiple health benefits including improved cardiovascular health and brain function (Council for Responsible Nutrition, 2005; Scollan et al., 2006; Simopoulos et al., 2000) and increased intake up to of 0.3 to 0.5 g/d have been recommended (Kris-Etherton et al., 2002) for EPA and DHA as dietary intakes are typically lower. Therefore,

knowing how improving the concentration of these FAs in beef relates to carcass quality traits is important. Except for a moderate negative phenotypic correlation of 18:3n3 with MARB (-0.31±0.08), phenotypic correlations with other carcass merit traits were generally weak (<0.2). Genetic correlations ranged between 0.01±0.46 and -0.82±0.11 but were mostly below 0.3 except for 22:6n3 and BFAT (-0.42±0.17), 18:3n3 and 22:6n3 with HCW (0.46±0.14, 0.47±0.17), 18:3n3 and 22:6n3 with MARB (-0.82±0.11, -0.60±0.17). This result indicates that selection for larger framed cattle would be expected to also increase 18:3n3 and 22:6n3 concentration. However, selection for increased marbling in cattle would result in lower concentration of 18:3n3 and 22:6n3. In addition, management practices structured to increase the concentration of 18:3n3 in beef would tend to reduce marbling score.

Interpretation of phenotypic and genetic correlations between minor FA and carcass merit traits should be made with caution due to low concentrations of minor FA in beef tissues, which are subject to more random error assocated with their quantification process. Nevertheless, the estimate of phenotypic and genetic correlations of FA and carcass merit traits in LL muscle will not only help us understand the biology of fatty acid deposition in LL muscle and their effects on carcass merit traits but also help design multiple selection indices to mitigate the unfavorable effects of improving fatty acid profile in beef on carcass merit traits.

4.4 Conclusion

Overall, phenotypic correlations of FA in the LL muscle with carcass merit traits were mostly low. However, there was a moderate antagonistic phenotypic correlation between health related FAs 10t-18:1, 11t-18:1, 18:3n3, total n-3, n-6/n-3 ratio with marbling score. This means that the concentration of these FAs in a well-marbled carcass is expected to be low. Marbling is a key factor in determining the quality grade of a carcass in Canada and the United States and it may attract premium to the producer especially when considered with respect to other carcass merit traits. Therefore, in order to create more healthy beef with sufficient marbling to attract a premium, management practices to increase the concentration of n-3 fatty acids, like adding linseed when finishing cattle should be explored. However, because polyunsaturated fatty acids have the tendency to oxidize resulting in rancidity and color change of beef at display leading to shortened shelf life, Vitamin E should be supplemented in the diet as it has been shown to delay this process.

The lack of a cheap non-invasive on-line technology for measuring FAs in beef carcasses is a limiting factor for direct breeding and selection of beneficial FA in beef. Therefore, indirect selection through routinely measured carcass quality traits may be an alternative. However, genetic correlations of FAs with carcass merit traits were weak to strong with a number of antagonistic relationships including the unfavorable genetic relationships between 9c-16:1 with YG, 16:0, 18:0, and SFA with HCW, 22:6n3 and BFAT, 18:3n3, 22:6n3, n-3 and n-6/n-3

with MARB. These relationships, will not permit simultaneous improvement of FAs and carcass merit traits, and will not allow for FAs to be altered using easier to measure carcass merit traits. Direct improvements of beneficial FAs using genomic technology is increasingly becoming viable and is an attractive option for improvement of beneficial FA in beef.

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Table 4-1. Descriptive statistics and heritability (±SE) for 6 carcass quality traits in the longissimus lumborum tissue.

Traits	N	Mean	SD	Range	CV	h^2±SE
Carcass quality						
HCW(Kg)	1351	349.13	41.08	207.11 - 581.51	11.77	0.52 ± 0.09
BFAT mm	1337	10.64	4.22	0.68 - 29.46	39.68	0.43 ±0.09
REA cm2	1337	86.88	10.57	58.00 - 121.94	12.16	0.34 ± 0.08
MARB	1323	387.42	74.11	185.00 - 760.00	19.13	0.44 ± 0.10
LMY %	1366	58.49	4.24	39.43 - 71.93	7.24	0.36 ± 0.09
YG	1335	2.68	0.77	0.49 - 5.98	28.79	0.37 ± 0.09

HCW= Hot Carcass Weight
BFAT= Back fat thickness
REA= Rib eye area
CMAR = Carcass Marbling
LMY=Lean Meat Yield
YG= Yield Grade

Table 4-2. Phenotypic and genetic correlation (±SE) of major fatty acids (concentration > 0.5% FAME) in the longissium lomborum muscle tissue of beef cattle with carcass merit traits.

	Hot Carcass Weight		Subcutaneous Fat Thickness		Rib Eye Area		Marbling		Lean Meat Yield		Calculated Yield Grade	
Fatty acid %	r_p	r_g	r_p	r_g	r_p	r_g	r_p	r_g	r_p	r_g	r_p	r_g
Saturated												
14:0	0.05±0.08	0±0.12	-0.09±0.09	-0.15±0.14	-0.03±0.1	0.07±0.13	0.08±0.1	-0.11±0.13	0±0.07	0.15±0.14	0±0.08	-0.16±0.14
15:0	-0.11±0.12	-0.01±0.14	-0.11±0.11	-0.13±0.15	-0.12±0.14	0.12±0.15	0.27±0.1	0.02±0.15	0.01±0.1	0.13±0.17	-0.02±0.13	-0.19±0.16
16:0	0.09±0.06	0.38±0.12	0.02±0.06	-0.02±0.14	0.01±0.09	0.18±0.14	-0.3±0.06	-0.31±0.13	-0.04±0.05	0.08±0.15	0.05±0.07	0.01±0.14
17:0	-0.05±0.11	0.1±0.13	0±0.12	0.17±0.14	-0.08±0.13	0.09±0.16	0.36±0.08	0.16±0.15	-0.08±0.1	-0.12±0.16	0.05±0.12	0.06±0.15
18:0	0.16±0.08	0.42±0.15	0.02±0.08	0.09±0.17	0.09±0.11	0.12±0.17	-0.11±0.08	0.03±0.17	0.02±0.07	-0.03±0.18	0.03±0.08	0.16±0.17
Monounsaturated												
9c-14:1	-0.11±0.05	-0.22±0.14	-0.08±0.04	-0.28±0.14	0.01±0.05	-0.03±0.16	-0.01±0.05	0.07±0.16	0.07±0.04	0.25±0.15	-0.08±0.05	-0.26±0.15
9c-16:1	-0.07±0.05	-0.15±0.13	-0.12±0.05	-0.44±0.13	-0.02±0.07	0±0.14	0.02±0.06	-0.04±0.14	0.07±0.04	0.33±0.14	-0.08±0.05	-0.33±0.13
9c-17:1	-0.12±0.11	-0.23±0.15	-0.05±0.12	-0.06±0.18	-0.13±0.14	0.1±0.18	0.35±0.09	0.14±0.17	-0.05±0.1	0.08±0.19	0.02±0.13	-0.23±0.18
9c-18:1	0.06±0.06	-0.19±0.14	0.18±0.06	0.08±0.15	0.01±0.09	-0.13±0.15	-0.05±0.06	0.18±0.16	-0.15±0.05	-0.09±0.16	0.15±0.06	0.08±0.16
11c-18:1	-0.12±0.1	-0.49±0.14	-0.16±0.09	-0.15±0.16	0.04±0.14	-0.18±0.17	0.31±0.08	0.19±0.16	0.17±0.08	-0.03±0.18	-0.21±0.1	-0.17±0.17
10t-18:1	-0.12±0.09	-0.26±0.15	-0.1±0.1	0.1±0.17	-0.03±0.12	0.07±0.18	0.3±0.08	0.13±0.18	0.06±0.08	-0.03±0.19	-0.12±0.1	-0.17±0.18
Polyunsaturated												
18:2n-6	-0.14±0.06	-0.24±0.18	-0.13±0.06	0.22±0.2	0.01±0.12	-0.19±0.2	0.18±0.07	-0.05±0.22	0.17±0.06	-0.3±0.22	-0.18±0.08	0.2±0.21
20:4n-6	-0.08±0.07	0.06±0.2	-0.15±0.08	0.09±0.22	-0.01±0.12	-0.08±0.21	-0.02±0.08	-0.43±0.21	0.18±0.08	-0.14±0.23	-0.14±0.1	0.18±0.23
Branched												
ai17:0	-0.18±0.11	-0.45±0.13	-0.28±0.1	-0.37±0.15	-0.17±0.15	0±0.16	0.18±0.11	-0.05±0.16	0.12±0.1	0.24±0.17	-0.1±0.13	-0.42±0.15

Table 4-2. Phenotypic and genetic correlation (±SE) of major fatty acids (concentration > 0.5% FAME) in LL muscle tissue of beef cattle with carcass merit traits Cont'd

	Hot Carcass Weight		Subcutaneous Fat Thickness		Rib Eye Area		Marbling		Lean Meat Yield		Calculated Yield Grade	
Fatty acid %	r_p	r_g	r_p	r_g	r_p	r_g	r_p	r_g	r_p	r_g	r_p	r_g
Groups												
[a]sumtrans18:1	-0.13±0.08	-0.29±0.15	-0.11±0.1	0.07±0.18	-0.03±0.11	0.07±0.18	0.26±0.08	0.15±0.18	0.08±0.07	-0.02±0.19	-0.12±0.09	-0.19±0.19
[b]SFA	0.16±0.06	0.44±0.12	0.01±0.06	0±0.14	0.03±0.09	0.16±0.15	-0.21±0.07	-0.23±0.14	-0.03±0.05	0.06±0.15	0.06±0.07	0.06±0.15
[c]MUFA	-0.04±0.04	-0.4±0.13	0.11±0.04	-0.01±0.15	-0.01±0.06	-0.15±0.15	0.15±0.04	0.3±0.15	-0.11±0.04	-0.01±0.16	0.08±0.05	-0.07±0.16
[d]PUFA	-0.14±0.07	-0.07±0.19	-0.15±0.07	0.14±0.21	-0.02±0.13	-0.1±0.21	0.07±0.08	-0.29±0.23	0.18±0.08	-0.19±0.23	-0.16±0.1	0.16±0.22
[e]BFA	-0.26±0.09	-0.31±0.15	-0.31±0.08	-0.4±0.16	-0.2±0.14	0.07±0.18	0.1±0.11	-0.04±0.18	0.14±0.1	0.3±0.18	-0.11±0.13	-0.42±0.17
[f]SFA+BFA	0.14±0.06	0.42±0.12	-0.01±0.06	-0.02±0.14	0.01±0.08	0.17±0.15	-0.2±0.07	-0.23±0.14	-0.01±0.05	0.07±0.16	0.05±0.06	0.04±0.15
[g]n-3	-0.12±0.12	0.56±0.13	-0.11±0.12	-0.14±0.16	-0.1±0.16	0.27±0.15	-0.33±0.09	-0.84±0.11	0.08±0.12	0.16±0.16	-0.04±0.14	-0.02±0.17
[h]n-6	-0.14±0.07	-0.17±0.19	-0.14±0.06	0.18±0.21	0±0.13	-0.15±0.21	0.12±0.08	-0.17±0.22	0.18±0.07	-0.25±0.22	-0.17±0.09	0.18±0.22
[i]n-6/n-3	-0.04±0.12	-0.69±0.1	0.04±0.09	0.2±0.14	0.17±0.13	-0.28±0.14	0.49±0.06	0.49±0.11	0.06±0.09	-0.21±0.15	-0.12±0.1	0.02±0.15
[j]Health Index	-0.11±0.06	-0.25±0.12	0.02±0.06	0.09±0.14	-0.02±0.08	-0.12±0.14	0.15±0.07	0.22±0.14	0.02±0.05	-0.11±0.15	-0.04±0.06	0.05±0.14

r_g = genetic correlation
r_p=phenotypic correlation
The concentrations of fatty acids were expressed as a percentage of total fatty acid methyl esters (FAME) quantified. Only fatty acids with a concentration greater than 0.5% of total FAME are presented. c=cis, t=trans. [a]Sum trans18:1 = 6t/8t-18:1 + 9t-18:1 + 10t-18:1 + 11t-18:1 + 12t-18:1 + 13t/14t-18:1 + 15t-18:1 +16t-18:1. [b]SFA (sum of saturated fatty acid) = 10:0 + 12:0 + 13:0 + 14:0 + 15:0 + 16:0 + 17:0 + 18:0 + 20:0 + 23:0. [c]MUFA (sum of monounsaturated fatty acid)= 9c-14:1 + 9c-15:1 + 7c-16:1 + 9c-16:1 + 9c-17:1 + 6t/7t/8t-18:1 + 9t-18:1 + 10t-18:1 +11t-18:1 + 12t-18:1 + 13t/14t-18:1 + 15t-18:1 + 16t-18:1 + 9c-18:1 + 11c-18:1 + 12c-18:1 + 13c-18:1 + 14c-18:1 + 16c-18:1 + 9c-20:1 + 11c-20:1. [d]PUFA (sum of polyunsaturated fatty acid) = 18:2n-6 + 18:3n-6 + 18:3n-3 + 20:2n-6 + 20:3n-9 + 20:3n-6 + 20:4n-6 + 22:4n-6 + 22:5n-3. [e]BCFA (sum of branched-chain fatty acid) = iso-14:0 + iso-15:0 + anteiso-15:0 + iso-16:0 + iso-17:0 + anteiso-17:0 + iso-18:0. [f]SFA+BCFA: sum of saturated and branched chain fatty acids, [g]n-3 (sum of omega 3 fatty acids) = 18:3n-3 + 22:5n-3. . [h]n-6 (sum of omega 6 fatty acids) = 18:2n-6 + 18:3n-6 + 20:2n-6 + 20:3n-6 +20:4n-6 + 22:4n-6. [i]n-6/n-3: ratio between n-6 and n-3 PUFA. jHealth Index: (total MUFA + total PUFA) / (4 x 14:0 + 16:0).

CHAPTER 5

PHENOTYPIC AND GENETIC CORRELATION OF FATTY ACID CONCENTRATION IN THE LONGISSIMUS LUMBORUM MUSCLE WITH MEAT QUALITY TRAITS IN BEEF CATTLE[4]

5.1 Introduction

Beef is a good source of high quality protein with several other beneficial nutrients including high amounts of easily absorbable heme iron, but it also contains high amounts of saturated fatty acids (SFA) (Aaslyng, 2009). However, beef also contains vaccenic acid (11t-18:1) and conjugated linoleic acid (9c-11t-18:2) which have been shown to have anticarcinogenic properties among their health benefits (Bassett et al., 2010; Belury, 2002; Wang et al., 2008). The pleasure derived from eating meat comes from its sensory attributes which are of major importance to aspects of meat quality (Webb and O'Neill, 2008). The sensory attributes of meat are influenced by its fatty acid (FA) composition (Suzuki, 2006). The fatty acid composition of a tissue also affects muscle color and meat flavor because it determines the firmness and oxidative stability of the tissue (Nieto and Ros, 2012, Webb and O'Neill, 2008). Consumers are increasingly demanding healthier beef having reduced SFA with enhanced concentration of beneficial FAs like MUFAs, n-3 PUFAs, CLAs, and high eating quality (Webb and O'Neill, 2008). Meeting this demand would require that we understand the relationship between fatty acid composition in beef and meat quality traits. This will help to design diet supplements and/or genetic selection and breeding programs to produce meat with improved fatty acids while not compromising meat quality or vice versa. Although phenotypic relationships between meat

[4] Manuscript in preparation

quality traits and fatty acid composition in beef have been reported (Garmyn et al., 2011; Sevane et al., 2014; Dryden and Maechello, 1970; Melton et al., 1982; Westerling and Hedrick, 1979; O'Quinn, 2012), they are scarce and only limited to major FAs in beef and in a few beef cattle populations. There are presently no reports on the genetic relationship of beef fatty acid content with meat quality. Therefore the aim of this study is to estimate both the genetic and phenotypic correlations of FA content with meat quality traits in the longissimus tissue of Canadian beef cattle in order to gain further insight into how modifying beef FA will affect meat quality traits in beef.

5.2 Material and Methods

5.2.1 Animals and Management

Spring-born crossbred heifers and steers (n=1366) from the Lacombe Research Center, Lacombe and three commercial cow-calf herds from Three Cross Ranches in Airdire, Deseret Ranch near Lethbridge, Raymond and Namaka Farms, Strathmore located in Alberta were used in this study. They were cared for according to the Canadian Council of Animal Care (1993) guidelines and the management of these herds have been described in (Basarab et al., 2011; Basarab et al., 2007; López-Campos et al., 2013; McKeown et al., 2013). In brief, the beef cattle were produced from multi-sire mating groups with cow: bull ratio ranging from 25:1 to 40:1. The population consisted of Aberdeen Angus crossbred (ANAN, n=6), Charolais–Red Angus crossbred (CHAR, n=93), Hereford–Angus crossbred (HEAN, n=120), Hereford-Angus-Gelbvieh crossbred (HEANGV, n=209) and Hereford × Black Angus × Red Angus × Limousin crossbred (TXX, n=934).

The calves were weaned at an average age of 182 days, and were randomly assigned to one of four beef production systems which were a combination of finishing systems and the use of

growth implants. The animals were assigned a production system based on breed cross, birth date, calf weight and dam age. Calf fed animals entered the feedlot immediately after weaning while yearling fed animals were backgrounded to build frame before entering the feedlot. On entering the feedlot, calf fed animals were adjusted from a high forage diet to a high grain finishing diet over a 27-42 day period. The finishing diet consisted of (on dry mater basis) 81.4% barley grain and protein premix, 8.9% barley silage, and 7.9% grass silage and was fed for 76 - 112 days. A subset of calf-fed cattle (n=1198) were implanted with 200mg progesterone (Synovex -S) and 20mg estradiol benzoate at weaning and re-implanted with 120 mg trenbolone acetate and 24 mg estradiol (Revalor-S) 90-100 days before slaughter. The yearling fed animals rotationally grazed alfalfa (*Medicago sativa L*) /meadow bromegrass (*Bromus riparius* Rehm) fall pasture for 52 days, after which they were fed a grower diet comprising 43.1% barley silage, 41.1% grass hay, 15.8% rolled barley:oat grain mix (60:40) (on dry matter basis) for 192 days because the snow prevented them from grazing. They returned to pasture (summer pasture) afterwards for 90 days before entering the feedlot where they were allowed a 21 - 23 day adjustment period before finishing on a high concentrate diet composed (on dry matter basis) of 79% barley grain, and 21% barley silage for 86 days. 56 of the yearling finished cattle (n=56) were implanted with Synovex-S at weaning, re-implanted a second time 83 days after weaning, a third time, 71 days after the second implant and a fourth time, 86 days after the third implant. Finally, 90 days before slaughter, Revalor-S was implanted.

5.2.2 Slaughter, Meat Quality Measurement and Sensory Analysis

The animals were targeted to be slaughtered at a constant back fat thickness of 9-10 mm over the right longissimus thoracis muscle between the 12^{th} and 13^{th} rib as determined by ultra sound

using an Aloka 500V diagnostic real time ultrasound machine with a 17cm 3.5Mhz linear array transducer (Overseas Monitor Corporation Ltd., Richmond BC). Calf fed animals were 11-14 months of age and year fed animals were 19-23 months of age. The animals were slaughtered in a batch of 14 consisting 7 implanted and 7 non-implanted cattle at 1-2 week interval. Carcasses were assessed according to the Canadian beef carcass grading system (AgricultureCanada, 1992) by trained personnel after which carcass number and slaughter data were recorded. After slaughter, (48h post mortem), the left longissimus muscle (striploin;longissimus lumborum, LL) was removed, vacuum packed, chilled at 2^{o}C and transported the same day to the Lacombe Research Center by a refrigerated truck. The next day, four steaks, each 2.5 cm thick, were fabricated from the anterior portion of each muscle. The first steak was used to determine shear force. A spear point temperature probe (10 cm) was inserted into the midpoint of the steak which was then grilled (Garland Grill ED30B, Condon Barr Food Equipment Ltd., Edmonton, AB) to an internal temperature of 35^{o}C and then turned and cooked to a final internal temperature of 71ºC (Hewlett Packard HP34970 Data Logger, Hewlett Packard Co., Boise, ID). To prevent further cooking, the steaks were placed in polyethylene bags, sealed and immediately immersed into an ice water bath. They were then transferred to a 4^{o}C cooler and held for 24h. The next day, six cores from each steak 1.9 cm in diameter were removed parallel to the fiber grain. Peak shear force was determined on each core perpendicular to the fiber grain using a TA-XTplus Texture Analyzer equipped with a Warner-Bratzler shear head at a cross head speed of 200 mm min^{-1} and a 30 kg load cell using texture Exponent 32 software (Texture Technologies Corp., Hamiliton, MA). Maximum shear force was estimated as the average of the 6 cores. All thaw and cook-losses, end point temperature and cook times were recorded.

The second steak was used for sensory analysis. They were cooked to a final internal temperature of 71^{o}C and then cut into 1.3cm cubes, avoiding connective tissues and large areas of fat. Eight

cubes from each sample were randomly assigned to an eight member trained sensory panel. Samples were placed in glass jars in a circulating water bather (Lindberg/Blue Model WB1120A-1, Kendro laboratory products, Asheville, NC) and allowed to equilibrate to 71ºC prior to evaluation. Sensory descriptors were defined on a nine point scale, from 1 to 9. Initial tenderness was perceived from the initial bite through the cut center (1= extremely tough and 9=extremely tender) and initial juiciness was perceived within the first three to five chews (1=extremely dry and, 9= extremely juicy). Flavor desirability (1=extremely undesirable, 9=extremely desirable), Flavor intensity (1= extremely bland beef and 9=extremely intense beef flavor), off flavor (1= extremely intense off flavor and 9 = no off-flavor) and the amount of connective tissue (1=abundant amount of connective tissue and 9= no connective tissue detected) , were perceived within 10 to 20 chews. Just before expelling the sample, overall tenderness (1= extremely tough and 9=extremely tender), overall juiciness (1=extremely dry and, 9= extremely juicy), overall palatability (1= extremely undesirable and 9= extremely desirable) attributes were collected. Flavor (metallic, off-sour, livery, grainy, bloody or other) and texture (typical, mushy, meaty, spongy, rubbery or crumbly) descriptors were assigned to each cube of meat. Flavor descriptors were reported as a percentage of panelists attributing that descriptor to that sample. All panel evaluations were completed in well ventilated partitioned booths under red filtered lighting (124 lux). To cleanse their palate of residual flavor notes between sampling, the panelist were provided with distilled water and unsalted soda crackers

The third steak was used to assess retail storage life/retail color stability by placing the steak in a polystyrene tray on a dri-loc pad, overwrapped with oxygen permeable film (8000 mL m^{-2} 24 h^{-1}; vitafilm choice wrap; Goodyear Canada Inc.) and stored in a retail display case at 1^{o}C. Objective color score for L* (measures brightness, where 0 is black and 100 is white) a^{*} (red-green axis measures relative redness) and b^{*} (yellow-blue axis, measures relative yellowness) were

determined on days 0, 2 and 4 from three surface locations after exposure to atmospheric oxygen for 20mins using the Minolta CR-300 with Spectra QC-300 Software (Minolta Canada Inc., Missisauga, ON) and the average was used for analysis. Color measurements were converted to hue and chroma. Spectral reflectance readings were also collected concurrently and the relative contents of met-myoglobin, myoglobin and oxy-myoglobin were calculated.

From the fourth steak, objective color scores were collected as previously described after 20 min of blooming following which the steaks were placed on a polystyrene tray on a dri-loc pad and overwrapped with oxygen permeable film to determine drip loss gravimetrically.

The remaining portion of the LL was labeled, vacuum packed (Multivar AGW, Multivac Inc., Kansas City, MO) and aged in a cooler at 2ºC (wind speed of 0.5 m s^{-1}) for 26 d. After the 26 d ageing period, four steaks were cut and 29 d post slaughter and shear force, proximate analysis, color and sensory analysis were analyzed as described above.

5.2.3 Fatty Acid Analysis

Lipid extraction and fatty acid analysis has been previously reported in Chapter 3. In summary, longissimus lumborum muscle was sampled from the 12^{th} rib, to determine fatty acid composition, Before fatty acids were analysed, muscle was ground and fat extracted using chloroform methanol (2:1, v/v) solvent and directly methylated with sodium methoxide to fatty acid methyl esters (FAME). To determine FA composition , FAME was analyzed by gas chromatography (GC) and silver-ion high performance liquid chromatography (Ag-HPLC) according to methods outlined by (Cruz-Hernandez et al., 2004). Individual and groups of fatty acids quantified in the longissimus lumborum muscle (n=83) and were expressed as percentages of total fatty acids detected and group fatty acids were calculated by summing the appropriate

components. The health index (HI) (Zhang et al., 2008), is an adjusted version of the atherogenicity index (AI) proposed by Ulbricht & Southgate (1991). It was calculated as

$$HI = \frac{(Total\ MUFA + Total\ PUFA)}{4 * 14{:}0 + 16{:}0}.$$

5.2.4 Statistical Analysis

The 83 fatty acids in the longissimus lumborum muscle were initially adjusted for marbling so that relationships of FAs in the muscle with meat quality traits is assessed at constant intramuscular fat A bivariate animal model was fitted to estimate phenotypic and genetic variance and covariance components for each of the adjusted 83 fatty acid against 13 meat quality traits in ASReml (Gilmour et al., 2009). The model consisted of fixed effects of gender, breed (HEANGV, TXX, CHAR, ANAN, HEAN), production system (calf fed, yearling fed), growth implant use (yes, no), and gender. Diet energy content, growth implant use (Yes , no), finishing system (Calf fed, Yearling fed), number of days between slaughter and fatty acid extraction for fatty acid traits and kill age as a covariate.

The model also included Random effects of contemporary groups (combinations of feedlot test locations, feedlot pens and feedlot test years, (c1 and c2), random additive polygenic effects of animals (a1 and a2), and the random residual effects. The model equation can be written as follows

$$\begin{bmatrix} \boldsymbol{y_1} \\ \boldsymbol{y_2} \end{bmatrix} = \begin{bmatrix} \boldsymbol{X}_1 & 0 \\ 0 & \boldsymbol{X}_2 \end{bmatrix} \begin{bmatrix} \boldsymbol{b_1} \\ \boldsymbol{b_2} \end{bmatrix} + \begin{bmatrix} \boldsymbol{Z}_1 & 0 \\ 0 & \boldsymbol{Z}_2 \end{bmatrix} \begin{bmatrix} \boldsymbol{a_1} \\ \boldsymbol{a_2} \end{bmatrix} + \begin{bmatrix} \boldsymbol{W}_1 & 0 \\ 0 & \boldsymbol{W}_2 \end{bmatrix} \begin{bmatrix} \boldsymbol{c_1} \\ \boldsymbol{c_2} \end{bmatrix} + \begin{bmatrix} \boldsymbol{e_1} \\ \boldsymbol{e_2} \end{bmatrix}$$

in which **y_1 and y_2** are vectors of phenotypic measurements of fatty acids adjusted for marbling and meat quality traits, respectively, **b_1** and **b_2** are vectors of fixed effects for traits 1 and 2, **a_1** and **a_2**, **c_1** and **c_2**, **e_1** and **e_2** are vectors of random additive effects, random contemporary group and random residual effect for traits 1 and 2 respectively. **X**, **Z and W** are incidence matrices relating phenotypic observations to fixed effects, random additive genetic effects and random contemporary group effects respectively. The random vectors a c and e were assumed to follow a multivariate normal distribution with a mean of 0 and variance $A\sigma_a^2$, $I\sigma_c^2$, $I\sigma_e^2$. The resulting variance-covariance matrix are described as

$$\text{var}\begin{bmatrix} a_1 \\ a_2 \\ c_1 \\ c_2 \\ e_1 \\ e_2 \end{bmatrix} = \begin{bmatrix} A\sigma_{a1}^2 & A\sigma_{a1a2} & 0 & 0 & 0 & 0 \\ A\sigma_{a1a2} & A\sigma_{a2}^2 & 0 & 0 & 0 & 0 \\ 0 & 0 & I_{nc}\sigma_{c1}^2 & I_{nc}\sigma_{c1c2} & 0 & 0 \\ 0 & 0 & I_{nc}\sigma_{c1c2} & I_{nc}\sigma_{c2}^2 & 0 & 0 \\ 0 & 0 & 0 & 0 & I_{nc}\sigma_{e1}^2 & I_{nc}\sigma_{e1e2} \\ 0 & 0 & 0 & 0 & I_{nc}\sigma_{e1e2} & I_{nc}\sigma_{e2}^2 \end{bmatrix},$$

.where A is the additive genetic relationship matrix constructed from the pedigree and σ_{a1}^2, σ_{a2}^2 and σ_{a1a2} are additive genetic variances and covariance respectively for trait 1 and 2. I_{n_c} is an $n_c x\, n_c$ identity matrix where n_c is the number of random contemporary groups and σ_{c1}^2, σ_{c2}^2 are variances of contemporary group effect for traits 1 and 2 while σ_{c1c2} is the covariance between the two traits due to the same contemporary group. Between different contemporary groups, the covariance of both traits was assumed to be 0. I_{n_e} is an $n_e x\, n_e$ identity matrix where n_e is the number of animals with records and σ_{e1}^2, σ_{e2}^2 and σ_{e1e2} are residual variances and covariance respectively between the two traits. Phenotypic variance and covariance were calculated as $\sigma_p^2 = \sigma_a^2 + \sigma_c^2 + \sigma_e^2$ and $\sigma_{p_1p_2} = \sigma_{a_1a_2} + \sigma_{c_1c_2} + \sigma_{e_1e_2}$. Heritability estimates for carcass merit traits

was the average of corresponding pairwise bivariate analysis. Phenotypic and genetic correlations were estimated as:

$$r_p = \frac{\sigma_{p1p2}}{\sqrt{(\sigma^2_{p1}) \quad (\sigma^2_{p2})}}$$

$$r_a = \frac{\sigma_{a1a2}}{\sqrt{(\sigma^2_{a1}) \quad (\sigma^2_{a2})}}$$

Standard error for genetic correlation coefficients was approximated as described in Falconer and Mackay (1996).

5.3 Results and Discussion

5.3.1 Variability in Meat Quality Traits

The descriptive statistics for 13 beef quality traits including sensory attributes of major importance, and their heritability estimates in beef are presented in Table 5-1. The 13 meat quality traits are drip loss (DL), shear force 3d (measured 3 days post mortem) (WBSF_3d), shear force 29d (measured 29 days postmortem after 26 days of ageing) (WBSF_29d), muscle color L*_3d (lightness/brightness) (MCL*3d), MCL* 29d, beef flavor intensity 3d (BFI_3d), BFI_29d, off beef flavor 3d (OF_3d), OF_29d, overall tenderness 3d (OT_3d), OT_29d, overall juiciness 3d (OJ_3d), OJ_29d. Considerable variation was evident for all meat quality traits with coefficient of variation (CV) ranging from 6.35% for OF_29d to 39.3% for DL. Estimates of heritability ranged from 0.02±0.03 for SJ_3d to 0.36±0.09 for OT_3d. With the exception of OT_3d (0.36±0.09), heritability estimates for meat quality traits were generally low (below 0.25). Higher heritability for subjective measure of tenderness OT_3d and 29d (0.36±0.09, 0.21±0.07)

compared to objective measure WBSF_3d and 29d (0.16±0.07, 0.17±0.07) is probably due to higher phenotypic variation in the WBSF values.

Variation in sensory attributes, off flavor, beef flavor intensity, overall tenderness and overall juiciness ranged from 6.35% for OF_3d to 19.4% for OT_29d. All sensory traits, had more variation in measurements taken 3days post mortem compared to those taken 29 days postmortem. The same was true for WBSF and MCL*. Between 3 days and 29 days post slaughter, average WBSF dropped from 7.82kg to 4.86kg suggesting the meat became more tender possibly due to the aging process where proteolytic enzymes loosen up the meat structure by degrading the protein (Aaslyng, 2009). This is somewhat reflected in the increase in taste panel subjective measures of tenderness as OT increased from 5.73 to 6.65 between 3 and 29 days post mortem. There was a very slight increase in BFI and MCL* and a slight decrease in SJ and OF between 3 and 29 days postmortem.

Average values of 7.83kg (WBSF_3d) and 4.86kg (WBSF_29d) for WBSF determination of tenderness obtained in this study are higher than unacceptable tenderness threshold values (>3.85kg and >4.54kg) defined by Wulf et al. (1996) and Tatum et al. (1999). Values in the defined range is reported by Boukha et al. (2010) and Boukha et al. (2011), in the longissimus thoracis muscle of Piedmontese young bulls measured 8 days after slaughter (2.65kg and 2.69kg respectively), Nephawe et al. (2004) in steaks from the longissimus dorsi muscle of crossbred cattle (4.17kg), Garmyn et al. (2011) for longissimus thoracis muscle of Angus cattle aged for 14 days (3.67kg) and Johnston et al. (2003) in the longissimus thoracis muscle of temperate cattle (Angus, Hereford, Shorthorn, Murray Grey) (4.12kg) aged for 14 days. However, higher values have been reported (4.61kg to 5.42kg) (Fernandes et al., 2002; Devitt et al., 2002; Dikeman et al., 2005) in the longissimus and semitendinosus muscle aged for up to 14 days. Low heritability for

WBSF 3d and 29d of 0.16±0.07 and 0.17±0.07 reported here is similar to low estimates (0.09 to 0.29) previously reported (Devitt et al., 2002; Fernandes et al., 2002; Allais et al., 2014; Tizioto et al., 2013; Nephawe et al., 2004; Van Vleck et al., 1992) in different breeds and muscles aged for up to 14 days. However, Dikeman et al. (2005) reported a higher WBSF heritability of 0.4 in steaks from longissimus muscle of 14 breeds of cattle aged for 14 days and Johnston et al. (2003) also reported higher estimates in the longissimus dorsi (0.30) and semitendinosus (0.42) muscle of tropical cattle (Brahman, Belmont Red, Santa Gertrudis). Interestingly, Johnston et al. (2003) also reported low WBSF heritability estimates (longissimus dorsi 0.09, semitendinosus 0.11) in temperate cattle.

Average DL of 3.11% in this study was lower than 4.21% and 4.24% reported by Boukha et al. (2010, 2011) in longissimus thorasis muscle of Piedmontese cattle measured 8 days after slaughter and 4.28% reported by Ribeca et al. (2014) in the same muscle and breed of cattle. In the longissimus muscle of Nelore bulls, Bonin et al. (2014) reported average DL of 2.80%, 3.70% and 4.50% in longissimus muscle aged for 7, 14 and 21 days respectively. Heritability for DL in this study (0.18±0.08) was lower than estimates (0.32, 0.24) reported by Boukha et al. (2010) and Boukha et al.(2011) respectively.

The average MCL*3d and 29d (37.17 and 38.82), was within the range (34 - 40) considered as normal (Chambaz et al., 2001). Values within this range were reported by Tizioto et al. (2013) and Bonin et al. (2014) in Nelore cattle (37.83 to 38.73) in longissimus muscle aged for 0 to 14 days. Lower values (darker muscle color) in the range of 32.79 to 34.75 were however reported by Boukha et al. (2010), Boukha et al (2011), Ribeca et al. (2014) and Allais et al. (2014). Johnston et al. (2003) however reported higher values (47.07, 39.57) in the longissimus thoracis and semitendinosus muscle in temperate cattle. Heritability for MCL* 3d and 29d was low

(0.21±0.08 and 0.09±0.05) and in agreement with low heritabilities (0.10 – 0.23) reported in other studies (Johnston et al., 2003; Tizioto et al., 2013; Allais et al., 2014). Low heritability for MCL* suggests that selection for improved muscle color might not result in much progress.

Mean values for taste panel scores for sensory attributes, BFI 3d and 29d, OF_3d and_29d, OT_3d and 29d, SJ_3d and 29d corresponded to slightly to moderately flavorful, tender and juicy beef. The heritability estimate obtained in this study for flavor associated traits, BFI and OF (0.05±0.04 to 0.10±0.05) is similar to low estimates (not more than 0.25) previously reported (Dikeman et al., 2005, Nephawe et al., 2004, Van Vleck et al., 1992, Johnston et al., 2003) suggesting that selection and breeding for flavor would result in little genetic progress.

For tenderness, our moderate estimate of 0.36±0.09 for OT_3d is similar to 0.37 reported by Dikeman et al. (2005) in the longissimus muscle of 14 breeds of cattle aged for 14 days while our estimate of 0.21±0.07 for OT29_d is similar to 0.26±0.08 reported by Nephawe et al. (2004) in the longissimus dorsi muscle of crossbred cattle. Low heritability estimate of 0.10 for tenderness was reported by Van Vleck et al. (1992) in the longissimus dorsi muscle of crossbred cattle and Johnston et al. (2003) reported an estimate of 0.10 in the longissimus thoracis muscle of temperate cattle aged for 14 days. Johnston et al. (2003) however reported a higher heritability of 0.3 for the same tissue in tropical cattle.

Heritability estimates for juiciness obtained in this study 0.02±0.03 to 0.10±0.06 is higher than 0.01±0.08 reported by Nephawe et al. (2004) but lower than 0.15 to 0.46 reported by Dikeman et al. (2005), Van Vleck et al. (1992) and Johnston et al. (2003) in temperate and tropical breeds of cattle.

Aside from analytical differences, breed differences, sample size, pedigree depth, which can result in differences in heritability estimates across studies, the different ways in which the traits ae defined, different ways in which the trait is measured and post slaughter practices (electrical stimulation of the carcass, ageing) might also influence heritability estimates and contribute significantly to the range observed.

5.3.2 Phenotypic and Genetic Relationships of Fatty Acids with Meat Quality Traits

The descriptive statistics for all FAs quantified were provided previously (Chapter 3). Of the 83 fatty acids quantified, 24 major individuals and groups of FAs including 14 individual FAs - 5 saturated fatty acids (SFA) (14:0, 15:0, 16:0, 17:0 and 18:0), 6 monounsaturated fatty acids (MUFA) (9c-14:1, 9c-16:1, 9c-17:1, 9c-18:1, 10t-18:1, 11c-18:1), 1 branched-chain fatty acid (BCFA), ai17:0 (anteiso), 2 polyunsaturated fatty acid (PUFA) (18:2n-6, 20:4n6), and 10 groups of fatty acids, SFA, MUFA, PUFA, BCFA, SFA+BCFA, sumtrans18:1, n-3, n-6, n-6/n-3 ratio and Health Index had a concentration equal to or greater than 0.5% of FAME. Phenotypic and genetic correlations of these major FAs with meat quality traits are reported in Table 5-2. Genetic and phenotypic correlations of other minor FAs (59) with concentration less than 0.5% in the muscle with the 13 meat quality traits are presented in Appendix S5-1.

Overall, phenotypic correlation of the 83 individual and groups of total fatty acids quantified in the longissimus lumborum muscle with meat quality traits were low to moderately high ranging from 0 between 20:2n-6 and DL, 20:3n-6 and 7t,9c-18:2 with WBSF_3d, 11c-20:1 and MCL*3d, 13c-18:1 and 15t-18:1 with MCL*29d, 8t,10c-18:2 and iso17:0 with BFI_3d, 9c-18:1 and BFI_29d, 9c-16:1 and OF_3d, 9c-14:1 and OT_3d, 7c-17:1 and OT_29d, 13:0, 14:0 and 11c-

18:1 with SJ_29, to -0.6±0.14 between 22:5n3 and SJ_3d. There was a wide range of genetic correlations with magnitude ranging from 0 for 20:0, 11c-20:1, 18:2n6 and 18:3n6 with WBSF_3d, 20:4n6 and WBSF_29d, ai17:0 and MCL*3d, SFA+BFA and MCL*29d, 9c,11t/9t,11c-18:2 and BFI_29d, 9c-15:1 and OF_3d, 6t,8t-18:2 and 9c,11t/9t,11c -18:2 with OT_3d to ±1±0 between 14c-18:1 and 11t,13c/11c,13t 18:2 with SJ_29d (Appendix S5-1).

Phenotypic correlation of major individual SFA with meat quality traits were low to moderate in magnitude ranging from 0 for 14:0 and SJ_29 to -0.36±0.19 for 15:0 and DL. The moderate phenotypic correlations (>0.3) involved a negative correlation of 14:0, 15:0, 17:0 with WBSF_3d (-0.31±0.13, -0.32±0.15, -0.3±0.13), 15:0 and 17:0 with DL (-0.36±0.19, -0.35±0.17), 15:0 with BFI_29d and 16:0 with BFI_3d (0.32±0.18, -0.32±0.11). Due to perceived negative effects of 14:0 and 16:0 on human health, reduction in the concentration of these FAs in beef as a step towards meeting consumers expectation of healthier beef product would not significantly affect the meat quality traits considered in this study as the relationships are mostly weak except for a tendency towards tougher steaks with decrease in concentration of 14:0 three days after slaughter (-0.31±0.13). This moderate phenotypic relationship however disappeared after 26 days of aging as seen in the very low phenotypic correlation between 14:0 and WBSF_29d (-0.03±0.09). Due to the recommendation to reduce total SFA content in the diet (Daley et al., 2010), steaks with reduced odd numbered FAs 15:0 and 17:0 might have lesser water holding capacity as seen in the moderate negative correlation of these FAs with DL. In the longissimus muscle of Angus cattle aged for 14 days, Garmyn et al. (2011) also found a weak negative phenotypic correlation (-0.06, -0.02) between 14:0 and 16:0 in total muscle lipid with WBSF. Dryden et al. (1970) obtained favourable phenotypic correlations between 14:0 and 16:0 in intramuscular lipid with WBSF in the triceps brachii (0.05, 0.16) and longissimus dorsi (-0.04, 0.50) muscle of Hereford steers aged

for 7 days. However, in the intramuscular lipid of semimembranosus muscle aged for 7 days, there was a moderately high unfavourable phenotypic correlation between 14:0 and WBSF (-0.52, -0.10). Phenotypic correlations across studies of Garmyn et al. (2011) and Westerling et al. (1979), Dryden et al. (1970), O'Quinn et al. (2012), Melton et al. (1982) suggest that reducing the concentration of 14:0 and 16:0 would not impact negatively on beef flavor (0.02 to -0.52). Garmyn et al. (2011), Dryden et al. (1970), and Westerling et al. (1979) also show that reducing the concentration of 14:0 and 16:0 in longissimus muscle would not have an adverse effect on beef tenderness (0.00 to -0.45) and juiciness (-0.2 to 0.06). However, reducing the concentration of 14:0 might be associated with reduction in tenderness and juiciness (0.35 0.45) in the semimembranosus muscle and reduction in juiciness in the triceps brachii (0.40). Reducing the concentration of 16:0 in the triceps brachii is also expected to reduce tenderness and juiciness (0.33 0.63). Stearic acid, 18:0, is said to have a neutral effect on serum cholesterol and does not impact either low density lipoprotein (LDL) or high density lipoprotein (HDL) (Daley et al., 2010). Phenotypic correlations between 18:0 and meat quality traits were low, ranging from 0.01 to 0.29. In particular, with eating quality, 18:0 was negatively associated with BFI_3d and 29_d (-0.14±0.16, -0.16±0.15), OF_3d and OF_29d (-0.02±0.15, -0.02±0.11), OT_3d and 29d (-0.09±0.09, -0.03±0.03). Higher negative estimates between 18:0 and beef flavor (-0.24 to -0.60) is reported in other studies (O'Quinn, 2012; Westerling and Hedrick, 1979; Dryden and Maechello, 1970; Melton et al., 1982). Garmyn et al. (2011) however reported a low positive phenotypic correlation of 0.04 with 18:0 and beef flavor. With tenderness, Garmyn et al. (2011), Westerling et al. (1976) and Dryden et al. (1970) all reported negative associations with 18:0, in alignment with what we observed but to different extents (-0.08 to -0.33). In agreement with the negative phenotypic correlation between 18:0 and juiciness reported in this study, Westerling et al. (1976) in longissimus muscle and Dryden et al. (1970) in Semimembranosus and triceps

brachii also reported negative association between 18:0 and tenderness (-0.06 to -0.58), although Dryden et al. (1970) found no relationship between 18:0 and juiciness in longissimus muscle. In contrast to our finding, Garmyn et al. (2011) observed a low positive correlation between 18:0 and juiciness (0.07).

For individual SFAs, genetic correlations with meat quality traits were very weak to moderately strong and ranged from 0.01 for 14:0 and WBSF_29, 15:0 with OT_29d and OF_3d to -0.86 for 16:0 and SJ_3d. Moderately strong (≥ 0.5) favorable positive genetic relationship was seen between 16:0 and DL (0.57±0.14), strong favorable negative association between, 14:0, 16:0 and 18:0 with BFI_3d (-0.5±0.22, -0.57±0.20, -0.55±0.23), SJ_3d (-0.68±0.23, -0.86±0.11, -0.56±0.38), 18:0 with BFI_29d and OT_3d (-0.5±0.22, -0.54±0.14). This suggests that selection for reduced amount of 14:0 in beef would indirectly result in improved beef flavor intensity and juiciness and reducing 16:0 would reduce drip loss while increasing beef flavor intensity. Margaric acid, 17:0 had a moderate antagonistic relationship with MCL*29d and SJ_29d (0.35±0.29, 0.41±0.25) suggesting that a reduction in 17:0 in beef might tend to result in darker and less juicy steak. Similar to findings in the current study, Tait et al. (2008) reported low genetic correlations, between WBSF and 16:0 (-0.04) and 18:0 (-0.07) in the longissimus dorsi muscle of Angus sired cattle aged for 14 days except for a moderate positive relationship of 0.31 with 14:0.

The magnitude of phenotypic relationships between major individual MUFAs and meat quality attributes were generally low to moderate ranging from 0 for 9c-14:1 and OT_3d, 9c-16:1 and OF_3d, 9c-18:1 and BFI_29d, 11c-18:1 and SJ_29d to -0.38± and DL. Most phenotypic correlations were below 0.3 in magnitude suggesting the concentration of these FAs does not influence the meat quality traits and in particular eating quality traits of beef - flavor tenderness

and juiciness to any significant extent. There were however moderate negative phenotypic correlations between 9c-17:1 and 11c-18:1 with DL (-0.38±0.17, -0.36±0.19). A similar trend was reported by Garmyn et al. (2011) where phenotypic correlations of individual MUFAs 9c-16:1, 9c-17:1, 9c-18:1, 10t/11t-18:1, 15t-18:1, with WBSF, sustained juiciness, overall tenderness and beef flavor ranged from 0.01 for 9c-17:1 and beef flavor to 0.17 for 9c-17:1 and sustained juiciness in total lipid from the strip loin of Angus sired beef cattle aged for 14 days. Dryden et al. (1970) found a wider range of correlations for individual MUFAs, 9c-14:1, 9c-16:1 and 9c-18:1 with tenderness, juiciness, flavor and WBSF (0.03 to 0.78 in magnitude) in the intramuscular fat of the longissimus dorsi, triceps brachii, and semimembranous muscle of Hereford steers aged for 7 days. In particular, they found moderately strong negative correlation between 9c-14:1 with juiciness and flavor (-0.50, -0.43), moderate negative association between 9c-16:1 and WBSF (-0.35) and a moderate to strong positive correlation of 9c-18:1 with tenderness, juiciness, flavor and a negative relationship with WBSF (0.48, 0.37, 0.66, -0.39) in the intramuscular fat of longissimus dorsi muscle of Hereford cattle. Their result suggests that 9c-14:1 and 9c-18:1 play a significant role in the development of flavor and juiciness, 9c-18:1 plays a role in determining tenderness, 9c-16:1 and 9c-18:1 influence WBSF in the longissimus dorsi muscle. In the triceps brachii, and semimembranous muscle, 9c-14:1 and 9c-16:1 have a moderate to very strong phenotypic relationship with juiciness (0.46 to 0.78), 9c-14:1 is moderately associated with tenderness in the semimembranous muscle (0.42) while 9c-16:1 has a moderate phenotypic relationship with tenderness and flavor (0.41, 0.39). Westerling and Hedrick (1979), found weak negative phenotypic correlation between 9c-16:1 with flavor, tenderness and juiciness scores (-0.17, -0.03 and -0.19 respectively) and a weak to strong positive relationship between 9c-18:1 with flavor, juiciness and tenderness scores (0.67, 0.04, 0.26 respectively). In the beef strip loins of Angus, Holstein and American Wagyu cattle, O'Quinn et al. (2012)

reported moderate phenotypic correlation of 9c-14:1 (0.40), 9c-16:1 (0.35) and 9c-18:1 (0.49) with overall flavor desirability. Melton et al. (1982), estimated phenotypic correlations of fatty acids within the neutral and polar lipid fraction of ground beef containing approximately 20% fat prepared from semimembranosus muscle and subcutaneous fat from the brisket with beef flavor score and found low to moderate phenotypic correlation between 9c-14:1 (0.08, -0.33), 9c-16:1 (0.05, -0.04), and 9c-18:1 (0.05, 0.29) in the polar and neutral lipid fraction respectively. The variation in magnitude and direction of phenotypic correlation estimates across studies might be reflective of the differences in tissues sampled, and how they were sampled (i.e. as total muscle lipid or as phospholipids or triacylglycerol/neutral lipid fractions), breed differences, different post slaughter practices (different ageing periods), sample size, differences in the methods of parameter estimation, effects fitted/accounted for /adjusted for in the statistical model

Oleic acid, 9c-18:1 makes up about one-third of the fatty acids in beef and have been shown to has a positive relationship with flavor (0.67, 0.66, 0.11, 0.29 in neutral lipid, 0.49) by Westerling et al. (1979), Dryden et al. (1970), Garmyn et al. (2011), Melton et al. (1982) and O'Quinn et al. (2012) respectively. In this study however, phenotypic correlation of 9c-18:1 with BFI_3d and 29d was 0.02±0.12 and 0±0.11 respectively. The difference between our estimate and that of other studies might be because we adjusted FAs for intramuscular fat content so that relationships of FAs with meat quality traits is assessed at constant marbling to take into account differences in FA composition with increase in intramuscular fat (De Smet et al., 2004). Beef marbling has been associated with juiciness, tenderness and flavor (Scollan et al., 2006) therefore, it is possible that these relationships established with 9c-18:1 across the different studies are dependent on the various degrees of marbling in the animals

The magnitude of genetic correlations between individual MUFAs and meat quality ranged from 0.01 for 10t-18:1 and MCL*29d, 9c-14:1 and DL, 9c-16:1 with WBSF_29 and SJ_29, 9c-18:1 and WBSF_3d to 0.84 for 9c-14:1 and BFI_29d. Majority of the genetic relationship between individual MUFAs with meat quality traits were low (below 0.3) and most of the moderate to strong relationships were favorable except for a moderate positive unfavorable genetic correlation between 10t-18:1 with SJ_3 (0.38±0.44), SJ_29 (0.35±0.27), OT_29 (0.32±0.23). This result suggests that increase increasing juiciness, tenderness after 26 days of ageing through breeding will tend to be accompanied with increased levels of 10t-18:1 which is not desirable since 10t-18:1 was shown to have detrimental effects on plasma lipids and the metabolism of lipoproteins in rabbits (Roy et al., 2007). Genetic correlation of 9c-18:1 with BFI_3d and 29d, independent of marbling was moderately high (0.64±0.18, 0.53±0.18 respectively). Taken together, the low phenotypic and moderately high genetic correlation of 9c-18:1 with BFI_3d and 29d independent of marbling suggests that breeding for increased flavor would yield a correlated response of increased 9c-18:1 concentration in steaks. However, 9c-18:1concentration independent of marbling in the steak would not be perceived to be flavorful.

Phenotypic correlation of major BCFA, ai17:0, was low to moderate, ranging from -0.02±0.13 for ai17:0 and OT_3d to -0.49±0.15 for ai17:0 and DL. Except for the moderate negative phenotypic correlation between DL and ai17:0, all the phenotypic relationship of ai17:0 with meat quality is low. Branched chain FAs endogenously result from replacement of malonyl-CoA by methylmalonylCoA in the elongation reaction of odd chain FA formed from propionate. They are also synthesized by the microbes in the rumen (Drackley, 2000). This result indicates that management practices that increase the concentration of branched chain FA, like feeding high grain diet (Christie, 2012) might tend to result in decreased drip loss. Except for a very high

positive genetic correlation of ai17:0 with SJ_3 (0.83±0.16), all phenotypic correlation estimates of ai17:0 with meat quality traits were below 0.3 suggesting that selection for increased juiciness in beef would also increase ai17:0 concentrations.

For phenotypic correlations of major individual PUFAs, 18:2n:6 and 20:4n6 considered in this study, estimates were low to moderate ranging from 0.01 to 0.41 in magnitude. 18:2n6 with OT_3d and OF_3d had phenotypic correlations of 0.01±0.09 and 0.01±0.15 while 20:4n6 and SJ_3d had a phenotypic correlation of -0.41±0.14. Phenotypic correlation estimates were mostly below 0.3. However, there was a moderate negative relationship between 20:4n6 with BFI_3d and 29d (-0.35±0.12, -0.34±0.11) and OF_3d and 29d (-0.35±0.13, -0.32±0.1). 18:2n6, linoleic acid, also had a moderate negative phenotypic correlation with DL (-0.35±0.14). This suggests steaks having a high concentration of 18:2n6 would tend to have less drip loss and that the concentration of 20:4n6, arachidonic acid, plays a modest role in the development of beef off flavor, causing it have a less desirable flavor and thus less acceptable by the panelists. Garymn et al. (2011) and Westerling et al. (1979) also reported negative correlation between 20:4n6 and beef flavor, but of a lesser magnitude (-0.08, -0.29). Westerling et al. (1979) also reported a strong negative correlation between 18:2n6 and flavor (-0.63). Dryden et al. (1970) observed a low to moderate negative phenotypic correlation between 18:2n6 and flavor in the longissimus dorsi and semimembranosus muscle (-0.13, -0.32) but a weak positive correlation in the triceps brachii (0.08). However, Melton et al. (1982) reported a low positive correlation of 0.11 for 20:4n6 in the polar lipid fraction of ground beef with flavor score. In line with the current study, Garmyn et al. (2011) reported low negative phenotypic correlations between tenderness and juiciness with 18:2n6 and 20:4n6 (-0.02 to -0.20). Westerling et al. (1979) also reported low phenotypic correlations between 18:2n6 with juiciness and tenderness (-0.06, -0.09) and between

20:4n6 and tenderness (0.05). They however observed a moderate positive phenotypic correlation between 20:4n6 and juiciness (0.41). Dryden et al. (1970) reported low to high phenotypic correlation between tenderness and juiciness with 18:2n6 in the longissimus, semimembranosus and triceps brachii muscle (-0.14 to -0.74). They however observed weak positive phenotypic relationship of 0.10 between 18:2n6 and tenderness in the semimembranosus muscle. Meat flavor is a combination of aroma and taste (Elmore and Mottram, 2009) and is derived through cooking since fresh meat has no aroma and has a bloodlike taste (Mottram, 1998). When meat is cooked, FAs in the lipids are attacked by oxygen and they decompose forming volatile aroma compounds such as aldehydes and ketones with susceptibility to oxidation increasing with increase in FA unsaturation (Grosch, 1987). Development of flavors would most likely depend on the relative concentration of FAs present in the heated system (Melton et al., 1982). Genetic correlations between 18:2n6 with meat quality traits ranged from 0 for 18:2n6 and WBSF_3, 20:4n6 and WBSF_29 to -0.88±0.08 for 20:4n6 and SJ_3d. Negative moderate to high estimates were observed for 20:4n6 with BFI_3d and 29d (-0.52±0.3, -0.49±0.27), OF_3d and 29d (-0.35±0.29, -0.49±0.27), OT 3d and 29d (-0.38±0.19, -0.39±0.23) and with 18:2n6 with BF I_29d (-0.37±0.29), OF_3d and 29d (-0.39±0.26, -0.76±0.15) and SJ_3d and 29d (-0.69±0.35, -0.37±0.29). These results indicate that breeding for more desirable beef flavor would tend to reduce 20:4n6 concentration in beef and its precursor 18:2n6. Genetic improvement for tenderness and juiciness might also result in moderate reductions in the concentration of 20:4n6 and 18:2n6 respectively.

For group fatty acids, phenotypic correlation with meat quality traits were low to moderately high, ranging from 0.01 to 0.58 in magnitude. Lowest phenotypic relationships were between SFA and OT_3d (0.01±0.09), n-6 and WBSF_3d (0.01±0.15), HI and OT_29d (0.01±0.09) and

the strongest was between n-3 and SJ_3d (-0.58±0.13). Moderate negative unfavorable phenotypic correlation estimates were observed between total n3 with BFI_3d and 29d (-0.53±0.14,-0.47±0.16), OF_3d and 29d (-0.49±0.14, -0.44±0.1), SJ_3d and 29d (-0.58±0.13, -0.38±0.09), OT_3d (-0.34±0.11), MCL*3d (0.34±0.15), total n6 with SJ_3d (-0.3±0.16), DL (-0.37±0.14), BFA with DL (-0.4±0.15), SFA and SFA+BFA with DL (0.35±0.13) and PUFA with SJ_3d (-0.36±0.15), DL (-0.38±0.15). These results suggest that steaks with the most desirable flavor, juiciness and tenderness tended to have lower concentrations of n-3 FAs. However, beef with brighter color also tended to have more n-3. Also steaks that had the least drip loss tended to have higher amounts of PUFA and lower SFA concentration. Similar to our report, Garmyn et al. (2011) found a negative but lower phenotypic correlation for total n3, total n6 and PUFA with flavor, juiciness and tenderness (-0.03 to -0.22) and Cho et al. (2005) also reported similar results (-0.04 to -0.34) for phenotypic correlation between total n-3, n-6 and PUFA with tenderness juiciness and flavor in the triceps brachii, longissimus dorsi and semimembranosus muscle from Australian Angus and Korean Hanwoo cattle except for a low positive correlation between n-3 and flavor (0.17). In the Longissimus thoracis muscle from bulls of 15 European breeds of cattle, Sevane et al. (2014) also reported negative phenotypic correlation between flavor and juiciness with proportions of 18:2n6, PUFA, n-3 and n-6 in the muscle (-0.01 to -0.27) except for a positive relationship between juiciness and total n3 proportion (0.08). In contrast to our observation that SFA had weak negative correlation with flavor and juiciness, Cho et al. reported a low positive correlation between SFA with flavor (0.24) and juiciness (0.15) and tenderness (0.29)

Genetic correlation between group FAs and all meat quality traits ranged from 0 for SFA+BFA with MCL*29d to -0.98±0.01 for n-3 and SJ_29d. Several moderate (>0.3) to high phenotypic

correlations with meat quality traits were favorable. However, there were antagonistic relationships between MCL*29d with n-3 and PUFA (-0.3±0.32, -0.34±0.3), DL with n-3, n-6/n-3 and PUFA (0.79±.012, -0.51±0.18, 0.48±0.25), BFI_3d with n-3, n-6/n-3 and PUFA (-0.84±0.12, 0.62±0.2, -0.33±0.36), BFI_29d with n-3, n-6/n-3 and PUFA (-0.83±0.1, 0.36±0.23, -0.56±0.24), OT_3d with n-3, n-6/n-3 (-0.43±0.19, 0.41±0.15), OT_29d with n-3, n-6/n-3 (-0.64±0.16, 0.60±0.13), SJ_3 with n-3 n-6/n-3 (-0.68±0.36, 0.41±0.41) , SJ_29d with n-3, n-6/n-3 and PUFA (-0.98±0.01, 0.79±0.1, -0.82±0.12). These results indicate that with genetic improvement for meat eating quality, flavor, tenderness and juiciness in beef would be expected to result in less total n-3, less PUFA and an unfavorable n-6/n-3 ratio. The positive phenotypic and negative genetic correlation of total n-3 with color suggests that a positive environmental correlation between n-3 and color.

Although not categorized as a major individual MUFA in this study (Appendix S 5-1), vaccenic acid, 11t-18:1 is of interest because of the positive health effects it is associated with including its metabolite, conjugated linoleic acid (9c, 11t-18:2) (Corl et al., 2003; Field et al., 2009; Bhattacharya et al., 2006). Phenotypic correlations of 11t-18:1 with meat quality were weak to moderate ranging from -0.01±0.17 (MCL*3d) to -0.4±0.16 (OF_3d), with negative correlations for all sensory traits, especially moderate phenotypic correlations with FI_3d, FI_29d, OF_3d, OF_29d, SJ_3d (-0.38±0.18, -0.39±0.17, 0.4±0.16 , -0.31±0.13, -0.34±0.2) while for 9c,11t-18:2, it ranged from 0.01±0.26 (WBSF_3d) to -0.5±0.16 (SJ_3d) with negative phenotypic correlations for all sensory traits particularly moderate to moderately high negative phenotypic correlations with OF_3d, OF_29d, SJ_3d (-0.34±0.14, -0.39±0.17, -0.50±0.16). This result suggests that steaks with more desirable flavors tend to have lower concentration of 11t-18:1 and 9c, 11t-18:2. Genetic correlations for 11t-18:1 with meat quality was from -0.03±0.34 to 0.49.

The weakest genetic correlation was between 11t-18:1 and FI_29d (-0.03±0.34) and the strongest was between 11t-18:1 and WBSF_29d (0.49±0.22). Genetic correlations with meat quality traits were mostly low, below (0.3). Moderate genetic unfavorable genetic correlations were observed for 11t-18:1 with FI_3d and WBSF_29d (-0.37±0.32, 0.49±0.22). Genetic correlations between major CLA isomer 9c, 11t-18:2 and meat quality ranged from 0 for 9c, 11t-18:2 with FI_29d and OT_3d to 0.66 for 9c, 11t-18:2 and SJ_3d. Except for a moderate negative unfavorable correlation between 9c, 11t-18:2 and OF_29d, SJ_29d, MCL*3d (-0.32±0.34, -0.34±0.3, -0.44±0.29) and a positive favorable genetic correlation with SJ_3d (0.66±0.39) other relationships were low.

With the increasing awareness for the need of more omega-3 PUFAs in the human diet, especially because of its positive effect on health (Wood et al., 2003; Simopoulos, 1999; Simopoulos, 2006; Swanson et al., 2012), it is important to understand how modifying the concentration of this FA in beef will influence meat quality. Our data shows that the phenotypic relationship of alpha linoleic acid (18:3n3) and its metabolites eicosapentanoic acid (EPA 20:5n3), and docosahexaenoic acid (DHA 22:6n3) with all meat quality traits were weak to moderate with a range of -0.02±0.1 for 20:5n3 and OT_29d to -0.50±0.14 for 22:6n3 and SJ_3d. Moderate phenotypic correlation was observed for 18:3n3 with BFI_3d and 29d (-0.35±0.16, -0.31±0.17), OF 3d and 29d (-0.33±0.14, -0.36±0.1) and SJ 3d and 29d (-0.44±0.14, -0.3±0.08), 20:5n3 with BFI_29d, OF_3d, SJ_3d (-0.32±0.14, -0.32±0.15, -0.32±0.17), 22:6n3 with BFI_3d and 29d (-0.43±0.12, -0.37±0.13), OF_3d and 29_d (-0.37±0.14,-0.32±0.11) SJ_3d and 29d (-0.50±0.14, -0.31±0.1), 22:6n3 with MCL*29d (0.31±0.14). This data indicates that there is a tendency for reduction in eating quality of beef with increased concentration of 18:3n3 and its metabolites. In accordance with our observation, Garmyn et al. (2011) and Westerling et al.

(1979) found negative but lower phenotypic correlation for 18:3n3 with flavor (-0.01 and -0.17) and Melton et al. (1982) reported a negative and larger correlation for the concentration of 18:3n3 in both the neutral and phospholipid fraction of lipids in ground beef (-0.51, -0.41) with flavor. O'Quin et al. (2012) also reported negative but large phenotypic correlation between 18:3n3 with the overall flavor desirability of beef (-0.65). For juiciness and tenderness, with 18:3n3, Garmyn et al. (2011) also reported negative correlation (-0.08, -0.02) while Westerling (1979) reported a low positive correlation of 0.06 with juiciness. Genetic correlations for 18:3n3 and its metabolites, 20:5n3 and 22:6n3 with meat quality traits ranged from -0.02±0.5 for 20:5n3 and OT_29d to -0.98±0.01 for 22:6n3 with SJ_29d. There was moderate to high negative unfavorable genetic correlation between 18:3n3 and BFI_3d and 29d (-0.65±0.2, -0.87±0.08), OF_29d (-0.92±0.05), SJ_3d and 29d (-0.3±0.51, -0.94±0.04) and DL (0.66±0.15). 20:5n3 had moderate to high unfavorable genetic correlation with MCL*3d and 29d (-0.4±0.45, -0.83±0.22), DL (0.42±0.39), BFI_29d (-0.32±0.56), OT_3d (-0.53±0.31). There was however a moderate positive genetic correlation for 20:5n3 with SJ_3d and 29d (0.55±.0.44, 0.54±0.54). 22:5n3 also had moderate to high negative genetic antagonistic correlation with all meat quality traits except color. These results suggests that genetic improvement of meat quality traits in beef cattle, especially eating quality is expected to yield undesired responses such that meat will contain less of these beneficial FAs.

Interpretation of the relationship of these minor FAs with meat quality traits should be made with caution due to the small concentration of these FAs in beef, which may be subject to more random errors in fatty acid quantification process. Nevertheless, information on the estimates of phenotypic correlations of FA with meat quality traits is lacking and there is presently no study on genetic correlations between FAs and meat traits in LL muscle. These results will not only

help us understand the acceptability of meat with enhanced beneficial FA profile, but will help design multiple selection index to alleviate undesirable effects of improving fatty acid profiles in beef on meat quality.

5.4 Conclusion

Heritability for meat quality traits in this study was low, except for tenderness measured 3 days post mortem, suggesting that selection to improve attributes of meat quality traits through breeding may be difficult due to low additive genetic variation.

Ideally, improving the FA profile of beef would involve reducing the concentration of harmful saturated fatty acids 14:0 and 16:0, while simultaneously improving the concentration of beneficial MUFAs like 9c-18:1 and 11t-18:1 and beneficial PUFAs like 9c,11t-18:2, 18:3n3, 20:5n3, and 22:6n3 without sacrificing meat quality. Reducing the concentration of harmful SFA, 14:0 and 16:0 is expected to have no antagonistic effect on meat quality except that there was a tendency for steaks having less 14:0 to be perceived as less tender. This perception appeared to disappear after steaks were aged. However, increasing the concentration of beneficial PUFAs is expected to have undesirable effects on meat quality as there was moderate to strong phenotypic and genetic antagonistic relationships between beneficial 9c,11t-18:2, 18:3n3, 20:5n3, and 22:6n3 with several meat quality traits including tenderness, juiciness and flavor. More work is required to validate these relationships and to determine appropriate selection index weights for genetic selection and breeding programs and to design diet supplements to produce meat with beneficial FA without compromising quality.

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Table 5-1. Mean, standard deviation (SD), range , coefficient of variation (CV) and heritability (h^2±SE) for meat quality traits.

Traits	N	Mean	SD	Range	CV	h^2±SE
Drip Loss mg/g-[1]	n=1025	31.10	12.22	9.20 - 59.50	39.30	0.18±0.08
Shear force_ 3d, Kg	n=1136	7.83	1.91	3.37 - 15.95	24.36	0.16±0.07
Shear force _29d, kg	n=1139	4.86	0.97	2.64 - 8.95	19.95	0.17±0.07
Color L*_3d	n=1137	37.17	4.18	25.26 - 47.42	11.25	0.21±0.08
Color L*_ 29d	n=1145	38.82	2.98	28.24 - 48.92	7.68	0.09±0.05
Beef Flavor Intensity_3d[1]	n=1133	5.37	0.69	4.25 - 6.63	12.82	0.05±0.04
Beef Flavor Intensity_29d[1]	n=1144	5.49	0.47	4 - 11.67	8.62	0.09±0.05
Off Flavor_3d[1]	n=1133	7.92	0.96	5.84 - 9	12.09	0.10±0.05
Off Flavor_29d[1]	n=1144	7.89	0.50	5.83 - 9	6.35	0.09±0.06
Overall Tenderness_3d[1]	n=1133	5.73	1.12	2.63 - 7.75	19.50	0.36±0.09
Overall Tenderness_29d[1]	n=1144	6.65	0.71	1.38 - 8.25	10.63	0.21±0.07
Sustained Juiciness_3d[1]	n=1133	5.45	0.70	4 - 6.88	12.85	0.02±0.03
Sustained Juiciness_29d[1]	n=1144	5.39	0.48	3.71 - 6.71	8.83	0.10±0.06

[1]Scale : 9 = Extremely tender, juicy, intense :7 = very tender, juicy, intense; 6 =moderately tender, juicy, intense, or slight;
5 = slightly tender, juicy, intense, or slight; 4 = slightly tough, dry, bland: 1=Exremely tough, dry, bland.
Trait_3d _ trait measured 3 days post mortem
Trait_29d_trait measured 29 days post mortem

Table 5-2. Phenotypic and genetic correlation (±SE) of major fatty acids (concentration > 0.5% FAME) in the longissium lomborum muscle tissue of beef cattle with meat quality traits.

	Driploss		Color_L3d		Color_L29d		Shear_3d		Shear_29d	
	rp	Rg	rp	rg	Rp	rg	rp	rg	rp	rg
Saturated										
14:0	-0.22±0.14	0.18±0.2	0.16±0.11	0.06±0.19	-0.04±0.14	-0.27±0.22	-0.31±0.13	-0.06±0.21	-0.03±0.09	0.01±0.2
15:0	-0.36±0.19	0.04±0.3	0.23±0.14	0.1±0.28	-0.03±0.2	0.12±0.36	-0.32±0.15	-0.05±0.29	-0.04±0.13	-0.21±0.28
16:0	0.15±0.19	0.57±0.14	0.2±0.1	0.21±0.17	0.23±0.11	-0.1±0.23	0.2±0.13	-0.06±0.21	0.1±0.09	-0.17±0.19
17:0	-0.35±0.17	-0.08±0.27	0.23±0.13	0.1±0.26	-0.01±0.18	0.35±0.29	-0.3±0.13	-0.07±0.26	-0.08±0.12	-0.25±0.25
18:0	0.29±0.15	0.33±0.21	-0.12±0.11	0.28±0.22	0.01±0.13	0.24±0.29	0.18±0.12	0.11±0.24	0.02±0.09	0.3±0.23
Monounsaturated										
9c-14:1	-0.05±0.08	0.01±0.19	0.04±0.05	0.24±0.17	-0.01±0.06	-0.09±0.24	-0.04±0.06	-0.2±0.18	0.02±0.05	-0.17±0.19
9c-16:1	-0.12±0.11	-0.06±0.18	0.08±0.06	0.16±0.16	-0.01±0.08	-0.2±0.2	-0.1±0.08	-0.21±0.17	0.01±0.05	-0.01±0.18
9c-17:1	-0.38±0.17	-0.25±0.28	0.24±0.13	0.13±0.29	0.02±0.19	0.48±0.29	-0.28±0.15	-0.18±0.29	-0.04±0.12	-0.31±0.27
9c-18:1	0.17±0.1	-0.68±0.11	-0.12±0.07	-0.18±0.18	0.01±0.09	0.13±0.24	0.06±0.09	0.01±0.2	-0.07±0.06	0.07±0.21
11c-18:1	-0.36±0.19	-0.61±0.19	0.07±0.16	-0.04±0.28	-0.17±0.18	-0.02±0.35	-0.25±0.16	-0.09±0.28	-0.01±0.12	-0.11±0.29
10t-18:1	-0.28±0.16	0.02±0.27	0.11±0.12	-0.31±0.23	-0.07±0.15	-0.01±0.33	-0.24±0.12	0.02±0.26	-0.03±0.1	-0.08±0.27
Branched										
ai17:0	-0.49±0.15	-0.11±0.28	0.28±0.13	0±0.28	0.11±0.19	0.06±0.37	-0.12±0.19	-0.01±0.31	0.15±0.11	0.11±0.3
Polyunsaturated										
18:2n-6	-0.35±0.14	0.2±0.3	0.12±0.13	-0.08±0.26	-0.1±0.13	-0.24±0.31	-0.08±0.13	0±0.28	0.1±0.09	-0.14±0.27
20:4n-6	-0.21±0.18	0.63±0.19	0.15±0.14	0.04±0.27	0.19±0.14	-0.24±0.33	0.26±0.12	0.39±0.25	0.24±0.08	0±0.29
Group										
sumtrans18:1	-0.24±0.14	0.07±0.26	0.09±0.11	-0.29±0.23	-0.08±0.13	-0.03±0.31	-0.22±0.11	0.02±0.25	-0.02±0.09	0.03±0.26
SFA	0.35±0.13	0.62±0.15	-0.11±0.14	0.27±0.19	0.12±0.12	0.01±0.24	0.16±0.11	-0.03±0.2	0.03±0.08	-0.06±0.2
SFA+BFA	0.35±0.13	0.63±0.15	-0.05±0.14	0.27±0.19	0.15±0.11	0±0.24	0.18±0.12	-0.02±0.21	0.08±0.08	-0.04±0.19
BFA	-0.4±0.15	0.18±0.28	0.23±0.12	-0.02±0.29	0.06±0.18	-0.04±0.38	-0.1±0.18	0.01±0.31	0.17±0.11	0.23±0.3
MUFA	0.02±0.05	-0.82±0.07	-0.06±0.04	-0.24±0.17	-0.04±0.07	0.08±0.24	-0.1±0.06	-0.06±0.2	-0.1±0.06	0.01±0.2
PUFA	-0.38±0.15	0.48±0.25	0.18±0.17	0.01±0.3	0.12±0.16	-0.3±0.32	0.07±0.16	0.12±0.28	0.22±0.1	-0.11±0.29
n-3	-0.26±0.24	0.79±0.12	0.26±0.16	0.18±0.27	0.34±0.15	-0.34±0.3	0.31±0.18	0.24±0.28	0.31±0.1	0.16±0.29
n-6	-0.37±0.14	0.36±0.29	0.16±0.15	-0.02±0.28	0.05±0.15	-0.28±0.32	0.01±0.15	0.1±0.28	0.19±0.09	-0.13±0.28
n-6/n-3	0.02±0.19	-0.51±0.18	-0.21±0.1	-0.22±0.21	-0.29±0.1	0.14±0.26	-0.14±0.13	-0.05±0.23	-0.15±0.09	-0.15±0.23
Health Index	0.12±0.12	-0.43±0.18	-0.17±0.08	-0.2±0.17	-0.17±0.1	0.09±0.23	0.11±0.13	0.03±0.19	-0.06±0.07	0.09±0.18

Table 5-2. Phenotypic and genetic correlation (±SE) of major fatty acids (concentration > 0.5% FAME) in longissium lomborum muscle tissue of beef cattle with meat quality traits cont'd.

	Off flavor_3d		Off flavor_29d		Beef flavor intensity_3d		Beef flavor intensity_29d	
	rp	rg	rp	Rg	rp	rg	rp	rg
Saturated								
14:0	0.02±0.16	-0.08±0.23	0.03±0.11	-0.15±0.25	0.18±0.16	-0.5±0.22	0.22±0.15	-0.23±0.23
15:0	0.07±0.22	-0.01±0.35	0.05±0.16	-0.05±0.37	0.29±0.2	-0.18±0.4	0.32±0.18	-0.12±0.35
16:0	-0.23±0.12	-0.12±0.23	-0.2±0.1	-0.45±0.18	-0.32±0.11	-0.57±0.2	-0.29±0.12	-0.43±0.19
17:0	0.12±0.2	0.16±0.3	0.08±0.14	-0.12±0.34	0.28±0.19	0.16±0.36	0.28±0.18	-0.09±0.32
18:0	-0.02±0.15	-0.08±0.29	-0.02±0.11	-0.31±0.27	-0.14±0.16	-0.55±0.23	-0.16±0.15	-0.5±0.22
Monounsaturated								
9c-14:1	0.02±0.07	-0.31±0.21	0.02±0.05	0.15±0.24	0.02±0.07	0.22±0.26	0.08±0.07	0.84±0.07
9c-16:1	0±0.09	0.05±0.2	0.04±0.06	0.37±0.19	0.02±0.1	0.09±0.24	0.06±0.09	0.34±0.18
9c-17:1	0.06±0.21	0.21±0.34	0.07±0.15	0.44±0.3	0.27±0.2	0.68±0.22	0.29±0.19	0.3±0.33
9c-18:1	0.06±0.1	0.22±0.23	0.11±0.07	0.63±0.16	0.02±0.12	0.64±0.18	0±0.11	0.53±0.18
11c-18:1	0.14±0.21	0.09±0.33	0.09±0.15	0.8±0.13	0.24±0.21	0.71±0.2	0.25±0.2	0.37±0.3
10t-18:1	0.08±0.17	0.09±0.31	0.04±0.12	-0.33±0.31	0.23±0.16	0.19±0.36	0.22±0.16	-0.14±0.32
Branched								
ai17:0	-0.16±0.2	-0.05±0.35	-0.14±0.14	0.09±0.39	0.09±0.23	-0.09±0.41	0.12±0.22	0.01±0.36
Polyunsaturated								
18:2n-6	-0.01±0.15	-0.39±0.26	-0.11±0.12	-0.76±0.15	0.02±0.17	-0.09±0.37	0.02±0.16	-0.37±0.29
20:4n-6	-0.35±0.13	-0.35±0.29	-0.32±0.1	-0.49±0.27	-0.35±0.12	-0.52±0.3	-0.34±0.11	-0.49±0.26
Group								
sumtrans18:1	0.08±0.15	0.12±0.29	0.03±0.11	-0.34±0.29	0.22±0.14	0.12±0.35	0.2±0.14	-0.18±0.3
SFA	-0.14±0.12	-0.13±0.23	-0.09±0.1	-0.47±0.19	-0.2±0.14	-0.77±0.12	-0.2±0.14	-0.54±0.17
SFA+BFA	-0.17±0.11	-0.14±0.23	-0.14±0.09	-0.48±0.18	-0.22±0.14	-0.78±0.12	-0.22±0.13	-0.55±0.17
BFA	-0.16±0.19	-0.16±0.35	-0.16±0.14	-0.27±0.37	0.07±0.22	-0.38±0.35	0.1±0.21	-0.27±0.34
MUFA	0.14±0.06	0.28±0.21	0.17±0.05	0.61±0.17	0.19±0.06	0.87±0.08	0.18±0.07	0.48±0.2
PUFA	-0.27±0.15	-0.42±0.28	-0.29±0.12	-0.81±0.13	-0.26±0.15	-0.33±0.36	-0.25±0.15	-0.56±0.24
n-3	-0.49±0.14	-0.45±0.27	-0.44±0.1	-0.87±0.09	-0.53±0.14	-0.84±0.12	-0.47±0.16	-0.83±0.1
n-6	-0.19±0.16	-0.38±0.28	-0.24±0.12	-0.74±0.16	-0.16±0.17	-0.22±0.37	-0.16±0.16	-0.46±0.27
n-6/n-3	0.29±0.11	0.3±0.25	0.25±0.09	0.31±0.28	0.32±0.14	0.62±0.2	0.25±0.14	0.36±0.23
Health Index	0.16±0.11	0.1±0.22	0.14±0.09	0.32±0.21	-0.24±0.13	0.57±0.2	-0.23±0.13	0.37±0.21

Table 5-2. Phenotypic and genetic correlation (±SE) of major fatty acids (concentration > 0.5% FAME) in longissium lomborum muscle tissue of beef cattle with meat quality traits cont'd.

	Overall juiciness_3d		Overall juiciness_29d		Overall tenderness_3d		Overall tenderness_29d	
	rp	rg	rp	Rg	rp	rg	rp	rg
Saturated								
14:0	-0.13±0.17	-0.68±0.23	0±0.08	-0.03±0.24	0.09±0.1	-0.03±0.16	-0.09±0.09	-0.13±0.19
15:0	-0.08±0.26	-0.17±0.63	0.03±0.11	0.25±0.32	0.14±0.13	0.03±0.23	-0.09±0.12	-0.01±0.28
16:0	-0.29±0.1	-0.86±0.11	-0.16±0.08	-0.47±0.17	-0.15±0.09	-0.13±0.15	-0.13±0.07	-0.2±0.17
17:0	-0.01±0.24	0.19±0.52	0.05±0.1	0.41±0.25	0.17±0.11	0.11±0.21	-0.03±0.12	0.13±0.25
18:0	0.11±0.17	-0.56±0.38	0.01±0.07	-0.03±0.3	-0.09±0.09	-0.54±0.14	-0.03±0.09	-0.36±0.2
Monounsaturated								
9c-14:1	-0.03±0.08	0.09±0.41	-0.04±0.04	-0.06±0.23	0±0.05	0.23±0.14	-0.01±0.05	0.15±0.18
9c-16:1	-0.09±0.09	-0.29±0.31	-0.01±0.04	0.01±0.21	0.03±0.06	0.09±0.14	0.01±0.06	0.03±0.17
9c-17:1	-0.09±0.24	0.34±0.57	0.04±0.11	0.36±0.31	0.14±0.13	0.24±0.23	-0.03±0.12	0.44±0.23
9c-18:1	0.1±0.11	NE	0.08±0.05	0.31±0.23	0.03±0.07	0.17±0.16	0.11±0.06	0.15±0.19
11c-18:1	0.03±0.25	0.66±0.37	0±0.11	0.29±0.32	0.11±0.12	0.27±0.21	-0.05±0.12	0.18±0.26
10t-18:1	0.02±0.2	0.38±0.44	0.04±0.09	0.35±0.27	0.14±0.1	0.23±0.2	-0.02±0.1	0.32±0.23
Branched								
ai17:0	-0.21±0.21	0.83±0.16	-0.08±0.1	0.14±0.36	-0.02±0.13	0.05±0.24	-0.13±0.09	0.28±0.26
Polyunsaturated								
18:2n-6	-0.13±0.18	-0.69±0.35	-0.13±0.08	-0.37±0.29	0.01±0.09	0.12±0.21	-0.14±0.09	0.03±0.25
20:4n-6	-0.41±0.14	NE	-0.26±0.09	-0.88±0.08	-0.24±0.1	-0.38±0.19	-0.2±0.1	-0.39±0.23
Group								
sumtrans18:1	0.04±0.17	0.39±0.43	0.05±0.08	0.4±0.24	0.13±0.09	0.2±0.19	-0.03±0.09	0.3±0.22
SFA	-0.06±0.17	-0.89±0.1	-0.02±0.08	-0.32±0.21	-0.11±0.08	-0.28±0.14	0.01±0.09	-0.29±0.18
SFA+BFA	-0.17±0.12	-0.83±0.14	-0.05±0.08	-0.33±0.21	-0.13±0.08	-0.27±0.14	-0.15±0.06	-0.29±0.16
BFA	-0.2±0.2	0.8±0.22	-0.09±0.1	-0.11±0.37	-0.05±0.13	-0.07±0.25	-0.17±0.09	0.14±0.29
MUFA	0.14±0.07	0.8±0.15	0.12±0.04	0.56±0.17	0.11±0.04	0.33±0.14	0.14±0.04	0.31±0.17
PUFA	-0.36±0.15	NE	-0.24±0.09	-0.82±0.12	-0.15±0.11	-0.08±0.23	-0.21±0.1	-0.17±0.26
n-3	-0.58±0.13	-0.68±0.36	-0.38±0.09	-0.98±0.01	-0.34±0.11	-0.43±0.19	-0.23±0.08	-0.64±0.16
n-6	-0.3±0.16	NE	-0.21±0.08	-0.6±0.22	-0.09±0.1	-0.02±0.22	-0.19±0.1	-0.08±0.26
n-6/n-3	0.4±0.12	0.41±0.41	0.19±0.07	0.79±0.1	0.2±0.08	0.41±0.15	0.1±0.06	0.6±0.13
Health Index	0.21±0.09	NE	0.06±0.07	0.25±0.21	0.01±0.09	0.14±0.15	0.11±0.07	0.22±0.17

The concentrations of fatty acids (FAs) were expressed as a percentage of fatty acid methyl esters (FAME) quantified. Only FAs > 0.5% of total FAME are presented. c=cis, t=trans. SFA: sum of saturated fatty acids; SFA+BCFA: sum of saturated and branched chain fatty acids; Sum trans18:1: sum of trans-18:1; MUFA: sum of all cis and all trans mono-unsaturated fatty acids.; SumCLA: sum of conjugated linoleic acids; PUFA: sum of polyunsaturated fatty acids; n-6/n-3: ratio between n-6 and n-3 PUFA; [b] Sum trans18:1 = 6t/8t-18:1 + 9t-18:1 + 10t-18:1 + 11t-18:1 + 12t-18:1 + 13t/14t-18:1 + 15t-18:1 +16t-18:1 ; [c] SumCLA = 8t,10c-18:2 + 9c,11t-18:2 + 7t,9c-18:2 + 9t,11c-18:2 + 10t,12c-18:2 + 11c,13t-18:2 + 11t,13c-18:2 +12t,14c-18:2+12c,14t-18:2+ 9c,11c-18:2 + 10c,12c-18:2 +6t,8t-18:2+ 9t,11t-18:2 + 11t,13t-18:2 + 12t,14t-18:2 + 10t,12t-18:2 + 8t,10t-18:2 + 7t,9t-18:2 ;[d]SFA = 10:0 + 12:0 + 13:0 + 14:0 + 15:0 + 16:0 + 17:0 + 18:0 + 20:0 + 23:0[e] MUFA = 9c-14:1 + 9c-15:1 + 7c-16:1 + 9c-16:1 + 9c-17:1 + 6t/7t/8t-18:1 + 9t-18:1 + 10t-18:1 +11t-18:1 + 12t-18:1 + 13t/14t-18:1 + 15t-18:1 + 16t-18:1 + 9c-18:1 + 11c-18:1 + 12c-18:1 + 13c-18:1 + 14c-18:1 + 16c-18:1 + 9c-20:1 + 11c-20:1 ; [f]PUFA = 18:2n-6 + 18:3n-6 + 18:3n-3 + 20:2n-6 + 20:3n-9 + 20:3n-6 + 20:4n-6 + 22:4n-6 + 22:5n-3.[g]BCFA iso-14:0 + iso-15:0 + anteiso-15:0 + iso-16:0 + iso-17:0 + anteiso-17:0 + iso-18:0 ; [h]n-6 PUFA = 18:2n-6 + 18:3n-6 + 20:2n-6 + 20:3n-6 +20:4n-6 + 22:4n-6 ; [i]n-3 PUFA = 18:3n-3 + 22:5n-3 ; [j]Health Index: (total MUFA + total PUFA) / (4 x C14:0 + C16:0).

* Descriptive statistics of these FAs have been reported in a previous study

CHAPTER 6

A MULTIPLE CANDIDATE GENE ASSOCIATION APPROACH TO IDENTIFYING SINGLE NUCLEOTIDE POLYMORPHISMS (SNP) ASSOCIATED WITH FATTY ACID COMPOSITION IN BRISKET ADIPOSE OF COMMERCIAL CROSSBRED BEEF STEERS

6.1 Introduction

Excessive animal fat intake has been considered to be associated with cardiovascular diseases, obesity and various forms of cancers (Rule et al., 1995; Whetsell et al., 2003. ; Wood et al., 2008) which has led to concerns on the consumption of red meats including beef (Aalhus et al., 2014). However, there is a growing body of evidence indicating that the type of fatty acids (FA) has more profound implications on human health than the amount of fat in the diet (Hu et al., 2001; Woodside and Kromhout, 2005). Some FA that are produced naturally by ruminant animals, such as trans-11 18:1 (vaccenic acid), and, cis-9, trans-11 18:2 (rumenic acid), an isomer of conjugated linoleic acid (CLA), has a number of potential health benefits (Bassett et al., 2010; Wang et al., 2008). In ruminants including beef cattle, FA composition in tissues is a complex trait. It is believed to be influenced by multiple factors including host animal gene, diets, rumen microflora composition and environment, management practices, (Jenkins, 1993; Malau-Aduli et al., 2000a; Mapiye et al., 2012; Wood et al., 2003), and possibly interactions among all factors. Variation associated with host animal genes offer a great potential to further improve content of beneficial FAs by capitalizing on the

natural genetic differences between animals through genetic selection and/or through gene-assisted management.

Genetic variation due to direct genetic effects in beef cattle have been investigated for some FAs, with heritability ranging from low to high (0.00 to 0.86) depending on the FA (Inoue et al., 2011; Kelly et al., 2013; Malau-Aduli et al., 2000b; Nogi et al., 2011; Pitchford et al., 2002; Tait et al., 2007; Yokota et al., 2012). Chromosomal regions or Quantitative trait loci (QTL) that are associated with FA composition in beef cattle have also been identified on multiple chromosomes based on low density DNA markers (Abe et al., 2008; Alexander et al., 2007; Gutierrez-Gil et al., 2010; Morris et al., 2010; Morris et al., 2007b), and based on the Illumina BovineSNP50 BeadChip (Ishii et al., 2013; Kelly et al., 2013; Saatchi et al., 2013; Uemoto et al., 2011). In addition to the QTL scan for FA, SNP markers of several genes including SCD (stearoyl-CoA desaturase (delta-9-desaturase), FASN (fatty acid synthase), SREBP-1 (sterol regulatory element binding transcription factor 1), FABP4 (fatty acid binding protein 4), LXR-alpha (liver X nuclear receptor alpha) also known as NR1H3 (nuclear receptor subfamily 1, group H, member 3), ACACA (acetyl-CoA carboxylase alpha), LEP (leptin), FADS1 (fatty acid desaturase 1), PPARG (peroxisome proliferative activated receptor gamma), THRSP (thyroid hormone responsive), PPARGC1A (peroxisome proliferator-activated receptor-gamma coactivator 1 alpha), have been reported to have associations with fatty acid composition in beef cattle populations (Abe et al., 2009; Barton et al., 2010; Han et al., 2013; Hoashi et al., 2007; Hoashi et al., 2008; Oh et al., 2014; Ohsaki et al., 2009; Orru et al., 2011;

Sevane et al., 2013; Taniguchi et al., 2004; Zhang et al., 2010). In this study, we assembled a gene SNP panel of 1463 single nucleotide polymorphisms (SNPs) in 556 genes genome-wide that have potential functions in growth and fat metabolism with the objective to evaluate their associations with 25 individual and groups of FAs in the brisket adipose tissue of commercial cross-bred beef steers.

6.2 Materials and methods

6.2.1 Animals and management

The animals were part of a study that was designed to assess the effect of nonionophore antimicrobial treatment on FA composition in beef cattle (Aldai et al. 2008). The animals are Angus and Charolais based Canadian commercial crossbred steers (n=223), originating from Deseret Ranches near Lethbridge, Alberta, Canada (Aldai et al., 2008), with no pedigree information available. The animal management, diets, and nonionophore antibiotic treatments were outlined previously in Aldai et al. 2008. In brief, steers of similar body weight (198±20 kg) were randomly assigned to 24 feedlot pens and 1 of 5 nonionophore antibiotic treatment, which was administered throughout the feeding period and was withdrawn 21 days prior to slaughter. The animals received a barley silage-based grower diet for 80 days consisting 53.9% barley silage, 37.1% barley, 6.8% supplement, and 2.2% antibiotic premix and were subsequently adapted from the grower diet to a grain-based finishing diet over 21 days using 4 transition diets (Aldai et al. 2008). The grain-based finishing diet was made up of 81.1% barley,

9.1% barley silage, 7.5% supplement, and 2.3% antibiotic premix and was fed the animals for 120 days.

6.2.2 Animal Tissue Collection and Fatty Acid Analysis

The steers were slaughtered at 580±34kg and, samples of brisket adipose tissues were collected within 48 hrs, placed in plastic bags, frozen on dry ice and stored at -80ºC for FA analyses. Details on FA analyses were previously described by Aldai et al. (2008). In summary, brisket adipose tissue samples were freeze-dried and directly methylated with sodium methoxide. Using the methods outlined by Cruz-Hernandez et al. (2004), the fatty acid methyl esters (FAME) were analyzed by gas chromatography (GC) and silver-ion high performance liquid chromatography (Ag-HPLC). The trans-18:1 isomers were further separated using two complementary GC temperature programs instead of a preparatory silver-ion thin layer chromatography (Ag-TLC) separation combined with GC analyses at 120ºC (Kramer et al., 2008).

FA concentrations were expressed as a proportion of total FAME quantified. Twenty-five FAs (15 individual and 10 grouped FAs including FAs indices) with a concentration greater than 0.5% were selected and analyzed in this study. The 15 individual FAs included 5 saturated fatty acids (SFA) (14:0, 15:0, 16:0, 17:0 and 18:0), 8 monounsaturated fatty acids (MUFA) (9c-14:1, 9c-16:1, 9c-17:1, 9c C18:1, 10t-18:1, 11c-18:1, 11t-18:1, 13c-18:1), 1 branched-chain fatty acid (BCFA), 17:0 ai (anteiso); and 1 PUFA (18:2n-6) while the 10 groups of FAs calculated by adding up proportions of the relevant components as described in

Table 6-1 were SFA, MUFA, PUFA, BCFA, SFA+BCFA, sumCLA (sum of conjugated linoleic acid), sumtrans18:1, n-6, n-6/n-3 ratio and Health Index. The health index (HI) (Zhang et al., 2008), a modified version of the atherogenic index (IA) proposed by Ulbricht & Southgate (1991), was calculated as: HI = (Total MUFA + Total PUFA)÷(4×C14:0+C16:0).

6.2.3 DNA Marker Genotyping

DNA was extracted from the brisket adipose tissue of each steer using the phenol/chloroform/isoamyl alcohol method as described by Sambrook and Russel (2001). Single nucleotide polymorphisms (SNP) of positional candidate genes under the reported QTL regions for fatty acids and functional candidate genes related to growth and fat metabolism were compiled from public databases and from data of in-house gene SNP discovery. In total, 1536 SNPs of 556 genes were compiled and a custom SNP chip was designed by Illumina and genotyped on all the 223 steers using an Illumina Goldengate Assay. SNPs with minor allele frequency less than 5% and animals with a genotype missing rate larger than 5% were excluded from the analyses. Also, to avoid genotyping errors, SNPs that departed from Hardy-Weinberg equilibrium ($P<0.0001$) were discarded. After data editing, 947 polymorphic markers were used for the SNP association analyses.

6.2.4 Statistical Analysis

A preliminary analysis was carried out to evaluate the effect of contemporary group (CG, combinations of antimicrobial treatment by feedlot pen) among all

individuals and groups of FAs. CG effect was significant for 9c-14:1, 9c-16:1, 9c-18:1 and 10t-18:1 and the phenotypic values of these FAs were pre-adjusted appropriately for subsequent two step SNP association analyses. In the first step, all 947 SNPs were fitted for each of the 25 individual and groups of FAs in a model which can be written as

$$y_i = \mu + \sum_{j=1}^{m} x_{ij}\, a_j + e_i$$

where y is the phenotypic value or adjusted phenotypes of fatty acid concentration for the i^{th} animal (i = 1,…,n), μ is the general mean, x_{ij} is the genotype on the j^{th} SNP locus (j=1,…,m) for the i^{th} animal coded as 0, 1, or 2 according to the numbers of an arbitrary specified allele, a_j is the regression coefficient (allele substitution effect) on the j^{th} SNP genotype and e_i is the random residual error for the i^{th} animal. A Bayesian approach was used for the analyses and the model assumes a flat prior distribution for μ while each SNP effect, a_j, follows a mixture of a normal distribution, $N(0,\sigma^2_a)$ with a weight of (1- π) and a point mass distribution concentrated at zero denoted as ($\delta_0(a_j)$) with a weight of π written as,

$$aj \mid \pi, \sigma^2_a \sim (1-\pi)\, N(0,\sigma^2_a) + \pi\delta_0(a_j)$$

in which π is the prior knowledge of the proportion of SNP markers that have no effects on the traits, and was arbitrarily set at 0.95 for all traits . The prior distributions for variances σ^2_a and σ^2_e is a scaled inverse Chi-square distribution with degree of freedom v_a and v_e set to 4 and 10 respectively. The scale factor S_a^2 and S_e^2, were determined as $(v_a - 2)\, \hat{\sigma}^2_a / (1-\pi)\overline{2pq}N$ and $(v_e - 2)\, \hat{\sigma}^2_e / v_e$, where

$\hat{\sigma}_a^2$ and $\hat{\sigma}_e^2$ are additive genetic variance and residual variance estimates obtained from ASreml (Gilmour et al., 2009) using a genomic relationship matrix defined based on the method proposed by VanRaden et al (2009). p and q are allele frequency of the SNP under investigation and N is the population size (n=223).A Markov-Chain Monte Carlo (MCMC) approach was used to sample the unknown parameters and this procedure involved 5000 iteration burn-in period, results of which were discarded, followed by 45000 iterations with which the posterior mean effect of each SNP, additive genetic variance and phenotypic variance was obtained. SNP effect was estimated as an average of all the MCMC samples after the burn-in period. SNP variance was estimated from SNP effects and allele frequency and the proportion of phenotypic variance explained by SNP was calculated as the ratio of SNP variance over posterior phenotypic variance. Proportion of additive variance explained by SNP was calculated as the ratio of SNP variance to additive genetic variance obtained from Asreml.

In the second step, linkage disequilibrium (LD) between SNPs within and across genes was estimated as pair-wise correlation. Average r^2 between SNPS in a gene was 0.58 and between SNPs across genes was 0.08 across gene . When SNPs within a gene were in LD with the r^2 greater than 0.80, the one with greater effect on the trait was kept for further analyses (Ehret et al., 2012; Espigolan et al., 2013). After eliminating correlated SNPs, 469 SNPs representing 437 genes were re-fitted simultaneously in the Bayesisn model to estimate their allele substitution effect and genetic variance caused by gene SNPs as described above.

6.2.5 Permutation Analysis for Hypothesis Testing

To determine the level of statistical significance for SNP effects, empirical thresholds were determined by permutation (Churchill and Doerge, 1994). This involved shuffling the phenotypic values for each trait among individuals while keeping the marker genotypes intact. The shuffled data for each trait was analyzed for SNP associations. The procedure was repeated one thousand times in the Bayesian model with parameters set as above, and the estimates of SNP effects were stored. To determine the significance level of SNP association for each trait, SNP-wise and genome-wise significance thresholds were obtained based on the distributions of the estimated SNP effects with the shuffled data sets. For the SNP-wise significance threshold, a critical value was determined for each SNP by sorting the one thousand estimated SNP effects from largest to smallest, and by selecting the 1^{st} (α=0.001), 10^{th} (α=0.01) and 50^{th} (α=0.05) largest estimated effect value as the SNP-wise significance threshold. For the genome-wise threshold, SNP effects of all SNPs estimated one thousand times were ordered from largest to smallest and the 50^{th} largest value of SNP effects (α=0.05) was selected as the critical value for the genome wise threshold for the trait. SNP associations were declared significant at the various p-values of SNP-wise significance and/or at α=0.05 of genome-wise significance if their effects of SNPs estimated from the original data (unshuffled) exceeded the respective critical values from the permutated data (shuffled).

6.3 Results

Summary statistics of the 25 individual and grouped FAs, number of gene SNPs associated with each FA at various P values, and the genes showing the strongest association with each FA are presented in Table 6-1. At SNP-wise α=0.05, the number of significant SNPs across FAs ranged from 28 to 152. SNPs associated with one or more FAs at SNP-wise significance threshold $\alpha \leq 0.05$ are provided in the supplementary Table (Appendix S6-1). As the significance thresholds move to lower SNP-wise p-values (α=0.01 and 0.001) and to genome-wide α=0.05, the number of SNPs significantly associated with the fatty acid traits reduced due to a more stringent significance threshold (Table 6-1). At the genome-wise α=0.05, the total number of SNPs associated with one or more fatty acids traits is ranged from 0 to 7, and some genes showing the strongest association with the traits did not pass the genome-wide threshold (Table 6-1). Table 6-2 lists SNP markers genes and chromsomes in which they are located, SNP ID and alleles, functional class, type of enzyme, allele substitution effect and phenotypic variance explained by each SNP and by all SNPs within the gene for SNPs that are associated with the trait at the genome-wise P <0.05. In total, 25 SNPs in 22 genes on 14 chromosomes showed significant associations with 6 individual and 2 groups of FA (Table 6-2). The total phenotypic variance explained by the significant SNPs at the genome-wide threshold for each FA ranged from 0.0001% for MUFA to 19.61% for 13c-18:1 with rs41255693 SNP in the SCD gene (stearoyl Co-A desaturase) on chromosome 26 explaining the largest phenotypic variance (19.61%). Phenotypic variance explained by individual SNPs varied largely

across FAs and there was no obvious correlation between the number of gene SNPs and the amount of phenotypic variance explained. For instance, one SNP of the SCD gene explained about 19.61% of the phenotypic variation in 13c-18:1, while 7 SNPs in 7 genes accounted for only 0.02% of the phenotypic variation in 9c-18:1 (Table 6-2). At the genome-wise P <0.05, multiple gene SNPs were found to be associated with 5 fatty acids including 14:0, 18:0, 9c-14:1, 9c-18:1 and sumtrans18:1 with SNPs jointly explained phenotype variance between 0.0183% for 9c-18:1 to 15.4812% for 9c-14:1. Figure 7. provides a schematic view of gene SNPs and fatty acid traits associations at genome-wide threshold. It showed that SNP of one gene have effects on multiple fatty acids and each fatty acid is influenced by multiple genes, indicating that fatty acid traits are likely polygenic and some host genes have effects on multiple fatty acid traits.

6.4 Discussion

Fatty acids in beef tissues have a complex origin. They can arise from dietary lipids absorbed from the digestive tract after having undergone partial or complete biohydrogenation in the rumen, or they are synthesized denovo by rumen bacteria or within the animal itself (Christie, 1981). The primary product of the lipogenic pathway is palmitic acid, 16:0. Further elongation and desaturation of 16:0 is carried out by accessory enzyme systems- fatty acids elongase and fatty acid desaturase, in the endoplasmic reticulum (Nelson et al., 2008). The stearoyl Co-A desaturase enzyme coded by the stearoyl Co-A desaturase (SCD) gene or delta-9-desaturase gene, is the rate limiting factor in the desaturation of saturated fatty

acids (Ntambi and Kim, 1999). The SCD enzyme introduces a cis double bond in the delta 9 position of the hydrocarbon chain of a spectrum of saturated fatty acids (Bernard et al., 2001) and also vaccenic acid, 11t-18:1 (Bauman et al., 2000). As is evident from our results, two SNPs, rs41255692 T > C and rs41255693 T > C in the SCD gene (with LD of 1) had the largest influence on beef FA profile. Both SNPs accounted for approximately 15% and 20% phenotypic variance in 9c-14:1 and 13c-18:1 respectively. SNP rs41255692 T > C is a synonymous variant and at SNP-wise level, it was associated with 14:0 but explained only 0.05% of the phenotypic variance among animals. This is understandable since the genetic correlation between 14:0 and 14:1 is 0.12±0.37 from an earlier study (Ekine-Dzivenu et al., 2014). SNP rs41255693 T > C is a functional variant with alanine (GCG) becoming valine (GTG) (p.Ala190Val). Taniguchi et al. (2004) detected this SNP in the open reading frame (ORF) of the SCD gene in the Musculus trapezius muscle of a population of Japanese Black cattle. The "C" allele was associated with an increase of 0.805% in total MUFA, which was defined as the sum of 9c-14:1, 9c-16:1 and 9c-18:1. In this study, the "C" allele was associated with increased concentration of 13c-18:1 by 0.14%. At SNP-wise level, other non-functional/silent SNPs in the SCD gene, rs41255696 A > C, rs41255700 A > G, rs41255703 T > C, rs41255689 A > G, rs41255690 A > G, were associated with 9c-16:1, 18:0, SFA, SFA+BFA, MUFA, n6_n3, sumtrans 18:1 (Appendix S6-1). It is very likely that the effect of these markers, including previously mentioned rs41255692 T > C is due to a linkage disequilibrium relationship with the functional variant rs41255693 T > C as the average LD of markers in the SCD

gene in this study was 0.8. When there were multiple SNPs in high LD within a gene, the one with the largest effect was chosen for each trait, such that different SCD SNPs other than the functional variant had the largest effect for some FAs and were chosen for analysis. In a previous study, Li et al. 2011 found an association of this non-synonymous SNP marker with 14:0, 9c-14:1, 13c-18:1 in the same population using a mixed model regression analsyes. In addition, several other studies (Barton et al., 2010; Li et al., 2011; Matsuhashi et al., 2011; Narukami et al., 2011; Oh et al., 2011; Ohsaki et al., 2009) have confirmed the effect of this SCD gene variant on 14:0, 9c-16:1, 18:0, 9c-18:1, 13c-18:1, MUFA and in particular 9c-14:1 in different tissues of different breeds of cattle, albeit with varying magnitudes indicating that the effect of this marker is not tissue or breed specific. This marker partially accounts for variation in the associated traits suggesting that there are possibly other SNPs in the SCD gene that have an influence on the profile of FA in beef. Accordingly, Maharani et al. (2013) reported another marker, g.8586 C > T, which was associated with differences in 9c-14:1 in the longissimus thoracis muscle of Hanwoo cattle. Mutations in genes that regulate the SCD enzyme might also explain some variation in the associated traits. One of such genes is the leptin gene (LEP) which is said to down regulate SCD activity (Biddinger et al., 2006). In their study, Orru et al. (2011) suggested that the effect of the SCD gene in their study was somewhat modulated by the SNPs in the LEP gene. One of the SNPs examined in their study, which we also examined here is rs29004508 C > T in the LEP gene, a missense mutation resulting in the substitution of alanine, (GCG) for valine (GTG) (p.Ala80Val).

The association of this SNP with 9c-14:1 which was confirmed in this study. In addition, we also observed that this marker rs29004508 C > T, in the LEP gene, was associated with some FAs in common with the SCD markers. Thus, this mutation might have influenced the effect of the SCD SNPs observed in this study. Another missense mutation in the LEP gene rs29004488 T > C in which cysteine (TGC) is substituted for arginine (CGC) (p.Cys25Arg) was associated with differences in 9c-18:1 in this study.

Insulin-like growth factor 2 (IGF2), located on chromosome 29, is an imprinted gene only expressed when derived paternally (Curchoe et al., 2005; Dindot et al., 2004). It has been implicated in influencing body composition (Goodall and Schmutz, 2007) and is found to be associated with lean muscle growth and percent fat (Goodall and Schmutz, 2007). In the present study, IGF 2 SNP, rs42196904 A > G, an intronic variant was associated with proportions of 14:0, 9c-14:1, and 9c-18:1 at genome-wide level and explained 2.62%, 0.36%, 0.001% phenotypic variance respectively in the FAs. The "G" allele reduced the concentration of 9c-18:1 by 0.05% and increased the concentration of 14:0 and 9c-14:1 by 0.17% and 0.05% respectively. At SNP-wise level, the "GG" homozygotes had less MUFA, n6_n3 and health Index (HI) but more 16:0, 9c-16:1, SFA, SFA+BFA than the "AA" individuals. There has been no report of association studies linking SNPs in the IGF2 gene with fatty acids in beef cattle. However, Saatchi et al. (2013) associated the 18th 1-Mb SNP window (rs42375315 to rs43770775) on bovine chromosome 29, harboring 14 SNPs to 14:0, 9c-14:1, 16:0, 16:1, 18:0, 9c-18:1, LCFA (long chain FA, sum of all FA

with 14 carbons or more), MCFA (medium chain FA, sum of 12:0 and 13:0) and Atherogenic Index (AI) and the region explained 17.1 %, 14%, 14%, 8%, 11.2%, 6.7%, 15.7%, 15.8%, 13.8%, of the genetic variance in the traits respectively. In this study, the SNP rs42196904 in the IGF2 gene was located on the same chromosome 29 but it is in the 50th Mb window (29:50059692). This suggests that even though the window defined by Saatchi et al. (2013) and the IGF2 gene SNP marker identified in this study are on the same chromosome, the IGF2 gene SNP has independent effects on the same FAs - 14:0, 9c-14:1, 16:0, 9c-16:1, 9c-18:1, MUFA, HI (inverse of AI) in comparion to the SNP effects in the 1-Mb SNP window identified by Saatchi et al. (2013), since the extent of linkage disequilibrium in cattle genome is not expected to exceed 0.5Mb on average (McKay et al., 2007).

Phosphoinositide-3-Kinase, Regulatory Subunit 1 alpha (PIK3R1), located on bovine chromosome 20 encodes the phosphatidylinositol 3-kinase enzyme (PI3K) which plays an important role in many cellular functions by acting as secondary messengers in binding to and activating several different target proteins in the cell (Katso et al., 2001). PI3K is necessary for insulin action on fat cells by inhibiting lipid breakdown and promoting triacylglycerol formation (Wijkander et al., 1998). In humans with impaired fat metabolism, who show symptoms of hyperglycemia and insulin resistance, PI3K activity seems to be suppressed (Dib et al., 1998). In this study, rs42589207 T > G, a synonymous SNP variant in the PIK3R1 gene was associated with sumtrans18:1 and explained 1.63% phenotypic variation in the trait. There is presently no report associating this marker to FAs in beef cattle.

Nonetheless, in the FA composition of longissimus muscle in a population of Angus-sired cattle, Saatchi et al. (2013) associated 28 SNPs in the 39th Mb window of bovine chromosome 20 (rs110243640 to rs110201922) to 12t-18:1, with that window explaining 13.4% genetic variation in the trait. 12t-18:1 is a component trait of sumtrans18:1 in this study with a genetic correlation of 0.91±0.18 and the SNP we identified, rs42589207 T > G, in the PIK3R1 gene is located in the 11th Mb window (20:11331735) and so might not be in linkage disequilibrium with markers in the window specified by Saatchi et al. (2013). Other SNPs associated with sumtrans18:1 at genome-wide level in this study includes rs43702942 T > C in the UGDH gene (UDP-glucose 6-dehydrogenase), rs41745644 T > C in the PAFAH1B2 gene (platelet activating factor acetylhydrolase 1b catalytic subunit 2), rs41899395 T > G in the MYH1 gene (myosin heavy chain 1), rs41687544 A > C in the DSTN gene (destrin), rs42767950 A > C in the ANKRD1 gene (ankyrin repeat domain containing protein 1) on bovine chromosome 6, 15, 19, 13, 26. Effect of these markers on beef FA has not been previously reported. However, on the 39th Mb window of bovine chromosome 13, bearing 26 SNPs and on the 20th Mb window of bovine chromosome 19 bearing 20 SNPs, Saatchi et al. (2013) reported association for 6t/9t-18:1 and 15t-18:1, which are component traits of sumtrans18:1 in this study with genetic correlation of > 0.7. The reported region explained 13.1% and 16.4% genetic variation in the FAs respectively. SNP rs41899395 in the MYHI gene identified in this study is located in the 30th Mb window on chromosome 19 (19:30110044) and it explained 0.35% phenotypic variation in sumtrans18:1

while SNP rs41687544 in the DSTN gene located in the 13th Mb window on chromosome 13 (13:38260976) explained 0.13% phenotypic variation in sumtrans18:1. Collectively, the SNP markers associated with sumtrans18:1 identified in this study explained approximately 4% of the phenotypic variance in sumtrans18:1.

Thyroid hormone responsive spot 14 protein (THRSP), gene located on bovine chromosome 19 is a transcription factor that has been reported to influence fatty acid composition in beef cattle by controlling transcription of genes involved in lipid metabolism (Cunningham et al., 1998; Harvatine and Bauman, 2006; Kinlaw et al., 1995). This gene has been associated with intramuscular fat content in beef cattle (Wang et al., 2009). In this study, SNP rs42714483 C > T, a missense mutation in the THRSP gene, where isoleucine, (ATC) substitutes for valine, (GTC) (p.Ile16Val) was associated with 14:0 and 18:0 at genome wide level explaining phenotypic variance of 0.60% and 0.07% respectively. The "T" allele reduced 14:0 concentrations by 0.07% and increased the 18:0 concentrations by approximately 0.07%. Other FAs, 15:0, 16:0, ai-17:0, 9c-14:1, 9c-18:1, 13c-18:1, 11t-18:1, n6_n3, SumCLA, BFA, HI were associated with this marker at SNP-wise $\alpha = 0.05$. Another missense mutation in this gene, rs42714482 T > C (29:18090403) which changes valine (GTC) to alanine (GCG) (p.Val51Ala) was significantly associated with sumCLA at snp-wise level $\alpha = 0.05$. In Korean cattle, La et al. (2013), found two non-synonymous SNPs c.88G>A and c.194C>T (AC_000186.1) located in the Leu-zipper domain of the THRSP gene with the first mutation changing valine (GTC) to isoleucine (ATC) (p.Val16Ile) and the

second changing alanine (GCG) to valine (GTG) (p.Ala56Val). Both SNPs were significantly associated with group SFA and MUFA content in the longissimus dorsi muscle of Korean (Hanwoo) cattle. In another study with Korean cattle (Oh et al., 2014) found two non-synonymous SNPs in the THRSP gene, SNPs g.78 G > A where isoleucine replaced valine and g.184 C > T where alanine is substituted for valine, was associated with 14:0, 16:0, 9c-14:1, 9c-18:1, 18:2n6, 18:3n3, SFA and MUFA. Based on the function of this gene as a transcription factor, the effect of the non-synonymous variants on FAs is likely indirect by its influence on the expression of genes directly involved in FA and lipid metabolism like the fatty acid synthase gene (FASN) (Zhu et al., 2001).

Genetic variants of the fatty acid synthase gene (FASN), which catalysis fatty acid synthesis, have been reported to be associated with several individual and group of fatty acids including 14:0, 16:0, 18:0, 9c-14:1, 9c-16:1, 9c-18:1, SFA, MUFA, HI (Abe et al., 2009; Bhuiyan et al., 2009; Li et al., 2012; Matsuhashi et al., 2011; Morris et al., 2007a; Uemoto et al., 2011; Zhang et al., 2008). In the present study, rs419199993 T > C, a missense mutation in which tyrosine (TAC) replaces histidine (CAC), (p.Tyr1390His) in the FASN protein was associated with 14:0 and 9c-18:1 at genome wide level of <0.05. Homozygous "CC" individuals had more 14:0 and less 9c-18:1 than "TT" individuals, with the marker explaining phenotypic variance of 0.16% and 0.002% for the FAs respectively (Table 6-2). Fatty acids 15:0, 18:0, 9c-14:1, and HI were influenced by this marker at SNP-wise α=0.05 (Appendix S6-1).

Glycerol-3-phosphate dehydrogenase 1 (GPD1) located on bovine chromosome 5, catalyses the reversible conversion of dihydroxyacetone phosphate (DHAP), an intermediate of the glycolytic pathway, to glycerol (glycerol-3-phosphate) used in the esterification of fatty acids into triacylglycerol for storage (Lehninger et al., 2008). SNP rs41256865 A > G in the GPD1 gene was associated with 14:0 and 9c-18:1 and explained phenotypic variance of 0.64% and 0.003% respectively.

Monoglyceride lipase (MGLL) frees FAs from the secondary and tertiary ester bonds during the hydrolysis of free fatty acids from the glycerol backbone (Fredrikson et al., 1986; Haemmerle et al., 2002; Zimmermann et al., 2009) in response to signal for energy. At genome-wide level, rs43724308 T > C, a synonymous variant in the MGLL gene on chromosome 22 influenced 9c-18:1 with "CC" individuals having a higher concentration of 9c-18:1 than "TT" individuals, and this marker explaining 0.001% phenotypic variation among individual animals. Since this marker is a synonymous variant, its effect is likely from linkage or linkage disequilibrium with a functional variant in the MGLL gene or close by. Other markers associated with 9c-18:1 are rs41780423 T > C, a missence mutation in the peptidase domain containing associated with muscle regeneration (PAMR1) gene also called regeneration-associated muscle protease (RAMP), which plays a role in regenerating skeletal muscle(Nakayama et al., 2004) and Annexin A11 (ANXA11) annexins which encodes a group of calcium dependent phospholipid binding proteins (Gerke and Moss, 2002), Association of beef FAs with these polymorphisms are reported for the first time here. Therefore, further studies are needed to validate their effects.

In the ankyrin repeat domain containing protein 1 (ANKRD1) gene, rs42767950 A > C was associated with sumtrans 18:1 at genome-wise level with the "AA" individuals having more sumtrans18:1 than the "CC" individuals. At SNP-wise α=0.05, the SNP was associated with 10t-18:1, 18:2n-6, SFA, PUFA and n6. Another marker in the same gene, rs4125567 A > G, was associated with n6_n3. ANKRD1, also called cardiac ankyrin repeat protein (CARP) is a family of conserved genes coding for proteins involved in muscle stress response like injury, stretch or hypertrophy (Mestroni, 2009). Amid other functions, it acts as a transcription co-factor regulating the activity of matrix metalloproteinase family of genes (MMP) which regulate extracellular matrix remodeling (Almodóvar-García et al., 2014). Dunner et al, (2013) associated ss77831914 G > C, ss77831916 G > A, ss77831919 G > C, ss7783192 G > T, ss77831923 C > T, ss77831924 C > T in the MMP1 gene with several fatty acids including 12:0, 14:0, 9c, 11t-18:2, 22:6n3 in the muscle of 15 European bos taurus cattle. In the current study, we associated SNP rs41744058 A > C, and rs41744055 T > C in the MMP3 gene with FAs 11c-18:1, 9c-17:1, 13c-18:1, and SFA at the snp-wise level.

Sirtuin1 (SIRT1) is a nuclear NAD^+ dependent deacetylase (Smith et al., 2000) which plays an important role in regulating energy homeostasis and lipid metabolism by regulating transcriptional regulators including peroxisome proliferator-activated receptor (PPARs), peroxisome proliferator-activated receptor-gamma coactivator 1alpha (PPARGC1A) and liver X receptor alpha (LXR alpha) (Li et al., 2007; Purushotham et al., 2009). Peroxisome proliferator-

activated receptor (PPARs) is a ligand activated family of transcription factor that modulates the expression of lipogenic and adipogenic pathways amongst other physiological activities that they regulate (Mandard et al., 2004; Tontonoz and Spiegelman, 2008). In the current study, SNP rs41652470 A > G, an upstream gene variant in the SIRT1 gene was associated with 9c-18:1 at genome-wide level with the "AA" individuals having more 9c-18:1 than "GG" individuals. At SNP-wise level, 14:0, 15:0, 16:0, 17:0, 11c-18:1, SFA, SFA+BFA, MUFA, and HI were associated with this marker. In the Peroxisome proliferator-activated receptor alpha (PPARA) gene, rs110625700, G > T, a synonymous variant influenced the concentration of 14:0, 18:0, 9c-14:1, 9c-16:1, 13c-18:1 and sumtrans 18:1. SNP rs42661650 T > C, an intronic variant in the PPARG was linked with 15:0 concentration and rs43464396 A > G an intronic variant in the PPARGC1A gene was associated with differences in 14:0, 9c-14:1 and 9c-18:1. Oh et al. (2012) reported a missense mutation NM_181024.2:c.1523G>T where histidine (CAT) replaced glutamine (CAG) in the PPARG gene, which influenced total MUFA and SFA concentration and Sevane et al. (2013) reported that ss62850198 G > A , a 5' UTR variant in the PPARG gene was associated with 20:2n3, 22:5n3, 22:6n3 omega 3 fatty acid concentration in different European beef breeds, with the "AA" individuals having increases of 15%, 9% and 18% respectively compared to the "GG" homozygous. They also found that c.5314 C > T in PPARGC1A gene, a transcription co-activator, was associated with 12:0, 14:0 in neutral lipid fraction and 18:0 in total lipid fraction of fatty acids in muscle.

Several SNPs in genes previously reported to be associated with beef FA were detected at SNP-wise thresholds in this study. Zhang et al. (2010) reported SNPs g.2350 T > C and g.2203 G > T in the promoter 1 region of the ACACA gene was associated with 14:0, 9c-14:1, 9c-16:1, 9c-17:1, 18:1n7 (presumably 11c-18:1) and 18:1trans (presumably sum of trans 18:1 fatty acids) in the longissimus dorsi muscle of crossbred cattle. Matsumoto et al. (2012) associated differences in 18:2 concentrations in the diaphragm muscle of Japanese Black cattle to a haplotype of two SNPs in the promoter III region of the ACACA gene. Acetyl-CoA carboxylase (ACAC) catalyses the carboxylation reaction converting acetyl Co-A to malonyl Co-A, in the FA biosynthetic process (Drackley, 2000). The ACAC enzyme exists in two forms, α and β (ACACA, ACACB) with the alpha form regulating the rate of FA synthesis in lipogenic tissues, while the beta form, majorly found in non-lipogenic tissues, controls FA oxidation by producing malonyl Co-A which inhibits FA oxidation (Drackley, 2000). In this study, genotypes of SNPs rs110241790 A > G and rs108968268 T > C in the ACACA gene and rs41844490 T > C and rs41844482 C > G in the ACACB gene were associated with 14:0, 9c-14:1, 9c-16:1, 9c-18:1 and SFA at snp-wise level (Appendix S6-1). SNP rs41844490 T > C is a synonymous variant while the others are intronic variants so that the effect of these markers might be as a result of linkage or linkage disequilibrium with a causative mutation in the gene or close by. There are presently no reports of association of SNPs in the ACACB gene with FA in beef cattle.

Fatty acid binding proteins (FABP) bind to free non esterified FAs in the cell for intracellular transport to where they are processed or catabolized (Furuhashi and Hotamisligil, 2008; Lee et al., 2006). Hoashi et al. (2008) associated a missense variant c.280 A > G, in which isoleucine substitutes valine in the fatty acid binding protein 4 (FABP4) gene with differences in 9c-16:1 concentration in the intramuscular fat of longissimus dorsi muscle of Japanese Black cattle while Narukami et al. (2011) found this marker was associated with content of 16:0 in the intramuscular fat of the diaphragm of Holstein cattle. Oh et al. (2012) associated this marker to 18:1, MUFA and SFA but not with 16:0 or 9c-16:1. They also associated another missense mutations, c.388 G > A with 9c-18:1 and MUFA, and two synonymous mutations, c.408 G > C and c.456 A > G with 16:0, 9c-18:1, 18:2n6, SFA, MUFA and 9c-18:1, 18:2n6, SFA, MUFA, respectively. Maharani et al. (2012) linked another SNP g.3691G > A in the FABP4 gene with 14:0, 16:0 and 20:4 in the longissimus thoracis of Korean Hanwoo cattle. In the present study, intronic variants, rs111014258 T > C and rs109346428 T > C was associated with 17:0, 18:0, SFA, and MUFA respectively but not 16:0 or 9c-16:1(Appendix S6-1). Some other members of the FABP family (FABP 5 and FABP 9) considered in this study were significantly associated with several fatty acids including 16:0 but not 9c-16:1 (Appendix S6-1). Inconsistencies between results of FAs associated with FABP4, especially for the c.280 A > G might be linked to differences in tissue or breeds used in the studies. Mutations in the regulatory region or exons of these genes have the potential of altering the binding site of transcription factors or the function of the gene, thus resulting in

altered substrate preference or specificity and the accumulation (or lack) of certain FAs in the cell.

Lipoprotein lipase (LPL) is an extracellular enzyme which plays a key role in the catabolism of triacylglycerol (TAG) (Nelson et al., 2008). In hydrolyzing TAG, LPL produces smaller molecules of chylomicron and very low density lipoprotein alongside free FAs, and monoacylglycerol (Mead et al., 2002). Oh et al. (2013) associated 3 SNPs, c.322G>A, c.329A>T in exon 2 and c.1591G>A in exon 9 of the LPL gene with FAs in the longissimus dorsi muscle of Korean (Hanwoo) cattle. They reported that SNP c.329A>T, in exon 9 is a missense mutation which changes threonine (ACA) to serine (TCA). This marker was linked with 16:0, 9c-18:1, 18:2n6, 18:3n3, total SFA, and total MUFA. SNP c.1591G>A was associated with 18:0, 18:2n6, 18:3n3, total SFA, and total MUFA while SNP c.322G>A was associated with 14:0, 16:0, 9c-18:1, 18:2n6, 18:3n3, total SFA and total MUFA. Sevane et al. (2013) associated ss65478732 C > T, a synonymous variant in this gene with omega 6 FAs in the muscle of 15 European cattle breeds and in this Study, we observed that SNP rs43560146 T > C located upstream of the transcription start site of the LPL gene was associated with 14:0, 9c-14:1, 9c-18:1, 10t-18:1, 18:2n6, n6, sumtrans 18:1 and PUFA at snp-wide level $\alpha = 0.05$ (Appendix S6-1). The different markers across all studies were commonly associated with omega 6 FAs suggesting a preference of the LPL enzyme for omega 6 FAs. Association of different SNPs in this gene with different types of FA suggest that depending on the location of the SNP, its phenotypic effect is different. A mutation in or near this gene might alter its preferred FAs. On the

other hand, a mutation in this gene might result in a defective protein leading to a non functional enzyme and the accumulation of TAG in the plasma. Further studies focusing on functional analysis of the polymorphisms in the LPL gene will throw more light on how this gene influences fatty acid composition in beef cattle.

Acyl Co-A synthase gene long chain (ACSL) catalyses the formation of free long chain FAs to fatty acyl-CoA esters thereby playing an important role in FA biosynthesis and degradation (Smith, 1995). Widmann et al., (2011) associated c.481-233A > G in intron 5 of the ACSL1 gene with n-3 FAs, MUFAs, PUFAs, sumtrans18:1 in the longissimus dorsi muscle of Chairolais–Holstein crossbred cattle. In the current study, rs42115578 C > G, a synonymous variant in the ACSL1 gene was associated with 18:0, 9c-17:1, 11c-18:1, 11t-18:1, n6_n3, SFA, SFA+BFA. Several other SNP markers in the acyl Co-A synthase long chain 1 and 6 and short chain 2 gene (ACSL1 and 6, ACSS 2) passed the SNP-wise α=0.05 threshold (Appendix S6-1).

Sterol regulatory element binding transcription factor 1 (SREBF1), which encodes the sterol regulatory element binding protein 1 (SREBP1), and Nuclear receptor subfamily 1, group H, member 3 (NR1H3) also called liver X receptor alpha (LXR alpha), are transcription factors involved in regulating the genes encoding enzymes involved in fatty acid and lipid metabolism. Hoashi et al. 2007 reported an 84bp indel (insertion, L and deletion S) (rs133958066) in intron 5 of the SREBP1 gene of Japanese Black cattle is associated with the concentration of MUFA, with the S type having more MUFA. Using this same population of Canadian commercial crossbred steer as in this study, Han et al. (2013) detected

this 84bp indel polymorphism and reported that it was significantly associated with the concentration of 9c-17:1. In Korean Hanwoo cattle, Bhuiyan (2009) associated this indel with 18:0, 18:2n6 and PUFA in the FA composition of muscle tissue. Xu et al. (2013) detected this polymorphism in Simmental and Snow Dragon black cattle and it was associated to 9c-16:1, 18:0 and SFA in muscle FA. In this study, SNP rs41912288, an intron variant in the SREPF1 gene was associated with 9c-16:1 concentration at SNP-wise $\alpha = 0.05$(Appendix S6-1). Hoashi et al (2008) reported a non-synonymous mutation (rs109428603) in the LXRα gene that causes an amino acid change from valine to isoleucine was associated with 18:2n6 in the intramuscular fat of Japanese black cattle. Han et al. (2013) found that same SNP was associated wih 9c, 11t-18:2, sum of conjugated linoleic acid and 11c-20:1. In the current study, a synonymous variant rs17870648 G > T in the NR1H3 gene was associated with concentration of 9c-16:1, MUFA and SFA+BFA (Appendix S6-1).

Taken together, the large number of SNPs in multiple genes identified in this study show that fatty acid and lipid metabolism is a complex and dynamic process which is affected directly or indirectly by a diverse and large family of genes. Some of these genes code for enzymes, transcription and translation factors, transcription coactivators, transporters, receptors and several other proteins involved in cellular biological functions. These results demonstrate that variation in the concentration of fatty acids in tissues of individual animals could results from a mutation in the genes involved in various cellular processes in the fatty acid and lipid metabolic pathways or from a combination of them as well as other

metabolic pathways implying that fatty acid concentrations in brisket adipose tissue of beef catle are affected by multiple genes (Figure 7).

Apart from the large effect of the SCD gene detected in this study, most of the identified SNPs were of small effects, thereby accounting for only a small proportion of the phenotypic variation in most FAs considered. One reason for this is the fact that most of the markers might not be causative. Another even more important reason is that concentration of each fatty acid is also largely influenced by environmental factors and/or by interaction of genes that were not evaluated in this study due to a relatively small sample size used. Moreover, only a small fraction of the candidates genes potentially influencing beef FA have been assessed for associations in this study. Further studies are needed to validate novel markers identified in this study especially those at lower SNP-wise threshold and more studies are needed to identify more genetic variants of more genes associated with the various FAs, as there is still a lot more genetic and phenotypic variation to be captured.

6.5 Conclusion

Using a Bayesian analytical approach that assesses all SNP effects simultaneously, we identified 19 novel SNPs assoiated with several FAs at genome wide level. At other thresholds, several novel and promising SNPs influencing beef FA profile were also identified and some previously described associations were confirmed. This study serves to improve our understanding of

the molecular mechanisms behind the variation of FA in beef tissues and extends our knowledge on how fatty acids are regulated at the molecular level.

Previous research has associated a number of candidate genes with beef fatty acid profiles but, to our knowledge, this is the first study which has attempted to comprehensively evaluate the effect of SNPs in this large number of candidate genes with several individual and groups of fatty acid in beef cattle. Even though several of the uncovered markers have small effects, the results show that several genes work in concert to influence the fatty acid composition in beef

As the demand for healthy food options increases, SNPs uncovered in this study may potentially provide a means for the beef industry to perpetually and accumulatively increase the amount of healthy fatty acids and reduce the amount of non beneficial ones while enhancing the palatability of beef through SNP marker selection and/or SNP marker assised diet management.

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Table 6-1. Summary statistics of 25 individual and group of fatty acid and genes showing strongest association for each fatty acid

Fatty acid	Mean±SD[1]	Snp-wise α=0.05	Snp-wise α=0.01	Snp-wise α=0.001	Genome-wise α=0.05	SNPs	Genes
Saturated							
14:0	3.55±0.65	127	66	31	7	rs42196904	IGF2
15:0	0.62±0.11	40	9	2	0	rs41793400	GNA14
16:0	25.56±1.86	41	11	3	0	rs42660323	PFN2
17:0	1.40±0.23	30	9	3	0	rs134451630	SIAT4A
18:0	8.92±1.5	151	75	21	4	rs43575364	MUSK
Monounsaturated							
9c-14:1	1.48±0.51	102	57	16	3	rs41255692	SCD
9c-16:1	5.60±1.11	110	49	15	1	rs41819943	HSD11B1
9c-17:1	1.49±0.25	38	12	1	0	rs41694130	CTSZ
9c-18:1	40.13±2.89	152	74	25	7	rs42196904	IGF2
11c-18:1	2.47±0.37	59	23	4	0	rs17871529	CGN
11t-18:1	0.54±0.16	36	13	1	0	rs43648117	SNWI
13c-18:1	0.75±0.21	50	13	5	1	rs41255693	SCD
10t-18:1	0.82±0.5	35	10	4	0	rs42767950	ANKRDI
Branched							
17:1 ai	0.59±0.07	40	4	0	0	rs29004508	LEP
Polyunsaturated							
18:2n-6	1.26±0.21	50	15	2	0	rs43702942	UGDH
Group fatty acids							
Σtrans18:1[b]	2.30±0.6	117	57	26	6	rs42589207	PIK3R1
ΣCLA[d]	0.59±0.11	28	6	2	0	rs41257366	POMC
SFA	40.29±2.94	100	37	0	0	rs43663565	DGUOK
MUFA[c]	55.41±2.96	94	32	6	1	rs43663540	DGUOK
BCFA	1.49±0.21	39	3	0	0	rs43734541	STAR
SFA+BCFA[a]	41.788±3.04	71	19	2	0	rs43663565	DGUCK
n-6	1.46±0.22	44	9	2	0	rs43702942	UGDH
n-6/n-3[f]	7.99±1.21	88	26	5	0	rs43315204	PRKAG3
PUFA[e]	2.81±0.33	60	14	1	0	rs109450360	IGF2R
Health Index[g]	1.49±0.23	39	8	3	0	rs42196904	IGF2

[1] The mean (SD) of each fatty acid was presented in another report (Ekine et al. 2013, submitted) but is also listed in Table 1 as a reference. The concentrations of fatty acids were expressed as a percentage of total fatty acid methyl esters (FAME) quantified. Only fatty acids with a concentration greater than 0.5% of total FAME are presented. c=cis, t=trans. [b]Sum trans18:1 = 6t/8t-18:1 + 9t-18:1 + 10t-18:1 + 11t-18:1 + 12t-18:1 + 13t/14t-18:1 + 15t-18:1 +16t-18:1. [d]SumCLA (sum of conjugated linoleic acid) = 8t,10c-18:2 + 9c,11t-18:2 + 7t,9c-18:2 + 9t,11c-18:2 + 10t,12c-18:2 + 11c,13t-18:2 + 11t,13c-18:2 +12t,14c-18:2+12c,14t-18:2+ 9c,11c-18:2 + 10c,12c-18:2 +6t,8t-18:2+ 9t,11t-18:2 + 11t,13t-18:2 + 12t,14t-18:2 + 10t,12t-18:2 + 8t,10t-18:2 + 7t,9t-18:2. SFA (sum of saturated fatty acid) = 10:0 + 12:0 + 13:0 + 14:0 + 15:0 + 16:0 + 17:0 + 18:0 + 20:0 + 23:0. [c]MUFA (sum of monounsaturated fatty acid)= 9c-14:1 + 9c-15:1 + 7c-16:1 + 9c-16:1 + 9c-17:1 + 6t/7t/8t-18:1 + 9t-18:1 + 10t-18:1 +11t-18:1 + 12t-18:1 + 13t/14t-18:1 + 15t-18:1 + 16t-18:1 + 9c-18:1 + 11c-18:1 + 12c-18:1 + 13c-18:1 + 14c-18:1 + 16c-18:1 + 9c-20:1 + 11c-20:1. [e]PUFA (sum of polyunsaturated fatty acid) = 18:2n-6 + 18:3n-6 + 18:3n-3 + 20:2n-6 + 20:3n-9 + 20:3n-6 + 20:4n-6 + 22:4n-6 + 22:5n-3. BCFA (sum of branched-chain fatty acid) = iso-14:0 + iso-15:0 + anteiso-15:0 + iso-16:0 + iso-17:0 + anteiso-17:0 + iso-18:0. [a]SFA+BCFA: sum of saturated and branched chain fatty acids. n-6 (sum of omega 6 fatty acids) = 18:2n-6 + 18:3n-6 + 20:2n-6 + 20:3n-6 +20:4n-6 + 22:4n-6. n-3 (sum of omega 3 fatty acids) = 18:3n-3 + 22:5n-3. [f] n-6/n-3: ratio between n-6 and n-3 PUFA. [g]Health Index: (total MUFA + total PUFA) / (4 x C14:0 + C16:0).
ankyrin repeat domain 1 (cardiac muscle) (ANKRD1), cingulin (CGN), cathespin Z(CTSZ), deoxyguanosine kinase (DGUOK), guanine nucleotide binding protein alpha 14 (GNA14), hydroxysteroid (11-beta) dehydrogenase 1 (HSD11B1), cingulin (CGN), insulin-like growth factor 2 (IGF2), insulin-like growth factor 2 receptor (IGF2R), leptin (LEP), muscle skeletal receptor tyrosine kinase (MUSK), profilin 2 (PFN2), phosphoinositide-3-kinase, regulatory subunit 1 (alpha) (PIK3R1), proopiomelanocortin (POMC), protein kinase, AMP-activated, gamma 3 non-catalytic subunit (PRKAG3), stearoyl-CoA desaturase (SCD), ST3 beta-galactoside alpha-2,3-sialyltransferase 1 (SIAT4A) , SNW domain containing 1 (SNW1), steroidogenic acute regulatory protein (STAR,), UDP-glucose 6-dehydrogenase (UGDH)

Table 6-2. Gene SNPs associated with fatty acids at genome-wide threshold P < 0.05

Trait	Gene	Chr	SNP ID	Allele*	Functional Class	Type	Allele Substitution effect	Phenotypic Variance per SNP	Phenotypic Variance all SNPs
14:0	IGF2	29	rs42196904	A/G	intron_variant	Growth factor	0.168	2.6200%	
	GPD1	5	rs41256865	A/G	3_prime_UTR_variant	Enzyme	0.075	0.6434%	
	THRSP	29	rs42714483	T/C	missense_variant	Transcription regulator	-0.071	0.5944%	
	ANXA11	28	rs42147575	A/G	splice_region_variant, intron_variant	Phospholipid binding protein	-0.066	0.5073%	
	TNS	2	rs43315761	C/G	missense_variant	Cross links actin filament	-0.045	0.0754%	
	PFN2	1	rs42660323	C/G	intergenic_variant	Actin monomer binding protein	0.044	0.2134%	
	FASN	19	rs41919993	T/C	missense_variant	Enzyme	0.037	0.1610%	4.8149%
18:0	MUSK	29	rs43575364	T/C	upstream_gene_variant	Kinase	0.066	0.0746%	
	THRSP	8	rs42714483	T/C	missense_variant	Transcription regulator	0.046	0.0476%	
	SCD	26	rs41255690	A/G	intron_variant	Enzyme	0.029	0.0176%	
	GAP43	1	rs43242960	A/T	intron_variant	Growth or plasticity protein	0.025	0.0103%	0.1501%
9c-14:1	SCD	26	rs41255693	T/C	missense_variant, splice_region_variant	Enzyme	-0.290	14.9951%	
	COPZ1	5	rs41654804	C/G	downstream_gene_variant	Transporter	-0.050	0.1285%	
	IGF2	29	rs42196904	A/G	intron_variant	Growth factor	0.049	0.3576%	15.4812%
9c-16:1	HSD11B1	16	rs41819943	T/C	downstream_gene_variant	Enzyme	0.038	0.0475%	0.0475%
9c-18:1	IGF2	29	rs42196904	A/G	intron_variant	Growth factor	-0.046	0.0099%	
	GPD1	5	rs41256865	A/G	3_prime_UTR_variant	Enzyme	-0.022	0.0028%	
	FASN	19	rs41919983	T/C	intergenic_variant	Enzyme	-0.018	0.0019%	
	MGLL	22	rs43724308	T/C	synonymous_variant	Enzyme	0.015	0.0008%	
	SIRT1	28	rs41652470	A/G	upstream_gene_variant	Transcription regulator	-0.014	0.0012%	
	PAMR1	15	rs41780423	T/C	missense_variant	Peptidase	0.013	0.001%	

	ANXA11	28	rs42147575	A/G	splice_region_variant, intron_variant	Phospholipid binding protein	0.011	0.0007%	0.0183%
13c-18:1	SCD	26	rs41255693	T/C	missense_variant, splice_region_variant	Enzyme	0.136	19.6129%	19.6129%
sumtrans18:1	PIK3R1	20	rs42589207	T/G	synonymous_variant	Kinase	-0.112	1.633%	
	UGDH	6	rs43702942	T/C	intron_variant	Enzyme	-0.110	1.611%	
	PAFAH1B2	15	rs41745644	T/C	intron_variant	Enzyme	0.057	0.0995%	
	MYH1	19	rs41899395	T/G	downstream_gene_variant	Enzyme	0.053	0.3539%	
	ANKRD1	26	rs42767950	A/C	downstream_gene_variant	Transcription regulator	-0.043	0.1300%	
	DSTN	13	rs41687544	A/C	intron_variant	Actin binding protein	0.037	0.1219%	3.94930%
MUFA	DGUOK	11	rs43663540	T/G	intron_variant	Kinase	0.007	0.0001%	0.0001%

*Allele a/b coded as aa = 0, ab=1, bb=2
ANKRD1 = ankyrin repeat domain 1; ANXA11 = annexin A11; COPZ = coatomer protein complex, subunit zeta 1 ; DGUOK = deoxyguanosine kinase ; DSTN = destrin (actin depolymerizing factor) ; FASN = fatty acid synthase ; GAP43 = growth associated protein 43 ; GPD1 = glycerol-3-phosphate dehydrogenase 1; HSD11B1 = hydroxysteroid (11-beta) dehydrogenase 1; IGF2 = insulin-like growth factor 2; MGLL = monoglyceride lipase ; MUSK = muscle, skeletal, receptor tyrosine kinase ; MYH1= myosin, heavy chain 1; PAFAH1B2 = platelet-activating factor acetylhydrolase 1b, catalytic subunit 2 ; PAMR1 = peptidase domain containing associated with muscle regeneration PFN2 = profilin 2; PIK3R1 = phosphoinositide-3-kinase, regulatory subunit 1; SCD = stearoyl-CoA desaturase (delta-9-desaturase) ; SIRT1 = sirtuin 1 ; THRSP = thyroid hormone responsive ; TNS1= tensin 1 ; UGDH = UDP-glucose 6-dehydrogenase

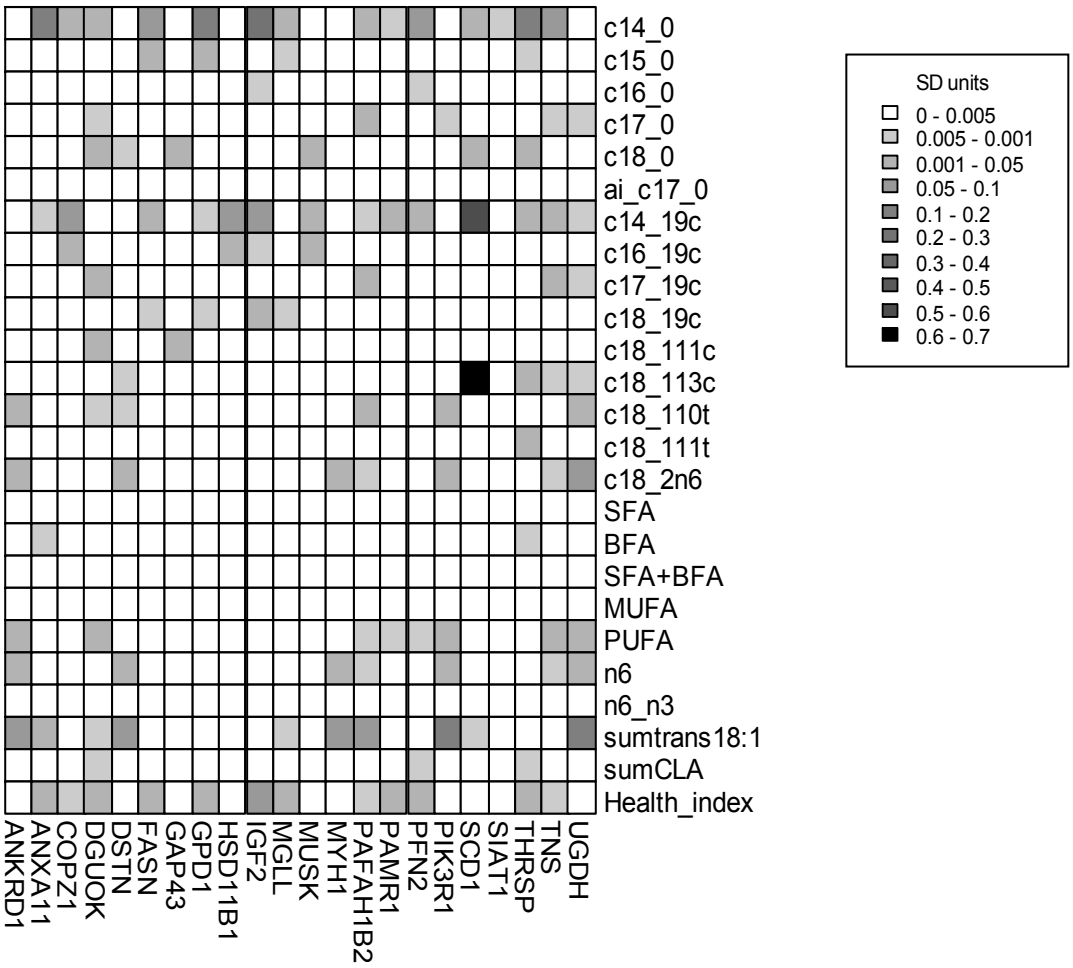

Fig 7. Schematic overview of the polygenic nature of each fatty acid and the pleiotropic effect of each gene.

Effect of particular genes vary depending on the fatty acids. The color key indicates the degree to which individual gene SNPs influence the various fatty acids, i.e. magnitude of gene SNP effect expressed as the allele substitution effect in standard devriation (SD) on the fatty acid trait, for which at leadt on gene SNP showed significant association with the trait at the genome-wide significance level of $P<0.05$.

CHAPTER 7

GENERAL DISCUSSION

7.1. General Discussion

Consumers are becoming more interested in the nutritional quality of food as they gain more understanding of the relationship between diet and health, and, in particular, saturated fats which are said to increase low density lipoproetein (LDL) and have been linked with a number of diseases, in particular, cardiovascular diseases (Hocquette et al., 2010; Scollan et al., 2006). Nutritional guidelines recommend reduction in SFA consumption (not more than 10% of total energy intake), with increase in the intake of Omega 3's, particularly EPA and DHA which have been shown to play important roles in reducing the risk of cardiovascular diseases, diabetes and cancers amongst other benefits (World Health Organization, 2003). This has resulted in increasing the concentration of these FAs in food sources like eggs and efforts are presently directed towards improving the FA profile in beef by enhancing the concentration of these important omega-3's and conjugated linoleic acids (CLAs). The latter are a group of fatty acids found in ruminants with the predominant isomer, rumenic acid, 9c,11t-18:2 and its precursor 11t-18:1 identified as having health effects related to reducing the risk of cardiovascular diseases, obesity and several types of cancers (Givens, 2010; Salter, 2013; Wang et al., 2012). Feeding animals ruminally-

protected lipid supplements has been the traditional way of enriching beef with omega 3s, however, this often involves the use of formaldehyde which is prohibited by some regulatory authorities for use in meat animals (Scollan et al., 2014). However, changes achieved are not permanent and do not accumulate. With genetic improvement via selection and breeding, changes are permanent and accumulate. In order to make genetic changes through selection and breeding, for FA in beef cattle, heritability values quantifying the amount of genetic variability has to be estimated. Presently, studies on heritability estimates in beef cattle are few, and estimates for a number of FAs found in beef have not been previously reported. This study is presenting information on heritability estimates for several FAs in beef cattle for the first time. With genetic selection, care has to be taken not to compromise on other traits of economic importance, with modification on a particular trait, thus the relationship of the various FAs quantified in the muscle (longissimus lumborum) with carcass and meat quality traits of economic importance were also assessed. Beef FAs are difficult and expensive to measure, and it involves the loss of potential parental animals having beneficial FA profile when fatty acid concentrations are measured in carcasses. In response to this gene SNP markers were examined for associations with FA in order to identify DNA markers that can be used as a tool for marker assisted selection to identify animals that will produce meat with healthier FA profile.

In the first study, chapter 2, we estimated the heritability, phenotypic and genetic correlations between 15 individuals and 10 groups of fatty acids having a concentration greater than 0.5% in the brisket adipose tissue of 223 Angus and

Charolais based crossbred commercial steers using univariate and bivariate animal models. The results showed that heritability for most of the fatty acids were low (below 0.2) except for 15:0, 9c-14:1, 13c-18:1 with heritability estimates of 0.31, 0.51 and 0.43 respectively. This result suggested the presence of genetic variation in the fatty acid profile of brisket fat but also showed that environmental factors have a stronger influence on their concentration. This means that reducing the concentration of harmful 14:0 and 16:0 and increasing the concentration of beneficial PUFA, 11t-18:1 and 9c,11t-18:2 might be difficult to achieve in brisket fat by breeding and selection. However, estimates of heritability for these FAs associated with health varied across studies. For example, heritability for 14:0 ranged from 0.18 (Pitchford et al., 2002) to 0.82 (Inoue et al., 2011). This variation suggested difference in the genetic control of FAs across different breeds and tissues used in the various studies. Nonetheless, other factors such as sample size, pedigree depth and techniques used in analyzing the data may have been responsible for the differences. Individual FAs, 9c-17:1, 10t-18:1,11c-18:1, 11t-18:1, and 13c-18:1 and FA group and ratios n-6, n-6/n-3, total branched chain FA (BCFA), total saturated and branched chain FA, total trans fatty acids (SFA+BCFA), total conjugated FA (sumCLA) and health index (HI) were reported for the first time in this study which has been published in the Meat Science journal - Volume 96, Issue 4, April 2014, Pages 1517–1526.

In chapter 3, we estiamted the variability and heritability of 81 and 83 individual and groups of fatty acids quantified in the subcutaneous adipose (SQ) and longissimus lumborum (LL) muscle of 1366 crossbred beef steers using a

univariate animal model. In general, the concentration of individual FAs varied from 0.0023% (18:3n-6, gammalinoleic acid) to 37.92% (9c-18:1, oleic acid) in SQ and from 0.0017% (8t,10t-18:2, 8-*trans*,10-*cis*-octadecadienoic) to 36.68% (9c-18:1, oleic acid) in the LL tissue. Oleic acid (9c-18:1 - 37.9% SQ, 36.7% LL), palmitic acid (16:0 – 25.09% SQ, 24.61% LL), and stearic acid (10.54% SQ, 12.41% LL), were the three most abundant FA in both tissue. This is consistent with the fatty acid profile reported in other studies for subcutaneous adipose tissue, muscle tissues and brisket adipose of beef cattle.

Concentration of beneficial FAs 11t-18:1 (0.55% SQ, 0.44% LL) and 9c,11t-18:2 (0.47% SQ, 0.26% LL), were low in both tissues, eicosapentaenoic acid, (EPA, 20:5n3), docosahexaenoic acid, (DHA, 22:5n3) were not detected in the SQ but in the LL. The concentration of EPA was 0.029% and that of DHA was 0.046%. Total PUFA was higher in the muscle 6.67%, almost three times that in the SQ 2.29%. Nutritional experts recommend the n6/n3 ratio be less than 4 and the PS ratio to be greater than 0.4 (Wood et al., 2008). In this study, average n6/n3 and P/S ratio was higher (9.26% SQ, 8.63% LL) and lower (0.06% SQ, 0.16% LL) than recommended in both tissues. It is important to keep the n6/n3 ratio in the recommended range because high n6/n3 ratio has been associated with cardiovascular diseases and cancers (Simopoulos, 1999a, 2006). Increased consumption of 18:2n6 FAs cause larger quantities of metabolic products of 18:2n6, particularly arachidonic acid (20:4n6) to be formed than metabolic products of 18:3n3 especially eicosapentaenoic acid (20:5n3) since the same enzymes are needed for their conversion. Because metabolic products from

arachidonic acid are biologically active even in small quantities, when they are formed in large amounts, they contribute to the formation of thrombi (blood clot) and atheroma (atherosclerosis – hardening of the arteries) (Simopoulos, 1999a, 2003, 1999b, 2006) which is the root cause of a number of cardiovascular diseases. Heritability estimates for the 81 and 83 individual and groups of fatty acids were quantified in the subcutaneous adipose (SQ) and longissimus lumborum (LL) muscle. In the muscle, heritability of FAs were estimated at constant marbling to determine if there was genetic variation for fatty acids independent of marbling. Heritability of several FAs is reported for the first time in this study and estimates ranged from 0 for several FAs to 0.64 ± 0.11 (12:0) in the SQ with 34 fatty acids having heritability estimates below 0.20 and from 0 (7c-17:1) to 0.68 ± 0.1 (9c-16:1) in the LL with heritability estimates of 45 FAs below 0.20 (Table 2). Heritability of FAs 14:0 (0.5 ± 0.16 SQ, 0.61 ±0.13 LL), 16:0 (0.28 ± 0.09 SQ, 0.54 ±0.1 LL), 11t-18:1(0.16 ± 0.07 SQ, 0.24 ±0.08 LL), 9c,11t-18:2(0.24 ± 0.08 SQ, 0.16 ±0.07 LL), EPA (0.03 ±0.04 LL), DHA (0.15 ±0.06 LL) associated with health and 9c-18:1 (0.17 ± 0.07 SQ, 0.42 ±0.09 LL) associated with palatability of beef were very low to moderately high in both tissues. Excess subcutaneous fat is trimmed off when the carcass is processed however, intramuscular fat is consumed with the muscle and so this result suggests that selection and breeding for beef cuts with lower concentration of 14:0 and 16:0 could result in significant progress, even in lean beef, since this estimate is independent of marbling. However increasing the concentration of 11t-18:1, 9c,11t-18:2, EPA, DHA through breeding would not result in much progress

because it appears that they are more influenced by environmental factors. Heritability estimates for these FAs previously reported were equally low, and ranged from 0 to 0.24 (Ekine-Dzivenu et al., 2014; Kelly et al., 2003; Saatchi et al., 2013; Tait et al., 2007) suggesting that improving the FA profile in beef would require both genetic and management strategies. Phenotypic and genetic relationships between fatty acids in the brisket adipose tissue estimated in chapter 2 showed that there was no serious antagonistic relationship between FAs of health interest, suggesting that they can be simultaneously improved. Genetic and phenotypic correlation estimates ranged between 0 and 1 depending on the pair of FAs. Vaccenic acid, 11t-18:1 and total CLA had a moderate to high favorable genetic correlation with 14:0 (−0.36±0.49, −0.74±0.41), 16:0 −0.84±0.24, −0.65±0.82), SFA (0.9±0.13, 0.63±0.75), MUFA (−0.94±0.09, −0.47±0.99) and PUFA (−0.55±0.4, −0.94±0.11).

In chapter 4, the phenotypic and genetic correlation between 83 FAs in the longissimus lumborum muscle was evaluated with 6 carcass merit traits, namely hot carcass weight (HCW), average back fat thickness (BFAT), rib eye area (REA), carcass marbling (MARB), lean meat yield (LMY), and YG (yield grade) measured on 1366 animals were evaluated using a bivariate animal model The estimates of phenotypic correlation between fatty acids and carcass merit traits ranged from 0 between several pairs of FA and carcass trait including 14:0 with lean meat yield (LMY) and yield grade (YG) to 0.49±0.06 between n-6/n-3 and (MARB).There were moderate negative unfavorable phenotypic correlations between 11t-18:1 (-0.39±0.09), 18:3n3 (-0.31±0.08), n3 (-0.33±0.09) with

MARB. Genetic correlation ranged from 0 for several pairs of FA and carcass merit trait including 14:0 with HCW, 9c-16:1 with (REA) to -0.84±0.11 between n-3 and MARB and there was a moderate unfavorable genetic correlation between MUFA and HCW (-0.4±0.13), 18:3n3, 22:3n6, and n-3 with MARB (-0.82±0.11, -0.60±0.17, -0.84±0.11). These results suggest that leaner meat will have higher content of n-3 fatty acids and genetic improvement for marbling will reduce the concentration of total n3 in beef cuts. In addition, the results suggest heavier carcasses will have less MUFA. In contrast however, Nogi et al. (2011) reported very weak negative genetic correlations of -0.02 for MUFA and HCW. Pitchford et al. (2002) also reported a weak phenotypic and genetic correlation for MUFA and HCW (0.04, -0.10). Estimates of phenotypic and genetic parameters between beef FA and carcass quality are very scarce. More studies are needed to validate these findings.

In chapter 5, fatty acids and meat quality traits were measured on muscle tissues of 1366 crossbred animals and a bivariate animal model was used to estimate phenotypic and genetic correlations between 83 FAs in the longissimus lumborum muscle with 13 major meat quality traits – namely drip loss (DL), shear force 3d (measured 3 days post mortem) (WBSF_3d), shear force 29d (measured 29 days postmortem after 26 days of ageing) (WBSF_29d), muscle color L*_3d (lightness/brightness) (MCL*3d), MCL* 29d, beef flavor intensity 3d (BFI_3d), BFI_29d, off beef flavor 3d (OF_3d), OF_29d, overall tenderness 3d (OT_3d), OT_29d, overall juiciness 3d (OJ_3d), OJ_29d.

The phenotypic correlations between all fatty acids and meat quality traits ranged from 0 to -0.6±0.14 between 22:5n3 and SJ_3d. and genetic correlations ranged from 0 to 1 in magnitude between 14c-18:1 and 11t,13c/11c,13t 18:2 with SJ_29d. There was a moderate unfavorable negative relationship between 14:0 and WBSF_3d (-0.31±0.13) and a moderate favorable negative phenotypic correlation between 16:0 and BFI_3d (-0.32±0.11) suggesting that steaks that had more 14:0 in the LL muscle were perceived as less tender while steaks that had less 16:0 had more desirable flavor. Genetic correlations between 14:0 and 16:0 with meat quality traits were mostly low and moderately strong favorable positive genetic relationship was seen between 16:0 and DL (0.57±0.14), 14:0 and 16:0 with BFI_3d (-0.5±0.22, -0.57±0.20), SJ_3d (-0.68±0.23, -0.86±0.11) indicating that decreasing the concentration of 14:0 and 16:0 through breeding would be expected to yield steaks that have more flavor and water holding capacity. Monounsaturated fatty acids had mostly favorable genetic and phenotypic correlations with meat quality traits except for antagonistic phenotypic and genetic correlation between 11t-18:1 and flavor (-0.37±0.32 to -0.39±0.17). There was moderate to strong antagonistic phenotypic and genetic relationship (-0.31 to -0.87) between beneficial polyunsaturated fatty acids, 18:3n3, 20:5n3, 22:6n3 and n3 with meat quality traits particularly flavor tenderness and juiciness.

These results suggest that modifying the beneficial polyunsaturated fatty acids in beef through selection would yield unfavorable changes in meat quality attributes. There are only a few published studies on the estimates of phenotypic correlation of FA with meat quality traits and there is good agreement that steaks with

increased concentrations of n-3 have less desirable flavor (Melton et al., 1982; O'Quinn, 2012). There is however no reports on genetic correlation of FAs with meat quality trait. It is reported for the first time here. More studies on genetic parameter estimation for fatty acids with meat quality are needed to validate these relationships.

In chapter 6, a DNA marker panel of 1463 single nucleotide polymorphisms (SNPs) in 556 growth and fat metabolism related genes was developed and genotyped on 223 commercial crossbred beef steers that had fatty acid profiles measured in brisket adipose tissue. After data editing, 947 polymorphic SNPs with minor allele frequency (MAF) greater than 0.05 were evaluated for their associations with the concentration of each of 15 individual and 10 grouped fatty acids using a two step Bayesian analysis approach. The analyses identified 24 SNPs in 22 genes involved in various cellular processes significantly associated with 8 fatty acids at a genome-wise threshold of $P<0.05$. Phenotypic variance explained by significant SNPs at genome-wise threshold for each trait ranged from 0.0001% for monounsaturated fatty acids (MUFA) to approximately 20% for cis-13-octadecenoic acid (13c-18:1). The results show that fatty acid concentrations in brisket adipose tissue of beef cattle are influenced by multiple bovine genes with different functional roles in the cell.

Previous research has associated a number of candidate genes with beef fatty acid profiles but, to our knowledge, this is the first study which has attempted to comprehensively evaluate the effect of SNPs in this large number of candidate genes with several individual and groups of fatty acid in beef cattle.

This study serves to improve our understanding on the molecular mechanisms behind the variation of FA in beef tissues and extends our knowledge on how fatty acids are regulated at the molecular level. Majority of the SNPs found were of small effect, except the SCD gene SNP, such that only a small proportion of the variation in the FAs was accounted for. Further studies are needed to validate novel markers identified in this study especially those at lower SNP-wise threshold and more studies are needed to identify more genetic variants associated with the various FAs, as there is still a lot more genetic and phenotypic variation to be captured.

7.2 Summary, Limitations of the Study and Recommendations for Future Research

In summary, this study revealed strong host direct genetic effects for harmful FAs 14:0 and 16:0 in beef longissimus lumborum muscle and suggests that selection and breeding to reduce the concentration of these FAs would be successful and will not have a negative impact on other beneficial FA like vaccenic acid, total CLA, MUFA and PUFA. However, heritability of other beneficial FA were low and improving them in beef muscle will require nutritional strategies.

Antagonistic relationships exists between fatty acids associated with health, 14:0, 16:0 , MUFA, 11t-18:1, 9c, 11t-18:2, 18:3n3, 20:5n3, 22:6n3, with carcass and meat quality traits, which should be considered when selection for the fatty acids is made.

The results of the study will not only help us gain more insight into the genetic influence of host animals on fatty acid composition in beef cattle tissues but also provide genetic parameters and DNA markers for more effective genetic evaluation and selection and DNA marker assisted diet management to improve fatty acids profiles in beef cattle.

However, it should be pointed out that there are some limitations of this study. One of such is the relatively small sample size especially for fatty acids sampled in the brisket asipose tissue (n=223). This is reflected to some extent in the large standard errors of some of the estimates. However, this limitation should be viewed in perspective with the fact that this trait is difficult and expensive to measure and is in its infancy compared to other traits of economic importance in beef cattle.

Another possible limitation is quantifying fatty acids on a relative term as a percentage of fatty acid methyl esters and not on an absolute term. Although this has a potential of affecting the direction of some estiamtes of correlations coefficients, its degree is debatable since quantifying fatty acids as a percentage of fatty acid methyl esters is less prone to fatty acid quantification errors and a lot of the estimates of heritability and genetic correlations from this study make sense in the biological context of the traits.

In comparison to other economically relevant traits in beef cattle, genetic studies on fatty acids are still at an early stage. Moreover, fatty acids are complex traits that are also highly influenced by rumen microbes and interaction of host genes

and rumen environments. Therefore, more research is needed in order to further delineate host genetic controls on fatty acids in beef tissues and to provide the industry with more effective genetic/genomic tools to improve beneficial fatty acids in beef tissues. Future research may include:

(1). Genetic and genomic studies on rumen microbes and on how rumen microbes interact with host genes under different management levels such as diet and diet supplements in determining contents of fatty acids in beef tissues.

(2). Increasing the animal populations by combining fatty acid datasets from different research groups and/or by analysing fatty acids on more animals in order to improve the accuracy of genetic parameter estimates for fatty acids.

(3). For unfavorable correlations between fatty acids and carcass and meat quality traits, further studies are needed to investigate how genes and gene variants affect both traits, i.e. gene linkage or gene pleiotropic effects, which will help design a selection index and/or other management methods to improve contents of beneficial fatty acid without significantly compromising carcass and meat quality traits.

(4). The gene SNP markers associated with fatty acids also provide a resource to investigate potential benefit of gene-based diet management to further improve contents of beneficial fatty acids in beef. In addition, gene SNP markers associated with fatty acids may alse needed to be evaluated for their predictability on fatty acid concentrations in various beef tissues.

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Appendix S4-1

Phenotypic and genetic correlation between minor fatty acids (concentration <0.5% FAME) with carcass quality traits

Fatty Acids	Hot Carcass Weight		Subcutaneous Fat Thickness		Rib Eye Area		Marbling		Lean Meat Yield		Calculated Yield Grade	
Saturated	rp	rg	rp	rg	Rp	rg	rp	rg	rp	rg	rp	rg
10:0	-0.1±0.1	0.15±0.13	0.04±0.08	-0.05±0.15	-0.14±0.11	-0.02±0.15	-0.07±0.1	0.12±0.14	-0.09±0.07	-0.05±0.15	0.09±0.09	0.05±0.15
12:0	-0.08±0.08	-0.02±0.12	-0.01±0.06	-0.02±0.14	-0.15±0.09	-0.06±0.14	0.03±0.06	0.12±0.13	-0.06±0.05	0.01±0.14	0.07±0.06	0±0.14
13:0	-0.16±0.1	-0.08±0.18	-0.04±0.09	-0.27±0.19	-0.08±0.12	-0.07±0.19	0.12±0.11	0.32±0.18	0.01±0.08	0.08±0.21	-0.04±0.1	-0.14±0.2
19:0	0.16±0.14	-0.53±0.12	0.13±0.12	-0.01±0.16	0.31±0.14	-0.23±0.15	0.01±0.13	0.34±0.15	0.1±0.1	-0.09±0.16	-0.14±0.13	-0.05±0.16
20:0	-0.01±0.1	0.54±0.14	0.02±0.09	-0.09±0.17	-0.13±0.13	0.12±0.18	-0.12±0.11	0.42±0.16	-0.04±0.09	0.03±0.18	0.13±0.12	0.13±0.17
22:0	-0.25±0.11	0.18±0.21	-0.15±0.11	-0.08±0.22	-0.28±0.14	-0.32±0.21	-0.09±0.13	-0.2±0.23	-0.04±0.12	-0.31±0.23	0.08±0.15	0.33±0.22
24:0	-0.04±0.1	0.45±0.14	-0.03±0.12	-0.12±0.17	0.05±0.13	0.17±0.16	-0.22±0.09	-0.62±0.15	0.12±0.1	0.11±0.17	-0.1±0.11	0.02±0.17
Monounsaturated												
9c-15:1	-0.2±0.1	-0.12±0.33	-0.1±0.09	-0.63±0.3	-0.19±0.13	0.13±0.34	0.13±0.11	0.19±0.34	-0.01±0.09	0.53±0.31	0±0.12	-0.53±0.32
7c-16:1	-0.28±0.07	-0.49±0.15	-0.31±0.08	-0.15±0.18	-0.18±0.14	-0.13±0.18	-0.01±0.1	0.13±0.19	0.16±0.09	0.04±0.19	-0.13±0.12	-0.17±0.18
11t-16:1	-0.09±0.11	-0.12±0.29	0.1±0.11	0.04±0.3	0.12±0.13	0±0.3	-0.01±0.12	0.7±0.32	0.03±0.09	0.05±0.31	-0.07±0.11	-0.09±0.3
12c-16:1	-0.1±0.04	-0.13±0.15	-0.04±0.04	-0.22±0.15	-0.03±0.05	-0.03±0.16	0.05±0.04	0.06±0.16	0.01±0.04	0.19±0.16	-0.03±0.04	-0.17±0.16
7c-17:1	0.01±0.11	8.2±3.67	0.1±0.12	0.49±0.57	-0.05±0.13	4.3±6	-0.26±0.1	-2.6±6.46	-0.08±0.11	-0.25±0.52	0.1±0.12	0.31±0.52
12c-18:1	-0.03±0.11	-0.55±0.16	0.09±0.1	0.13±0.19	0.07±0.14	-0.34±0.2	0.08±0.13	0.49±0.15	0.01±0.09	-0.28±0.2	-0.03±0.11	0.08±0.2
13c-18:1	-0.08±0.04	-0.23±0.14	0.05±0.04	-0.1±0.15	-0.02±0.06	-0.08±0.15	0.1±0.05	0.03±0.15	-0.06±0.04	0.07±0.16	0.03±0.04	-0.11±0.15
14c-18:1	0.04±0.05	-0.16±0.19	-0.12±0.05	-0.29±0.2	-0.09±0.1	0.3±0.19	0.12±0.05	0.28±0.2	0.06±0.05	0.45±0.18	-0.03±0.06	-0.47±0.18
15c-18:1	-0.01±0.08	-0.12±0.16	-0.17±0.1	-0.32±0.18	-0.01±0.11	0.34±0.18	0.24±0.08	-0.02±0.19	0.12±0.07	0.37±0.19	-0.12±0.08	-0.5±0.17
9c-20:1	-0.08±0.12	0.5±0.16	0.03±0.09	-0.05±0.21	-0.22±0.13	-0.01±0.2	-0.06±0.11	0.23±0.2	-0.13±0.08	-0.01±0.21	0.17±0.11	0.19±0.2

Fatty Acids	Hot Carcass Weight		Subcutaneous Fat Thickness		Rib Eye Area		Marbling		Lean Meat Yield		Calculated Yield Grade	
	rp	rg	rp	rg	rp	rg	rp	rg	rp	rg	rp	rg
11c-20:1	-0.06±0.07	-0.08±0.14	0.03±0.06	0.04±0.15	-0.12±0.11	-0.04±0.15	0.01±0.06	0.22±0.15	-0.06±0.06	-0.09±0.16	0.07±0.07	0.01±0.16
6t/8t-18:1	0.12±0.1	0.17±0.2	-0.03±0.09	0±0.21	0.15±0.12	0.27±0.21	0.21±0.09	0.04±0.21	0.1±0.08	0.11±0.22	-0.11±0.1	-0.14±0.21
9t-18:1	0.12±0.1	0.09±0.2	0.01±0.09	0.05±0.21	0.15±0.11	0.26±0.21	0.19±0.09	0.03±0.21	0.07±0.08	0.1±0.22	-0.08±0.1	-0.15±0.21
11t-18:1	-0.14±0.1	-0.26±0.15	0.01±0.1	0.01±0.17	-0.16±0.13	-0.14±0.17	-0.39±0.09	-0.06±0.18	-0.03±0.1	-0.09±0.18	0.07±0.12	0.04±0.18
12t-18:1	0.08±0.09	0.04±0.21	-0.02±0.1	-0.61±0.18	-0.05±0.12	0.24±0.22	-0.06±0.1	0.39±0.23	0.03±0.09	0.54±0.2	0.05±0.12	-0.53±0.21
15t-18:1	-0.04±0.04	-0.82±1.01	0±0.04	-0.01±0.51	0.04±0.05	-0.41±0.71	0.05±0.04	0.34±0.57	0.02±0.03	-0.32±0.71	-0.03±0.04	0.03±0.52
16t-18:1	0.04±0.12	-0.17±0.17	0.07±0.13	-0.42±0.16	-0.02±0.15	-0.03±0.18	-0.31±0.1	0.27±0.2	-0.04±0.11	0.35±0.17	0.1±0.13	-0.27±0.17
Polyunsaturated												
9c,13t/8t,12c-18:2	0.04±0.1	-0.21±0.16	0.1±0.09	-0.46±0.15	-0.13±0.13	0.02±0.18	-0.01±0.12	0.05±0.18	-0.1±0.09	0.29±0.17	0.16±0.12	-0.38±0.16
9c,15c-18:2	-0.15±0.09	-0.11±0.15	0.02±0.1	0±0.17	-0.19±0.11	0.04±0.17	0.16±0.1	0.07±0.17	-0.12±0.07	0.03±0.18	0.1±0.09	-0.08±0.17
8t,13c-18:2	-0.02±0.09	-0.57±0.3	0.04±0.09	-0.64±0.25	-0.15±0.12	-0.42±0.39	-0.14±0.09	-0.09±0.3	-0.05±0.09	0.33±0.3	0.1±0.11	-0.3±0.28
11t,15c-18:2	-0.15±0.08	-0.01±0.15	-0.06±0.08	0.13±0.16	-0.1±0.1	0.14±0.17	0.02±0.08	-0.23±0.17	0.01±0.07	-0.09±0.17	-0.03±0.08	-0.04±0.17
6t,8t-18:2	0.01±0.08	-0.37±0.68	-0.02±0.06	-0.53±0.86	0.09±0.09	-0.1±0.62	0.02±0.07	0.61±0.83	0.07±0.06	0.49±0.8	-0.08±0.08	-0.37±0.75
7t,9c-18:2	0.11±0.05	-0.16±0.34	0.02±0.04	-0.17±0.39	0.1±0.06	-0.08±0.36	0.07±0.04	-0.12±0.39	0.02±0.04	0.13±0.41	-0.02±0.04	-0.13±0.4
7t,9t-18:2	-0.14±0.11	-0.08±0.25	-0.18±0.1	-0.1±0.27	-0.26±0.12	-0.24±0.25	0.05±0.11	0.13±0.26	-0.01±0.09	0.19±0.28	0.07±0.11	0.1±0.26
8t,10c-18:2	-0.05±0.11	0.32±0.14	-0.04±0.07	0.12±0.16	-0.14±0.12	-0.14±0.16	-0.11±0.08	-0.34±0.15	-0.04±0.07	-0.22±0.16	0.07±0.09	0.3±0.16
8t,10t-18:2	0.13±0.08	-0.27±0.31	0±0.06	-0.19±0.37	0.14±0.09	-0.33±0.37	0.12±0.06	0.36±0.36	0.05±0.05	0.05±0.38	-0.05±0.07	0.09±0.38
9c,11t/9t,11c -18:2	-0.21±0.11	0.17±0.16	-0.05±0.1	0.18±0.18	-0.22±0.13	-0.02±0.18	-0.18±0.1	-0.15±0.18	-0.06±0.09	-0.2±0.18	0.07±0.12	0.19±0.18
9t,11t-18:2	0.04±0.06	0.17±0.21	0.02±0.06	0.16±0.22	0.06±0.08	-0.25±0.22	0.06±0.06	0.03±0.23	0.01±0.05	-0.28±0.22	-0.01±0.07	0.35±0.21
10t,12c-18:2	-0.01±0.11	0±0.28	-0.17±0.1	0.09±0.3	0.04±0.14	-0.43±0.26	0.37±0.08	0.59±0.22	0.16±0.09	-0.39±0.29	-0.16±0.11	0.4±0.29
10t,12t-18:2	0.12±0.1	-0.33±0.18	-0.02±0.08	0.03±0.21	0.17±0.12	-0.59±0.17	0.38±0.06	0.55±0.16	0.08±0.08	-0.38±0.19	-0.1±0.1	0.36±0.2
11t,13c/11c,13t -18:2	-0.11±0.11	0.5±0.18	-0.03±0.09	-0.03±0.21	-0.16±0.13	-0.01±0.21	-0.23±0.09	-0.76±0.14	-0.04±0.08	0.01±0.21	0.07±0.11	0.21±0.2
11t,13t-18:2	0.15±0.09	0.24±0.17	0.07±0.08	0.05±0.18	0.11±0.1	-0.15±0.18	-0.08±0.07	-0.23±0.18	-0.02±0.06	-0.2±0.18	0.03±0.07	0.24±0.18

	Hot Carcass Weight		Subcutaneous Fat Thickness		Rib Eye Area		Marbling		Lean Meat Yield		Calculated Yield Grade	
Fatty Acids	rp	Rg	rp	rg	rp	rg	**Fatty acids**	rp	rg	rp	rg	rp
12t,14c/12c,14t -18:2	-0.01±0.08	0±0.19	-0.06±0.07	-0.18±0.21	-0.09±0.1	-0.28±0.2	0.13±0.06	0.13±0.21	0.01±0.05	-0.01±0.22	0.01±0.07	0.12±0.21
12t,14t-18:2	0.34±0.12	-0.07±0.17	0.32±0.12	-0.12±0.19	0.26±0.16	-0.28±0.18	0.06±0.15	0.41±0.16	-0.09±0.11	-0.12±0.19	0.06±0.15	0.11±0.19
18:3n-3	-0.12±0.1	0.46±0.14	-0.1±0.09	0.02±0.16	-0.08±0.14	0.23±0.16	-0.31±0.08	-0.82±0.11	0.08±0.09	0.02±0.17	-0.06±0.11	0.04±0.16
18:3n-6	-0.16±0.11	0.17±0.19	-0.16±0.09	-0.23±0.2	-0.15±0.15	0.09±0.2	-0.03±0.11	-0.01±0.22	0.08±0.11	0.17±0.21	-0.03±0.13	-0.11±0.21
20:2n-6	-0.19±0.07	-0.25±0.27	-0.06±0.07	-0.01±0.31	-0.08±0.12	-0.26±0.29	0.04±0.09	-0.2±0.34	0.06±0.07	-0.23±0.33	-0.06±0.08	0.13±0.32
20:3n-6	-0.07±0.09	0.13±0.17	-0.07±0.11	-0.13±0.18	-0.02±0.14	0.13±0.17	-0.11±0.09	-0.48±0.18	0.14±0.1	0.19±0.18	-0.1±0.12	-0.08±0.18
20:3n-9	-0.14±0.1	0.38±0.18	-0.17±0.09	-0.2±0.19	-0.14±0.13	0.04±0.2	-0.29±0.08	-0.47±0.18	0.1±0.1	0.09±0.21	-0.05±0.12	0.06±0.21
20:5n-3	-0.06±0.09	0.02±0.38	0.15±0.09	0.14±0.42	-0.1±0.11	0.15±0.41	-0.08±0.11	-0.24±0.44	-0.11±0.08	0.01±0.46	0.11±0.1	-0.09±0.44
22:4n-6	-0.16±0.1	0.05±0.18	-0.15±0.1	-0.16±0.19	-0.18±0.14	0.03±0.19	-0.1±0.11	-0.19±0.2	0.04±0.11	0.08±0.21	0.01±0.13	-0.06±0.2
22:5n-3	-0.1±0.12	0.53±0.14	-0.1±0.12	-0.18±0.16	-0.1±0.15	0.26±0.16	-0.29±0.09	-0.72±0.13	0.08±0.12	0.21±0.17	-0.03±0.14	-0.04±0.17
22:6n-3	-0.07±0.09	0.47±0.17	-0.09±0.1	-0.42±0.17	-0.03±0.13	0.16±0.19	-0.2±0.08	-0.6±0.17	0.11±0.09	0.25±0.19	-0.09±0.11	-0.12±0.2
Branched												
iso14:0	-0.18±0.08	-0.15±0.24	-0.16±0.07	-0.2±0.25	-0.17±0.11	-0.41±0.28	-0.14±0.07	0.31±0.28	0.06±0.07	-0.15±0.29	-0.03±0.09	0.16±0.28
iso15:0	-0.15±0.08	0.02±0.16	-0.18±0.07	-0.49±0.15	-0.17±0.12	0.16±0.17	-0.23±0.07	-0.03±0.18	0.08±0.08	0.44±0.16	-0.03±0.11	-0.38±0.16
ai15:0	-0.24±0.09	-0.27±0.13	-0.36±0.08	-0.51±0.13	-0.22±0.13	-0.01±0.15	0.11±0.1	0.18±0.15	0.16±0.09	0.35±0.15	-0.11±0.12	-0.39±0.14
iso16:0	-0.24±0.1	0.08±0.16	-0.35±0.09	-0.3±0.17	-0.21±0.14	0.22±0.17	0±0.11	-0.09±0.18	0.16±0.09	0.29±0.17	-0.1±0.12	-0.29±0.17
iso17:0	-0.26±0.09	-0.28±0.19	-0.26±0.09	-0.25±0.21	-0.17±0.15	0.16±0.23	0.04±0.12	-0.2±0.23	0.13±0.11	0.19±0.23	-0.11±0.14	-0.37±0.21
iso18:0	-0.24±0.09	-0.06±0.15	-0.2±0.11	0.07±0.16	-0.15±0.13	0.09±0.17	0.19±0.1	-0.06±0.17	0.11±0.08	-0.04±0.18	-0.1±0.11	-0.06±0.17
Groups												
[a]sumCLA	-0.13±0.1	0.26±0.17	-0.05±0.08	0.13±0.19	-0.16±0.12	-0.05±0.19	-0.05±0.09	-0.04±0.2	-0.02±0.07	-0.19±0.19	0.04±0.09	0.21±0.19

Fatty Acids	Hot Carcass Weight		Subcutaneous Fat Thickness		Rib Eye Area		Marbling		Lean Meat Yield		Calculated Yield Grade	
	rp	rg	rp	rg	rp	rg	rp	rg	rp	rg	rp	rg
[b]PUFA	-0.14±0.07	-0.07±0.19	-0.15±0.07	0.14±0.21	-0.02±0.13	-0.1±0.21	0.07±0.08	-0.29±0.23	0.18±0.08	-0.19±0.23	-0.16±0.1	0.16±0.22
[c]n-3	-0.12±0.12	0.56±0.13	-0.11±0.12	-0.14±0.16	-0.1±0.16	0.27±0.15	-0.33±0.09	-0.84±0.11	0.08±0.12	0.16±0.16	-0.04±0.14	-0.02±0.17
[d]n-6	-0.14±0.07	-0.17±0.19	-0.14±0.06	0.18±0.21	0±0.13	-0.15±0.21	0.12±0.08	-0.17±0.22	0.18±0.07	-0.25±0.22	-0.17±0.09	0.18±0.22
[e]n-6/n-3	-0.04±0.12	-0.69±0.1	0.04±0.09	0.2±0.14	0.17±0.13	-0.28±0.14	0.49±0.06	0.49±0.11	0.06±0.09	-0.21±0.15	-0.12±0.1	0.02±0.15
[f]P/S	-0.17±0.08	-0.23±0.18	-0.15±0.07	0.16±0.21	-0.06±0.14	-0.14±0.2	0.08±0.09	-0.14±0.21	0.15±0.09	-0.22±0.22	-0.14±0.11	0.14±0.21
[g]P/(S+B)	-0.17±0.08	-0.22±0.18	-0.14±0.07	0.17±0.21	-0.05±0.14	-0.14±0.2	0.08±0.09	-0.15±0.21	0.15±0.08	-0.23±0.22	-0.14±0.11	0.15±0.21

c=cis, t=trans, NE= Not Estimable

SumCLA: sum of conjugated linoleic acids, SFA (sum of saturated fatty acids), PUFA (sum of polyunsaturated fatty acids), BCFA (Branched chain fatty acid)

[a]SumCLA = 8t,10c-18:2 + 9c,11t-18:2 + 7t,9c-18:2 + 9t,11c-18:2 + 10t,12c-18:2 + 11c,13t-18:2 + 11t,13c-18:2 +12t,14c-18:2+12c,14t-18:2+ 9c,11c-18:2 + 10c,12c-18:2 +6t,8t-18:2+ 9t,11t-18:2 + 11t,13t-18:2 + 12t,14t-18:2 + 10t,12t-18:2 + 8t,10t-18:2 + 7t,9t-18:2 ; [b]PUFA= 18:2n-6 + 18:3n-6 + 18:3n-3 + 20:2n-6 + 20:3n-9 + 20:3n-6 + 20:4n-6 + 22:4n-6 + 22:5n-3 ; SFA = 10:0 + 12:0 + 13:0 + 14:0 + 15:0 + 16:0 + 17:0 + 18:0 + 20:0 + 23:0 ; MUFA = 9c-14:1 + 9c-15:1 + 7c-16:1 + 9c-16:1 + 9c-17:1 + 6t/7t/8t-18:1 + 9t-18:1 + 10t-18:1 +11t-18:1 + 12t-18:1 + 13t/14t-18:1 + 15t-18:1 + 16t-18:1 + 9c-18:1 + 11c-18:1 + 12c-18:1 + 13c-18:1 + 14c-18:1 + 16c-18:1 + 9c-20:1 + 11c-20:1; BCFA iso-14:0 + iso-15:0 + anteiso-15:0 + iso-16:0 + iso-17:0 + anteiso-17:0 + iso-18:0 ;[c]n-3 PUFA = 18:3n-3 + 22:5n-3; [d]n-6 PUFA = 18:2n-6 + 18:3n-6 + 20:2n-6 + 20:3n-6 +20:4n-6 + 22:4n-6 ; [e]n-6/n-3: ratio between n-6 and n-3 PUFA ; [f]P/S: ratio of PUFA to SFA ; [g]P/(S+B): ratio of PUFA to SFA+BFA

Appendix S5-1

Phenotypic and genetic correlation between minor fatty acids (concentration <0.5% FAME) with meat quality traits

Fatty acids	DRIPLOSS		COLOR_L1_3d		COLOR_L1_29d		SHEAR_3d	
Saturated	r_p	r_g	r_p	r_g	r_p	r_g	r_p	r_g
10:0	-0.35±0.16	0.07±0.26	0.33±0.14	0.19±0.22	0.33±0.15	0.44±0.22	0.19±0.17	0.15±0.23
12:0	-0.12±0.11	0.05±0.18	0.12±0.08	0.05±0.17	0.09±0.1	-0.11±0.21	-0.04±0.09	0.15±0.17
13:0	-0.45±0.17	-0.09±0.33	0.25±0.16	-0.14±0.28	-0.1±0.2	0.11±0.36	-0.34±0.16	0.14±0.3
19:0	0.29±0.21	-0.46±0.25	-0.24±0.14	-0.04±0.3	-0.12±0.18	0.38±0.32	0.09±0.19	0.27±0.3
20:0	0.24±0.21	0.42±0.24	-0.06±0.15	0.05±0.26	0.16±0.17	-0.21±0.32	0.32±0.13	0±0.28
22:0	-0.4±0.2	0.18±0.35	0.3±0.15	0.43±0.27	0.23±0.18	0.1±0.42	0.06±0.23	-0.27±0.33
24:0	0.25±0.22	0.74±0.13	-0.04±0.17	0.16±0.24	0.45±0.17	-0.19±0.33	0.35±0.16	0.18±0.27
Monounsaturated								
9c-15:1	-0.39±0.17	-0.18±0.44	0.27±0.13	0.3±0.38	0.06±0.19	-0.03±0.55	-0.17±0.17	-0.35±0.39
7c-16:1	-0.32±0.17	-0.28±0.27	0.14±0.14	-0.16±0.27	-0.01±0.17	-0.11±0.35	-0.1±0.16	0.3±0.26
11t-16:1	0.2±0.23	-0.55±0.31	-0.13±0.16	-0.08±0.38	-0.05±0.19	-0.15±0.47	0.05±0.2	0.17±0.39
12c-16:1	-0.05±0.09	0.05±0.2	0.03±0.05	0.06±0.19	-0.01±0.05	-0.28±0.23	-0.05±0.06	-0.3±0.18
7c-17:1	0.1±0.22	0.56±0.4	0.05±0.14	NE	0.24±0.14	NE	0.27±0.14	0.46±0.47
11c-18:1	-0.36±0.19	-0.61±0.19	0.07±0.16	-0.04±0.28	-0.17±0.18	-0.02±0.35	-0.25±0.16	-0.09±0.28
12c-18:1	0.47±0.17	-0.23±0.31	-0.36±0.14	-0.23±0.28	-0.16±0.2	-0.17±0.36	0.17±0.2	0.08±0.3
13c-18:1	-0.01±0.08	-0.28±0.19	-0.01±0.06	-0.12±0.18	0±0.05	-0.18±0.23	-0.03±0.06	-0.22±0.18
14c-18:1	-0.07±0.11	-0.1±0.28	-0.01±0.08	0.08±0.25	0.01±0.08	-0.27±0.31	-0.01±0.08	-0.15±0.27
15c-18:1	-0.16±0.16	0.19±0.26	0.08±0.12	0.3±0.22	-0.11±0.14	0.36±0.26	-0.26±0.13	-0.21±0.25
9c-20:1	-0.12±0.25	0.19±0.31	0.16±0.16	-0.12±0.29	0.26±0.16	-0.35±0.32	0.21±0.17	-0.3±0.28
11c-20:1	-0.1±0.12	-0.21±0.2	0±0.09	-0.52±0.13	0.04±0.09	-0.21±0.23	0.1±0.09	0±0.2
6t/8t-18:1	0.23±0.18	0.77±0.12	-0.19±0.11	0.11±0.27	-0.22±0.13	-0.01±0.36	-0.11±0.16	-0.23±0.29
9t-18:1	0.28±0.16	0.64±0.17	-0.2±0.11	0.01±0.28	-0.2±0.13	-0.08±0.36	-0.09±0.16	-0.26±0.28
10t-18:1	-0.28±0.16	0.02±0.27	0.11±0.12	-0.31±0.23	-0.07±0.15	-0.01±0.33	-0.24±0.12	0.02±0.26
11t-18:1	0.07±0.26	-0.13±0.28	-0.01±0.17	-0.21±0.25	0.26±0.17	-0.23±0.33	0.39±0.15	0.22±0.28
12t-18:1	0.14±0.18	0.19±0.3	-0.02±0.13	0.45±0.22	0.13±0.13	0.23±0.35	0.24±0.11	-0.34±0.27

	DRIPLOSS		COLOR_L1_3d		COLOR_L1_29d		SHEAR_3d	
Fatty acids	r_p	r_g	r_p	r_g	r_p	r_g	r_p	r_g
15t-18:1	-0.15±0.08	-0.73±0.33	0.07±0.08	0.17±0.86	0±0.07	0.18±0.74	-0.09±0.07	-0.15±0.55
16t-18:1	0.26±0.22	0.01±0.31	-0.11±0.16	0.06±0.29	0.16±0.18	-0.07±0.37	0.35±0.13	0.16±0.29
Polyunsaturated								
9c,13t/8t,12c-18:2	0.04±0.2	0.06±0.28	0.03±0.13	-0.1±0.26	0.19±0.13	-0.03±0.33	0.23±0.12	-0.24±0.25
9c,15c-18:2	-0.26±0.14	-0.3±0.22	0.2±0.12	-0.15±0.24	0.06±0.15	0.01±0.33	-0.12±0.15	-0.14±0.26
8t,13c-18:2	0.06±0.18	-0.19±0.42	-0.02±0.12	-0.15±0.36	0.14±0.13	-0.14±0.48	0.23±0.11	-0.19±0.38
11t,15c-18:2	-0.22±0.12	0.17±0.21	0.15±0.09	-0.29±0.2	0.01±0.12	-0.08±0.28	-0.14±0.11	0.08±0.23
6t,8t-18:2	0.21±0.14	-0.55±0.49	-0.14±0.1	0.32±0.57	-0.15±0.1	NE	-0.06±0.12	0.24±0.75
7t,9c-18:2	0.14±0.06	0.3±0.43	-0.02±0.06	-0.39±0.38	-0.05±0.05	-0.93±0.08	0±0.06	-0.59±0.32
7t,9t-18:2	-0.17±0.2	-0.15±0.36	0.12±0.14	-0.07±0.35	0.02±0.02	-0.78±0.18	-0.12±0.16	-0.15±0.36
8t,10c-18:2	-0.16±0.19	0.23±0.24	0.18±0.12	-0.03±0.23	0.15±0.13	-0.58±0.17	-0.03±0.15	-0.29±0.22
8t,10t-18:2	0.17±0.12	0.55±0.33	-0.15±0.09	-0.69±0.22	-0.13±0.09	-0.37±0.46	-0.06±0.1	-0.03±0.47
9c,11t/9t,11c -18:2	-0.37±0.2	0.05±0.32	0.3±0.16	-0.18±0.28	0.26±0.19	-0.44±0.29	0.01±0.22	-0.1±0.3
9t,11t-18:2	0.21±0.1	0.35±0.25	-0.07±0.08	-0.12±0.28	-0.11±0.09	-0.4±0.31	-0.02±0.09	-0.49±0.24
10t,12c-18:2	-0.03±0.22	0.1±0.41	0.03±0.15	-0.09±0.39	-0.22±0.14	-0.49±0.39	-0.29±0.13	-0.42±0.34
10t,12t-18:2	0.19±0.21	-0.05±0.31	-0.11±0.15	-0.37±0.25	-0.24±0.15	-0.44±0.29	-0.27±0.12	-0.19±0.29
11t,13c/11c,13t -18:2	-0.27±0.22	0.72±0.16	0.24±0.15	-0.14±0.29	0.25±0.17	-0.61±0.22	0.04±0.2	-0.1±0.31
11t,13t-18:2	0.17±0.14	0.7±0.13	0.02±0.11	-0.06±0.24	0.13±0.09	-0.3±0.26	0.15±0.1	-0.19±0.24
12t,14c/12c,14t -18:2	-0.08±0.15	0.23±0.27	0.09±0.1	-0.01±0.27	-0.14±0.08	-0.42±0.28	-0.11±0.1	-0.45±0.22
12t,14t-18:2	0.33±0.22	0.25±0.33	-0.17±0.17	0.21±0.32	0.04±0.22	0.28±0.4	0.25±0.18	-0.22±0.34
18:3n-3	-0.25±0.18	0.66±0.15	0.21±0.13	0.04±0.24	0.21±0.14	-0.28±0.28	0.11±0.17	-0.11±0.26
18:3n-6	-0.38±0.19	0.58±0.23	0.32±0.15	0.4±0.25	0.27±0.16	0.02±0.38	0.04±0.21	0±0.32
20:2n-6	-0.33±0.14	0.06±0.41	0.16±0.12	-0.53±0.28	0.11±0.13	-0.75±0.2	0±0.14	-0.07±0.4
20:3n-6	0±0.23	0.6±0.18	0.05±0.16	0.08±0.25	0.26±0.16	-0.4±0.27	0.32±0.13	0.25±0.25
20:3n-9	-0.26±0.22	0.46±0.25	0.24±0.15	0.55±0.2	0.33±0.16	-0.21±0.33	0.32±0.15	0.23±0.3
20:4n-6	-0.21±0.18	0.63±0.19	0.15±0.14	0.04±0.27	0.19±0.14	-0.24±0.33	0.26±0.12	0.39±0.25

	DRIPLOSS		COLOR_L1_3d		COLOR_L1_29d		SHEAR_3d	
Fatty acids	r_p	r_g	r_p	r_g	r_p	r_g	r_p	r_g
20:5n-3	0.07±0.21	0.42±0.39	0.03±0.13	-0.4±0.45	0.23±0.15	-0.83±0.22	0.24±0.13	-0.02±0.55
22:4n-6	-0.3±0.21	0.24±0.3	0.2±0.16	0.3±0.27	0.28±0.15	-0.27±0.32	0.17±0.19	0.22±0.29
22:5n-3	-0.23±0.26	0.69±0.17	0.24±0.17	0.25±0.27	0.38±0.19	-0.34±0.32	0.39±0.16	0.35±0.26
22:6n-3	-0.16±0.22	0.72±0.14	0.23±0.14	0.22±0.25	0.31±0.14	-0.16±0.33	0.27±0.14	0.53±0.2
Branched								
iso14:0	-0.28±0.13	0.1±0.4	0.22±0.12	-0.25±0.33	0.11±0.14	-0.2±0.4	-0.07±0.14	0.13±0.34
iso15:0	-0.16±0.2	0.44±0.22	0.14±0.14	0.1±0.25	0.11±0.15	-0.08±0.31	0.13±0.16	0.19±0.25
ai15:0	-0.29±0.16	0.08±0.26	0.17±0.13	0.18±0.25	0.02±0.16	0.09±0.33	-0.08±0.16	0.02±0.27
iso16:0	-0.25±0.18	0.61±0.18	0.13±0.13	-0.02±0.27	-0.02±0.16	-0.22±0.33	-0.1±0.15	0.02±0.28
iso17:0	-0.43±0.15	0.24±0.32	0.25±0.12	0.03±0.33	0.11±0.18	0.05±0.42	-0.07±0.19	0.16±0.34
ai17:0	-0.49±0.15	-0.11±0.28	0.28±0.13	0±0.28	0.11±0.19	0.06±0.37	-0.12±0.19	-0.01±0.31
iso18:0	-0.28±0.17	0.17±0.27	0.12±0.13	-0.29±0.24	-0.08±0.16	-0.28±0.32	-0.18±0.13	-0.18±0.26
Group								
sumtrans18:1	-0.24±0.14	0.07±0.26	0.09±0.11	-0.29±0.23	-0.08±0.13	-0.03±0.31	-0.22±0.11	0.02±0.25
sumCLA	-0.18±0.2	0.25±0.28	0.15±0.13	-0.23±0.26	0.05±0.16	-0.63±0.21	-0.07±0.16	-0.3±0.26
PUFA	-0.38±0.15	0.48±0.25	0.18±0.17	0.01±0.3	0.12±0.16	-0.3±0.32	0.07±0.16	0.12±0.28
n-3	-0.26±0.24	0.79±0.12	0.26±0.16	0.18±0.27	0.34±0.15	-0.34±0.3	0.31±0.18	0.24±0.28
n-6	-0.37±0.14	0.36±0.29	0.16±0.15	-0.02±0.28	0.05±0.15	-0.28±0.32	0.01±0.15	0.1±0.28
n-6/n-3	0.02±0.19	-0.51±0.18	-0.21±0.1	-0.22±0.21	-0.29±0.1	0.14±0.26	-0.14±0.13	-0.05±0.23
P/S	-0.41±0.15	0.11±0.31	0.2±0.16	-0.07±0.29	0.12±0.17	-0.27±0.33	0.02±0.18	0.09±0.28
P/(S+B)	-0.42±0.15	0.12±0.31	0.2±0.16	-0.07±0.29	0.13±0.17	-0.27±0.33	0.03±0.18	0.1±0.28
Health Index	0.12±0.12	-0.43±0.18	-0.17±0.08	-0.2±0.17	-0.17±0.1	0.09±0.23	0.11±0.13	0.03±0.19

Fatty acids	SHEAR_29d		OF_3d		OF_29d		FI_3d		FI_29d	
Saturated	rp	rg	rp	rg	rp	rg	rp	rg	rp	rg
10:0	0.2±0.11	0.25±0.22	-0.33±0.16	-0.08±0.27	-0.21±0.15	-0.01±0.28	-0.39±0.15	-0.12±0.32	-0.35±0.16	0.11±0.28
12:0	0.07±0.07	0.24±0.17	-0.11±0.09	0.07±0.2	-0.06±0.08	-0.18±0.21	-0.02±0.11	-0.13±0.24	0.05±0.11	-0.09±0.2
13:0	-0.04±0.13	0.05±0.29	0.14±0.22	-0.18±0.33	0.06±0.16	-0.25±0.35	0.19±0.23	0.14±0.4	0.25±0.21	0.17±0.35
19:0	-0.13±0.11	0.41±0.27	0.17±0.2	0.07±0.36	0.08±0.15	0.51±0.29	-0.1±0.24	-0.12±0.43	-0.16±0.23	0.42±0.31
20:0	0.18±0.11	0.33±0.25	-0.25±0.18	0.06±0.33	-0.18±0.14	-0.52±0.26	-0.28±0.19	-0.49±0.28	-0.28±0.18	-0.53±0.24
22:0	0.28±0.1	-0.25±0.34	-0.36±0.17	-0.21±0.39	-0.29±0.13	-0.18±0.45	-0.09±0.27	-0.23±0.47	-0.05±0.27	-0.25±0.41
24:0	0.34±0.14	0.14±0.27	-0.25±0.21	-0.52±0.22	-0.49±0.14	-0.7±0.18	-0.57±0.13	-0.94±0.04	-0.52±0.14	-0.62±0.21
Monounsaturated										
9c-15:1	0.09±0.12	0.07±0.45	-0.07±0.21	0±0.52	-0.05±0.15	0.37±0.5	0.16±0.22	0.83±0.22	0.23±0.2	0.97±0.03
7c-16:1	0.18±0.11	0.7±0.16	-0.14±0.19	-0.49±0.24	-0.13±0.14	0.98±0.02	0.1±0.2	-0.37±0.33	0.15±0.19	0.63±0.22
11t-16:1	-0.09±0.13	-0.02±0.4	0.18±0.2	-0.45±0.35	0.12±0.15	0.57±0.35	-0.18±0.22	0.66±0.32	-0.15±0.22	0.72±0.23
12c-16:1	0.02±0.04	-0.25±0.19	0.03±0.06	-0.12±0.24	0.02±0.05	0.01±0.26	0.02±0.06	0.38±0.24	0.06±0.06	0.81±0.08
7c-17:1	0.14±0.09	0.36±0.53	-0.26±0.15	-0.38±0.56	-0.24±0.11	-0.23±0.68	-0.37±0.14	-0.33±0.65	-0.33±0.14	-0.29±0.63
11c-18:1	-0.01±0.12	-0.11±0.29	0.14±0.21	0.09±0.33	0.09±0.15	0.8±0.13	0.24±0.21	0.71±0.2	0.25±0.2	0.37±0.3
12c-18:1	-0.11±0.13	-0.21±0.28	0.2±0.22	0.51±0.27	0.17±0.16	0.16±0.37	-0.02±0.27	0.78±0.18	-0.07±0.25	0.11±0.35
13c-18:1	-0.02±0.04	-0.24±0.18	0.08±0.05	0.03±0.22	0.06±0.04	0.27±0.23	0.05±0.06	0.65±0.15	0.07±0.05	0.92±0.04
14c-18:1	0.05±0.06	0.04±0.27	-0.05±0.09	0.52±0.24	-0.02±0.07	-0.04±0.34	0.09±0.1	0.55±0.29	0.07±0.1	-0.12±0.32
15c-18:1	-0.02±0.09	-0.27±0.24	0.08±0.16	0.25±0.28	0.05±0.12	-0.27±0.3	0.31±0.14	0.2±0.35	0.29±0.14	-0.1±0.31
9c-20:1	0.19±0.12	-0.16±0.3	-0.35±0.17	0.04±0.35	-0.26±0.14	-0.4±0.33	-0.13±0.23	0.34±0.39	-0.08±0.23	-0.01±0.37
11c-20:1	0.1±0.06	0.03±0.21	-0.16±0.08	-0.02±0.22	-0.05±0.07	0.15±0.25	-0.13±0.09	0.47±0.21	-0.06±0.1	0.2±0.22
6t/8t-18:1	-0.13±0.08	-0.05±0.3	0.25±0.15	0.45±0.28	0.16±0.11	-0.36±0.32	0.28±0.15	0.19±0.39	0.22±0.16	-0.46±0.28
9t-18:1	-0.12±0.08	-0.08±0.3	0.23±0.15	0.6±0.23	0.15±0.11	-0.36±0.31	0.25±0.16	0.33±0.37	0.19±0.16	-0.42±0.29
10t-18:1	-0.03±0.1	-0.08±0.27	0.08±0.17	0.09±0.31	0.04±0.12	-0.33±0.31	0.23±0.16	0.19±0.36	0.22±0.16	-0.14±0.32
11t-18:1	0.28±0.11	0.49±0.22	-0.4±0.16	-0.14±0.32	-0.31±0.13	-0.14±0.35	-0.38±0.18	-0.37±0.32	-0.39±0.17	-0.03±0.34
12t-18:1	0.11±0.09	-0.28±0.29	-0.18±0.14	0.39±0.33	-0.11±0.11	0.21±0.39	-0.21±0.15	0.33±0.42	-0.22±0.15	0.06±0.37
15t-18:1	0.03±0.05	0.83±0.2	0.03±0.07	0.43±0.57	-0.08±0.06	NE	-0.06±0.07	NE	-0.09±0.07	NE
16t-18:1	0.13±0.12	0.31±0.28	-0.2±0.2	-0.14±0.35	-0.14±0.15	0.13±0.38	-0.35±0.18	-0.45±0.32	-0.35±0.17	-0.17±0.35
Polyunsaturated										

	SHEAR_29d		OF_3d		OF_29d		FI_3d		FI_29d	
Fatty Acids	rp	rg	rp	rg	rp	rg	rp	rg	rp	rg
9c,13t/8t,12c-18:2	0.12±0.09	-0.27±0.25	-0.24±0.13	0.32±0.29	-0.1±0.11	-0.02±0.34	-0.22±0.15	0.55±0.29	-0.18±0.15	-0.4±0.26
9c,15c-18:2	0.05±0.1	-0.25±0.25	-0.05±0.17	0.03±0.31	-0.02±0.12	0.03±0.34	0.12±0.19	0.81±0.13	0.16±0.18	0.6±0.21
8t,13c-18:2	0.14±0.08	0.01±0.41	-0.25±0.13	0.6±0.33	-0.14±0.11	0.3±0.49	-0.23±0.14	0.97±0.04	-0.19±0.14	-0.34±0.4
11t,15c-18:2	0.02±0.08	0.11±0.23	-0.04±0.13	-0.32±0.25	-0.11±0.1	-0.9±0.06	0.06±0.15	-0.46±0.26	0.06±0.14	-0.39±0.24
6t,8t-18:2	-0.1±0.07	-0.17±0.72	0.17±0.12	NE	0.11±0.08	NE	0.17±0.12	NE	0.13±0.12	NE
7t,9c-18:2	0.01±0.04	-0.66±0.28	0.05±0.06	0.45±0.44	-0.01±0.05	-0.65±0.34	0.05±0.06	0.25±0.62	0.02±0.06	-0.93±0.09
7t,9t-18:2	0.06±0.11	0.07±0.37	-0.03±0.18	0.52±0.31	-0.03±0.13	0.08±0.46	0.19±0.18	-0.83±0.14	0.21±0.16	-0.57±0.29
8t,10c-18:2	0.17±0.1	-0.06±0.24	-0.25±0.14	0.37±0.23	-0.2±0.11	-0.23±0.29	0±0.19	-0.35±0.28	0.03±0.18	-0.35±0.25
8t,10t-18:2	-0.07±0.07	0.26±0.43	0.18±0.1	0.96±0.05	0.07±0.08	0.47±0.45	0.17±0.1	-0.11±0.62	0.09±0.12	0.22±0.54
9c,11t/9t,11c -18:2	0.3±0.13	0.06±0.3	-0.39±0.17	0.02±0.35	-0.34±0.14	-0.32±0.34	-0.04±0.27	0.05±0.41	0.01±0.26	0±0.35
9t,11t-18:2	0.01±0.06	-0.02±0.31	0.14±0.1	0.43±0.3	0.07±0.07	-0.23±0.37	0.13±0.11	-0.07±0.41	0.09±0.11	-0.23±0.34
10t,12c-18:2	-0.16±0.09	-0.15±0.39	0.24±0.15	0.11±0.46	0.18±0.11	-0.37±0.42	0.35±0.15	0.4±0.52	0.34±0.14	-0.01±0.48
10t,12t-18:2	-0.22±0.12	0.13±0.29	0.36±0.14	0.55±0.25	0.23±0.13	-0.05±0.39	0.18±0.19	0.17±0.4	0.14±0.19	-0.31±0.31
11t,13c/11c,13t -18:2	0.26±0.13	-0.25±0.29	-0.41±0.15	0.07±0.33	-0.27±0.14	-0.85±0.1	-0.03±0.26	-0.79±0.16	0.01±0.25	-0.95±0.03
11t,13t-18:2	0.14±0.06	-0.02±0.24	-0.1±0.12	0.16±0.28	-0.23±0.07	-0.79±0.11	-0.25±0.1	-0.61±0.2	-0.25±0.1	-0.9±0.05
12t,14c/12c,14t -18:2	0.01±0.08	-0.4±0.24	0.03±0.12	0.36±0.28	0.03±0.09	-0.5±0.26	0.16±0.1	0.17±0.38	0.18±0.1	-0.53±0.24
12t,14t-18:2	-0.03±0.14	-0.07±0.36	0.04±0.24	0.43±0.34	0.04±0.18	-0.49±0.36	-0.25±0.22	0.17±0.48	-0.28±0.2	-0.49±0.32
18:3n-3	0.19±0.09	-0.13±0.26	-0.33±0.14	-0.23±0.28	-0.36±0.1	-0.92±0.05	-0.35±0.16	-0.65±0.2	-0.31±0.17	-0.87±0.08
18:3n-6	0.2±0.1	-0.13±0.31	-0.37±0.16	-0.27±0.33	-0.31±0.13	-0.33±0.35	-0.15±0.24	-0.5±0.35	-0.08±0.24	-0.27±0.35
20:2n-6	0.13±0.08	-0.33±0.36	-0.19±0.14	-0.19±0.44	-0.17±0.11	-0.7±0.25	-0.15±0.15	-0.03±0.55	-0.13±0.15	-0.71±0.25
20:3n-6	0.28±0.11	-0.05±0.28	-0.41±0.14	-0.24±0.3	-0.37±0.12	-0.73±0.17	-0.42±0.13	-0.57±0.26	-0.41±0.13	-0.64±0.19
20:3n-9	0.3±0.12	0.33±0.27	-0.44±0.14	-0.73±0.17	-0.38±0.13	-0.27±0.36	-0.45±0.14	-0.91±0.07	-0.43±0.14	-0.12±0.36
20:4n-6	0.24±0.08	0±0.29	-0.35±0.13	-0.35±0.29	-0.32±0.1	-0.49±0.27	-0.35±0.12	-0.52±0.3	-0.34±0.11	-0.49±0.26
20:5n-3	0.21±0.1	0.17±0.53	-0.32±0.15	0.4±0.61	-0.27±0.12	-0.16±0.68	-0.31±0.14	NE	-0.32±0.14	-0.32±0.56
22:4n-6	0.28±0.12	-0.4±0.28	-0.39±0.14	0.26±0.31	-0.34±0.13	-0.06±0.4	-0.17±0.23	0.24±0.39	-0.15±0.23	-0.21±0.35
22:5n-3	0.35±0.33	0.22±0.29	-0.51±0.14	-0.54±0.24	-0.44±0.12	-0.74±0.18	-0.55±0.13	-0.95±0.04	-0.5±0.14	-0.71±0.17
22:6n-3	0.25±0.09	0.39±0.23	-0.37±0.14	-0.39±0.27	-0.32±0.11	-0.62±0.2	-0.43±0.12	-0.74±0.17	-0.37±0.13	-0.7±0.16
Branched										
iso14:0	0.17±0.09	0.34±0.31	-0.2±0.14	-0.28±0.36	-0.21±0.11	0.24±0.41	-0.01±0.18	-0.55±0.31	0.04±0.17	-0.09±0.41

	SHEAR_29d		OF_3d		OF_29d		FI_3d		FI_29d	
Fatty Acids	rp	rg	rp	rg	rp	rg	rp	rg	rp	rg
iso15:0	0.29±0.1	0.3±0.25	-0.29±0.14	-0.29±0.27	-0.27±0.12	-0.25±0.31	-0.06±0.21	-0.81±0.11	-0.02±0.2	-0.33±0.28
ai15:0	0.16±0.1	0.19±0.27	-0.13±0.17	-0.11±0.31	-0.1±0.13	0.03±0.35	0.09±0.19	-0.33±0.32	0.14±0.18	0.02±0.32
iso16:0	0.14±0.1	0.28±0.26	-0.12±0.17	-0.3±0.3	-0.12±0.13	-0.98±0.01	0.11±0.19	-0.84±0.11	0.13±0.17	-0.73±0.16
iso17:0	0.18±0.1	0.37±0.3	-0.2±0.19	-0.25±0.37	-0.21±0.13	-0.36±0.38	0±0.24	-0.37±0.41	0.03±0.23	-0.37±0.36
ai17:0	0.15±0.11	0.11±0.3	-0.16±0.2	-0.05±0.35	-0.14±0.14	0.09±0.39	0.09±0.23	-0.09±0.41	0.12±0.22	0.01±0.36
iso18:0	0.06±0.11	-0.01±0.29	-0.01±0.18	-0.04±0.32	-0.03±0.13	-0.71±0.18	0.19±0.17	-0.13±0.37	0.21±0.16	-0.46±0.26
Group										
sumtrans18:1	-0.02±0.09	0.03±0.26	0.08±0.15	0.12±0.29	0.03±0.11	-0.34±0.29	0.22±0.14	0.12±0.35	0.2±0.14	-0.18±0.3
sumCLA	0.15±0.11	0.01±0.28	-0.17±0.16	0.21±0.31	-0.17±0.13	-0.5±0.26	0.09±0.19	0.13±0.39	0.12±0.18	-0.23±0.31
PUFA	0.22±0.1	-0.11±0.29	-0.27±0.15	-0.42±0.28	-0.29±0.12	-0.81±0.13	-0.26±0.15	-0.33±0.36	-0.25±0.15	-0.56±0.24
n-3	0.31±0.1	0.16±0.29	-0.49±0.14	-0.45±0.27	-0.44±0.1	-0.87±0.09	-0.53±0.14	-0.84±0.12	-0.47±0.16	-0.83±0.1
n-6	0.19±0.09	-0.13±0.28	-0.19±0.16	-0.38±0.28	-0.24±0.12	-0.74±0.16	-0.16±0.17	-0.22±0.37	-0.16±0.16	-0.46±0.27
n-6/n-3	-0.15±0.09	-0.15±0.23	0.29±0.11	0.3±0.25	0.25±0.09	0.31±0.28	0.32±0.14	0.62±0.2	0.25±0.14	0.36±0.23
P/S	0.23±0.1	-0.09±0.29	-0.29±0.16	-0.34±0.29	-0.29±0.12	-0.54±0.26	-0.12±0.21	0.01±0.39	-0.09±0.21	-0.2±0.33
P/(S+B)	0.23±0.1	-0.1±0.29	-0.29±0.16	-0.34±0.3	-0.29±0.13	-0.55±0.26	-0.15±0.21	0.01±0.39	-0.12±0.21	-0.2±0.33
Health Index	-0.06±0.07	0.09±0.18	0.16±0.11	0.1±0.22	0.14±0.09	0.32±0.21	-0.24±0.13	0.57±0.2	-0.23±0.13	0.37±0.21

Fatty Acids	SJ_3d		SJ_29d		OT_3d		OT_29d	
Saturated	rp	rg	Rp	rg	rp	rg	rp	rg
10:0	-0.37±0.16	-0.47±0.42	-0.21±0.11	-0.19±0.27	-0.18±0.13	-0.1±0.19	-0.15±0.11	-0.21±0.21
12:0	-0.14±0.12	-0.46±0.32	-0.02±0.06	-0.21±0.2	-0.03±0.07	-0.05±0.14	-0.07±0.07	-0.21±0.16
13:0	-0.02±0.27	0.17±0.56	0±0.12	0.07±0.35	0.18±0.13	-0.06±0.24	-0.06±0.13	-0.09±0.27
19:0	0.32±0.19	0.15±0.64	0.07±0.12	0.51±0.27	0.08±0.13	-0.12±0.25	0.15±0.1	-0.07±0.3
20:0	-0.11±0.23	0.25±0.51	-0.06±0.1	-0.19±0.32	-0.22±0.11	-0.33±0.2	-0.13±0.12	-0.33±0.23
22:0	-0.49±0.16	-0.82±0.26	-0.22±0.1	-0.65±0.26	-0.18±0.13	0.06±0.28	-0.23±0.09	-0.3±0.3
24:0	-0.13±0.26	-0.95±0.05	-0.34±0.11	-0.85±0.08	-0.34±0.13	-0.43±0.18	0.07±0.12	-0.66±0.14
Monounsaturated								
9c-15:1	-0.18±0.22	-0.37±0.76	-0.05±0.1	0.71±0.3	0.02±0.13	0.21±0.34	-0.13±0.11	0.37±0.37
7c-16:1	-0.19±0.2	0.33±0.55	-0.07±0.1	-0.34±0.29	-0.07±0.13	-0.24±0.22	-0.22±0.09	-0.12±0.27
11t-16:1	0.26±0.21	0.43±0.69	0.02±0.11	0.46±0.39	0.07±0.14	-0.03±0.32	0.23±0.11	0.16±0.36
12c-16:1	-0.05±0.07	0.19±0.42	-0.01±0.04	0.08±0.24	0.01±0.04	0.33±0.14	0.01±0.05	0.22±0.18
7c-17:1	-0.19±0.2	-0.25±0.8	-0.15±0.09	-0.54±0.5	-0.22±0.1	-0.51±0.35	0±0.11	-0.35±0.51
11c-18:1	0.03±0.25	0.66±0.37	0±0.11	0.29±0.32	0.11±0.12	0.27±0.21	-0.05±0.12	0.18±0.26
12c-18:1	0.39±0.19	0.52±0.45	0.19±0.11	0.61±0.23	0.05±0.15	0.38±0.2	0.15±0.11	0.47±0.22
13c-18:1	0.01±0.06	0.38±0.4	0.02±0.04	0.15±0.23	0.03±0.04	0.37±0.13	0.08±0.04	0.34±0.16
14c-18:1	-0.02±0.11	NE	0.07±0.05	1±0	-0.02±0.06	0.03±0.22	-0.08±0.07	0.32±0.24
15c-18:1	0.02±0.18	0.2±0.53	0.1±0.08	0.48±0.23	0.19±0.11	0.3±0.19	-0.1±0.09	0.33±0.22
9c-20:1	-0.38±0.18	0.97±0.03	-0.09±0.11	-0.1±0.36	-0.25±0.12	0.01±0.25	-0.19±0.11	-0.03±0.29
11c-20:1	-0.17±0.13	0.93±0.04	-0.04±0.06	0.18±0.24	-0.11±0.06	0.24±0.14	-0.07±0.06	0.07±0.19
6t/8t-18:1	0.35±0.15	-0.05±0.63	0.15±0.08	0.35±0.31	0.13±0.1	0.12±0.24	0.08±0.09	0.21±0.27
9t-18:1	0.34±0.15	0.15±0.62	0.15±0.07	0.41±0.29	0.13±0.1	0.24±0.23	0.1±0.09	0.32±0.26
10t-18:1	0.02±0.2	0.38±0.44	0.04±0.09	0.35±0.27	0.14±0.1	0.23±0.2	-0.02±0.1	0.32±0.23
11t-18:1	-0.34±0.2	0.15±0.61	-0.12±0.11	-0.18±0.32	-0.28±0.11	-0.28±0.2	-0.16±0.12	-0.11±0.26

	SJ_3d		SJ_29d		OT_3d		OT_29d	
Fatty Acids	rp	rg	Rp	rg	rp	rg	rp	rg
12t-18:1	-0.04±0.19	NE	-0.03±0.08	0.71±0.21	-0.14±0.08	0.19±0.24	-0.03±0.09	0.36±0.26
15t-18:1	-0.1±0.09	NE	-0.02±0.05	0.68±0.3	0.02±0.05	0.19±0.4	0.03±0.05	-0.02±0.53
16t-18:1	-0.03±0.26	0.61±0.47	-0.05±0.11	0.17±0.37	-0.22±0.11	-0.39±0.2	-0.02±0.13	-0.23±0.27
Polyunsaturated								
9c,13t/8t,12c-18:2	-0.13±0.18	0.99±0.01	-0.01±0.09	0.25±0.31	-0.14±0.1	0.36±0.19	-0.02±0.09	0.36±0.23
9c,15c-18:2	-0.2±0.18	0.77±0.21	-0.03±0.08	0.35±0.27	0.01±0.11	0.33±0.19	-0.05±0.1	0.31±0.23
8t,13c-18:2	-0.2±0.16	0.47±0.46	-0.03±0.08	0.88±0.13	-0.16±0.09	0.23±0.32	-0.06±0.08	0.63±0.24
11t,15c-18:2	-0.11±0.15	-0.13±0.51	-0.05±0.07	-0.45±0.24	0.05±0.08	-0.05±0.19	-0.04±0.08	-0.01±0.22
6t,8t-18:2	0.27±0.13	NE	0.09±0.09	0.17±0.94	0.09±0.08	0±0.6	0.08±0.07	0.44±0.52
7t,9c-18:2	0.13±0.07	0.55±0.43	0.03±0.04	0.6±0.36	0.04±0.04	0.64±0.22	0.04±0.05	0.53±0.32
7t,9t-18:2	-0.15±0.2	0.32±0.69	0.04±0.1	-0.3±0.38	-0.01±0.12	-0.15±0.28	-0.22±0.1	0.32±0.31
8t,10c-18:2	-0.29±0.15	-0.19±0.47	-0.06±0.09	-0.57±0.18	-0.13±0.1	0.29±0.17	-0.16±0.12	0.26±0.23
8t,10t-18:2	0.25±0.11	0.94±0.11	0.09±0.07	0.2±0.56	0.07±0.07	-0.32±0.32	0.08±0.07	-0.17±0.41
9c,11t/9t,11c -18:2	-0.5±0.16	0.66±0.39	-0.18±0.12	-0.34±0.3	-0.2±0.14	0±0.24	-0.2±0.11	0.14±0.27
9t,11t-18:2	0.19±0.11	-0.15±0.63	0.08±0.05	0±0.37	0.02±0.07	0.17±0.24	0.06±0.07	0.41±0.24
10t,12c-18:2	0.22±0.2	0.25±0.76	0.15±0.09	0.13±0.47	0.21±0.09	0.28±0.29	-0.01±0.11	0.33±0.34
10t,12t-18:2	0.42±0.15	0.42±0.45	0.15±0.1	0.11±0.35	0.21±0.11	0.15±0.23	0.19±0.11	0.11±0.28
11t,13c/11c,13t -18:2	-0.45±0.16	0.01±0.62	-0.13±0.12	-1±0	-0.25±0.12	-0.03±0.24	-0.24±0.13	-0.07±0.3
11t,13t-18:2	-0.02±0.15	-0.2±0.53	-0.12±0.06	-0.52±0.2	-0.15±0.07	-0.11±0.19	0.02±0.09	-0.05±0.23
12t,14c/12c,14t -18:2	-0.05±0.14	0.3±0.5	0.04±0.06	0.26±0.31	0.02±0.08	0.17±0.21	-0.15±0.09	0.24±0.25
12t,14t-18:2	0.2±0.26	0.47±0.54	0.04±0.13	0.03±0.43	-0.04±0.16	-0.14±0.28	0.17±0.11	-0.1±0.33
18:3n-3	-0.44±0.14	-0.3±0.51	-0.3±0.08	-0.94±0.04	-0.2±0.1	-0.14±0.2	-0.14±0.09	-0.22±0.23
18:3n-6	-0.43±0.16	-0.32±0.58	-0.27±0.11	-0.55±0.28	-0.18±0.13	-0.15±0.25	-0.2±0.1	-0.17±0.29
20:2n-6	-0.26±0.15	-0.08±0.82	-0.13±0.07	-0.26±0.46	-0.05±0.09	0.48±0.23	-0.06±0.08	0.29±0.33
20:3n-6	-0.48±0.15	-0.55±0.4	-0.25±0.1	-0.74±0.14	-0.3±0.11	-0.33±0.19	-0.14±0.11	-0.3±0.22
20:3n-9	-0.49±0.15	-0.83±0.22	-0.27±0.1	-0.85±0.09	-0.34±0.12	-0.63±0.15	-0.27±0.1	-0.88±0.07
20:4n-6	-0.41±0.14	NE	-0.26±0.09	-0.88±0.08	-0.24±0.1	-0.38±0.19	-0.2±0.1	-0.39±0.23

	SJ_3d		SJ_29d		OT_3d		OT_29d	
Fatty Acids	rp	rg	Rp	rg	rp	rg	rp	rg
20:5n-3	-0.32±0.17	0.55±0.44	-0.12±0.09	0.54±0.54	-0.2±0.1	-0.53±0.31	-0.02±0.1	-0.02±0.5
22:4n-6	-0.46±0.16	0.45±0.5	-0.19±0.1	-0.28±0.35	-0.25±0.11	-0.08±0.24	-0.2±0.1	0.06±0.28
22:5n-3	-0.6±0.14	-0.65±0.37	-0.34±0.1	-0.96±0.03	-0.38±0.11	-0.48±0.18	-0.26±0.09	-0.73±0.13
22:6n-3	-0.5±0.14	-0.81±0.23	-0.31±0.1	-0.98±0.01	-0.25±0.1	-0.58±0.14	-0.14±0.1	-0.78±0.1
Branched								
iso14:0	-0.27±0.15	0.05±0.69	-0.16±0.08	-0.29±0.38	-0.11±0.1	-0.21±0.26	-0.19±0.09	-0.24±0.3
iso15:0	-0.28±0.17	0.66±0.29	-0.1±0.1	-0.46±0.23	-0.26±0.1	-0.41±0.17	-0.32±0.1	-0.33±0.23
ai15:0	-0.13±0.19	0.55±0.37	-0.02±0.09	0.13±0.32	-0.04±0.12	-0.1±0.21	-0.2±0.08	-0.01±0.26
iso16:0	-0.15±0.19	0.09±0.58	-0.04±0.1	-0.44±0.28	-0.04±0.12	-0.19±0.22	-0.2±0.08	-0.19±0.26
iso17:0	-0.27±0.19	0.43±0.57	-0.16±0.08	-0.43±0.33	-0.08±0.13	-0.2±0.27	-0.16±0.09	0.18±0.31
ai17:0	-0.21±0.21	0.83±0.16	-0.08±0.1	0.14±0.36	-0.02±0.13	0.05±0.24	-0.13±0.09	0.28±0.26
iso18:0	-0.05±0.2	0.52±0.39	0.02±0.09	-0.05±0.33	0.06±0.11	0.21±0.21	-0.1±0.1	0.28±0.24
Group								
sumtrans18:1	0.04±0.17	0.39±0.43	0.05±0.08	0.4±0.24	0.13±0.09	0.2±0.19	-0.03±0.09	0.3±0.22
sumCLA	-0.24±0.18	0.66±0.34	-0.03±0.1	-0.27±0.3	-0.09±0.11	0.15±0.22	-0.19±0.13	0.28±0.26
PUFA	-0.36±0.15	NE	-0.24±0.09	-0.82±0.12	-0.15±0.11	-0.08±0.23	-0.21±0.1	-0.17±0.26
n-3	-0.58±0.13	-0.68±0.36	-0.38±0.09	-0.98±0.01	-0.34±0.11	-0.43±0.19	-0.23±0.08	-0.64±0.16
n-6	-0.3±0.16	NE	-0.21±0.08	-0.6±0.22	-0.09±0.1	-0.02±0.22	-0.19±0.1	-0.08±0.26
n-6/n-3	0.4±0.12	0.41±0.41	0.19±0.07	0.79±0.1	0.2±0.08	0.41±0.15	0.1±0.06	0.6±0.13
P/S	-0.38±0.16	-0.24±0.57	-0.24±0.09	-0.56±0.24	-0.14±0.12	0.04±0.23	-0.21±0.1	-0.02±0.27
P/(S+B)	-0.39±0.16	-0.26±0.56	-0.24±0.09	-0.56±0.24	-0.15±0.12	0.04±0.23	-0.21±0.1	-0.03±0.27
Health Index	0.21±0.09	NE	0.06±0.07	0.25±0.21	0.01±0.09	0.14±0.15	0.11±0.07	0.22±0.17

c=cis, t=trans. NE: not estimated.

SumCLA: sum of conjugated linoleic acids, SFA (sum of saturated fatty acids), PUFA (sum of polyunsaturated fatty acids), BCFA (Branched chain fatty acid)

[a]SumCLA = 8t,10c-18:2 + 9c,11t-18:2 + 7t,9c-18:2 + 9t,11c-18:2 + 10t,12c-18:2 + 11c,13t-18:2 + 11t,13c-18:2 +12t,14c-18:2+12c,14t-18:2+ 9c,11c-18:2 + 10c,12c-18:2 +6t,8t-18:2+ 9t,11t-18:2 + 11t,13t-18:2 + 12t,14t-18:2 + 10t,12t-18:2 + 8t,10t-18:2 + 7t,9t-18:2 ; [b]PUFA= 18:2n-6 + 18:3n-6 + 18:3n-3 + 20:2n-6 + 20:3n-9 + 20:3n-6 + 20:4n-6 + 22:4n-6 + 22:5n-3 ; SFA = 10:0 + 12:0 + 13:0 + 14:0 + 15:0 + 16:0 + 17:0 + 18:0 + 20:0 + 23:0 ; MUFA = 9c-14:1 + 9c-15:1 + 7c-16:1 + 9c-16:1 + 9c-17:1 + 6t/7t/8t-18:1 + 9t-18:1 + 10t-18:1 +11t-18:1 + 12t-18:1 + 13t/14t-18:1 + 15t-18:1 + 16t-18:1 + 9c-18:1 + 11c-18:1 + 12c-18:1 + 13c-18:1 + 14c-18:1 + 16c-18:1 + 9c-20:1 + 11c-20:1; BCFA iso-14:0 + iso-15:0 + anteiso-15:0 + iso-16:0 + iso-17:0 + anteiso-17:0 + iso-18:0 ;[c]n-3 PUFA = 18:3n-3 + 22:5n-3; [d]n-6 PUFA = 18:2n-6 + 18:3n-6 + 20:2n-6 + 20:3n-6 +20:4n-6 + 22:4n-6 ; [e]n-6/n-3: ratio between n-6 and n-3 PUFA ; [f]P/S: ratio of PUFA to SFA ; [g]P/(S+B): ratio of PUFA to SFA+BF

Appendix S6-1

Gene SNP significant at snp-wise alpha 0.05

Fatty acid	SNP	Gene	Chr	Alleles*	Functional Class	Codon	SNP_EFFECT	Phenotypic Variance	Total variance
c14_0	rs42196904	IGF2	29	A/G	intron_variant	-	0.168029	2.620030%	
c14_0	rs41256865	GPD1	5	A/G	3_prime_UTR_variant	-	0.075153	0.643390%	
c14_0	rs42714483	THRSP	29	T/C	missense_variant	Atc/Gtc	-0.07122	0.594420%	
c14_0	rs42147575	ANXA11	28	A/G	splice_region_variant,intron_variant	-	-0.06601	0.507268%	
c14_0	rs43315761	TNS	2	C/G	missense_variant	gGt/gCt	-0.04486	0.075374%	
c14_0	rs42660323	PFN2	1	C/G	intergenic_variant	-	0.04381	0.213435%	
c14_0	rs41919993	FASN	19	T/C	missense_variant	Tac/Cac	0.037335	0.160971%	
c14_0	rs41613043	BDH1	1	T/C	intron_variant	-	-0.03006	0.085517%	
c14_0	rs43440606	PTPRR	5	T/G	intron_variant	-	-0.02769	0.047752%	
c14_0	rs41255692	SCD1	26	T/C	synonymous_variant	-	0.020434	0.045463%	
c14_0	rs380876224	SOAT1	16	T/C	upstream_gene_variant	-	-0.01973	0.032122%	
c14_0	rs43724308	MGLL	22	T/C	synonymous_variant	agT/agC	0.017643	0.022311%	
c14_0	rs41652470	SIRT1	28	A/G	upstream_gene_variant	-	0.01701	0.033290%	
c14_0	rs43675525	FSHR	11	A/G	intron_variant	-	-0.01564	0.020861%	
c14_0	#N/A	COX5B	11	COX5B	-	-	-0.01378	0.021345%	
c14_0	rs41255193	HADHB	11	T/C	3_prime_UTR_variant	-	-0.01371	0.021599%	
c14_0	rs109450360	IGF2R	9	A/G	intron_variant	-	-0.01348	0.021362%	
c14_0	rs41255257	AP2B1	19	T/C	intergenic_variant	-	-0.01341	0.009891%	
c14_0	rs41793400	GNA14	8	T/C	5_prime_UTR_variant	-	-0.01272	0.008291%	
c14_0	rs41844655	PRKAB1	17	A/G	downstream_gene_variant	-	-0.01249	0.009821%	
c14_0	rs41884788	CEBPG	18	A/G	downstream_gene_variant	-	-0.01227	0.009903%	

c14_0	rs42605121	SLC8A1	11	A/G	intron_variant	-	0.012226	0.006712%	
c14_0	rs43663565	DGUCK	11	T/C	intron_variant	-	-0.01146	0.012777%	
c14_0	rs41654804	COPZ1	5	C/G	downstream_gene_variant	-	-0.01137	0.004066%	
c14_0	rs41844490	ACACB	17	T/C	synonymous_variant	ttC/ttT	-0.01106	0.005591%	
c14_0	rs41783612	ACAD8	15	A/C	intron_variant	-	-0.01065	0.006324%	
c14_0	rs110241790	ACACA	19	A/G	intron_variant	-	-0.01034	0.002691%	
c14_0	rs42176298	SLC37A2	29	T/C	intron_variant	-	0.010216	0.005664%	
c14_0	rs41845683	OASL	17	A/G	3_prime_UTR_variant	-	0.009694	0.009181%	
c14_0	rs41567325	TG	14	T/C	-	-	-0.00947	0.003285%	
c14_0	rs41900392	PRKCG	18	T/C	downstream_gene_variant	-	0.00935	0.010272%	
c14_0	rs42114536	FAT	27	T/G	missense_variant	gaG/gaT	0.009326	0.002030%	
c14_0	rs43289839	PRKRA	2	T/G	intron_variant	-	0.008865	0.002120%	
c14_0	rs41781896	LRP4	15	T/C	missense_variant	aTg/aCg	0.008497	0.002141%	
c14_0	rs17871427	PPM1B	11	A/G	downstream_gene_variant	-	0.008344	0.001690%	
c14_0	rs43649423	TSHR	10	A/G	synonymous_variant	ttG/ttA	0.007947	0.002472%	
c14_0	rs29004508	LEP	4	T/C	missense_variant	gCg/gTg	0.00781	0.003624%	
c14_0	rs42183386	DCPS	29	T/C	3_prime_UTR_variant	-	-0.00776	0.005931%	
c14_0	rs41745642	PAFAH1B2	15	C/G	intron_variant	-	-0.00738	0.001419%	
c14_0	#N/A	ACSF3	18	C/T	-	-	-0.00711	0.003962%	
c14_0	rs41755155	APOC3	15	T/C	3_prime_UTR_variant	-	0.006953	0.002364%	
c14_0	#N/A	OLR1	5	A/G	-	-	-0.00691	0.002028%	
c14_0	rs43267303	AGTR1	1	T/C	intergenic_variant	-	-0.00678	0.002829%	
c14_0	rs29024247	SLCO2B1	15	A/G	synonymous_variant	gtA/gtG	-0.00649	0.003523%	
c14_0	rs42973901	LARG	15	T/C	downstream_gene_variant	-	0.006434	0.004780%	
c14_0	rs42413973	EIF3S6	14	T/G	intron_variant	-	0.006397	0.002959%	

c14_0	rs42522205	IL4	7	T/C	intron_variant	-	0.0063	0.004646%	
c14_0	rs42742546	SERPINI2	1	A/G	downstream_gene_variant	-	-0.00627	0.002766%	
c14_0	rs41642657	ACADVL	19	T/C	downstream_gene_variant	-	0.006178	0.003274%	
c14_0	rs42090456	TNFRSF6	26	C/G	intron_variant	-	0.00616	0.003722%	
c14_0	rs41780423	PAMR1	15	T/C	missense_variant	aCg/aTg	-0.00611	0.004037%	
c14_0	rs384562096	ACADM	3	A/G	3_prime_UTR_variant	-	-0.00597	0.003927%	
c14_0	rs43710977	GSTM1	3	A/C	intron_variant	-	-0.00585	0.003861%	
c14_0	rs42269059	NFE2L2	2	A/G	intron_variant	-	0.005826	0.001469%	
c14_0	rs43369271	SIAT6	3	A/G	synonymous_variant	gcT/gcC	0.005694	0.001243%	
c14_0	rs43494871	PROM1	6	T/G	missense_variant	ttA/ttC	-0.00563	0.001528%	
c14_0	rs43709215	TXNIP	3	C/G	intron_variant	-	-0.00554	0.002200%	
c14_0	rs43251315	CDH9	20	A/G	missense_variant	Cca/Tca	-0.00543	0.001669%	
c14_0	rs41850479	ADRBK2	17	T/C	intergenic_variant	-	0.005421	0.002106%	
c14_0	rs29003543	TIMP3	5	A/G	intron_variant	-	-0.00537	0.002970%	
c14_0	rs41707705	HNF4A	13	A/G	downstream_gene_variant	-	-0.00533	0.001276%	
c14_0	rs29016220	JAM1	3	A/C	intron_variant	-	-0.00518	0.001673%	
c14_0	rs41667443	CAPNS1	18	T/C	downstream_gene_variant	-	-0.00502	0.000703%	
c14_0	rs29026551	FAM13A1	6	T/C	missense_variant	aGa/aAa	-0.00495	0.002522%	
c14_0	rs43707854	SOCS2	5	A/C	3_prime_UTR_variant	-	-0.00494	0.002609%	
c14_0	rs43560146	LPL	8	T/C	upstream_gene_variant	-	-0.00485	0.001396%	
c14_0	rs43390314	PRKAR2B	4	T/C	intron_variant	-	-0.00483	0.001210%	
c14_0	rs41634890	TXNRD2	17	A/G	intron_variant	-	-0.00479	0.002691%	
c14_0	rs211686954	HADHSC	26	A/G	synonymous_variant	-	-0.00468	0.002256%	
c14_0	rs41926529	GRB2	19	A/C	upstream_gene_variant	-	-0.00466	0.002512%	
c14_0	rs41568467	ACADSB	26	T/C	intron_variant	-	0.004504	0.001206%	

c14_0	rs42102756	TCF7L2	26	T/C	intron_variant	-	0.004464	0.000797%	
c14_0	rs42156960	EED	29	A/G	intron_variant	-	-0.00442	0.001076%	
c14_0	rs43706516	AGXT2L1	6	T/G	3_prime_UTR_variant	-	-0.00437	0.000515%	
c14_0	rs17870352	THBS	4	A/G	missense_variant	Att/Gtt	-0.00435	0.002117%	
c14_0	rs42022871	KISS1	16	T/C	downstream_gene_variant	-	-0.00435	0.002016%	
c14_0	rs208474334	TIEG2	11	T/C	missense_variant	-	-0.00433	0.000573%	
c14_0	rs41648757	FDPS	3	T/G	intron_variant	-	0.004123	0.000750%	
c14_0	rs43476247	CXCL10	6	T/G	downstream_gene_variant	-	-0.00411	0.000689%	
c14_0	rs42176279	SLC37A2	29	C/G	intron_variant	-	-0.0041	0.001194%	
c14_0	rs41655877	NPFF	5	A/C	intron_variant	-	-0.00404	0.001429%	
c14_0	rs42411170	FGFR3	6	T/C	intron_variant	-	-0.00404	0.001335%	
c14_0	rs42905009	MOGAT2	15	T/G	intron_variant	-	0.00393	0.001812%	
c14_0	rs41662474	SLC2A12	9	T/C	intron_variant	-	0.003908	0.001791%	
c14_0	rs43306652	GRB14	2	C/G	intron_variant	-	0.003903	0.000684%	
c14_0	rs43359070	EPS15	3	A/G	intron_variant	-	-0.00374	0.000768%	
c14_0	rs42099883	SIAT1	1	T/C	intron_variant	-	0.003715	0.001622%	
c14_0	rs43464396	PPARGC1A	6	A/G	intron_variant	-	-0.00361	0.001532%	
c14_0	rs41600452	FGF12	1	T/G	intron_variant	-	0.003611	0.000616%	
c14_0	rs43493654	PROM1	6	C/G	missense_variant	Ccc/Gcc	-0.00361	0.000783%	
c14_0	rs208631210	APM 1	1	C/G	upstream_gene_variant	-	-0.0036	0.001481%	
c14_0	rs29026524	RPGRIP1	10	C/G	downstream_gene_variant	-	0.003585	0.001459%	
c14_0	rs41810735	PRKCZ	16	A/T	intron_variant	-	-0.00353	0.001410%	
c14_0	rs41915705	STAT3	19	T/C	intron_variant	-	-0.00353	0.001464%	
c14_0	rs110953296	FABP5	14	T/C	downstream_gene_variant	-	-0.00353	0.001048%	
c14_0	rs43379951	IGF2BP3	4	A/C	intron_variant	-	-0.00353	0.001281%	

c14_0	rs43720497	RARA	19	A/G	intron_variant	-	-0.0035	0.000656%	
c14_0	rs43624642	RYR3	10	T/C	missense_variant	Gca/Aca	0.003481	0.001379%	
c14_0	rs42185608	OPCML	29	A/G	intron_variant	-	0.003423	0.001029%	
c14_0	rs43644340	PSEN1	10	T/G	splice_region_variant,intron_variant	-	-0.00333	0.001246%	
c14_0	rs41883755	FFAR3	18	T/C	downstream_gene_variant	-	0.003304	0.000524%	
c14_0	rs109368962	SAA3	29	A/G	intron_variant	-	0.003152	0.000364%	
c14_0	rs41668638	SLC1A4	11	T/G	intron_variant	-	-0.00314	0.000670%	
c14_0	rs17871740	PCK1	13	A/G	intron_variant	-	0.003138	0.000855%	
c14_0	rs43702510	DDEF1	14	T/C	synonymous_variant	ggT/ggC	-0.00307	0.000489%	
c14_0	rs41657132	AGPAT4	9	T/C	intron_variant	-	-0.00305	0.000447%	
c14_0	rs41654029	ATP2B1	5	T/G	intron_variant	-	-0.003	0.000313%	
c14_0	rs41746520	NCAM1	15	A/G	-	-	-0.00294	0.000501%	
c14_0	rs43717462	ACSS2	13	A/G	synonymous_variant	atT/atC	0.002894	0.000195%	
c14_0	rs41730630	BIG1	14	A/G	intron_variant	-	-0.00288	0.000320%	
c14_0	rs42844528	CAPN10	3	C/G	downstream_gene_variant	-	0.002874	0.000397%	
c14_0	rs41647951	FNTA	27	T/G	intron_variant	-	0.002741	0.000571%	
c14_0	rs135700617	ANXA9	3	A/G	missense_variant	-	0.002693	0.000787%	
c14_0	rs41649195	F11	27	A/T	intron_variant	-	0.002681	0.000604%	
c14_0	rs41974999	MFGE8	21	T/C	intron_variant	-	-0.00247	0.000237%	
c14_0	rs43407600	GHRHR	4	T/C	intron_variant	-	-0.00245	0.000183%	
c14_0	rs43727187	FGFR1	27	T/C	intron_variant	-	-0.00241	0.000324%	
c14_0	rs110625700	PPARA	5	C/G	synonymous_variant	-	-0.0024	0.000221%	
c14_0	rs43317366	CRYBA2	3	A/C	intron_variant	-	0.00236	0.000282%	
c14_0	rs29011369	COL4A3	2	A/C	intron_variant	-	-0.00236	0.000134%	
c14_0	rs41904271	ACADVL	19	T/C	-	-	0.002347	0.000174%	

c14_0	rs109224524	GHRH	13	A/G	intron_variant	-	-0.00231	0.000282%	
c14_0	rs42044790	MYOM1	24	A/G	missense_variant,splice_region_variant	Aga/Gga	0.002286	0.000275%	
c14_0	rs41639432	AOX1	2	C/G	intron_variant	-	0.002163	0.000172%	
c14_0	rs42197376	PC	29	A/G	3_prime_UTR_variant	-	0.002152	0.000153%	
c14_0	rs41926990	PRKCA	19	C/G	intron_variant	-	-0.00209	0.000113%	5.418%
c15_0	rs41793400	GNA14	8	T/C	5_prime_UTR_variant	-	-0.00203	0.007603%	
c15_0	rs41919993	FASN	19	T/C	missense_variant	Tac/Cac	0.001924	0.015375%	
c15_0	rs41667445	CAPNS1	18	A/G	downstream_gene_variant	-	0.001649	0.002450%	
c15_0	rs41652470	SIRT1	28	A/G	upstream_gene_variant	-	0.001309	0.007096%	
c15_0	rs41256865	GPD1	5	A/G	3_prime_UTR_variant	-	0.001304	0.006927%	
c15_0	rs41800886	PLOD	16	T/C	downstream_gene_variant	-	0.001269	0.005237%	
c15_0	rs41729168	FABP9	14	A/G	intron_variant	-	-0.00117	0.002027%	
c15_0	rs43359039	EPS15	3	C/G	intron_variant	-	0.001161	0.002703%	
c15_0	rs41257187	ITGB5	5	T/C	synonymous_variant	ccC/ccT	0.001102	0.004900%	
c15_0	rs43406303	ADCY1	4	A/G	intron_variant	-	0.001083	0.004900%	
c15_0	rs134451630	SIAT4A	14	A/G	intron_variant	-	0.001025	0.004393%	
c15_0	rs42185608	OPCML	29	A/G	intron_variant	-	0.000988	0.003041%	
c15_0	rs109697714	GHRH	13	T/C	intron_variant	-	-0.00096	0.003886%	
c15_0	rs43289839	PRKRA	2	T/G	intron_variant	-	0.000919	0.000845%	
c15_0	rs42149515	NEUROG3	28	A/T	downstream_gene_variant	-	-0.00088	0.001014%	
c15_0	rs42421976	PPP1R3A	4	A/C	downstream_gene_variant	-	0.000858	0.001098%	
c15_0	rs43576438	IL7	14	T/C	intron_variant	-	0.000838	0.002450%	
c15_0	rs43717437	GSS	13	A/G	missense_variant	aaC/aaA	-0.00082	0.002619%	
c15_0	rs41916426	CRHR1	19	C/G	intron_variant	-	0.000817	0.002534%	

c15_0	rs41915673	STAT5B	19	T/C	intron_variant	-	0.000816	0.002788%	
c15_0	rs43727187	FGFR1	27	T/C	intron_variant	-	-0.00081	0.001352%	
c15_0	rs42714483	THRSP	29	T/C	missense_variant	Atc/Gtc	-0.00081	0.002703%	
c15_0	rs29024247	SLCO2B1	15	A/G	synonymous_variant	gtA/gtG	-0.0008	0.001943%	
c15_0	rs42269059	NFE2L2	2	A/G	intron_variant	-	0.000776	0.000929%	
c15_0	rs41897480	EMP3	18	C/G	intron_variant	-	-0.00077	0.002281%	
c15_0	rs43490031	HTT	6	C/G	upstream_gene_variant	-	0.000748	0.001521%	
c15_0	rs41900392	PRKCG	18	T/C	downstream_gene_variant	-	0.000748	0.002365%	
c15_0	rs41632689	ACAD8	15	T/C	missense_variant	Gtc/Atc	-0.00075	0.002365%	
c15_0	rs41600007	TGFA	11	T/C	intergenic_variant	-	0.000746	0.001605%	
c15_0	rs17870222	IGFBP3	4	T/C	intron_variant	-	0.000742	0.000929%	
c15_0	rs110055647	AFABP	15	A/G	upstream_gene_variant	-	0.000732	0.001858%	
c15_0	rs41847805	MVK	17	T/C	synonymous_variant	gcC/gcT	0.000692	0.000422%	
c15_0	rs41974998	MFGE8	21	T/G	3_prime_UTR_variant	-	-0.00069	0.001943%	
c15_0	rs41597184	LIAS	6	T/C	intron_variant	-	-0.00066	0.001352%	
c15_0	rs42020773	MGLL	22	T/G	intergenic_variant	-	0.000655	0.001183%	
c15_0	rs41781896	LRP4	15	T/C	missense_variant	aTg/aCg	0.000654	0.000422%	
c15_0	rs109285736	MYF6	5	C/G	3_prime_UTR_variant	-	-0.00063	0.001183%	
c15_0	rs41897291	CNOT3	18	A/G	upstream_gene_variant	-	0.000587	0.001352%	
c15_0	rs42661650	PPARG	22	T/C	intron_variant	-	-0.00058	0.001267%	
c15_0	rs42973901	LARG	15	T/C	downstream_gene_variant	-	0.000563	0.001352%	0.114%
c16_0	rs42660323	PFN2	1	C/G	intergenic_variant	-	0.013604	0.002545%	
c16_0	rs42196904	IGF2	29	A/G	intron_variant	-	0.011519	0.001523%	
c16_0	rs43663565	DGUCK	11	T/C	intron_variant	-	-0.00768	0.000709%	

c16_0	rs41844655	PRKAB1	17	A/G	downstream_gene_variant	-	-0.00637	0.000316%	
c16_0	rs41780423	PAMR1	15	T/C	missense_variant	aCg/aTg	-0.00627	0.000526%	
c16_0	rs42714483	THRSP	29	T/C	missense_variant	Atc/Gtc	-0.00588	0.000500%	
c16_0	rs42113899	FAT	27	T/C	missense_variant	Gtg/Atg	0.004683	0.000245%	
c16_0	rs43493654	PROM1	6	C/G	missense_variant	Ccc/Gcc	-0.00321	0.000076%	
c16_0	rs29024247	SLCO2B1	15	A/G	synonymous_variant	gtA/gtG	-0.00314	0.000102%	
c16_0	rs42413973	EIF3S6	14	T/G	intron_variant	-	0.003086	0.000085%	
c16_0	rs41256865	GPD1	5	A/G	3_prime_UTR_variant	-	0.00295	0.000123%	
c16_0	rs42243023	NEUROD1	2	T/G	upstream_gene_variant	-	-0.00291	0.000121%	
c16_0	rs41652470	SIRT1	28	A/G	upstream_gene_variant	-	0.002598	0.000096%	
c16_0	rs43707854	SOCS2	5	A/C	3_prime_UTR_variant	-	-0.00257	0.000087%	
c16_0	rs43315761	TNS	2	C/G	missense_variant	gGt/gCt	-0.00248	0.000028%	
c16_0	rs41708480	HNF4A	13	A/C	intron_variant	-	0.002453	0.000085%	
c16_0	rs41987132	COL4A4	2	T/C	intron_variant	-	-0.00244	0.000086%	
c16_0	rs42115578	FACL2	27	C/G	synonymous_variant	-	-0.0024	0.000077%	
c16_0	#N/A	COX5B	11	T/G	-	-	-0.00234	0.000076%	
c16_0	rs41613049	BDH1	1	T/C	intron_variant	-	0.002299	0.000062%	
c16_0	rs42411170	FGFR3	6	T/C	intron_variant	-	-0.00229	0.000053%	
c16_0	rs29017040	ACP6	3	T/C	intron_variant	-	0.002272	0.000072%	
c16_0	rs41800886	PLOD	16	T/C	downstream_gene_variant	-	0.002265	0.000057%	
c16_0	rs43494871	PROM1	6	T/G	missense_variant	ttA/ttC	-0.00224	0.000030%	
c16_0	rs41863915	CDH1	18	A/G	intergenic_variant	-	-0.00213	0.000065%	
c16_0	#N/A	ACSF3	18	C/T	-	-	-0.0021	0.000043%	
c16_0	rs43703893	ACAT2	9	A/G	downstream_gene_variant	-	0.002023	0.000049%	
c16_0	rs42742546	SERPINI2	1	A/G	downstream_gene_variant	-	-0.00193	0.000033%	

c16_0	rs110953296	FABP5	14	T/C	downstream_gene_variant	-	-0.00187	0.000036%	
c16_0	rs110730017	IGF2R	9	T/C	missense_variant	-	0.001858	0.000034%	
c16_0	rs41755155	APOC3	15	T/C	3_prime_UTR_variant	-	0.001854	0.000021%	
c16_0	rs43724308	MGLL	22	T/C	synonymous_variant	agT/agC	0.00179	0.000028%	
c16_0	rs43359099	EPS15	3	C/G	intron_variant	-	-0.00167	0.000019%	
c16_0	rs41781896	LRP4	15	T/C	missense_variant	aTg/aCg	0.001629	0.000010%	
c16_0	rs43289839	PRKRA	2	T/G	intron_variant	-	0.001622	0.000009%	
c16_0	rs43477493	FGF5	5	A/T	intron_variant	-	0.00153	0.000016%	
c16_0	rs43649421	TSHR	10	T/C	synonymous_variant	atC/atT	0.001422	0.000008%	
c16_0	rs41745642	PAFAH1B2	15	C/G	intron_variant	-	-0.00142	0.000006%	
c16_0	rs208317364	DGAT1	14	A/G	intron_variant	-	0.001368	0.000007%	
c16_0	rs43369271	SIAT6	3	A/G	synonymous_variant	gcT/gcC	0.00134	0.000008%	
c16_0	rs41654804	COPZ1	5	C/G	downstream_gene_variant	-	-0.00128	0.000006%	0.008%
c17_0	rs134451630	SIAT4A	14	A/G	intron_variant	-	0.006456	0.038099%	
c17_0	rs17871529	CGN	3	C/G	upstream_gene_variant	-	-0.00641	0.035987%	
c17_0	rs41255193	HADHB	11	A/G	3_prime_UTR_variant	-	0.003969	0.014138%	
c17_0	rs41648836	RYR2	28	A/G	synonymous_variant	gcT/gcC	-0.00363	0.004205%	
c17_0	rs43727174	FGFR1	27	T/C	synonymous_variant	acC/acT	0.003526	0.005563%	
c17_0	rs41257187	ITGB5	5	T/C	synonymous_variant	ccC/ccT	0.003218	0.009070%	
c17_0	rs41613049	BDH1	1	T/C	intron_variant	-	-0.00302	0.006738%	
c17_0	rs42176295	SLC37A2	29	T/C	intron_variant	-	-0.00273	0.003085%	
c17_0	rs41745642	PAFAH1B2	15	C/G	intron_variant	-	0.00268	0.001469%	
c17_0	rs43649423	TSHR	10	A/G	synonymous_variant	ttG/ttA	-0.00244	0.001818%	
c17_0	rs29002485	IDH1	2	G/A	intron_variant	-	0.002289	0.004131%	

c17_0	rs41932864	BTF3	20	A/G	intron_variant	-	0.002263	0.003764%	
c17_0	rs41255352	TNS	2	C/G	3_prime_UTR_variant	-	-0.00221	0.004370%	
c17_0	rs110055647	AFABP	15	A/G	upstream_gene_variant	-	0.002193	0.003654%	
c17_0	rs43387500	PNPLA8	4	A/G	missense_variant	aGc/aAc	-0.0021	0.004039%	
c17_0	rs43734541	STAR	27	A/G	intron_variant	-	-0.0021	0.004003%	
c17_0	rs41963466	BG1	21	T/C	downstream_gene_variant	-	0.002053	0.003305%	
c17_0	rs43508512	ACSL6	7	A/T	intron_variant	-	0.002037	0.000808%	
c17_0	rs43663563	DGUCK	11	A/G	intron_variant	-	0.001937	0.000900%	
c17_0	rs43235355	CASR	1	A/C	intron_variant	-	0.001903	0.001763%	
c17_0	rs41793400	GNA14	8	T/C	5_prime_UTR_variant	-	-0.00185	0.001359%	
c17_0	rs135871423	TIEG2	11	A/G	missense_variant	-	-0.00184	0.002662%	
c17_0	rs42589207	PIK3R1	20	T/G	synonymous_variant	acC/acA	-0.00182	0.002901%	
c17_0	rs41652470	SIRT1	28	A/G	upstream_gene_variant	-	0.001765	0.002809%	
c17_0	rs110097521	PCK2	10	T/C	downstream_gene_variant	-	0.001633	0.002442%	
c17_0	rs43702942	UGDH	6	T/C	intron_variant	-	-0.00163	0.002387%	
c17_0	rs111014258	FABP4	14	T/C	intron_variant	-	0.001599	0.001983%	
c17_0	rs41606036	PCDHA13	7	A/G	missense_variant	aCa/aTa	-0.00146	0.001083%	
c17_0	rs41667443	CAPNS1	18	T/C	downstream_gene_variant	-	-0.00144	0.000441%	
c17_0	rs43407618	ADCYAP1R1	4	T/C	intron_variant	-	0.001286	0.000294%	0.169%
c18_0	rs43575364	MUSK	8	T/C	upstream_gene_variant	-	0.066118	0.074471%	
c18_0	rs42714483	THRSP	29	T/C	missense_variant	Atc/Gtc	0.046442	0.047633%	
c18_0	rs41255690	SCD1	26	A/G	intron_variant	-	0.02926	0.017569%	
c18_0	rs43242960	GAP43	1	A/T	intron_variant	-	0.025031	0.010315%	
c18_0	rs43513961	VDAC1	7	A/G	downstream_gene_variant	-	0.020249	0.006946%	

c18_0	rs43663558	DGUCK	11	A/C	intron_variant	-	0.016542	0.003494%	
c18_0	rs41974998	MFGE8	21	T/G	3_prime_UTR_variant	-	0.014497	0.004512%	
c18_0	rs41890171	SDR3	18	A/T	intron_variant	-	0.014298	0.003889%	
c18_0	rs41641851	IDH1	2	T/G	intron_variant	-	0.013042	0.003198%	
c18_0	rs42127354	DCTN6	27	C/G	upstream_gene_variant	-	-0.01225	0.003149%	
c18_0	rs43380663	IL6	4	T/C	intron_variant	-	-0.0114	0.002722%	
c18_0	rs43720497	RARA	19	A/G	intron_variant	-	0.010734	0.001162%	
c18_0	rs43707861	THBS	4	A/G	missense_variant	Atc/Gtc	-0.01034	0.002255%	
c18_0	rs41687553	DSTN	13	T/C	intron_variant	-	-0.00982	0.001805%	
c18_0	rs17871529	CGN	3	C/G	upstream_gene_variant	-	-0.00936	0.001852%	
c18_0	rs41601769	CS	5	A/G	intron_variant	-	0.009329	0.001334%	
c18_0	rs42194738	INS	29	T/C	downstream_gene_variant	-	-0.00918	0.001087%	
c18_0	rs41640705	IGF1R	21	A/G	intron_variant	-	0.009055	0.001108%	
c18_0	rs43382870	SCIN	4	A/G	3_prime_UTR_variant	-	-0.00881	0.001409%	
c18_0	rs43235355	CASR	1	A/C	intron_variant	-	0.008526	0.000853%	
c18_0	rs43332985	OVGP1	3	T/C	-	-	-0.00821	0.001344%	
c18_0	rs41793396	GNA14	8	C/G	synonymous_variant	gcC/gcG	-0.00815	0.001432%	
c18_0	rs110625700	PPARA	5	C/G	synonymous_variant	-	0.008092	0.000473%	
c18_0	rs43703893	ACAT2	9	A/G	downstream_gene_variant	-	-0.00783	0.001114%	
c18_0	rs41987137	COL4A4	2	T/C	intron_variant	-	-0.00783	0.000820%	
c18_0	rs42910826	ATP2C1	1	A/T	intron_variant	-	0.00774	0.000969%	
c18_0	rs41642657	ACADVL	19	T/C	downstream_gene_variant	-	-0.00765	0.000945%	
c18_0	rs43429822	SYT1	5	A/G	intron_variant	-	-0.00759	0.001154%	
c18_0	rs41635843	MTRR	20	T/G	intron_variant	-	-0.00731	0.001079%	
c18_0	rs41694130	CTSZ	13	T/G	-	-	-0.00721	0.001139%	

c18_0	rs41600007	TGFA	11	T/C	intergenic_variant	-	-0.00719	0.000764%	
c18_0	rs43648117	SNW1	10	T/C	downstream_gene_variant	-	-0.0071	0.001108%	
c18_0	rs42197376	PC	29	A/G	3_prime_UTR_variant	-	0.007058	0.000311%	
c18_0	rs43707870	ITGA5	4	T/C	3_prime_UTR_variant	-	-0.00687	0.000971%	
c18_0	rs29023213	LDHB	5	T/C	intron_variant	-	-0.00681	0.001027%	
c18_0	rs41915673	STAT5B	19	T/C	intron_variant	-	-0.00659	0.000946%	
c18_0	rs41662474	SLC2A12	9	T/C	intron_variant	-	-0.00656	0.000953%	
c18_0	rs110097521	PCK2	10	T/C	downstream_gene_variant	-	0.006515	0.000935%	
c18_0	rs43498004	PIK3R2	7	T/C	synonymous_variant	atC/atT	0.006469	0.000915%	
c18_0	rs43707854	SOCS2	5	A/C	3_prime_UTR_variant	-	-0.00611	0.000752%	
c18_0	rs41899395	MYH1	19	T/G	downstream_gene_variant	-	0.006007	0.000733%	
c18_0	rs41906356	PAFAH1B1	19	C/G	intron_variant	-	-0.00594	0.000685%	
c18_0	rs43387500	PNPLA8	4	A/G	missense_variant	aGc/aAc	-0.00593	0.000778%	
c18_0	rs42115578	FACL2	27	C/G	synonymous_variant	-	-0.00564	0.000648%	
c18_0	rs42868313	DGAT2	15	A/G	intron_variant	-	0.005631	0.000509%	
c18_0	rs42399155	NOX4	29	T/C	synonymous_variant	aaT/aaC	-0.00558	0.000503%	
c18_0	rs41592941	GUCY2C	5	T/C	splice_region_variant,intron_variant	-	0.005493	0.000645%	
c18_0	rs41632203	WISP1	14	T/C	upstream_gene_variant	-	-0.00548	0.000608%	
c18_0	rs42115578	ACSL1	27	C/G	synonymous_variant	tcG/tcC	-0.00532	0.000577%	
c18_0	rs43476247	CXCL10	6	T/G	downstream_gene_variant	-	-0.00516	0.000205%	
c18_0	rs42861142	ADAM18	27	A/C	intergenic_variant	-	-0.00512	0.000569%	
c18_0	rs42742546	SERPINI2	1	A/G	downstream_gene_variant	-	-0.00489	0.000317%	
c18_0	rs109368962	SAA3	29	A/G	intron_variant	-	0.004874	0.000164%	
c18_0	rs42185608	OPCML	29	A/G	intron_variant	-	-0.00477	0.000376%	
c18_0	rs42311181	HSD17B12	15	T/G	missense_variant	aaT/aaG	0.004633	0.000427%	

c18_0	rs42096946	FGF8	26	A/C	downstream_gene_variant	-	0.004632	0.000225%	
c18_0	rs41627981	ATP6V1H	14	A/G	intron_variant	-	-0.0046	0.000429%	
c18_0	rs43267303	AGTR1	1	T/C	intergenic_variant	-	-0.0045	0.000235%	
c18_0	rs43433318	MYF5	5	A/G	intron_variant	-	-0.00433	0.000394%	
c18_0	rs109663333	GHRH	13	A/T	5_prime_UTR_variant	-	-0.00428	0.000405%	
c18_0	rs43379084	IGF2BP3	4	T/C	3_prime_UTR_variant	-	-0.00427	0.000400%	
c18_0	rs133934411	RDH5	5	T/C	synonymous_variant	-	0.004259	0.000374%	
c18_0	rs42660323	PFN2	1	C/G	intergenic_variant	-	-0.00421	0.000372%	
c18_0	rs43463543	UGDH	6	T/G	intron_variant	-	0.004197	0.000167%	
c18_0	rs43489995	DGKQ	6	A/G	downstream_gene_variant	-	0.004191	0.000376%	
c18_0	rs43709215	TXNIP	3	C/G	intron_variant	-	-0.00406	0.000223%	
c18_0	rs43502114	CSF2	7	A/G	downstream_gene_variant	-	0.003896	0.000147%	
c18_0	rs41886803	ATP1A3	18	A/C	intron_variant	-	-0.0038	0.000271%	
c18_0	rs41583157	TCF7L2	26	A/C	intron_variant	-	0.0038	0.000167%	
c18_0	rs43500802	SLC27A1	7	T/C	intron_variant	-	-0.00376	0.000312%	
c18_0	rs43706499	CCL2	19	A/G	-	-	0.003676	0.000277%	
c18_0	rs43369255	SIAT6	3	T/C	downstream_gene_variant	-	0.003671	0.000095%	
c18_0	rs43319556	EGF	6	T/C	intergenic_variant	-	-0.00365	0.000138%	
c18_0	rs109221039	CAST	7	A/G	3_prime_UTR_variant	-	-0.0036	0.000215%	
c18_0	rs43707575	PNPLA2	29	T/C	downstream_gene_variant	-	0.003543	0.000178%	
c18_0	rs41767628	UCP3	15	T/C	intron_variant	-	0.003524	0.000076%	
c18_0	rs41568652	FGF14	12	A/T	intergenic_variant	-	-0.00345	0.000233%	
c18_0	rs41849828	ADRBK2	17	T/C	downstream_gene_variant	-	0.003435	0.000209%	
c18_0	rs208618783	APM 1	1	C/G	3_prime_UTR_variant	-	-0.00342	0.000234%	
c18_0	rs41932210	MAP2K6	19	A/C	downstream_gene_variant	-	0.00342	0.000256%	

c18_0	rs41656364	VIL2	9	T/C	intron_variant	-	-0.00342	0.000090%	
c18_0	rs43477493	FGF5	5	A/T	intron_variant	-	-0.00341	0.000120%	
c18_0	rs41897473	EMP3	18	T/C	downstream_gene_variant	-	-0.00335	0.000244%	
c18_0	rs43508512	ACSL6	7	A/T	intron_variant	-	0.003343	0.000053%	
c18_0	rs41648650	FGFR2	26	A/T	intron_variant	-	0.003333	0.000224%	
c18_0	rs43493654	PROM1	6	C/G	missense_variant	Ccc/Gcc	0.003312	0.000124%	
c18_0	rs29026551	FAM13A1	6	T/C	missense_variant	aGa/aAa	0.003284	0.000209%	
c18_0	rs41845704	OASL	17	C/G	downstream_gene_variant	-	-0.00316	0.000196%	
c18_0	rs43242931	GAP43	1	C/G	synonymous_variant	ccG/ccC	-0.00305	0.000093%	
c18_0	rs43724308	MGLL	22	T/C	synonymous_variant	agT/agC	0.003049	0.000126%	
c18_0	rs41890291	SLC1A5	18	A/G	3_prime_UTR_variant	-	-0.00299	0.000196%	
c18_0	rs41917436	CRHR1	19	A/C	intron_variant	-	-0.00298	0.000103%	
c18_0	rs43229098	SIAT10	1	T/C	3_prime_UTR_variant	-	-0.00295	0.000054%	
c18_0	rs41576373	UGALT2	19	A/G	downstream_gene_variant	-	-0.00292	0.000082%	
c18_0	rs41646367	ANXA11	28	A/G	synonymous_variant	gcC/gcT	-0.00284	0.000177%	
c18_0	rs43346270	PRKACB	3	A/G	intron_variant	-	0.002836	0.000151%	
c18_0	rs41747063	IL18	15	T/C	intron_variant	-	-0.00283	0.000103%	
c18_0	rs29014813	GPLD1	23	T/C	intron_variant	-	-0.00282	0.000132%	
c18_0	rs42114536	FAT	27	T/G	missense_variant	gaG/gaT	0.002815	0.000035%	
c18_0	rs43562598	AQP7	8	T/C	downstream_gene_variant	-	-0.00278	0.000155%	
c18_0	rs42827372	ACADM	3	T/C	upstream_gene_variant	-	-0.00269	0.000127%	
c18_0	rs41922087	SPHK1	19	A/G	upstream_gene_variant	-	0.002662	0.000042%	
c18_0	rs42761489	SLC27A2	10	A/G	intron_variant	-	-0.00265	0.000153%	
c18_0	rs41809799	PRKCZ	16	T/C	downstream_gene_variant	-	0.002635	0.000029%	
c18_0	rs42413973	EIF3S6	14	T/G	intron_variant	-	-0.00262	0.000093%	

c18_0	rs42176298	SLC37A2	29	T/C	intron_variant	-	0.002618	0.000070%	
c18_0	rs43715243	JAM1	3	C/G	-	-	-0.0026	0.000132%	
c18_0	rs41648836	RYR2	28	A/G	synonymous_variant	gcT/gcC	-0.0026	0.000052%	
c18_0	rs41710349	TGM2	13	A/T	3_prime_UTR_variant	-	0.002592	0.000047%	
c18_0	rs41734016	THRAP6	14	A/G	5_prime_UTR_variant	-	-0.00259	0.000148%	
c18_0	rs41919993	FASN	19	A/G	missense_variant	Tac/Cac	0.002552	0.000127%	
c18_0	rs41630327	SPON1	15	A/G	-	-	-0.00254	0.000081%	
c18_0	rs42211560	ADRP	15	T/C	missense_variant	-	-0.00254	0.000081%	
c18_0	rs42102755	TCF7L2	26	C/G	intron_variant	-	0.002534	0.000048%	
c18_0	rs109513400	ACBP	2	A/T	upstream_gene_variant	-	0.00252	0.000084%	
c18_0	rs41255521	STARD3	19	C/G	3_prime_UTR_variant	-	-0.0025	0.000074%	
c18_0	rs41909257	ALOX12	19	A/T	splice_region_variant,intron_variant	-	-0.00249	0.000124%	
c18_0	rs43317359	CRYBA2	3	T/G	intron_variant	-	-0.00248	0.000057%	
c18_0	rs41569368	IFNGR1	9	T/G	synonymous_variant	acA/acC	-0.00246	0.000133%	
c18_0	rs111014258	FABP4	14	T/C	intron_variant	-	0.00244	0.000111%	
c18_0	rs42427751	COL6A1	1	C/G	missense_variant	Gca/Cca	0.00242	0.000120%	
c18_0	rs42794062	FGF18	18	T/C	intron_variant	-	-0.00237	0.000121%	
c18_0	rs110953296	FABP5	14	T/C	downstream_gene_variant	-	-0.00237	0.000089%	
c18_0	rs41771527	SLCO2B1	15	A/C	synonymous_variant	gtA/gtC	-0.00229	0.000115%	
c18_0	rs211552420	SAA3	29	A/G	intron_variant	-	-0.00228	0.000040%	
c18_0	rs29022551	HABP2	26	T/C	intron_variant	-	0.002275	0.000105%	
c18_0	rs110757796	AFABP	15	A/G	missense_variant	-	0.002266	0.000096%	
c18_0	rs17871681	POMC	11	T/C	synonymous_variant	ttC/ttT	0.002242	0.000062%	
c18_0	rs43727174	FGFR1	27	T/C	synonymous_variant	acC/acT	0.002236	0.000054%	
c18_0	rs42211560	ADFP	8	A/G	missense_variant	gCt/gTt	-0.00223	0.000062%	

c18_0	rs43702510	DDEF1	14	T/C	synonymous_variant	ggT/ggC	-0.00221	0.000048%	
c18_0	rs208317364	DGAT1	14	A/G	intron_variant	-	0.002189	0.000027%	
c18_0	rs29011323	CDC10	4	T/C	intron_variant	-	-0.00217	0.000041%	
c18_0	rs43254867	COL6A2	1	A/C	intron_variant	-	0.002154	0.000056%	
c18_0	rs109285736	MYF6	5	C/G	3_prime_UTR_variant	-	0.002132	0.000071%	
c18_0	rs41755155	APOC3	15	T/C	3_prime_UTR_variant	-	0.00211	0.000041%	
c18_0	rs41746484	NCAM1	15	A/G	synonymous_variant	acC/acT	0.002091	0.000035%	
c18_0	rs42421976	PPP1R3A	4	A/C	downstream_gene_variant	-	-0.00209	0.000035%	
c18_0	rs41963475	BG1	21	T/C	intron_variant	-	0.002063	0.000050%	
c18_0	rs43710327	ACADS	17	A/G	missense_variant	cGg/cAg	0.002055	0.000047%	
c18_0	rs41634660	CAPN2	16	T/C	intron_variant	-	-0.00205	0.000023%	
c18_0	rs42844528	CAPN10	3	C/G	downstream_gene_variant	-	-0.00201	0.000037%	
c18_0	rs41916108	DCT	12	A/C	synonymous_variant	Cga/Aga	-0.00199	0.000063%	
c18_0	rs43326496	COL4A3	2	A/G	missense_variant	aGa/aAa	-0.00198	0.000019%	
c18_0	rs41707704	HNF4A	13	T/G	downstream_gene_variant	-	0.001954	0.000028%	
c18_0	#N/A	LEP	4	T/C	-	-	0.001952	0.000052%	
c18_0	rs41730630	BIG1	14	A/G	intron_variant	-	0.001894	0.000026%	
c18_0	rs41914856	UGALT2	19	T/C	downstream_gene_variant	-	0.001832	0.000029%	
c18_0	rs42500029	FAT3	29	A/G	missense_variant	Gtg/Atg	-0.00179	0.000036%	
c18_0	rs43649423	TSHR	10	A/G	synonymous_variant	ttG/ttA	-0.00176	0.000023%	
c18_0	rs43306652	GRB14	2	C/G	intron_variant	-	0.001713	0.000025%	0.230%
SFA	rs43663565	DGUCK	11	T/C	intron_variant	-	-0.00607	0.000176%	
SFA	rs43724308	MGLL	22	T/C	synonymous_variant	agT/agC	0.004046	0.000058%	
SFA	rs42113899	FAT	27	T/C	missense_variant	Gtg/Atg	0.003627	0.000058%	

SFA	rs41256865	GPD1	5	A/G	3_prime_UTR_variant	-	0.003291	0.000061%	
SFA	rs42196904	IGF2	29	A/G	intron_variant	-	0.00327	0.000049%	
SFA	rs42090456	TNFRSF6	26	C/G	intron_variant	-	0.003112	0.000047%	
SFA	rs110953296	FABP5	14	T/C	downstream_gene_variant	-	-0.00293	0.000035%	
SFA	rs41255703	SCD1	26	T/C	downstream_gene_variant	-	0.002922	0.000046%	
SFA	rs43494871	PROM1	6	T/G	missense_variant	ttA/ttC	-0.00291	0.000020%	
SFA	rs42115578	ACSL1	27	C/G	synonymous_variant	tcG/tcC	-0.00279	0.000041%	
SFA	rs43707854	SOCS2	5	A/C	3_prime_UTR_variant	-	-0.00279	0.000041%	
SFA	rs29024247	SLCO2B1	15	A/G	synonymous_variant	gtA/gtG	-0.00264	0.000029%	
SFA	rs42742546	SERPINI2	1	A/G	downstream_gene_variant	-	-0.00262	0.000024%	
SFA	rs17871529	CGN	3	C/G	upstream_gene_variant	-	-0.00249	0.000034%	
SFA	rs43648117	SNW1	10	T/C	downstream_gene_variant	-	-0.00237	0.000032%	
SFA	rs42115578	FACL2	27	C/G	synonymous_variant	-	-0.00233	0.000029%	
SFA	#N/A	ACSF3	18	C/T	-	-	-0.00231	0.000021%	
SFA	rs43476247	CXCL10	6	T/G	downstream_gene_variant	-	-0.0023	0.000011%	
SFA	rs41652470	SIRT1	28	A/G	upstream_gene_variant	-	0.002299	0.000030%	
SFA	rs109763947	IGF1	5	T/C	upstream_gene_variant	-	0.002241	0.000029%	
SFA	rs41844655	PRKAB1	17	A/G	downstream_gene_variant	-	-0.00223	0.000015%	
SFA	rs41646367	ANXA11	28	A/G	synonymous_variant	gcC/gcT	-0.00217	0.000027%	
SFA	rs41755155	APOC3	15	T/C	3_prime_UTR_variant	-	0.002072	0.000010%	
SFA	rs41907825	ALOX15	19	C/G	intron_variant	-	0.002061	0.000024%	
SFA	rs42243023	NEUROD1	2	T/G	upstream_gene_variant	-	-0.00203	0.000024%	
SFA	rs41900392	PRKCG	18	T/C	downstream_gene_variant	-	0.002024	0.000024%	
SFA	rs41694130	CTSZ	13	T/G	-	-	-0.002	0.000023%	
SFA	rs380876224	SOAT1	16	T/C	upstream_gene_variant	-	-0.002	0.000016%	

SFA	rs41708480	HNF4A	13	A/C	intron_variant	-	0.001985	0.000022%	
SFA	rs41744783	CASP1	15	T/C	intergenic_variant	-	0.001954	0.000021%	
SFA	rs43709215	TXNIP	3	C/G	intron_variant	-	-0.00195	0.000013%	
SFA	rs41744058	MMP3	15	A/C	intron_variant	-	0.001934	0.000014%	
SFA	rs29002484	IDH1	2	A/C	intron_variant	-	-0.0019	0.000021%	
SFA	rs41909257	ALOX12	19	A/T	splice_region_variant,intron_variant	-	-0.00188	0.000018%	
SFA	rs41640705	IGF1R	21	A/G	intron_variant	-	0.001864	0.000012%	
SFA	rs41886799	ATP1A3	18	A/G	intron_variant	-	0.001858	0.000017%	
SFA	rs109450360	IGF2R	9	A/G	intron_variant	-	-0.00183	0.000019%	
SFA	rs41800886	PLOD	16	T/C	downstream_gene_variant	-	0.001821	0.000015%	
SFA	rs43710977	GSTM1	3	A/C	intron_variant	-	-0.00182	0.000018%	
SFA	rs29022551	HABP2	26	T/C	intron_variant	-	0.001782	0.000017%	
SFA	rs43267303	AGTR1	1	T/C	intergenic_variant	-	-0.00174	0.000009%	
SFA	rs29017040	ACP6	3	T/C	intron_variant	-	0.001724	0.000017%	
SFA	rs41897473	EMP3	18	T/C	downstream_gene_variant	-	-0.0017	0.000016%	
SFA	rs109697714	GHRH	13	T/C	intron_variant	-	-0.00169	0.000017%	
SFA	rs41849828	ADRBK2	17	T/C	downstream_gene_variant	-	0.001687	0.000013%	
SFA	rs210864945	APM 1	1	A/G	intron_variant	-	-0.00166	0.000015%	
SFA	rs42149515	NEUROG3	28	A/T	downstream_gene_variant	-	-0.00165	0.000005%	
SFA	rs41613043	BDH1	1	T/C	intron_variant	-	-0.00163	0.000012%	
SFA	rs43289839	PRKRA	2	T/G	intron_variant	-	0.001611	0.000003%	
SFA	rs41890207	SDR3	18	T/C	downstream_gene_variant	-	0.001585	0.000012%	
SFA	rs41845684	OASL	17	C/G	3_prime_UTR_variant	-	0.001564	0.000014%	
SFA	rs109346428	FABP4	14	T/C	intron_variant	-	0.001542	0.000012%	
SFA	rs41800338	NME7	16	A/G	intron_variant	-	-0.00154	0.000010%	

SFA	rs43429822	SYT1	5	A/G	intron_variant	-	-0.00154	0.000012%	
SFA	rs42767950	ANKRD1	26	A/C	downstream_gene_variant	-	0.001495	0.000007%	
SFA	rs42311181	HSD17B12	15	T/G	missense_variant	aaT/aaG	0.001483	0.000011%	
SFA	rs41975002	MFGE8	21	T/C	intron_variant	-	0.001482	0.000010%	
SFA	rs41780423	PAMR1	15	T/C	missense_variant	aCg/aTg	-0.00147	0.000012%	
SFA	rs41781896	LRP4	15	T/C	missense_variant	aTg/aCg	0.00147	0.000003%	
SFA	rs43498004	PIK3R2	7	T/C	synonymous_variant	atC/atT	0.001468	0.000012%	
SFA	rs29021775	TG	14	A/G	intergenic_variant	-	-0.00146	0.000012%	
SFA	rs42660323	PFN2	1	C/G	intergenic_variant	-	0.001462	0.000012%	
SFA	rs43720495	RARA	19	T/C	intron_variant	-	0.001459	0.000008%	
SFA	rs43649421	TSHR	10	T/C	synonymous_variant	atC/atT	0.001455	0.000003%	
SFA	rs41579640	MTHFR	16	T/C	intron_variant	-	0.001446	0.000012%	
SFA	rs43317359	CRYBA2	3	T/G	intron_variant	-	-0.00144	0.000005%	
SFA	rs43707575	PNPLA2	29	T/C	downstream_gene_variant	-	0.001439	0.000008%	
SFA	rs42905005	MOGAT2	15	A/G	intron_variant	-	0.001432	0.000012%	
SFA	rs43315761	TNS	2	C/G	missense_variant	gGt/gCt	-0.00142	0.000004%	
SFA	rs42183365	DCPS	29	T/C	synonymous_variant	acC/acT	-0.00142	0.000010%	
SFA	rs137564647	LEP	4	C/G	upstream_gene_variant	-	0.001419	0.000012%	
SFA	rs41691188	ACAS2L	13	T/G	downstream_gene_variant	-	0.001403	0.000010%	
SFA	rs43407618	ADCYAP1R1	4	T/C	intron_variant	-	0.001373	0.000002%	
SFA	rs41687553	DSTN	13	T/C	intron_variant	-	-0.00137	0.000009%	
SFA	rs208317364	DGAT1	14	A/G	intron_variant	-	0.001367	0.000003%	
SFA	rs43675525	FSHR	11	A/G	intron_variant	-	-0.00137	0.000008%	
SFA	rs42436359	HGF	4	A/G	synonymous_variant	caA/caG	0.001332	0.000008%	
SFA	rs17871740	PCK1	13	A/G	intron_variant	-	0.001329	0.000008%	

SFA	rs110055647	AFABP	15	A/G	upstream_gene_variant	-	0.001324	0.000008%	
SFA	rs42411170	FGFR3	6	T/C	intron_variant	-	-0.00131	0.000007%	
SFA	rs42194738	INS	29	T/C	downstream_gene_variant	-	-0.0013	0.000006%	
SFA	rs43235355	CASR	1	A/C	intron_variant	-	0.001282	0.000005%	
SFA	rs43429740	SYT1	5	T/G	intron_variant	-	-0.00127	0.000005%	
SFA	rs384562096	ACADM	3	A/G	3_prime_UTR_variant	-	-0.0012	0.000008%	
SFA	rs41255521	STARD3	19	C/G	3_prime_UTR_variant	-	-0.00119	0.000004%	
SFA	rs43229098	SIAT10	1	T/C	3_prime_UTR_variant	-	-0.00116	0.000002%	
SFA	rs43242931	GAP43	1	C/G	synonymous_variant	ccG/ccC	-0.00116	0.000003%	
SFA	rs43359099	EPS15	3	C/G	intron_variant	-	-0.00112	0.000003%	
SFA	rs41654029	ATP2B1	5	T/G	intron_variant	-	-0.0011	0.000002%	
SFA	rs41853829	ISCU	17	A/G	upstream_gene_variant	-	-0.00107	0.000004%	
SFA	rs42176295	SLC37A2	29	T/C	intron_variant	-	0.001051	0.000003%	
SFA	rs42500029	FAT3	29	A/G	missense_variant	Gtg/Atg	-0.00104	0.000003%	
SFA	rs41809799	PRKCZ	16	T/C	downstream_gene_variant	-	0.00102	0.000001%	
SFA	rs109368962	SAA3	29	A/G	intron_variant	-	0.001002	0.000002%	
SFA	rs42605121	SLC8A1	11	A/G	intron_variant	-	0.000932	0.000002%	
SFA	rs43369271	SIAT6	3	A/G	synonymous_variant	gcT/gcC	0.000912	0.000002%	
SFA	rs41729168	FABP9	14	A/G	intron_variant	-	-0.00084	0.000001%	
SFA	rs42197376	PC	29	A/G	3_prime_UTR_variant	-	0.000774	0.000001%	
SFA	rs108968268	ACACA	19	T/C	intron_variant	-	-0.00076	0.000001%	
SFA	rs42102079	GPAM	26	A/G	intron_variant	-	-0.00065	0.000000%	0.002%
ai_c17_0	rs29004508	LEP	4	T/C	missense_variant	gCg/gTg	0.000587	0.001916%	
ai_c17_0	rs42188426	EEF1G	29	T/C	intron_variant	-	-0.00057	0.000852%	

ai_c17_0	rs41884788	CEBPG	18	A/G	downstream_gene_variant	-	-0.00056	0.001916%	
ai_c17_0	rs42098336	HABP2	26	T/C	downstream_gene_variant	-	-0.00055	0.001490%	
ai_c17_0	rs41255193	HADHB	11	T/C	3_prime_UTR_variant	-	-0.00053	0.002980%	
ai_c17_0	rs43734541	STAR	27	A/G	intron_variant	-	0.000521	0.002767%	
ai_c17_0	rs41922087	SPHK1	19	A/G	upstream_gene_variant	-	0.000514	0.000852%	
ai_c17_0	rs42176298	SLC37A2	29	T/C	intron_variant	-	0.000504	0.001277%	
ai_c17_0	rs41583801	DNMT2	13	A/G	intron_variant	-	-0.0005	0.001064%	
ai_c17_0	#N/A	LEP	4	#N/A	-	-	0.0005	0.001703%	
ai_c17_0	rs41883756	FFAR3	18	T/C	synonymous_variant	ggG/ggA	-0.00047	0.000852%	
ai_c17_0	rs43649423	TSHR	10	A/G	synonymous_variant	ttG/ttA	0.000465	0.000852%	
ai_c17_0	rs110953296	FABP5	14	T/C	downstream_gene_variant	-	-0.00046	0.001703%	
ai_c17_0	rs41634660	CAPN2	16	T/C	intron_variant	-	0.000456	0.000639%	
ai_c17_0	rs42421329	PPP1R3A	4	A/G	synonymous_variant	gaC/gaT	0.000452	0.001490%	
ai_c17_0	rs43317366	CRYBA2	3	A/C	intron_variant	-	0.000451	0.000852%	
ai_c17_0	rs41255587	CAST	7	A/G	3_prime_UTR_variant	-	0.000441	0.001916%	
ai_c17_0	rs41746484	NCAM1	15	A/G	synonymous_variant	acC/acT	0.000433	0.000639%	
ai_c17_0	rs41579049	5-OPASE	14	T/C	splice_region_variant,intron_variant	-	0.000427	0.000852%	
ai_c17_0	rs41597184	LIAS	6	T/C	intron_variant	-	-0.00042	0.001277%	
ai_c17_0	rs42358344	CALCR	4	A/G	downstream_gene_variant	-	-0.00042	0.000639%	
ai_c17_0	rs41610128	UGP2	11	A/G	intron_variant	-	0.000412	0.001703%	
ai_c17_0	rs109513400	ACBP	2	A/T	upstream_gene_variant	-	0.000411	0.001064%	
ai_c17_0	rs17871740	PCK1	13	A/G	intron_variant	-	0.000408	0.001277%	
ai_c17_0	rs42868252	DGAT2	15	A/G	intron_variant	-	0.000395	0.001277%	
ai_c17_0	rs41576422	THRA	19	A/G	intron_variant	-	0.000395	0.001490%	
ai_c17_0	rs43489995	DGKQ	6	A/G	downstream_gene_variant	-	0.000391	0.001490%	

ai_c17_0	rs41845704	OASL	17	C/G	downstream_gene_variant	-	-0.00039	0.001490%	
ai_c17_0	rs29012945	FBLN5	21	A/G	intron_variant	-	0.00039	0.001703%	
ai_c17_0	rs41890207	SDR3	18	T/C	downstream_gene_variant	-	0.00038	0.001277%	
ai_c17_0	rs43706509	NFE2L2	2	C/G	missense_variant	caG/caC	-0.00036	0.001277%	
ai_c17_0	rs41588659	COL1A2	4	A/G	intron_variant	-	-0.00035	0.001064%	
ai_c17_0	rs29022551	HABP2	26	T/C	intron_variant	-	0.000348	0.001277%	
ai_c17_0	rs41643443	GPLD1	23	A/G	intron_variant	-	0.000341	0.001064%	
ai_c17_0	rs43372452	SCIN	4	T/C	synonymous_variant	agC/agT	0.000329	0.001064%	
ai_c17_0	rs41909257	ALOX12	19	A/T	splice_region_variant,intron_variant	-	-0.00032	0.001064%	
ai_c17_0	rs109763947	IGF1	5	T/C	upstream_gene_variant	-	0.000311	0.001064%	
ai_c17_0	rs29026038	ANXA4	11	A/G	intron_variant	-	-0.00031	0.000852%	
ai_c17_0	rs42714482	THRSP	29	A/G	missense_variant	gTg/gCg	0.000284	0.000852%	
ai_c17_0	rs41580467	TG	14	A/C	missense_variant	Cgg/Ggg	0.000276	0.000852%	0.052%
BFA	rs43734541	STAR	27	A/G	intron_variant	-	0.004853	0.026684%	
BFA	rs41255193	HADHB	11	A/G	3_prime_UTR_variant	-	-0.0047	0.024694%	
BFA	rs29022551	HABP2	26	T/C	intron_variant	-	0.003275	0.011204%	
BFA	rs43706509	NFE2L2	2	C/G	missense_variant	caG/caC	-0.0028	0.008254%	
BFA	rs41847581	ACADS	17	T/C	intron_variant	-	0.002584	0.007340%	
BFA	rs41845704	OASL	17	C/G	downstream_gene_variant	-	-0.00248	0.006196%	
BFA	rs41793400	GNA14	8	T/C	5_prime_UTR_variant	-	-0.00348	0.006059%	
BFA	rs41884788	CEBPG	18	A/G	downstream_gene_variant	-	-0.00284	0.005168%	
BFA	rs41849830	ADRBK2	17	A/G	3_prime_UTR_variant	-	-0.00238	0.005145%	
BFA	rs41652470	SIRT1	28	A/G	upstream_gene_variant	-	0.001993	0.004436%	
BFA	rs29002484	IDH1	2	A/C	intron_variant	-	-0.00194	0.004207%	

BFA	rs29012945	FBLN5	21	A/G	intron_variant	-	0.001728	0.003407%	
BFA	rs17871740	PCK1	13	A/G	intron_variant	-	0.001983	0.003315%	
BFA	rs42868252	DGAT2	15	A/G	intron_variant	-	0.001969	0.003224%	
BFA	rs41632202	WISP1	14	A/T	intron_variant	-	0.001952	0.003087%	
BFA	rs109513400	ACBP	2	A/T	upstream_gene_variant	-	0.002008	0.002767%	
BFA	rs43649423	TSHR	10	A/G	synonymous_variant	ttG/ttA	0.002663	0.002698%	
BFA	rs110953296	FABP5	14	T/C	downstream_gene_variant	-	-0.00178	0.002607%	
BFA	rs42714482	THRSP	29	A/G	missense_variant	gTg/gCg	0.001503	0.002584%	
BFA	rs42147575	ANXA11	28	A/G	splice_region_variant,intron_variant	-	-0.00148	0.002492%	
BFA	rs43317366	CRYBA2	3	A/C	intron_variant	-	0.002225	0.002424%	
BFA	rs29026038	ANXA4	11	A/G	intron_variant	-	-0.00159	0.002378%	
BFA	rs41597184	LIAS	6	T/C	intron_variant	-	-0.00166	0.002309%	
BFA	rs17870507	ACSL6	7	A/G	intron_variant	-	0.001741	0.002195%	
BFA	rs41909257	ALOX12	19	A/T	splice_region_variant,intron_variant	-	-0.00145	0.002149%	
BFA	rs41255587	CAST	7	A/G	3_prime_UTR_variant	-	0.001384	0.002035%	
BFA	rs43254867	COL6A2	1	A/C	intron_variant	-	-0.00178	0.001966%	
BFA	rs42164253	FGFBP1	6	T/G	upstream_gene_variant	-	0.001342	0.001944%	
BFA	rs29004508	LEP	4	T/C	missense_variant	gCg/gTg	0.001828	0.001921%	
BFA	rs41884944	APOE	18	A/C	upstream_gene_variant	-	0.001581	0.001921%	
BFA	rs42421329	PPP1R3A	4	A/G	synonymous_variant	gaC/gaT	0.001545	0.001783%	
BFA	rs41579049	5-OPASE	14	T/C	splice_region_variant,intron_variant	-	0.001965	0.001738%	
BFA	rs41987137	COL4A4	2	T/C	intron_variant	-	-0.00154	0.001623%	
BFA	rs41630327	SPON1	15	A/G	-	-	-0.00143	0.001326%	
BFA	rs42176295	SLC37A2	29	T/C	intron_variant	-	0.001481	0.001120%	
BFA	rs43306652	GRB14	2	C/G	intron_variant	-	0.001548	0.001052%	

BFA	rs41922087	SPHK1	19	A/G	upstream_gene_variant	-	0.00165	0.000823%	
BFA	rs41634660	CAPN2	16	T/C	intron_variant	-	0.001279	0.000457%	
BFA	rs42114536	FAT	27	T/G	missense_variant	gaG/gaT	0.001379	0.000434%	0.167%
c14_19c	rs41255692	SCD1	26	T/C	synonymous_variant	-	-0.29043	14.995081%	
c14_19c	rs42196904	IGF2	29	A/G	intron_variant	-	0.048583	0.357598%	
c14_19c	rs41654804	COPZ1	5	C/G	downstream_gene_variant	-	-0.05004	0.128459%	
c14_19c	rs41819943	HSD11B1	16	T/C	downstream_gene_variant	-	0.028509	0.124986%	
c14_19c	rs29023213	LDHB	5	T/C	intron_variant	-	0.021286	0.086841%	
c14_19c	rs43500802	SLC27A1	7	T/C	intron_variant	-	0.021287	0.086818%	
c14_19c	rs29026524	RPGRIP1	10	C/G	downstream_gene_variant	-	0.018897	0.066200%	
c14_19c	rs41919983	FASN	19	T/C	intergenic_variant	-	0.01855	0.064876%	
c14_19c	rs41635843	MTRR	20	T/G	intron_variant	-	0.017915	0.056140%	
c14_19c	rs110625700	PPARA	5	C/G	synonymous_variant	-	-0.02868	0.051447%	
c14_19c	rs42714483	THRSP	29	T/C	missense_variant	Atc/Gtc	-0.01586	0.048102%	
c14_19c	rs41780423	PAMR1	15	T/C	missense_variant	aCg/aTg	-0.01319	0.030752%	
c14_19c	rs43734541	STAR	27	A/G	intron_variant	-	0.0126	0.030176%	
c14_19c	rs42185608	OPCML	29	A/G	intron_variant	-	0.013387	0.025710%	
c14_19c	rs42660323	PFN2	1	C/G	intergenic_variant	-	0.0118	0.025280%	
c14_19c	rs133934411	RDH5	5	T/C	synonymous_variant	-	-0.01043	0.019437%	
c14_19c	rs43387500	PNPLA8	4	A/G	missense_variant	aGc/aAc	0.010027	0.019272%	
c14_19c	rs43575364	MUSK	8	T/C	upstream_gene_variant	-	-0.00982	0.014227%	
c14_19c	rs41613049	BDH1	1	T/C	intron_variant	-	0.009511	0.013977%	
c14_19c	rs42127354	DCTN6	27	C/G	upstream_gene_variant	-	0.00861	0.013490%	
c14_19c	rs17872093	CAPN1	29	T/C	synonymous_variant	ggT/ggC	-0.00835	0.013367%	

c14_19c	rs41583801	DNMT2	13	A/G	intron_variant	-	0.013058	0.011821%	
c14_19c	rs43560146	LPL	8	T/C	upstream_gene_variant	-	-0.01089	0.011503%	
c14_19c	rs42761489	SLC27A2	10	A/G	intron_variant	-	0.007657	0.011027%	
c14_19c	rs41601769	CS	5	A/G	intron_variant	-	-0.00887	0.010448%	
c14_19c	rs41634890	TXNRD2	17	A/G	intron_variant	-	-0.0068	0.008836%	
c14_19c	rs41915673	STAT5B	19	T/C	intron_variant	-	0.006143	0.007129%	
c14_19c	rs43715243	JAM1	3	C/G	-	-	0.006397	0.006891%	
c14_19c	rs41639260	GHR	20	A/C	intron_variant	-	-0.00611	0.006845%	
c14_19c	rs43508512	ACSL6	7	A/T	intron_variant	-	-0.01281	0.006745%	
c14_19c	rs42861142	ADAM18	27	A/C	intergenic_variant	-	0.005917	0.006576%	
c14_19c	rs109221039	CAST	7	A/G	3_prime_UTR_variant	-	0.00657	0.006227%	
c14_19c	rs41783612	ACAD8	15	A/C	intron_variant	-	-0.00826	0.006208%	
c14_19c	rs41907823	ALOX15	19	T/C	intron_variant	-	-0.00583	0.006066%	
c14_19c	rs41890171	SDR3	18	A/T	intron_variant	-	-0.00574	0.005433%	
c14_19c	rs384562096	ACADM	3	A/G	3_prime_UTR_variant	-	-0.00526	0.004972%	
c14_19c	rs41932210	MAP2K6	19	A/C	downstream_gene_variant	-	-0.00499	0.004723%	
c14_19c	rs41647951	FNTA	27	T/G	intron_variant	-	0.006003	0.004474%	
c14_19c	rs41642657	ACADVL	19	T/C	downstream_gene_variant	-	0.00535	0.004009%	
c14_19c	rs43720497	RARA	19	A/G	intron_variant	-	-0.00656	0.003756%	
c14_19c	rs43380663	IL6	4	T/C	intron_variant	-	0.004524	0.003714%	
c14_19c	rs41576373	UGALT2	19	A/G	downstream_gene_variant	-	0.006517	0.003553%	
c14_19c	rs42413973	EIF3S6	14	T/G	intron_variant	-	0.005483	0.003549%	
c14_19c	rs41747063	IL18	15	T/C	intron_variant	-	0.005543	0.003434%	
c14_19c	rs17871427	PPM1B	11	A/G	downstream_gene_variant	-	0.009163	0.003326%	
c14_19c	#N/A	ACSF3	18	C/T	-	-	-0.005	0.003208%	

c14_19c	rs41932860	BTF3	20	A/T	intron_variant	-	-0.00454	0.003204%	
c14_19c	rs41256865	GPD1	5	A/G	3_prime_UTR_variant	-	0.00414	0.003188%	
c14_19c	rs42194738	INS	29	T/C	downstream_gene_variant	-	0.005328	0.003173%	
c14_19c	rs41767628	UCP3	15	T/C	intron_variant	-	-0.00766	0.003092%	
c14_19c	rs29024247	SLCO2B1	15	A/G	synonymous_variant	gtA/gtG	-0.00475	0.003077%	
c14_19c	rs41916426	CRHR1	19	C/G	intron_variant	-	0.004055	0.002878%	
c14_19c	rs42147575	ANXA11	28	A/G	splice_region_variant,intron_variant	-	-0.00389	0.002874%	
c14_19c	rs43502114	CSF2	7	A/G	downstream_gene_variant	-	-0.00573	0.002747%	
c14_19c	rs43440606	PTPRR	5	T/G	intron_variant	-	-0.00511	0.002651%	
c14_19c	rs42269059	NFE2L2	2	A/G	intron_variant	-	0.006102	0.002632%	
c14_19c	rs41844490	ACACB	17	T/C	synonymous_variant	ttC/ttT	-0.00582	0.002528%	
c14_19c	rs41974998	MFGE8	21	T/G	3_prime_UTR_variant	-	-0.00368	0.002517%	
c14_19c	rs43315761	TNS	2	C/G	missense_variant	gGt/gCt	-0.00627	0.002402%	
c14_19c	rs41897480	EMP3	18	C/G	intron_variant	-	-0.00362	0.002321%	
c14_19c	rs110061082	UCP7	15	T/C	intron_variant	-	-0.00553	0.002283%	
c14_19c	rs42605121	SLC8A1	11	A/G	intron_variant	-	0.005522	0.002237%	
c14_19c	rs43464396	PPARGC1A	6	A/G	intron_variant	-	-0.00336	0.002156%	
c14_19c	rs41906356	PAFAH1B1	19	C/G	intron_variant	-	0.003535	0.002103%	
c14_19c	rs41668638	SLC1A4	11	T/G	intron_variant	-	-0.00427	0.002018%	
c14_19c	rs441492140	ACAT2	5	A/C	downstream_gene_variant	-	0.003548	0.001991%	
c14_19c	rs211686954	HADHSC	26	A/G	synonymous_variant	-	-0.0032	0.001730%	
c14_19c	rs43051819	DPP4	2	A/C	upstream_gene_variant	-	-0.00294	0.001654%	
c14_19c	rs41648836	RYR2	28	A/G	synonymous_variant	gcT/gcC	0.00494	0.001627%	
c14_19c	rs42868313	DGAT2	15	A/G	intron_variant	-	-0.00337	0.001581%	
c14_19c	rs41793396	GNA14	8	C/G	synonymous_variant	gcC/gcG	0.002817	0.001481%	

c14_19c	rs42102756	TCF7L2	26	T/C	intron_variant	-	0.00468	0.001431%	
c14_19c	rs41710349	TGM2	13	A/T	3_prime_UTR_variant	-	-0.00486	0.001412%	
c14_19c	rs109944439	HADHB	11	C/G	intron_variant	-	-0.00442	0.001404%	
c14_19c	rs41657132	AGPAT4	9	T/C	intron_variant	-	-0.00398	0.001243%	
c14_19c	rs42183537	CD5	29	A/G	3_prime_UTR_variant	-	0.004078	0.001239%	
c14_19c	rs43463543	UGDH	6	T/G	intron_variant	-	-0.00384	0.001212%	
c14_19c	rs41730630	BIG1	14	A/G	intron_variant	-	-0.00438	0.001197%	
c14_19c	#N/A	LEP	4	#N/A	-	-	-0.00306	0.001116%	
c14_19c	rs444777683	SOAT1	16	T/C	upstream_gene_variant	-	-0.00352	0.001113%	
c14_19c	rs43369271	SIAT6	3	A/G	synonymous_variant	gcT/gcC	0.004048	0.001024%	
c14_19c	rs43493654	PROM1	6	C/G	missense_variant	Ccc/Gcc	-0.0032	0.001005%	
c14_19c	rs43383602	CROT	4	T/C	intron_variant	-	0.002809	0.000978%	
c14_19c	rs29004508	LEP	4	T/C	missense_variant	gCg/gTg	0.002775	0.000748%	
c14_19c	rs43649423	TSHR	10	A/G	synonymous_variant	ttG/ttA	0.003241	0.000671%	
c14_19c	rs41900388	PRKCG	18	T/C	intron_variant	-	0.003165	0.000641%	
c14_19c	rs41781896	LRP4	15	T/C	missense_variant	aTg/aCg	0.003631	0.000637%	
c14_19c	rs42102079	GPAM	26	A/G	intron_variant	-	0.003861	0.000545%	
c14_19c	rs42844528	CAPN10	3	C/G	downstream_gene_variant	-	0.002508	0.000495%	
c14_19c	rs41648757	FDPS	3	T/G	intron_variant	-	0.0026	0.000487%	
c14_19c	rs41810747	PRKCZ	16	C/G	intron_variant	-	0.003551	0.000460%	
c14_19c	#N/A	OLR1	5	A/G	-	-	-0.00238	0.000391%	
c14_19c	rs42821718	API5	15	A/G	intron_variant	-	-0.00268	0.000388%	
c14_19c	rs41922087	SPHK1	19	A/G	upstream_gene_variant	-	-0.00269	0.000372%	
c14_19c	rs41745642	PAFAH1B2	15	C/G	intron_variant	-	-0.00278	0.000330%	
c14_19c	rs43477496	FGF5	5	T/G	downstream_gene_variant	-	0.002171	0.000299%	

c14_19c	rs42436380	HGF	4	A/G	intron_variant	-	0.002432	0.000269%	
c14_19c	rs41634660	CAPN2	16	T/C	intron_variant	-	0.002218	0.000230%	
c14_19c	rs42165955	PROM1	6	A/C	missense_variant	caT/caG	-0.00194	0.000223%	
c14_19c	rs42197376	PC	29	A/G	3_prime_UTR_variant	-	-0.00201	0.000219%	
c14_19c	rs41847805	MVK	17	T/C	synonymous_variant	gcC/gcT	0.00234	0.000207%	
c14_19c	rs41678692	CPT2	3	T/C	3_prime_UTR_variant	-	0.002027	0.000203%	16.509%
c16_19c	rs41819943	HSD11B1	16	T/C	downstream_gene_variant	-	0.038248	0.047447%	
c16_19c	rs43387500	PNPLA8	4	A/G	missense_variant	aGc/aAc	0.02452	0.024309%	
c16_19c	rs41932210	MAP2K6	19	A/C	downstream_gene_variant	-	-0.01949	0.015174%	
c16_19c	rs43502114	CSF2	7	A/G	downstream_gene_variant	-	-0.02863	0.014456%	
c16_19c	rs41890171	SDR3	18	A/T	intron_variant	-	-0.01905	0.012615%	
c16_19c	rs43380663	IL6	4	T/C	intron_variant	-	0.01673	0.010718%	
c16_19c	rs43500802	SLC27A1	7	T/C	intron_variant	-	0.015714	0.009978%	
c16_19c	rs42127354	DCTN6	27	C/G	upstream_gene_variant	-	0.015671	0.009424%	
c16_19c	rs43575364	MUSK	8	T/C	upstream_gene_variant	-	-0.01172	0.004278%	
c16_19c	rs41915673	STAT5B	19	T/C	intron_variant	-	0.010359	0.004277%	
c16_19c	rs17871529	CGN	3	C/G	upstream_gene_variant	-	0.009845	0.003742%	
c16_19c	rs29026524	RPGRIP1	10	C/G	downstream_gene_variant	-	0.00964	0.003633%	
c16_19c	rs110625700	PPARA	5	C/G	synonymous_variant	-	-0.01596	0.003358%	
c16_19c	rs109663333	GHRH	13	A/T	5_prime_UTR_variant	-	0.009014	0.003284%	
c16_19c	rs41583801	DNMT2	13	A/G	intron_variant	-	0.01455	0.003096%	
c16_19c	rs42185608	OPCML	29	A/G	intron_variant	-	0.009517	0.002740%	
c16_19c	rs41601769	CS	5	A/G	intron_variant	-	-0.00966	0.002614%	
c16_19c	rs133934411	RDH5	5	T/C	synonymous_variant	-	-0.00815	0.002502%	

c16_19c	rs41641851	IDH1	2	T/G	intron_variant	-	-0.00823	0.002326%	
c16_19c	rs29023213	LDHB	5	T/C	intron_variant	-	0.007562	0.002311%	
c16_19c	rs42194738	INS	29	T/C	downstream_gene_variant	-	0.009386	0.002076%	
c16_19c	rs41635843	MTRR	20	T/G	intron_variant	-	0.007458	0.002052%	
c16_19c	rs109221039	CAST	7	A/G	3_prime_UTR_variant	-	0.008189	0.002041%	
c16_19c	rs41694130	CTSZ	13	T/G	-	-	0.006815	0.001856%	
c16_19c	rs17870648	NR1H3	15	A/C	synonymous_variant	ctG/ctT	-0.00693	0.001841%	
c16_19c	rs41897480	EMP3	18	C/G	intron_variant	-	-0.00696	0.001806%	
c16_19c	rs42761489	SLC27A2	10	A/G	intron_variant	-	0.006618	0.001737%	
c16_19c	rs42196904	IGF2	29	A/G	intron_variant	-	0.007178	0.001646%	
c16_19c	rs41654804	COPZ1	5	C/G	downstream_gene_variant	-	-0.01192	0.001537%	
c16_19c	rs43734541	STAR	27	A/G	intron_variant	-	0.00603	0.001457%	
c16_19c	rs43702942	UGDH	6	T/C	intron_variant	-	0.005975	0.001412%	
c16_19c	rs43242960	GAP43	1	A/T	intron_variant	-	-0.00637	0.001221%	
c16_19c	rs41583157	TCF7L2	26	A/C	intron_variant	-	-0.00713	0.001078%	
c16_19c	rs43508512	ACSL6	7	A/T	intron_variant	-	-0.01102	0.001052%	
c16_19c	rs41909257	ALOX12	19	A/T	splice_region_variant,intron_variant	-	0.005114	0.000956%	
c16_19c	rs41630327	SPON1	15	A/G	-	-	0.005984	0.000819%	
c16_19c	rs41910301	MYH8	19	A/G	downstream_gene_variant	-	0.004497	0.000814%	
c16_19c	rs41719131	FBXO32	14	T/G	upstream_gene_variant	-	0.00631	0.000744%	
c16_19c	rs41257258	TGM2	13	T/C	3_prime_UTR_variant	-	0.004569	0.000734%	
c16_19c	rs41648836	RYR2	28	A/G	synonymous_variant	gcT/gcC	0.007187	0.000726%	
c16_19c	rs41974998	MFGE8	21	T/G	3_prime_UTR_variant	-	-0.00422	0.000699%	
c16_19c	rs43300154	SCN7A	2	A/G	missense_variant	atG/atA	0.004163	0.000698%	
c16_19c	rs41793397	GNA14	8	C/G	5_prime_UTR_variant	-	0.004183	0.000691%	

c16_19c	rs43720497	RARA	19	A/G	intron_variant	-	-0.00608	0.000681%	
c16_19c	rs42910826	ATP2C1	1	A/T	intron_variant	-	-0.00479	0.000678%	
c16_19c	rs41916426	CRHR1	19	C/G	intron_variant	-	0.004252	0.000667%	
c16_19c	rs41576373	UGALT2	19	A/G	downstream_gene_variant	-	0.006102	0.000656%	
c16_19c	rs41766285	ILK	15	A/G	intron_variant	-	-0.00404	0.000641%	
c16_19c	rs41955844	MYO10	5	A/G	synonymous_variant	ccG/ccA	0.004508	0.000636%	
c16_19c	rs41747063	IL18	15	T/C	intron_variant	-	0.005147	0.000624%	
c16_19c	rs42861142	ADAM18	27	A/C	intergenic_variant	-	0.003924	0.000610%	
c16_19c	rs42522206	IL4	7	A/T	intron_variant	-	0.004283	0.000606%	
c16_19c	rs43727174	FGFR1	27	T/C	synonymous_variant	acC/acT	-0.00554	0.000604%	
c16_19c	rs41847571	ACADS	17	T/C	intron_variant	-	0.00395	0.000600%	
c16_19c	rs41641695	LOXL1	21	A/G	downstream_gene_variant	-	0.004097	0.000587%	
c16_19c	rs110097521	PCK2	10	T/C	downstream_gene_variant	-	-0.00366	0.000539%	
c16_19c	rs109300983	GHR	20	A/G	missense_variant	-	0.004164	0.000536%	
c16_19c	rs43463543	UGDH	6	T/G	intron_variant	-	-0.00556	0.000536%	
c16_19c	rs137748130	SORBS1	26	T/C	synonymous_variant	-	0.005169	0.000527%	
c16_19c	rs108993696	IGFBP5	2	T/C	synonymous_variant	-	-0.00382	0.000481%	
c16_19c	rs41662474	SLC2A12	9	T/C	intron_variant	-	0.003428	0.000475%	
c16_19c	rs41912288	SREBF1	19	T/C	intron_variant	-	-0.00343	0.000475%	
c16_19c	rs41255696	SCD1	26	A/C	downstream_gene_variant	-	-0.00334	0.000441%	
c16_19c	rs42660323	PFN2	1	C/G	intergenic_variant	-	0.003353	0.000430%	
c16_19c	rs43406303	ADCY1	4	A/G	intron_variant	-	-0.00326	0.000429%	
c16_19c	rs43489995	DGKQ	6	A/G	downstream_gene_variant	-	-0.0033	0.000426%	
c16_19c	rs109513400	ACBP	2	A/T	upstream_gene_variant	-	-0.00417	0.000422%	
c16_19c	rs42193357	POLA2	29	T/C	missense_variant	cAt/cGt	0.003198	0.000407%	

c16_19c	rs43707870	ITGA5	4	T/C	3_prime_UTR_variant	-	0.003271	0.000402%	
c16_19c	rs41647951	FNTA	27	T/G	intron_variant	-	0.003884	0.000395%	
c16_19c	rs41780423	PAMR1	15	T/C	missense_variant	aCg/aTg	-0.00322	0.000386%	
c16_19c	rs41613049	BDH1	1	T/C	intron_variant	-	0.003428	0.000383%	
c16_19c	rs43706499	CCL2	19	A/G	-	-	-0.00316	0.000372%	
c16_19c	rs42195889	SLC3A2	29	T/C	intron_variant	-	-0.00394	0.000369%	
c16_19c	rs43706466	IGFBP4	19	T/C	3_prime_UTR_variant	-	-0.0033	0.000356%	
c16_19c	rs41883758	FFAR3	18	A/G	downstream_gene_variant	-	-0.00345	0.000333%	
c16_19c	rs43251315	CDH9	20	A/G	missense_variant	Cca/Tca	-0.00403	0.000316%	
c16_19c	rs378738877	APM 1	1	A/G	upstream_gene_variant	-	0.003748	0.000315%	
c16_19c	rs43738103	CART	20	T/C	intron_variant	-	-0.00311	0.000312%	
c16_19c	rs41932860	BTF3	20	A/T	intron_variant	-	-0.00296	0.000287%	
c16_19c	rs43379086	IGF2BP3	4	A/G	3_prime_UTR_variant	-	-0.00286	0.000267%	
c16_19c	rs41730630	BIG1	14	A/G	intron_variant	-	-0.00445	0.000261%	
c16_19c	rs41640705	IGF1R	21	A/G	intron_variant	-	-0.00317	0.000248%	
c16_19c	rs43493654	PROM1	6	C/G	missense_variant	Ccc/Gcc	-0.00315	0.000206%	
c16_19c	rs42413973	EIF3S6	14	T/G	intron_variant	-	0.002812	0.000197%	
c16_19c	rs42821720	API5	15	A/G	intron_variant	-	-0.00415	0.000197%	
c16_19c	rs109944439	HADHB	11	C/G	intron_variant	-	-0.00342	0.000177%	
c16_19c	rs43235355	CASR	1	A/C	intron_variant	-	-0.00284	0.000172%	
c16_19c	rs43649423	TSHR	10	A/G	synonymous_variant	ttG/ttA	0.003501	0.000165%	
c16_19c	rs43306652	GRB14	2	C/G	intron_variant	-	-0.00315	0.000153%	
c16_19c	#N/A	LEP	4	T/C	-	-	-0.00246	0.000151%	
c16_19c	rs29011323	CDC10	4	T/C	intron_variant	-	0.003056	0.000150%	
c16_19c	rs42180931	SIAE	29	A/G	intron_variant	-	-0.00297	0.000146%	

c16_19c	rs41746520	NCAM1	15	A/G	-	-	-0.00262	0.000138%	
c16_19c	rs41900388	PRKCG	18	T/C	intron_variant	-	0.003193	0.000138%	
c16_19c	rs41255492	LGALS9	19	T/C	synonymous_variant	gcC/gcT	-0.0027	0.000123%	
c16_19c	rs42844528	CAPN10	3	C/G	downstream_gene_variant	-	0.002679	0.000119%	
c16_19c	rs43242931	GAP43	1	C/G	synonymous_variant	ccG/ccC	0.002455	0.000109%	
c16_19c	rs42096946	FGF8	26	A/C	downstream_gene_variant	-	-0.00226	0.000098%	
c16_19c	rs42605121	SLC8A1	11	A/G	intron_variant	-	0.002445	0.000092%	
c16_19c	rs42436380	HGF	4	A/G	intron_variant	-	0.002867	0.000078%	
c16_19c	rs41844482	ACACB	17	C/G	intron_variant	-	-0.00201	0.000064%	
c16_19c	rs41767622	UCP3	15	T/C	intron_variant	-	-0.00278	0.000062%	
c16_19c	rs42102079	GPAM	26	A/G	intron_variant	-	0.002492	0.000048%	
c16_19c	rs43326496	COL4A3	2	A/G	missense_variant	aGa/aAa	0.002153	0.000040%	
c16_19c	rs43663547	DGUCK	11	T/C	-	-	-0.00193	0.000040%	
c16_19c	rs43407618	ADCYAP1R1	4	T/C	intron_variant	-	-0.0022	0.000039%	
c16_19c	rs41904273	ACADVL	19	A/G	downstream_gene_variant	-	0.002039	0.000033%	
c16_19c	rs41718865	DDEF1	14	T/C	3_prime_UTR_variant	-	0.001772	0.000024%	
c16_19c	rs41810747	PRKCZ	16	C/G	intron_variant	-	0.001784	0.000024%	0.234%
c17_19c	rs41694130	CTSZ	13	T/G	-	-	0.005879	0.027633%	
c17_19c	rs29022551	HABP2	26	T/C	intron_variant	-	-0.00536	0.021239%	
c17_19c	rs134451630	SIAT4A	14	A/G	intron_variant	-	0.004727	0.018001%	
c17_19c	rs41632202	WISP1	14	A/T	intron_variant	-	-0.00473	0.012789%	
c17_19c	rs43707861	THBS	4	A/G	missense_variant	Atc/Gtc	0.004045	0.012611%	
c17_19c	rs43720495	RARA	19	T/C	intron_variant	-	-0.00477	0.012578%	
c17_19c	rs42176298	SLC37A2	29	T/C	intron_variant	-	-0.00499	0.009308%	

c17_19c	rs43576438	IL7	14	T/C	intron_variant	-	0.003632	0.008952%	
c17_19c	rs41975002	MFGE8	21	T/C	intron_variant	-	-0.00366	0.008450%	
c17_19c	rs43429822	SYT1	5	A/G	intron_variant	-	0.003273	0.007835%	
c17_19c	rs29026524	RPGRIP1	10	C/G	downstream_gene_variant	-	-0.00304	0.007236%	
c17_19c	rs42311181	HSD17B12	15	T/G	missense_variant	aaT/aaG	-0.00308	0.006896%	
c17_19c	rs43663558	DGUCK	11	A/C	intron_variant	-	-0.00357	0.005957%	
c17_19c	rs41255352	TNS	2	C/G	3_prime_UTR_variant	-	-0.00268	0.005682%	
c17_19c	rs41744055	MMP3	15	T/C	intron_variant	-	-0.00353	0.004792%	
c17_19c	rs41916109	DCT	12	T/C	3_prime_UTR_variant	-	0.002644	0.004112%	
c17_19c	rs43406303	ADCY1	4	A/G	intron_variant	-	0.002159	0.003756%	
c17_19c	rs42115578	FACL2	27	C/G	synonymous_variant	-	0.00223	0.003707%	
c17_19c	rs41741743	EIF3S3	14	A/G	intron_variant	-	-0.00218	0.003578%	
c17_19c	rs42115578	ACSL1	27	C/G	synonymous_variant	tcG/tcC	0.002074	0.003205%	
c17_19c	rs41613043	BDH1	1	T/C	intron_variant	-	0.00211	0.002898%	
c17_19c	rs41257186	ITGB5	5	C/G	synonymous_variant	ggC/ggG	0.001932	0.002865%	
c17_19c	rs41255193	HADHB	11	A/G	3_prime_UTR_variant	-	0.001837	0.002671%	
c17_19c	rs384562096	ACADM	3	A/G	3_prime_UTR_variant	-	0.001781	0.002412%	
c17_19c	rs43710288	NOD2	18	A/T	missense_variant	cAg/cTg	0.001846	0.001975%	
c17_19c	rs41897480	EMP3	18	C/G	intron_variant	-	-0.00161	0.001943%	
c17_19c	rs42096946	FGF8	26	A/C	downstream_gene_variant	-	-0.00223	0.001910%	
c17_19c	rs41640705	IGF1R	21	A/G	intron_variant	-	-0.00185	0.001684%	
c17_19c	rs41567325	TG	14	T/C	-	-	0.002306	0.001344%	
c17_19c	rs43463543	UGDH	6	T/G	intron_variant	-	-0.00191	0.001263%	
c17_19c	rs41745644	PAFAH1B2	15	T/C	intron_variant	-	0.002511	0.001133%	
c17_19c	rs41746484	NCAM1	15	A/G	synonymous_variant	acC/acT	-0.00187	0.001020%	

c17_19c	rs41718866	DDEF1	14	C/G	3_prime_UTR_variant	-	-0.00158	0.000971%	
c17_19c	rs209255077	SST	1	A/G	upstream_gene_variant	-	0.00171	0.000761%	
c17_19c	rs42197376	PC	29	A/G	3_prime_UTR_variant	-	-0.00181	0.000745%	
c17_19c	rs43649421	TSHR	10	T/C	synonymous_variant	atC/atT	-0.00172	0.000648%	
c17_19c	rs42436380	HGF	4	A/G	intron_variant	-	0.001685	0.000550%	
c17_19c	rs41667445	CAPNS1	18	A/G	downstream_gene_variant	-	0.00166	0.000486%	0.216%
c18_19c	rs42196904	IGF2	29	A/G	intron_variant	-	-0.0456	0.009860%	
c18_19c	rs41256865	GPD1	5	A/G	3_prime_UTR_variant	-	-0.02207	0.002834%	
c18_19c	rs41919983	FASN	19	T/C	intergenic_variant	-	-0.01772	0.001853%	
c18_19c	rs41652470	SIRT1	28	A/G	upstream_gene_variant	-	-0.01435	0.001210%	
c18_19c	rs41780423	PAMR1	15	T/C	missense_variant	aCg/aTg	0.013186	0.000962%	
c18_19c	rs43724308	MGLL	22	T/C	synonymous_variant	agT/agC	0.014927	0.000816%	
c18_19c	rs42147575	ANXA11	28	A/G	splice_region_variant,intron_variant	-	0.01048	0.000653%	
c18_19c	#N/A	COX5B	11	T/G	-	-	0.007619	0.000333%	
c18_19c	rs41900392	PRKCG	18	T/C	downstream_gene_variant	-	-0.00614	0.000226%	
c18_19c	rs29024247	SLCO2B1	15	A/G	synonymous_variant	gtA/gtG	0.006773	0.000196%	
c18_19c	rs41793400	GNA14	8	T/C	5_prime_UTR_variant	-	0.008135	0.000173%	
c18_19c	rs43717437	GSS	13	A/G	missense_variant	aaC/aaA	0.005549	0.000168%	
c18_19c	rs43663558	DGUCK	11	A/C	intron_variant	-	-0.00646	0.000145%	
c18_19c	rs109450360	IGF2R	9	A/G	intron_variant	-	0.004866	0.000142%	
c18_19c	rs41845683	OASL	17	A/G	3_prime_UTR_variant	-	-0.00487	0.000119%	
c18_19c	rs41916426	CRHR1	19	C/G	intron_variant	-	-0.00459	0.000116%	
c18_19c	rs41634890	TXNRD2	17	A/G	intron_variant	-	0.004277	0.000109%	
c18_19c	rs41632689	ACAD8	15	T/C	missense_variant	Gtc/Atc	0.004101	0.000100%	

c18_19c	rs42413973	EIF3S6	14	T/G	intron_variant	-	-0.00519	0.000100%	
c18_19c	rs43227622	ARL6	1	T/C	upstream_gene_variant	-	-0.00391	0.000089%	
c18_19c	rs41569386	ANXA4	11	A/G	intron_variant	-	-0.00386	0.000088%	
c18_19c	#N/A	ACSF3	18	C/T	-	-	0.004455	0.000080%	
c18_19c	rs41597184	LIAS	6	T/C	intron_variant	-	0.004182	0.000078%	
c18_19c	rs43315799	TNS	2	A/G	intron_variant	-	0.003584	0.000076%	
c18_19c	rs41884793	CEBPG	18	A/T	intron_variant	-	-0.00364	0.000075%	
c18_19c	rs41844655	PRKAB1	17	A/G	downstream_gene_variant	-	0.00471	0.000071%	
c18_19c	rs43500802	SLC27A1	7	T/C	intron_variant	-	-0.00344	0.000071%	
c18_19c	rs41613049	BDH1	1	T/C	intron_variant	-	-0.00381	0.000070%	
c18_19c	rs43644340	PSEN1	10	T/G	splice_region_variant,intron_variant	-	0.003376	0.000065%	
c18_19c	rs41847581	ACADS	17	T/C	intron_variant	-	-0.00335	0.000065%	
c18_19c	rs380876224	SOAT1	16	T/C	upstream_gene_variant	-	0.003744	0.000059%	
c18_19c	rs42102756	TCF7L2	26	T/C	intron_variant	-	-0.00532	0.000058%	
c18_19c	rs41755155	APOC3	15	T/C	3_prime_UTR_variant	-	-0.00466	0.000054%	
c18_19c	rs384562096	ACADM	3	A/G	3_prime_UTR_variant	-	0.003015	0.000051%	
c18_19c	rs43624642	RYR3	10	T/C	missense_variant	Gca/Aca	-0.00291	0.000049%	
c18_19c	rs43289839	PRKRA	2	T/G	intron_variant	-	-0.00595	0.000049%	
c18_19c	rs43235983	NR1I2	1	A/G	intron_variant	-	0.002828	0.000047%	
c18_19c	rs41635843	MTRR	20	T/G	intron_variant	-	-0.00291	0.000046%	
c18_19c	rs110953296	FABP5	14	T/C	downstream_gene_variant	-	0.003263	0.000046%	
c18_19c	rs17870352	THBS	4	A/G	missense_variant	Att/Gtt	0.002829	0.000046%	
c18_19c	rs135700617	ANXA9	3	A/G	missense_variant	-	-0.00285	0.000045%	
c18_19c	rs43707854	SOCS2	5	A/C	3_prime_UTR_variant	-	0.002836	0.000044%	
c18_19c	rs110442528	IGFBP2	2	A/G	intron_variant	-	0.002735	0.000039%	

c18_19c	rs41912299	RAI1	19	T/C	synonymous_variant	ccG/ccT	0.002543	0.000039%	
c18_19c	rs29022551	HABP2	26	T/C	intron_variant	-	-0.00264	0.000038%	
c18_19c	rs42905005	MOGAT2	15	A/G	intron_variant	-	-0.00246	0.000036%	
c18_19c	rs42761489	SLC27A2	10	A/G	intron_variant	-	-0.00244	0.000035%	
c18_19c	rs42742546	SERPINI2	1	A/G	downstream_gene_variant	-	0.00304	0.000033%	
c18_19c	rs41974998	MFGE8	21	T/G	3_prime_UTR_variant	-	0.002331	0.000032%	
c18_19c	rs29021775	TG	14	A/G	intergenic_variant	-	0.002355	0.000031%	
c18_19c	rs29004488	LEP	4	T/C	missense_variant	-	0.00224	0.000030%	
c18_19c	rs134204153	HADHSC	26	A/C	intron_variant	-	-0.00223	0.000029%	
c18_19c	rs42176279	SLC37A2	29	C/G	intron_variant	-	0.002794	0.000028%	
c18_19c	rs43675525	FSHR	11	A/G	intron_variant	-	0.002523	0.000028%	
c18_19c	rs41257187	ITGB5	5	T/C	synonymous_variant	ccC/ccT	-0.00219	0.000027%	
c18_19c	rs41907825	ALOX15	19	C/G	intron_variant	-	-0.00218	0.000027%	
c18_19c	rs42660323	PFN2	1	C/G	intergenic_variant	-	-0.00218	0.000027%	
c18_19c	rs208631210	APM 1	1	C/G	upstream_gene_variant	-	0.002131	0.000027%	
c18_19c	rs41804173	SLC2A5	16	T/C	intron_variant	-	0.002254	0.000027%	
c18_19c	rs17870202	IGFBP3	4	C/G	upstream_gene_variant	-	0.002445	0.000026%	
c18_19c	rs41584658	IGFBP5	2	T/C	intron_variant	-	0.002253	0.000026%	
c18_19c	rs41654804	COPZ1	5	C/G	downstream_gene_variant	-	0.004023	0.000026%	
c18_19c	rs42183365	DCPS	29	T/C	synonymous_variant	acC/acT	0.002234	0.000025%	
c18_19c	rs42522205	IL4	7	T/C	intron_variant	-	-0.00203	0.000025%	
c18_19c	rs43464396	PPARGC1A	6	A/G	intron_variant	-	0.002001	0.000024%	
c18_19c	#N/A	OLR1	5	A/G	-	-	0.003316	0.000024%	
c18_19c	rs42714483	THRSP	29	T/C	missense_variant	Atc/Gtc	0.001995	0.000024%	
c18_19c	rs43617150	RPGRIP1	10	A/G	intron_variant	-	-0.00202	0.000023%	

c18_19c	rs41255193	HADHB	11	A/G	3_prime_UTR_variant	-	0.001964	0.000023%	
c18_19c	rs42973901	LARG	15	T/C	downstream_gene_variant	-	-0.00192	0.000022%	
c18_19c	rs41691208	ACAS2L	13	A/G	intron_variant	-	-0.00204	0.000021%	
c18_19c	rs42193752	PC	29	A/G	intron_variant	-	-0.00255	0.000021%	
c18_19c	rs41800886	PLOD	16	T/C	downstream_gene_variant	-	-0.00211	0.000021%	
c18_19c	rs41844482	ACACB	17	C/G	intron_variant	-	0.002945	0.000020%	
c18_19c	rs29003543	TIMP3	5	A/G	intron_variant	-	0.001959	0.000020%	
c18_19c	rs41567325	TG	14	T/C	-	-	0.003262	0.000020%	
c18_19c	rs41708480	HNF4A	13	A/C	intron_variant	-	-0.00183	0.000020%	
c18_19c	rs41909257	ALOX12	19	A/T	splice_region_variant,intron_variant	-	0.001898	0.000020%	
c18_19c	rs43649423	TSHR	10	A/G	synonymous_variant	ttG/ttA	-0.00311	0.000019%	
c18_19c	rs43709215	TXNIP	3	C/G	intron_variant	-	0.002271	0.000019%	
c18_19c	rs109697714	GHRH	13	T/C	intron_variant	-	0.001766	0.000019%	
c18_19c	rs41593883	PRKCH	10	T/C	intron_variant	-	-0.00177	0.000018%	
c18_19c	rs43455066	BDH2	6	C/G	intron_variant	-	0.001961	0.000018%	
c18_19c	rs43576438	IL7	14	T/C	intron_variant	-	-0.00191	0.000018%	
c18_19c	rs43440606	PTPRR	5	T/G	intron_variant	-	0.002344	0.000018%	
c18_19c	rs42243023	NEUROD1	2	T/G	upstream_gene_variant	-	0.001702	0.000017%	
c18_19c	rs41863915	CDH1	18	A/G	intergenic_variant	-	0.001695	0.000017%	
c18_19c	rs41592953	CLEC7A	5	T/C	intron_variant	-	-0.00168	0.000017%	
c18_19c	rs41255646	HMGCL	2	A/G	3_prime_UTR_variant	-	0.001677	0.000017%	
c18_19c	rs42156960	EED	29	A/G	intron_variant	-	0.002433	0.000017%	
c18_19c	rs41668638	SLC1A4	11	T/G	intron_variant	-	0.002175	0.000016%	
c18_19c	rs41846570	NOS1	17	A/C	upstream_gene_variant	-	0.001985	0.000016%	
c18_19c	rs43703893	ACAT2	9	A/G	downstream_gene_variant	-	-0.00181	0.000016%	

c18_19c	rs17871740	PCK1	13	A/G	intron_variant	-	-0.00187	0.000015%	
c18_19c	rs42605121	SLC8A1	11	A/G	intron_variant	-	-0.0026	0.000015%	
c18_19c	rs41657132	AGPAT4	9	T/C	intron_variant	-	0.0025	0.000015%	
c18_19c	rs42099881	SIAT1	1	A/G	intron_variant	-	-0.0016	0.000015%	
c18_19c	rs41574477	UGDH	6	A/G	intron_variant	-	0.001596	0.000015%	
c18_19c	rs29002484	IDH1	2	A/C	intron_variant	-	0.001562	0.000014%	
c18_19c	rs41932864	BTF3	20	A/G	intron_variant	-	0.00173	0.000014%	
c18_19c	rs29016220	JAM1	3	A/C	intron_variant	-	0.002118	0.000014%	
c18_19c	rs41256890	MGP	5	T/C	synonymous_variant	taT/taC	-0.00166	0.000014%	
c18_19c	rs41897476	EMP3	18	T/C	downstream_gene_variant	-	-0.00159	0.000014%	
c18_19c	rs42185605	OPCML	29	T/C	intron_variant	-	0.001621	0.000014%	
c18_19c	rs41687553	DSTN	13	T/C	intron_variant	-	0.001657	0.000014%	
c18_19c	rs42269059	NFE2L2	2	A/G	intron_variant	-	-0.00251	0.000014%	
c18_19c	rs41582028	ACVR2	2	C/G	intron_variant	-	0.001711	0.000014%	
c18_19c	rs41963518	BG1	21	T/G	intron_variant	-	-0.00156	0.000014%	
c18_19c	rs42861142	ADAM18	27	A/C	intergenic_variant	-	-0.00151	0.000013%	
c18_19c	rs42411170	FGFR3	6	T/C	intron_variant	-	0.001786	0.000013%	
c18_19c	rs43300154	SCN7A	2	A/G	missense_variant	atG/atA	-0.00148	0.000013%	
c18_19c	rs42085437	DNTT	26	A/G	intron_variant	-	-0.00156	0.000013%	
c18_19c	rs43560146	LPL	8	T/C	upstream_gene_variant	-	0.002039	0.000013%	
c18_19c	rs41647951	FNTA	27	T/G	intron_variant	-	-0.0018	0.000012%	
c18_19c	rs43710977	GSTM1	3	A/C	intron_variant	-	0.001473	0.000012%	
c18_19c	rs17871529	CGN	3	C/G	upstream_gene_variant	-	0.001453	0.000012%	
c18_19c	rs41819943	HSD11B1	16	T/C	downstream_gene_variant	-	-0.00154	0.000012%	
c18_19c	rs42321611	PRLR	20	A/G	upstream_gene_variant	-	-0.00152	0.000011%	

c18_19c	rs17871427	PPM1B	11	A/G	downstream_gene_variant	-	-0.00288	0.000010%	
c18_19c	rs43476247	CXCL10	6	T/G	downstream_gene_variant	-	0.002222	0.000010%	
c18_19c	rs41610128	UGP2	11	A/G	intron_variant	-	-0.00137	0.000010%	
c18_19c	rs29015653	ILF3	7	T/C	intron_variant	-	-0.00159	0.000010%	
c18_19c	rs43493654	PROM1	6	C/G	missense_variant	Ccc/Gcc	0.001753	0.000009%	
c18_19c	rs43477479	FGF5	5	A/C	intron_variant	-	0.00181	0.000009%	
c18_19c	rs29014396	PTPRR	5	T/C	intron_variant	-	0.001462	0.000009%	
c18_19c	rs134754797	SLC27A3	3	A/G	intron_variant	-	-0.00184	0.000009%	
c18_19c	rs41255257	AP2B1	19	T/C	intergenic_variant	-	0.001705	0.000008%	
c18_19c	rs109285736	MYF6	5	C/G	3_prime_UTR_variant	-	0.001356	0.000008%	
c18_19c	rs43429740	SYT1	5	T/G	intron_variant	-	0.001514	0.000008%	
c18_19c	rs42211557	ADFP	8	A/T	intron_variant	-	-0.00169	0.000008%	
c18_19c	rs208510799	GDF3	2	T/C	downstream_gene_variant	-	0.001153	0.000007%	
c18_19c	rs41781896	LRP4	15	T/C	missense_variant	aTg/aCg	-0.00211	0.000007%	
c18_19c	rs43359039	EPS15	3	C/G	intron_variant	-	-0.00154	0.000007%	
c18_19c	rs41642340	MVK	17	T/C	intron_variant	-	-0.0015	0.000006%	
c18_19c	rs43707575	PNPLA2	29	T/C	downstream_gene_variant	-	-0.00128	0.000006%	
c18_19c	rs43294227	SLC25A12	2	A/T	upstream_gene_variant	-	0.001236	0.000005%	
c18_19c	rs43727187	FGFR1	27	T/C	intron_variant	-	0.001366	0.000005%	
c18_19c	rs109368962	SAA3	29	A/G	intron_variant	-	-0.00162	0.000005%	
c18_19c	rs108968268	ACACA	19	T/C	intron_variant	-	0.001707	0.000004%	
c18_19c	rs43502114	CSF2	7	A/G	downstream_gene_variant	-	0.00105	0.000003%	
c18_19c	rs41639432	AOX1	2	C/G	intron_variant	-	-0.00119	0.000003%	
c18_19c	rs43369271	SIAT6	3	A/G	synonymous_variant	gcT/gcC	-0.00115	0.000003%	
c18_19c	rs41600452	FGF12	1	T/G	intron_variant	-	-0.00103	0.000003%	

c18_19c	rs41729168	FABP9	14	A/G	intron_variant	-	0.001099	0.000003%	
c18_19c	rs43317372	CRYBA2	3	A/G	downstream_gene_variant	-	-0.001	0.000003%	
c18_19c	rs41654029	ATP2B1	5	T/G	intron_variant	-	0.001077	0.000002%	
c18_19c	rs41926990	PRKCA	19	C/G	intron_variant	-	0.001212	0.000002%	
c18_19c	rs43717462	ACSS2	13	A/G	synonymous_variant	atT/atC	-0.00116	0.000002%	
c18_19c	rs41667443	CAPNS1	18	T/C	downstream_gene_variant	-	0.000987	0.000001%	
c18_19c	rs42821719	API5	15	A/G	intron_variant	-	0.00106	0.000001%	
c18_19c	rs43326496	COL4A3	2	A/G	missense_variant	aGa/aAa	0.000922	0.000001%	
c18_19c	rs42114536	FAT	27	T/G	missense_variant	gaG/gaT	-0.00089	0.000001%	0.023%
c18_111c	rs17871529	CGN	3	C/G	upstream_gene_variant	-	0.018921	0.124315%	
c18_111c	rs41909257	ALOX12	19	A/T	splice_region_variant,intron_variant	-	0.014943	0.073446%	
c18_111c	rs43648117	SNW1	10	T/C	downstream_gene_variant	-	0.013359	0.064399%	
c18_111c	rs41907825	ALOX15	19	C/G	intron_variant	-	-0.01022	0.036450%	
c18_111c	rs41884788	CEBPG	18	A/G	downstream_gene_variant	-	0.0109	0.024186%	
c18_111c	rs42742546	SERPINI2	1	A/G	downstream_gene_variant	-	0.009461	0.019513%	
c18_111c	rs41890171	SDR3	18	A/T	intron_variant	-	-0.00729	0.016602%	
c18_111c	rs41643443	GPLD1	23	A/G	intron_variant	-	-0.00638	0.012643%	
c18_111c	rs41844655	PRKAB1	17	A/G	downstream_gene_variant	-	0.007583	0.011216%	
c18_111c	rs41897473	EMP3	18	T/C	downstream_gene_variant	-	0.005401	0.010379%	
c18_111c	rs42115578	ACSL1	27	C/G	synonymous_variant	tcG/tcC	0.005032	0.008472%	
c18_111c	#N/A	ACSF3	18	C/T	-	-	0.005895	0.008443%	
c18_111c	rs41886803	ATP1A3	18	A/C	intron_variant	-	0.005202	0.008326%	
c18_111c	rs42115578	FACL2	27	C/G	synonymous_variant	-	0.004919	0.008101%	
c18_111c	rs42113899	FAT	27	T/C	missense_variant	Gtg/Atg	-0.00508	0.007220%	

c18_111c	rs43317359	CRYBA2	3	T/G	intron_variant	-	0.006897	0.007198%	
c18_111c	rs110953296	FABP5	14	T/C	downstream_gene_variant	-	0.005072	0.006703%	
c18_111c	rs43663540	DGUCK	11	T/G	intron_variant	-	0.005197	0.005888%	
c18_111c	rs42149515	NEUROG3	28	A/T	downstream_gene_variant	-	0.007116	0.005604%	
c18_111c	rs43498004	PIK3R2	7	T/C	synonymous_variant	atC/atT	-0.0038	0.005182%	
c18_111c	rs41652470	SIRT1	28	A/G	upstream_gene_variant	-	-0.00381	0.005160%	
c18_111c	rs43489995	DGKQ	6	A/G	downstream_gene_variant	-	-0.00337	0.004003%	
c18_111c	rs41744783	CASP1	15	T/C	intergenic_variant	-	-0.00322	0.003508%	
c18_111c	rs41974998	MFGE8	21	T/G	3_prime_UTR_variant	-	-0.00311	0.003414%	
c18_111c	rs109763947	IGF1	5	T/C	upstream_gene_variant	-	-0.00304	0.003348%	
c18_111c	rs43268388	MRAS	1	A/G	downstream_gene_variant	-	-0.00321	0.003188%	
c18_111c	rs43715243	JAM1	3	C/G	-	-	0.003128	0.003130%	
c18_111c	rs41587421	PIK4CB	3	T/C	intron_variant	-	-0.003	0.003093%	
c18_111c	rs43429822	SYT1	5	A/G	intron_variant	-	0.002984	0.002926%	
c18_111c	rs109221039	CAST	7	A/G	3_prime_UTR_variant	-	0.003256	0.002904%	
c18_111c	rs41641849	IDH1	2	A/G	intron_variant	-	0.00308	0.002904%	
c18_111c	rs43664478	DYSF	11	T/C	missense_variant	cGc/cAc	-0.00299	0.002686%	
c18_111c	rs43242931	GAP43	1	C/G	synonymous_variant	ccG/ccC	0.004003	0.002620%	
c18_111c	rs41606409	ELF5	15	A/G	intron_variant	-	0.002823	0.002526%	
c18_111c	rs41849828	ADRBK2	17	T/C	downstream_gene_variant	-	-0.00294	0.002518%	
c18_111c	rs42197376	PC	29	A/G	3_prime_UTR_variant	-	-0.00488	0.002446%	
c18_111c	rs43229098	SIAT10	1	T/C	3_prime_UTR_variant	-	0.004393	0.001980%	
c18_111c	rs41255257	AP2B1	19	T/C	intergenic_variant	-	0.003355	0.001914%	
c18_111c	rs41900388	PRKCG	18	T/C	intron_variant	-	0.003882	0.001827%	
c18_111c	rs41691188	ACAS2L	13	T/G	downstream_gene_variant	-	-0.00228	0.001623%	

c18_111c	rs380876224	SOAT1	16	T/C	upstream_gene_variant	-	0.002446	0.001528%	
c18_111c	rs41632202	WISP1	14	A/T	intron_variant	-	-0.00238	0.001456%	
c18_111c	rs41987137	COL4A4	2	T/C	intron_variant	-	0.002553	0.001434%	
c18_111c	rs41744058	MMP3	15	A/C	intron_variant	-	-0.00246	0.001427%	
c18_111c	rs43513961	VDAC1	7	A/G	downstream_gene_variant	-	-0.00217	0.001303%	
c18_111c	rs43727174	FGFR1	27	T/C	synonymous_variant	acC/acT	-0.0027	0.001288%	
c18_111c	rs43709215	TXNIP	3	C/G	intron_variant	-	0.002327	0.001201%	
c18_111c	rs42500029	FAT3	29	A/G	missense_variant	Gtg/Atg	0.002507	0.001157%	
c18_111c	rs109368962	SAA3	29	A/G	intron_variant	-	-0.00307	0.001070%	
c18_111c	rs42180931	SIAE	29	A/G	intron_variant	-	-0.00263	0.001034%	
c18_111c	rs41917436	CRHR1	19	A/C	intron_variant	-	0.002212	0.000932%	
c18_111c	rs41781896	LRP4	15	T/C	missense_variant	aTg/aCg	-0.00307	0.000866%	
c18_111c	rs43267303	AGTR1	1	T/C	intergenic_variant	-	0.002128	0.000866%	
c18_111c	rs43502114	CSF2	7	A/G	downstream_gene_variant	-	-0.00214	0.000728%	
c18_111c	rs41710349	TGM2	13	A/T	3_prime_UTR_variant	-	-0.0025	0.000706%	
c18_111c	rs41691161	ABHD12	13	C/G	downstream_gene_variant	-	-0.00208	0.000502%	
c18_111c	rs43289839	PRKRA	2	T/G	intron_variant	-	-0.00233	0.000451%	
c18_111c	rs208317364	DGAT1	14	A/G	intron_variant	-	-0.00208	0.000400%	
c18_111c	rs43326496	COL4A3	2	A/G	missense_variant	aGa/aAa	0.001879	0.000277%	0.541%
c18_113c	rs41255693	SCD1	26	T/C	missense_variant,splice_region_variant	-	0.135453	19.612924%	
c18_113c	rs42714483	THRSP	29	T/C	missense_variant	Atc/Gtc	-0.00864	0.085822%	
c18_113c	rs41974998	MFGE8	21	T/G	3_prime_UTR_variant	-	-0.00596	0.039658%	
c18_113c	rs41890171	SDR3	18	A/T	intron_variant	-	-0.00397	0.015596%	
c18_113c	rs43720497	RARA	19	A/G	intron_variant	-	-0.0041	0.008836%	

c18_113c	rs43235355	CASR	1	A/C	intron_variant	-	-0.00365	0.008144%	
c18_113c	rs41884788	CEBPG	18	A/G	downstream_gene_variant	-	0.00355	0.008121%	
c18_113c	rs42761489	SLC27A2	10	A/G	intron_variant	-	0.002596	0.007613%	
c18_113c	rs109221039	CAST	7	A/G	3_prime_UTR_variant	-	0.002833	0.006967%	
c18_113c	rs43513961	VDAC1	7	A/G	downstream_gene_variant	-	-0.00275	0.006690%	
c18_113c	rs29022551	HABP2	26	T/C	intron_variant	-	-0.00252	0.006667%	
c18_113c	rs41694130	CTSZ	13	T/G	-	-	0.002154	0.005283%	
c18_113c	rs17871529	CGN	3	C/G	upstream_gene_variant	-	0.002188	0.005260%	
c18_113c	rs41601769	CS	5	A/G	intron_variant	-	-0.00256	0.005237%	
c18_113c	rs42742546	SERPINI2	1	A/G	downstream_gene_variant	-	0.002755	0.005237%	
c18_113c	rs42211560	ADRP	15	T/C	missense_variant	-	0.002812	0.005214%	
c18_113c	rs42211560	ADFP	8	A/G	missense_variant	gCt/gTt	0.002662	0.004660%	
c18_113c	rs29023213	LDHB	5	T/C	intron_variant	-	0.001679	0.003253%	
c18_113c	rs41922087	SPHK1	19	A/G	upstream_gene_variant	-	-0.00315	0.003045%	
c18_113c	rs378738877	APM 1	1	A/G	upstream_gene_variant	-	0.00213	0.002907%	
c18_113c	rs41744055	MMP3	15	T/C	intron_variant	-	-0.0023	0.002884%	
c18_113c	rs41653368	UGDH	6	A/G	intron_variant	-	-0.00187	0.002676%	
c18_113c	rs41906356	PAFAH1B1	19	C/G	intron_variant	-	0.001623	0.002676%	
c18_113c	rs43727187	FGFR1	27	T/C	intron_variant	-	0.0022	0.002653%	
c18_113c	rs41932210	MAP2K6	19	A/C	downstream_gene_variant	-	-0.00139	0.002192%	
c18_113c	rs110953296	FABP5	14	T/C	downstream_gene_variant	-	0.001624	0.002169%	
c18_113c	rs41900388	PRKCG	18	T/C	intron_variant	-	0.002236	0.001915%	
c18_113c	rs41687544	DSTN	13	A/C	-	-	-0.00157	0.001892%	
c18_113c	rs379096458	ACAT2	5	C/G	downstream_gene_variant	-	0.001598	0.001799%	
c18_113c	rs41583157	TCF7L2	26	A/C	intron_variant	-	-0.00161	0.001569%	

c18_113c	rs41255521	STARD3	19	C/G	3_prime_UTR_variant	-	0.001549	0.001477%	
c18_113c	rs42197376	PC	29	A/G	3_prime_UTR_variant	-	-0.00208	0.001407%	
c18_113c	rs43229098	SIAT10	1	T/C	3_prime_UTR_variant	-	0.002033	0.001338%	
c18_113c	rs41764378	MRVI1	15	A/G	3_prime_UTR_variant	-	-0.00147	0.001315%	
c18_113c	rs109452913	ACBP	2	T/C	upstream_gene_variant	-	0.001595	0.001269%	
c18_113c	rs109368962	SAA3	29	A/G	intron_variant	-	-0.00174	0.001084%	
c18_113c	rs42096946	FGF8	26	A/C	downstream_gene_variant	-	-0.0014	0.001061%	
c18_113c	rs41767622	UCP3	15	T/C	intron_variant	-	-0.00209	0.001015%	
c18_113c	rs41926528	GRB2	19	A/G	upstream_gene_variant	-	-0.00153	0.000992%	
c18_113c	rs110625700	PPARA	5	C/G	synonymous_variant	-	-0.00158	0.000946%	
c18_113c	rs41576373	UGALT2	19	A/G	downstream_gene_variant	-	0.001361	0.000923%	
c18_113c	rs41767628	UCP3	15	T/C	intron_variant	-	-0.00168	0.000900%	
c18_113c	rs43369255	SIAT6	3	T/C	downstream_gene_variant	-	-0.00149	0.000807%	
c18_113c	rs42436380	HGF	4	A/G	intron_variant	-	0.001641	0.000738%	
c18_113c	rs43315761	TNS	2	C/G	missense_variant	gGt/gCt	-0.00142	0.000738%	
c18_113c	rs133786352	GHRH	13	A/G	intron_variant	-	-0.00143	0.000692%	
c18_113c	rs42175961	CSRP3	29	T/C	upstream_gene_variant	-	-0.00134	0.000508%	
c18_113c	rs17871427	PPM1B	11	A/G	downstream_gene_variant	-	0.001336	0.000415%	
c18_113c	rs43407618	ADCYAP1R1	4	T/C	intron_variant	-	-0.00127	0.000369%	
c18_113c	rs43508512	ACSL6	7	A/T	intron_variant	-	-0.00115	0.000323%	19.888%
c18_110t	rs43702942	UGDH	6	T/C	intron_variant	-	-0.01807	0.063222%	
c18_110t	rs42767950	ANKRD1	26	A/C	downstream_gene_variant	-	-0.02437	0.060161%	
c18_110t	rs42589207	PIK3R1	20	T/G	synonymous_variant	acC/acA	-0.01508	0.042966%	
c18_110t	rs41696920	ATRN	13	A/G	missense_variant	aGg/aAg	-0.00611	0.007249%	

c18_110t	rs134451630	SIAT4A	14	A/G	intron_variant	-	0.005963	0.007008%	
c18_110t	rs41910301	MYH8	19	A/G	downstream_gene_variant	-	-0.00436	0.003745%	
c18_110t	rs41729168	FABP9	14	A/G	intron_variant	-	-0.00736	0.003730%	
c18_110t	rs29015741	ACVR1	2	A/G	intron_variant	-	-0.0043	0.003342%	
c18_110t	rs41613049	BDH1	1	T/C	intron_variant	-	-0.00451	0.003247%	
c18_110t	rs41745642	PAFAH1B2	15	C/G	intron_variant	-	0.008468	0.003152%	
c18_110t	rs41768431	UCP3	15	T/G	intron_variant	-	0.003982	0.003100%	
c18_110t	rs41932855	BTF3	20	T/G	intron_variant	-	-0.00439	0.003061%	
c18_110t	rs43406303	ADCY1	4	A/G	intron_variant	-	0.003921	0.003033%	
c18_110t	rs29012834	FGF12	1	T/C	intron_variant	-	0.005001	0.002938%	
c18_110t	rs41686830	DSTN	13	T/G	intron_variant	-	-0.00429	0.002799%	
c18_110t	rs110953296	FABP5	14	T/C	downstream_gene_variant	-	-0.00398	0.002253%	
c18_110t	rs41974998	MFGE8	21	T/G	3_prime_UTR_variant	-	-0.00322	0.001995%	
c18_110t	rs43346270	PRKACB	3	A/G	intron_variant	-	-0.0033	0.001825%	
c18_110t	rs41640705	IGF1R	21	A/G	intron_variant	-	-0.00371	0.001659%	
c18_110t	rs43576438	IL7	14	T/C	intron_variant	-	0.00313	0.001627%	
c18_110t	rs43663540	DGUCK	11	T/G	intron_variant	-	0.003554	0.001497%	
c18_110t	rs41601769	CS	5	A/G	intron_variant	-	0.003189	0.001394%	
c18_110t	rs42089635	LOXL4	26	A/G	downstream_gene_variant	-	0.002898	0.001358%	
c18_110t	rs17871529	CGN	3	C/G	upstream_gene_variant	-	-0.00262	0.001295%	
c18_110t	rs41718866	DDEF1	14	C/G	3_prime_UTR_variant	-	-0.00348	0.001136%	
c18_110t	rs41606036	PCDHA13	7	A/G	missense_variant	aCa/aTa	-0.00291	0.000926%	
c18_110t	rs208474334	TIEG2	11	T/C	missense_variant	-	0.003948	0.000804%	
c18_110t	rs41600452	FGF12	1	T/G	intron_variant	-	0.003025	0.000729%	
c18_110t	rs43560146	LPL	8	T/C	upstream_gene_variant	-	0.002683	0.000721%	

c18_110t	rs41793400	GNA14	8	T/C	5_prime_UTR_variant	-	-0.00262	0.000594%	
c18_110t	rs42180931	SIAE	29	A/G	intron_variant	-	-0.00259	0.000542%	
c18_110t	rs42605121	SLC8A1	11	A/G	intron_variant	-	-0.00245	0.000455%	
c18_110t	rs208317364	DGAT1	14	A/G	intron_variant	-	-0.00286	0.000408%	
c18_110t	rs42358344	CALCR	4	A/G	downstream_gene_variant	-	-0.002	0.000305%	
c18_110t	rs109368962	SAA3	29	A/G	intron_variant	-	-0.0021	0.000273%	0.235%
c18_111t	rs43648117	SNW1	10	T/C	downstream_gene_variant	-	-0.00311	0.018801%	
c18_111t	rs41847581	ACADS	17	T/C	intron_variant	-	0.002657	0.013317%	
c18_111t	rs42115578	ACSL1	27	C/G	synonymous_variant	tcG/tcC	-0.00208	0.007795%	
c18_111t	rs42115578	FACL2	27	C/G	synonymous_variant	-	-0.00206	0.007599%	
c18_111t	rs41567825	INSR	7	C/G	intron_variant	-	-0.00207	0.006933%	
c18_111t	rs42714483	THRSP	29	T/C	missense_variant	Atc/Gtc	0.001875	0.006854%	
c18_111t	rs41784334	ACAD8	15	C/G	3_prime_UTR_variant	-	0.002329	0.006776%	
c18_111t	rs41642657	ACADVL	19	T/C	downstream_gene_variant	-	-0.00216	0.006659%	
c18_111t	rs41255193	HADHB	11	T/C	3_prime_UTR_variant	-	-0.00174	0.005797%	
c18_111t	rs43707854	SOCS2	5	A/C	3_prime_UTR_variant	-	-0.00176	0.005523%	
c18_111t	rs109221039	CAST	7	A/G	3_prime_UTR_variant	-	-0.00177	0.004583%	
c18_111t	rs43576438	IL7	14	T/C	intron_variant	-	-0.00154	0.003917%	
c18_111t	rs43707861	THBS	4	A/G	missense_variant	Atc/Gtc	-0.00145	0.003917%	
c18_111t	rs42268967	TWIST1	4	T/C	downstream_gene_variant	-	-0.00154	0.003799%	
c18_111t	rs41718866	DDEF1	14	C/G	3_prime_UTR_variant	-	0.001913	0.003408%	
c18_111t	rs41796045	GNA14	8	T/G	intron_variant	-	0.001207	0.002859%	
c18_111t	rs43267303	AGTR1	1	T/C	intergenic_variant	-	-0.00157	0.002546%	
c18_111t	rs43706499	CCL2	19	A/G	-	-	0.001167	0.002468%	

c18_111t	rs42188426	EEF1G	29	T/C	intron_variant	-	-0.00223	0.002389%	
c18_111t	rs41594003	SIAT8A	5	A/G	intron_variant	-	0.001083	0.002115%	
c18_111t	rs43687642	SCD5	6	T/G	3_prime_UTR_variant	-	-0.00105	0.002076%	
c18_111t	rs17871740	PCK1	13	A/G	intron_variant	-	0.00116	0.001958%	
c18_111t	rs43724661	ACLY	23	T/C	synonymous_variant	ggT/ggC	0.001221	0.001919%	
c18_111t	rs41741805	EIF3S3	14	T/C	intron_variant	-	-0.00119	0.001880%	
c18_111t	rs41641850	IDH1	2	A/G	intron_variant	-	0.001042	0.001802%	
c18_111t	rs41847792	MVK	17	A/G	intron_variant	-	-0.00096	0.001763%	
c18_111t	rs41987137	COL4A4	2	T/C	intron_variant	-	-0.00122	0.001763%	
c18_111t	rs43251315	CDH9	20	A/G	missense_variant	Cca/Tca	-0.00128	0.001528%	
c18_111t	rs42197376	PC	29	A/G	3_prime_UTR_variant	-	0.001576	0.001371%	
c18_111t	rs41916108	DCT	12	A/C	synonymous_variant	Cga/Aga	-0.00096	0.001332%	
c18_111t	rs43407600	GHRHR	4	T/C	intron_variant	-	-0.00156	0.001253%	
c18_111t	rs41583801	DNMT2	13	A/G	intron_variant	-	-0.00128	0.001175%	
c18_111t	rs42176298	SLC37A2	29	T/C	intron_variant	-	0.001038	0.000979%	
c18_111t	rs41656364	VIL2	9	T/C	intron_variant	-	-0.00117	0.000940%	
c18_111t	rs110061082	UCP3	15	T/C	intron_variant	-	-0.00107	0.000862%	
c18_111t	rs43229098	SIAT10	1	T/C	3_prime_UTR_variant	-	-0.00097	0.000509%	0.141%
MUFA	rs43663540	DGUCK	11	T/G	intron_variant	-	0.006462	0.000143%	
MUFA	rs41256865	GPD1	5	A/G	3_prime_UTR_variant	-	-0.00314	0.000055%	
MUFA	rs110953296	FABP5	14	T/C	downstream_gene_variant	-	0.003508	0.000050%	
MUFA	rs42196904	IGF2	29	A/G	intron_variant	-	-0.00304	0.000042%	
MUFA	rs42113899	FAT	27	T/C	missense_variant	Gtg/Atg	-0.00306	0.000041%	
MUFA	rs42115578	FACL2	27	C/G	synonymous_variant	-	0.002738	0.000039%	

MUFA	rs17871529	CGN	3	C/G	upstream_gene_variant	-	0.002595	0.000037%	
MUFA	rs41646367	ANXA11	28	A/G	synonymous_variant	gcC/gcT	0.002408	0.000033%	
MUFA	rs41255690	SCD1	26	A/G	intron_variant	-	-0.00247	0.000032%	
MUFA	rs43707854	SOCS2	5	A/C	3_prime_UTR_variant	-	0.002477	0.000032%	
MUFA	rs109763947	IGF1	5	T/C	upstream_gene_variant	-	-0.00236	0.000032%	
MUFA	rs29024247	SLCO2B1	15	A/G	synonymous_variant	gtA/gtG	0.00276	0.000031%	
MUFA	rs41652470	SIRT1	28	A/G	upstream_gene_variant	-	-0.00232	0.000030%	
MUFA	rs41909257	ALOX12	19	A/T	splice_region_variant,intron_variant	-	0.002332	0.000028%	
MUFA	rs42742546	SERPINI2	1	A/G	downstream_gene_variant	-	0.002794	0.000027%	
MUFA	rs43494871	PROM1	6	T/G	missense_variant	ttA/ttC	0.003349	0.000026%	
MUFA	rs41694130	CTSZ	13	T/G	-	-	0.002134	0.000026%	
MUFA	rs29022551	HABP2	26	T/C	intron_variant	-	-0.00218	0.000025%	
MUFA	rs41884788	CEBPG	18	A/G	downstream_gene_variant	-	0.002769	0.000024%	
MUFA	rs41653368	UGDH	6	A/G	intron_variant	-	-0.00249	0.000024%	
MUFA	rs43235355	CASR	1	A/C	intron_variant	-	-0.00278	0.000023%	
MUFA	rs43648117	SNW1	10	T/C	downstream_gene_variant	-	0.002018	0.000023%	
MUFA	#N/A	ACSF3	18	C/T	-	-	0.00238	0.000022%	
MUFA	rs109697714	GHRH	13	T/C	intron_variant	-	0.001939	0.000021%	
MUFA	rs41907825	ALOX15	19	C/G	intron_variant	-	-0.00197	0.000021%	
MUFA	rs43724308	MGLL	22	T/C	synonymous_variant	agT/agC	-0.00246	0.000021%	
MUFA	rs41708480	HNF4A	13	A/C	intron_variant	-	-0.0019	0.000020%	
MUFA	rs41800338	NME7	16	A/G	intron_variant	-	0.002119	0.000019%	
MUFA	rs42243023	NEUROD1	2	T/G	upstream_gene_variant	-	0.001844	0.000019%	
MUFA	rs43710977	GSTM1	3	A/C	intron_variant	-	0.001793	0.000018%	
MUFA	rs29002484	IDH1	2	A/C	intron_variant	-	0.001766	0.000017%	

MUFA	rs210864945	APM 1	1	A/G	intron_variant	-	0.001724	0.000016%	
MUFA	rs41845684	OASL	17	C/G	3_prime_UTR_variant	-	-0.00166	0.000016%	
MUFA	rs41687553	DSTN	13	T/C	intron_variant	-	0.001747	0.000015%	
MUFA	rs41844655	PRKAB1	17	A/G	downstream_gene_variant	-	0.002191	0.000015%	
MUFA	rs42090456	TNFRSF6	26	C/G	intron_variant	-	-0.00176	0.000015%	
MUFA	rs41900392	PRKCG	18	T/C	downstream_gene_variant	-	-0.0016	0.000015%	
MUFA	rs29021775	TG	14	A/G	intergenic_variant	-	0.001647	0.000014%	
MUFA	rs43315799	TNS	2	A/G	intron_variant	-	0.001537	0.000013%	
MUFA	rs42905009	MOGAT2	15	T/G	intron_variant	-	-0.00152	0.000013%	
MUFA	rs43717444	GSS	13	T/G	intron_variant	-	-0.00151	0.000013%	
MUFA	rs43709215	TXNIP	3	C/G	intron_variant	-	0.001892	0.000012%	
MUFA	rs41890207	SDR3	18	T/C	downstream_gene_variant	-	-0.00151	0.000011%	
MUFA	rs17870648	NR1H3	15	A/C	synonymous_variant	ctG/ctT	-0.00141	0.000011%	
MUFA	rs43489995	DGKQ	6	A/G	downstream_gene_variant	-	-0.00139	0.000011%	
MUFA	rs17871740	PCK1	13	A/G	intron_variant	-	-0.00158	0.000011%	
MUFA	rs41800886	PLOD	16	T/C	downstream_gene_variant	-	-0.00155	0.000011%	
MUFA	rs41849827	ADRBK2	17	T/C	downstream_gene_variant	-	-0.00152	0.000011%	
MUFA	rs41886803	ATP1A3	18	A/C	intron_variant	-	0.001482	0.000011%	
MUFA	rs41863915	CDH1	18	A/G	intergenic_variant	-	0.001359	0.000010%	
MUFA	rs41255193	HADHB	11	A/G	3_prime_UTR_variant	-	0.001357	0.000010%	
MUFA	rs42183386	DCPS	29	T/C	3_prime_UTR_variant	-	0.001411	0.000009%	
MUFA	rs380876224	SOAT1	16	T/C	upstream_gene_variant	-	0.001519	0.000009%	
MUFA	rs43267303	AGTR1	1	T/C	intergenic_variant	-	0.001755	0.000009%	
MUFA	rs43382870	SCIN	4	A/G	3_prime_UTR_variant	-	0.001311	0.000008%	
MUFA	rs41632202	WISP1	14	A/T	intron_variant	-	-0.0014	0.000008%	

MUFA	rs42411170	FGFR3	6	T/C	intron_variant	-	0.001399	0.000008%	
MUFA	rs43476247	CXCL10	6	T/G	downstream_gene_variant	-	0.001948	0.000008%	
MUFA	rs43720495	RARA	19	T/C	intron_variant	-	-0.00139	0.000008%	
MUFA	rs41755155	APOC3	15	T/C	3_prime_UTR_variant	-	-0.00177	0.000007%	
MUFA	rs43429740	SYT1	5	T/G	intron_variant	-	0.001518	0.000007%	
MUFA	rs384562096	ACADM	3	A/G	3_prime_UTR_variant	-	0.001137	0.000007%	
MUFA	rs43675525	FSHR	11	A/G	intron_variant	-	0.001279	0.000007%	
MUFA	rs42211560	ADFP	8	A/G	missense_variant	gCt/gTt	0.001354	0.000006%	
MUFA	rs109346428	FABP4	14	T/C	intron_variant	-	-0.00109	0.000006%	
MUFA	rs41961336	IGF1R	21	T/C	synonymous_variant	gaT/gaC	-0.00132	0.000006%	
MUFA	rs42176298	SLC37A2	29	T/C	intron_variant	-	-0.00148	0.000006%	
MUFA	rs43707575	PNPLA2	29	T/C	downstream_gene_variant	-	-0.00125	0.000006%	
MUFA	rs41255257	AP2B1	19	T/C	intergenic_variant	-	0.001439	0.000005%	
MUFA	rs42194738	INS	29	T/C	downstream_gene_variant	-	0.001283	0.000005%	
MUFA	rs41597184	LIAS	6	T/C	intron_variant	-	0.00113	0.000005%	
MUFA	rs43575364	MUSK	8	T/C	upstream_gene_variant	-	-0.0011	0.000005%	
MUFA	rs29004508	LEP	4	T/C	missense_variant	gCg/gTg	-0.00131	0.000005%	
MUFA	rs43242931	GAP43	1	C/G	synonymous_variant	ccG/ccC	0.001295	0.000004%	
MUFA	rs41567325	TG	14	T/C	-	-	0.00152	0.000004%	
MUFA	rs42149515	NEUROG3	28	A/T	downstream_gene_variant	-	0.001482	0.000004%	
MUFA	rs43289839	PRKRA	2	T/G	intron_variant	-	-0.00163	0.000004%	
MUFA	rs43359099	EPS15	3	C/G	intron_variant	-	0.001155	0.000004%	
MUFA	rs43702510	DDEF1	14	T/C	synonymous_variant	ggT/ggC	0.00117	0.000003%	
MUFA	rs42500029	FAT3	29	A/G	missense_variant	Gtg/Atg	0.001043	0.000003%	
MUFA	rs42767950	ANKRD1	26	A/C	downstream_gene_variant	-	-0.00105	0.000003%	

MUFA	rs43229098	SIAT10	1	T/C	3_prime_UTR_variant	-	0.001415	0.000003%	
MUFA	rs43306652	GRB14	2	C/G	intron_variant	-	-0.00113	0.000003%	
MUFA	rs41600452	FGF12	1	T/G	intron_variant	-	-0.00106	0.000003%	
MUFA	rs43317359	CRYBA2	3	T/G	intron_variant	-	0.001043	0.000003%	
MUFA	rs43727187	FGFR1	27	T/C	intron_variant	-	0.000959	0.000003%	
MUFA	rs109368962	SAA3	29	A/G	intron_variant	-	-0.00104	0.000002%	
MUFA	rs42605121	SLC8A1	11	A/G	intron_variant	-	-0.00093	0.000002%	
MUFA	rs41654029	ATP2B1	5	T/G	intron_variant	-	0.000968	0.000002%	
MUFA	rs43649421	TSHR	10	T/C	synonymous_variant	atC/atT	-0.00102	0.000002%	
MUFA	rs41729168	FABP9	14	A/G	intron_variant	-	0.000873	0.000001%	
MUFA	rs43326496	COL4A3	2	A/G	missense_variant	aGa/aAa	0.001119	0.000001%	
MUFA	rs41781896	LRP4	15	T/C	missense_variant	aTg/aCg	-0.00095	0.000001%	
MUFA	#N/A	DGAT1	14	T/G	-	-	0.000887	0.000001%	0.001%
c18_2n6	rs43702942	UGDH	6	T/C	intron_variant	-	-0.01168	0.156317%	
c18_2n6	rs17872093	CAPN1	29	T/C	synonymous_variant	ggT/ggC	0.004082	0.019484%	
c18_2n6	rs41640705	IGF1R	21	A/G	intron_variant	-	-0.0049	0.017142%	
c18_2n6	rs43051819	DPP4	2	A/C	upstream_gene_variant	-	0.003148	0.011545%	
c18_2n6	rs41255232	TMEM175	6	A/G	downstream_gene_variant	-	-0.00332	0.010702%	
c18_2n6	rs41899395	MYH1	19	T/G	downstream_gene_variant	-	0.003072	0.010140%	
c18_2n6	rs29014633	CACNG2	5	A/T	intron_variant	-	0.002699	0.008009%	
c18_2n6	rs42589207	PIK3R1	20	T/G	synonymous_variant	acC/acA	-0.00268	0.008009%	
c18_2n6	rs29015741	ACVR1	2	A/G	intron_variant	-	-0.00273	0.007939%	
c18_2n6	rs42399155	NOX4	29	T/C	synonymous_variant	aaT/aaC	0.0028	0.006698%	
c18_2n6	rs41910301	MYH8	19	A/G	downstream_gene_variant	-	-0.00227	0.006019%	

c18_2n6	rs41764713	TRPC2	15	A/G	synonymous_variant	acT/acC	-0.00227	0.005995%	
c18_2n6	rs135871423	TIEG2	11	A/G	missense_variant	-	-0.00241	0.005855%	
c18_2n6	rs42767950	ANKRD1	26	A/C	downstream_gene_variant	-	-0.0029	0.005035%	
c18_2n6	rs42827362	ACADM	3	T/C	synonymous_variant	-	0.001972	0.004520%	
c18_2n6	rs42794062	FGF18	18	T/C	intron_variant	-	0.001991	0.004496%	
c18_2n6	rs41687544	DSTN	13	A/C	-	-	0.002388	0.004426%	
c18_2n6	rs41772033	SLCO2B1	15	A/G	3_prime_UTR_variant	-	-0.0023	0.004028%	
c18_2n6	rs41610128	UGP2	11	A/G	intron_variant	-	0.001942	0.003981%	
c18_2n6	rs43372452	SCIN	4	T/C	synonymous_variant	agC/agT	0.0019	0.003817%	
c18_2n6	rs41768494	P4HA3	15	A/G	intron_variant	-	-0.0017	0.003396%	
c18_2n6	rs43560146	LPL	8	T/C	upstream_gene_variant	-	0.002397	0.003396%	
c18_2n6	rs109452913	ACBP	2	T/C	upstream_gene_variant	-	-0.00259	0.003372%	
c18_2n6	rs41907824	ALOX15	19	T/C	intron_variant	-	-0.00166	0.003208%	
c18_2n6	rs42358344	CALCR	4	A/G	downstream_gene_variant	-	-0.00267	0.003208%	
c18_2n6	rs109450360	IGF2R	9	A/G	intron_variant	-	0.001647	0.003185%	
c18_2n6	rs41768431	UCP3	15	T/G	intron_variant	-	0.001606	0.002974%	
c18_2n6	rs380876224	SOAT1	16	T/C	upstream_gene_variant	-	0.001896	0.002951%	
c18_2n6	rs41632203	WISP1	14	T/C	upstream_gene_variant	-	0.001662	0.002951%	
c18_2n6	rs43562598	AQP7	8	T/C	downstream_gene_variant	-	0.001612	0.002763%	
c18_2n6	rs42421976	PPP1R3A	4	A/C	downstream_gene_variant	-	0.002432	0.002506%	
c18_2n6	rs43299525	SCN7A	2	A/G	missense_variant	Tat/Cat	-0.00156	0.002365%	
c18_2n6	rs43433318	MYF5	5	A/G	intron_variant	-	-0.00146	0.002365%	
c18_2n6	rs17871529	CGN	3	C/G	upstream_gene_variant	-	-0.00144	0.002318%	
c18_2n6	rs43347904	LEPR	3	T/C	missense_variant	tTc/tCc	-0.00141	0.002295%	
c18_2n6	rs41906365	PAFAH1B1	19	T/C	intron_variant	-	-0.00146	0.002178%	

c18_2n6	rs43306652	GRB14	2	C/G	intron_variant	-	0.002185	0.002131%	
c18_2n6	rs211581461	LIPE	18	A/G	missense_variant	-	0.002104	0.002061%	
c18_2n6	rs109368962	SAA3	29	A/G	intron_variant	-	-0.00234	0.001991%	
c18_2n6	rs41809801	PRKCZ	16	A/C	intron_variant	-	-0.00149	0.001756%	
c18_2n6	rs41634418	PISD	17	A/G	intron_variant	-	0.001575	0.001733%	
c18_2n6	rs41915684	STAT5B	19	T/G	intron_variant	-	-0.0014	0.001592%	
c18_2n6	rs137651874	GH1	19	T/C	intron_variant	-	0.001482	0.001499%	
c18_2n6	rs43383602	CROT	4	T/C	intron_variant	-	0.001411	0.001499%	
c18_2n6	rs43476247	CXCL10	6	T/G	downstream_gene_variant	-	0.001806	0.001335%	
c18_2n6	rs110061082	UCP4	15	T/C	intron_variant	-	0.001498	0.001030%	
c18_2n6	rs43315761	TNS	2	C/G	missense_variant	gGt/gCt	0.001578	0.000937%	
c18_2n6	rs41745644	PAFAH1B2	15	T/C	intron_variant	-	0.001874	0.000913%	
c18_2n6	rs43407600	GHRHR	4	T/C	intron_variant	-	0.001632	0.000820%	
c18_2n6	rs42821720	API5	15	A/G	intron_variant	-	0.001415	0.000656%	0.370%
PUFA	rs109450360	IGF2R	9	A/G	intron_variant	-	0.016645	0.128329%	
PUFA	rs43702942	UGDH	6	T/C	intron_variant	-	-0.01209	0.066258%	
PUFA	rs41961336	IGF1R	21	T/C	synonymous_variant	gaT/gaC	-0.0115	0.035001%	
PUFA	rs41768494	P4HA3	15	A/G	intron_variant	-	-0.00692	0.022197%	
PUFA	rs29014633	CACNG2	5	A/T	intron_variant	-	0.006968	0.021095%	
PUFA	rs42399155	NOX4	29	T/C	synonymous_variant	aaT/aaC	0.007828	0.020724%	
PUFA	rs41257366	POMC	11	A/G	synonymous_variant	ggC/ggT	-0.00584	0.015258%	
PUFA	rs42794062	FGF18	18	T/C	intron_variant	-	0.005766	0.014953%	
PUFA	rs42589207	PIK3R1	20	T/G	synonymous_variant	acC/acA	-0.00544	0.013081%	
PUFA	rs43663565	DGUCK	11	T/C	intron_variant	-	0.005691	0.012423%	

PUFA	rs43562598	AQP7	8	T/C	downstream_gene_variant	-	0.005212	0.011404%	
PUFA	rs42767950	ANKRD1	26	A/C	downstream_gene_variant	-	-0.00665	0.010487%	
PUFA	rs41744783	CASP1	15	T/C	intergenic_variant	-	-0.00451	0.008773%	
PUFA	rs41255352	TNS	2	C/G	3_prime_UTR_variant	-	-0.00392	0.006967%	
PUFA	rs41886799	ATP1A3	18	A/G	intron_variant	-	-0.00373	0.005410%	
PUFA	rs41910301	MYH8	19	A/G	downstream_gene_variant	-	-0.00341	0.005355%	
PUFA	rs41632203	WISP1	14	T/C	upstream_gene_variant	-	0.003493	0.005151%	
PUFA	rs41255193	HADHB	11	A/G	3_prime_UTR_variant	-	-0.00332	0.004984%	
PUFA	rs41610128	UGP2	11	A/G	intron_variant	-	0.003368	0.004734%	
PUFA	rs380876224	SOAT1	16	T/C	upstream_gene_variant	-	0.003748	0.004567%	
PUFA	rs41764713	TRPC2	15	A/G	synonymous_variant	acT/acC	-0.0031	0.004456%	
PUFA	rs41796045	GNA14	8	T/G	intron_variant	-	0.002963	0.004067%	
PUFA	rs41772033	SLCO2B1	15	A/G	3_prime_UTR_variant	-	-0.00361	0.003947%	
PUFA	rs41588659	COL1A2	4	A/G	intron_variant	-	-0.00319	0.003826%	
PUFA	rs43051819	DPP4	2	A/C	upstream_gene_variant	-	0.002732	0.003437%	
PUFA	rs43476247	CXCL10	6	T/G	downstream_gene_variant	-	0.004605	0.003409%	
PUFA	rs42311181	HSD17B12	15	T/G	missense_variant	aaT/aaG	-0.00284	0.003363%	
PUFA	rs42436359	HGF	4	A/G	synonymous_variant	caA/caG	-0.00299	0.003307%	
PUFA	rs41809801	PRKCZ	16	A/C	intron_variant	-	-0.0032	0.003215%	
PUFA	rs43667662	ALMS1	11	T/C	downstream_gene_variant	-	0.002774	0.002928%	
PUFA	rs42089635	LOXL4	26	A/G	downstream_gene_variant	-	0.002757	0.002872%	
PUFA	rs43499691	INSL3	7	T/G	downstream_gene_variant	-	-0.00253	0.002816%	
PUFA	rs41600007	TGFA	11	T/C	intergenic_variant	-	0.002898	0.002594%	
PUFA	rs41853830	ISCU	17	T/C	upstream_gene_variant	-	-0.00228	0.002399%	
PUFA	rs43560146	LPL	8	T/C	upstream_gene_variant	-	0.003173	0.002362%	

PUFA	rs42660323	PFN2	1	C/G	intergenic_variant	-	-0.00231	0.002344%	
PUFA	rs41915684	STAT5B	19	T/G	intron_variant	-	-0.00251	0.002029%	
PUFA	rs41780349	PAMR1	15	T/C	intron_variant	-	0.002393	0.001983%	
PUFA	rs42358344	CALCR	4	A/G	downstream_gene_variant	-	-0.00331	0.001946%	
PUFA	rs109452913	ACBP	2	T/C	upstream_gene_variant	-	-0.00309	0.001899%	
PUFA	rs110512442	ANXA9	3	A/G	intron_variant	-	-0.00213	0.001881%	
PUFA	rs109300983	GHR	20	A/G	missense_variant	-	0.002301	0.001871%	
PUFA	rs17872093	CAPN1	29	T/C	synonymous_variant	ggT/ggC	0.001962	0.001779%	
PUFA	rs42821720	API5	15	A/G	intron_variant	-	0.00362	0.001714%	
PUFA	rs41255759	IL8	6	T/C	3_prime_UTR_variant	-	-0.00211	0.001362%	
PUFA	rs137651874	GH1	19	T/C	intron_variant	-	0.002162	0.001260%	
PUFA	rs41255492	LGALS9	19	T/C	synonymous_variant	gcC/gcT	0.002455	0.001158%	
PUFA	rs42421976	PPP1R3A	4	A/C	downstream_gene_variant	-	0.002623	0.001149%	
PUFA	rs43365624	SFPQ	3	A/G	upstream_gene_variant	-	-0.00242	0.001121%	
PUFA	rs43306652	GRB14	2	C/G	intron_variant	-	0.002289	0.000926%	
PUFA	rs43317359	CRYBA2	3	T/G	intron_variant	-	0.00214	0.000880%	
PUFA	rs41691161	ABHD12	13	C/G	downstream_gene_variant	-	-0.00228	0.000769%	
PUFA	rs109368962	SAA3	29	A/G	intron_variant	-	-0.00229	0.000750%	
PUFA	rs41745644	PAFAH1B2	15	T/C	intron_variant	-	0.002579	0.000686%	
PUFA	rs42605121	SLC8A1	11	A/G	intron_variant	-	-0.00197	0.000686%	
PUFA	rs17870222	IGFBP3	4	T/C	intron_variant	-	0.001748	0.000537%	
PUFA	rs42883159	CAPZB	2	T/C	-	-	0.002083	0.000537%	
PUFA	rs208317364	DGAT1	14	A/G	intron_variant	-	-0.00208	0.000500%	
PUFA	rs41904273	ACADVL	19	A/G	downstream_gene_variant	-	0.001684	0.000259%	
PUFA	rs41849704	ASCC2	17	A/G	intron_variant	-	0.001694	0.000250%	0.496%

n6	rs43702942	UGDH	6	T/C	intron_variant	-	-0.00845	0.070628%	
n6	rs41640705	IGF1R	21	A/G	intron_variant	-	-0.00591	0.021508%	
n6	rs17872093	CAPN1	29	T/C	synonymous_variant	ggT/ggC	0.004344	0.019062%	
n6	rs42589207	PIK3R1	20	T/G	synonymous_variant	acC/acA	-0.00335	0.010794%	
n6	rs29014633	CACNG2	5	A/T	intron_variant	-	0.003089	0.009056%	
n6	rs43051819	DPP4	2	A/C	upstream_gene_variant	-	0.002836	0.008086%	
n6	rs41255232	TMEM175	6	A/G	downstream_gene_variant	-	-0.00297	0.007419%	
n6	rs42794062	FGF18	18	T/C	intron_variant	-	0.002691	0.007115%	
n6	rs29015741	ACVR1	2	A/G	intron_variant	-	-0.00272	0.006792%	
n6	rs42767950	ANKRD1	26	A/C	downstream_gene_variant	-	-0.00356	0.006570%	
n6	rs41899395	MYH1	19	T/G	downstream_gene_variant	-	0.002486	0.005721%	
n6	rs42358344	CALCR	4	A/G	downstream_gene_variant	-	-0.00383	0.005660%	
n6	rs41686830	DSTN	13	T/G	intron_variant	-	-0.00257	0.005134%	
n6	rs41768494	P4HA3	15	A/G	intron_variant	-	-0.00207	0.004326%	
n6	rs43560146	LPL	8	T/C	upstream_gene_variant	-	0.002873	0.004225%	
n6	rs41910301	MYH8	19	A/G	downstream_gene_variant	-	-0.00202	0.004103%	
n6	rs109450360	IGF2R	9	A/G	intron_variant	-	0.001968	0.003922%	
n6	rs41632203	WISP1	14	T/C	upstream_gene_variant	-	0.002045	0.003861%	
n6	rs41764713	TRPC2	15	A/G	synonymous_variant	acT/acC	-0.00195	0.003820%	
n6	rs380876224	SOAT1	16	T/C	upstream_gene_variant	-	0.002286	0.003699%	
n6	rs41772033	SLCO2B1	15	A/G	3_prime_UTR_variant	-	-0.00235	0.003639%	
n6	rs41768431	UCP3	15	T/G	intron_variant	-	0.001891	0.003558%	
n6	rs41906365	PAFAH1B1	19	T/C	intron_variant	-	-0.00186	0.003073%	
n6	rs135871423	TIEG2	11	A/G	missense_variant	-	-0.00185	0.002971%	

n6	rs109452913	ACBP	2	T/C	upstream_gene_variant	-	-0.00257	0.002870%	
n6	rs43372452	SCIN	4	T/C	synonymous_variant	agC/agT	0.001757	0.002810%	
n6	rs43433318	MYF5	5	A/G	intron_variant	-	-0.00163	0.002567%	
n6	rs41610128	UGP2	11	A/G	intron_variant	-	0.001671	0.002547%	
n6	rs43707854	SOCS2	5	A/C	3_prime_UTR_variant	-	0.001664	0.002547%	
n6	rs42089635	LOXL4	26	A/G	downstream_gene_variant	-	0.001749	0.002527%	
n6	rs42399155	NOX4	29	T/C	synonymous_variant	aaT/aaC	0.001734	0.002224%	
n6	rs43687642	SCD5	6	T/G	3_prime_UTR_variant	-	0.001472	0.002122%	
n6	rs41255762	IL8	6	A/G	3_prime_UTR_variant	-	0.001732	0.002001%	
n6	rs42421976	PPP1R3A	4	A/C	downstream_gene_variant	-	0.002262	0.001860%	
n6	rs137651874	GH1	19	T/C	intron_variant	-	0.001743	0.001779%	
n6	rs43306652	GRB14	2	C/G	intron_variant	-	0.002129	0.001759%	
n6	rs43476247	CXCL10	6	T/G	downstream_gene_variant	-	0.002142	0.001617%	
n6	rs41634418	PISD	17	A/G	intron_variant	-	0.001588	0.001516%	
n6	rs42096946	FGF8	26	A/C	downstream_gene_variant	-	-0.00164	0.001294%	
n6	rs109368962	SAA3	29	A/G	intron_variant	-	-0.00192	0.001152%	
n6	rs110061082	UCP6	15	T/C	intron_variant	-	0.001694	0.001132%	
n6	rs211581461	LIPE	18	A/G	missense_variant	-	0.001426	0.000809%	
n6	rs43315761	TNS	2	C/G	missense_variant	gGt/gCt	0.001543	0.000768%	
n6	rs41745642	PAFAH1B2	15	C/G	intron_variant	-	0.001588	0.000566%	0.261%
n6_n3	rs43315204	PRKAG3	2	T/C	intron_variant	-	0.013993	0.006425%	
n6_n3	rs42268967	TWIST1	4	T/C	downstream_gene_variant	-	0.013818	0.005364%	
n6_n3	rs41644756	RABGGTA	10	A/G	intron_variant	-	-0.01154	0.004229%	
n6_n3	rs41916109	DCT	12	T/C	3_prime_UTR_variant	-	0.012571	0.003907%	

n6_n3	rs41582778	SP2	19	T/C	intron_variant	-	0.010277	0.003115%	
n6_n3	rs42115578	FACL2	27	C/G	synonymous_variant	-	0.009788	0.003002%	
n6_n3	rs42115578	ACSL1	27	C/G	synonymous_variant	tcG/tcC	0.009549	0.002857%	
n6_n3	rs41755156	APOC3	15	T/C	intron_variant	-	0.009628	0.002482%	
n6_n3	rs41774218	UCP2	15	T/C	intron_variant	-	-0.00894	0.002320%	
n6_n3	rs42243023	NEUROD1	2	T/G	upstream_gene_variant	-	0.008121	0.002229%	
n6_n3	rs110061082	UCP9	15	T/C	intron_variant	-	0.010631	0.001498%	
n6_n3	rs29020989	TGM1	10	A/G	intron_variant	-	0.007441	0.001435%	
n6_n3	rs41696920	ATRN	13	A/G	missense_variant	aGg/aAg	-0.00626	0.001311%	
n6_n3	rs42827372	ACADM	3	T/C	upstream_gene_variant	-	0.006359	0.001088%	
n6_n3	rs42714483	THRSP	29	T/C	missense_variant	Atc/Gtc	-0.00556	0.001052%	
n6_n3	rs42127354	DCTN6	27	C/G	upstream_gene_variant	-	0.005594	0.001011%	
n6_n3	rs41897477	EMP3	18	A/G	downstream_gene_variant	-	0.00849	0.000973%	
n6_n3	rs41768494	P4HA3	15	A/G	intron_variant	-	0.004938	0.000831%	
n6_n3	rs43707854	SOCS2	5	A/C	3_prime_UTR_variant	-	0.005159	0.000825%	
n6_n3	rs41845704	OASL	17	C/G	downstream_gene_variant	-	0.005021	0.000759%	
n6_n3	rs43485037	PTPN13	6	A/T	intergenic_variant	-	-0.00468	0.000727%	
n6_n3	rs43623727	RYR3	10	T/G	missense_variant	gaC/gaA	0.007046	0.000715%	
n6_n3	rs17870507	ACSL6	7	A/G	intron_variant	-	-0.0057	0.000700%	
n6_n3	rs41892122	POLD1	18	A/G	synonymous_variant	ctA/ctC	0.00502	0.000671%	
n6_n3	rs29015741	ACVR1	2	A/G	intron_variant	-	-0.00463	0.000666%	
n6_n3	rs41636478	PDE6D	2	A/G	intron_variant	-	-0.00418	0.000593%	
n6_n3	rs43407600	GHRHR	4	T/C	intron_variant	-	0.008009	0.000568%	
n6_n3	rs41741743	EIF3S3	14	A/G	intron_variant	-	-0.0042	0.000556%	
n6_n3	rs41603759	PAFAH1B2	15	A/G	intron_variant	-	0.004403	0.000545%	

n6_n3	rs43734541	STAR	27	A/G	intron_variant	-	-0.00381	0.000489%	
n6_n3	rs211581461	LIPE	18	A/G	missense_variant	-	0.005767	0.000448%	
n6_n3	rs41847581	ACADS	17	T/C	intron_variant	-	-0.00362	0.000431%	
n6_n3	rs110097521	PCK2	10	T/C	downstream_gene_variant	-	-0.00351	0.000418%	
n6_n3	rs41601769	CS	5	A/G	intron_variant	-	0.004105	0.000397%	
n6_n3	rs41613043	BDH1	1	T/C	intron_variant	-	0.003698	0.000376%	
n6_n3	rs208618783	APM 1	1	C/G	3_prime_UTR_variant	-	0.003431	0.000363%	
n6_n3	rs41844655	PRKAB1	17	A/G	downstream_gene_variant	-	0.004364	0.000348%	
n6_n3	rs41768431	UCP3	15	T/G	intron_variant	-	0.00321	0.000346%	
n6_n3	rs43490031	HTT	6	C/G	upstream_gene_variant	-	0.003941	0.000343%	
n6_n3	rs41577445	RPH3A	17	A/G	intron_variant	-	-0.00317	0.000339%	
n6_n3	rs41632202	WISP1	14	A/T	intron_variant	-	-0.00374	0.000337%	
n6_n3	rs41634890	TXNRD2	17	A/G	intron_variant	-	0.003133	0.000333%	
n6_n3	rs41848862	MYO18B	17	T/C	missense_variant	cAg/cGg	0.003835	0.000332%	
n6_n3	rs41569367	IFNGR1	9	A/C	3_prime_UTR_variant	-	-0.00311	0.000326%	
n6_n3	rs41630327	SPON1	15	A/G	-	-	0.00411	0.000325%	
n6_n3	rs41907823	ALOX15	19	T/C	intron_variant	-	0.003192	0.000323%	
n6_n3	rs41255700	SCD1	26	A/G	downstream_gene_variant	-	-0.00308	0.000320%	
n6_n3	rs42196904	IGF2	29	A/G	intron_variant	-	-0.00343	0.000317%	
n6_n3	rs41594003	SIAT8A	5	A/G	intron_variant	-	-0.00313	0.000305%	
n6_n3	rs41847802	MVK	17	T/C	missense_variant,splice_region_variant	gCc/gTc	0.003027	0.000305%	
n6_n3	rs42973904	LARG	15	A/G	downstream_gene_variant	-	-0.00308	0.000299%	
n6_n3	rs43499691	INSL3	7	T/G	downstream_gene_variant	-	0.002984	0.000288%	
n6_n3	rs41568467	ACADSB	26	T/C	intron_variant	-	-0.00399	0.000275%	
n6_n3	rs41932864	BTF3	20	A/G	intron_variant	-	0.003109	0.000264%	

n6_n3	rs42868252	DGAT2	15	A/G	intron_variant	-	-0.00325	0.000262%	
n6_n3	rs43706499	CCL2	19	A/G	-	-	-0.00283	0.000252%	
n6_n3	rs109697714	GHRH	13	T/C	intron_variant	-	0.002712	0.000250%	
n6_n3	rs41884788	CEBPG	18	A/G	downstream_gene_variant	-	0.003563	0.000242%	
n6_n3	rs41255193	HADHB	11	A/G	3_prime_UTR_variant	-	0.002603	0.000226%	
n6_n3	rs42189263	GNG3	29	A/C	intron_variant	-	-0.00295	0.000222%	
n6_n3	rs43707870	ITGA5	4	T/C	3_prime_UTR_variant	-	-0.00264	0.000221%	
n6_n3	rs208510799	GDF3	2	T/C	downstream_gene_variant	-	0.00263	0.000211%	
n6_n3	rs42096946	FGF8	26	A/C	downstream_gene_variant	-	-0.00357	0.000207%	
n6_n3	rs41588659	COL1A2	4	A/G	intron_variant	-	0.002635	0.000192%	
n6_n3	rs41255671	ANKRD1	26	A/G	intron_variant	-	0.002818	0.000178%	
n6_n3	rs17871681	POMC	11	T/C	synonymous_variant	ttC/ttT	0.003007	0.000170%	
n6_n3	rs41915481	CACNA1G	19	T/C	synonymous_variant	aaC/aaT	0.003351	0.000162%	
n6_n3	rs43315810	TNS	2	T/C	-	-	-0.00287	0.000157%	
n6_n3	rs42400583	NOX4	29	A/G	synonymous_variant	ccA/ccG	-0.00259	0.000155%	
n6_n3	rs43428730	SYT1	5	A/G	intron_variant	-	0.002784	0.000154%	
n6_n3	rs43319555	EGF	6	T/C	intergenic_variant	-	0.003075	0.000151%	
n6_n3	rs17871740	PCK1	13	A/G	intron_variant	-	-0.00238	0.000142%	
n6_n3	rs41793400	GNA14	8	T/C	5_prime_UTR_variant	-	0.002776	0.000114%	
n6_n3	rs41783612	ACAD8	15	A/C	intron_variant	-	0.002578	0.000108%	
n6_n3	rs109368962	SAA3	29	A/G	intron_variant	-	-0.00312	0.000104%	
n6_n3	rs41583157	TCF7L2	26	A/C	intron_variant	-	0.002257	0.000091%	
n6_n3	rs43345375	PRKACB	3	T/G	synonymous_variant	ggA/ggC	0.002192	0.000082%	
n6_n3	rs43369271	SIAT6	3	A/G	synonymous_variant	gcT/gcC	-0.00248	0.000068%	
n6_n3	rs29011323	CDC10	4	T/C	intron_variant	-	0.002222	0.000067%	

n6_n3	rs42605121	SLC8A1	11	A/G	intron_variant	-	-0.00224	0.000065%	
n6_n3	rs41718866	DDEF1	14	C/G	3_prime_UTR_variant	-	-0.00198	0.000063%	
n6_n3	rs42114536	FAT	27	T/G	missense_variant	gaG/gaT	-0.00299	0.000061%	
n6_n3	rs43476247	CXCL10	6	T/G	downstream_gene_variant	-	0.002046	0.000050%	
n6_n3	rs43508528	ACSL6	7	T/C	intron_variant	-	-0.0022	0.000031%	
n6_n3	#N/A	DGAT1	14	T/G	-	-	-0.00204	0.000028%	
n6_n3	rs41781896	LRP4	15	T/C	missense_variant	aTg/aCg	0.001761	0.000027%	
n6_n3	rs17871427	PPM1B	11	A/G	downstream_gene_variant	-	-0.00181	0.000023%	
n6_n3	rs41926990	PRKCA	19	C/G	intron_variant	-	-0.00156	0.000018%	0.066%
Health_index	rs42196904	IGF2	29	A/G	intron_variant	-	-0.01399	0.145459%	
Health_index	rs42714483	THRSP	29	T/C	missense_variant	Atc/Gtc	0.005586	0.029280%	
Health_index	rs43663540	DGUCK	11	T/G	intron_variant	-	0.006746	0.025648%	
Health_index	rs42660323	PFN2	1	C/G	intergenic_variant	-	-0.00439	0.017199%	
Health_index	rs109450360	IGF2R	9	A/G	intron_variant	-	0.004122	0.015995%	
Health_index	rs41256865	GPD1	5	A/G	3_prime_UTR_variant	-	-0.00404	0.014885%	
Health_index	rs41780423	PAMR1	15	T/C	missense_variant	aCg/aTg	0.003412	0.010086%	
Health_index	rs41844655	PRKAB1	17	A/G	downstream_gene_variant	-	0.00429	0.009277%	
Health_index	rs42147575	ANXA11	28	A/G	splice_region_variant,intron_variant	-	0.002791	0.007264%	
Health_index	rs43724308	MGLL	22	T/C	synonymous_variant	agT/agC	-0.00347	0.006925%	
Health_index	rs29024247	SLCO2B1	15	A/G	synonymous_variant	gtA/gtG	0.003094	0.006417%	
Health_index	0	ACSF3	18	C/T	-	-	0.003052	0.005852%	
Health_index	rs41919983	FASN	19	T/C	intergenic_variant	-	-0.00231	0.004930%	
Health_index	rs41652470	SIRT1	28	A/G	upstream_gene_variant	-	-0.00218	0.004366%	
Health_index	rs41900392	PRKCG	18	T/C	downstream_gene_variant	-	-0.00215	0.004347%	

Health_index	rs42113899	FAT	27	T/C	missense_variant	Gtg/Atg	-0.0021	0.003199%	
Health_index	rs41845683	OASL	17	A/G	3_prime_UTR_variant	-	-0.002	0.003143%	
Health_index	rs42183386	DCPS	29	T/C	3_prime_UTR_variant	-	0.001953	0.003011%	
Health_index	rs109763947	IGF1	5	T/C	upstream_gene_variant	-	-0.00179	0.002992%	
Health_index	rs380876224	SOAT1	16	T/C	upstream_gene_variant	-	0.002103	0.002917%	
Health_index	rs41613043	BDH1	1	T/C	intron_variant	-	0.001946	0.002860%	
Health_index	rs43315799	TNS	2	A/G	intron_variant	-	0.001723	0.002766%	
Health_index	rs42742546	SERPINI2	1	A/G	downstream_gene_variant	-	0.002142	0.002578%	
Health_index	rs43494871	PROM1	6	T/G	missense_variant	ttA/ttC	0.002575	0.002559%	
Health_index	rs110953296	FABP5	14	T/C	downstream_gene_variant	-	0.001807	0.002202%	
Health_index	rs42905009	MOGAT2	15	T/G	intron_variant	-	-0.00142	0.001901%	
Health_index	rs42411170	FGFR3	6	T/C	intron_variant	-	0.00169	0.001882%	
Health_index	rs42413973	EIF3S6	14	T/G	intron_variant	-	-0.00174	0.001769%	
Health_index	rs41793400	GNA14	8	T/C	5_prime_UTR_variant	-	0.002041	0.001712%	
Health_index	rs42605121	SLC8A1	11	A/G	intron_variant	-	-0.00195	0.001374%	
Health_index	rs41781896	LRP4	15	T/C	missense_variant	aTg/aCg	-0.00227	0.001223%	
Health_index	rs43649421	TSHR	10	T/C	synonymous_variant	atC/atT	-0.00216	0.001204%	
Health_index	rs41567325	TG	14	T/C	-	-	0.001917	0.001073%	
Health_index	rs43369257	SIAT6	3	A/G	3_prime_UTR_variant	-	0.001833	0.001054%	
Health_index	#N/A	OLR1	5	A/G	-	-	0.001745	0.001035%	
Health_index	rs43289839	PRKRA	2	T/G	intron_variant	-	-0.00199	0.000847%	
Health_index	rs43476247	CXCL10	6	T/G	downstream_gene_variant	-	0.001543	0.000772%	
Health_index	rs41654804	COPZ1	5	C/G	downstream_gene_variant	-	0.001555	0.000602%	
Health_index	rs41745644	PAFAH1B2	15	T/C	intron_variant	-	0.00143	0.000433%	0.353%

SFA+BFA	rs43663565	DGUCK	11	T/C	intron_variant	-	-0.00419	0.000079%	
SFA+BFA	rs41256865	GPD1	5	A/G	3_prime_UTR_variant	-	0.002654	0.000037%	
SFA+BFA	rs42115578	FACL2	27	C/G	synonymous_variant	-	-0.00242	0.000029%	
SFA+BFA	rs42113899	FAT	27	T/C	missense_variant	Gtg/Atg	0.002557	0.000027%	
SFA+BFA	rs109763947	IGF1	5	T/C	upstream_gene_variant	-	0.002218	0.000027%	
SFA+BFA	rs42115578	ACSL1	27	C/G	synonymous_variant	tcG/tcC	-0.0023	0.000026%	
SFA+BFA	rs43707854	SOCS2	5	A/C	3_prime_UTR_variant	-	-0.00232	0.000026%	
SFA+BFA	rs41652470	SIRT1	28	A/G	upstream_gene_variant	-	0.00221	0.000026%	
SFA+BFA	rs41646367	ANXA11	28	A/G	synonymous_variant	gcC/gcT	-0.00206	0.000023%	
SFA+BFA	rs41255703	SCD1	26	T/C	downstream_gene_variant	-	0.002104	0.000022%	
SFA+BFA	rs43724308	MGLL	22	T/C	synonymous_variant	agT/agC	0.00252	0.000021%	
SFA+BFA	rs42196904	IGF2	29	A/G	intron_variant	-	0.002167	0.000020%	
SFA+BFA	rs42090456	TNFRSF6	26	C/G	intron_variant	-	0.002091	0.000020%	
SFA+BFA	rs110953296	FABP5	14	T/C	downstream_gene_variant	-	-0.00224	0.000020%	
SFA+BFA	rs42742546	SERPINI2	1	A/G	downstream_gene_variant	-	-0.00235	0.000018%	
SFA+BFA	rs43648117	SNW1	10	T/C	downstream_gene_variant	-	-0.00179	0.000017%	
SFA+BFA	rs41909257	ALOX12	19	A/T	splice_region_variant,intron_variant	-	-0.00182	0.000016%	
SFA+BFA	rs43710977	GSTM1	3	A/C	intron_variant	-	-0.0017	0.000015%	
SFA+BFA	rs41907825	ALOX15	19	C/G	intron_variant	-	0.001622	0.000014%	
SFA+BFA	rs17871529	CGN	3	C/G	upstream_gene_variant	-	-0.00161	0.000013%	
SFA+BFA	rs29022551	HABP2	26	T/C	intron_variant	-	0.001636	0.000013%	
SFA+BFA	rs109450360	IGF2R	9	A/G	intron_variant	-	-0.00155	0.000013%	
SFA+BFA	rs29024247	SLCO2B1	15	A/G	synonymous_variant	gtA/gtG	-0.0018	0.000012%	
SFA+BFA	rs41641849	IDH1	2	A/G	intron_variant	-	-0.00165	0.000012%	
SFA+BFA	rs42243023	NEUROD1	2	T/G	upstream_gene_variant	-	-0.00152	0.000012%	

SFA+BFA	rs41900392	PRKCG	18	T/C	downstream_gene_variant	-	0.001463	0.000012%	
SFA+BFA	rs43494871	PROM1	6	T/G	missense_variant	ttA/ttC	-0.00229	0.000012%	
SFA+BFA	#N/A	ACSF3	18	C/T	-	-	-0.00177	0.000011%	
SFA+BFA	rs41780423	PAMR1	15	T/C	missense_variant	aCg/aTg	-0.00143	0.000010%	
SFA+BFA	rs41694130	CTSZ	13	T/G	-	-	-0.00137	0.000010%	
SFA+BFA	rs41886799	ATP1A3	18	A/G	intron_variant	-	0.001483	0.000010%	
SFA+BFA	rs17870648	NR1H3	15	A/C	synonymous_variant	ctG/ctT	0.001375	0.000010%	
SFA+BFA	rs109697714	GHRH	13	T/C	intron_variant	-	-0.0013	0.000009%	
SFA+BFA	rs29017040	ACP6	3	T/C	intron_variant	-	0.001334	0.000009%	
SFA+BFA	rs41627981	ATP6V1H	14	A/G	intron_variant	-	-0.00136	0.000009%	
SFA+BFA	rs29021775	TG	14	A/G	intergenic_variant	-	-0.00132	0.000009%	
SFA+BFA	rs41744783	CASP1	15	T/C	intergenic_variant	-	0.00133	0.000009%	
SFA+BFA	rs41800338	NME7	16	A/G	intron_variant	-	-0.00148	0.000009%	
SFA+BFA	rs41849827	ADRBK2	17	T/C	downstream_gene_variant	-	0.0014	0.000009%	
SFA+BFA	rs43709215	TXNIP	3	C/G	intron_variant	-	-0.0016	0.000008%	
SFA+BFA	rs41897473	EMP3	18	T/C	downstream_gene_variant	-	-0.00125	0.000008%	
SFA+BFA	rs380876224	SOAT1	16	T/C	upstream_gene_variant	-	-0.00144	0.000008%	
SFA+BFA	rs41844655	PRKAB1	17	A/G	downstream_gene_variant	-	-0.00161	0.000008%	
SFA+BFA	rs43675525	FSHR	11	A/G	intron_variant	-	-0.00136	0.000007%	
SFA+BFA	rs41687553	DSTN	13	T/C	intron_variant	-	-0.00122	0.000007%	
SFA+BFA	rs43720495	RARA	19	T/C	intron_variant	-	0.001357	0.000007%	
SFA+BFA	rs41845683	OASL	17	A/G	3_prime_UTR_variant	-	0.001219	0.000007%	
SFA+BFA	rs41613043	BDH1	1	T/C	intron_variant	-	-0.00122	0.000007%	
SFA+BFA	rs43476247	CXCL10	6	T/G	downstream_gene_variant	-	-0.0018	0.000006%	
SFA+BFA	rs41800886	PLOD	16	T/C	downstream_gene_variant	-	0.001186	0.000006%	

SFA+BFA	rs43707575	PNPLA2	29	T/C	downstream_gene_variant	-	0.001298	0.000006%	
SFA+BFA	rs41961336	IGF1R	21	T/C	synonymous_variant	gaT/gaC	0.001357	0.000006%	
SFA+BFA	rs43267303	AGTR1	1	T/C	intergenic_variant	-	-0.00143	0.000006%	
SFA+BFA	rs17871740	PCK1	13	A/G	intron_variant	-	0.001141	0.000005%	
SFA+BFA	rs41255521	STARD3	19	C/G	3_prime_UTR_variant	-	-0.00132	0.000005%	
SFA+BFA	rs41755155	APOC3	15	T/C	3_prime_UTR_variant	-	0.00145	0.000005%	
SFA+BFA	rs42194738	INS	29	T/C	downstream_gene_variant	-	-0.0011	0.000004%	
SFA+BFA	rs43235355	CASR	1	A/C	intron_variant	-	0.001111	0.000004%	
SFA+BFA	rs42211560	ADFP	8	A/G	missense_variant	gCt/gTt	-0.00103	0.000003%	
SFA+BFA	rs42605121	SLC8A1	11	A/G	intron_variant	-	0.00124	0.000003%	
SFA+BFA	rs42176295	SLC37A2	29	T/C	intron_variant	-	0.001108	0.000003%	
SFA+BFA	rs43727187	FGFR1	27	T/C	intron_variant	-	-0.00098	0.000002%	
SFA+BFA	rs43289839	PRKRA	2	T/G	intron_variant	-	0.001244	0.000002%	
SFA+BFA	rs41654029	ATP2B1	5	T/G	intron_variant	-	-0.00108	0.000002%	
SFA+BFA	rs109368962	SAA3	29	A/G	intron_variant	-	0.000949	0.000002%	
SFA+BFA	rs43317359	CRYBA2	3	T/G	intron_variant	-	-0.0008	0.000001%	
SFA+BFA	rs43369271	SIAT6	3	A/G	synonymous_variant	gcT/gcC	0.000883	0.000001%	
SFA+BFA	rs41781896	LRP4	15	T/C	missense_variant	aTg/aCg	0.000893	0.000001%	
SFA+BFA	rs43229098	SIAT10	1	T/C	3_prime_UTR_variant	-	-0.00077	0.000001%	
SFA+BFA	rs43649421	TSHR	10	T/C	synonymous_variant	atC/atT	0.000753	0.000001%	
SFA+BFA	rs43407618	ADCYAP1R1	4	T/C	intron_variant	-	0.000786	0.000001%	0.001%
sumCLA	rs41257366	POMC	11	A/G	synonymous_variant	ggC/ggT	-0.00191	0.014582%	
sumCLA	rs41255193	HADHB	11	T/C	3_prime_UTR_variant	-	-0.00151	0.009227%	
sumCLA	rs109450360	IGF2R	9	A/G	intron_variant	-	0.00131	0.007085%	

sumCLA	rs42195007	PC	29	A/G	synonymous_variant	ctC/ctT	-0.00147	0.007003%	
sumCLA	rs42090456	TNFRSF6	26	C/G	intron_variant	-	-0.00099	0.003378%	
sumCLA	rs41847792	MVK	17	A/G	intron_variant	-	-0.00091	0.003295%	
sumCLA	rs43663565	DGUCK	11	T/C	intron_variant	-	0.000973	0.003213%	
sumCLA	rs41847571	ACADS	17	T/C	intron_variant	-	0.000864	0.002884%	
sumCLA	rs42660323	PFN2	1	C/G	intergenic_variant	-	-0.00084	0.002801%	
sumCLA	rs41887418	LIPE	18	A/G	stop_gained	taT/taA	0.000829	0.002554%	
sumCLA	rs43315204	PRKAG3	2	T/C	intron_variant	-	-0.00078	0.002472%	
sumCLA	rs41568467	ACADSB	26	T/C	intron_variant	-	0.001035	0.002224%	
sumCLA	rs41594003	SIAT8A	5	A/G	intron_variant	-	0.000755	0.002142%	
sumCLA	rs110061082	UCP8	15	T/C	intron_variant	-	-0.00112	0.002060%	
sumCLA	rs42436359	HGF	4	A/G	synonymous_variant	caA/caG	-0.00077	0.001977%	
sumCLA	rs43235983	NR1I2	1	A/G	intron_variant	-	0.000699	0.001977%	
sumCLA	rs41255157	SLC1A3	20	T/C	3_prime_UTR_variant	-	0.000661	0.001813%	
sumCLA	rs42399155	NOX4	29	T/C	synonymous_variant	aaT/aaC	0.00075	0.001730%	
sumCLA	rs41744783	CASP1	15	T/C	intergenic_variant	-	-0.00066	0.001648%	
sumCLA	rs41963518	BG1	21	T/G	intron_variant	-	0.000619	0.001483%	
sumCLA	rs42714482	THRSP	29	A/G	missense_variant	gTg/gCg	0.000593	0.001483%	
sumCLA	rs43562598	AQP7	8	T/C	downstream_gene_variant	-	0.000629	0.001483%	
sumCLA	rs41567825	INSR	7	C/G	intron_variant	-	-0.00064	0.001401%	
sumCLA	rs42861142	ADAM18	27	A/C	intergenic_variant	-	0.00059	0.001401%	
sumCLA	rs43407600	GHRHR	4	T/C	intron_variant	-	-0.00095	0.000989%	
sumCLA	rs41926990	PRKCA	19	C/G	intron_variant	-	0.000936	0.000824%	
sumCLA	rs41583801	DNMT2	13	A/G	intron_variant	-	-0.0007	0.000741%	
sumCLA	rs42188426	EEF1G	29	T/C	intron_variant	-	-0.00079	0.000659%	0.085%

sumtrans18:1	rs42589207	PIK3R1	20	T/G	synonymous_variant	acC/acA	-0.11158	1.633429%	
sumtrans18:1	rs43702942	UGDH	6	T/C	intron_variant	-	-0.10946	1.611111%	
sumtrans18:1	rs41899395	MYH1	19	T/G	downstream_gene_variant	-	0.052979	0.353873%	
sumtrans18:1	rs43406303	ADCY1	4	A/G	intron_variant	-	0.033505	0.153856%	
sumtrans18:1	rs42767950	ANKRD1	26	A/C	downstream_gene_variant	-	-0.04298	0.130023%	
sumtrans18:1	rs41687544	DSTN	13	A/C	-	-	0.036578	0.121910%	
sumtrans18:1	rs41745644	PAFAH1B2	15	T/C	intron_variant	-	0.057105	0.099496%	
sumtrans18:1	rs43051819	DPP4	2	A/C	upstream_gene_variant	-	0.022388	0.068584%	
sumtrans18:1	rs41255232	TMEM175	6	A/G	downstream_gene_variant	-	-0.02223	0.056511%	
sumtrans18:1	rs41640705	IGF1R	21	A/G	intron_variant	-	-0.02447	0.050225%	
sumtrans18:1	rs17870222	IGFBP3	4	T/C	intron_variant	-	0.02599	0.035493%	
sumtrans18:1	rs211581461	LIPE	18	A/G	missense_variant	-	0.025011	0.034047%	
sumtrans18:1	rs110953296	FABP5	14	T/C	downstream_gene_variant	-	-0.01537	0.023273%	
sumtrans18:1	rs41255492	LGALS9	19	T/C	synonymous_variant	gcC/gcT	0.018808	0.020234%	
sumtrans18:1	rs41793400	GNA14	8	T/C	5_prime_UTR_variant	-	-0.01766	0.018700%	
sumtrans18:1	rs41917438	CRHR1	19	A/G	intron_variant	-	-0.01123	0.016766%	
sumtrans18:1	rs43489990	DGKQ	6	A/G	downstream_gene_variant	-	-0.01625	0.015587%	
sumtrans18:1	rs134451630	SIAT4A	14	A/G	intron_variant	-	0.010292	0.014500%	
sumtrans18:1	rs29014633	CACNG2	5	A/T	intron_variant	-	0.010185	0.013381%	
sumtrans18:1	rs41610128	UGP2	11	A/G	intron_variant	-	0.009052	0.010153%	
sumtrans18:1	rs110121818	DGAT2	15	NA	3_prime_UTR_variant	-	-0.01009	0.010026%	
sumtrans18:1	rs43576438	IL7	14	T/C	intron_variant	-	0.008338	0.008016%	
sumtrans18:1	rs29013472	SLC8A1	11	T/C	intron_variant	-	-0.00736	0.007364%	
sumtrans18:1	rs17871529	CGN	3	C/G	upstream_gene_variant	-	-0.00706	0.006539%	

sumtrans18:1	rs41678692	CPT2	3	T/C	3_prime_UTR_variant	-	-0.01288	0.005932%	
sumtrans18:1	rs110625700	PPARA	5	C/G	synonymous_variant	-	0.01143	0.005857%	
sumtrans18:1	rs41634418	PISD	17	A/G	intron_variant	-	0.00846	0.005841%	
sumtrans18:1	rs41584659	IGFBP2	2	A/G	intron_variant	-	0.007539	0.005448%	
sumtrans18:1	rs43490031	HTT	6	C/G	upstream_gene_variant	-	0.007781	0.005387%	
sumtrans18:1	rs41630327	SPON1	15	A/G	-	-	-0.00829	0.005340%	
sumtrans18:1	rs42147600	ANXA11	28	A/G	5_prime_UTR_variant	-	0.006251	0.005274%	
sumtrans18:1	rs43560146	LPL	8	T/C	upstream_gene_variant	-	0.008656	0.005208%	
sumtrans18:1	rs43707575	PNPLA2	29	T/C	downstream_gene_variant	-	0.007608	0.005109%	
sumtrans18:1	rs41768431	UCP3	15	T/G	intron_variant	-	0.006122	0.005087%	
sumtrans18:1	rs208474334	TIEG2	11	T/C	missense_variant	-	0.011904	0.005065%	
sumtrans18:1	rs41907824	ALOX15	19	T/C	intron_variant	-	-0.006	0.004933%	
sumtrans18:1	rs42176279	SLC37A2	29	C/G	intron_variant	-	-0.00754	0.004730%	
sumtrans18:1	rs42605121	SLC8A1	11	A/G	intron_variant	-	-0.0094	0.004645%	
sumtrans18:1	rs41588659	COL1A2	4	A/G	intron_variant	-	-0.00641	0.004565%	
sumtrans18:1	rs41613043	BDH1	1	T/C	intron_variant	-	0.006399	0.004535%	
sumtrans18:1	rs43687642	SCD5	6	T/G	3_prime_UTR_variant	-	0.005801	0.004507%	
sumtrans18:1	rs29015741	ACVR1	2	A/G	intron_variant	-	-0.00577	0.004177%	
sumtrans18:1	rs41579063	BIG1	14	A/T	intron_variant	-	-0.00553	0.004147%	
sumtrans18:1	rs41650227	IRF2	27	T/C	intron_variant	-	0.007238	0.004029%	
sumtrans18:1	rs41255315	ATIC	16	T/C	synonymous_variant	caC/caT	0.005077	0.003501%	
sumtrans18:1	rs41910301	MYH8	19	A/G	downstream_gene_variant	-	-0.005	0.003426%	
sumtrans18:1	rs41619977	NRBF1	2	A/G	intron_variant	-	0.005113	0.003349%	
sumtrans18:1	rs43727187	FGFR1	27	T/C	intron_variant	-	-0.00715	0.003336%	
sumtrans18:1	rs208317364	DGAT1	14	A/G	intron_variant	-	-0.00967	0.003245%	

sumtrans18:1	rs41764379	MRVI1	15	A/G	3_prime_UTR_variant	-	-0.00617	0.003050%	
sumtrans18:1	rs42180931	SIAE	29	A/G	intron_variant	-	-0.00726	0.002962%	
sumtrans18:1	rs41600452	FGF12	1	T/G	intron_variant	-	0.007212	0.002879%	
sumtrans18:1	rs42761489	SLC27A2	10	A/G	intron_variant	-	-0.00462	0.002871%	
sumtrans18:1	rs43299525	SCN7A	2	A/G	missense_variant	Tat/Cat	-0.00489	0.002736%	
sumtrans18:1	rs41800886	PLOD	16	T/C	downstream_gene_variant	-	0.005042	0.002689%	
sumtrans18:1	rs43447360	GUCY2C	5	A/G	intron_variant	-	0.0044	0.002643%	
sumtrans18:1	rs41255689	SCD1	26	A/G	intron_variant	-	0.004363	0.002618%	
sumtrans18:1	rs41768578	GDPD5	5	A/G	intron_variant	-	0.008084	0.002403%	
sumtrans18:1	rs43706499	CCL2	19	A/G	-	-	0.004259	0.002304%	
sumtrans18:1	rs43706906	STAT1	2	C/G	intron_variant	-	0.004384	0.002236%	
sumtrans18:1	rs41600366	MAP2K6	19	A/G	intron_variant	-	-0.00404	0.002219%	
sumtrans18:1	rs29012338	SLC1A3	20	A/C	intron_variant	-	0.006311	0.002131%	
sumtrans18:1	rs41696920	ATRN	13	A/G	missense_variant	aGg/aAg	-0.00389	0.002043%	
sumtrans18:1	rs43562598	AQP7	8	T/C	downstream_gene_variant	-	0.004047	0.002040%	
sumtrans18:1	rs42821718	API5	15	A/G	intron_variant	-	0.007099	0.001955%	
sumtrans18:1	rs41932855	BTF3	20	T/G	intron_variant	-	-0.00411	0.001859%	
sumtrans18:1	rs42211560	ADFP	8	A/G	missense_variant	gCt/gTt	-0.00486	0.001856%	
sumtrans18:1	rs43707870	ITGA5	4	T/C	3_prime_UTR_variant	-	-0.00372	0.001768%	
sumtrans18:1	rs42211560	ADRP	15	T/C	missense_variant	-	-0.00474	0.001763%	
sumtrans18:1	rs43289839	PRKRA	2	T/G	intron_variant	-	0.007428	0.001741%	
sumtrans18:1	rs41691208	ACAS2L	13	A/G	intron_variant	-	0.003828	0.001724%	
sumtrans18:1	rs42101239	FGFR2	26	T/C	intron_variant	-	0.003485	0.001622%	
sumtrans18:1	rs211686954	HADHSC	26	A/G	synonymous_variant	-	0.003661	0.001617%	
sumtrans18:1	rs109944439	HADHB	11	C/G	intron_variant	-	0.005577	0.001606%	

sumtrans18:1	rs43379084	IGF2BP3	4	T/C	3_prime_UTR_variant	-	-0.00335	0.001529%	
sumtrans18:1	rs41764713	TRPC2	15	A/G	synonymous_variant	acT/acC	-0.00333	0.001526%	
sumtrans18:1	rs137748130	SORBS1	26	T/C	synonymous_variant	-	0.004726	0.001496%	
sumtrans18:1	rs43724308	MGLL	22	T/C	synonymous_variant	agT/agC	0.004118	0.001422%	
sumtrans18:1	rs41987132	COL4A4	2	T/C	intron_variant	-	0.003216	0.001411%	
sumtrans18:1	rs41576422	THRA	19	A/G	intron_variant	-	0.003238	0.001364%	
sumtrans18:1	rs41632203	WISP1	14	T/C	upstream_gene_variant	-	0.003207	0.001290%	
sumtrans18:1	rs41883750	FFAR3	18	T/C	downstream_gene_variant	-	0.003617	0.001246%	
sumtrans18:1	rs41772033	SLCO2B1	15	A/G	3_prime_UTR_variant	-	-0.00369	0.001224%	
sumtrans18:1	rs42193359	POLA2	29	C/G	5_prime_UTR_variant	-	-0.00556	0.001202%	
sumtrans18:1	rs41729168	FABP9	14	A/G	intron_variant	-	-0.00501	0.001196%	
sumtrans18:1	rs41887665	XRCC1	18	T/C	downstream_gene_variant	-	-0.00331	0.001191%	
sumtrans18:1	rs42358344	CALCR	4	A/G	downstream_gene_variant	-	-0.00459	0.001108%	
sumtrans18:1	rs41601769	CS	5	A/G	intron_variant	-	0.003397	0.001097%	
sumtrans18:1	rs43702459	ACSL1	2	A/T	intron_variant	-	0.003567	0.001048%	
sumtrans18:1	rs29024246	SLCO2B1	15	A/G	intron_variant	-	0.003587	0.001001%	
sumtrans18:1	rs43235355	CASR	1	A/C	intron_variant	-	0.003704	0.001001%	
sumtrans18:1	rs109513400	ACBP	2	A/T	upstream_gene_variant	-	0.00347	0.000993%	
sumtrans18:1	rs42044790	MYOM1	24	A/G	missense_variant,splice_region_variant	Aga/Gga	-0.00395	0.000965%	
sumtrans18:1	rs43289838	PRKRA	2	T/C	intron_variant	-	-0.0033	0.000962%	
sumtrans18:1	rs43663540	DGUCK	11	T/G	intron_variant	-	0.003409	0.000957%	
sumtrans18:1	rs42089635	LOXL4	26	A/G	downstream_gene_variant	-	0.002744	0.000847%	
sumtrans18:1	rs41887405	LIPE	18	A/G	missense_variant	-	0.00293	0.000811%	
sumtrans18:1	rs29012834	FGF12	1	T/C	intron_variant	-	0.00314	0.000806%	
sumtrans18:1	rs29023450	NDUFS2	3	A/G	intron_variant	-	0.003241	0.000704%	

sumtrans18:1	rs41719131	FBXO32	14	T/G	upstream_gene_variant	-	-0.00322	0.000657%	
sumtrans18:1	rs41803011	NME7	16	T/C	intron_variant	-	-0.0034	0.000594%	
sumtrans18:1	rs43493654	PROM1	6	C/G	missense_variant	Ccc/Gcc	0.002795	0.000550%	
sumtrans18:1	rs41579049	5-OPASE	14	T/C	splice_region_variant,intron_variant	-	0.003174	0.000547%	
sumtrans18:1	rs41922087	SPHK1	19	A/G	upstream_gene_variant	-	0.003778	0.000525%	
sumtrans18:1	rs41636983	SLC9A1	11	A/G	intron_variant	-	-0.00312	0.000445%	
sumtrans18:1	rs43508512	ACSL6	7	A/T	intron_variant	-	0.003855	0.000437%	
sumtrans18:1	rs109224524	GHRH	13	A/G	intron_variant	-	0.002622	0.000423%	
sumtrans18:1	rs109368962	SAA3	29	A/G	intron_variant	-	-0.00314	0.000421%	
sumtrans18:1	rs110061082	UCP5	15	T/C	intron_variant	-	0.002544	0.000346%	
sumtrans18:1	rs43317359	CRYBA2	3	T/G	intron_variant	-	0.002443	0.000341%	
sumtrans18:1	rs42149515	NEUROG3	28	A/T	downstream_gene_variant	-	-0.00267	0.000300%	
sumtrans18:1	rs41667443	CAPNS1	18	T/C	downstream_gene_variant	-	-0.00299	0.000291%	
sumtrans18:1	rs43717452	ACSS2	13	T/C	downstream_gene_variant	-	-0.00309	0.000261%	
sumtrans18:1	rs42421976	PPP1R3A	4	A/C	downstream_gene_variant	-	0.002225	0.000245%	
sumtrans18:1	rs41809799	PRKCZ	16	T/C	downstream_gene_variant	-	-0.00279	0.000203%	
sumtrans18:1	rs29011369	COL4A3	2	A/C	intron_variant	-	-0.00259	0.000190%	
sumtrans18:1	rs41847805	MVK	17	T/C	synonymous_variant	gcC/gcT	0.002597	0.000184%	4.720%

*Allele a/b coded as aa = 0, ab=1, bb=2