GENOMIC EFFECTS ON MILK FATTY ACID COMPOSITION OF BEEF COWS AND ITS INFLUENCES ON CALF PRE-WEANING GROWTH

By

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CHAPTER I

INTRODUCTION

The beef industry is a critical part of animal agriculture in American society (Otto et al., 2001) and world-wide (EPA's Ag Center, 2012). Of meats produced in the U.S., beef is the predominate product (Source: American Meat Institute, 2011). Beef is also greatest of the red meat consumed in the United States, representing approximately 33.24 percent (boneless weight) of the total red meat and poultry consumption in 2009 (Source: USDA, 2012). One important factor in determining the efficiency of beef production is the growth rate of the calf, in both pre-weaning and post-weaning periods. Therefore, calves' preweaning average daily gain (PRWADG) has great economic importance, and directly affects post-weaning gain and final carcass weight (Koch et al., 1973; Hyder et al., 2002). Accordingly, the quantity and quality of beef cows' milk production is of interest since milk yield and quality are associated with beef calves' growth not only in the pre-weaning phase but also in post-weaning period among different breeds (Brown et al., 2002; Reynolds et al., 1978; Christian et al., 1965). Fatty acid composition is one of the most important indicators for milk quality, and may influence calves' growth. Hence, it is reasonable to evaluate beef milk fatty acid composition in terms of its influence on suckling calf growth.

RESEARCH OBJECTIVES

The objectives of this research were: 1) Evaluate the influence of beef cow milk yield and quality on calf preweaning ADG in cows sired by Bonsmara, Brangus, Charolais, Gelbvieh, Hereford, and Romosinuano sires, and 2) Evaluate the association of several gene polymorphisms and milk fatty acid composition in beef cattle.

REVIEW OF LITERATURE

The summary of the six beef cattle breeds used in this study

There are hundreds of breeds of beef cattle including both pure breeds and composite breeds in the world. Six of them are described as below, and have variety of genetic backgrounds from breeds in Europe, Africa, Asia and South America.

Bonsmara

Bonsmara is a tropically-adapted, non-Zebu composite breed comprised of 5/8 Afrikaner and 3/8 British breeds (Shorthorn, Herford) and was developed in South Africa. The breed performs well under heat and drought conditions (Collins-Lusweti, 2000). In research conducted in Nebraska, cattle with Bonsmara blood had slower growth rate, lighter body weight at slaughter, lower carcass weight, and less desirable carcass traits including lower percentage of carcass classified as USDA Choice and lesser fat deposition compared to Angus cattle (Casas et al., 2010). This suggests the Bonsmara breed may have less utility in temperate regions. However, in the sub-tropical area of South Africa, Bonsmara were resistant to ticks, had heavier carcass weight, larger loin eye muscle area and higher dressing percentage than the Angus breed (Muchenje et al., 2008). Bonsmara are considered to have high quality meat and has been selected for economical production in sub-tropical regions (Porter, 1992). Long-term selection in Bonsmara has resulted in higher weaning weights and lower mortality compared to traditional breeds in South Africa. Moreover, focus on other economically

important traits such as reproductive efficiency have been emphasized in the selection of Bonsmara, which makes Bonsmara popular in the beef industry in South Africa.

Brangus

Brangus is also a composite breed consisting of 3/8 Brahman and 5/8 Angus, is solid black and polled. Brahman are *Bos indicus* cattle originally from Indian breeds and are adapted to regions of tropical and humid environments. Hundreds of years' of natural selection in the *Bos indicus* has resulted in adaptation to heat stress, insect pests, parasites, diseases and inadequate nutrition. Angus are originally from Scotland and have genetics and a reputation for desirable carcass traits. Both of these two parental breeds have good maternal traits. With the combined merits of the two breeds, Brangus cattle have a better adaptation to subtropical environments than Angus and a better adaptation to lower temperate environments than *Bos indicus* (reference?). Brangus benefit from the maternal abilities of both breeds and the carcass merit inherent in Angus. For example, in research conducted under semi desert climate of New Mexico State, Brangus cattle showed superior adaption in this environment compared to a traditional European breed (Hereford) (Winder et al., 1992). In addition, Brangus have been shown to have a more rapid growth rate than Brahman cattle (Thomas et al., 2002).

Charolais

Charolais cattle are white in color and are characterized by larger mature weights and slower rate of maturing compared to British breeds of cattle. Charolais cattle originate from France and were selected for size, muscling, bone and strength over many generations (Briggs, 1969). The larger frame size of the Charolais has attracted widespread use of this breed in the

beef industry in the United States due to superior weaning weights, postweaning growth and efficiency, and carcass weights. In previous studies, Charolais have been shown to have higher requirements for maintenance, and slower rates of maturing than Angus and Hereford (Nadarajah et al., 1984; Melton et al., 1967). In other studies, Charolais had a similar growth rate to Angus in spite of less fatness (Chambaz et al., 2003; Coleman et al., 1993). Furthermore, compared with the Angus breed, Charolais can be less fertile and wean fewer calves (Marshall et al., 1976). However, in some recent studies, Charolais-sired calves showed their superiority during pre-weaning (Brown and Lalman, 2008) and post-weaning (Brown et al., 2008) growth. Although they are considered a temperate breed, the light hair coat of Charolais can allow some adaptation to warmer, sunnier conditions compared to dark-coated cattle. Consequently, this breed has significantly influenced the North American beef industry.

Gelbvieh

Gelbvieh is a German breed and has been selected for meat, milk and draft. This breed is red in color and generally polled in the United States. Gelbvieh are larger mature weight cattle compared to British breeds with good maternal ability. In research of comparing different breeds, cows sired by Gelbvieh had heavier body weights than cows sired by breeds of British origin at different ages (Arango et al., 2002). Moreover, Gelbvieh is one of the continental breeds which have superior milk production (Arango et al., 2002). The lesser rate of maturing in the Gelbvieh also allows good feedlot gains and efficiency with good carcass merit (DeRouen et al., 2000). Although traits like tenderness, marbling scores and quality grades of Gelbvieh were inferior to Angus, Gelbvieh cattle had larger longissimus areas (DeRouen et al., 2000).

Hereford

Hereford is a traditional English breed originally selected for efficiency of the conversion of grass to beef. This breed has a red body, with white face and underline. Just like other British breeds, Herefords have a smaller body size compared to Continental breeds. However, Hereford crosses have been shown to have greater body condition scores than continental-cross breeds (Arango et al., 2002). After importation into the United States, Hereford became popular among American cattlemen for its traits of early maturity and fattening ability at a young age. In investigating calf gain and milk production (Melton et al., 1967), showed that Hereford calves were more efficient in transforming milk into body gain compared with Angus and Charolais. More recently, the lower milk yield in the Hereford, lower weaning weights, poor udders, and the problem of prevalence for cancer eye among Hereford have caused losses in breed popularity.

Romosinuano

Romosinuano is a tropically-adapted, non-Zebu breed from Colombia of South America. This breed is red-brown and polled. Since this breed is Criollo type from Colombia, it is well adapted in the tropical region. In a comparison between Romosinuano and Angus under heat challenge, the Romosinuano cattle expressed better adaption to heat (Scharf et al., 2010). However, compared with British breeds like Angus and Hereford, it has slower growth rate, lighter carcass weight, lesser quality score and lower marbling score during the finishing period (Casas et al., 2010; Phillips et al., 2006). Romosinuano cattle were imported into the United States to cross breed with local breeds to improve their resistances to heat and humid stress.

However, the problem of high calf birth weight of Romosinuano should be resolved during the process of breed improvement (Riley et al., 2007).

Factors affecting pre-weaning average daily gain

Both genetic and environmental factors work together to influence the calves' growth rate from birth to weaning (Schaeffer et al., 1974). Genetic factors can include both additive and non-additive genetic effects while environmental factors may include such factors as climate, management, nutrition, disease, and parasites.

Pre-weaning average daily gain is one of the complex traits which are influenced by multiple genes. The influence of additive genetic factors of polygenic traits are usually estimated using heritability (Goyache et al., 2003; Hyder et al., 2002), which can be calculated as the proportion of the phenotypic variation that is due to additive genetic variation for different traits such as birth weight or weaning weight. Additive genetic variation may come from breed of the dam and sire (Reynolds et al., 1978), the sex of calves (Goyache et al., 2003; Hyder et al., 2002; Schaeffer et al., 1974; Koch et al., 1973; Rutledge et al., 1971; Melton et al., 1967) and individual genetic merit of the sire and dam. Furthermore, for crossbred cattle, the effect of heterosis which is a non-additive genetic factor has a significant influence on average daily gain (Wiltbank et al., 1966).

Environmental factors are basically divided into outside conditional effects like climate and management, nutritional effects, and health effects like diseases and parasites. For example, year of birth (Rutledge et al., 1971) and seasonal effects have great influence on pre-weaning

average daily gain (Hyder et al., 2002). Month of calving and age at weaning also significantly affects average daily gain (Goyache et al., 2003). For the nutritional effects, milk quantity and quality (Brown et al., 2002), feeding system (Schaeffer et al., 1974), and other supplements of milk replacer (Cruywagen et al., 1995) have been shown to influence the pre-weaning growth. Moreover, when comparing healthy and diseased steers (Waggoner et al., 2007) showed that healthy steers had greater average daily gain. It has also been shown that strategic parasite control helped the growth of calves in a beef cow/calf herd (Stromberg et al., 1997).

Maternal effects represent the influences on offspring's phenotypic traits from both maternal environmental and genotypic effects from its mother. One maternal effect influencing pre-weaning calf growth is from milk, an important nutritional source for calves before weaning. Milk yield greatly affects calf pre-weaning average daily gain (Brown et al., 2002; Rutledge et al., 1971). Other factors such as age of dam (Reynolds et al., 1978; Schaeffer et al., 1974), breed (Brown et al., 2002; Notter et al., 1978; Totusek et al., 1973), forage environment for cows, and maternal heterosis (Brown et al., 2002), may influence the quantity and quality of beef cows' milk production and are considered indirect effects. Moreover, the milk composition or quality (milk fat, milk protein, solid-not-fat, total solids, somatic cell count) influences calves' average daily gain (Melton et al., 1967, Brown et al., 2002). Additionally, in research with lambs, certain fatty acid proportions, such as saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA), and conjugated linoleic acid (CLA), in ewes' milk significantly affected lambs' growth (Valvo et al., 2005). Therefore, similar influences from milk fatty acid composition may also exist in beef species.

Genes related to FA composition

Fatty acid composition, especially and specifically changes of proportions of SFA, MUFA, PUFA and ratios of a few key fatty acids (e.g., omega-6 to omega-3 ratio), are important indicators for the fat quality in both meat and milk. Fatty acid composition is controlled by multiple genes with different pathways and is also greatly influenced by nutritional factors (Bouwman et al., 2011; Garnsworthy et al., 2010). There are many proteins from multiple genes working in fatty acids composition pathways. However, genes like diacylglycerol O-acyltransferase 1 (DGAT1), stearoyl-CoA desaturase 1 (SCD1), and fatty acid synthase (FASN) tend to have significant influence on fatty acid composition. The specific discussions of the functions and studies of these three genes are following.

Diacylglycerol O-acyltransferase 1

Diacylglycerol O-acyltransferase 1 is located on bovine chromosome 14 and is a functional gene for milk fat content in cattle (Winter et al., 2002) in breeds like Italian Brown Cattle (Conte et al., 2010), Brazilian Cattle (Lacorte et al., 2006), Holstein Cattle (Rincon et al., 2012), and Dutch Dairy cattle (Bouwman et al., 2011), and also in other species like sheep (Crisa et al., 2010) and mice (Smith et al., 2000). Diacylglycerol O-acyltransferase 1 is a microsomal enzyme, which has been considered as a key enzyme to catalyze triglyceride (TAG) synthesis from diacylglycerol (DAG) and fatty acyl-coenyzme A at the final step in the glycerol phosphate pathway, which is essential to fat formation both in body adipose tissue and mammary glands (Smith et al., 2000; Conte et al., 2009). In the *de novo* glycerolipid metabolic pathway, DAG can

be derived from the hydrolyzed phosphatidic acid (PA) which is derived from glycerol-3-phosphate (Glycerol 3-P), from esterified monoacylglycerol (MAG), or from hydrolyzed TAG or phospholipid (PL). With the involvement of DGAT1, TAG is synthesized by DAG and fatty acyl-coenyzme A as its substrates, which is an important energy storage form in eukaryotic cells (Figure 1.1). Given the important functions of DGAT1 in TAG synthesis, this gene is critical for body energy storage and fat deposition in muscles, milk and oocytes for mammals (Cases et al., 1998).

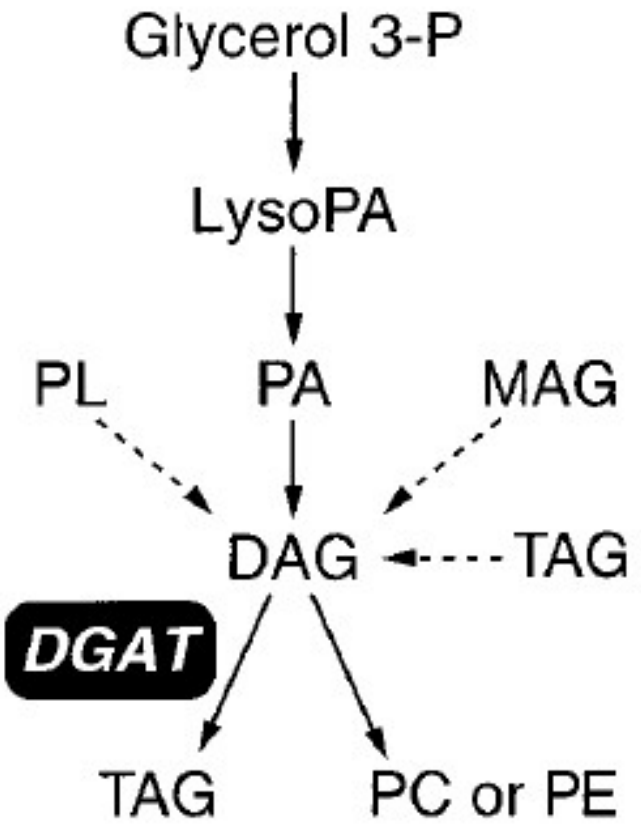

Figure 1.1. Glycerolipid metabolism pathway.

Diacylglycerol (DAG) can be derived from the hydrolyzed phosphatidic acid (PA) which is derived from glycerol-3-phosphate (Glycerol 3-P), from esterified monoacylglycerol (MAG), or

from hydrolyzed TAG or phospholipid (PL). With the involvement of DGAT1, triglyceride (TAG) is synthesized by DAG and fatty acyl-coenyzme A as its substrates.

Adapted from Cases et al., 1998.

A polymorphism of the DGAT1 gene mostly from a change of base pairs from GC to AA which translate into a lysine to alanine substitution (K232A) at the protein level (Winter et al., 2002) was shown to have an important effect on milk production traits (Lacorte et al., 2006). In Lacorte's research, the A allele (alanine) could improve milk protein content and milk yield but diminished milk fat percentage. Additionally, this polymorphism also had strong influences on milk fat composition in Dutch dairy cattle and in Holstein populations (Bouwman et al., 2011, Rincon et al., 2012).

SCD1

Stearoyl-CoA desaturase 1 (SCD1) gene has been mapped to bovine chromosome 26, and has effects on controlling milk and muscle fat composition in different breeds of cattle (Taniguchi et al., 2004; Macciotta et al., 2007; Jiang et al., 2008; Conte et al., 2009; Garnsworthy et al., 2010; Barton et al., 2010; Bouwman et al., 2011; Rincon et al., 2012). It is an endoplasmic reticulum enzyme, which synthesizes *cis*-double bonds between carbon 9 and 10 of saturated fatty acids with a chain length from 10 to 18 carbons in the mammary gland and adipose tissues, and primarily uses palmitoyl- CoA and stearoyl-CoA as substrates to form palmitoleoyl-CoA and oleoyl-CoA (Dobrzyn et al., 2005). For this reason, SCD is also named delta-9-desaturase (Soyeurt et al., 2008). In the presence of an oxygen atom, SCD gets an electron which has been passed from NADPH, NADH-cytochrome *b5* reductase and cytochrome *b5* and then introduces the *cis* bond (Figure 1.2). At this desaturating step, the reaction is rate-limited.

The products of SCD are major MUFAs among a variety of fatty acids, which are important components for lipid storage and strongly influence the body and milk fatty acid composition; some of those MUFAs also play critical roles on regulating biological functions and cell growth, controlling some cell metabolisms and mediating signal transductions by affecting cell-membrane fluidity with changing the ratio of SFA to MUFA (Zhang et al., 1999).

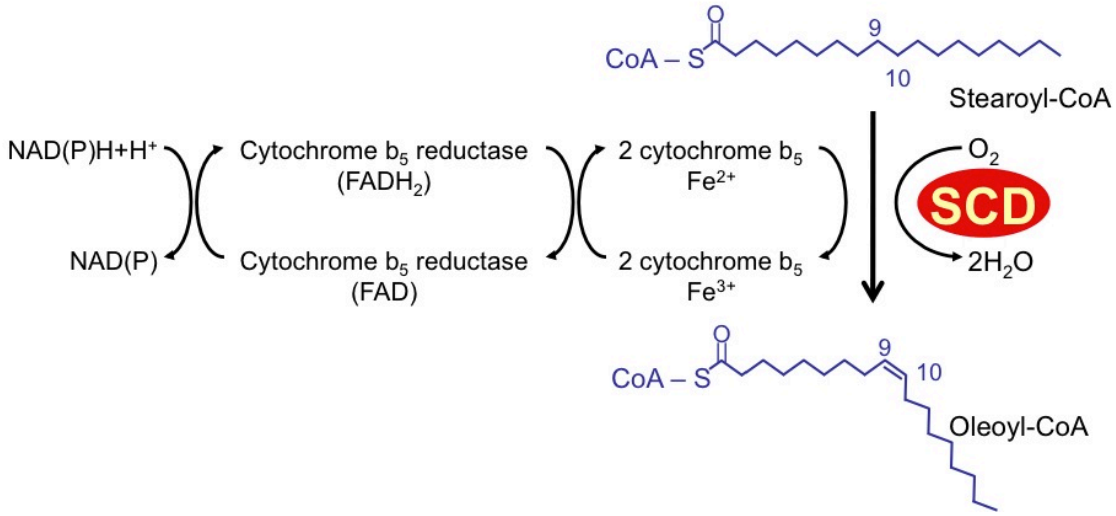

Figure 1.2. SCD involved Desaturation of Stearoyl-CoA.

In the presence of an oxygen atom, SCD gets an electron which has been passed from NADPH, NADH-cytochrome *b5* reductase and cytochrome *b5* and then works to introduce the *cis* bond on stearoyl-CoA.

Adapted from Website resource (http://www.biochem.wisc.edu/faculty/ntambi/).

Polymorphisms in SCD gene have accounted for differences of milk and fat traits in cattle, including fatty acid composition of milk and meat. A single nucleotide polymorphism on SCD1 caused the replacement of valine (allele V) with alanine (allele A) on the 293rd site. The VV cows yielded more milk and more milk protein rather than AA cows (Macciotta et al., 2007). This same mutation also influenced fatty acid composition in muscle. Bulls with allele A including AA and AV genotypes had lower SFA, higher MUFA and higher MUFA/SFA ratio

compared with VV bulls (Barton et al., 2010). Similarly, in Italian Holsteins, AA cows had higher C18:1 cis-9 and total MUFA proportions and higher C14:1/C14:0 ratio in milk fatty acid (Mele et al., 2007). However, the VV genotype was associated with higher C14:1 cis-9 and lower C14:0 content than the AA genotype in the milk of Italian Brown cattle (Conte et al., 2009). Furthermore, in some other studies, SNPs rs41255700 and rs41255691 on SCD1 changed the fatty acid composition by affecting the desaturation index in milk of Holstein cattle (Rincon et al., 2012); an SNP in the open reading frame (ORF) accounted for part of fatty acid composition variation in meat of Japanese Black cattle (Taniguchi et al., 2004) and the SNP g.8586C>T in SCD 1 gene had significant effects on the proportion of myristoleic acid (C14:1) in Korean cattle (Maharani et al., 2012).

FASN

The fatty acid synthase (FASN) gene is located on bovine chromosome 19 and codes a multifunctional protein complex which effectively catalyzes *de novo* fatty acid synthesis in mammals, not only during the adult stage, but also during embryonic development (Maharani et al., 2012). The enzyme has seven active sites: β-ketoacyl synthase, malonyl/acetyl transferase, and dehydrase are three N-terminal domains; and the enoyl reductase, β-ketoacyl reductase, acyl carrier protein and thiosterase are four C-terminal domains. These two kinds of terminal domains are divided by a core structure (Figure 1.3). The malonyl/acetyl transferase leads the substrates loading reaction before condensation reactions; β-ketoacyl synthase works on chain condensation reactions and is also one of three β-carbon processing enzymes with dehydrase and enoyl reductase; after specifying elongated products, thioesterase catalyzes the

chain terminating reaction to make palmitate as the major product (Smith et al., 2003). All of them together help acetyl-coenzyme A and malonyl-coenzyme A to form palmitate with the presence of NADPH (Roy et al., 2006). Therefore, FASN is a key gene for the fat content and composition.

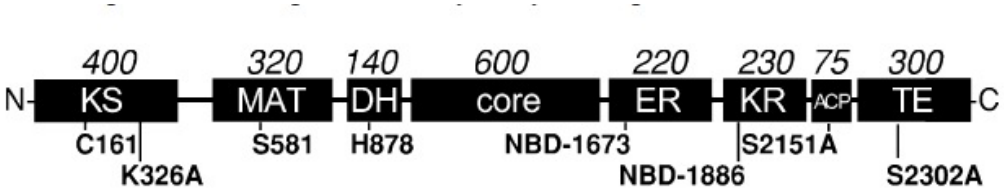

Figure 1.3. Linear domain order map of FASN.

Three N-terminal domains: β-ketoacyl synthase (KS), malonyl/acetyl transferase (MAT), and dehydrase (DH) and four C-terminal domains: the enoyl reductase (ER), β-ketoacyl reductase (KR), acyl carrier protein (ACP) and thiosterase (TE) are divided by a core structure.

Adapted from Smith et al., 2003.

Some SNPs in the FASN gene have been shown to control differences of milk fat content in beef cows (Roy et al., 2006; Ordovas et al., 2008). In another study, SNP's at several sites on the FASN gene also have been shown to have strong associations with different fatty acid proportions in Dutch dairy cattle (Bouwman et al., 2011). Interestingly, the influences from the same SNP in FASN gene on C14:0 percentage are not the same for adipose tissue compared to milk fat in bovine. It may indicate that the effect of FASN is tissue specific (Morris et al., 2007). Additionally, the proportion of myristic acid (C14:0) in meat was higher for the AA genotype rather than GG genotype at the 17924 position in Korean cattle (Maharani et al., 2012).

Candidate gene approaches/analysis

Genetic association studies have been prevalent in recent decades and enable scientists to determine relationships between specific genotypes and phenotypic traits in all kinds of organisms. In animal agriculture, association studies can aid selection programs for traits in populations more accurately and efficiently. Genome-wide association studies rely on numerous genetic markers evenly spaced throughout the whole genome, and do not specially focus on genes that are suspected to be related to certain traits from the biological and physiological perspectives (Tabor et al., 2002). However, the candidate gene analyses use selected genes that have been shown to account for major variances of phenotypes. The basic methodology of candidate gene approach has the following steps:

1. Choosing the candidate gene based on biological functions and physiological position.

2. Uncovering a DNA polymorphism in the candidate gene, for example, single nucleotide polymorphism (SNP), variable number tandem repeats (VNTRs), insertion/deletions, (Tabor et al., 2002; Lewis et al., 2012)

3. Developing a convenient procedure for candidate gene detection on a large scale in specific families such PCR-based genotyping method.

4. Carrying out a statistical association study of the phenotypic records with candidate gene information.

5. Verifying the association result using related experiments such as constructing transgenic or knockout animal models.

The candidate gene approach is widely used in genetic association studies, gene disease research, and drug responses from animal models to human beings (Zhu et al., 2010).

A polymorphism is defined as a variation in DNA sequence that has a more than 1% allele frequency in a large population. Various types of polymorphisms exist in the genome. Examples are Restriction Fragment Length Polymorphisms (RFLPs); Variable Number Tandem Repeats (VNTRs) including Satellites with the size of 100kb to 1Mb, Minisatellites with the total size of 0.5 to 30kb, Microsatellites with the total length of less than 100bp; and the most commonly used polymorphism: single nucleotide polymorphism (SNP) containing single-base substitutions and single-base insertions or deletions (Tabor et al., 2002). Compared with other types of polymorphisms, SNPs have several advantages for candidate gene studies. Firstly, SNPs are distributed throughout the genome with high density. Secondly, SNPs are more stable and easy to assay using GeneChip Microarrays for a large number of different SNPs. Moreover, SNPs are a main cause of genetic diversity among different individuals. Consequently, they are preferred in large scale genetic association studies as genetic markers.

However, the traditional candidate gene approach has met some challenges with the increase in genomic database resources (Tabor et al., 2002). To address this, some strategies have been developed and applied when choosing candidate genes. Position-dependent strategy uses genomic information and selects candidate genes based on the physical linkage information in a QTL-mapped region. Comparative genomics strategy takes advantage of the comparative functional and positional information from other related species. Function-dependent strategy has been verified by gene knock-out, transgenic animal and cellular models to determine gene associations with phenotypes (Zhu et al., 2007). Furthermore, a novel approach to addressing the challenge of large amounts of genomic information is the digital candidate gene approach (DigiCGA), which is supported by the technologies and principal

knowledge of the combination of the fundamentals and applications of biology, computer and bioinformatics (Zhu et al., 2010).

Conclusion

The maternal effects from cows have significant influence on calf growth and milk fatty acid composition is one of the important indicators that influence calf pre-weaning average daily gain. Among numerous factors contributing to fatty acid composition, genomic polymorphisms and especially SNPs, have been identified as having significant associations with certain fatty acid proportions in both meat and milk fatty acid profile. To evaluate these genes, the candidate gene approach can be employed to confirm the association between the complex traits of milk fatty acid composition and SNPs.

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CHAPTER II

PREDICTION OF PREWEANING AVERAGE DAILY GAIN IN BEEF CALVES FROM MILK FATTY ACID COMPOSITION OF THEIR DAMS

ABSTRACT: Research has shown milk yield (MWT) has an important influence on calf pre-weaning ADG (PRWADG), but MWT accounts for only a moderate amount of variation in PRWADG. The objective of this study was to determine if milk fatty acid methyl esters (FAME), alone and in combination with MWT, could improve accuracy of prediction of PRWADG. Forty-five beef cows sired by Bonsmara, Brangus, Charolais, Gelbvieh, Hereford and Romosinuano bulls were used in a 2-year study. Spring-calving cows were milked 6 times per year every 28 days beginning late May, and milk samples were analyzed for milk fat and protein. Milk samples collected in May, July and September each year were analyzed for FAME. Percentages of 42 FAME in each milk sample were acquired using a gas chromatograph flame ion detector. Milk weights, quality data, and FAME were averaged over collection dates prior to analyses. Stepwise regression was used to identify linear models to predict PRWADG using MWT, age of dam (AOD), and percent FAME. The R^2 and associated condition index (CI, an indicator of collinearity) were used in model evaluation. Condition indexes less than or close to 30 were considered to have low collinearity. Regression of PRWADG on MWT resulted in an R^2 of 0.35 with a CI of 9.4 while inclusion of AOD gave an R^2 of 0.4 and a CI of 21.6. A regression equation using 8 FAME accounted 54% of the variation in calf ADG with a CI of 33. When MWT and AOD were included with FAME as predictors, a prediction equation with 8 FAME, MWT, and AOD accounted for 69% of the variation in PRWADG with a CI of 29. Partial least squares regression (PLS) was also used to predict PRWADG from FAME, MWT, and AOD. Results from PLS analyses yielded a dependent variable R^2 of 0.61 using all 42 FAME with 7 extracted factors and a dependent variable R^2 of 0.78

when MWT and AOD were included with the 42 FAME with 7 extracted factors. Results from these preliminary analyses suggest that FAME composition of milk influences calf ADG and that data on percent FAME in combination with MWT and AOD can improve the accuracy of prediction of calf PRWADG compared to MWT and AOD alone.

Key words: beef cattle, milk fatty acids, pre-weaning ADG, stepwise regression, partial least squares (PLS)

INTRODUCTION

Maternal effects play an important role in calf growth, which is phenotypic expression of effects from the dam expressed in the offspring. One maternal effect influencing pre-weaning calf growth is from milk, an important nutritional source for calves before weaning. Milk yield significantly affects calf pre-weaning average daily gain (Brown et al., 2002; Rutledge et al., 1971). Other factors such as age of dam (Reynolds et al., 1978; Schaeffer et al., 1974), breed (Brown et al., 2002; Notter et al., 1978; Totusek et al., 1973), forage environment for cows, and maternal heterosis (Brown et al., 2002), may influence the quantity and quality of beef cows' milk production and are considered indirect effects. However, milk yield alone accounts for only a moderate amount of variation in PRWADG. Moreover, the milk composition or quality (milk fat, milk protein, solid-not-fat, total solids, somatic cell count) influences calves' average daily gain (Melton et al., 1967, Brown et al., 2002). Additionally, in research with lambs, certain fatty acid proportions, such as saturated fatty acids (SFA),

monounsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA), and conjugated linoleic acid (CLA), in ewes' milk significantly affected lambs' growth (Valvo et al., 2005). Therefore, similar influences from milk fatty acid composition may also exist in beef species. Therefore, the objective of this research was to determine if maternal effects from milk fatty acid composition of cows influence calf pre-weaning growth.

MATERIALS AND METHODS

Animals

The animal population for this research was from United States Department of Agriculture Agricultural Research Service (USDA-ARS) Grazinglands Research Laboratory (El Reno, Oklahoma) and all experimental procedures were reviewed and accepted by the ARS Animal Care and Use Committee. A total 45 beef cows sired by 6 different breeds (Bonsmara, Brangus, Charolais, Gelbvieh, Hereford and Romosinuano) by AI out of registered Brangus dams were used to collect milk samples and conduct milk fatty acid profiles in 2009 and 2010. The calving dates of these cows were from March to April, and the weaning date was in October of each year. The PRWADG of calves were calculated using PRWADG=(WWT-BWT)/DAY; WWT is the actual weaning weight of each calf, BWT is the birth weight of each calf and DAY is the days from birth date to weaning date.

Milk sample collection

Cows were milked 6 times each year by the method of milking machine every 28 days starting approximately a month after parturition and milk samples were analyzed for milk fat and protein among other milk quality traits. Cows were separated from calves at about 7:00 pm in the evening before milking day provided with water overnight approximately 14 hours and there was no milk-out before separation. Cows were given a shot of 1.5 mL acepromazine maleate (10 mg/mL, i.m.) ten minutes before milking and a shot of 1.0 mL of oxytocin (20 USP units/mL) immediately before milking to facilitate milk let down. Milk yield was measured by digital platform scale after milking and adjusted to 24-hour milk yield using [(milk weight/14)*24]. Milk fat and milk protein were analyzed by a commercial dairy laboratory (Brown and Lalman, 2010). Milk weights and quality data were averaged over collection dates prior to analyses.

Fatty acid methyl esters (FAME) preparation

Milk samples collected in May, July and September of each year were analyzed for FAME using the methyl ester derivatization method. Milk samples were immediately frozen at -80°C freezer after acquiring. Frozen milk samples were thawed in a water bath at 38°C and transferred to a centrifuge tube. Milk fatty acids were extracted according to the Rose-Gottlieb Method (Secchiari et al., 2003). Ammonia and ethanol were added to precipitate milk protein, and hexane was added as a solvent for milk fat. An internal standard of C23:0 was added for latter fatty acid analysis. After centrifugation, the supernatant containing the hexane solvent with milk fatty acids was transferred to another tube and sodium sulfate was used to eliminate residual water.

The extracted milk fatty acids were esterified with sodium hydroxide in methanol to form fatty acids methyl esters (FAME).

The percentages of 42 different FAME, from short-chain to long-chain, in each milk sample were acquired using a gas chromatograph flame ion detector, which analyses were conducted in the FAPC Analytical Services Lab (Oklahoma State University). The percent of each FAME in milk fat was calculated by ChemStation Revision B.03.02. (341) software (Agilent Technologies, Santa Clara, Calif.) based on the area of each FAME peak and confirmed using a calibration table. Milk FAME data were averaged over collection dates prior to analyses.

Statistical analysis.

Multiple linear stepwise regression models were used to identify linear models to predict PRWADG using milk weight (MWT), age of dam (AOD), each of 42 FAME percentages and weights. These 42 fatty acids were: C4:0, C6:0, C8:0, C10:0, C11:0, C12:0, C13:0, C14:0, C14:1, C15:0, C15:1, C16:0, C16:1, C17:0, C17:1, C18:0, C18:1n9t, Vaccenic, C18:1n9c, C18:2n6t, C18:2n6c, C20:0, C18:3n6, C20:1, C18:3n3, CLA1, C21:0, CLA2, CLA3, CLA4, C20:2n6, C22:0, C20:3n6, C22:1n9, C20:3n3, C20:4n6, C22:2n6, C24:0, C20:5n3, C24:1, C22:5n3,and C22:6n3. In the stepwise regression model, the coefficient of determination (R^2), residual mean square error (RMSE), and the associated condition index (CI) were used for model evaluation. Coefficient of determination evaluates how accurate the prediction is, the RMSE indicates precision of the estimate, and the condition index is an indicator of collinearity. A condition index less than or close to 30 was considered to have relatively low collinearity, those over 50 were deemed to have

moderate to high levels of collinearity, and those more than 100 were deemed unacceptable. Models in which CI were close to 30 and 50 were selected to be reported.

Partial least squares regression (PLS) models were also used to predict PRWADG from FAME, MWT, and AOD in order to determine the accuracy of prediction from linearly independent extracted factors using all 42 FAME.

RESULTS AND DISCUSSION

Milk quality and fatty acid composition profile

The basic statistics for milk yield, milk fat, milk protein, 13 kinds of important fatty acid composition, the proportions of SumC12C14C16, SFA, MUFA, PUFA, N6, N3 and the ratios of N6/N3, PUFA/SFA, N3/SFA of 45 beef cows among six breeds in two years were shown in table 2.1. Results indicated that SFA (53.51%) were the main constituent of total milk fat compared with MUFA and PUFA. Of the SFA, C14, C16 and C18, comprised 43.60% of total milk fat. Besides of SFA, vaccenic acid (4.6%) and CLAc9t11 (1.62%) greater proportions than others.

Table 2.1. Milk quality data and fatty acid composition in beef cows

	Min	Max	Mean	SD
MWT	3.3	10.9	7.48	1.78
MF	2.91	4.94	3.77	0.52
MP	2.8	6.38	3.5	0.6
C14:0	5.56	11.05	8.63	0.98
C16:0	19.62	26.92	23.01	1.63
C18:0	8.48	15.96	11.96	1.40
C18:1n9c	0.30	1.89	0.86	0.48
Vaccenic	3.32	7.30	4.60	0.93

C20:0	0.17	0.37	0.26	0.04
CLA c9t11	1.01	2.78	1.62	0.41
C21:0	0.00	0.21	0.06	0.05
C22:0	0.07	0.15	0.11	0.02
C22:1n9	0.01	0.05	0.02	0.01
C20:4n6	0.01	0.12	0.05	0.03
C20:5n3	0.05	0.11	0.08	0.01
C22:5n3	0.09	0.29	0.14	0.04
SumC12C14C16	29.13	39.84	34.27	2.39
SFA	45.78	61.06	53.51	3.09
MUFA	17.93	31.02	22.16	2.76
PUFA	1.90	2.85	2.28	0.24
N6	1.12	1.72	1.32	0.15
N3	0.77	1.26	0.96	0.11
N6/N3	1.18	1.85	1.40	0.14
PUFA/SFA	0.03	0.06	0.04	0.01
N3/SFA	0.01	0.03	0.02	0.00

MWT: milk yield, MF: milk fat, MP: milk protein, SFA: saturated fatty acid, MUFA: monounsaturated fatty acid, PUFA: polyunsaturated fatty acid, N6: omega-6 fatty acid, N3: omega-3 fatty acid

Prediction Model of calves' pre-weaning average daily gain

Stepwise regression of PRWADG on MWT (Model 1) resulted in a moderate R^2 of 0.35 with a CI of 9.4 while inclusion of AOD (Model 2) gave an R^2 of 0.4 and a CI of 21.6. Evaluation of milk fat (MF) (Model 3 and Model 5) and milk protein (MP) (Model 4 and Model 6) showed that MF and MP were only moderately accurate indicators to predict PRWADG of calves (Table 2.2).

Table 2.2. The R^2 and CI from stepwise regression models of PRWADG on MWT, MF, MP with AOD.

PRWADG vs.	MWT Model 1	MWT&AOD Model 2	MF Model 3	MF&AOD Model 4	MP Model 5	MP&AOD Model 6
R^2	0.35	0.4	0.28	0.33	0.29	0.36
CI	9.4	21.6	8.8	21.6	8.8	21.6
RMSE	0.015	0.014	0.016	0.015	0.016	0.015

PRWADG: pre-weaning average daily gain, MWT: milk yield, AOD: age of dam, MF: milk fat, MP: milk protein

Results from stepwise regression demonstrated that a regression equation using 8 fatty acids accounted 54% of the variation in calf ADG with a CI of 33.2 (Model 7) while 11 FAME proportions accounted for 59% with a CI of 49.3 (Model 8) (Table 2.3). When only AOD was included with fatty acids as predictors, the R^2 of prediction equations increased to 0.61 with a CI of 29.3 (Model 9) and to 0.76 with a CI of 42.6 (Model 10) (Table 2.4). When MWT and AOD were included with fatty acids as predictors, a prediction equation with 8 fatty acids, MWT, and AOD accounted for 69% of the variation in calf ADG with a CI of 29.5 (Model 11) and a prediction equation with 11 fatty acids, MWT, and AOD accounted for 80% of the variation with a CI of 50.8 (Model 12) (Table 2.5).

Table 2.3. The R^2 and CI from stepwise regression models of PRWADG on FAME proportion.

Predictor	Model 7	Model 8
Intercept	*1.74*	*1.74*
C14:1		*-0.33*
C15:0		*0.36*
C18:1n11t	*0.05*	
C18:1n9c	*-0.02*	*-0.03*
C20:0	*-3.27*	*-3.46*
C18:3n6	*2.64*	*3.05*
C18:3n3		*-0.23*
CLA c9t11		*0.23*
C21:0	*0.95*	*1.41*
CLA c9c11	*2.57*	*2.89*
C22:0	*3.32*	*2.8*
C22:6n3	*-13.16*	*-16*
R^2	0.54	0.59
CI	33.2	49.3
RMSE	0.012	0.012

PRWADG: pre-weaning average daily gain, FAME: fatty acid methyl esters, R^2: the coefficient of determination, CI: condition index

Table 2.4. The R^2 and CI from stepwise regression models of PRWADG on FAME proportion with AOD.

Predictor	Model 9	Model 10
Intercept	*0.96*	*-0.16*
AOD	*0.12*	*0.14*
C10:0		*1.12*
C12:0		*-0.79*
C17:1	*1.53*	*0.89*
C18:1n11t	*0.07*	*0.09*
C18:1n9c	*-0.04*	
C20:0	*-1.97*	*-1.83*
C20:1		*1.16*
CLA 9t11t	*2.82*	*2.12*
C22:0	*2.69*	*2.86*
C20:4n6	*-4.74*	*-6.85*
C20:5n3		*2.29*
R^2	0.61	0.76
CI	29.3	42.6
RMSE	0.011	0.0070

PRWADG: pre-weaning average daily gain, FAME: fatty acid methyl esters, AOD: age of dam, R^2: the coefficient of determination, CI: condition index

Table 2.5. The R^2 and CI from stepwise regression models of PRWADG on FAME proportion with AOD & MWT.

Predictor	Model 11	Model 12
Intercept	*-0.99*	-1.64
MWT	*0.04*	0.04
AOD	*0.1*	0.13
C8:0	*0.64*	0.83
C17:1	*1.41*	2.58
C18:1n11t	*0.09*	0.15
C20:0		-0.96
CLA c9t11		-0.18
C21:0	*1.48*	1.77
CLA c9c11		-0.69
C22:0		3.27
C20:4n6	*-5.52*	-7.76
C20:5n3	*3.63*	3.84
C22:6n3		6.47
R^2	0.69	0.8

CI	29.5	50.8
RMSE	0.0082	0.0061

PRWADG: pre-weaning average daily gain, FAME: fatty acid methyl esters, AOD: age of dam, MWT: milk yield, R^2: the coefficient of determination, CI: condition index

FAME weight/day (g/d) was calculated as the product of milk fat secretion (g/d) and FAME percent with the assumption that free fatty acids were minimal. To evaluate the effects of milk FAME g/day on the pre-weaning growth of calves, FAME g/day for each FAME were set as predictors with MWT and AOD in stepwise regression models (Model 13-18). In these models, FAME g/day had similar accuracy of prediction of PRWADG as FAME proportion (Table 2.6, 2.7, 2.8).

Table 2.6. The R^2 and CI from stepwise regression models of PRWADG on FAME g/day.

Predictor	Model 13	Model 14
Intercept	*0.76*	0.73
C10:0	*0.06*	0.06
C14:1		-0.04
C16:0	*0*	-0.01
C17:1	*0.27*	0.44
C18:1n11t	*0.03*	0.03
C20:0	*-0.88*	-0.71
C20:1	*0.77*	
C21:0		0.89
C20:2n6	*-1.8*	-2.08
C22:0	*1.38*	0.98
C22:1n9	*3.41*	4.98
C20:4n6		-1.56
C20:5n3		1.07
C22:6n3	*-5.39*	-3.28
R^2	0.63	0.68
CI	29	43
RMSE	0.010	0.0098

PRWADG: pre-weaning average daily gain, FAME: fatty acid methyl esters, R^2: the coefficient of determination, CI: condition index

Table 2.7. The R^2 and CI from stepwise regression models of PRWADG on FAME g/day with AOD.

Predictor	Model 15	Model 16
Intercept	*0.16*	0.11
AOD	*0.14*	0.13
C8:0	*0.17*	0.31
C13:0		-1.4
C16:0	*-0.01*	-0.01
C17:1	*0.63*	0.81
C18:1n11t	*0.03*	0.04
C21:0		0.9
CLA 9t11t		-1.14
C20:2n6	*-1.57*	-3.38
C22:1n9	*2.88*	7.83
C20:4n6	*-1.84*	-3.21
C20:5n3		1.7
R^2	0.64	0.78
CI	31	50
RMSE	0.0097	0.0064

PRWADG: pre-weaning average daily gain, FAME: fatty acid methyl esters, AOD: age of dam, R^2: the coefficient of determination, CI: condition index

Table 2.8. The R^2 and CI from stepwise regression models of PRWADG on FAME g/day with AOD & MWT.

Predictor	Model 17	Model 18
Intercept	*0.23*	0.21
MWT	*0.02*	0.04
AOD	*0.11*	0.11
C8:0		0.47
C12:0		-0.08
C16:0		-0.01
C18:1n11t	*0.01*	
C21:0		0.34
CLA 9t11t	*0.62*	
C22:1n9	*1.31*	2.17
C20:4n6	*-1.76*	-2.39
C20:5n3		1.38
C22:5n3	*0.51*	0.5
R^2	0.58	0.69
CI	25	49.6
RMSE	0.011	0.0087

PRWADG: pre-weaning average daily gain, FAME: fatty acid methyl esters, AOD: age of dam, MWT: milk yield, R^2: the coefficient of determination, CI: condition index

Results from PLS analyses yielded a dependent variable R^2 of 0.61 using all 42 fatty acids with 7 extracted factors (Model 19), a dependent variable R^2 of 0.69 when AOD was included with the 42 FAME with 7 extracted factors (Model 20) and a dependent variable R^2 of 0.78 when MWT and AOD were included with the 42 FAME with 7 extracted factors (Model 21) (Table 2.9). FAME used in the analyses were both percentage of total FAME and g/day.

Table 2.9. The R^2 of PLS models for PRWADG based on FAME with AOD & MWT.

PRWADG vs.	FAME Model 19	FAME&AOD Model 20	FAME&AOD&MWT Model 21
R^2	0.61	0.69	0.78

PRWADG: pre-weaning average daily gain, FAME: fatty acid methyl esters, AOD: age of dam, MWT: milk yield, PLS: partial least squares regression, R^2: the coefficient of determination

In the first 6 models, traditional factors used to predict pre-weaning ADG (milk yield, milk fat and milk protein) combined with age of dam accounted only moderate variation in prediction of pre-weaning average daily gain. Models 7 to 12 contained fatty acid methyl ester proportions combined with age of dam and milk yield, which accounted for much more variation for the prediction than previous 6 models. Models 13 to 18 which mainly expressed the effects from fatty acid methyl ester concentrations had similar results as the previous 6 models using proportions. The last 3 partial least squares regression models (19 to 21) using all 42 fatty acids combined with age of dam and milk yield had relatively high R-squares, which were improvements over linear combinations of FAME derived from the step-wise regression analyses.

CONCLUSION

Results from these preliminary analyses suggest that fatty acid composition of milk influences calf preweaning ADG and that data on percent fatty acids in combination with milk yield and age of dam can improve the accuracy of prediction of calf pre-weaning ADG compared to milk yield and age of dam alone. The partial least squares regression model appears to be the most accurate predictor of calf pre-weaning ADG using fatty acid composition data when information of milk yield and age of dam is included in the model. More detailed analysis and interpretation of the FAME most important in prediction of calf preweaning ADG is needed. If a few FAME are found to be most influential in predicting preweaning ADG and genetic variants of genes affecting FA secretion can be identified, it may be possible to identify cows with lower milk yield potential that can raise calves at a similar or better preweaning ADG compared to cows with higher milk yield potential and a less favorable FAME profile. If this is possible, preweaning production efficiency could be improved through lower cow maintenance and feed requirements in cows with a favorable milk FA profile and lower milk potential.

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CHAPTER III

EFFECTS OF GENOTYPE ON MILK FATTY ACID COMPOSITION OF BEEF COWS

ABSTRACT: The milk fatty acid composition of beef cows is markedly influenced by nutritional factors and also significantly controlled by a few major genes effects. In this study, three genes, diacylglycerol O-acyltransferase 1 (DGAT1), stearoyl-CoA desaturase 1 (SCD1), and fatty acid synthase (FASN), were selected to determine their associations with milk fatty acids of beef cows. A total 59 beef cows sired by 6 different breeds (Bonsmara, Brangus, Charolais, Gelbvieh, Hereford and Romosinuano) out of Brangus dams were used to collect milk samples and conduct milk fatty acid profiles in 2009, 2010 and 2011. One SNP was identified in each gene and nucleotide substitution was determined by the methods of sequencing and High Resolution Melt (HRM) melting curve analysis. Results showed genotypic differences in variants of the DGAT1 gene for saturated fatty acid (SFA), the ratio of omega-6 to omega-3 fatty acids (N6/N3), C14:0, C18:1n9c, C22:1n9 and C22:5n3 ($P < 0.05$), and for omega-3 fatty acids (N3) and the ratio of polyunsaturated fatty acid to saturated fatty acid (PUFA/SFA) ($P < 0.10$). The variation in the SCD1 gene also influenced vaccenic acid, C20:0 and C21:0 ($P<0.10$). For FASN gene, genotypic differences affected the composition of C22:1n9 ($P<0.05$) and C22:0 ($P<0.10$). However, genotypic differences for each fatty acid category were not consistent among the different sire breeds.

Key Words: milk fatty acid composition, DGAT1, SCD1, FASN, SNP, beef breed

INTRODUCTION

Fatty acid composition, especially and specifically changes of proportions of SFA, MUFA, PUFA and ratios of a few important fatty acids (e.g., omega-6 to omega-3 ratio), are important indicators for the fat quality in both meat and milk. In our previous research determining the relationship of fatty acid profile to calf pre-weaning average daily gain, it showed that the fatty acid profile was important to improve the accuracy of prediction of calf growth. To select cows at an early age that will product calves with a superior pre-weaning average daily gain, it is necessary to identify specific genotypes of cows that may influence specific milk fatty acids or combinations.

Fatty acid expression is controlled by multiple genes with different pathways and is also greatly influenced by nutritional factors (Bouwman et al., 2011; Garnsworthy et al., 2010). There are many proteins from thousands of genes working in fatty acids composition pathways. However, genes like diacylglycerol O-acyltransferase 1 (DGAT1), stearoyl-CoA desaturase 1 (SCD1), and fatty acid synthase (FASN) tend to have significant influence on fatty acid composition.

Diacylglycerol O-acyltransferase 1 (DGAT1) is located on bovine chromosome 14 and is a functional gene for milk fat content in cattle (Winter et al., 2002) in breeds like Italian Brown Cattle (Conte et al., 2010), Brazilian Cattle (Lacorte et al., 2006), Holstein Cattle (Rincon et al., 2012), and Dutch Dairy cattle (Bouwman et al., 2011), and also in other species like sheep (Crisa et al., 2010) and mice (Smith et al., 2000). Diacylglycerol O-acyltransferase 1is a microsomal enzyme, and is considered a key enzyme to catalyze triglyceride (TAG) synthesis from

diacylglycerol (DAG) and fatty acyl-coenyzme A at the final step in the glycerol phosphate pathway, which is essential to fat formation both in body adipose tissue and mammary glands (Smith et al., 2000; Conte et al., 2010).

Stearoyl-CoA desaturase 1 (SCD1) gene has been detected on bovine chromosome 26, and has been shown to control milk and muscle fat composition in different breeds of cattle (Taniguchi et al., 2003; Macciotta et al., 2007; Jiang et al., 2008; Conte et al., 2009; Garnsworthy et al., 2010; Barton et al., 2010; Bouwman et al., 2011; Rincon et al., 2012). It is an endoplasmic reticulum enzyme, which synthesizes *cis*-double bonds between carbon 9 and 10 of saturated fatty acids with a chain length from 10 to 18 carbons in the mammary gland and adipose tissues, and primarily uses palmitoyl- CoA and stearoyl-CoA as substrates to form palmitoleoyl-CoA and oleoyl-CoA (Dobrzyn et al., 2005). For this reason, SCD is also named delta-9 desaturase (Soyeurt et al., 2008).

The fatty acid synthase (FASN) gene is on bovine chromosome 19 and codes a multifunctional protein complex which effectively catalyzes *de novo* fatty acid synthesis in mammals not only during the adult stage, but also during embryonic development (Maharani et al., 2012). FASN is a key gene for the fat content and composition.

Furthermore, based on the different genetic backgrounds from different breeds, these specific genotypic effects from above three genes may not be same since the epistatic effects from other loci of the genome on these genes. Therefore, the interaction effects of genotype and breed for each fatty acid or combination is valuable to determine if the genotypic differences are similar or not among sire breeds.

MATERIALS AND METHODS

Animals

The animal population for this research was from United States Department of Agriculture, Agricultural Research Service (USDA-ARS) Grazinglands Research Laboratory (El Reno, Oklahoma). A total of 59 beef cows sired by 6 different breeds (Bonsmara, Brangus, Charolais, Gelbvieh, Hereford and Romosinuano) from Brangus dams were used in this study in 2009, 2010 and 2011. The calving dates of these cows were from March to April in each year. The ages of these cows were distributed from 3 to 9 years of age. Milk samples of each cow were collected in May, July and August of each year for fatty acid profile analyses. Blood samples were collected in the year of 2011 for genomic analysis for cows milked in each of the three years and stored at -20°C.

Fatty acid methyl esters (FAME) preparation and detection

Milk samples collected in May, July and September of each year were analyzed for FAME using the methyl ester derivatization method. Frozen milk samples were thawed in a water bath at 38°C and transferred to a centrifuge tube. Milk fatty acids were extracted according to the Rose-Gottlieb Method (Secchiari et al., 2003). Ammonia and ethanol were added to precipitate milk protein, and hexane was added as a solvent for milk fat. An internal standard of C23:0 was added for latter fatty acid analysis. After centrifugation, the supernatant containing the hexane solvent with milk fatty acids was transferred to another tube and sodium sulfate was used to eliminate residual water. The extracted milk fatty acids were esterified with sodium hydroxide in methanol to form fatty acids methyl esters (FAME).

The percentages of 42 different FAME, from short-chain to long-chain, in each milk sample were acquired using a gas chromatograph flame ion detector, which was conducted in FAPC Analytical Services Lab (Oklahoma State University). The percent of each FAME in milk fat was calculated by ChemStation Revision B.03.02. (341) software (Agilent Technologies, Santa Clara, Calif.) based on the area of each FAME peak and confirmed using a calibration table.

Genotype detection

All 55 beef cows' genomic DNA was extracted from whole blood samples with FlexiGene DNA Kits (Qiagen, Valencia, CA). Whole blood samples were thawed at room temperature and were mixed with lysis Buffer FG1 to lyse cell membranes in a labeled tube. After centrifuging, the supernatant was discarded leaving leucocytes and lymphocytes, containing whole genomic DNA. Buffer FG2/QIAGEN protease was added in each tube and incubated at 65°C to catalyze proteolysis to eliminate proteins combined with the DNA. Genomic DNA was then precipitated using isopropanol. Seventy percent ethanol was used to wash the isolated DNA precipitate to eliminate cations like Mg^{2+} and Na^{+}. The liquid ethanol supernatant was discarded and volatilized after centrifuging. Hydration Buffer FG3 was added to the DNA pellet and incubated at 65°C to dissolve the isolated genomic DNA. A NanoDrop spectrophotometer (Thermo-Scientific Wilmington, DE) was used to measure the quantity, quality and purity of genomic DNA.

Published single nucleotide polymorphisms for Diacylglycerol O-acyltransferase 1 (DGAT1), Stearoyl-CoA Desaturase 1 (SCD1), and Fatty acid Synthase (FASN) genes were selected to identify the genotypic effects on milk fatty acids composition of beef cows. The polymorphisms of these sites were pre-confirmed by pooled sequencing. A total 10 SNPs of the

three genes were selected based on the information on the website of the National Center for Biotechnology Information (SNP: GeneView). Primers were designed with Primer3 online software (v. 0.4.0) and integrated by Integrated DNA Technologies, Inc. (Coralville, IA). Two forward and one reverse primers were used for each DGAT1 gene and SCD1 gene, and two pairs of primers were used for the FASN gene based on the SNPs positions of each gene (Table 3.1). Three samples from each breed were randomly selected for pooled sequencing. The polymerase chain reaction (PCR) was used to amplify designed pooled sequencing fragments. Eighteen PCR products amplified by each pair of primers shown in Table 3.1 from the 6 breeds were mixed together for agarose gel electrophoresis. The Wizard SV Gel and PCR Clean-Up System kit (Promega Co., Madison, WI) was used to recollect the PCR product in the cut gel. The clean mixed PCR products were sent for sequencing in DNA sequencing facility (Oklahoma State University) (Figure 3.1).

Table 3.1. Primers for DGAT1, SCD1 and FASN genes pooled sequencing.

GENE	NAME	SEQUENCE	LENGTH(bp)	START POSITION
DGAT	*DGAT forward1*	TCCTCAAGCTGTTCTCCTACC	21	224
	DGAT forward2	GTCCCCAACCACCTCATCT	19	1077
	DGAT reverse	TTGACACATTCAGACCCTTG	20	2144
SCD	*SCD forward1*	ATGGCGTTCCAGGTAAGAAG	20	239
	SCD forward2	GTTGCTTTTCCACTTATGCTTC	22	1516
	SCD reverse	CAGTCTCCCTCCCTTTTGTG	20	2301
FASN	*FASN forward1*	AAGTAAGCAAGCGCAAGTCC	20	171
	FASN reverse1	CAATTTCCATGTTCCCCAGT	20	2424
	FASN	GGCTGTTCTGTGGGATATGG	20	4704

forward2			
FASN reverse2	CGAAGCCAAAGGAGTTGATG	20	5472

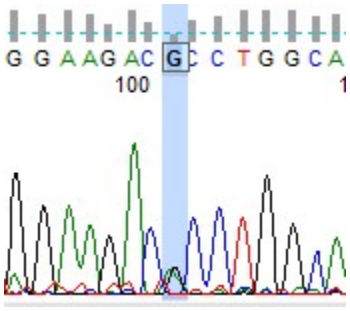

Figure 3.1. The pooled sequencing result for one SNP on SCD1 gene.
A G/A single nucleotide polymorphism at position 102 with two peaks: Guanine (G, black) and Adenine (A, green).

High Resolution Melt (HRM) curve analysis: Ten pairs of primers for amplifying short fragments for each SNP were designed with Primer3 online software (v. 0.4.0) (Table 3.2) and integrated by Integrated DNA Technologies, Inc. (Coralville, IA). Gradient PCR was applied for each pair of primers before HRM melt curve analysis to determine a best annealing temperature for real-time PCR (RT-PCR) (Figure 3.2). EvaGreen dye was combined with replicated PCR products in RT-PCR using SsoFast™ EvaGreen® Supermix and Bio-Rad CFX Touch™ Real-Time PCR Dectection System (Bio-Rad Laboratories Inc., Hercules, CA). High Resolution Melt curve analysis was applied directly after RT-PCR using Precision Melt Analysis™ Software (Bio-Rad Laboratories Inc., Hercules, CA), which identified and collected fluorescence units data released from single strand DNA denatured from original double-strand DNA of PCR products every 0.2°C from 70°C to 90°C. Differences of melt curves, which represented different genotypes resulting from same sized fragments with different SNPs of PCR products, were acquired (Figure 3.3).

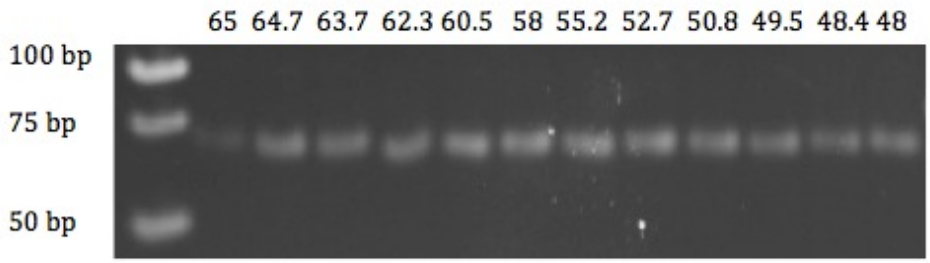

Figure 3.2. Agarose gel image of gradient PCR.
100 bp, 75 bp and 50 bp sizes are shown on the vertical axis. The horizontal axis (65 to 48 ° C) are the different annealing temperatures applied in gradient PCR. The brightest band in the lane of 60.5°C represented the best annealing temperature for the PCR program.

Table 3.2. Primers for SNPs of DGAT1, SCD1 and FASN genes.

GENE	NAME	SEQUENCE	SIZE (bp)	SNP
DGAT	*DGAT1-F*	CGCTTGCTCGTAGCTTTGG	19	rs109234250
	DGAT1-R	AGGTCAGGTTGTCGGGGTAG	20	rs109326954 (AA232)
	DGAT2-F	GAACTCCGAGTCCATCACCT	20	rs134083952
	DGAT2-R	ACCTGATGCACCACTTGTGA	20	
	DGAT3-F	GTGGCTGTCACTCATCATCG	20	rs135329220
	DGAT3-R	TCACGGTTGAGCACGTAGTAG	21	
SCD	*SCD1-F*	AGGACTTGTCAACATGAGCTG	21	rs41255690
	SCD1-R	AAACAACAGTCTATGGCTCTGG	22	
	SCD2-F	CTCCTTTGGAGCACCAACTC	20	rs41255689
	SCD2-R	AGACACCCTCTCAGGGGAAT	20	
	SCD3-F	CTGGACAGCCACTTCACTTTC	21	rs43740732
	SCD3-R	CCTACTTGCCTCTGCCAGTC	20	
FASN	*FASN1-F*	TGGCACTGTTGAGGAGACC	19	rs110674576
	FASN1-R	ACTGGACTAGCTGGCTCTGC	20	
	FASN2-F	TTTGTTGCAGGGCTTTCTG	19	rs137372738
	FASN2-R	TGAACTCCCTCCTCCATCTG	20	
	FASN3-F	TCACCCCAGTTTCCTCACTC	20	rs137117849
	FASN3-R	AGATCCTGCCTCCTGCTCTG	20	
	FASN4-F	GAGAGGAGACAGAGCATGTGG	21	rs137684230
	FASN4-R	GCTGCAAGCAATTTCATTCTC	21	

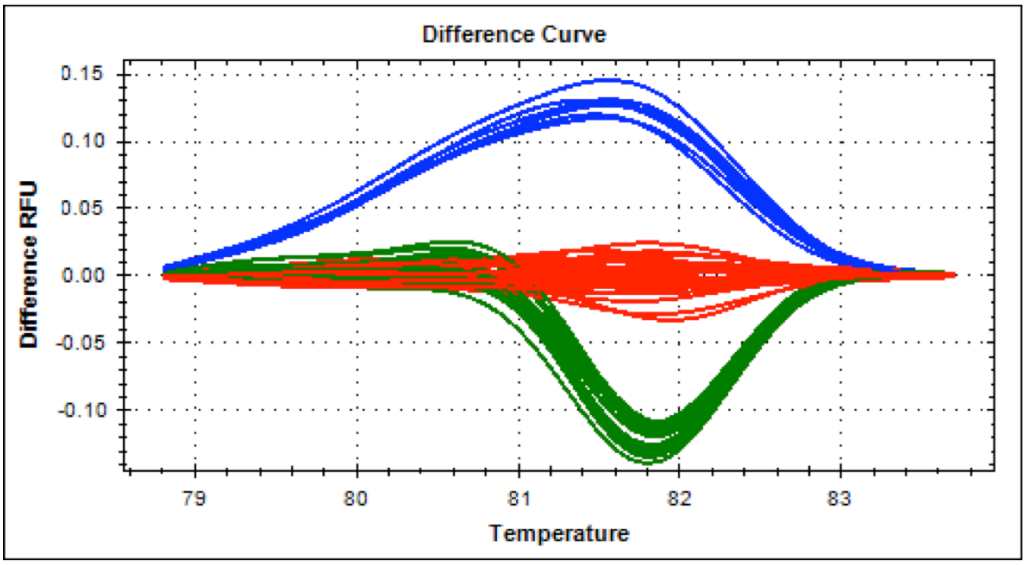

Figure 3.3. The melting curves of samples with SNP of SCD1 gene.
The melting profile curves represent different genotypes. RFU: relative fluorescence units.

Forty uL PCR products of each selected sample containing verified polymorphisms identified in HRM melt curve analysis were amplified with the same primers used for HRM melt curve analysis by normal PCR. The PCR products were separated by agarose gel electrophoresis and cut for DNA purification using Wizard SV Gel and PCR Clean-Up System kit (Promega Co., Madison, WI). Purified PCR products were sent for sequencing in DNA sequencing facility (Oklahoma State University) to identify the specific substitutions of different nucleotides (Figure 3.4).

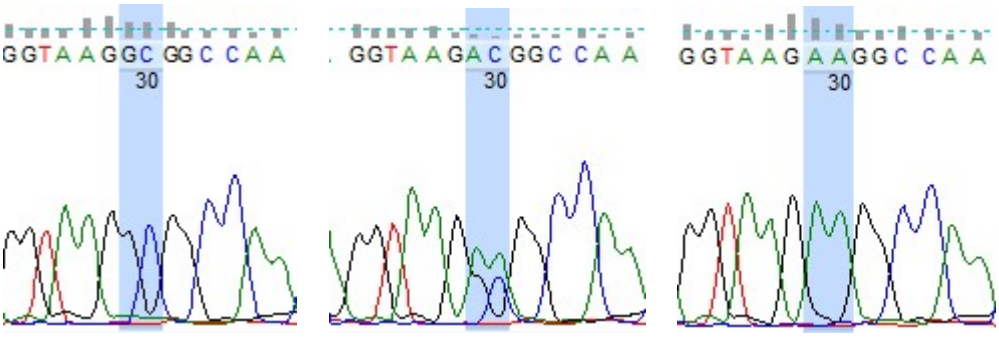

Figure 3.4. The sequencing results of DGAT1 gene fragments selected from different clusters approved in HRM.
The SNP: GC/GC, GC/AA, AA/AA, were determined from different clusters shown in HRM by

sequencing.

Statistical analysis.

Fatty acid methyl ester data analyzed included saturated fatty acid (SFA), monounsaturated fatty acid (MUFA), polyunsaturated fatty acid (PUFA), omega-6 fatty acid (N6), omega-3 fatty acid (N3) and the sum of C12:0, C14:0 and C16:0 (SumC12C14C16). Additionally, ratios of the percentages of N6 to N3 (N6/N3), PUFA to SFA (PUFA/SFA), N3 to SFA (N3/SFA) were also analyzed. The percentages of biologically important fatty acids, CLAc9t11, C14:0, C16:0, C18:0, Vaccenic, C18:1n9c and C22:5n3 and other fatty acids C20:0, C21:0, C22:0, C20:4n6, C20:5n3 and C22:1n9, which were determined to have important effects on calves' pre-weaning average daily gain in a previous study were also selected for further analysis. Data for each gene was analyzed using mixed model least squares procedures (SAS Institute Inc., Cary, NC). The initial linear models included year (fixed), sire breed (fixed), genotype (fixed), sire breed x genotype (fixed), year x sire breed (fixed), year x genotype (fixed), year x sire breed x genotype (fixed), cow nested in year, sire breed, and genotype (random), month (repeated, fixed), month x year (fixed), month x sire breed (fixed), month x genotype (fixed), month x sire breed x genotype (fixed), month x sire breed x year (fixed), month x genotype x year (fixed), month x sire breed x genotype x year (fixed) and month x cow nested in year, sire breed, and genotype (random). Of particular interest in these analyses were genotype and sire breed x genotype effects. Models were reduced according to accepted procedures for model reduction with the exception that effects with missing cells that resulted in estimability issues for sire breed x genotype least squares means were also eliminated. With minor exceptions, such effects were not significant ($P > 0.10$) and could be eliminated without bias. Contrasts among

least squares means were done using t statistics at both the $P < 0.05$ and $P < 0.10$ levels, with $P < 0.10$ denoting trends (Saxton, 1998).

RESULTS AND DISCUSSION

The genomic effects on milk fatty acid composition

One single nucleotide polymorphism was identified in each of the 3 genes in this study using methods of HRM melt curve analysis and sequencing in the cow populations of this research. Specifically, SNP's were identified at AA232 of the DGAT1 gene (AA/GC) (Figure 3.5), rs41255689 of the SCD1 gene (A/G) (Figure 3.6) and rs137372738 of the FASN gene (C/T) (Figure 3.7). The genotype and gene frequencies of these 3 SNPs among six sire breeds Bonsmara, Brangus, Charolais, Gelbvieh, Hereford and Romosinuano are shown in table 3.3-5.

Among the three SNPs, the AA232 of DGAT1 showed effects on C14:0, C18:1n9c, C22:1n9, C22:5n3, SFA, and N6/N3 ($P<0.05$), and on N3 and PUFA/SFA ($P<0.1$). Additionally, the SNP rs41255689 of the SCD1 gene influenced the proportions of C21:0 ($P< 0.05$), and vaccenic acid and C21:0 ($P<0.10$). The SNP rs137372738 of the FASN gene, showed significant association with C22:1n9 ($P<0.05$) and C22:0 ($P<0.10$). (Table 3.6).

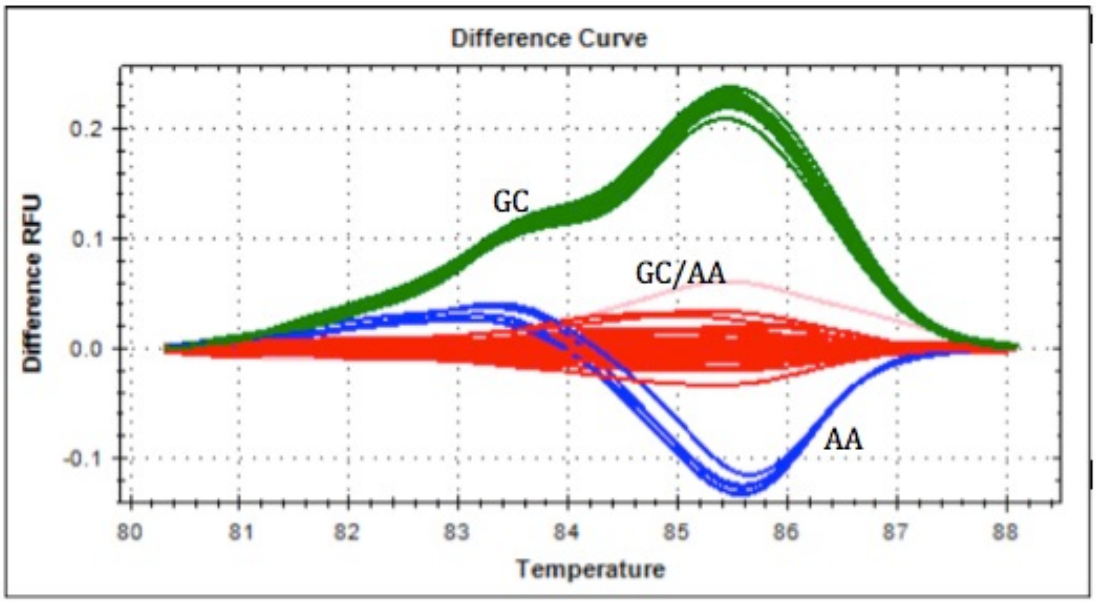

Figure 3.5. The polymorphisms of SNP AA232 of DGAT1 gene shown in HRM melting curve analysis.

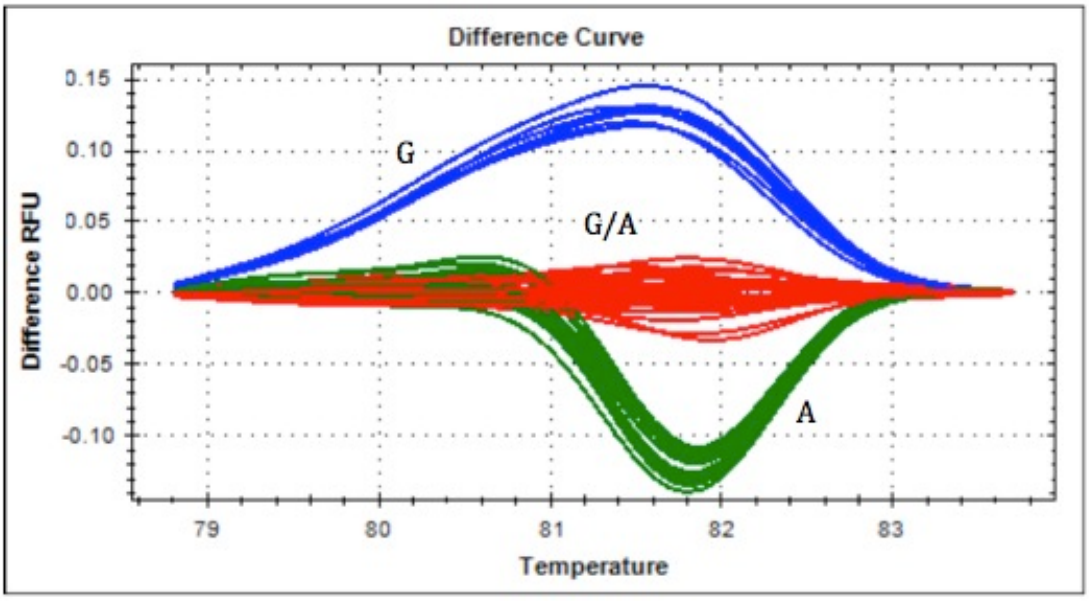

Figure 3.6. The polymorphisms of SNP rs41255689 of SCD1 gene shown in HRM melting curve analysis.

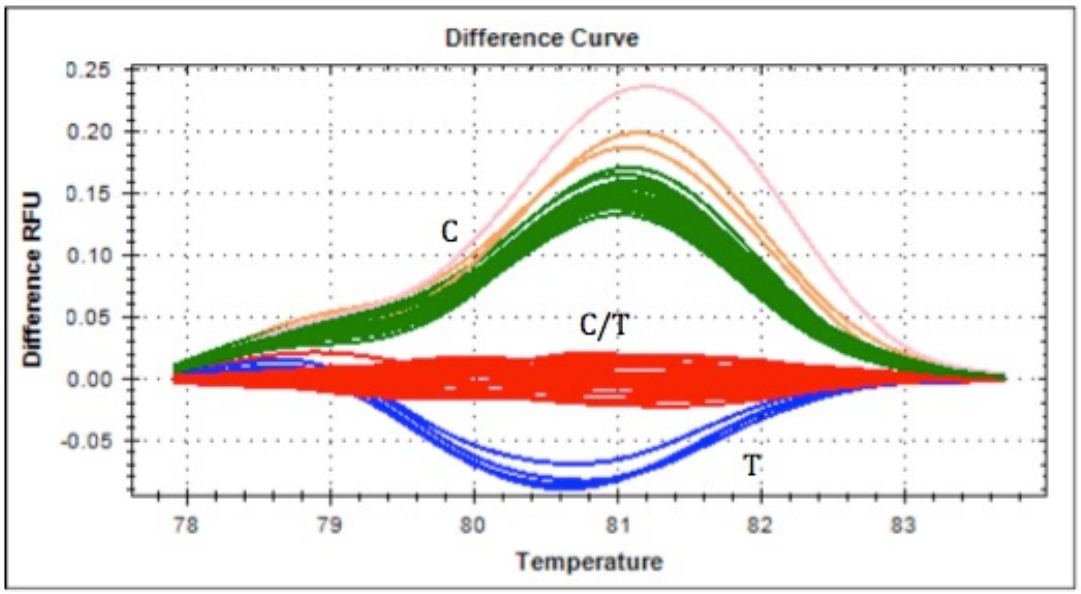

Figure 3.7. The polymorphisms of SNP rs137372738 of FASN gene shown in HRM melting curve analysis.

Table 3.3 Genotype and gene frequencies of DGAT1 gene in six breeds

BREED	Genotype Frequency			Gene Frequency	
	AA/AA	GC/AA	GC/GC	AA	GC
Bonsmara	*0.22*	0.44	*0.33*	0.44	0.56
Brangus	*0.20*	0.70	*0.10*	0.55	0.45
Charolais	*0.00*	1.00	*0.00*	0.50	0.50
Gelbvieh	*0.00*	0.38	*0.63*	0.19	0.81
Hereford	*0.00*	0.56	*0.44*	0.28	0.72
Romosinuano	*0.11*	0.67	*0.22*	0.44	0.56
TOTAL	0.09	0.64	0.27	0.41	0.59

Table 3.4. Genotype and gene frequencies of SCD1 gene in six breeds.

BREED	Genotype Frequency			Gene Frequency	
	AA	AG	GG	A	G
Bonsmara	*0.44*	0.44	*0.11*	0.67	0.33
Brangus	*0.44*	0.44	*0.11*	0.67	0.33
Charolais	*0.33*	0.56	*0.11*	0.61	0.39
Gelbvieh	*0.25*	0.75	*0.00*	0.63	0.38
Hereford	*0.67*	0.22	*0.11*	0.78	0.22
Romosinuano	*0.13*	0.50	*0.38*	0.38	0.63
TOTAL	0.38	0.48	0.13	0.62	0.38

Table 3.5. Genotype and gene frequencies of FASN gene in six breeds.

BREED	Genotype Frequency			Gene Frequency	
	CC	CT	TT	C	T
Bonsmara	*0.67*	0.33	*0.00*	0.83	0.17
Brangus	*0.50*	0.50	*0.00*	0.75	0.25
Charolais	*0.30*	0.60	*0.10*	0.60	0.40
Gelbvieh	*0.38*	0.63	*0.00*	0.69	0.31
Hereford	*0.44*	0.56	*0.00*	0.72	0.28
Romosinuano	*0.44*	0.22	*0.33*	0.56	0.44
TOTAL	0.45	0.47	0.07	0.69	0.31

Table 3.6. The P-values of the each effect of the SNP on fatty acid composition.

	DGAT1	SCD1	FASN
C14:0	0.00	0.62	0.27
C16:0	0.85	0.97	0.48
C18:0	0.17	0.41	0.12
C18:1n9c	0.00	0.55	0.89
Vaccenic	0.17	0.07	0.18
C20:0	0.55	0.06	0.11
CLA c9t11	0.23	0.39	0.18
C21:0	0.69	0.05	0.83
C22:0	0.86	0.30	0.09
C22:1n9	0.01	0.88	0.00
C20:4n6	0.14	0.27	0.61
C20:5n3	0.67	0.37	0.72
C22:5n3	0.04	0.19	0.95
SumC12C14C16	0.93	0.80	0.29
SFA	0.00	0.73	0.71
MUFA	0.40	0.44	0.87
PUFA	0.13	0.61	0.92
N6	0.17	0.26	0.97
N3	0.10	0.50	0.74
N6/N3	0.05	0.16	0.52
PUFA/SFA	0.08	0.60	0.77
N3/SFA	0.62	0.65	0.38

The replacement of the amino acid alanine to threonine at the position 232 of DGAT1

gene was caused by the nucleotides substitution of GC to AA at DNA level (Source: NCBI). In published studies, increased alanine could improve milk protein content and milk yield but diminished milk fat percentage (Lacorte et al., 2006); it also affected the ratio of SFA to unsaturated fatty acid (UFA) (Rincon et al., 2012). The amino acid change in this study resulted in increases in the proportion of C14:0, C20:0 and SFA of all milk fat, and decreases in the proportions of C18:1n9c, C21:0, C22:5n3, MUFA, N3 and the ratios of N6/N3 and PUFA/SFA (Table 3.8-9).

Saturated FA levels were higher in the GC genotype of the DGAT1 gene compared to GC/AA and AA genotypes ($P<0.05$). Monounsaturated FA were greater in GC genotypes than GC/AA and AA ($P<0.05$) and MUFA were greater in GC/AA genotypes compared to AA genotypes ($P<0.05$). Levels of N6 were lesser in the AA genotype of the DGAT1 gene compared to GC/AA and GC ($P<0.05$) while there was little evidence of genotypic differences in N3 ($P>0.05$). The ratios of N6/N3 were greater in the AA genotype than GC/AA and GC ($P<0.05$), and ratios in GC/AA genotype were greater than GC ($P<0.05$). The ratios of PUFA/SFA in AA genotype were lesser than GC/AA and GC ($P<0.05$). The level of C14:0 were higher in GC genotype than GC/AA and AA ($P<0.05$), while C18:1n9c was lesser in GC genotype than GC/AA and AA ($P<0.05$). C22:5n3 in GC/AA genotype was greater than GC and AA ($P<0.05$), and AA genotype was greater than GC ($P<0.05$). However, there was little evidence of genotypic differences in PUFA, N3/SFA, SumC12C14C16, CLAc9t11, C16:0, C18:0 and Vaccenic for the DGAT1 gene ($P>0.05$).

Interaction between breed and genotype for percent fatty acid methyl esters

Analyses indicated that genotypic differences for some percent fatty acid methyl esters were not the same for each sire breed of cow. Observed significance levels for the F test of the sire breed x genotype means square are given in Table 3.7. There were indications of interactions of sire breed of cow for individual FAME in each gene with the preponderance occurring in FASN where 11 of the 22 FAME reported showed such interaction ($P < 0.05$). The genotypic differences in proportions of CLA c9t11, C21:0, C22:1n9 and MUFA for DGAT1 were not the same for each sire breed of cow ($P < 0.05$). There were also trends of genotypic differences for C14:0, C18:1n9c, and N3 to SFA ration to vary among sire breeds for DGAT1 ($P \leq 0.12$). Breed x genotype interactions were evident for vaccenic acid and C20:4n6 for the SCD1 gene ($P<0.05$). For the FASN gene there was evidence that genotypic differences for C14:0, C16:0, C20:4n6, C22:5n3, SumC12C14C16, SFA, PUFA, N6, N3, the ratio of PUFA to SFA and the ratio of N3 to SFA varied among sire breeds. ($P<0.05$) and trends of sire breed x genotype interactions for C18:0 and CLA c9t11 ($P\leq0.12$).

Table 3.7. The P-values of the interaction between breed of sire and SNP from each gene on fatty acid composition.

	DGAT1	SCD1	FASN
C14:0	0.11	0.20	0.01
C16:0	0.44	0.97	0.01
C18:0	0.17	0.65	0.12
C18:1n9c	0.09	0.27	0.20
Vaccenic	0.83	0.05	0.59
C20:0	0.45	0.66	0.87
CLA c9t11	0.00	0.90	0.09
C21:0	0.03	0.28	0.14
C22:0	0.64	0.97	0.73
C22:1n9	0.01	0.49	0.16
C20:4n6	0.87	0.03	0.01
C20:5n3	0.85	0.45	0.58
C22:5n3	0.66	0.35	0.04
SumC12C14C16	0.22	0.81	0.01

SFA	0.15	0.50	0.03
MUFA	0.04	0.21	0.21
PUFA	0.26	0.28	0.02
N6	0.37	0.39	0.01
N3	0.50	0.22	0.04
N6/N3	0.56	0.39	0.31
PUFA/SFA	0.50	0.22	0.01
N3/SFA	0.12	0.19	0.01

DGAT1

Least squares means, standard errors, and genotypic comparisons for sire breed by genotype subclasses are given in Tables 3.8 and Appendix A, Table A.1 for comparisons at the P < 0.05 and P< 0.10 levels (significant level showed as A, B and C), respectively. Discussion is limited to Table 3.8 but Table A.1 is included in the Appendix to record potentially important trends not discussed. Genotype differences in DGAT1 were not consistent across sire breeds (P<0.05) for C14:0 with little evidence of sire breed differences for Bonsmara, Brangus, Charolais, or Romosinuano. However, the GC genotype for C14:0 was greater than GC/AA and AA in Gelbvieh and Hereford (P<0.05). The AA genotype for C18:0 was lesser than GC/AA and GC only in Brangus (P<0.05). For C18:1n9c, AA genotype was greater than GC/AA in Brangus and greater than GC in Hereford (P<0.05) but no differences were evident in other breeds. For CLA c9t11, the AA genotype was greater than GC/AA and GC in Brangus and greater than GC/AA only in Romosinuano (P<0.05) (Figure 3.8). For C21:0, the AA genotype was greater than GC/AA and GC genotype only in Brangus (P<0.05) (Figure 3.9). For C22:1n9, the heterozygous genotype GC/AA was lesser than homozygous genotype GC in Brangus but greater than genotype GC and AA in Romosinuano (P<0.05). For C22:5n3, only in Hereford, the genotype GC was lesser than

GC/AA (P<0.05). For SFA, there was little evidence of sire breed differences for Bonsmara, Charolais, Gelbvieh, or Romosinuano (P>0.05). However, levels of SFA were greater for GC genotypes in Brangus than AA genotypes (P<0.05) and SFA in GC genotypes in Hereford were greater than GC/AA and AA genotypes (P<0.05). For MUFA, there was little evidence of genotypic differences for Bonsmara, Charolais, Gelbvieh, or Romosinuano, but MUFA in AA genotype were greater than GC/AA and GC in Brangus and in GC genotype were lesser than GC/AA and AA genotype in Hereford (P<0.05) (Figure 3.10). The N6 in genotype AA was greater than GC/AA in Brangus and greater than GC/AA and GC in Hereford (P<0.05). For the ratios of N6/N3, genotype AA was greater than GC in Bonsmara, Brangus and Hereford (P<0.05), but no significant differences in Charolais, Gelbvieh, or Romosinuano. For PUFA, N3, PUFA/SFA and N3/SFA, only AA genotype in Hereford was higher than GC (P<0.05), but no significant differences were noted in other sire breeds. There was little evidence of interaction effects between genotype and breed for SumC12C14C16, C16:0 and vaccenic among six sired-breeds (P>0.05).

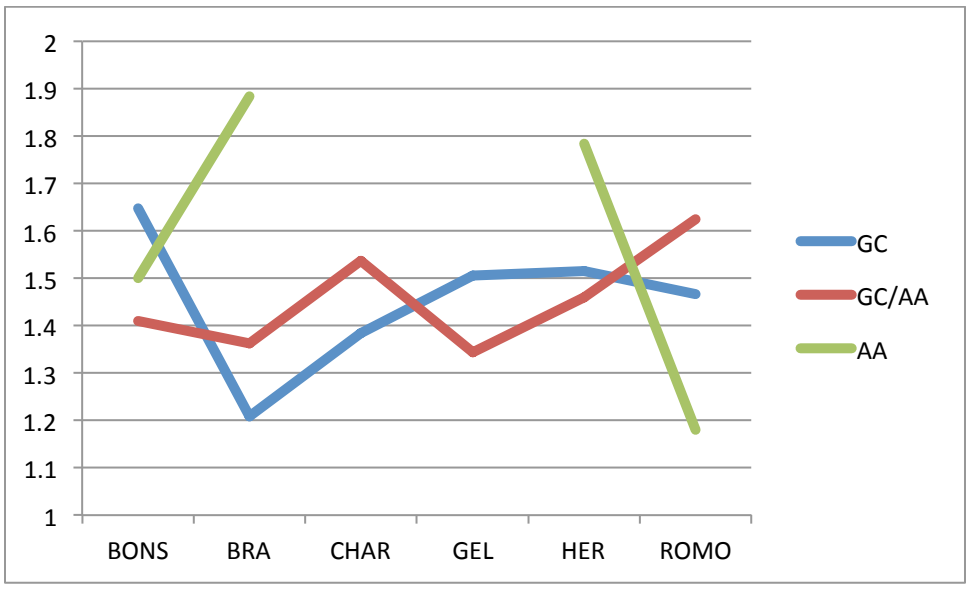

Figure 3.8. The interaction effects between sire of breed and genotypes of DGAT1 gene on CLAc9t11.

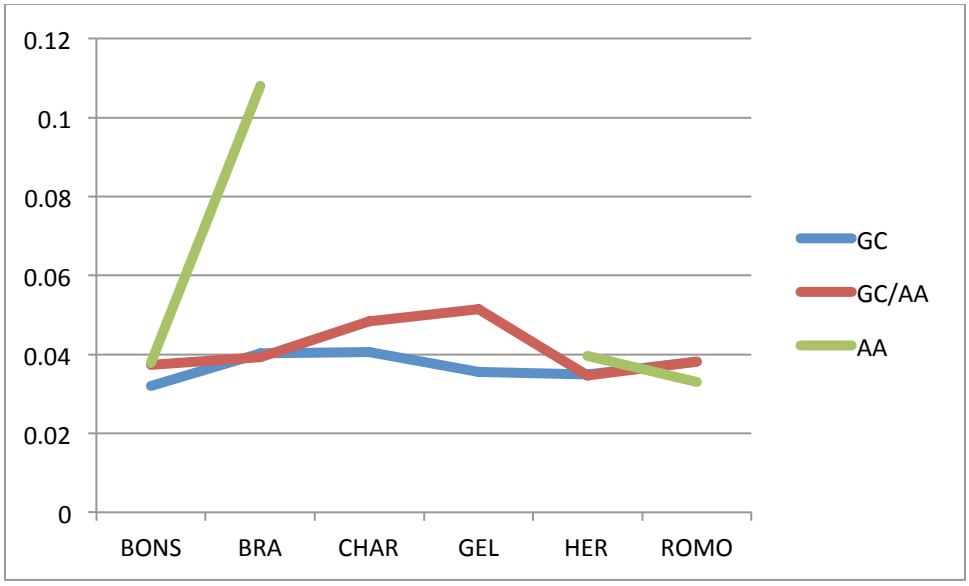

Figure 3.9. The interaction effects between sire of breed and genotypes of DGAT1 gene on C21:0.

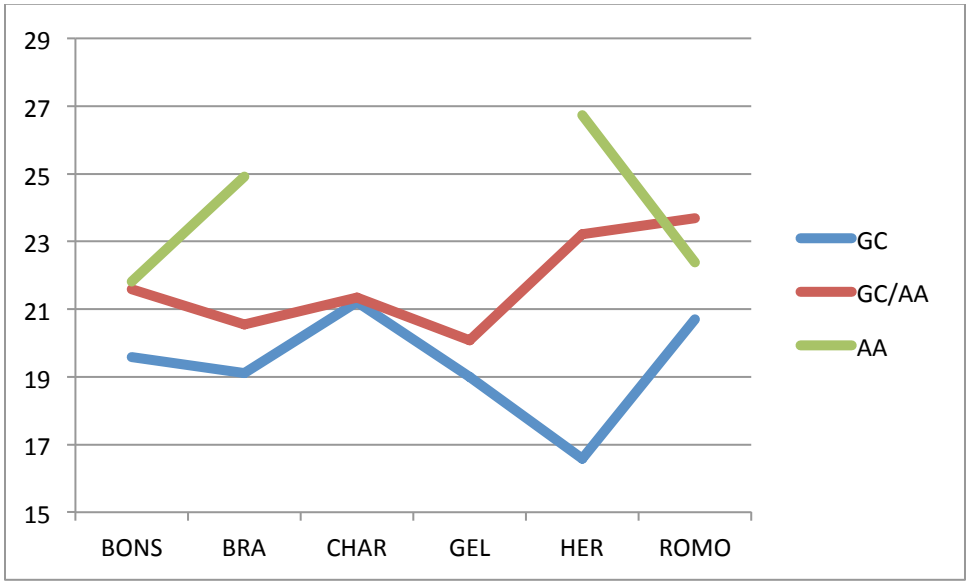

Figure 3.10. The interaction effects between sire of breed and genotypes of DGAT1 gene on MUFA.

SCD1

Least squares means, standard errors, and genotypic comparisons for sire breed by genotype subclasses are given in Tables 3.9 and Appendix A, Table A.2 for comparisons at the P < 0.05 and P< 0.10 levels, respectively. Discussion is limited to Table 3.10 but Table A.2 is included in the Appendix to record potentially important trends not discussed. There was little evidence of interaction effects between genotype and breed for SCD1 for SFA, MUFA, SumC12C14C16, CLAc9t11, C16:0, C18:0 and C18:1n9c among six sire breeds (P>0.05). For PUFA, N3 and N3/SFA, only G genotype in Hereford were greater than A/G and G (P<0.05). For N6 and N6/N3, only A genotype in Brangus were lesser than A/G (P<0.05). For the ratios of PUFA/SFA and C14:0, only G genotype in Hereford were greater than A/G (P<0.05). For vaccenic, only A genotype in Charolais were greater than A/G (P<0.05) (Figure 3.11). For C20:4n6, genotype A was greater than genotype A/G in Hereford and genotype G was less than genotype A and A/G in Romosinuano (P<0.05) (Figure 3.12). For C22:5n3, A genotype was greater than A/G in Hereford and greater than G in Romosinuano (P<0.05) but no differences were evident in other breeds.

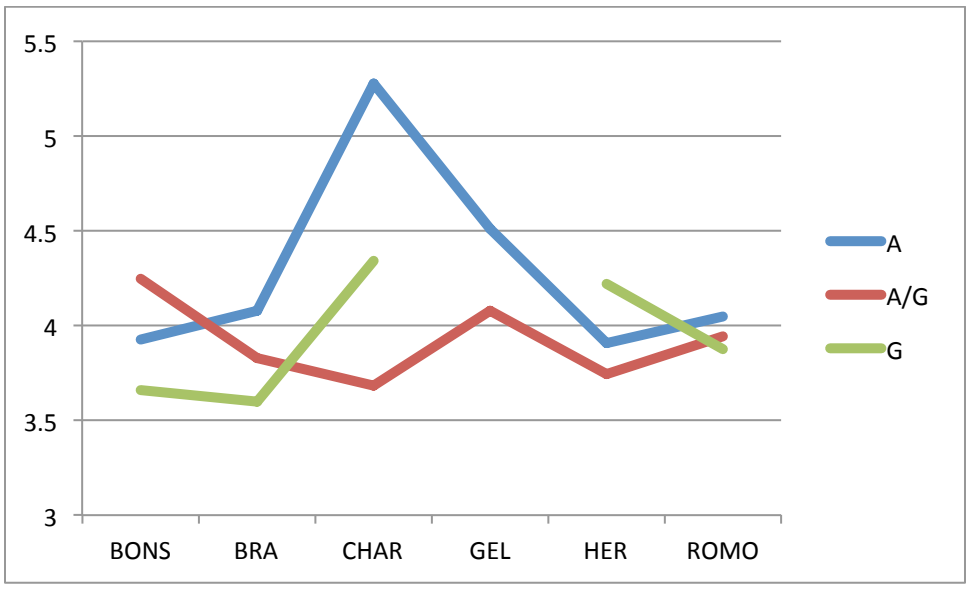

Figure 3.11. The interaction effects between sire of breed and genotypes of SCD1 gene on Vaccenic acid.

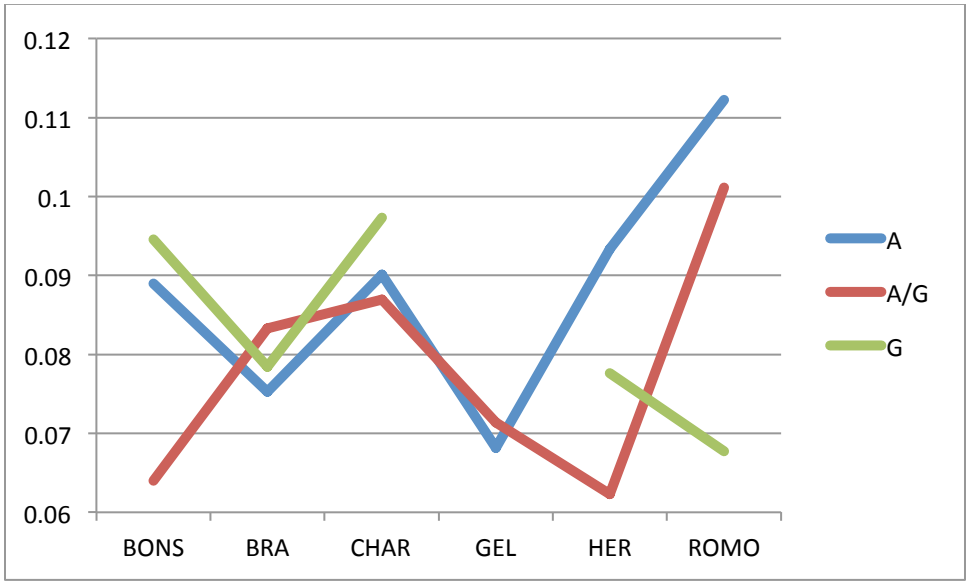

Figure 3.12. The interaction effects between sire of breed and genotypes of SCD1 gene on C20:4n6.

FASN

Least squares means, standard errors, and genotypic comparisons for sire breed by genotype subclasses are given in Tables 3.10 and Appendix A, Table A.3 for comparisons at the P < 0.05 and P< 0.10 levels, respectively. Discussion is limited to Table 3.10 but Table A.3 is included in the Appendix to record potentially important trends not discussed. For C14:0, C/T was lesser than C in Gelbvieh, T lesser than C in Hereford, and C lesser than T in Romosinuano (P<0.05) (Figure 3.13). For C16:0, C was greater than C/T in Bonsmara but T was greater than C/T and C in Gelbvieh (P<0.05) (Figure 3.14). For C18:0, C/T was greater than T in both Gelbvieh and Hereford (P<0.05). For C18:1n9c, only C was greater than T in Romosinuano (P<0.05). For vaccenic, only T in Gelbvieh was lesser than C/T and C (P<0.05). For CLAc9t11, C was lesser than C/T in Bonsmara but T was greater than C/T in Romosinuano (P<0.05) while there was no

evidence of genotypic differences among other sire breeds. For C20:4n6, C was greater than T in both Charolais and Romosinuano (P<0.05) (Figure 3.15). For C22:5n3, T was lesser than C/T and C in Charolais and C was greater than C/T in Romosinuano (P<0.05) (Figure 3.16). For SumC12C14C16, T was greater than C in Gelbvieh and Romosinuano but lesser than C in Hereford (P<0.05) (Figure 3.17). For SFA, C was greater than T in Hereford but lesser than T in Romosinuano (P<0.05), and no differences among the three genotypes were noted in Bonsmara, Brangus, Charolais and Gelbvieh (Figure 3.18). For MUFA, only C in Romosinuano was greater than T (P<0.05). For PUFA, only T in Hereford was greater than C/T and T (P<0.05) (Figure 3.19). The level of N6 in T was lesser than A for Hereford and Romosinuano (P<0.05) (Figure 3.20) but the level of N3 in T was greater than A for Hereford (P<0.05) (Figure 3.21). The ratio of N6/N3 was greater in C/T than C and T for Hereford (P<0.05). For ratios of PUFA/SFA and N3/SFA, T was greater than C/T and C in Hereford but lesser than C in Romosinuano (P<0.05) (Figure 3.22-23).

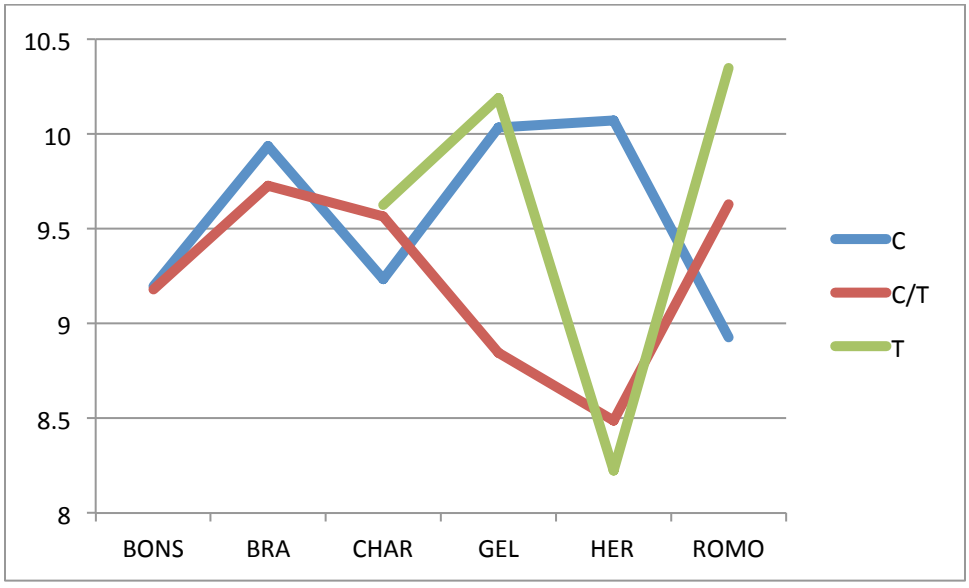

Figure 3.13. The interaction effects between sire of breed and genotypes of FASN gene on C14:0.

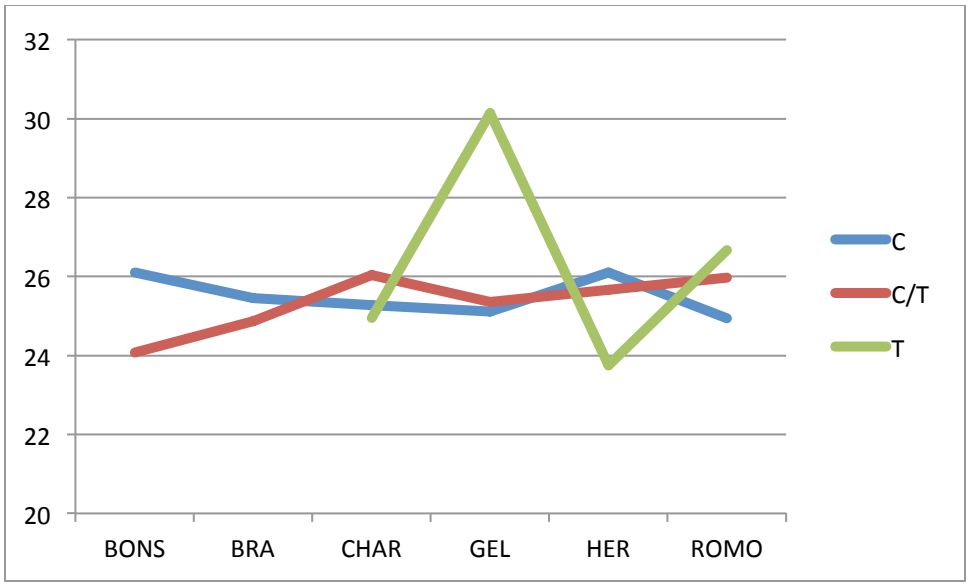

Figure 3.14. The interaction effects between sire of breed and genotypes of FASN gene on C16:0.

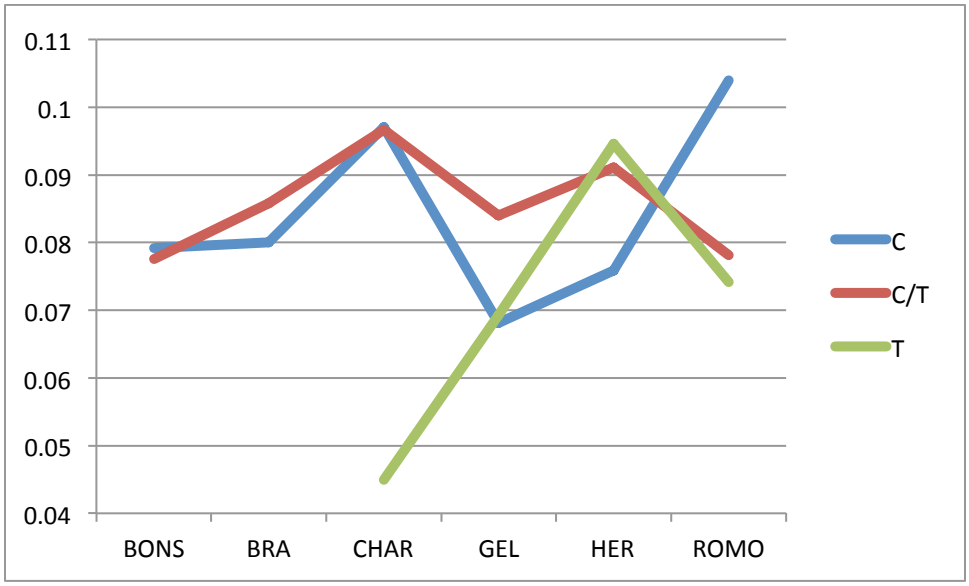

Figure 3.15. The interaction effects between sire of breed and genotypes of FASN gene on C20:4n6.

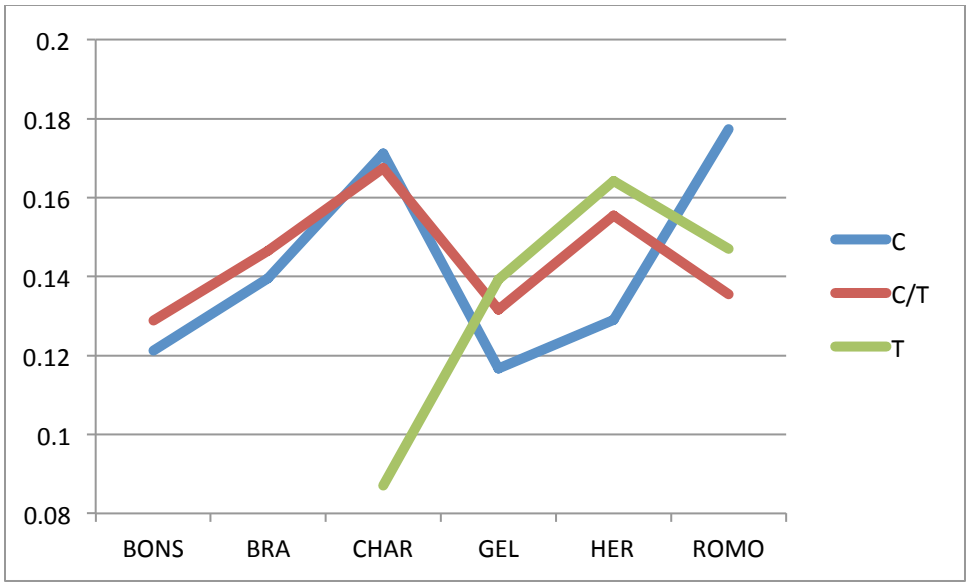

Figure 3.16. The interaction effects between sire of breed and genotypes of FASN gene on C20:5n3.

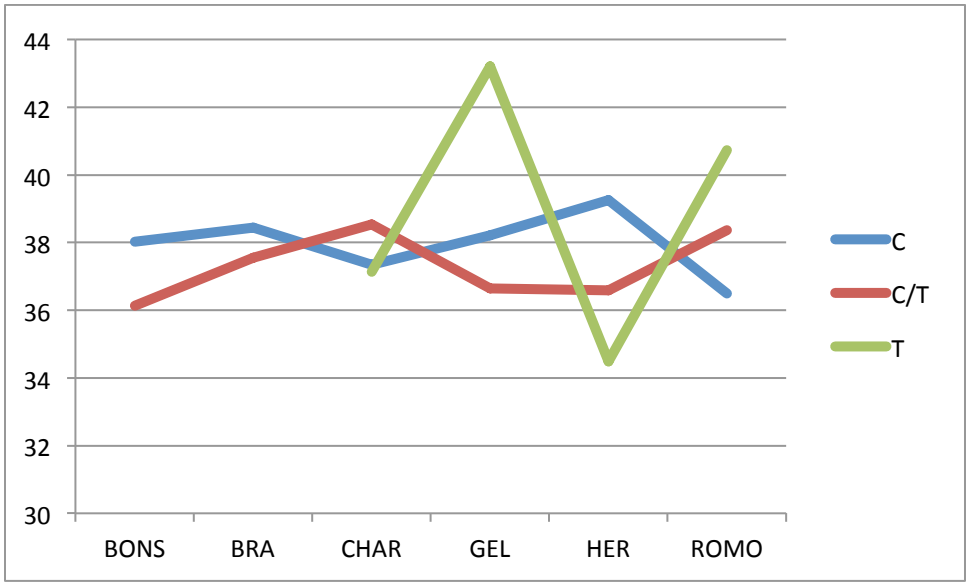

Figure 3.17. The interaction effects between sire of breed and genotypes of FASN gene on SumC12C14C16.

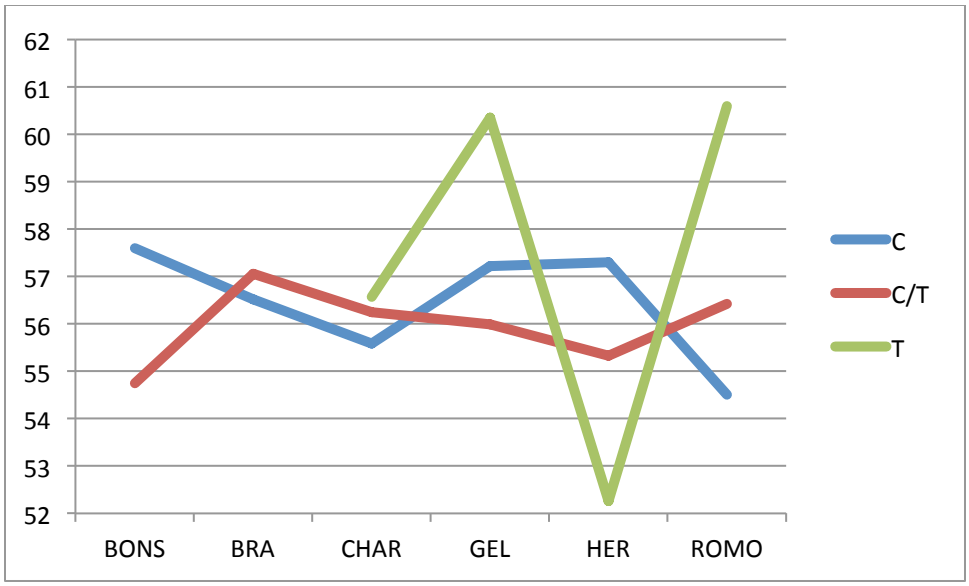

Figure 3.18. The interaction effects between sire of breed and genotypes of FASN gene on SFA.

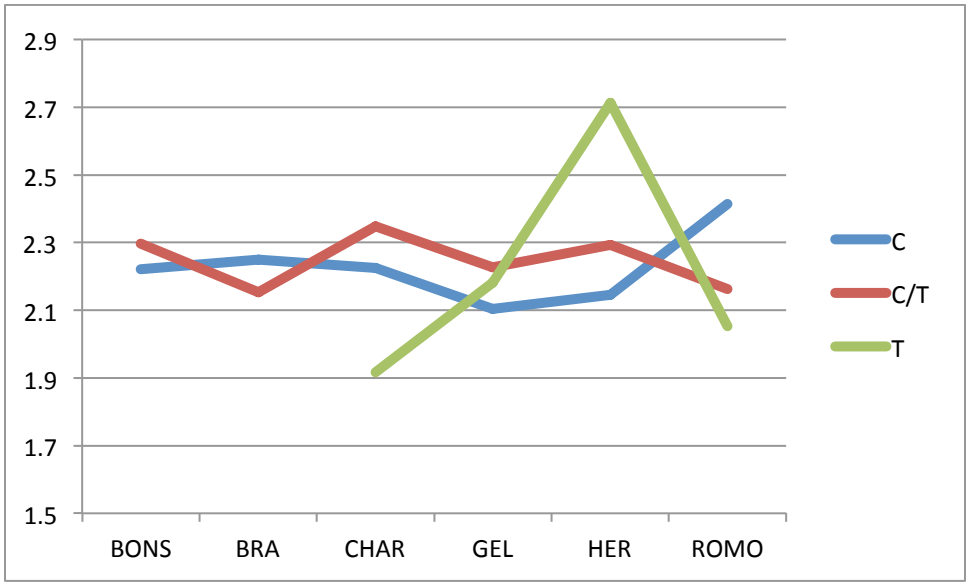

Figure 3.19. The interaction effects between sire of breed and genotypes of FASN gene on PUFA.

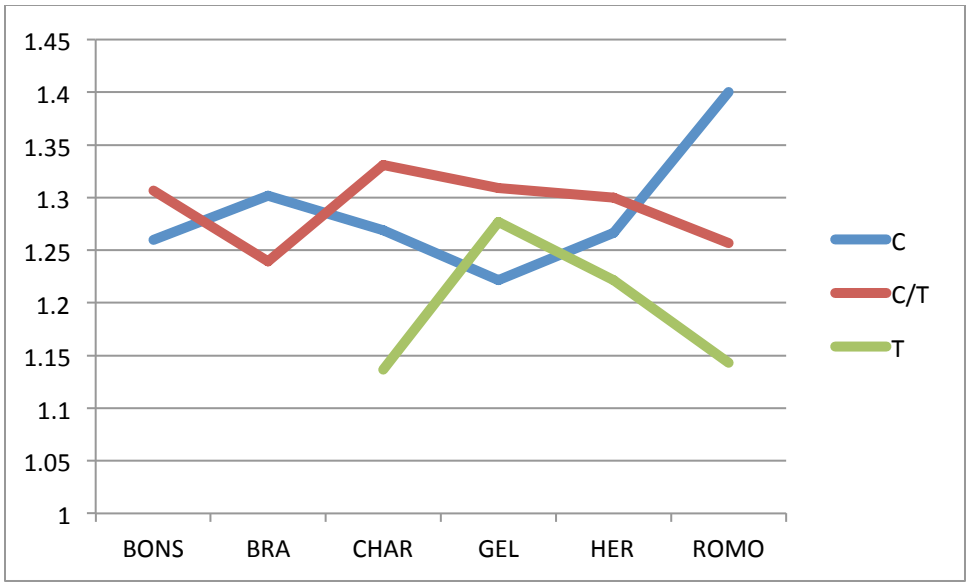

Figure 3.20. The interaction effects between sire of breed and genotypes of FASN gene on N6.

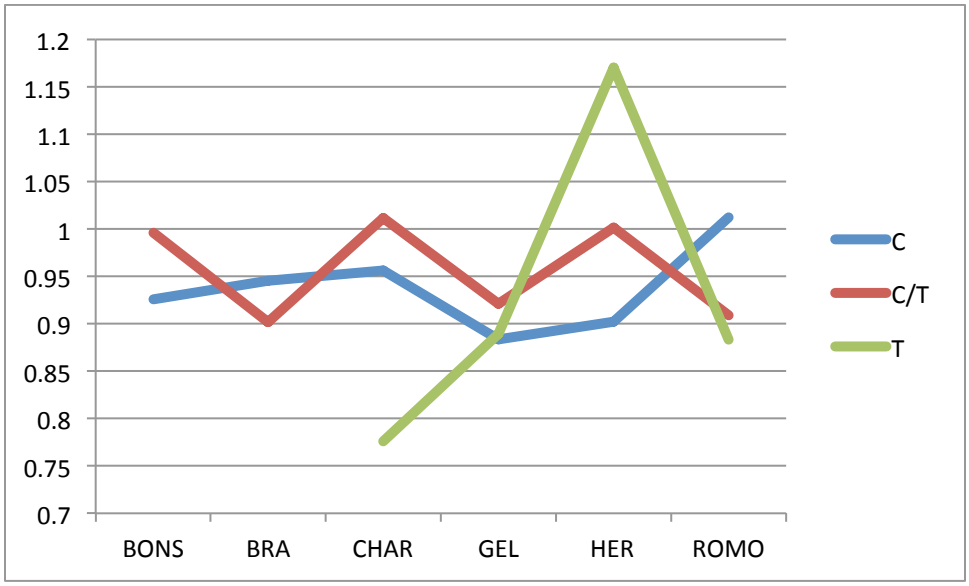

Figure 3.21. The interaction effects between sire of breed and genotypes of FASN gene on N3.

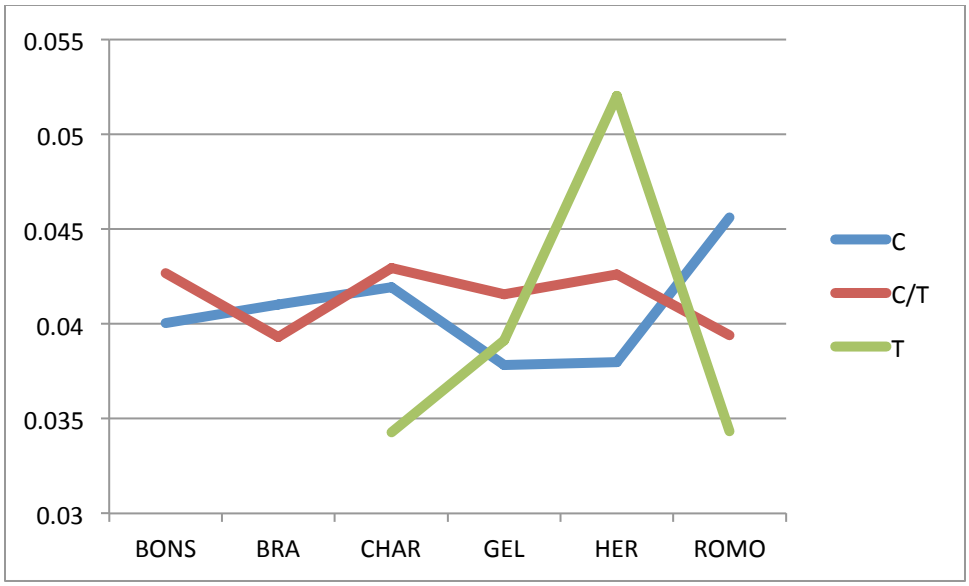

Figure 3.22. The interaction effects between sire of breed and genotypes of FASN gene on PUFA/SFA.

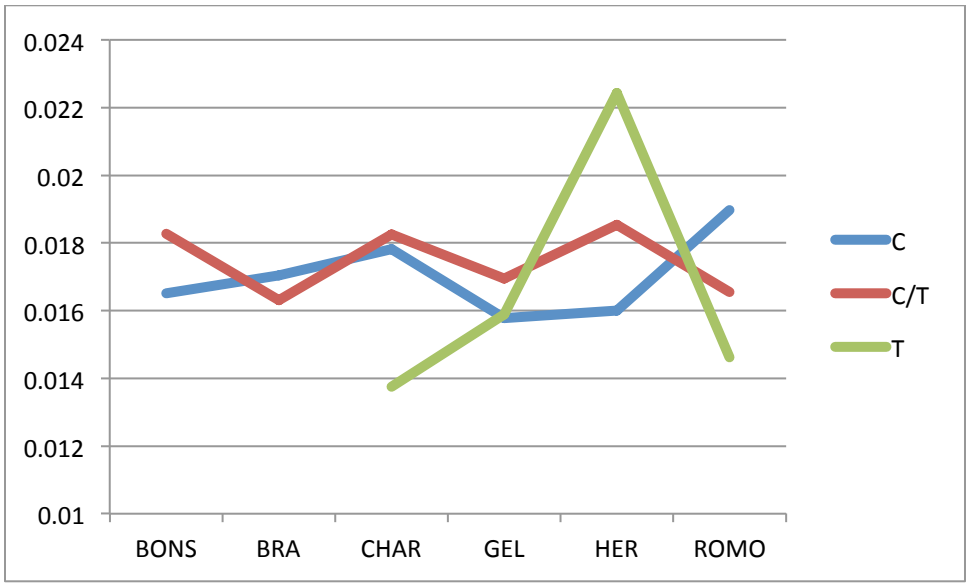

Figure 3.23. The interaction effects between sire of breed and genotypes of FASN gene on N3/SFA.

Table 3.8. The multiple comparisons of LS-means of fatty acid composition data under the effects of DGAT1 gene within certain breed (P<0.05).

	(P<0.05)						
	BONS	BRA	CHAR	GEL	HER	ROMO	ALL

	GC	10.25	A	11.22	A	10.30	A	11.51	A	11.01	A	10.03	A	10.87	A
C14:0	GC/AA	9.47	A	10.06	A	9.73	A	9.79	B	8.93	B	9.29	A	9.54	B
	AA	9.69	A	10.08	A					7.93	B	8.27	A	9.39	B
	GC	25.37	A	25.82	A	24.46	A	24.79	A	26.16	A	25.46	A	25.28	A
C16:0	GC/AA	25.10	A	24.99	A	26.05	A	26.11	A	25.41	A	25.09	A	25.43	A
	AA	24.62	A	24.79	A					23.81	A	26.61	A	25.11	A
	GC	11.45	A	11.65	A	11.15	A	11.11	A	11.98	A	12.05	A	11.49	A
C18:0	GC/AA	11.86	A	11.58	A	10.70	A	12.37	A	11.20	A	10.72	A	11.32	A
	AA	11.71	A	9.04	B					11.38	A	10.26	A	10.49	A
	GC	17.32	A	16.87	AB	18.91	A	16.76	A	14.72	A	18.49	A	16.60	A
C18:1n9c	GC/AA	19.49	A	18.27	A	18.96	A	18.09	A	20.77	B	21.01	A	19.47	B
	AA	19.50	A	21.66	B					23.63	B	20.29	A	20.79	B
	GC	4.86	A	3.84	A	3.87	A	4.30	A	4.45	A	4.21	A	4.34	A
Vaccenic	GC/AA	3.99	A	3.95	A	4.33	A	4.21	A	3.88	A	3.88	A	4.04	A
	AA	4.18	A	3.53	A					4.35	A	3.39	A	3.86	A
	GC	0.29	A	0.29	AB	0.25	A	0.28	A	0.27	A	0.30	A	0.28	A
C20:0	GC/AA	0.27	A	0.31	A	0.28	A	0.30	A	0.29	A	0.30	A	0.30	B
	AA	0.30	A	0.22	B					0.24	A	0.26	A	0.25	AB
	GC	1.65	A	1.21	A	1.38	A	1.51	A	1.52	A	1.47	AB	1.51	A
CLA c9t11	GC/AA	1.41	A	1.36	A	1.54	A	1.34	A	1.46	A	1.62	A	1.46	A
	AA	1.50	A	1.88	B					1.78	A	1.18	B	1.59	A
	GC	0.03	A	0.04	A	0.04	A	0.04	A	0.03	A	0.04	A	0.04	A
C21:0	GC/AA	0.04	A	0.04	A	0.05	A	0.05	A	0.03	A	0.04	A	0.04	A
	AA	0.04	A	0.11	B					0.04	A	0.03	A	0.06	B
	GC	0.11	A	0.12	AB	0.12	A	0.11	A	0.12	A	0.11	A	0.12	A
C22:0	GC/AA	0.11	A	0.13	A	0.11	A	0.11	A	0.12	A	0.12	A	0.12	A
	AA	0.12	A	0.10	B					0.11	A	0.11	A	0.11	A
	GC	0.02	A	0.03	A	0.03	A	0.02	A	0.03	A	0.02	A	0.03	A
C22:1n9	GC/AA	0.02	A	0.02	B	0.02	A	0.02	A	0.03	A	0.03	B	0.02	A
	AA	0.03	A	0.03	AB					0.03	A	0.02	A		
	GC	0.08	A	0.08	A	0.09	A	0.07	A	0.07	A	0.09	A	0.08	A
C20:4n6	GC/AA	0.08	A	0.09	A	0.09	A	0.07	A	0.09	A	0.10	A	0.09	A
	AA	0.08	A	0.07	A					0.08	A	0.08	A	0.08	A
	GC	0.08	A	0.08	A	0.09	A	0.07	A	0.07	A	0.08	A	0.08	A
C20:5n3	GC/AA	0.08	A	0.08	A	0.09	A	0.08	A	0.09	A	0.09	A	0.08	A
	AA	0.09	A	0.07	A					0.08	A	0.08	A	0.08	A
	GC	0.11	A	0.14	A	0.14	A	0.12	A	0.12	A	0.13	A	0.13	A
C22:5n3	GC/AA	0.13	A	0.15	A	0.17	A	0.12	A	0.16	B	0.16	A	0.15	B
	AA	0.13	A	0.12	A					0.18	AB	0.16	A	0.14	AB
SUM	GC	37.00	A	39.24	A	38.45	A	37.12	A	38.32	A	37.02	A	37.41	A
C12C14C16	GC/AA	37.02	A	37.84	A	36.22	A	38.76	A	37.02	A	37.06	A	37.67	A
	AA	36.90	A	37.19	A					33.64	A	40.81	A	37.54	A

SFA	GC	59.70	A	60.59	A	58.93	A	60.42	A	63.21	A	58.61	A	60.76	A
	GC/AA	57.31	A	58.21	AB	56.80	A	58.68	A	55.50	B	55.04	A	56.88	B
	AA	56.89	A	53.97	B					50.67	B	55.60	A	54.92	B
MUFA	GC	19.58	A	19.12	A	21.21	A	18.99	A	16.58	A	20.71	A	18.97	A
	GC/AA	21.58	A	20.54	A	21.34	A	20.08	A	23.21	B	23.69	A	21.81	B
	AA	21.81	A	24.92	B					26.73	B	22.39	A	23.98	C
PUFA	GC	2.38	A	1.98	A	2.15	A	2.17	A	2.40	AB	2.45	A	2.27	A
	GC/AA	2.07	A	2.07	A	2.31	A	2.15	A	2.28	A	2.36	A	2.21	A
	AA	2.33	A	2.41	A					2.93	B	2.15	A	2.42	A
N6	GC	1.12	A	1.29	AB	1.12	A	1.05	A	1.15	A	1.27	A	1.16	A
	GC/AA	1.23	A	1.22	A	1.30	A	1.21	A	1.29	A	1.33	A	1.26	A
	AA	1.33	A	1.45	B					1.74	B	1.41	A	1.43	B
N3	GC	1.07	A	0.89	A	1.04	A	0.88	A	1.00	A	1.13	A	1.00	A
	GC/AA	0.90	A	0.87	A	1.00	A	0.87	A	0.95	A	1.00	A	0.94	A
	AA	0.99	A	1.02	A					1.32	B	0.93	A	1.02	A
N6/N3	GC	1.10	A	1.25	A	1.20	A	1.29	A	1.15	A	1.27	A	1.20	A
	GC/AA	1.26	AB	1.32	A	1.38	A	1.36	A	1.39	B	1.35	A	1.34	B
	AA	1.39	B	1.60	B					1.47	AB	1.29	A	1.48	C
PUFA/SFA	GC	0.03	A	0.05	A	0.04	A	0.03	A	0.03	A	0.04	A	0.03	A
	GC/AA	0.04	A	0.04	A	0.04	A	0.04	A	0.04	A	0.04	A	0.04	A
	AA	0.04	A	0.04	A					0.06	B	0.05	A	0.05	B
N3/SFA	GC	0.02	A	0.02	A	0.02	A	0.02	A	0.02	A	0.02	A	0.02	A
	GC/AA	0.02	A	0.02	A	0.02	A	0.02	A	0.02	A	0.02	A	0.02	A
	AA	0.02	A	0.02	A					0.02	B	0.01	A	0.02	A

Table 3.9. The multiple comparisons of LS-means of fatty acid composition data under the effects of SCD1 gene within certain breed (P<0.05).

		(P<0.05)													
		BONS		BRA		CHAR		GEL		HER		ROMO		ALL	
C14:0	A	8.83	A	10.04	A	9.15	A	9.92	A	8.89	AB	9.08	AB	9.39	A
	A/G	9.56	A	9.75	A	9.42	A	9.53	A	10.15	A	8.84	A	9.35	A
	G	9.39	A	9.63	A	9.80	A			8.30	B	10.23	B	9.67	A
C16:0	A	25.28	A	25.49	A	24.86	A	25.63	A	25.03	A	26.40	A	25.41	A
	A/G	24.78	A	25.12	A	26.09	A	25.66	A	25.74	A	25.10	A	25.35	A
	G	25.27	A	25.71	A	25.29	A			25.61	A	25.53	A	25.49	A
C18:0	A	11.67	A	11.93	A	10.60	A	12.28	A	11.24	A	11.45	A	11.52	A
	A/G	12.00	A	10.60	A	10.86	A	11.33	A	10.78	A	11.32	A	11.16	A
	G	10.07	A	11.16	A	10.69	A			11.68	A	10.34	A	10.96	A
C18:1n9c	A	21.70	A	18.67	A	18.68	A	18.99	A	20.97	A	18.99	A	19.77	A
	A/G	18.96	A	19.97	A	20.46	A	19.86	A	19.55	A	21.44	A	20.37	A
	G	21.62	A	18.87	A	19.71	A			21.34	A	19.21	A	19.78	A

Vaccenic	A	3.93	A	4.08	A	5.28	A	4.51	A	3.91	A	4.05	A	4.20	A
	A/G	4.25	A	3.83	A	3.68	B	4.08	A	3.74	A	3.94	A	4.03	A
	G	3.66	A	3.60	A	4.34	AB			4.22	A	3.88	A	3.99	A
C20:0	A	0.28	A	0.29	A	0.26	A	0.29	A	0.30	A	0.30	A	0.29	A
	A/G	0.31	A	0.29	A	0.28	A	0.29	A	0.22	A	0.29	A	0.29	A
	G	0.25	A	0.31	A	0.28	A			0.25	A	0.28	A	0.27	A
CLA c9t11	A	1.38	A	1.38	A	1.63	A	1.43	A	1.47	A	1.54	A	1.46	A
	A/G	1.55	A	1.56	A	1.50	A	1.42	A	1.55	A	1.56	A	1.52	A
	G	1.50	A	1.32	A	1.51	A			1.49	A	1.61	A	1.49	A
C21:0	A	0.05	A	0.04	A	0.04	A	0.06	A	0.03	A	0.04	A	0.04	A
	A/G	0.03	A	0.07	A	0.06	A	0.04	A	0.02	A	0.04	A	0.04	A
	G	0.02	A	0.02	A	0.04	A			0.04	A	0.04	A	0.04	A
C22:0	A	0.11	A	0.13	A	0.11	A	0.11	A	0.13	A	0.12	A	0.12	A
	A/G	0.12	A	0.12	A	0.11	A	0.11	A	0.11	A	0.12	A	0.12	A
	G	0.10	A	0.12	A	0.12	A			0.12	A	0.12	A	0.12	A
C22:1n9	A	0.02	A	0.02	A	0.03	A	0.02	A	0.03	A	0.02	A	0.02	AB
	A/G	0.03	A	0.03	A	0.03	A	0.02	A	0.04	A	0.03	A	0.03	A
	G	0.03	A	0.02	A	0.03	A			0.02	B	0.03	A	0.02	B
C20:4n6	A	0.09	A	0.08	A	0.09	A	0.07	A	0.09	A	0.11	A	0.09	A
	A/G	0.06	A	0.08	A	0.09	A	0.07	A	0.06	B	0.10	A	0.08	B
	G	0.09	A	0.08	A	0.10	A			0.08	AB	0.07	B		
C20:5n3	A	0.08	A	0.07	A	0.09	A	0.07	A	0.09	A	0.08	A	0.08	A
	A/G	0.08	A	0.08	A	0.08	A	0.07	A	0.07	A	0.09	A	0.08	A
	G	0.10	A	0.07	A	0.09	A			0.08	A	0.08	A	0.08	A
C22:5n3	A	0.13	A	0.14	A	0.17	A	0.13	A	0.13	A	0.20	A	0.15	A
	A/G	0.12	A	0.14	A	0.15	A	0.12	A	0.11	B	0.18	A	0.14	A
	G	0.13	A	0.16	A	0.18	A			0.12	AB	0.13	B	0.14	A
SUM C12C14C16	A	36.48	A	38.52	A	37.03	A	38.31	A	36.74	A	38.14	A	37.60	A
	A/G	37.31	A	37.74	A	38.18	A	38.12	A	39.46	A	36.49	A	37.53	A
	G	37.53	A	38.68	A	37.90	A			36.16	A	39.14	A	38.11	A
SFA	A	54.15	A	57.99	A	55.99	A	57.15	A	55.17	A	57.44	A	56.33	A
	A/G	57.04	A	55.79	A	55.72	A	56.75	A	58.68	A	54.95	A	55.86	A
	G	55.24	A	57.91	A	56.49	A			54.68	A	57.14	A	56.55	A
MUFA	A	24.20	A	21.01	A	21.03	A	21.10	A	23.44	A	21.44	A	22.14	A
	A/G	21.26	A	22.80	A	23.05	A	22.03	A	22.05	A	24.03	A	22.87	A
	G	24.12	A	21.46	A	22.03	A			23.66	A	21.58	A	22.10	A
PUFA	A	2.34	A	2.11	A	2.25	A	2.13	A	2.23	A	2.36	A	2.21	A
	A/G	2.15	A	2.27	A	2.24	A	2.16	A	2.16	A	2.36	A	2.25	A
	G	2.21	A	1.96	A	2.29	A			2.67	B	2.21	A	2.29	A
N6	A	1.31	A	1.19	A	1.26	A	1.20	A	1.30	A	1.31	A	1.25	A
	A/G	1.23	A	1.35	B	1.29	A	1.25	A	1.31	A	1.39	A	1.32	A

	G	1.36	A	1.15	AB	1.26	A			1.48	A	1.26	A	1.29	A
	A	1.03	A	0.92	A	0.98	A	0.90	A	0.94	A	1.00	A	0.94	A
N3	A/G	0.92	A	0.94	A	0.95	A	0.89	A	0.88	A	0.99	A	0.94	A
	G	0.87	A	0.82	A	1.02	A			1.18	B	0.95	A	0.99	A
	A	1.34	A	1.33	A	1.41	A	1.40	A	1.40	A	1.35	A	1.37	A
N6/N3	A/G	1.33	A	1.50	B	1.38	A	1.43	A	1.40	A	1.38	A	1.41	A
	G	1.46	A	1.35	AB	1.31	A			1.28	A	1.35	A	1.34	A
	A	0.04	A	0.04	A	0.04	A	0.04	A	0.04	AB	0.04	A	0.04	A
PUFA/SFA	A/G	0.04	A	0.04	A	0.04	A	0.04	A	0.04	A	0.05	A	0.04	A
	G	0.04	A	0.04	A	0.04	A			0.05	B	0.04	A	0.04	A
	A	0.02	A	0.02	A	0.02	A	0.02	A	0.02	A	0.02	A	0.02	A
N3/SFA	A/G	0.02	A	0.02	A	0.02	A	0.02	A	0.02	A	0.02	A	0.02	A
	G	0.02	A	0.01	A	0.02	A			0.02	B	0.02	A	0.02	A

Table 3.10. The multiple comparisons of LS-means of fatty acid composition data under the effects of FASN gene within certain breed (P<0.05).

		(P<0.05)													
		BONS		BRA		CHAR		GEL		HER		ROMO		ALL	
	C	9.20	A	9.94	A	9.23	A	10.03	A	10.07	A	8.93	A	9.52	A
C14:0	C/T	9.18	A	9.73	A	9.57	A	8.85	B	8.49	B	9.63	AB	9.25	A
	T					9.63	A	10.19	AB	8.22	B	10.35	B	9.51	A
	C	26.10	A	25.46	A	25.28	A	25.11	A	26.10	A	24.94	A	25.46	A
C16:0	C/T	24.07	B	24.87	A	26.04	A	25.35	A	25.67	A	25.97	A	25.29	A
	T					24.95	A	30.14	B	23.75	A	26.68	A	25.76	A
	C	11.84	A	10.42	A	10.53	A	11.53	AB	10.26	A	10.97	A	10.99	A
C18:0	C/T	11.52	A	11.47	A	10.56	A	12.70	A	12.20	B	11.02	A	11.58	A
	T					12.68	A	9.07	B	10.00	A	10.22	A	10.63	A
	C	20.32	A	19.65	A	20.07	A	19.19	A	18.97	A	21.56	A	20.05	A
C18:1n9c	C/T	20.50	A	19.39	A	19.75	A	20.29	A	21.01	A	19.72	AB	20.13	A
	T					19.74	A	18.92	A	22.29	A	17.44	B	19.66	A
	C	3.95	A	3.87	A	4.33	A	4.32	A	3.75	A	3.79	A	4.02	A
Vaccenic	C/T	4.21	A	3.99	A	4.38	A	4.46	A	4.39	A	4.19	A	4.24	A
	T					4.25	A	2.32	B	3.38	A	3.66	A	3.73	A
	C	0.29	A	0.26	A	0.27	A	0.29	A	0.27	A	0.29	A	0.28	A
C20:0	C/T	0.29	A	0.31	B	0.27	A	0.32	A	0.29	A	0.31	A	0.30	B
	T					0.28	A	0.24	A	0.28	A	0.26	A	0.27	AB
	C	1.31	A	1.56	A	1.52	A	1.49	A	1.47	A	1.57	AB	1.48	A
CLA c9t11	C/T	1.62	B	1.38	A	1.60	A	1.33	A	1.47	A	1.64	A	1.51	A
	T					1.26	A	1.23	A	1.55	A	1.26	B	1.34	A

C21:0	C	0.03	A	0.07	A	0.04	A	0.03	A	0.04	A	0.04	A	0.04	A
	C/T	0.04	A	0.04	B	0.06	A	0.06	A	0.03	A	0.04	A	0.04	A
	T					0.01	A	0.04	A	0.04	A	0.03	A	0.04	A
C22:0	C	0.11	A	0.12	A	0.11	A	0.11	AB	0.12	A	0.12	A	0.12	A
	C/T	0.11	A	0.12	A	0.11	A	0.13	A	0.12	A	0.12	A	0.12	A
	T					0.12	A	0.08	B	0.09	A	0.15	A	0.11	A
C22:1n9	C	0.03	A	0.03	A	0.03	A	0.02	A	0.03	A	0.03	A	0.03	A
	C/T	0.02	A	0.02	B	0.03	A	0.03	A	0.03	A	0.03	A	0.02	A
	T					0.02	A	0.02	A	0.01	A	0.03	A	0.02	A
C20:4n6	C	0.08	A	0.08	A	0.10	A	0.07	A	0.08	A	0.10	A	0.08	A
	C/T	0.08	A	0.09	A	0.10	A	0.08	A	0.09	A	0.08	B	0.09	A
	T					0.04	B	0.07	A	0.09	A	0.07	B	0.08	A
C20:5n3	C	0.07	A	0.08	A	0.09	A	0.08	A	0.08	A	0.09	A	0.08	A
	C/T	0.09	A	0.08	A	0.09	A	0.07	A	0.09	A	0.09	A	0.08	A
	T					0.06	A	0.07	A	0.09	A	0.08	A	0.08	A
C22:5n3	C	0.12	A	0.14	A	0.17	A	0.12	A	0.13	A	0.18	A	0.14	A
	C/T	0.13	A	0.15	A	0.17	A	0.13	A	0.16	A	0.14	B	0.15	A
	T					0.09	B	0.14	A	0.16	A	0.15	AB	0.14	A
SUM C12C14C16	C	38.03	A	38.44	A	37.35	A	38.21	A	39.25	A	36.50	A	37.87	A
	C/T	36.13	A	37.55	A	38.53	A	36.64	A	36.60	AB	38.37	AB	37.29	A
	T					37.14	A	43.22	B	34.49	B	40.73	B	38.18	A
SFA	C	57.60	A	56.51	A	55.59	A	57.22	A	57.30	A	54.51	A	56.37	A
	C/T	54.75	A	57.05	A	56.24	A	55.99	A	55.33	AB	56.43	AB	55.97	A
	T					56.57	A	60.35	A	52.26	B	60.60	B	56.91	A
MUFA	C	22.61	A	22.36	A	22.63	A	21.34	A	21.46	A	24.25	A	22.52	A
	C/T	22.86	A	21.88	A	22.23	A	22.37	A	23.28	A	22.12	AB	22.48	A
	T					21.75	A	21.33	A	24.92	A	19.58	B	21.99	A
PUFA	C	2.22	A	2.25	A	2.23	A	2.10	A	2.15	A	2.41	A	2.23	A
	C/T	2.30	A	2.15	A	2.35	A	2.23	A	2.29	A	2.16	A	2.25	A
	T					1.92	A	2.18	A	2.71	B	2.05	A	2.30	A
N6	C	1.26	A	1.30	A	1.27	A	1.22	A	1.27	A	1.40	A	1.29	A
	C/T	1.31	A	1.24	A	1.33	A	1.31	A	1.30	A	1.26	AB	1.29	A
	T					1.14	A	1.28	A	1.22	B	1.14	B	1.32	A
N3	C	0.93	A	0.95	A	0.96	A	0.88	A	0.90	A	1.01	A	0.94	A
	C/T	1.00	A	0.90	A	1.01	A	0.92	A	1.00	AB	0.91	A	0.96	A
	T					0.78	A	0.89	A	1.17	B	0.88	A	0.98	A
N6/N3	C	1.29	A	1.42	A	1.38	A	1.38	A	1.50	A	1.37	A	1.40	A
	C/T	1.35	A	1.35	A	1.36	A	1.46	A	1.27	B	1.38	A	1.37	A
	T					1.56	A	1.45	A	1.51	AB	1.32	A	1.43	A
PUFA/SFA	C	0.04	A	0.04	A	0.04	A	0.04	A	0.04	A	0.05	A	0.04	A

	C/T	0.04	A	0.04	A	0.04	A	0.04	A	0.04	A	0.04	AB	0.04	A
	T					0.03	A	0.04	A	0.05	B	0.03	B	0.04	A
	C	0.02	A	0.02	A	0.02	A	0.02	A	0.02	A	0.02	A	0.02	A
N3/SFA	C/T	0.02	A	0.02	A	0.02	A	0.02	A	0.02	A	0.02	AB	0.02	A
	T					0.01	A	0.02	A	0.02	B	0.01	B	0.02	A

It is clear from these results that genotypic differences in percent FAME for DGAT1, SCD1, and especially FASN can depend on the sire breed of cow. We hypothesize that the failure of genotypic differences for these genes to be constant among the sire breeds of cow may be due to differences in epistatic effects. In other words, the alleles of these genes affecting fatty acids may interact with other alleles at other loci and these “other alleles” can differ from sire breed to sire breed, particularly given the genetic diversity of the sire breeds used. The results have use in determining the genotypes useful in alter fatty acid percentages, as may be desirable, but within the sire breeds used in this study. These results do not address what genotypic differences might be observed in sire breeds not present in this study. Further research is warranted to determine mechanisms of interactions of genotype with sire breed to facilitate interpretation of results from candidate gene analyses.

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APPENDIX A

Table A.1. The multiple comparisons of LS-means of fatty acid composition data under the effects of DGAT1 gene within certain breed (P<0.1).

		(P<0.10)													
		BONS		BRA		CHAR		GEL		HER		ROMO		ALL	
C14:0	GC	10.25	A	11.22	A	10.30	A	11.51	A	11.01	A	10.03	A	10.87	A
	GC/AA	9.47	A	10.06	A	9.73	A	9.79	B	8.93	B	9.29	A	9.54	B
	AA	9.69	A	10.08	A					7.93	B	8.27	A	9.39	B
C16:0	GC	25.37	A	25.82	A	24.46	A	24.79	A	26.16	A	25.46	A	25.28	A
	GC/AA	25.10	A	24.99	A	26.05	A	26.11	A	25.41	A	25.09	A	25.43	A
	AA	24.62	A	24.79	A					23.81	A	26.61	A	25.11	A
C18:0	GC	11.45	A	11.65	A	11.15	A	11.11	A	11.98	A	12.05	A	11.49	A
	GC/AA	11.86	A	11.58	A	10.70	A	12.37	A	11.20	A	10.72	B	11.32	A
	AA	11.71	A	9.04	B					11.38	A	10.26	AB	10.49	B
C18:1n9c	GC	17.32	A	16.87	A	18.91	A	16.76	A	14.72	A	18.49	A	16.60	A
	GC/AA	19.49	A	18.27	A	18.96	A	18.09	A	20.77	B	21.01	A	19.47	B
	AA	19.50	A	21.66	B					23.63	B	20.29	A	20.79	B
Vaccenic	GC	4.86	A	3.84	A	3.87	A	4.30	A	4.45	A	4.21	A	4.34	A
	GC/AA	3.99	A	3.95	A	4.33	A	4.21	A	3.88	A	3.88	A	4.04	AB
	AA	4.18	A	3.53	A					4.35	A	3.39	A	3.86	B
C20:0	GC	0.29	A	0.29	A	0.25	A	0.28	A	0.27	A	0.30	A	0.28	A
	GC/AA	0.27	A	0.31	A	0.28	A	0.30	A	0.29	A	0.30	A	0.30	B
	AA	0.30	A	0.22	B					0.24	A	0.26	A	0.25	AB
CLA c9t11	GC	1.65	A	1.21	A	1.38	A	1.51	A	1.52	A	1.47	AB	1.51	A
	GC/AA	1.41	B	1.36	A	1.54	A	1.34	A	1.46	A	1.62	A	1.46	A
	AA	1.50	AB	1.88	B					1.78	A	1.18	B	1.59	A
C21:0	GC	0.03	A	0.04	A	0.04	A	0.04	A	0.03	A	0.04	A	0.04	A
	GC/AA	0.04	A	0.04	A	0.05	A	0.05	A	0.03	A	0.04	A	0.04	A
	AA	0.04	A	0.11	B					0.04	A	0.03	A	0.06	B
C22:0	GC	0.11	A	0.12	AB	0.12	A	0.11	A	0.12	A	0.11	A	0.12	A
	GC/AA	0.11	A	0.13	A	0.11	A	0.11	A	0.12	A	0.12	A	0.12	A
	AA	0.12	A	0.10	B					0.11	A	0.11	A	0.11	A
C22:1n9	GC	0.02	A	0.03	A	0.03	A	0.02	A	0.03	A	0.02	A	0.03	A
	GC/AA	0.02	A	0.02	B	0.02	A	0.02	A	0.03	A	0.03	B	0.02	A
	AA	0.03	A	0.03	A					0.03	A	0.02	A		
C20:4n6	GC	0.08	A	0.08	A	0.09	A	0.07	A	0.07	A	0.09	A	0.08	A
	GC/AA	0.08	A	0.09	A	0.09	A	0.07	A	0.09	B	0.10	A	0.09	A
	AA	0.08	A	0.07	A					0.08	AB	0.08	A	0.08	A
C20:5n3	GC	0.08	A	0.08	A	0.09	A	0.07	A	0.07	A	0.08	A	0.08	A

	GC/AA	0.08	A	0.08	A	0.09	A	0.08	A	0.09	A	0.09	A	0.08	A
	AA	0.09	A	0.07	A					0.08	A	0.08	A	0.08	A
	GC	0.11	A	0.14	A	0.14	A	0.12	A	0.12	A	0.13	A	0.13	A
C22:5n3	GC/AA	0.13	A	0.15	A	0.17	A	0.12	A	0.16	B	0.16	A	0.15	B
	AA	0.13	A	0.12	A					0.18	B	0.16	A	0.14	AB
SUM	GC	37.00	A	39.24	A	38.45	A	37.12	A	38.32	A	37.02	A	37.41	A
C12C14C16	GC/AA	37.02	A	37.84	A	36.22	A	38.76	A	37.02	AB	37.06	A	37.67	A
	AA	36.90	A	37.19	A					33.64	B	40.81	B	37.54	A
	GC	59.70	A	60.59	A	58.93	A	60.42	A	63.21	A	58.61	A	60.76	A
SFA	GC/AA	57.31	A	58.21	A	56.80	A	58.68	A	55.50	B	55.04	B	56.88	B
	AA	56.89	A	53.97	B					50.67	C	55.60	AB	54.92	C
	GC	19.58	A	19.12	A	21.21	A	18.99	A	16.58	A	20.71	A	18.97	A
MUFA	GC/AA	21.58	A	20.54	A	21.34	A	20.08	A	23.21	B	23.69	B	21.81	B
	AA	21.81	A	24.92	B					26.73	B	22.39	AB	23.98	C
	GC	2.38	A	1.98	A	2.15	A	2.17	A	2.40	A	2.45	A	2.27	AB
PUFA	GC/AA	2.07	A	2.07	A	2.31	A	2.15	A	2.28	A	2.36	A	2.21	A
	AA	2.33	A	2.41	A					2.93	B	2.15	A	2.42	B
	GC	1.12	A	1.29	AB	1.12	A	1.05	A	1.15	A	1.27	A	1.16	A
N6	GC/AA	1.23	A	1.22	A	1.30	A	1.21	A	1.29	A	1.33	A	1.26	A
	AA	1.33	A	1.45	B					1.74	B	1.41	A	1.43	B
	GC	1.07	A	0.89	A	1.04	A	0.88	A	1.00	A	1.13	A	1.00	AB
N3	GC/AA	0.90	A	0.87	A	1.00	A	0.87	A	0.95	A	1.00	A	0.94	A
	AA	0.99	A	1.02	A					1.32	B	0.93	A	1.02	B
	GC	1.10	A	1.25	A	1.20	A	1.29	A	1.15	A	1.27	A	1.20	A
N6/N3	GC/AA	1.26	AB	1.32	A	1.38	A	1.36	A	1.39	B	1.35	A	1.34	B
	AA	1.39	B	1.60	B					1.47	B	1.29	A	1.48	C
	GC	0.03	A	0.05	AB	0.04	A	0.03	A	0.03	A	0.04	A	0.03	A
PUFA/SFA	GC/AA	0.04	A	0.04	A	0.04	A	0.04	A	0.04	B	0.04	A	0.04	B
	AA	0.04	A	0.04	B					0.06	C	0.05	A	0.05	C
	GC	0.02	A	0.02	A	0.02	A	0.02	A	0.02	A	0.02	AB	0.02	A
N3/SFA	GC/AA	0.02	A	0.02	A	0.02	A	0.02	A	0.02	A	0.02	A	0.02	A
	AA	0.02	A	0.02	A					0.02	B	0.01	B	0.02	A

Table A.2. The multiple comparisons of LS-means of fatty acid composition data under the effects of SCD1 gene within certain breed (P<0.1).

		(P<0.10)													
		BONS		BRA		CHAR		GEL		HER		ROMO		ALL	
C14:0	A	8.83	A	10.04	A	9.15	A	9.92	A	8.89	AB	9.08	AB	9.39	A
	A/G	9.56	A	9.75	A	9.42	A	9.53	A	10.15	A	8.84	A	9.35	A

	G	9.39	A	9.63	A	9.80	A			8.30	B	10.23	B	9.67	A
	A	25.28	A	25.49	A	24.86	A	25.63	A	25.03	A	26.40	A	25.41	A
C16:0	A/G	24.78	A	25.12	A	26.09	A	25.66	A	25.74	A	25.10	A	25.35	A
	G	25.27	A	25.71	A	25.29	A			25.61	A	25.53	A	25.49	A
	A	11.67	A	11.93	A	10.60	A	12.28	A	11.24	A	11.45	A	11.52	A
C18:0	A/G	12.00	A	10.60	B	10.86	A	11.33	A	10.78	A	11.32	A	11.16	A
	G	10.07	A	11.16	AB	10.69	A			11.68	A	10.34	A	10.96	A
	A	21.70	A	18.67	A	18.68	A	18.99	A	20.97	A	18.99	AB	19.77	A
C18:1n9c	A/G	18.96	B	19.97	A	20.46	A	19.86	A	19.55	A	21.44	A	20.37	A
	G	21.62	AB	18.87	A	19.71	A			21.34	A	19.21	B	19.78	A
	A	3.93	A	4.08	A	5.28	A	4.51	A	3.91	A	4.05	A	4.20	A
Vaccenic	A/G	4.25	A	3.83	A	3.68	B	4.08	A	3.74	A	3.94	A	4.03	A
	G	3.66	A	3.60	A	4.34	B			4.22	A	3.88	A	3.99	A
	A	0.28	A	0.29	A	0.26	A	0.29	A	0.30	A	0.30	A	0.29	A
C20:0	A/G	0.31	A	0.29	A	0.28	A	0.29	A	0.22	B	0.29	A	0.29	A
	G	0.25	A	0.31	A	0.28	A			0.25	AB	0.28	A	0.27	A
	A	1.38	A	1.38	A	1.63	A	1.43	A	1.47	A	1.54	A	1.46	A
CLA c9t11	A/G	1.55	A	1.56	A	1.50	A	1.42	A	1.55	A	1.56	A	1.52	A
	G	1.50	A	1.32	A	1.51	A			1.49	A	1.61	A	1.49	A
	A	0.05	A	0.04	AB	0.04	A	0.06	A	0.03	A	0.04	A	0.04	A
C21:0	A/G	0.03	A	0.07	A	0.06	A	0.04	A	0.02	A	0.04	A	0.04	A
	G	0.02	A	0.02	B	0.04	A			0.04	A	0.04	A	0.04	A
	A	0.11	A	0.13	A	0.11	A	0.11	A	0.13	A	0.12	A	0.12	A
C22:0	A/G	0.12	A	0.12	A	0.11	A	0.11	A	0.11	A	0.12	A	0.12	A
	G	0.10	A	0.12	A	0.12	A			0.12	A	0.12	A	0.12	A
	A	0.02	A	0.02	A	0.03	A	0.02	A	0.03	A	0.02	A	0.02	A
C22:1n9	A/G	0.03	A	0.03	A	0.03	A	0.02	B	0.04	B	0.03	A	0.03	B
	G	0.03	A	0.02	A	0.03	A			0.02	C	0.03	A	0.02	A
	A	0.09	A	0.08	A	0.09	A	0.07	A	0.09	A	0.11	A	0.09	A
C20:4n6	A/G	0.06	B	0.08	A	0.09	A	0.07	A	0.06	B	0.10	A	0.08	B
	G	0.09	AB	0.08	A	0.10	A			0.08	AB	0.07	B		
	A	0.08	A	0.07	A	0.09	A	0.07	A	0.09	A	0.08	A	0.08	A
C20:5n3	A/G	0.08	A	0.08	A	0.08	A	0.07	A	0.07	B	0.09	A	0.08	A
	G	0.10	A	0.07	A	0.09	A			0.08	AB	0.08	A	0.08	A
	A	0.13	A	0.14	A	0.17	A	0.13	A	0.13	A	0.20	A	0.15	A
C22:5n3	A/G	0.12	A	0.14	A	0.15	A	0.12	A	0.11	B	0.18	A	0.14	A
	G	0.13	A	0.16	A	0.18	A			0.12	AB	0.13	B	0.14	A
SUM	A	36.48	A	38.52	A	37.03	A	38.31	A	36.74	A	38.14	A	37.60	A
C12C14C16	A/G	37.31	A	37.74	A	38.18	A	38.12	A	39.46	A	36.49	A	37.53	A
	G	37.53	A	38.68	A	37.90	A			36.16	A	39.14	A	38.11	A
SFA	A	54.15	A	57.99	A	55.99	A	57.15	A	55.17	A	57.44	A	56.33	A

	A/G	57.04	A	55.79	A	55.72	A	56.75	A	58.68	A	54.95	A	55.86	A
	G	55.24	A	57.91	A	56.49	A			54.68	A	57.14	A	56.55	A
	A	24.20	A	21.01	A	21.03	A	21.10	A	23.44	A	21.44	AB	22.14	A
MUFA	A/G	21.26	B	22.80	A	23.05	A	22.03	A	22.05	A	24.03	A	22.87	A
	G	24.12	AB	21.46	A	22.03	A			23.66	A	21.58	B	22.10	A
	A	2.34	A	2.11	A	2.25	A	2.13	A	2.23	A	2.36	A	2.21	A
PUFA	A/G	2.15	A	2.27	A	2.24	A	2.16	A	2.16	A	2.36	A	2.25	A
	G	2.21	A	1.96	A	2.29	A			2.67	B	2.21	A	2.29	A
	A	1.31	A	1.19	A	1.26	A	1.20	A	1.30	A	1.31	A	1.25	A
N6	A/G	1.23	A	1.35	B	1.29	A	1.25	A	1.31	AB	1.39	A	1.32	A
	G	1.36	A	1.15	AB	1.26	A			1.48	B	1.26	A	1.29	A
	A	1.03	A	0.92	A	0.98	A	0.90	A	0.94	A	1.00	A	0.94	A
N3	A/G	0.92	A	0.94	A	0.95	A	0.89	A	0.88	A	0.99	A	0.94	A
	G	0.87	A	0.82	A	1.02	A			1.18	B	0.95	A	0.99	A
	A	1.34	A	1.33	A	1.41	A	1.40	A	1.40	A	1.35	A	1.37	AB
N6/N3	A/G	1.33	A	1.50	B	1.38	A	1.43	A	1.40	A	1.38	A	1.41	A
	G	1.46	A	1.35	AB	1.31	A			1.28	A	1.35	A	1.34	B
	A	0.04	A	0.04	A	0.04	A	0.04	A	0.04	A	0.04	AB	0.04	A
PUFA/SFA	A/G	0.04	A	0.04	A	0.04	A	0.04	A	0.04	A	0.05	A	0.04	A
	G	0.04	A	0.04	A	0.04	A			0.05	B	0.04	B	0.04	A
	A	0.02	A	0.02	A	0.02	A	0.02	A	0.02	A	0.02	A	0.02	A
N3/SFA	A/G	0.02	A	0.02	A	0.02	A	0.02	A	0.02	A	0.02	A	0.02	A
	G	0.02	A	0.01	A	0.02	A			0.02	B	0.02	A	0.02	A

Table A.3. The multiple comparisons of LS-means of fatty acid composition data under the effects of FASN gene within certain breed (P<0.1).

		(P<0.10)													
		BONS		BRA		CHAR		GEL		HER		ROMO		ALL	
	C	9.20	A	9.94	A	9.23	A	10.03	A	10.07	A	8.93	A	9.52	A
C14:0	C/T	9.18	A	9.73	A	9.57	A	8.85	B	8.49	B	9.63	AB	9.25	A
	T					9.63	A	10.19	AB	8.22	B	10.35	B	9.51	A
	C	26.10	A	25.46	A	25.28	A	25.11	A	26.10	A	24.94	A	25.46	A
C16:0	C/T	24.07	B	24.87	A	26.04	A	25.35	A	25.67	AB	25.97	A	25.29	A
	T					24.95	A	30.14	B	23.75	B	26.68	A	25.76	A
	C	11.84	A	10.42	A	10.53	A	11.53	A	10.26	A	10.97	A	10.99	A
C18:0	C/T	11.52	A	11.47	B	10.56	A	12.70	A	12.20	B	11.02	A	11.58	B
	T					12.68	A	9.07	B	10.00	A	10.22	A	10.63	AB
C18:1n9c	C	20.32	A	19.65	A	20.07	A	19.19	A	18.97	A	21.56	A	20.05	A
	C/T	20.50	A	19.39	A	19.75	A	20.29	A	21.01	AB	19.72	AB	20.13	A

	T					19.74	A	18.92	A	22.29	B	17.44	B	19.66	A
Vaccenic	C	3.95	A	3.87	A	4.33	A	4.32	A	3.75	A	3.79	A	4.02	A
	C/T	4.21	A	3.99	A	4.38	A	4.46	A	4.39	A	4.19	A	4.24	A
	T					4.25	A	2.32	B	3.38	A	3.66	A	3.73	A
C20:0	C	0.29	A	0.26	A	0.27	A	0.29	A	0.27	A	0.29	A	0.28	A
	C/T	0.29	A	0.31	B	0.27	A	0.32	A	0.29	A	0.31	A	0.30	B
	T					0.28	A	0.24	A	0.28	A	0.26	A	0.27	AB
CLA c9t11	C	1.31	A	1.56	A	1.52	A	1.49	A	1.47	A	1.57	A	1.48	AB
	C/T	1.62	B	1.38	A	1.60	A	1.33	A	1.47	A	1.64	A	1.51	A
	T					1.26	A	1.23	A	1.55	A	1.26	B	1.34	B
C21:0	C	0.03	A	0.07	A	0.04	AB	0.03	A	0.04	A	0.04	A	0.04	A
	C/T	0.04	A	0.04	B	0.06	A	0.06	A	0.03	A	0.04	A	0.04	A
	T					0.01	B	0.04	A	0.04	A	0.03	A	0.04	A
C22:0	C	0.11	A	0.12	A	0.11	A	0.11	AB	0.12	A	0.12	A	0.12	A
	C/T	0.11	A	0.12	A	0.11	A	0.13	A	0.12	A	0.12	A	0.12	A
	T					0.12	A	0.08	B	0.09	A	0.15	A	0.11	A
C22:1n9	C	0.03	A	0.03	A	0.03	A	0.02	A	0.03	A	0.03	A	0.03	A
	C/T	0.02	A	0.02	B	0.03	A	0.03	A	0.03	A	0.03	A	0.02	A
	T					0.02	A	0.02	A	0.01	A	0.03	A	0.02	A
C20:4n6	C	0.08	A	0.08	A	0.10	A	0.07	A	0.08	A	0.10	A	0.08	A
	C/T	0.08	A	0.09	A	0.10	A	0.08	A	0.09	A	0.08	B	0.09	A
	T					0.04	B	0.07	A	0.09	A	0.07	B	0.08	A
C20:5n3	C	0.07	A	0.08	A	0.09	A	0.08	A	0.08	A	0.09	A	0.08	A
	C/T	0.09	A	0.08	A	0.09	A	0.07	A	0.09	A	0.09	A	0.08	A
	T					0.06	A	0.07	A	0.09	A	0.08	A	0.08	A
C22:5n3	C	0.12	A	0.14	A	0.17	A	0.12	A	0.13	A	0.18	A	0.14	A
	C/T	0.13	A	0.15	A	0.17	A	0.13	A	0.16	A	0.14	B	0.15	A
	T					0.09	B	0.14	A	0.16	A	0.15	AB	0.14	A
SUM C12C14C16	C	38.03	A	38.44	A	37.35	A	38.21	A	39.25	A	36.50	A	37.87	A
	C/T	36.13	A	37.55	A	38.53	A	36.64	A	36.60	B	38.37	AB	37.29	A
	T					37.14	A	43.22	B	34.49	B	40.73	B	38.18	A
SFA	C	57.60	A	56.51	A	55.59	A	57.22	A	57.30	A	54.51	A	56.37	A
	C/T	54.75	B	57.05	A	56.24	A	55.99	A	55.33	AB	56.43	A	55.97	A
	T					56.57	A	60.35	A	52.26	B	60.60	B	56.91	A
MUFA	C	22.61	A	22.36	A	22.63	A	21.34	A	21.46	A	24.25	A	22.52	A
	C/T	22.86	A	21.88	A	22.23	A	22.37	A	23.28	AB	22.12	AB	22.48	A
	T					21.75	A	21.33	A	24.92	B	19.58	B	21.99	A
PUFA	C	2.22	A	2.25	A	2.23	AB	2.10	A	2.15	A	2.41	A	2.23	A
	C/T	2.30	A	2.15	A	2.35	A	2.23	A	2.29	A	2.16	B	2.25	A
	T					1.92	B	2.18	A	2.71	B	2.05	B	2.30	A
N6	C	1.26	A	1.30	A	1.27	A	1.22	A	1.27	A	1.40	A	1.29	A

	C/T	1.31	A	1.24	A	1.33	A	1.31	A	1.30	A	1.26	B	1.29	A
	T					1.14	A	1.28	A	1.22	B	1.14	B	1.32	A
	C	0.93	A	0.95	A	0.96	AB	0.88	A	0.90	A	1.01	A	0.94	A
N3	C/T	1.00	A	0.90	A	1.01	A	0.92	A	1.00	A	0.91	A	0.96	A
	T					0.78	B	0.89	A	1.17	B	0.88	A	0.98	A
	C	1.29	A	1.42	A	1.38	A	1.38	A	1.50	A	1.37	A	1.40	A
N6/N3	C/T	1.35	A	1.35	A	1.36	A	1.46	A	1.27	B	1.38	A	1.37	A
	T					1.56	A	1.45	A	1.51	B	1.32	A	1.43	A
	C	0.04	A	0.04	A	0.04	A	0.04	A	0.04	A	0.05	A	0.04	A
PUFA/SFA	C/T	0.04	A	0.04	A	0.04	A	0.04	A	0.04	A	0.04	B	0.04	A
	T					0.03	A	0.04	A	0.05	B	0.03	B	0.04	A
	C	0.02	A	0.02	A	0.02	A	0.02	A	0.02	A	0.02	A	0.02	A
N3/SFA	C/T	0.02	A	0.02	A	0.02	AB	0.02	A	0.02	A	0.02	AB	0.02	A
	T					0.01	B	0.02	A	0.02	B	0.01	B	0.02	A

APPENDIX B

Common name	C: D
Lauric acid	C12:0
Myristic acid	C14:0
Myristoleic acid	C14:1
Palmitic acid	C16:0
Palmitoleic acid	C16:1
Stearic acid	C18:0
Oleic acid	C18:1n9c
Vaccenic acid	C18:1n11
Arachidic acid	C20:0
Arachidonic acid	C20:4n6
Behenic acid	C22:0
Erucic acid	C22:1n9

VITA

Zhuo Deng

Candidate for the Degree of

Master of Science

Thesis: GENOMIC EFFECTS ON MILK FATTY ACID COMPOSITION OF BEEF COWS AND ITS INFLUENCES ON CALF PRE-WEANING GROWTH

Major Field: Animal Science

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Education:

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