POLYMORPHIC VARIATION OF THE DGAT1 GENE IN CAL POLY HOLSTEIN
AND JERSEY BREEDS AND THE CORRELATION OF MILK FAT CONTENT,
AND GENETIC VALUES

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Abstract

Polymorphic Variation of the DGAT1 Gene in Cal Poly Holstein and Jersey Breeds and the Correlation of Milk Fat Content, and Genetic Values

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The selection of dairy cattle is dictated by improvement of yield and composition of milk. Genotyping tests give breeders the opportunity to use previously unavailable information to increase the frequency of beneficial alleles in their herds. A QTL influencing milk yield and composition is located in the centromeric end of chromosome 14. The polymorphism being analyzed is a K232A substitution (lysine to alanine amino acid change) in exon VII of the diacylglycerol acyl transferase 1 (DGAT1) gene. DGAT1 is a key acyltransferase in triglyceride biosynthesis. DGAT1 encodes an enzyme which catalyzes the final step of mammalian triglyceride synthesis from carbohydrates. The K232A substitution in the DGAT1 gene has an effect on milk production traits by having a higher milk fat content. The two nucleotide polymorphisms in exon VII (aa → gc) give rise to an MwoI restriction fragment length polymorphism (RFLP) which is used to identify the three DGAT1 genotypes (AA, AG, GG). Research has shown that the genotype AA of the DGAT1 gene is associated with higher milk fat. Since the United States has been selecting for milk volume and protein, herds in the US should have a higher proportion of the G allele.

The objective of this study was to examine the association of DGAT1 genotypes with fat content, lipid profiles, citrate levels, and MFGM proteins in the Cal Poly Herd. Our results confirmed that allele A is correlated with higher average percent milk fat content in Cal Poly Holstein and Jersey cows and shows a link between genes and fat composition. The changes were also reflected in the variability of MFGM proteins and lipid profiles. No statistically significant results were seen in citrate levels. In addition to examining the correlation between

genetic variants and milk composition, there was a desire to examine the DGAT1 expression in epithelial mammary cells. These live epithelial cells were obtained via the novel technique of magnetic separation, which had questionable results. Microscopic evidence shows binding has limited success, and the inconclusive correlation between DGAT1 genotype and gene expression could be due to either the small sample size or limited extraction of viable epithelial cells.

Bovine genomic DNA has been successfully extracted from milk cells, and this technique was tested with the examination of cells in milk powder. This technique had great success and shows great promise for future research in determining point of origin.

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Chapter 1

Introduction

During the last 15 years, US dairy milk production has increased by approximately 20% ((IDFA), 2005), making it an important agricultural product that, based on a farm value of production, ranks second only to beef within livestock industries (Hoskin, 2007). In 2004, US dairies produced 170 billion pounds of milk ((IDFA), 2005). Dairy products include more than fluid milk, cheese, yogurt, butter, and ice cream, it also includes others that are often found in processed foods such as dry and condensed milk and whey products. All 50 states produce milk, with California being the biggest producer. Milk production per cow has reached 19,000 pounds per year in the year 2000, a huge increase over the 9,700 pounds per year average during the 1970's (Hoskin, 2007). Although milk production has increased per cow during this time period, the number of dairy operations in the United States has decreased from 650,000 to 90,000 during the same time period (Hoskin, 2007). With more milk per cow, there are fewer cows required to produce the same amount of milk for profit. This trend is mainly due to genetic improvement of the dairy cows, with the use of genetic markers aiding in selection of cows.

Genetic markers have been identified in dairy cattle, including quantitative trait loci (QTL) or genomic regions that affect quantitative traits. Determining the animals' genotype at these loci helps to improve traits that cannot be improved by conventional selection, for example those traits which have a low heritability (Dekkers, 2004). It gives producers the opportunity to know an animal's potential before it enters the milk string. For example, Europe and New Zealand are actively genotyping their cattle for specific

genetics to improve their milk processing production (ie. cheese) and profits. New Zealand researchers have predicted what a United States dairy herd should look like based on their herd results and knowledge of prior US selection goals (Spelman et al., 2002), but the United States needs to analyze its own herds to look at the coding and regulatory regions of genes of genetic variants between and within dairy cattle breeds to determine what is best for the United States dairy industry. This should include familiar genes such as β -lactoglobulin and κ -casein, but also expand to new possibilities based on current research focused on milk composition and quality.

One new possibility involves DGAT enzymes, which use diacylglycerol and fatty acid CoA as substrates (Cases et al., 1998). The role DGAT plays with regards to triacylglycerol metabolism leads into its involvement with intestinal fat absorption, lipoprotein assembly, adipose tissue formation, and lactation (Cases et al., 1998). It is believed that DGAT has a significant effect on lipid metabolism (Moore et al., 2003).

Glucose and insulin have a positive regulation on transcription of genes when energy is in surplus, because fatty acid synthesis and triglyceride formation are stimulated to store the excess energy (Meegalla et al., 2002). Looking into the DGAT effect on lipid metabolism, two types of the DGAT enzymes were found – DGAT1 and DGAT2. Glucose enhances DGAT1 mRNA expression and insulin both enhances DGAT2 mRNA expression and has an additive effect on DGAT activation (Meegalla, 2002). A fasting/re-feeding study performed by Meegalla et al. in 2002 found that DGAT1 was more involved in fat absorption in the intestine and DGAT2 was more involved in assembly of VLDL particles in the liver.

DGAT-deficient transgenic mice were found to be resistant to high-fat diet induced obesity through increased energy expenditure (Chen and Ladha, 2002). DGAT-deficient mice were found to have a reduced rate of triacylglycerol absorption (Buhman et al., 2002) and insulin sensitivity (Meegalla et al., 2002). These results may be useful in fighting human obesity with those individuals with insulin and leptin resistance (Chen et al., 2002b). With consumers becoming more health-conscious, the demand for food products that deliver nutritional and functional benefits has increased.

Determining if the DGAT1 gene is a functional gene for increasing fat content in milk, or if it is simply a genetic marker, will require examination at the molecular level. Genetic information is transferred from DNA to functional proteins by way of messenger RNA (mRNA) within cells (Alberts et al., 2002). The mRNA is extracted from cells believed to be involved in the trait of interest. With milk composition traits, cells from the mammary gland would be used. The difficulty of obtaining a tissue sample will be avoided by examining the novel technique of obtaining live mammary epithelial cells from fresh raw milk (Boutinaud and Jammes, 2002). Milk from healthy mammary glands contains 100,000-500,000/mL of somatic cells, representing mostly leucocytes, with less than 2% epithelial cells (Safarik and Safarikova, 1999). Magnetic separation is a technique used to purify cells from crude samples by isolating target cells, and will be used to obtain the epithelial mammary cells needed for the high quality mRNA. Bovine genomic DNA has been successfully extracted from milk cells, and this technique will also be tested with the examination of milk powder for use in tracing a product.

To summarize, the objectives of this study were:

1. To examine the association of DGAT1 genotypes with fat content, lipid profile, citrate levels, and MFGM proteins in the Cal Poly Herd.
2. To determine the ability to extract epithelial cells with viable RNA through a novel, non-invasive technique.
3. To create a molecular test for milk powder, using genetic markers, that aids in the identification of origin.

Chapter 2

Literature Review

2.1 *Milk and By-Products*

2.1.1 *Milk*

Raw cow's milk is a very nutritious and complex food, containing three basic components (fat, solids-not-fat (SNF), and a fluid carrier) and a wide array of chemicals (Walstra et al., 1999; Patton, 2004). A natural oil-in-water emulsion that contains lipids, proteins, lactose and minerals (approximately 12% of milk's weight) and water holds all of these components in solution and in colloidal form. The white appearance of raw milk is due to light scattered by two types of particles in suspension in whey; fat globules and casein micelles (Ribadeau-Dumas and Grappin, 1989).

Milk proteins are some of the most widely consumed food proteins. They are very important in human nutrition and influence the properties of dairy products (Huth et al., 2006). The major milk proteins are casein and whey proteins, and milk fat globule membrane proteins (MFGM) represent approximately 1% of total milk protein. Casein proteins constitute approximately 80% of milk proteins with the main fractions of α -s₁ and α -s₂ caseins, β -casein, and κ -casein (Walstra et al., 2006). Whey proteins are known as the serum or globular proteins and the principle whey proteins are β -lactoglobulin, α -lactalbumin, bovine serum albumin (BSA), and immunoglobulins (Ig). Of the total milk proteins, the MFGM proteins are present at low concentration (approximately 0.35 g/L of bovine milk), but they are of interest from many standpoints including their role in mechanisms of milk secretion, structure, and enzymatic activities of the lactating cell

membranes and their interactions with serum proteins in whole milk during processing (Cavaletto et al., 2004).

The lipids in milk exist in the form of fat globules. In milk, the less dense fat, in comparison to water, tends to rise due to the difference in density between the milk plasma (milk minus fat) and the fat globules (Walstra et al., 1999). This is the natural creaming of milk.

Table 1. Milk composition of various mammals. (Source: (Patton, 2004))

Nutrient	Cow	Goat	Sheep	Buffalo	Human
Water (%)	87.99	88.9	83	81.1	87.5
Fat (g)	3.34	3.5	6	8	4.38
Protein (g)	3.29	3.1	5.4	4.5	1.03
Carbohydrate (g)	4.66	4.4	5.1	4.9	6.89
Energy (kcal)	61	60	95	110	70
Lactose (g)	4.8	4.4	5.1	4.9	7
Cholesterol (g)	14	10	11	8	14
Calcium (mg)	119	100	170	195	32

2.1.2 Skim Milk Powder

Milk is extremely perishable, and yet it is desirable to preserve it for later consumption. Milk is skimmed and standardized for fat content before heat treatment (low, medium, or high) to remove 95-97% of water, giving it the ability to retain a much longer shelf life (3 years) than its aqueous equivalent (Walstra et al., 1999). The spray drying process is a very successful method of preservation, converting the skim milk to powder without any loss in nutritional value, and gained importance worldwide in the 1930s and has been an essential part of the dairy industry since (Varnan and Sutherland, 1994). The disadvantage is the high-energy demand per ton of finished product due to the high water content (approximately 90%) of milk.

The average consumer usually considers skim milk powder (SMP) to only be used as reconstituted imitation milk. SMP is also used as an ingredient for several food products domestically and internationally such as in baked goods, infant formulas, meat products, and prepared foods. Currently the United States is the largest SMP producer in the world, with California being the largest producing state (Hoskin, 2007). Even though much of the SMP produced is used and sold domestically, approximately 148,000 metric tons was exported in 2003 ((IDFA), 2005).

The longer shelf life makes SMP important to developing and economically challenged countries where refrigeration is limited (Murillo, 2004). SMP remains in short supply globally, and thus prices for now remain strong. There is great potential for expansion into the Pacific Rim and China, areas that are growing economically with currently low dairy consumption and an interest in Western products and diets (Hoskin, 2007). With the demand and expansion surrounding SMP, the need to ensure the purity of the product and control of market-share has developed. The polymerase chain reaction (discussed later in this chapter) is one of the most promising techniques available for rapid detection of food and environmental microorganisms, lending to the potential of tracking and protecting our food sources.

2.1.3 Buttermilk

Buttermilk is the main by-product of the butter-making process. Milk fat globules are mechanically disrupted during the cream churning, displacing the milk fat globule membrane (MFGM) and water-soluble components in cream into the aqueous phase. This aqueous phase is known as sweet buttermilk. In 2008, approximately 1600 billion pounds of butter and buttermilk was produced in the United States.

Sweet buttermilk is commonly discarded as it is not desirable for direct consumption (Walstra et al., 1999). However, in 2003, the United States used buttermilk in food products such as baked products (39%), dairy products such as ice cream and yogurt (23%), dry mixes (33%), and about 1% was used for animal feed. It is a beneficial ingredient, adding flavor, firmness, moisture retention, and improving whipping and emulsion properties of products. Due to high spoilage, buttermilk is often dried and sold as buttermilk powder (Walstra et al., 1999). The health benefits ascribed to MFGM components such as proteins and phospholipids (PL), concentrations of 32.9 and 2.03 grams per 100 grams of dry buttermilk respectively, give good reason to increase use of this by-product.

2.2 The milk fat globule membrane

2.2.1 Origin

Milk lipids (mainly triglycerides) are synthesized in the endoplasmic reticulum of the mammary epithelial cell. Lipids are first assembled into very small microlipid droplets (diameter size of less than 0.5 μm to more than 4 μm) surrounded by a monolayer membrane consisting of polar phospholipids and proteins (Keenan, 2001). These microlipid droplets can fuse together to form cytoplasmic lipid droplets (CLD). The CLD then approach the apical cell surface and are gradually enveloped in the membrane and extruded from the mammary gland secreting cells. These spherical droplets can range in diameter from 0.2 – 15 μm with the majority at 1 – 8 μm (Bylund, 1995). These final fat globules are surrounded by a membrane known as the milk fat globule membrane (MFGM).

Figure 1. Electron micrograph of apical region of milk-secreting mammary cell from guinea pig showing lipid droplet emerging from the cell. (Source: (Keenan and Mather, 2002))

2.2.2 Composition and Structure

The MFGM is viewed as a triple layer membrane with a total thickness of 10 – 50 nm with components distributed asymmetrically (Singh, 2006). The first membrane, derived from the endoplasmic reticulum, is a monolayer containing phospholipids and proteins. The second membrane, derived from the mammary gland secretory cells, is a phospholipid bilayer containing proteins, phospholipids, enzymes, cholesterol, and other minor components. Between the first membrane and the bilayer, there is an electron-dense coat rich in proteins (Evers, 2004; Heid and Keenan, 2005).

Figure 2. Schematic representation of the structure of the milk fat globule membrane. Adapted from Lopez et al., 2008.

Proteins and lipids account for more than 90% of the dry matter of the MFGM (Danthine et al., 2000). Discrepancies in the gross composition of the MFGM observed in the literature may be due to variations in MFGM isolation and purification procedures used. The composition can be altered by chemical/enzymatic or physical/mechanical factors (such as cooling, drying, separation, agitation, heating, and homogenization) (Evers, 2004). The composition and structure of the MFGM can also change due to physiological factors such as stage of lactation, breed and age of the animal, diet, seasonal variation, and fat globule size (Mulder and Walstra, 1974). A study by Lopez in 2008 observed a significant decrease in saturated fatty acid content in milk from cows fed a diet rich in polyunsaturated fatty acids (Lopez et al., 2008).

2.2.2.1 Proteins

Proteins account for 25-70% of the MFGM, which equates to 1-2% of the total milk proteins, ranging in molecular weight (MW) from 15,000 to 240,000 Da (Danthine et al., 2000; Walstra et al., 2006; Fong, 2007). The main MFGM proteins are glycoproteins (Dewettinck, 2008). Recently, Reinhardt and Lippolis identified 120 proteins (Reinhardt and Lippolis, 2006). The most characterized are mucin 1, xanthine dehydrogenase/oxidase (XDH/XO), CD36, PAS 6/7, adipophilin, and butyrophilin.

The analysis of MFGM proteins is usually carried out by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE). Electrophoresis in the presence of SDS is widely used to determine the molecular weights of proteins since the logarithm of molecular weight is proportional to the distance of migration (Ribadeau-Dumas and Grappin, 1989). The SDS is an anionic detergent that denatures proteins by surrounding the polypeptide backbone and binds through hydrophobic interaction. The charge is made negative and thus negligible, and so in acrylamide gel electrophoresis, the larger a protein, the lower its electrophoretic mobility because of the gel matrix (Kim, 1994).

2.2.2.2 Lipids

Polar lipids, such as phospholipids and sphingolipids, are amphiphilic molecules comprised of a hydrophobic tail and a hydrophilic head, and account for less than 1% of the total lipids of the milk fat globule (Jensen et al., 1991; Jimenez-Flores and Brisson, 2008). Other lipids, such as triglycerides, diglycerides, monoglycerides, sterols, and sterol esters, are mainly present in the milk fat globule core. Triglycerides represent about 60% of the neutral lipids found in the milk fat globules, and cholesterol accounts for 90% of the sterols (Keenan and Mather, 2002).

Since phospholipids only represent a small fraction, separation of the total lipids from biological components (ie proteins and sugars) that could interfere in the determination of the phospholipid distribution is recommended. In this extraction, an organic, hydrophobic solvent is mixed with an aqueous, hydrophilic solvent. The lipids partition into the organic solvent, with neutral lipids extracted with non-polar organic solvents (such as hexane or light petroleum) and polar lipids with polar organic solvents (such as methanol or ethanol). Solid-phase extraction is used to separate phospholipids from neutral lipids by use of an organic solvent (such as methanol, chloroform, or hexane) to elute the polar lipids from the column (the solid-phase) made of silica (Peterson and Cummings, 2006).

Thin Layer Chromatography (TLC) is an easy and inexpensive way to qualitatively identify phospholipids in a sample (Peterson and Cummings, 2006). TLC separates compounds from a mixture for analysis and identification of simple and complex lipid classes, and this is based on polarity differing between phospholipids (Gunstone and Norris, 1983). It uses a strip of glass, plastic, or aluminum as a backing with a thin layer of silica gel as the absorbent. For visualization, exposure to iodine fumes is required on any type of TLC plate. The iodine fumes are absorbed by organic compounds causing the plate to change color to a yellow/brown at that particular spot on the plate. The color effect will fade once exposure to the iodine fumes has ceased (Christie, 1982).

2.2.3 *Isolation of MFGM*

A number of methods have been developed on a laboratory scale for the isolation of the MFGM from milk. Most methods use a common 4-step procedure: 1) cream separation, 2) cream washing, 3) washed cream disruption, and 4) MFGM recovery.

Cream separation is done through a physiological buffer layer. The use of a saline buffer for cream washing, instead of pure water, allows for better elimination of skim milk material without destabilization of the fat globules. Three repeated washings of the cream are sufficient to remove skim milk material and further washing can cause losses of MFGM material (Anderson and Brooker, 1975). Destabilizing of the fat globules is usually done by mechanical churning, but can also be done by freeze-thawing or detergent treatments (Danthine et al., 2000). The last step is the recovery of the MFGM fragments, found in the aqueous phase (buttermilk) after churning, by ultracentrifugation for complete collection of MFGM fragments as a dense pellet (Patton and Keenan, 1975). Sodium citrate can be used in the final step to disrupt the casein micelles. Citrate is a calcium chelating agent that can remove calcium from the micelles creating a breakdown of its structure allowing for caseins to remain in the supernatant after ultracentrifugation (Corredig and Dalgleish, 1997).

2.2.4 *Functional and biological properties*

MFGM is the native emulsifier in milk due to its amphiphilic nature (Kanno, 1989). The water distribution properties of the MFGM aid in thickening formulations and foaming of processed foods. MFGM components have also been used beyond milk fat globule stability and enhance the absorption of fat-soluble nutrients such as vitamin D₃ and vitamin A.

Progress in the knowledge of the composition and role of MFGM components has led to the realization that some possess biological properties beyond their nutritional significance. Research has found the MFGM to have antimicrobial proteins, antiviral properties (such as inhibition of the ulcer-forming bacterium *Helicobacter pylori* and rotavirus), illness suppressors, micronutrient-binding proteins that bind compounds such as iron, zinc, copper, folate, and vitamin B1, and other components with anti-carcinogenic properties (Spitsberg, 2005; Lopez et al., 2008). One clinical study found the complementation of infant food with MFGM and micronutrients lead to more copper and vitamin B12 uptake by infants (Lonnerdal et al., 2006). Another study found MFGM complementation has reduced the incidence and prevalence of diarrhea in infants (Zavaleta et al., 2006).

2.3 *Diacylglycerol acyltransferase*

Total content of lipids in milk varies among species. With regards to bovine milk, over 99% of the lipids were found within droplets and triacylglycerol accounted for at least 97% (Neville and Picciano, 1997). Within bovine milk, there is variation due to physiological factors such as stage of lactation, diet, and body lipid content (Neville and Picciano, 1997). Triacylglycerols are a storage form of energy, created from carbohydrates. They are quantitatively the most important storage form of energy for eukaryotic cells (Cases et al., 1998). Lipid secretion occurs across the mammary secretory epithelium from the blood side to milk (Neville and Picciano, 1997)(see chapter 2.2.1).

Biosynthesis of triglycerides occurs in the membrane of the endoplasmic reticulum. The main pathway for de novo biosynthesis of triglycerides in mammals is via

the glycerol-3-phosphate pathway. Through this pathway, glycerol-3-phosphate is derived from either glucose or glycerol from the plasma. With the glycerol-3-phosphate pathway and other pathways (for example the monoacylglycerol pathway), diacylglycerol is synthesized and converted to triacylglycerol by diacylglycerol acyltransferase (DGAT) (Coleman et al., 2000)(Figure 3).

Figure 3. De novo biosynthesis of triglycerides in mammals via the glycerol-3-phosphate pathway. Adapted from Winter et al., 2002.

Fatty acids drawn from the plasma contribute 50% of the total milk lipid synthesis in cows (Eckel, 1989). A characteristic feature of the triacylglycerols in the milk from ruminants is that they contain the short-chain acids butyric and hexanoic acid. Both short-chain acids are not found normally in the milk of non-ruminants (Marshall and Knudsen, 1977). DGAT catalyses the final step in the triglyceride biosynthesis and may be the rate-limiting step in triglyceride synthesis (Chen et al., 2002a). DGAT activity was identified in 1960 by Weiss et al. and two genes encoding DGAT activity are known at this time, DGAT1 and DGAT2 (Weiss, 1960).

A study with DGAT1 deficient mice showed that the expression of DGAT1 is not only necessary for lipid synthesis within the mammary gland, it is generally crucial for lactation, as DGAT deficient mice produce no milk at all (Winter et al., 2002). This study emphasized DGAT1 as a strong candidate gene for milk fat percentage. This observation highlights the determining role of DGAT1 in milk fat synthesis and milk production in general.

2.4 Genetic Analysis

2.4.1 History of genetics and selection

The philosophy of breeding animals and plants to improve selected traits has been present since prehistoric times. Gregor Mendel's work on inheritance was the basis for the science of modern genetics that began in the beginning of the 20th century when William Bateson coined the word *genetics* (Snustad and Simmons, 2003). Throughout the last one hundred years, the field continues to evolve with the understanding of heredity and the development of techniques to improve the accuracy of predicting it. The dairy industry has used this field to improve yield and composition (Hoskin, 2007).

For many dairy farmers, genetic selection has been primarily directed towards higher milk production in order to increase their profits. Selection used observable phenotypes to obtain superior animals for the herd. The commercial use of artificial insemination (AI) has accomplished the task of increasing milk production for most dairy herds since the 1950's and led to the genetic improvement of highly heritable traits. The breeding values of elite sires are measured by progeny testing. This is accomplished by evaluating the phenotypic performance of the elite sires' offspring. In dairy cattle progeny testing, new sires resulting from planned matings of superior parents are evaluated based on their daughters' average phenotypic performance (Georges et al., 1995). Progeny testing can be time consuming and expensive, taking an average of three and a half years and costing as much as \$25,000 per bull (Tassell, 2003). Also, only about 1 in every 9 bulls successfully passes progeny testing, so there is a substantial loss when bulls do not pass (Tassell, 2003). Selection based on phenotypic information is

limited, especially prior to reproductive age or when traits are expressed in only one gender. The use of molecular genetics can ease some of the problems with traditional quantitative selection. A great benefit of molecular genetics is the ability to evaluate the genetic worth of an animal, regardless of sex, at any stage of life.

Milk production and composition is a complex, polygenic trait, influenced by genetics and environment. For example, decreased milk production could also be due to environmental factors, such as poor management practices, and may be improved simply through better nutrition (Smaragdov, 2006). Milk yield as well as milk components are subject to considerable variation between breeds and within breeds. An example is the Holstein breed has an average milk fat percentage 3.66, whereas the Jersey breed has an average milk fat percentage 4.57 (Underwood, 2002). It is this genetic variability that is the basis for breeding. The aim is to concentrate as many of the positive gene variants in one animal to improve its genetic potential.

Table 2. Milk composition comparisons of the six dairy cattle breeds. (Source: (Patton, 2004))

Breed	Fat %	Protein %
Holstein	3.7	3.4
Jersey	4.6	3.8
Brown Swiss	4.1	3.5
Guernsey	5	3.7
Ayrshire	4	3.5
Milking Shorthorn	3.8	3.3

Milk fat percentage is a quantitative trait that is determined by the collective effect of multiple genes and environmental factors. The heritability (genetic contribution to the variation) of the milk fat percentage was estimated to be between 0.45 and 0.5 (Winter et al., 2002). A broad definition of heritability is the proportion of the animals' phenotype that is due to its genetics. A more narrow definition is the value which

represents how the genes an offspring receives from its parents will affect the expression of the phenotypes (Weller et al., 1990). This estimate can be useful in breeding programs to help predict how closely an offspring will perform compared to his or her parent. The heritability of milk production and composition traits are good, but non-traditional selection methods such as genotyping may prove to be a better approach for improving the traits due to the limits set by reproductive age and expression in only one gender.

2.4.2 *Analysis of Quantitative Traits*

Economically important traits are often influenced by a large number of genes with relatively small effects, called polygenes (Georges et al., 1995). Molecular genetics has already been used to benefit some quantitative traits by identifying the genetics behind them. Knowledge of the genes causing variation within a trait enable marker assisted selection (MAS), which is selection based on the presence or absence of genetic markers that are linked to desired or undesired characteristics (Smaragdov, 2006). An example of MAS with regard to milk composition, is the initial discovery of genetic polymorphism at the β -lactoglobulin locus in 1955 and the complete amino acid sequences of the genetic variants A and B of κ -casein in 1972 and 1973 helped dairy farmers better select bulls to improve milk production (Tsiaras et al., 2005). By applying MAS, animals can be tested for their genetic potential early in their development and independently from their gender (Winter et al., 2002).

One of the current focuses of molecular genetics is the search for quantitative trait loci (QTL). QTL have specific chromosomal positions and are polygenes that are traceable by markers, with the marker alleles being associated with a measurable effect on a quantitative characteristic (Geldermann, 1975; Smaragdov, 2006). Researchers

identify functional candidate genes based on knowledge about the underlying mechanism of the phenotypic trait. The main objective would be to determine if there is an association between the different alleles at the candidate gene and the phenotypic trait of interest in the population (Shorten et al., 2004). The first DNA markers were restriction fragment length polymorphisms (RFLP) (Botstein et al., 1980). RFLPs are caused by single nucleotide polymorphisms (SNPs), which are very easy to genotype today due to the knowledge of the sequences and the restriction enzymes available. SNPs are ideal DNA markers due to their relative high frequency and even distribution over the genome.

2.4.3 *DGAT1*

A previous study mapped *DGAT1* to the centromeric end of bovine chromosome 14, which falls within the region of a QTL for milk fat percentage and other milk performance traits (Grisart et al., 2004). Thus, both functional and positional data made *DGAT1* a promising candidate gene for milk fat percentage in cattle. A genetic variation in *DGAT1* has also been reported to be associated with a major effect on milk yield and composition. Specifically, the mutation is a two nucleotide polymorphism in exon VIII (aa to gc). This polymorphism in exon VIII of the *DGAT1* gene at position 232 produces a non-conservative lysine to alanine substitution in the protein (Grisart et al., 2004). It also gives rise to a *MwoI* restriction fragment length polymorphism (RFLP) (Rincon and Medrano, 2004). Research has shown that the lysine232 polymorphism of the *DGAT1* gene is associated with higher milk fat (Grisart et al., 2004).

Figure 4. *MwoI* Restriction Enzyme recognition site.
(Source: New England BioLabs, Beverly, MA)

2.4.4 *β -Lactoglobulin C Variant*

The major whey protein, β -lactoglobulin, is found in two major variant forms, A and B, in bovine milk. For some populations of Jersey cows, a third β -lactoglobulin variant, “C”, is present at low frequencies (Paterson et al., 1995). This unique variant is present at moderate frequencies in Australian and New Zealand milk and not in the United States (Creamer et al., 2004). There is one amino acid difference (substitution of glutamine-59 to histidine) between variants B and C (Paterson et al., 1995). Specifically, the mutation is a nucleotide polymorphism in exon II of the β -lactoglobulin gene. The low frequency of this mutation makes it a unique candidate for a rapid, specific, and sensitive screening test of origin.

2.4.5 *Methods for analysis*

Molecular methods are sensitive and rapid, proving useful for many industries. Many of these methods utilize techniques such as nucleic acid hybridization, restriction endonucleases, and restriction fragment length polymorphism (RFLP) to fingerprint and classify (Wang and Brown, 1999).

2.4.5.1 DNA Extraction

One of the easiest ways to obtain the genotype of a cow is through a blood sample. In 1868, Friedrich Miescher first discovered the ability to extract DNA from the nucleus of white blood cells (Weiss et al., 1960; Dahm, 2005). Today we continue to do this in the whole blood sample (the red blood cells have no nucleus and therefore no DNA). The goal of the DNA extraction is to obtain pure and high yield DNA with

minimal shearing. Cells can be disrupted in many ways, including chemical, enzymatic, mechanical, or combinations of these (Roose-Amsaleg et al., 2001). Many cell lysis protocols combine detergents and lytic enzymes. Common detergents include SDS and sarkosyl, while lytic enzymes may be lysozyme, Proteinase K, or other types.

Temperatures can vary depending on the protocol, and have ranged from -70 to 100C.

Many commercial DNA extraction kits are available and widely used, due to the entire procedure taking a short amount of time and producing clean genomic DNA ready to use (Malferrari et al., 2002). With pure, intact DNA extracted, the next step is to amplify a section of the target gene containing the nucleotide polymorphisms.

2.4.5.2 Polymerase Chain Reaction

One of the most ingenious developments in molecular biology has been a rapid method for enzymatic amplification of DNA called polymerase chain reaction (PCR). PCR is an in vitro method of amplifying specific nucleic acid sequences within complex genomic samples and was first discussed in a paper by Kleppe in 1971 (Kleppe et al., 1971). It was not until 1986 when Kary Mullis and coworkers at Cetus simplified the method using a combination of thermal cycles and a DNA polymerase to amplify replicated DNA segments (Sorscher, 1997). The basis for PCR comes from natural DNA replication and so requires similar molecular reagents and follows the same basic principles (Bej et al., 1991). During thermocycling, DNA is denatured, primers flanking the target region anneal, and new strands are extended by the heat stable Taq DNA polymerase (isolated from the thermophilic bacterium, *Thermus aquaticus*) (Coen and Scharf, 1991). Most PCR reactions are specific, and with regards to the DGAT1 gene, due to unequal amplification of the two alleles under standard conditions, 5% DMSO was

added to enforce the equal amplification of the two alleles (Rincon and Medrano, 2004). Each DNA molecule is doubled each successive cycle (Bej et al., 1991), so 30 cycles should result in a billion-fold amplification of the product). Thermocycling is carried out using precision, automated thermocycler machines with capacities of 24-96 sample tubes.

2.4.5.3 Restriction Enzyme Digestion

Digestion of generated PCR products must be given several considerations. Selection of 4-6 base pair cutters, digestion time, as well as DNA purity can greatly affect the results obtained. Enzymes can be selected based on the literature or sequence analysis via online molecular tools (NCBI). Enzyme concentration (empirical determination) and incubation time (at least 2 hours) must be sufficient for proper digestion and creation of the "fingerprint". The PCR products can then be size-fractionated by agarose-gel electrophoresis to produce specific DNA fingerprint patterns and the genotype is determined by the comparison of banding patterns.

2.5 *Identification of DGAT1 mRNA in milk cells*

A challenge by dairy scientists and the dairy industry is to understand which genes control the composition of milk, how these genes are regulated, and how they might be manipulated to enhance both the manufacturing and health properties of dairy products. Evaluation of gene expression of bovine tissues will permit identification of and will allow ascribing function to genes that are important regulators of milk component synthesis during lactation (Bionaz and Loores, 2007).

2.5.1 Cell Extraction

Most protocols analyze gene expression through mRNA extraction from tissue immediately processed as live cells are needed (little or no mRNA remains in dead cells) (Pfaffl et al., 2003). One goal of this research was an attempt to avoid the invasive technique of obtaining a tissue sample, but still obtain live mammary epithelial cells in which to analyze the DGAT1 gene expression. Mammary epithelial cells are exfoliated and shed into milk during lactation (Boutinaud and Jammes, 2002). The attempt will be made using magnetic separation technology to remove the target cells from the crude raw milk sample. Magnetic separation is relatively simple and fast (Gomm et al., 1995). Invitrogen (Carlsbad, CA) produces a magnetic bead that can be coated with an antibody or lectin. Lectins have been immobilized on magnetic particles by absorption or covalent immobilization on tosylactivated beads (Safarik and Safarikova, 1999). Many lectins can interact with saccharide residues on the cell surfaces (Boutinaud et al., 2004). Then a simple magnet is needed to select for the magnetic beads and whatever has attached to them.

Figure 5. Schematic representation of the extraction of epithelial cells via Dynabeads technology. Adapted from Invitrogen (Carlsbad, CA).

To increase the ability to obtain a pure sample of mammary epithelial cells, the choice of lectin is important. The apical membranes of the epithelia have binding sites for several lectins (Stumpf and Welsch, 2004). Lectins are designated in terms of their monosaccharide specificity (Kennedy et al., 1995). The binding of a lectin, or carbohydrate recognition protein, to specific saccharide ligands is typically a low-affinity interaction. However, the multivalent nature of lectin-saccharide interactions allows many low-affinity binding events to occur, resulting in high overall avidity (Brewer et al., 2002). Looking at the bovine mammary gland, a number of glycoproteins have been shown to contain N-linked oligosaccharides with the GalNAc β 1 $\rightarrow$ 4GlcNAc structure in their outer chain moieties (Kitamura et al., 2003). *Wistaria floribunda* agglutinin (WFA) interacts with oligosaccharides with N-acetylgalactosamine at their termini and has been used in previous studies with regard to bovine mammary epithelial cells (Kitamura et al., 2003).

2.5.2 RNA and RT-PCR

The extraction of RNA is complex and potentially more problematic than DNA extraction because RNA is more labile than DNA at elevated temperatures and pH conditions (Kimball, 1996). Thus, sample collection and handling to prevent RNA degradation is a crucial step and the main possibility for problems (Wintzingerode et al., 1997). Samples must be preserved as much as possible during the sampling period by storing on ice or refrigerated always. RNA is also prone to both endonucleolytic and exonucleolytic attack from a variety of ribonucleases (RNases), which are ubiquitous in the environment, cells, and tissues. RNases are small, extremely resilient enzymes that have minimal cofactor requirements for activity. Therefore, all RNA extraction protocols

must involve complete deactivation or elimination of all RNases throughout the procedure for success of the extraction (Farrel, 1993). To ensure DNA does not interfere and give false results, samples will be exposed to DNase for total DNA digestion and subject to PCR analysis of each sample to examine possible residual DNA contamination. Even though RNA extractions require more specialized procedures and attention to detailed technique than DNA extractions out of the same medium, RNA extraction protocols have become much more efficient and less complicated to perform.

Reverse transcription of RNA followed by cDNA amplification (RT-PCR) has been used as a tool to quantitate and monitor gene expression in eukaryotic cells (Wang et al., 1989). It can be used to compare the levels of mRNAs in different sample populations, to characterize patterns of mRNA expression, and to analyze RNA structure (Wang and Brown, 1999). The RT-PCR method consists of a series of protocols that ultimately renders profiles of desired samples. To identify which transcript is expressed, the technique involves 1) the collection of samples, 2) extraction of total RNA, 3) reverse transcription of RNA to cDNA, 4) PCR amplification of selected genes using a selected primer, 5) digestion of the products with specific 4-6 base pair-cutter restriction endonuclease, and 6) gel electrophoresis.

2.6 *Correlation with Citrate*

Citrate is known for its role in cellular energy metabolism, as an intermediate in the tricarboxylic acid cycle. It forms inside the mitochondria by the condensing of a molecule of acetyl CoA with a molecule of oxaloacetate (Alberts et al., 2002). This cycle does occur in the mammary gland and uses acetate, fatty acids, or pyruvate as energy sources (Faulkner and Peaker, 1982). Previous research has given strong evidence that

the citrate present in milk originates from what is formed in the mammary secretory cell, rather than the blood plasma, and is a major determinant of milk citrate concentration (Linzell et al., 1976). Citrate is important as its level is thought to be an indication of the amount of energy available to a cow and indirectly in de novo fatty acid synthesis (Baticz et al., 2002).

Citrate plays an indirect role in fat synthesis. For ruminants, they absorb large quantities of acetate from the diet for use as the major precursor for fat synthesis in the mammary gland (Linzell et al., 1976). The low amount of the citrate cleavage enzyme in ruminant mammary tissue suggests the intermediate role of citrate in fatty acid synthesis is not significant (Faulkner and Peaker, 1982). Although it may be not be a significant intermediate, citrate appears to be indirectly involved in providing the reducing equivalents in the form of NADPH needed for fat synthesis in the ruminant mammary gland. If there is a decrease in fatty acid synthesis, the requirement for NADPH decreases and there becomes some accumulation of NADPH, which increases citrate concentrations in the cytosol (Faulkner and Peaker, 1982). Research has found that dietary changes, for example feeding fresh pasture during the grazing season, that caused decreased fatty acid synthesis in the mammary gland have been shown to result in the production of milk with higher citrate content (Garnsworthy et al., 2006).

Large increases in the citrate concentration of mammary secretions occur at the time of parturition, followed by a slight decrease during the first week of lactation. The decrease in concentration continues in mid to late lactation (Baticz et al., 2002). It could be hard to attribute the change solely to stage of lactation as diet changes during this time are also common for dairy cattle, but a 2006 study confirmed that variation in milk citrate

with stage of lactation is independent of diet and milk yield (Garnsworthy et al., 2006). The changes in the citrate content of milk based on stage of lactation may be due to the concentration of citrate in the cytosol as well. In early lactation, fatty acid synthesis in the mammary gland is low due to the utilization of fat stores in adipose tissue and concentration of citrate in the milk is high. As lactation progresses, fatty acid synthesis in the mammary gland increases due to the decrease in the use of adipose tissue and the citrate content of milk decreases (Faulkner and Peaker, 1982).

Citrate is a normal constituent of milk, playing an important role as part of the buffering system (Braunschweig and Puhon, 1999). It is vital to milk production because it interacts with other components of milk to aid in protein coagulation, protein stability, flavor and aroma (Walstra et al., 1999). Calcium, when bound to phosphate in the cell (CaPO_4) is insoluble when heat is added; when citrate is present, it binds to calcium, removing some of the calcium from this equilibrium and reducing heat instability (Visser, 1979). Changes in milk calcium appear to be regulated by the amount of citrate and casein present (Linzell et al., 1976). In cheesemaking, rapid coagulation of protein is sought, but gelation must be avoided in processes such as the production of heat-sterilized milk (Faulkner and Peaker, 1982).

Previous research using cows from the Cal Poly Dairy, has indicated that high producing Jersey cows have a consistently higher level of citrate in the milk and blood compared to low producers of the breed as well as to the high producers of the Holsteins (with no significant difference within the breed) (Howell, 2009). This supports the knowledge that in milk processing, Jersey cow milk has better heat stability

(Braunschweig and Puhan, 1999). High producing Jerseys also had the widest range of data, showing that there is variation with the potential to be examined further.

A few studies have been done examining the correlation between κ -casein variants and citrate content in milk. (Braunschweig and Puhan, 1999) The results have proved inconsistent with some studies discovering a correlation between a variant and citrate content, and others finding no correlation. These results were found even when holding stage of lactation, nutrition, stress and age constant due to the significant variation in concentration of milk citrate by these variables (Garnsworthy et al., 2006). Citrate can be measured accurately and rapidly in milk by using capillary electrophoresis or infrared spectroscopy technology (Baticz et al., 2002). Due to the role DGAT plays in triglyceride synthesis, there is a possibility for correlation between DGAT1 variants and citrate content in milk that can be explored further.

Chapter 3

Materials and Methods

3.1 DGAT1 Genotyping

3.1.1 Collection of blood samples and handling

Blood samples were gathered from all milking Holsteins and Jerseys at the Cal Poly Dairy over a five year period using a 20 GA 1-1/2" thin wall double-sided sterile needle attached to a 5 mL sterile Monoject vacuum tube w/EDTA (purple top) (Hardy Diagnostics, Santa Maria, CA). The cows were locked in stanchions following milking and during feeding. The blood remained on ice during collection and was then stored at 4°C.

3.1.2 Preparation of genomic DNA from blood

Two hundred microliters of each blood sample was applied to the MoBio Blood Spin Kit (MoBio Laboratories, Inc., Solana Beach, CA) for genomic DNA isolation and used according to the manufacturer's instructions. The integrity of the DNA was examined on a 1% (w/v) agarose gel (Fisher Scientific, Tustin, CA) containing ethidium bromide (final concentration 0.5 µg/mL) (Fisher Scientific, Tustin, CA). A 10X TBE (0.89 M Tris base, 0.89 M Boric Acid, 0.02 M EDTA) buffer (pH 8.3) (Sigma, St. Louis, MO) diluted to 0.5X concentration was used as the electrophoresis buffer. Ten microliters of each sample was loaded per well and electrophoresis was conducted at room temperature for 40 minutes at 90V. Gels were visualized with a UV transilluminator (ChemiDoc XRS imaging device, Bio-Rad, Hercules, CA). The concentration and purity of DNA in a 1:20 dilution of the isolated samples were measured with the SpectroMax Plus UV spectrometer (Molecular Devices, Sunnyvale,

CA) at 260 and 280 nm, respectively. The following equations (Molecular Biology Lab Book, Chemistry Department, Cal Poly, San Luis Obispo, CA) were used to determine concentration:

$$\text{Percent nucleic acid} = \frac{(11.16 \times R - 6.32)}{2.16 - R}$$

(%N.A.)

Where $R = \text{Abs}_{260}/\text{Abs}_{280}$

$\text{Abs} = (\% \text{N.A.}/100)(A_{260})$

$(\text{Abs})(50)(\text{Dilution Factor}) = [\text{DNA}] \text{ ng}/\mu\text{L}$

All samples were adjusted with PCR-grade water (Fisher Scientific, Tustin, CA) to a concentration of 25 ng/ μL and stored at -20°C.

3.1.3 *Polymerase Chain Reaction*

PCR was performed on diluted genomic DNA (1 ng/ μL) with primers specific to bovine DGAT1 (5'-CCTGATGGTCTACACCATCC-3', 5'-CAGGATCCTCACCGCGGTAG-3') under the following conditions: 50 μL total volume containing 50 ng DNA, 1X AmpliTaq Gold PCR reaction buffer (Applied Biosystems, Branchburg, NJ), 0.2 mM dNTPs (Invitrogen, Carlsbad, CA), 5% DMSO (Sigma, St. Louis, MO), 1.5 mM MgCl_2 (Applied Biosystems, Branchburg, NJ), 0.4 μM of each primer (Integrated DNA Technologies, Coralville, IL), 0.625 units of AmpliTaq Gold DNA polymerase (Applied Biosystems, Branchburg, NJ) and quantity sufficient PCR-grade water (Fisher Scientific, Tustin, CA). Thermocycling was performed on a Perkin Elmer ® 2400 GeneAmp PCR System under the following conditions: initial denaturing at 94°C for 5 minutes; followed by 35 cycles at 94°C for 30 seconds, 58°C for 30 seconds, 72°C for 30 seconds; and final extension at 72°C for 10 minutes.

Ten microliters of the PCR products were separated on a 1.5% (w/v) agarose gel (Fisher Scientific, Tustin, CA) containing ethidium bromide (Fisher Scientific, Tustin, CA) (final concentration 0.5 µg/mL) in 0.5X TBE buffer (Sigma, St. Louis, MO) at 90V for 40 minutes to determine proper amplification of a 285 bp fragment from exon VII to intron VIII of the DGAT1 gene . Gels were visualized with a UV transilluminator (ChemiDoc XRS imaging device, Bio-Rad, Hercules, CA). The concentration of the PCR product in a 1:20 dilution was measured with the SpectroMax Plus UV spectrometer (Molecular Devices, Sunnyvale, CA) at 260nm and adjusted with PCR-grade water (Fisher Scientific) to a concentration of 1 ng/µL and stored at -20°C.

3.1.4 SNP genotyping by RFLP analysis

Restriction enzyme fingerprinting was used to compare banding patterns (Figure 6).

Figure 6. DGAT1 Genotype Fragment Pattern. Adapted from Rincon and Medrano (2005).

PCR products of each sample (20 ng) were digested with 5 units of *Mwo*I (New England Biolabs, Beverly, MA), 2.5 µL of the corresponding reaction buffer (New England Biolabs, Beverly, MA), and quantity sufficient PCR-grade water (Fisher Scientific, Tustin, CA) in a final volume of 25 µL at 60°C for 16 hours.

Ten microliters of the digested samples were separated on a 3.5% (w/v) agarose gel (Fisher Scientific, Tustin, CA) containing ethidium bromide (Fisher Scientific, Tustin, CA) (final concentration 0.5 µg/mL) in 0.5X TBE buffer (Sigma, St. Louis, MO) at 90V for 40 minutes to determine genotype (Rincon and Medrano, 2004). Gels were visualized with a UV transilluminator (ChemiDoc XRS imaging device, Bio-Rad, Hercules, CA).

3.2 *Identification of DGAT1 mRNA in milk cells*

3.2.1 *Collection of milk samples and handling*

Twelve hundred milliliters of raw milk was collected into sterile Falcon® tubes (BD Falcon, San Jose, CA) from 12 Holsteins and 12 Jerseys at the Cal Poly Dairy. The milk remained on ice during collection and throughout the extraction process. Cold milk was centrifuged at 1,800 RPM at 4°C for 10 minutes to pellet all cells. Cells were washed twice in the presence of Dulbecco's PBS (0.9 mM CaCl₂, 2.7 mM KCl, 1.47 mM KH₂PO₄, 0.5 mM MgCl₂, 137 mM NaCl, 8.1 mM Na₂HPO₄) buffer (pH 7.2) and 0.5 mM EDTA (Fisher Scientific, Tustin, CA) and reconstituted to a volume of one milliliter in PBS.

3.2.2 *Isolation of mammary epithelial cells*

Dynabeads M-450 (magnetic beads) Epoxy (Invitrogen, Carlsbad, CA), were resuspended and washed with PBS according to manufacturer's instructions in 1.5 mL eppendorf® tubes. Five micrograms of pure *Wisteria floribunda* (WFA) lectin from Japanese wisteria (EY Laboratories Inc., San Mateo, CA) were added to 25 µL of washed Dynabeads and brought up to a total volume of one milliliter with PBS, followed by rotation at room temperature overnight. After incubation, the WFA lectin-bound

Dynabeads were washed with PBS to remove any unbound WFA lectin. One milliliter of reconstituted cells extracted from raw milk was added to each tube of WFA lectin-bound Dynabeads, followed by rotation at 4°C for 30 minutes. After incubation, the Dynabeads (with attached mammary epithelial cells) were captured at the tube wall using a Dynal® Magnetic Particle Concentrator (MPC) magnet (Invitrogen, Carlsbad, CA) and were washed four times with PBS to remove any unbound cells. At this point, the pellet of Dynabeads with attached epithelial cells was ready for RNA extraction.

Binding between epithelial cells and lectin-coated Dynabeads was examined by mounting the cells onto microscope slides (Fisher Scientific, Tustin, CA) and Gram stained (Fisher Scientific, Tustin, CA) according to the manufacturer's protocol. Photographs were taken under the 100x oil immersion objective using a single lens camera (Canon, Lake Success, NY) attached to a vertical camera tube fitted to the microscope.

3.2.3 Total RNA Extraction

For the first method, total RNA extraction was performed using the RNeasy Mini Kit from Qiagen (Valencia, CA) according to manufacturer's instructions.

For the second method, eight hundred microliters of Trizol (Invitrogen, Carlsbad, CA) was added to the 1.5 mL tube containing a pellet of Dynabeads with attached epithelial cells to lyse the cells by careful mixing and incubation at room temperature for 5 minutes. One hundred-sixty microliters of chloroform (Fisher Scientific, Tustin, CA) was added and shaken vigorously by hand for 15 seconds, followed by incubation at room temperature for 3 minutes. The solution was centrifuged at 12,000 RPM at 4°C for 15 minutes to separate the aqueous phase, which was transferred to a fresh tube. The

RNA was precipitated from the aqueous phase with the addition of 400 μL isopropyl alcohol (Fisher Scientific, Tustin, CA) and incubated at room temperature for 10 minutes. The RNA was pelleted by centrifugation at 12,000 RPM at 4°C for 10 minutes. The pellet was washed with 800 μL of 75% ethanol (Fisher Scientific, Tustin, CA), centrifuged at 8,000 RPM at 4°C for 5 minutes. All ethanol was removed by decanting and evaporation. The isolated RNA was resuspended in 25 μL of depc- H_2O .

The quality of the RNA was examined on a 1% (w/v) agarose gel (Fisher Scientific, Tustin, CA) containing ethidium bromide (Fisher Scientific, Tustin, CA) (final concentration 0.5 $\mu\text{g/mL}$) in 0.5X TBE buffer (Sigma, St. Louis, MO). Ten microliters of each sample was loaded per well and electrophoresis was conducted at room temperature for 40 minutes at 90V. Gels were visualized with a UV transilluminator (ChemiDoc XRS imaging device, Bio-Rad, Hercules, CA). The concentration and purity of RNA in a 1:100 dilution of the isolated samples were measured with the SpectroMax Plus UV spectrometer (Molecular Devices, Sunnyvale, CA) at 260 and 280 nm, respectively. The following equations (Molecular Biology Lab Book, Chemistry Department, Cal Poly, San Luis Obispo, CA) were used to determine concentration:

$$\text{Percent nucleic acid} = \frac{(11.16 \times R - 6.32)}{2.16 - R}$$

(%N.A.)

$$\text{Where } R = \text{Abs}_{260} / \text{Abs}_{280}$$

$$\text{Abs} = (\% \text{N.A.} / 100)(A_{260})$$

$$(\text{Abs})(40)(\text{Dilution Factor}) = [\text{RNA}] \text{ ng}/\mu\text{L}$$

All samples were adjusted with PCR-grade water (Fisher Scientific, Tustin, CA) to a concentration of 1 $\mu\text{g}/\mu\text{L}$ and stored at -20°C.

3.2.4 *Reverse Transcription*

Reverse transcription (mRNA converted to cDNA) was carried out using SuperScript Preamplification System for First Strand Synthesis (Invitrogen, Carlsbad, CA) according to the manufacturer's instructions. All reagents and supplies used in this step were purchased from Invitrogen (Carlsbad, CA) unless otherwise stated. Two micrograms of RNA were mixed with one microliter of Amplification Grade DNase I and corresponding reaction buffer for 15 minutes at room temperature to remove unwanted DNA. The reaction was deactivated for 15 minutes at 65°C directly before reverse transcription.

Reverse transcription was immediately started on DNase treated RNA (2 µg) by the addition of 0.5 µg oligo dt (Integrated DNA Technologies, Coaralville, IL) and incubation for 10 minutes at 70°C with an ice quench. Corresponding reaction buffer, 25mM MgCl₂, and 10mM dNTPs were added in order and incubated at 42°C for 5 minutes. 200 units of Superscript II RT was then added to the contents. Single strand synthesis was performed by the Perkin Elmer 2400 GeneAmp PCR System at 42°C for 50 minutes. Reactions were then deactivated for 15 minutes at 70°C. After cooling down to 37°C, *E. coli* RNase H was added to remove RNA during incubation at 37°C for 20 minutes. All cDNA samples were stored at -20°C.

3.2.5 *Polymerase Chain Reaction*

PCR was performed on cDNA (1 ng/µL) with primers specific to bovine DGAT1 cDNA (5'-CACCATCCTCTTCCTCAAG-3', 5'-GGAAGCGCTTTCGGATG-3') under the following conditions: 50 µL total volume containing 50 ng cDNA, 1X AmpliTaq Gold PCR reaction buffer (Applied Biosystems, Branchburg, NJ), 0.2 mM dNTPs

(Invitrogen, Carlsbad, CA), 5% DMSO (Sigma, St. Louis, MO), 1.5 mM MgCl₂ (Applied Biosystems, Branchburg, NJ), 0.4 μM of each primer (Integrated DNA Technologies, Coralville, IL), 0.625 units of AmpliTaq Gold DNA polymerase (Applied Biosystems, Branchburg, NJ) and quantity sufficient PCR-grade water (Fisher Scientific, Tustin, CA). Thermocycling was performed on a Perkin Elmer ® 2400 GeneAmp PCR System under the following conditions: initial denaturing at 94°C for 5 minutes; followed by 35 cycles at 94°C for 30 seconds, 55°C for 30 seconds, 72°C for 30 seconds; and final extension at 72°C for 10 minutes.

Ten microliters of the PCR products were separated on a 1.5% (w/v) agarose gel (Fisher Scientific, Tustin, CA) containing ethidium bromide (Fisher Scientific, Tustin, CA) (final concentration 0.5 μg/mL) in 0.5X TBE buffer (Sigma, St. Louis, MO) at 90V for 40 minutes to determine proper amplification of a 232 bp fragment from exon VII to exon IX of the DGAT1 gene . Gels were visualized with a UV transilluminator (ChemiDoc XRS imaging device, Bio-Rad, Hercules, CA). The concentration of the PCR product in a 1:20 dilution was measured with the SpectroMax Plus UV spectrometer (Molecular Devices, Sunnyvale, CA) at 260nm and adjusted with PCR-grade water (Fisher Scientific, Tustin, CA) to a concentration of 1 ng/μL and stored at -20°C.

3.2.6 SNP genotyping by RFLP analysis

Restriction enzyme fingerprinting was used to compare banding patterns (Figure 7).

Figure 7. DGAT1 Genotype Fragment Pattern. Adapted from Rincon and Medrano (2005).

PCR products of each sample (20 ng) were digested by 5 units of *Mwo*I (New England Biolabs, Beverly, MA), 2.5 μ L of the corresponding reaction buffer (New England Biolabs, Beverly, MA), and quantity sufficient PCR-grade water (Fisher Scientific, Tustin, CA) in a final volume of 25 μ L at 60°C for 16 hours. Ten microliters of the digested samples were separated on a 3.5% (w/v) agarose gel (Fisher Scientific, Tustin, CA) containing ethidium bromide (Fisher Scientific, Tustin, CA) (final concentration 0.5 μ g/mL) in 0.5X TBE buffer (Sigma, St. Louis, MO) at 90V for 40 minutes to determine genotype (Rincon and Medrano, 2004). Gels were visualized with a UV transilluminator (ChemiDoc XRS imaging device, Bio-Rad, Hercules, CA).

3.3 Milk Fat Globule Membrane Analysis

3.3.1 MFGM Extraction

The amount of protein on the MFGM was measured to determine a possible difference between the DGAT1 genetic variants. A modified procedure of the method

developed by Patton and Basch was used to isolate the MFGM material from raw milk (Basch, 1985; Patton and Huston, 1986). All reagents and supplies used in this step were purchased from Fisher Scientific (Tustin, CA) unless otherwise stated.

Twelve hundred milliliters of raw milk was collected into sterile Falcon® tubes (BD Falcon, San Jose, CA) from 12 Holsteins and 12 Jerseys at the Cal Poly Dairy. The milk was pooled based on breed and DGAT1 genotype to give six samples. Raw milk was centrifuged at 4,000 RPM at 4°C for 20 minutes to obtain the cream. The cream layer was carefully removed and mixed well with 30 mL of PBS (0.1M NaCl, 0.08M Na₂HPO₄, 0.02 M NaH₂PO₄) buffer (pH 7.0) containing 2% (w/v) sodium citrate for casein dispersal. The cream was then centrifuged for 20 minutes at 4,000 RPM and 4°C. This washing step was repeated once. The cream layer was again removed and added to an appropriate amount of PBS buffer, containing Tween80 at 0.2% (v/v) for the removal of triglycerides, to create a 40% solution. The tubes were then shaken and vortexed for five minutes in order to break the fat emulsion. A water bath was heated to 42°C and the tubes were heated for 30 minutes. The tubes were centrifuged for 30 minutes at 4,000 RPM and 40°C. The aqueous cloudy middle layer was isolated and transferred to a 15 mL Falcon® tube (San Jose, CA) containing 2% (w/v) sodium citrate and incubated overnight at 4°C.

The isolated buttermilk was centrifuged at 25,000 RPM at 4°C for 2 hours to pellet the MFGM. The pellet was resuspended in cold PBS buffer containing 1% (v/v) Triton X-100 to purify the proteins. The solution was then centrifuged for 2 hours at 25,000 RPM and 4°C. The pellet was resuspended in cold PBS buffer containing 1% (v/v) Triton X-100 and homogenized with a Fisher Scientific Sonic Dismembrator

(Tustin, CA). One hundred microliters of the homogenized protein pellet was precipitated and de-fatted with methanol/chloroform/water (500:125:375, v/v/v) followed by centrifugation at 14,000 RPM at 4°C for 10 minutes. The upper layer was removed and 500 µL of methanol was added to the interface and lower layer. The tubes were centrifuged at 14,000 RPM at 4°C for 10 minutes to pellet the isolated MFGM protein. All methanol was removed by decanting and evaporation. The samples were then stored at -20°C prior to analysis using sodium dodecyl sulfate (SDS)/polyacrylamide gel electrophoresis (PAGE).

3.3.2 *SDS-PAGE Analysis*

The aqueous MFGM samples were prepared for SDS-PAGE with dilution of the samples in a 1:2 ratio (v/v) with SDS reducing buffer (Laemmli Sample Buffer, BioRad, Hercules, CA) containing 3-β-mercaptoethanol to create a final concentration of 5% (v/v), and heated at 100°C for five minutes to denature the proteins. BioRad's Precision Plus Protein Unstained Standard (Hercules, CA) was run as a molecular weight comparison for the samples. A sample size of 15 µL was loaded into each well of a standard pre-cast 12% Tris-HCl SDS-PAGE gel (BioRad, Hercules, CA) and the samples were run at 90 V through the stacking gel and 110 V through the main resolving gel. A 10X TGS (25 mM Tris base, 192 mM glycine, 0.1% w/v SDS) buffer (pH 8.3) (BioRad, Hercules, CA) diluted to 1X concentration was used as the electrophoresis buffer. The gels were stained with the Modified Silver Stain procedure (BioRad, Hercules, CA) and visualized under white light using BioRad's Gel Doc 1000 and Molecular Analyst version 2.1 software.

3.4 Lipid Analysis

3.4.1 Lipid extraction from raw milk

Twelve hundred milliliters of raw milk was collected into sterile Falcon® tubes (BD Falcon, San Jose, CA) from 12 Holsteins and 12 Jerseys at the Cal Poly Dairy. The lipid content of the samples were determined using the Mojonnier ether extraction method of fat analysis according to the procedure in the AOAC method 989.05 and instructions for raw milk. All reagents used were of analytical grade and purchased from Fisher Scientific (Tustin, CA). Samples were run in duplicate, individually and pooled based on breed and DGAT1 genotype to give six samples. Extracted lipids were resuspended in chloroform/methanol (2:1, v/v) at a concentration of 10 mg/mL and stored at -20°C.

3.4.2 Thin Layer Chromatography Analysis

For analysis of polar and non-polar lipids, extracted lipid samples were blotted approximately 2 inches from the bottom of silica gel plates coated with glass (Fisher Scientific, Tustin, CA) and one centimeter apart using a glass capillary tube at 10 mg/mL (~2 mm spot diameter). After sample application, the plates were dried in a vacuum oven for 10 minutes at room temperature. The polar plates were placed in the developing tanks that contained chloroform/methanol/water (65:25:4, v/v/v) and non-polar plates placed in the developing tanks that contained petroleum ether/ethyl ether/glacial acetic acid (85:15:2, v/v/v). Whatman filter paper was applied to the walls of both developing tanks to improve the performance of the vapor chamber. Plates were run until the solvent was approximately one centimeter from the top of the plate (approximately 1.5 - 2 hours). The plates were stained by iodine vapor overnight and visualized using a BioRad

Imaging Densitometer GS-700 and Molecular Analyst version 2.1 software (Hercules, CA).

3.4.3 Solid Phase Extraction Analysis

Strata silica SI-1 normal phase SPE cartridges (bed weight 2g, volume 12 mL) (Phenomenex, Torrance, CA) were conditioned with chloroform. Elution of the neutral lipids from the pooled lipid samples (by breed and DGAT1 genotype to give 4 samples) was performed with 40 mL of hexane:ethyl ether (1:1, v/v) followed by elution of the phospholipids with a 20 mL methanol wash and a 20 mL chloroform:methanol:water (3:5:2, v/v/v) wash. Solvents were evaporated and extracted phospholipids were resuspended in chloroform/methanol (2:1, v/v) at a concentration of 10 mg/mL and stored at -20°C. All reagents used were of HPLC grade and purchased from Fisher Scientific (Tustin, CA).

3.5 Citrate Analysis

Fifty milliliters of raw milk was collected into sterile Falcon® tubes (BD Falcon, San Jose, CA) from all milking Holsteins and Jerseys at the Cal Poly Dairy twice during a six month period. The amount of citric acid in each sample was determined by FT-IR using a Foss Milkoscan FT2 (Eden Prairie, MN) according to manufacturer's instructions.

3.6 Development of a Milk Powder Molecular Test

3.6.1 Primer Design

Bovine β -lactoglobulin gene sequences were obtained via a search of NCBI (<http://www.ncbi.nlm.nih.gov>). Primers were designed using Primer Quest from Integrated DNA Technologies (<http://www.idtdna.com>) to generate fragments between 200 and 300 bp. The estimated melting temperatures of primers were between 56.5°C and 60.5°C. The optimal primer size was 22 bases, with a range from 18 to 24 bases.

3.6.2 Preparation of genomic DNA from milk powder

Twenty domestic and international commercial skim milk powder samples were reconstituted to be 10% total solids with PCR-grade water overnight at 4°C. Reconstituted samples were then centrifuged in a Beckman L7-35 Ultracentrifuge at 7,000 RPM for 30 minutes. The supernatant was decanted and the cell pellet was resuspended in PCR-grade water and washed twice with ice-cold PBS. The supernatant was decanted and the cell pellet was resuspended in one milliliter of digestion buffer containing: 100mM NaCl, 10mM Tris-Cl pH 8, 25mM EDTA pH 8, 0.5% sodium dodecyl sulfate, and 0.1 mg/mL proteinase K, followed by rotation at 50°C for 16 hours. Phenol/Chloroform/Isoamyl Alcohol (25:24:1, v/v/v) was added at a rate of half-volume and centrifuged at 4,000 RPM at room temperature for 10 minutes to separate the aqueous phase. The aqueous phase was transferred to a fresh tube. 7.5M ammonium acetate was added at a rate of half-volume and 100% ethanol was added at a rate of twice volume, followed by centrifugation at 4,000 RPM at room temperature for 5 minutes to pellet the DNA. The supernatant was decanted and 70% ethanol was added followed by one more spin with the latter parameters to wash the pellet. All ethanol was removed by

decanting and allowing the residual ethanol to evaporate. Finally, the pellets were resuspended in 50 μ L of PCR-grade water and rotated at room temperature for 16 hours. All reagents used were of analytical grade and purchased from Fisher Scientific (Tustin, CA).

The integrity of the DNA was examined on a 1% (w/v) agarose gel (Fisher Scientific, Tustin, CA) containing ethidium bromide (Fisher Scientific, Tustin, CA) (final concentration 0.5 μ g/mL) in 0.5X TBE buffer (Sigma, St. Louis, MO). Ten microliters of each sample was loaded per well and electrophoresis was conducted at room temperature for 40 minutes at 90V. Gels were visualized with a UV transilluminator (ChemiDoc XRS imaging device, Bio-Rad, Hercules, CA). The concentration and purity of DNA in a 1:20 dilution of the isolated samples were measured with the SpectroMax Plus UV spectrometer (Molecular Devices, Sunnyvale, CA) at 260 and 280 nm, respectively. All samples were adjusted with PCR-grade water (Fisher Scientific, Tustin, CA) to a concentration of 1 ng/ μ L and stored at -20°C.

3.6.3 *Polymerase Chain Reaction*

PCR was performed on diluted genomic DNA (1 ng/ μ L) with primers specific to bovine beta-lactoglobulin (5'-TGAGAGTGTATGTGGAGGAGCTGA-3', 5'-TGGATAAGCAGCCTTGGGTGGA-3') under the following conditions: 50 μ L total volume containing 10 ng DNA, 1X AmpliTaq Gold PCR reaction buffer (Applied Biosystems, Branchburg, NJ), 0.1 mM dNTPs (Invitrogen, Carlsbad, CA), 1.5 mM MgCl₂ (Applied Biosystems, Branchburg, NJ), 0.3 μ M of each primer (Integrated DNA Technologies, Coralville, IL), 1.25 units of AmpliTaq Gold DNA polymerase (Applied Biosystems, Branchburg, NJ) and quantity sufficient PCR-grade water (Fisher Scientific,

Tustin, CA). Thermocycling was performed on a Perkin Elmer ® 2400 GeneAmp PCR System under the following conditions: initial denaturing at 94°C for 5 minutes; followed by 40 cycles at 94°C for 1 minute, 66°C for 1 minute, 72°C for 1 minute; and final extension at 72°C for 10 minutes.

Ten microliters of the PCR products were separated on a 3% (w/v) agarose gel (Fisher Scientific, Tustin, CA) containing ethidium bromide (Fisher Scientific, Tustin, CA) (final concentration 0.5 µg/mL) in 0.5X TBE buffer (Sigma, St. Louis, MO) at 90V for 40 minutes to determine proper amplification of a 222 bp fragment from exon II to intron III of the beta lactoglobulin gene. Gels were visualized with a UV transilluminator (ChemiDoc XRS imaging device, Bio-Rad, Hercules, CA).

Chapter 4

Results and Discussion

4.1 *DGAT1* Genotyping by RFLP

Genomic DNA was extracted using the MoBio Blood Spin Kit from 232 cows in 2005, 193 cows in 2007, and 182 cows in 2010. Ten microliters of sample was loaded onto a 1.0% agarose gel (0.5X TBE) containing ethidium bromide and separated by electrophoresis at 90 V for 40 minutes. The ethidium bromide allowed for immediate viewing and image capturing over UV light. DNA extraction proved to be successful with a high yield of DNA at good quality as seen by the bright high molecular weight band with no smearing (Figure 8).

Figure 8. A 1% agarose gel of extracted DNA stained with ethidium bromide. Samples in order from left to right. Lane 1: 100bp BioRad Ladder, Lane 2-8: DNA extracted from a bovine blood sample.

The amplification reaction used primers designed to amplify a PCR fragment within the *DGAT1* gene surrounding the mutation site and used the temperature and time cycles indicated in the Materials and Methods (Rincon and Medrano, 2004). Ten microliters of each sample was loaded onto a 1.5% agarose gel (0.5X TBE) containing

ethidium bromide and separated by electrophoresis at 90 V for 40 minutes. BioRad's EZ Load 100bp Molecular Ruler (Hercules, CA) was run as a standard to determine fragment size. The ethidium bromide allowed for immediate viewing and image capturing over UV light. PCR amplification proved to be successful with the correct base pair length of 285 in high yield and good quality (Figures 9 and 10).

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ccacgctgtc tcggccacgg gcagcgcggg gggcgtggcc tgagcttgcc tctcccacag
tgggctccgt gctggc cctg atggtctaca ccatcc tctt cctcaagctg ttctcctacc
gggacgtcaa cctctggtgc cgagagcgca gggctggggc caaggccaag gctggtgagg
gctgcctcgg gctggggcca ctgggctgcc acttgctcgg ggaccggcag gggctcggct
cacccccgac ccgccccctg ccgcttgctc gtagctttgg caggtaagaa ggccaacggg
ggagctgccc agcgcaccgt gagctacccc gacaacctga cctaccgcgg tgaggatcct
gccggggggc ggggggactg cccggcggcc tggcctgcta gccccgccct cccttcaga
tctctactac ttctctcttcg cccccaccct gtgctacgag ctcaacttcc cccgctcccc
ccgcatccga aagcgcttcc tgctgcggcg actcctggag atggtgaggc ggggcctcgc
gggccagggc gggcgggcct gccggcaccc gccaccgggg ctcagctcac tgtccgcttg

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Figure 9. Partial DGAT1 gene showing exons 7-9 (shaded gray), primer sites (yellow), and mutation site (purple) from NCBI on PubMed.

Figure 10. A 1.5% agarose gel of PCR fragments using DGAT1 primers stained with ethidium bromide. Samples in order from left to right. Lane 1: 100bp BioRad Ladder, Lane 2-8: PCR fragments at 285 bp, Lane 9: PCR quality control.

The products of the PCR amplifications were then digested with the *MwoI* restriction enzyme corresponding to the mutation site at 60°C for 16 hours. Ten microliters of sample was loaded onto a 3.5% agarose gel (0.5X TBE) containing

ethidium bromide and separated by electrophoresis at 90 V for 40 minutes. BioRad's EZ Load 20bp Molecular Ruler (Hercules, CA) was run as a standard to determine fragment size. The ethidium bromide allowed for immediate viewing and image capturing over UV light. The PCR-RFLP analysis allowed the identification of the three genotypes based on assessing the fragment fingerprint pattern (Figures 6 and 11). All DGAT1 genotypes for the Cal Poly Dairy Herd can be found in Appendix A, B, and C.

Figure 11. A 3.5% gel of PCR fragments digested with *MwoI* stained with ethidium bromide. Samples in order from left to right. Lane 1: 20 bp BioRad Ladder, Lane 2-30: Digested DGAT1 PCR fragments.

The allele frequencies were calculated using the following equations:

$$\text{frequency of the A allele} = \frac{\# \text{ of AG heterozygotes} + 2(\# \text{ of AA homozygotes})}{\text{total number of alleles in population of that locus}}$$

$$\text{frequency of the G allele} = 1 - \text{frequency of the A allele}$$

Since the population does not meet the Hardy-Weinberg assumptions (the population size is small, there was non-random mating, migration, and selection), the Hardy-Weinberg equation could not be used (Alberts et al., 2002). The following equations were used to determine the genotype distributions from the PCR-RFLP results:

$$\begin{aligned} \text{frequency of AA genotype} &= \# \text{ of AA individuals} / \text{population size} \\ \text{frequency of AG genotype} &= \# \text{ of AG individuals} / \text{population size} \\ \text{frequency of GG genotype} &= \# \text{ of GG individuals} / \text{population size} \end{aligned}$$

The 2005 observed allele frequencies for the DGAT1 gene in Cal Poly Holsteins were 0.265 for allele A and 0.735 for allele G. The calculated genotype distributions were 0.06 for AA, 0.41 for AG, and 0.53 for GG. The observed allele frequencies for the DGAT1 gene in Cal Poly Jerseys were 0.435 for allele A and 0.565 for allele G. The calculated genotype distributions were 0.19 for AA, 0.48 for AG, and 0.33 for GG.

Figure 12. DGAT1 genotype distributions for the Cal Poly Dairy Herd in 2005.

The 2007 observed allele frequencies for the DGAT1 gene in Cal Poly Holsteins were 0.307 for allele A and 0.693 for allele G. The calculated genotype distributions were 0.09 for AA, 0.43 for AG, and 0.48 for GG. The observed allele frequencies for the DGAT1 gene in Cal Poly Jerseys were 0.486 for allele A and 0.514 for allele G. The calculated genotype distributions were 0.25 for AA, 0.47 for AG, and 0.28 for GG.

Figure 13. DGAT1 genotype distributions for the Cal Poly Dairy Herd in 2007.

The 2010 observed allele frequencies for the DGAT1 gene in Cal Poly Holsteins were 0.17 for allele A and 0.83 for allele G. The calculated genotype distributions were 0.04 for AA, 0.26 for AG, and 0.70 for GG. The observed allele frequencies for the DGAT1 gene in Cal Poly Jerseys were 0.49 for allele A and 0.51 for allele G. The calculated genotype distributions were 0.22 for AA, 0.54 for AG, and 0.24 for GG.

Figure 14. DGAT1 Genotype distributions for the Cal Poly Dairy Herd in 2010.

For each time point of analysis, the genotypic breakdown is as expected with the Jersey breed having a higher distribution of the AA genotype which corresponds with their overall higher milk fat content in comparison to the Holstein breed. Comparing the 2007 Cal Poly herd to that from 2005, there is an increase in the frequency of the A allele primarily through the increase in the distribution of the AA genotype. Looking at the Cal Poly herd in 2010, the change was mostly seen in the Holstein breed with an increase in the frequency of the G allele through the increase in the distribution of the GG genotype. An explanation for this change could be due to the small herd reduction in late 2009 and the selection for higher milk producing Holsteins in the hopes of increasing income for the Cal Poly Dairy (Sneed, 2009). Milk yield is negatively correlated to fat and protein percentages, therefore higher milk yield would be related to a lower milk fat percentage (Grisart et al., 2004).

The Dairy Herd Improvement Association (DHIA) records for the 305-day average percent milk fat content during the first lactation were obtained and analyzed based on breed and DGAT1 genotype (raw data in Appendix D). Table 3 shows a trend of higher average percent milk content for the DGAT1 AA genotype and the lowest for the DGAT1 GG genotype within each breed, however when also considering the standard deviation of each mean, there no longer appears to be a difference between the genotypes.

Table 3. 305-day average % milk fat content in Holstein and Jersey cows in relation to DGAT1 genotype.

Breed	AA		AG		GG	
	N	Mean $\pm$ SD	N	Mean $\pm$ SD	N	Mean $\pm$ SD
Holstein	13	4.25 $\pm$ 0.59	82	3.9 $\pm$ 0.38	138	3.62 $\pm$ 0.36
Jersey	57	4.86 $\pm$ 0.57	126	4.64 $\pm$ 0.49	67	4.29 $\pm$ 0.40

For statistical analysis of the results, a General Linear Model (basic two-way analysis of variance) was used with 305-day average milk fat percent as the response, and breed and genotype as the model factors (Appendix E). The results of the ANOVA test indicate that the effect of the breed is significant ($p < 0.001$), the effect of the genotype is significant ($p < 0.001$), and the effect of the interaction term (breed and genotype) is not significant ($p = 0.626$). Removing the interaction term, the F-value for the breed factor increased ($F = 266.16$) suggesting responsibility for a higher proportion of variation in comparison to the genotype factor ($F = 45.16$).

The suggestion that the breed factor is responsible for more of the variation in milk fat content than the genotype factor was analyzed further (Appendix E). The correlation coefficient between breed and percent milk fat content was calculated to be $r = 0.662$ ($p < 0.001$), showing a linear relationship, whereas DGAT1 genotype and percent milk fat content was calculated to be $r = 0.028$ and not statistically significant ($p = 0.538$). The regression analysis, which represents the variation in percent milk fat that can be explained by the DGAT1 genotype and breed, gave an adjusted R^2 of 52.4%. This evidence shows that both variables had an effect on milk fat percent, but there still is roughly 48% of variation left unexplained. The results of an ANOVA test, with 305-day average milk fat percent as the response and breed as the model factor, indicate that the effect of the breed is significant ($p < 0.001$) and an adjusted R^2 at 43.69%. The increase in the adjusted R^2 due to the addition of the genotype factor can be explained by the effect of the factor or just through the addition of another variable.

4.2 Identification of DGAT1 mRNA in milk cells

To examine a difference at the molecular level of DGAT1, fresh epithelial cells were extracted from milk using the novel technique of magnetic Dynabeads. These live cells are needed to obtain mRNA, which could identify DGAT1 as more than a genetic marker. The technique appears to be successful, evidenced by the microscope images (Figure 15) showing binding between lectin coated Dynabeads and epithelial cells. These are believed to be epithelial cells due to the use of a specific lectin (*Wisteria floribunda* agglutinin) that recognizes an oligosaccharide (N-acetylgalactosamine) on the cell membrane, as well as staining gram negative. It is hard to determine the level of success of the technique as it was seen under the microscope many unbound epithelial cells and Dynabeads as well.

Figure 15. Microscope images at 100x oil immersion. Picture on left shows 1. epithelial cells extracted from milk bound to 2. lectin-coated dynabeads. Picture on right shows bacteria, debris and leukocytes not bound to dynabeads. Gram stained.

Two RNA extraction techniques were compared due to the difficulty of obtaining good quality RNA from a milk sample. This is due to the high amount of protein present and quick degradation of RNA. The RNA was extracted using either the Qiagen RNeasy Kit or Trizol method. Ten μL of sample was loaded onto a 1.0% agarose gel (0.5X TBE)

containing ethidium bromide and separated by electrophoresis at 90 V for 40 minutes. The ethidium bromide allowed for immediate viewing and image capturing over UV light. RNA extraction proved to have moderate success with both methods by yielding RNA at good quality, as seen by the clear appearance of the 28S and 18S bands with little to no smearing (Figure 16).

Figure 16. A 1% agarose gel of extracted RNA stained with ethidium bromide. Samples in order from left to right. Lane 1: 20bp BioRad Ladder, Lane 2-7: RNA extracted via the Trizol method, Lane 8-11: RNA extracted via the Qiagen RNeasy Kit (Valencia, CA).

The total RNA extracted was then transcribed into cDNA followed by DNA amplification and electrophoresis. The amplification reaction used primers designed to amplify a PCR fragment within the DGAT1 gene surrounding the mutation site and used the temperature and time cycles indicated in the Materials and Methods (Rincon and Medrano, 2004). Ten microliters of each sample was loaded onto a 1.5% agarose gel (0.5X TBE) containing ethidium bromide and separated by electrophoresis at 90 V for 40 minutes. BioRad's EZ Load 100bp Molecular Ruler (Hercules, CA) was run as a standard to determine fragment size. The ethidium bromide allowed for immediate viewing and image capturing over UV light. PCR amplification proved to be successful

with the correct base pair length of 232 in high yield and good quality (Figures 17 and 18).

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tggggtccgt gctggccctg atggtcta ca ccatacctctt cctcaagctg ttctcctacc
gggacgtcaa cctctggtgc cgagagcgca gggctggggc caaggccaag gctctttgg
caggtaagaa ggccaacggg ggagctgccc agcgcaccgt gagctacccc gacaacctga c
tctctactac ttctctctcg cccccaccct gtgctacgag ctcaacttcc cccgctcccc
ccgcatccga aagcgcttcc tgctgcggcg actcctggag atg

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Figure 17. Partial DGAT1 gene showing exons 7-9 (shaded gray), primer sites (yellow), and mutation site (purple) from NCBI on PubMed.

Figure 18. A 1.5% agarose gel of PCR fragments created using the DGAT1 cDNA primers stained with ethidium bromide. Samples in order from left to right. Lane 1: 100bp BioRad Ladder, Lane 2: PCR quality control, Lane 3-8: PCR fragments at 232 bp.

The products of the RT-PCR amplifications were then digested with the *MwoI* restriction enzyme corresponding to the mutation site at 60°C for 16 hours. Ten microliters of sample was loaded onto a 3.5% agarose gel (0.5X TBE) containing ethidium bromide and separated by electrophoresis at 90 V for 40 minutes. BioRad's EZ Load 20bp Molecular Ruler (Hercules, CA) was run as a standard to determine fragment size. The ethidium bromide allowed for immediate viewing and image capturing over UV light. The PCR-RFLP analysis allowed the identification of the genotypes based on assessing the fragment fingerprint pattern (Figure 7 and 19).

Figure 19. A 3.5% gel of PCR fragments digested with *MwoI* stained with ethidium bromide. Samples in order from left to right. Lane 1: 20 bp BioRad Ladder, Lane 2-6: Digested DGAT1 PCR fragments, Lane 7-8: Empty.

The samples in Figure 19 came from cows with the heterozygous DGAT1 genotype and both high and low milk fat levels. The desired result was to see a correlation between higher milk fat and a specific DGAT1 genotype. A previous study showed that the lysine232 polymorphism was associated with higher milk fat, so this was the expected result (Grisart et al., 2004). Imaging from the computer software (which had better picture quality than the exported image) gave banding patterns consistent with the heterozygous DGAT1 genotype. This meant that no correlation between milk fat levels and DGAT1 genotype could be determined. The results were difficult to determine because of faint bands. These faint bands could suggest difference in concentration of fragments and therefore alleles, but with faint bands in fragments found in all genotypes (an example would be the 83 base pair fragment in Lane 6 of Figure 19) and consistent with all fragments in each sample, it appears the faint bands are due to low concentration of original PCR product and digested fragments. It was decided to not repeat this experiment, mainly due to funding constraints, so a correlation between milk fat levels

and the DGAT1 genotype at the functional gene level could not be determined in this research.

4.3 *Milk Fat Globule Membrane Analysis*

The isolated MFGM proteins, from a 200 milliliter raw milk sample pooled by breed and DGAT1 genotype, were visually compared using SDS-PAGE Electrophoresis. The 12% acrylamide gel is shown as Figure 20 with a BioRad Precision Plus Protein Unstained standard.

Figure 20. MFGM extracted from raw milk samples taken from pooled Jersey and Holstein cows during their third lactation and run on a 12% SDS-Page gel and visualized through silver staining. Lane 1: Precision Plus Protein Standard (BioRad, Hercules, CA), Lane 2 and 3: Jersey AA, Lane 4 and 5: Jersey GG, Lane 6 and 7: Holstein AA, Lane 8 and 9: Holstein GG.

It was the intention of this project to use the densitometer and the Molecular Analyst software to measure the densities of the proteins on the gels. Using a colloidal Coomassie Blue stain would have allowed quantitative analysis, but the sensitivity of the stain was low (Westermeyer and Marouga, 2005). The silver stain reagent offered better sensitivity, but is considered to be inconsistent and non-quantitative (Fong et al., 2007).

Therefore, the intensity of each band does not necessarily reflect the concentration of the protein present in the sample (Fong et al., 2007). The Silver Stain method is also very sensitive and any disturbance to the gel such as fingerprints can cause darkening on the gel surface. The densitometer detects all of the shading on an SDS-PAGE gel that has been stained by the Silver Stain method due to the dark background.

Even though the silver stain reagent is considered non-quantitative, analysis was done using a ChemiDoc XRS imaging device (BioRad, Hercules, CA) and Quality One software (BioRad, Hercules, CA) due to a visual comparison showing possible differences between the isolated MFGM samples.

Figure 21. Figure 20 analyzed by the *Compare Lane Feature* within BioRad's Quality One software.

Using the Quality One software (Figure 21), no difference in the higher molecular weight (>75 kD) was observed. Intense bands at the molecular weights typical for caseins (19-25 kD) and whey proteins (14 and 18 kD) were not expected. Aggregation of MFGM proteins, loss of MFGM proteins, and incorporation of caseins and whey proteins into the MFGM have been reported (Evers, 2004). Washing of cream is usually

performed to remove residual casein, which can be difficult to remove. MFGM damage can be done due to the cream washing, even with attempts to minimize the damage, leaving exposed fragments and binding opportunities for caseins and β -lactoglobulin (Fong et al., 2007). Sample preparation also involved an extraction with chloroform and methanol, possibly causing some intrinsic proteins to be lost due to their precipitation or their difficulty to solubilize into solution prior to electrophoresis. The difference in band intensity at the molecular weight for casein (19-25 kD) may be due to other genetic profiles unrelated to the DGAT1. These other genetic profiles are currently unknown.

A visual comparison shows no difference in MFGM proteins (>75 kD) between the DGAT1 genotypes. Since this is only by visual comparison, expansion into two-dimensional gel electrophoresis might show a difference in MFGM proteins.

4.4 *Lipid Analysis*

Two hundred milliliters of a raw milk sample, pooled by breed and DGAT1 genotype, had lipids extracted by Mojonnier ether extraction method of fat analysis and run on TLC plates in polar and non-polar conditions at a concentration of 10 mg/mL. The standard used contained one mg/mL each of sphingomyelin (SM) isolated from bovine brain, phosphatidylethanolamine (PE) isolated from egg yolk, and phosphatidylcholine (PC) isolated from bovine brain. All standards were purchased from Sigma Chemical Company (St. Louis, MO).

Figure 22. Left - The polar lipid profiles run on a TLC plate and visualized using iodine and compared with the standards. Right - The non-polar lipid profiles run on a TLC plate and visualized using iodine.

The TLC plates were not evaluated quantitatively due to lack of proper equipment (e.g. densitometer). A visual comparison shows a difference between the breeds and a possible difference in phospholipids between the DGAT1 genotypes. To examine this possible difference quantitatively, a solid phase extraction was performed to determine the amount of phospholipids in each pooled sample based on DGAT1 genotype.

Table 4. Amount of phospholipids, extracted via SPE, based on DGAT1 genotype.

Sample	Recovered Phospholipids (g)	%Phospholipids (original sample)
H-AA	0.0075	37.50
H-GG	0.0073	33.18
J-AA	0.0068	30.91
J-GG	0.0063	27.39

Looking at the percentage of phospholipids extracted from each lipid sample, there appears to be a difference based on DGAT1 genotype for both breeds (Table 4). However, due to the lack of supplies to repeat this experiment, no statistical analysis can

be done and therefore no significant difference can be determined since the experiment was performed only once. Based on this preliminary trial, for both Holsteins and Jerseys, the DGAT1 genotype of GG has a lower amount of phospholipids extracted. This is consistent with previous research, as the DGAT1 genotype GG is correlated with lower fat content (Grisart et al., 2004). This also supports more selection for the DGAT1 genotype of AA, correlated with higher fat content, as phospholipids have been shown in research to have a positive effect on health (Spitsberg, 2005; Lopez et al., 2008). Since this was only a single trial, repeating this experiment may confirm these results and find a significant difference between the DGAT1 genotypes.

4.5 *Citrate Analysis*

The amount of citric acid in each 50 milliliter sample was determined by FT-IR using a Foss Milkoscan FT2 (Eden Prairie, MN) according to manufacturer's instructions. Samples were collected in April 2010 for Holsteins and Jerseys at the Cal Poly Dairy (Appendix G) and again in September 2010 for the same Holsteins (Appendix H).

The data was statistically analyzed using Minitab 16. Based on Analysis of Variance, there was no significant difference ($p>0.05$) in citrate levels (given as a percentage of the milk sample) between the DGAT1 genetic variants or Cal Poly Holstein and Jersey breeds when looking at the entire population in April 2010 (Appendix I). If we hold days in milk (DIM) and the lactation number constant due to the naturally large effect on citrate levels, which is difficult to do with the limited numbers of the AA genetic variant for both breeds and the GG genetic variant for the Cal Poly Jersey breed, there still is no significant difference ($p>0.05$) in citrate levels between the DGAT1

genetic variants (Appendix J). There was a significant difference ($p < 0.05$) in citrate levels between the Cal Poly Jersey and Holstein breeds, with levels higher in the Jersey breed. Similar results were found in previous research at Cal Poly (Howell, 2009). Since body condition scores were not taken, it is possible that the higher amount of citrate seen in the Cal Poly Jersey breed is due to utilization of fat stores in adipose tissue (Faulkner and Peaker, 1982) (Akerlind et al., 1999). Another possibility stems from the higher percentage of protein in the milk, and the fact that citrate aids in protein coagulation and stability (Walstra et al., 1999). Higher citrate levels could have been selected for in the Jersey breed due to the desire of better heat stability to aid in milk processing (Braunschweig and Puhon, 1999).

Figure 23. Citrate levels (given as percentage of milk sample) based on DGAT1 genotype and time of milk collection. Boxplot from Minitab software version 16.

Comparing the citrate levels in milk collected from Cal Poly Holsteins in April 2010 to that collected in September 2010, there was no significant difference ($p > 0.05$) in citrate levels. Looking at the above graph (Figure 23), it is hard to make any comparisons due to the limited number of samples from the AA genetic variant and high amount of variability among the other variants, shown by the size of the error bars for the standard

deviation of the mean. Overall, the trend appears to show lower levels of citrate in September, which correlates to a warmer climate and a higher DIM.

4.6 Analysis of the Milk Powder Molecular Test

The purpose of this study was to investigate the adaptation of a polymerase chain reaction (PCR) amplification assay for the detection of β -lactoglobulin C variant in bulk milk powder samples. Genomic DNA was extracted using a technique adapted from use with mammalian cell culture (1994) from 15 domestic and international skim milk powder samples. Ten microliters of sample was loaded onto a 1.0% agarose gel (0.5X TBE) containing ethidium bromide and separated by electrophoresis at 90 V for 40 minutes. The ethidium bromide allowed for immediate viewing and image capturing over UV light. DNA extraction proved to be successful with a high yield of DNA at good quality (Figure 24).

Figure 24. A 1% agarose gel of extracted DNA stained with ethidium bromide. Samples in order from left to right. Lane 1: 100bp BioRad Ladder, Lane 2-35: DNA extracted from domestic and international skim milk powders.

Several primer sets were designed using Primer Quest from Integrated DNA Technologies (<http://www.idtdna.com>) to generate fragments between 200 and 300 bp surrounding a single nucleotide polymorphism in exon II of the β -lactoglobulin gene. The successful amplification reaction used primers labeled BLF1 and BLR2 to amplify a PCR fragment within exon II of the β -lactoglobulin gene surrounding the mutation site and used the temperature and time cycles indicated in the Materials and Methods to give the correct fragment size and quantity. Ten microliters of each sample was loaded onto a 1.5% agarose gel (0.5X TBE) containing ethidium bromide and separated by electrophoresis at 90 V for 40 minutes. BioRad's EZ Load 100bp Molecular Ruler (Hercules, CA) was run as a standard to determine fragment size. The ethidium bromide allowed for immediate viewing and image capturing over UV light. PCR amplification proved to be successful with the correct base pair length of 222 in high yield and good quality (Figures 25 and 26).

```
cgccctgga gtgcagctca aggtccctcc ccagggtggcg gggacttggt actccttggc
catggcgggc agcgacatct ccctgctgga cgcccagagt gccccctga gagtgtatgt
ggaggagctg aagcccaccc ctgagggcga cctggagatc ctgctgCAGa aatgggtgggc
gtccccccca aaaaaagcat ggaaccccca ctccccaggg atatggaccc cccggggtgg
ggtgcaggag ggaccagggc cccagggctg gggaacgggg cttggagttt cctggtaccc
ctggaggtcc acccaaggct gcttatccag ggctttctct ttcttttttt cccccaactt
ttattaattt gatgcttcag
```

Figure 25. Partial β -lactoglobulin gene showing exon 2 (shaded gray), primer sites (yellow), and mutation site (purple) from NCBI on PubMed.

Figure 26. A 1.5% agarose gel of PCR fragments using β -lactoglobulin primers stained with ethidium bromide. Samples in order from left to right. Lane 1: 100bp BioRad Ladder, Lane 2: PCR quality control, Lane 3: Blank, Lane 4-23: PCR fragments at 222 bp, Lane 24: Blank, Lane 25: PCR quality control.

A restriction enzyme was examined for the ability to identify the β -lactoglobulin C variants in bulk skim milk powder samples. Unfortunately it was determined that there are currently no restriction enzymes that will cut at the mutation site to allow for genotyping. This means it is currently not possible to determine if the C variant is present in the powder sample. Presenting these results show the possibility of a test whenever a restriction enzyme is found that cuts at the mutation site.

To show the ability to genotype from a bulk powder sample, the extracted DNA was subjected to PCR amplification using primers for the DGAT1 gene. The products of the PCR amplifications were then digested with the *MwoI* restriction enzyme corresponding to the mutation site at 60°C for 16 hours. Ten microliters of sample was loaded onto a 3.5% agarose gel (0.5X TBE) containing ethidium bromide and separated by electrophoresis at 90 V for 40 minutes. BioRad's EZ Load 20bp Molecular Ruler (Hercules, CA) was run as a standard to determine fragment size. The ethidium bromide allowed for immediate viewing and image capturing over UV light. The PCR-RFLP

analysis allowed the identification of the heterozygous genotype based on assessing the fragment fingerprint pattern (Figure 6 and 27).

Figure 27. A 3.5% gel of PCR fragments digested with *MwoI* stained with ethidium bromide. Samples in order from left to right. Lane 1: 100 bp BioRad Ladder, Lane 2-38: Digested DGAT1 PCR fragments.

Showing all powders to be heterozygous for the DGAT1 genotype was as expected. These samples were commercial skim milk powders, basically dried bulk milk pooled from thousands of cows. This represents the problem of genotyping using a mutation that is common in a variety of locations; it doesn't help identify origin as no differences can be detected. These results show the possibility of obtaining a genotype from milk powder, and if a unique variant is used (such as β -lactoglobulin C) it could be possible to determine origin.

Chapter 5

Conclusion and Future Research

Previously, a QTL affecting milk fat percentage was mapped to the centromeric end of bovine chromosome 14. The DGAT1 gene was mapped to the same location and became promising candidate gene for milk fat percentage in cattle. The genetic variation in DGAT1 was found to be associated with a major effect on milk yield and composition. Further research has shown that an increase in the “A” allele will have an increase in fat yield, with a decrease in protein and milk volume (Spelman et al., 2002). With regards to the Cal Poly Dairy Herd, the results show that the lysine232 allele (allele A) has a significant effect increasing milk fat content in Holstein and Jersey cows. A New Zealand research team believes that US dairy herds will have more “Q” alleles due to past intense selection for milk volume and protein (Spelman et al., 2002). This proved to be true with the Cal Poly Dairy Herd. Change has been occurring with more selection towards the “A” allele, especially within the Jersey breed.

The lectin-coated Dynabeads proved successful in extracting mammary epithelial cells from a milk sample, and the mRNA is believed to be obtained from these isolated cells due to the size of the RT-PCR fragment. The desired result of the enzyme digestion was to see a correlation between DGAT1 genotypes and milk fat levels, which would help determine the functionality of the DGAT1 gene. Since all banding patterns were consistent with the heterozygous DGAT1 genotype, no correlation to milk fat levels could be determined. Faint bands in the digested fragments suggest a low concentration of product, either due to low amounts of extracted mammary epithelial cells or low levels of mRNA available for reverse transcription. Continuing to develop this method of using

lectin-coated Dynabeads, a correlation between DGAT1 genotypes and milk fat levels may be determined by increasing the concentration of extracted mammary epithelial cells and ensuring limited mRNA degradation.

Since a visual comparison showed possible differences between isolated MFGM samples based on the DGAT1 genotype, computer analysis was attempted even though the Silver Stain method does not allow for proper densitometry comparisons. There was no difference between the samples for proteins at higher molecular weights, but intense bands and differences at the molecular weights typical for caseins and whey proteins were found. It is believed that there could still be small differences at the higher molecular weights proteins due to the connection between MFGM and lipids, so analysis of isolated MFGM proteins via two-dimensional gel electrophoresis could be an area of future research. Preliminary work has been done in this area, but a lack of AA variants to allow for adequately pooled samples, has made proper comparisons difficult. Variation was seen in the area of lipids and the amount of phospholipids present varies between genotype and within both the Holstein and Jersey breeds in the preliminary trial performed. This should be repeated to confirm these results. Expanding on the analysis of MFGM proteins based on DGAT1 genotype, the phospholipids present on the MFGM could also be analyzed to determine if there is a difference in concentration.

A correlation between DGAT1 genetic variants and citrate levels in the milk proved to have no statistical significance. This held true even when DIM and the lactation number were considered, though the small number of AA genetic variants in the total population could have an effect. Looking at a comparison based on season, a trend of decreasing citrate levels can be seen in the warmer season but it also was not

statistically significant due to large variations, probably due to sample size. With DGAT1 appearing to have no effect on citrate levels, other genes that correlate with fat content in the milk (such as κ -casein) could be examined due to the indirect role citrate plays in fat synthesis in the mammary cell (Braunschweig and Puhon, 1999). Another possible area of future research with regards to citrate levels involves varying the dietary intake to observe the influence of nutrition on citrate production and de novo fatty acid synthesis in times of stress.

The ability to obtain bovine genomic DNA that has survived processing gives a very powerful tool to verify the purity or identify the origin of a milk powder. Starting with β -lactoglobulin C to test the ability to determine origin through molecular tools was ideal to the unique nature of the variant's presence limited to the Oceanic region. Unfortunately there is not a restriction enzyme able to cut at the single mutation site to complete this molecular test. To genotype these samples for the β -lactoglobulin C variant, one possibility is through specialty designed probes with fluorescent tags to identify the presence or absence of the single base pair mutation unique to the C variant. The value of this tool makes it worthwhile to create these fluorescent tags and explore the possibility of tagging US milk powder with a unique DNA sequence.

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Appendix

APPENDIX A. DGAT1 Genotypes from the Cal Poly Dairy Herd in 2005

Number	Breed	Genotype
80	Jersey	AG
81	Jersey	AG
376	Jersey	AA
413	Jersey	AG
479	Jersey	AA
486	Jersey	AA
497	Jersey	AG
503	Jersey	GG
517	Jersey	GG
521	Jersey	AA
537	Jersey	AA
550	Jersey	AG
551	Jersey	GG
553	Jersey	AG
557	Jersey	AG
567	Jersey	AG
573	Jersey	AG
582	Jersey	AG
584	Jersey	GG
589	Jersey	AG
596	Jersey	AG
597	Jersey	AG
600	Jersey	GG
601	Jersey	AG
603	Jersey	AG
605	Jersey	AA
609	Jersey	GG
614	Jersey	AG
619	Jersey	GG
628	Jersey	GG
629	Jersey	GG
633	Jersey	AG
634	Jersey	AG
635	Jersey	AG
636	Jersey	GG
640	Jersey	AG
642	Jersey	GG
643	Jersey	AG
644	Jersey	GG
650	Jersey	AG
651	Jersey	GG
654	Jersey	GG
657	Jersey	AG
658	Jersey	GG
660	Jersey	AG
673	Jersey	GG
675	Jersey	AA
678	Jersey	AA

Number	Breed	Genotype
680	Jersey	AA
687	Jersey	AG
689	Jersey	AG
690	Jersey	AA
694	Jersey	AA
701	Jersey	AG
703	Jersey	AG
707	Jersey	AG
708	Jersey	AG
709	Jersey	AG
710	Jersey	AG
711	Jersey	AA
712	Jersey	GG
714	Jersey	GG
715	Jersey	GG
718	Jersey	GG
719	Jersey	AG
722	Jersey	GG
724	Jersey	AG
729	Jersey	AG
731	Jersey	GG
733	Jersey	AA
734	Jersey	GG
735	Jersey	AA
739	Jersey	AG
740	Jersey	AG
741	Jersey	GG
745	Jersey	GG
748	Jersey	GG
749	Jersey	AA
750	Jersey	AG
751	Jersey	GG
753	Jersey	AG
755	Jersey	GG
757	Jersey	AG
762	Jersey	AG
763	Jersey	GG
764	Jersey	GG
766	Jersey	AG
768	Jersey	GG
771	Jersey	AG
772	Jersey	AG
773	Jersey	AG
775	Jersey	AG
777	Jersey	GG
778	Jersey	AG
779	Jersey	AG
781	Jersey	GG

Number	Breed	Genotype
783	Jersey	GG
787	Jersey	AG
788	Jersey	AG
790	Jersey	AG
791	Jersey	GG
792	Jersey	AA
793	Jersey	AG
794	Jersey	AG
796	Jersey	AG
798	Jersey	AA
800	Jersey	AG
801	Jersey	AG
803	Jersey	AG
805	Jersey	GG
806	Jersey	AA
808	Jersey	GG
812	Jersey	GG
815	Jersey	AG
817	Jersey	AA
842	Jersey	GG
8937	Jersey	AA
9011	Jersey	GG
9013	Jersey	AA
9062	Jersey	AG
9077	Jersey	AG
9140	Jersey	AA
9300	Jersey	AA
1689	Holstein	GG
1740	Holstein	AG
1778	Holstein	GG
1783	Holstein	AG
1786	Holstein	GG
1795	Holstein	AG
1800	Holstein	AG
1804	Holstein	AG
1807	Holstein	GG
1808	Holstein	AG
1809	Holstein	GG
1812	Holstein	AG
1816	Holstein	AG
1823	Holstein	AG
1839	Holstein	GG
1843	Holstein	AG
1844	Holstein	AG
1846	Holstein	GG
1848	Holstein	GG
1851	Holstein	GG
1855	Holstein	AG

Number	Breed	Genotype
1856	Holstein	AG
1860	Holstein	GG
1861	Holstein	GG
1866	Holstein	GG
1879	Holstein	GG
1887	Holstein	GG
1892	Holstein	GG
1896	Holstein	AG
1900	Holstein	AG
1901	Holstein	GG
1904	Holstein	GG
1905	Holstein	GG
1906	Holstein	GG
1907	Holstein	GG
1908	Holstein	AA
1911	Holstein	AG
1913	Holstein	AA
1918	Holstein	GG
1921	Holstein	GG
1922	Holstein	AG
1926	Holstein	GG
1927	Holstein	GG
1928	Holstein	GG
1933	Holstein	GG
1936	Holstein	AG
1939	Holstein	AG
1940	Holstein	AG
1941	Holstein	GG
1943	Holstein	AA
1946	Holstein	GG
1947	Holstein	GG
1948	Holstein	AG
1950	Holstein	AG
1951	Holstein	AG
1953	Holstein	GG
1957	Holstein	AG
1958	Holstein	AG
1959	Holstein	AG
1961	Holstein	GG
1962	Holstein	AG
1963	Holstein	GG
1964	Holstein	AG
1968	Holstein	GG
1969	Holstein	AA
1970	Holstein	AA
1971	Holstein	AG
1972	Holstein	AG
1974	Holstein	GG

Number	Breed	Genotype
1975	Holstein	GG
1976	Holstein	GG
1977	Holstein	GG
1978	Holstein	GG
1979	Holstein	AG
1980	Holstein	GG
1981	Holstein	AG
1982	Holstein	GG
1983	Holstein	AG
1985	Holstein	GG
1986	Holstein	AG
1990	Holstein	GG
1992	Holstein	AG
1993	Holstein	GG
1994	Holstein	GG
1995	Holstein	GG
1997	Holstein	GG
1998	Holstein	GG
1999	Holstein	AG
2001	Holstein	AG
2004	Holstein	GG
2005	Holstein	AA
2007	Holstein	AG
2008	Holstein	GG
2009	Holstein	AG
2010	Holstein	GG
2011	Holstein	AG
2012	Holstein	GG
2013	Holstein	GG
2017	Holstein	AG
2018	Holstein	AG
2019	Holstein	GG
2020	Holstein	AG
2021	Holstein	GG
2024	Holstein	AG
2027	Holstein	GG
2029	Holstein	GG
72	Holstein	AG
73	Holstein	GG
74	Holstein	GG

APPENDIX B. DGAT1 Genotypes from the Cal Poly Dairy Herd in 2007

Number	Breed	Genotype
103	Jersey	AA
104	Jersey	AG
105	Jersey	AG
108	Jersey	AA
110	Jersey	AG
111	Jersey	AG
113	Jersey	AG
116	Jersey	AG
118	Jersey	AG
119	Jersey	AG
121	Jersey	AG
122	Jersey	AG
128	Jersey	AG
130	Jersey	AG
135	Jersey	AA
136	Jersey	AG
139	Jersey	AA
141	Jersey	AA
145	Jersey	AG
147	Jersey	GG
148	Jersey	AA
149	Jersey	AA
150	Jersey	AG
152	Jersey	AG
154	Jersey	AG
155	Jersey	AG
156	Jersey	AA
157	Jersey	AA
158	Jersey	AG
159	Jersey	AG
160	Jersey	AG
161	Jersey	AA
171	Jersey	AG
174	Jersey	AA
533	Jersey	GG
551	Jersey	GG
600	Jersey	GG
605	Jersey	AA
636	Jersey	GG
638	Jersey	GG
643	Jersey	AG
654	Jersey	GG
657	Jersey	AG
675	Jersey	AG
703	Jersey	GG
708	Jersey	AG
711	Jersey	AA
724	Jersey	AG

Number	Breed	Genotype
729	Jersey	AG
733	Jersey	AA
735	Jersey	AA
741	Jersey	GG
748	Jersey	AA
749	Jersey	GG
751	Jersey	GG
753	Jersey	AG
772	Jersey	AG
775	Jersey	AG
781	Jersey	AA
783	Jersey	GG
790	Jersey	AG
792	Jersey	GG
794	Jersey	AG
802	Jersey	AA
803	Jersey	AG
807	Jersey	AG
812	Jersey	GG
815	Jersey	AG
817	Jersey	AA
818	Jersey	GG
833	Jersey	AG
835	Jersey	GG
836	Jersey	AA
850	Jersey	GG
857	Jersey	AG
861	Jersey	GG
865	Jersey	AG
867	Jersey	GG
870	Jersey	GG
871	Jersey	GG
872	Jersey	AA
873	Jersey	AG
876	Jersey	AG
878	Jersey	AA
879	Jersey	AA
880	Jersey	AG
882	Jersey	GG
884	Jersey	AA
886	Jersey	GG
889	Jersey	AA
890	Jersey	GG
891	Jersey	GG
896	Jersey	AG
897	Jersey	GG
902	Jersey	AG
903	Jersey	GG

Number	Breed	Genotype
907	Jersey	AG
908	Jersey	AA
910	Jersey	AG
916	Jersey	AA
918	Jersey	AG
926	Jersey	GG
931	Jersey	AG
932	Jersey	AG
934	Jersey	AA
935	Jersey	GG
936	Jersey	AG
937	Jersey	AG
939	Jersey	AG
940	Jersey	GG
1800	Holstein	AG
1812	Holstein	AG
1823	Holstein	AG
1848	Holstein	GG
1906	Holstein	GG
1908	Holstein	AA
1922	Holstein	AG
1926	Holstein	GG
1928	Holstein	GG
1940	Holstein	AG
1943	Holstein	AA
1945	Holstein	AG
1969	Holstein	AA
1979	Holstein	AG
1980	Holstein	AG
1982	Holstein	GG
1983	Holstein	GG
1985	Holstein	GG
2007	Holstein	AG
2009	Holstein	AG
2010	Holstein	AG
2023	Holstein	AG
2032	Holstein	AG
2033	Holstein	AG
2036	Holstein	AG
2040	Holstein	AG
2044	Holstein	AG
2052	Holstein	AA
2054	Holstein	GG
2056	Holstein	AG
2060	Holstein	GG
2062	Holstein	AG
2066	Holstein	GG
2068	Holstein	AG

Number	Breed	Genotype
2070	Holstein	GG
2072	Holstein	GG
2076	Holstein	GG
2077	Holstein	GG
2078	Holstein	GG
2081	Holstein	GG
2082	Holstein	GG
2083	Holstein	GG
2086	Holstein	AG
2090	Holstein	AA
2091	Holstein	GG
2093	Holstein	AA
2094	Holstein	AG
2095	Holstein	AG
2099	Holstein	AG
2102	Holstein	AG
2103	Holstein	GG
2104	Holstein	GG
2106	Holstein	GG
2108	Holstein	AG
2109	Holstein	GG
2115	Holstein	GG
2116	Holstein	AG
2119	Holstein	GG
2121	Holstein	AA
2122	Holstein	AG
2123	Holstein	AG
2127	Holstein	GG
2130	Holstein	GG
2138	Holstein	GG
2139	Holstein	GG
2201	Holstein	GG
2202	Holstein	GG
2203	Holstein	GG
2207	Holstein	AG
2212	Holstein	AG
2213	Holstein	GG
2214	Holstein	AG
2216	Holstein	GG
2220	Holstein	AA
2225	Holstein	GG
2226	Holstein	GG
2228	Holstein	GG
2229	Holstein	AG
2230	Holstein	GG
2232	Holstein	GG
2233	Holstein	GG
2237	Holstein	GG
2240	Holstein	GG

APPENDIX C. DGAT1 Genotypes from the Cal Poly Dairy Herd in 2010

Number	Breed	Genotype
105	Jersey	AG
106	Jersey	AG
108	Jersey	AA
110	Jersey	AG
111	Jersey	GG
118	Jersey	AG
128	Jersey	AG
130	Jersey	AG
135	Jersey	AA
139	Jersey	AA
145	Jersey	AG
154	Jersey	AG
155	Jersey	AG
158	Jersey	AG
166	Jersey	AG
167	Jersey	GG
168	Jersey	GG
170	Jersey	AA
172	Jersey	AG
174	Jersey	AA
179	Jersey	AG
187	Jersey	GG
189	Jersey	AG
190	Jersey	AA
192	Jersey	AA
193	Jersey	AA
203	Jersey	AG
205	Jersey	AA
209	Jersey	AG
210	Jersey	AA
212	Jersey	AG
213	Jersey	AG
221	Jersey	AG
227	Jersey	GG
228	Jersey	AG
229	Jersey	AA
232	Jersey	AG
234	Jersey	AA
237	Jersey	AA
238	Jersey	AG
240	Jersey	GG
241	Jersey	AG
243	Jersey	GG
246	Jersey	AG
248	Jersey	GG
250	Jersey	AG
251	Jersey	AG
253	Jersey	AA

Number	Breed	Genotype
255	Jersey	GG
256	Jersey	AA
258	Jersey	GG
259	Jersey	GG
265	Jersey	AA
266	Jersey	AG
267	Jersey	AG
268	Jersey	GG
271	Jersey	GG
273	Jersey	AA
275	Jersey	AG
276	Jersey	AG
277	Jersey	AA
282	Jersey	AA
284	Jersey	AG
285	Jersey	AG
286	Jersey	AG
287	Jersey	AG
288	Jersey	AA
292	Jersey	GG
293	Jersey	AG
294	Jersey	AG
303	Jersey	AG
636	Jersey	GG
643	Jersey	AG
703	Jersey	AG
714	Jersey	GG
803	Jersey	AG
807	Jersey	AG
814	Jersey	AG
844	Jersey	AG
852	Jersey	AG
870	Jersey	GG
876	Jersey	AG
880	Jersey	AG
886	Jersey	GG
890	Jersey	GG
891	Jersey	GG
897	Jersey	GG
903	Jersey	GG
923	Jersey	AG
939	Jersey	AG
1833	Holstein	GG
1864	Holstein	GG
1983	Holstein	AG
1985	Holstein	GG
2033	Holstein	AG
2054	Holstein	GG

Number	Breed	Genotype
2060	Holstein	GG
2066	Holstein	GG
2068	Holstein	AG
2070	Holstein	GG
2078	Holstein	GG
2082	Holstein	GG
2083	Holstein	GG
2116	Holstein	AG
2119	Holstein	GG
2123	Holstein	AG
2129	Holstein	GG
2136	Holstein	GG
2201	Holstein	GG
2206	Holstein	GG
2212	Holstein	AG
2214	Holstein	AG
2217	Holstein	GG
2220	Holstein	AG
2232	Holstein	GG
2237	Holstein	GG
2242	Holstein	AG
2245	Holstein	GG
2247	Holstein	AG
2248	Holstein	GG
2252	Holstein	GG
2253	Holstein	AG
2257	Holstein	GG
2259	Holstein	GG
2260	Holstein	GG
2264	Holstein	GG
2265	Holstein	AG
2266	Holstein	GG
2268	Holstein	GG
2273	Holstein	AA
2275	Holstein	AG
2278	Holstein	GG
2281	Holstein	GG
2284	Holstein	AG
2288	Holstein	GG
2289	Holstein	GG
2290	Holstein	AG
2296	Holstein	GG
2300	Holstein	GG
2302	Holstein	GG
2303	Holstein	AG
2305	Holstein	GG
2307	Holstein	GG
2308	Holstein	GG

Number	Breed	Genotype
2309	Holstein	GG
2310	Holstein	GG
2317	Holstein	GG
2318	Holstein	AA
2324	Holstein	GG
2328	Holstein	GG
2329	Holstein	AG
2331	Holstein	GG
2335	Holstein	GG
2337	Holstein	GG
2338	Holstein	GG
2339	Holstein	AG
2340	Holstein	GG
2341	Holstein	GG
2343	Holstein	GG
2346	Holstein	GG
2347	Holstein	AG
2348	Holstein	GG
2349	Holstein	AG
2352	Holstein	GG
2354	Holstein	GG
2356	Holstein	AA
2361	Holstein	AG
2362	Holstein	AG
2363	Holstein	AG
2364	Holstein	GG
2365	Holstein	AG
2366	Holstein	GG
2367	Holstein	GG
2369	Holstein	GG
2372	Holstein	GG
2768	Holstein	GG
2938	Holstein	AA
3021	Holstein	GG
3045	Holstein	GG
6860	Holstein	GG
15707	Holstein	GG
20194	Holstein	GG

APPENDIX D. 305-day average milk fat percent.

No.	Breed	Genotype	Fat %
80	J	AG	4.9
81	J	AG	3.9
376	J	AA	4.7
413	J	AG	4.6
479	J	AA	4.6
486	J	AA	4.3
497	J	AG	4.4
503	J	GG	3.7
517	J	GG	3.8
521	J	AA	4.9
537	J	AA	4.4
550	J	AG	4.5
551	J	GG	4.6
553	J	AG	4.5
557	J	AG	4.4
567	J	AG	4.9
573	J	AG	4.1
582	J	AG	4.4
589	J	AG	3.9
596	J	AG	4.5
600	J	GG	4.5
601	J	AG	4.4
603	J	AG	3.9
605	J	AA	4.2
609	J	GG	4.4
614	J	AG	4.3
619	J	GG	4
628	J	GG	4.2
629	J	GG	4.5
633	J	AG	4.2
634	J	AG	4.4
635	J	AG	4.3
636	J	GG	3.7
640	J	AG	3.5
642	J	GG	3.3
643	J	AG	4.4
644	J	GG	4.9
650	J	AG	5.1
651	J	GG	3.3
654	J	GG	3.8
657	J	AG	4.3
658	J	GG	3.7
660	J	AG	3.8
673	J	GG	4.1
675	J	AA	4.9

No.	Breed	Genotype	Fat %
678	J	AA	4.1
680	J	AA	4.2
687	J	AG	3.1
689	J	AG	3.9
690	J	AA	4.3
694	J	AA	4.6
701	J	AG	4.6
703	J	AG	4.2
707	J	AG	4.3
709	J	AG	4.5
710	J	AG	4.7
711	J	AA	4.2
712	J	GG	4.3
714	J	GG	4
715	J	GG	4.9
718	J	GG	4.1
719	J	AG	4.8
722	J	GG	4.6
724	J	AG	4.3
729	J	AG	4.3
731	J	GG	4
733	J	AA	4.1
734	J	GG	4.1
735	J	AA	4.8
739	J	AG	4.1
740	J	AG	4.6
741	J	GG	4.7
745	J	GG	3.6
748	J	GG	4.2
749	J	AA	5
750	J	AG	4.6
751	J	GG	4.4
753	J	AG	4.2
755	J	GG	4.1
757	J	AG	4.3
762	J	AG	3.7
763	J	GG	3.9
764	J	GG	4.4
766	J	AG	3.8
768	J	GG	4.6
771	J	AG	4.6
772	J	AG	4.6
773	J	AG	4.1
775	J	AG	4.7
777	J	GG	4.4

No.	Breed	Genotype	Fat %
778	J	AG	4.5
779	J	AG	4.7
781	J	GG	4.5
783	J	GG	4.8
787	J	AG	5.6
788	J	AG	5.2
790	J	AG	4.9
791	J	GG	4
792	J	AA	5.3
793	J	AG	5
794	J	AG	4.4
796	J	AG	4.8
798	J	AA	5.2
800	J	AG	4.8
801	J	AG	5
803	J	AG	4.9
808	J	GG	4.3
812	J	GG	5.3
815	J	AG	5.8
817	J	AA	4.9
842	J	GG	4.4
8937	J	AA	4.4
9011	J	GG	4.8
9013	J	AA	4.3
9062	J	AG	4.1
9077	J	AG	4
9140	J	AA	4.6
9300	J	AA	3.6
1689	H	GG	3.6
1740	H	AG	3.7
1778	H	GG	3
1783	H	AG	3.5
1786	H	GG	3
1795	H	AG	3.7
1800	H	AG	3
1804	H	AG	3.6
1807	H	GG	3.5
1808	H	AG	3.8
1809	H	GG	3
1812	H	AG	3.3
1816	H	AG	3.2
1823	H	AG	3.6
1839	H	GG	3.4
1843	H	AG	3.7
1844	H	AG	3.4

No.	Breed	Genotype	Fat %
1848	H	GG	3.2
1851	H	GG	3.4
1855	H	AG	3.8
1856	H	AG	4
1860	H	GG	3
1861	H	GG	3.3
1866	H	GG	4
1879	H	GG	3.4
1887	H	GG	3.7
1896	H	AG	3.8
1900	H	AG	4.3
1901	H	GG	3.7
1904	H	GG	3.5
1905	H	GG	3.6
1906	H	GG	3.6
1907	H	GG	3.6
1908	H	AA	3.7
1911	H	AG	3.4
1913	H	AA	3.6
1918	H	GG	3.3
1921	H	GG	3.5
1922	H	AG	3.6
1926	H	GG	3.5
1927	H	GG	3.3
1928	H	GG	3.4
1933	H	GG	4.4
1936	H	AG	3.4
1939	H	AG	3.8
1940	H	AG	3.8
1941	H	GG	3.4
1943	H	AA	5.4
1946	H	GG	3.2
1947	H	GG	3.2
1948	H	AG	3.7
1950	H	AG	4.1
1951	H	AG	3.7
1953	H	GG	3.8
1957	H	AG	3.9
1958	H	AG	3.7
1959	H	AG	3.5
1961	H	GG	3.6
1962	H	AG	3.8
1963	H	GG	3.2
1964	H	AG	3.5
1968	H	GG	3.8

No.	Breed	Genotype	Fat %
1846	H	GG	3.5
1970	H	AA	4.4
1971	H	AG	3.4
1972	H	AG	4.1
1974	H	GG	3.9
1975	H	GG	3.4
1976	H	GG	3.6
1977	H	GG	3.5
1978	H	GG	3.5
1979	H	AG	3.6
1980	H	GG	3.6
1981	H	AG	4
1982	H	GG	3.9
1983	H	AG	3.8
1985	H	GG	3.3
1986	H	AG	4
1990	H	GG	3.3
1992	H	AG	4
1993	H	GG	3.8
1994	H	GG	3.7
1995	H	GG	3.8
1997	H	GG	3.8
1998	H	GG	3.6
1999	H	AG	4.2
2001	H	AG	4.2
2004	H	GG	3.7
2005	H	AA	3.8
2007	H	AG	4.6
2009	H	AG	5
2010	H	GG	3.7
2011	H	AG	3.9
2012	H	GG	3.8
2013	H	GG	4
2017	H	AG	4.2
2019	H	GG	3.7
2020	H	AG	4.4
2021	H	GG	4
2027	H	GG	3.7
2029	H	GG	4.3
72	H	AG	3.6
73	H	GG	3.5
74	H	GG	3.2
103	J	AA	6.5
104	J	AG	5.2
105	J	AG	4.9

No.	Breed	Genotype	Fat %
1969	H	AA	3.4
119	J	AG	6.1
121	J	AG	5.6
122	J	AG	5.1
136	J	AG	4.2
141	J	AA	5.5
147	J	GG	4.1
148	J	AA	5.9
149	J	AA	5.6
157	J	AA	5.4
159	J	AG	4.3
160	J	AG	4.6
161	J	AA	5.4
171	J	AG	5.2
638	J	GG	3.9
802	J	AA	4.2
818	J	GG	4.8
833	J	AG	5
835	J	GG	4.6
836	J	AA	4.9
857	J	AG	5.1
861	J	GG	4.7
865	J	AG	4.6
867	J	GG	4.8
871	J	GG	4.7
872	J	AA	5
873	J	AG	4.8
878	J	AA	5.4
879	J	AA	5.8
882	J	GG	4.1
884	J	AA	4.7
889	J	AA	5.4
896	J	AG	4.6
902	J	AG	4.7
907	J	AG	5.2
908	J	AA	5.5
910	J	AG	4.8
916	J	AA	5.4
918	J	AG	4.8
926	J	GG	4.6
931	J	AG	5.4
932	J	AG	4.9
934	J	AA	5.4
935	J	GG	4.6
936	J	AG	5.6

No.	Breed	Genotype	Fat %
113	J	AG	4.8
116	J	AG	5.1
1945	H	AG	3.7
2023	H	AG	3.9
2032	H	AG	3.9
2036	H	AG	4.3
2040	H	AG	4.1
2044	H	AG	3.9
2052	H	AA	4.5
2054	H	GG	3.5
2056	H	AG	3.9
2062	H	AG	3.2
2072	H	GG	3.4
2076	H	GG	3.7
2077	H	GG	3.7
2081	H	GG	3.8
2086	H	AG	3.5
2090	H	AA	4.8
2091	H	GG	3.5
2093	H	AA	3.7
2094	H	AG	4
2095	H	AG	4.2
2099	H	AG	4.2
2102	H	AG	4.5
2103	H	GG	4
2104	H	GG	3.7
2106	H	GG	3.9
2108	H	AG	4
2109	H	GG	4.1
2115	H	GG	4.6
2121	H	AA	4.3
2122	H	AG	3.7
2127	H	GG	4.2
2130	H	GG	3.7
2138	H	GG	4.1
2139	H	GG	4.4
2202	H	GG	4.7
2203	H	GG	3.7
2207	H	AG	4.7
2213	H	GG	4
2216	H	GG	3.8
2225	H	GG	3.3
2226	H	GG	4
2228	H	GG	3.6
2229	H	AG	4

No.	Breed	Genotype	Fat %
937	J	AG	4.5
940	J	GG	5
110	J	AG	5.3
111	J	GG	4.6
118	J	AG	5.1
128	J	AG	4.9
130	J	AG	5.5
135	J	AA	4.9
139	J	AA	4.6
145	J	AG	4.9
154	J	AG	5.1
155	J	AG	5.1
158	J	AG	5
166	J	AG	4.5
167	J	GG	3.8
170	J	AA	4.8
172	J	AG	4.3
174	J	AA	4.7
179	J	AG	5
187	J	GG	4
189	J	AG	4.3
190	J	AA	4.9
192	J	AA	4.7
193	J	AA	5
203	J	AG	4.7
205	J	AA	4.2
209	J	AG	4.8
210	J	AA	5
212	J	AG	4.9
213	J	AG	4.3
221	J	AG	4.6
227	J	GG	4.4
228	J	AG	5.2
229	J	AA	5.1
232	J	AG	4.9
234	J	AA	4.8
237	J	AA	4.9
238	J	AG	5.2
240	J	GG	4.2
241	J	AG	4.7
243	J	GG	4.7
246	J	AG	3.4
248	J	GG	4.3
250	J	AG	4.5
251	J	AG	3.9

No.	Breed	Genotype	Fat %
105	J	AG	4.9
106	J	AG	5.2
108	J	AA	5.9
259	J	GG	4.4
265	J	AA	3.9
266	J	AG	4.8
267	J	AG	4.7
268	J	GG	4.4
271	J	GG	4.1
273	J	AA	4.3
275	J	AG	4.3
276	J	AG	4.8
277	J	AA	4.3
282	J	AA	5.3
284	J	AG	4.7
285	J	AG	3.5
286	J	AG	4.7
287	J	AG	4.1
288	J	AA	4.5
292	J	GG	4.5
294	J	AG	4.7
807	J	AG	4.9
814	J	AG	5.2
844	J	AG	4.6
852	J	AG	4.8
870	J	GG	4.4
876	J	AG	5
880	J	AG	4.6
886	J	GG	4.3
890	J	GG	4.3
891	J	GG	4.3
897	J	GG	4
903	J	AG	4.2
923	J	AG	4.9
939	J	AG	5.1
1833	H	GG	3
1864	H	GG	2.7
2033	H	AG	3.7
2054	H	GG	3.5
2060	H	GG	3.7
2066	H	GG	4.3
2068	H	AG	4.2
2070	H	GG	3.3
2078	H	GG	3.6
2082	H	GG	3.8

No.	Breed	Genotype	Fat %
253	J	AA	5.4
255	J	GG	4.1
258	J	GG	4.1
2129	H	GG	2.9
2136	H	GG	4.1
2201	H	GG	3.9
2206	H	GG	3
2212	H	AG	4.1
2214	H	AG	4.3
2217	H	GG	3.7
2220	H	AG	3.7
2232	H	GG	4
2237	H	GG	4
2242	H	AG	3.6
2245	H	GG	3.6
2247	H	AG	4
2248	H	GG	3.2
2252	H	GG	3.6
2253	H	AG	3.7
2257	H	GG	3.5
2259	H	GG	2.9
2260	H	GG	3.2
2264	H	GG	3.7
2265	H	AG	3.8
2266	H	GG	3.4
2268	H	GG	3.1
2273	H	AA	4.2
2275	H	AG	3.3
2278	H	GG	3.4
2281	H	GG	3.4
2284	H	AG	3.7
2288	H	GG	3.4
2290	H	AG	3.8
2296	H	GG	3.5
2300	H	GG	3.3
2302	H	GG	3.8
2303	H	AG	4
2305	H	GG	3.9
2307	H	GG	3.7
2308	H	GG	3.6
2309	H	GG	3.3
2310	H	GG	3.7
2317	H	GG	3.7
2318	H	AA	4.9
2324	H	GG	3.4

No.	Breed	Genotype	Fat %
2083	H	GG	3.4
2116	H	AG	4.5
2119	H	GG	3.8
2123	H	AG	4.5
2337	H	GG	3.9
2338	H	GG	4.1
2339	H	AG	4.4
2340	H	GG	3.6
2341	H	GG	3.6
2343	H	GG	3.8
2346	H	GG	3.4
2347	H	AG	3.9
2348	H	GG	4.3
2352	H	GG	3.7
2354	H	GG	3.7
2356	H	AA	4.5
2361	H	AG	4.1
2362	H	AG	4.3
2363	H	AG	4.4
2364	H	GG	3.9
2365	H	AG	4.7
2366	H	GG	3.5
2369	H	GG	3.5
2372	H	GG	4.1
3021	H	GG	4.1
3045	H	GG	3.2
6860	H	GG	3.6
15707	H	GG	4.5
20194	H	GG	3.5
2328	H	GG	3.5
2329	H	AG	4.1
2331	H	GG	3.3
2335	H	GG	3.4

APPENDIX E. Statistical results of 305-day average milk fat percent based on the DGAT1 genetic variants using Minitab software version 16.

Descriptive Statistics: Fat %

Results for Genotype = AA

Variable	Breed	N	N*	Mean	SE Mean	StDev	Minimum	Q1	Median
Fat %	H	13	0	4.246	0.163	0.588	3.400	3.700	4.300
	J	57	0	4.8561	0.0754	0.5692	3.6000	4.3500	4.9000

Variable	Breed	Q3	Maximum
Fat %	H	4.650	5.400
	J	5.3500	6.5000

Results for Genotype = AG

Variable	Breed	N	N*	Mean	SE Mean	StDev	Minimum	Q1	Median
Fat %	H	82	0	3.9000	0.0418	0.3781	3.0000	3.7000	3.9000
	J	126	0	4.6365	0.0440	0.4941	3.1000	4.3000	4.7000

Variable	Breed	Q3	Maximum
Fat %	H	4.1250	5.0000
	J	4.9000	6.1000

Results for Genotype = GG

Variable	Breed	N	N*	Mean	SE Mean	StDev	Minimum	Q1	Median
Fat %	H	138	0	3.6196	0.0303	0.3555	2.7000	3.4000	3.6000
	J	67	0	4.2940	0.0483	0.3950	3.3000	4.0000	4.3000

Variable	Breed	Q3	Maximum
Fat %	H	3.8000	4.7000
	J	4.6000	5.3000

General Linear Model: Fat % versus Breed, Genotype

Factor	Type	Levels	Values
Breed	fixed	2	H, J
Genotype	fixed	3	AA, AG, GG

Analysis of Variance for Fat %, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Breed	1	85.416	29.862	29.862	154.83	0.000
Genotype	2	17.383	16.251	8.126	42.13	0.000
Breed*Genotype	2	0.181	0.181	0.090	0.47	0.626
Error	477	92.000	92.000	0.193		
Total	482	194.980				

S = 0.439171 R-Sq = 52.82% R-Sq(adj) = 52.32%

Unusual Observations for Fat %

Obs	Fat %	Fit	SE Fit	Residual	St Resid
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34	3.50000	4.63651	0.03912	-1.13651	-2.60	R
35	3.30000	4.29403	0.05365	-0.99403	-2.28	R
39	3.30000	4.29403	0.05365	-0.99403	-2.28	R
48	3.10000	4.63651	0.03912	-1.53651	-3.51	R
81	3.70000	4.63651	0.03912	-0.93651	-2.14	R
95	5.60000	4.63651	0.03912	0.96349	2.20	R
108	5.30000	4.29403	0.05365	1.00597	2.31	R
109	5.80000	4.63651	0.03912	1.16349	2.66	R
118	3.60000	4.85614	0.05817	-1.25614	-2.89	R
125	3.00000	3.90000	0.04850	-0.90000	-2.06	R
153	3.70000	4.24615	0.12180	-0.54615	-1.29	X
155	3.60000	4.24615	0.12180	-0.64615	-1.53	X
167	5.40000	4.24615	0.12180	1.15385	2.73	RX
182	3.40000	4.24615	0.12180	-0.84615	-2.01	RX
183	4.40000	4.24615	0.12180	0.15385	0.36	X
208	3.80000	4.24615	0.12180	-0.44615	-1.06	X
210	5.00000	3.90000	0.04850	1.10000	2.52	R
224	6.50000	4.85614	0.05817	1.64386	3.78	R
229	6.10000	4.63651	0.03912	1.46349	3.35	R
230	5.60000	4.63651	0.03912	0.96349	2.20	R
235	5.90000	4.85614	0.05817	1.04386	2.40	R
256	5.80000	4.85614	0.05817	0.94386	2.17	R
272	5.60000	4.63651	0.03912	0.96349	2.20	R
281	4.50000	4.24615	0.12180	0.25385	0.60	X
290	4.80000	4.24615	0.12180	0.55385	1.31	X
292	3.70000	4.24615	0.12180	-0.54615	-1.29	X
302	4.60000	3.61957	0.03738	0.98043	2.24	R
303	4.30000	4.24615	0.12180	0.05385	0.13	X
309	4.70000	3.61957	0.03738	1.08043	2.47	R
320	5.90000	4.85614	0.05817	1.04386	2.40	R
360	3.40000	4.63651	0.03912	-1.23651	-2.83	R
368	3.90000	4.85614	0.05817	-0.95614	-2.20	R
379	3.50000	4.63651	0.03912	-1.13651	-2.60	R
400	2.70000	3.61957	0.03738	-0.91957	-2.10	R
436	4.20000	4.24615	0.12180	-0.04615	-0.11	X
453	4.90000	4.24615	0.12180	0.65385	1.55	X
470	4.50000	4.24615	0.12180	0.25385	0.60	X
482	4.50000	3.61957	0.03738	0.88043	2.01	R

R denotes an observation with a large standardized residual.

X denotes an observation whose X value gives it large leverage.

General Linear Model: Fat % versus Breed, Genotype

Factor	Type	Levels	Values
Breed	fixed	2	H, J
Genotype	fixed	3	AA, AG, GG

Analysis of Variance for Fat %, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Breed	1	85.416	51.220	51.220	266.16	0.000
Genotype	2	17.383	17.383	8.692	45.16	0.000
Error	479	92.180	92.180	0.192		
Total	482	194.980				

S = 0.438683 R-Sq = 52.72% R-Sq(adj) = 52.43%

Unusual Observations for Fat %

Obs	Fat %	Fit	SE Fit	Residual	St Resid
34	3.50000	4.62103	0.03477	-1.12103	-2.56 R
35	3.30000	4.30936	0.04203	-1.00936	-2.31 R
39	3.30000	4.30936	0.04203	-1.00936	-2.31 R
48	3.10000	4.62103	0.03477	-1.52103	-3.48 R
81	3.70000	4.62103	0.03477	-0.92103	-2.11 R
95	5.60000	4.62103	0.03477	0.97897	2.24 R
108	5.30000	4.30936	0.04203	0.99064	2.27 R
109	5.80000	4.62103	0.03477	1.17897	2.70 R
118	3.60000	4.87234	0.05303	-1.27234	-2.92 R
125	3.00000	3.92379	0.03994	-0.92379	-2.11 R
167	5.40000	4.17511	0.06293	1.22489	2.82 R
210	5.00000	3.92379	0.03994	1.07621	2.46 R
224	6.50000	4.87234	0.05303	1.62766	3.74 R
229	6.10000	4.62103	0.03477	1.47897	3.38 R
230	5.60000	4.62103	0.03477	0.97897	2.24 R
235	5.90000	4.87234	0.05303	1.02766	2.36 R
256	5.80000	4.87234	0.05303	0.92766	2.13 R
272	5.60000	4.62103	0.03477	0.97897	2.24 R
302	4.60000	3.61212	0.03367	0.98788	2.26 R
309	4.70000	3.61212	0.03367	1.08788	2.49 R
320	5.90000	4.87234	0.05303	1.02766	2.36 R
325	5.50000	4.62103	0.03477	0.87897	2.01 R
360	3.40000	4.62103	0.03477	-1.22103	-2.79 R
368	3.90000	4.87234	0.05303	-0.97234	-2.23 R
379	3.50000	4.62103	0.03477	-1.12103	-2.56 R
400	2.70000	3.61212	0.03367	-0.91212	-2.09 R
482	4.50000	3.61212	0.03367	0.88788	2.03 R

R denotes an observation with a large standardized residual.

Correlations: Fat %, Breed_H

Pearson correlation of Fat % and Breed_H = 0.662
P-Value = 0.000

Correlations: Fat %, G No.

Pearson correlation of Fat % and G No. = 0.028
P-Value = 0.538

Regression Analysis: Fat % versus Genotype_GG, Genotype_AG, Breed_H

The regression equation is

Fat % = 4.87 - 0.563 Genotype_GG - 0.251 Genotype_AG - 0.697 Breed_H

Predictor	Coef	SE Coef	T	P
Constant	4.87234	0.05303	91.88	0.000
Genotype_GG	-0.56298	0.06420	-8.77	0.000
Genotype_AG	-0.25132	0.06127	-4.10	0.000
Breed_H	-0.69724	0.04274	-16.31	0.000

S = 0.438683 R-Sq = 52.7% R-Sq(adj) = 52.4%

Analysis of Variance

Source	DF	SS	MS	F	P
Regression	3	102.800	34.267	178.06	0.000
Residual Error	479	92.180	0.192		
Total	482	194.980			

Source	DF	Seq SS
Genotype_GG	1	43.337
Genotype_AG	1	8.242
Breed_H	1	51.220

Unusual Observations

Obs	Genotype_GG	Fat %	Fit	SE Fit	Residual	St Resid
34	0.00	3.5000	4.6210	0.0348	-1.1210	-2.56R
35	1.00	3.3000	4.3094	0.0420	-1.0094	-2.31R
39	1.00	3.3000	4.3094	0.0420	-1.0094	-2.31R
48	0.00	3.1000	4.6210	0.0348	-1.5210	-3.48R
81	0.00	3.7000	4.6210	0.0348	-0.9210	-2.11R
95	0.00	5.6000	4.6210	0.0348	0.9790	2.24R
108	1.00	5.3000	4.3094	0.0420	0.9906	2.27R
109	0.00	5.8000	4.6210	0.0348	1.1790	2.70R
118	0.00	3.6000	4.8723	0.0530	-1.2723	-2.92R
125	0.00	3.0000	3.9238	0.0399	-0.9238	-2.11R
167	0.00	5.4000	4.1751	0.0629	1.2249	2.82R
210	0.00	5.0000	3.9238	0.0399	1.0762	2.46R
224	0.00	6.5000	4.8723	0.0530	1.6277	3.74R
229	0.00	6.1000	4.6210	0.0348	1.4790	3.38R
230	0.00	5.6000	4.6210	0.0348	0.9790	2.24R
235	0.00	5.9000	4.8723	0.0530	1.0277	2.36R
256	0.00	5.8000	4.8723	0.0530	0.9277	2.13R
272	0.00	5.6000	4.6210	0.0348	0.9790	2.24R
302	1.00	4.6000	3.6121	0.0337	0.9879	2.26R
309	1.00	4.7000	3.6121	0.0337	1.0879	2.49R
320	0.00	5.9000	4.8723	0.0530	1.0277	2.36R
325	0.00	5.5000	4.6210	0.0348	0.8790	2.01R
360	0.00	3.4000	4.6210	0.0348	-1.2210	-2.79R
368	0.00	3.9000	4.8723	0.0530	-0.9723	-2.23R
379	0.00	3.5000	4.6210	0.0348	-1.1210	-2.56R
400	1.00	2.7000	3.6121	0.0337	-0.9121	-2.09R
482	1.00	4.5000	3.6121	0.0337	0.8879	2.03R

R denotes an observation with a large standardized residual.

Main Effects Plot for Fat %

Interaction Plot for Fat %

One-way ANOVA: Fat % versus Breed

Source	DF	SS	MS	F	P
Breed	1	85.416	85.416	374.99	0.000
Error	481	109.563	0.228		
Total	482	194.980			

S = 0.4773 R-Sq = 43.81% R-Sq(adj) = 43.69%

				Individual 95% CIs For Mean Based on Pooled StDev			
Level	N	Mean	StDev	-----+-----+-----+-----			
H	233	3.7532	0.4175	(-*--)			
J	250	4.5948	0.5269	(---*-)			
				-----+-----+-----+-----			
				3.75	4.00	4.25	4.50

Pooled StDev = 0.4773

APPENDIX F. Data printout from Cal Poly Dairy's DairyComp software in October 2010

Cow	Breed	Dsf	Lactation	Age (mo)	Last % Fat	305 Milk
105	J	357	5	63.2	4.2	28610
110	J	79	4	62.3	4.6	13970
128	J	246	3	58	2.5	18170
135	J	234	3	57.1	5.5	20580
139	J	582	2	56.7	5.8	20500
145	J	197	3	56.3	4.3	20470
158	J	196	3	54.5	4.5	19290
164	J	102	3	53.6	3.8	17540
167	J	287	2	53.2	3.9	24150
170	J	353	2	52.8	7.5	22900
172	J	87	3	52.4	3.9	22870
174	J	70	3	52.1	4.8	20680
179	J	354	2	50.9	4.9	25120
183	J	116	3	50.2	4	25020
187	J	7	3	49.6		
190	J	267	2	49.3	5.1	20890
192	J	382	2	48.8	5.9	27570
193	J	33	3	48.6	4.6	20320
201	J	31	2	45.9	4.7	16560
209	J	270	2	44.5	3.8	21120
212	J	230	2	44.2	4.8	21110
213	J	331	2	44.1	4.2	25050
214	J	135	2	44.1	4.3	16920
221	J	356	1	42.8	7.5	20870
225	J	134	2	42.1	4.9	20060
227	J	60	2	42	3.7	16830
231	J	57	2	41.2	3.4	20010
232	J	40	2	41.2	4.2	18650
234	J	199	2	41.1	5.3	23180
236	J	85	2	40.3	4.4	16970
237	J	73	2	40.3	4.7	19950
238	J	378	1	40.3	5	25000
241	J	52	2	39.8	4.9	19930
243	J	99	2	39.1	4.4	20420
246	J	72	2	38.7	5.8	16010
247	J	37	2	38.6	5.3	21050
248	J	86	2	38.3	4.3	18970
250	J	397	1	38.1	5.2	20360
251	J	189	2	37.9	4.3	17440
252	J	91	2	37.8	3.9	18940
253	J	351	1	37.8	5.9	22550
255	J	402	1	37.6	4.1	26190
256	J	10	2	37.5		

Cow	Breed	Dsf	Lactation	Age (mo)	Last % Fat	305 Milk
258	J	353	1	37.1	5	21140
266	J	22	2	35.8		
268	J	352	1	35.7	3.9	21820
271	J	293	1	35.5	5.1	24970
273	J	394	1	35.1	6.4	26700
275	J	10	2	34.5		
283	J	117	1	32.6	6.2	18120
284	J	220	1	32.2	4.5	25020
285	J	273	1	32.1	6.3	22730
286	J	276	1	31.9	5.2	20210
287	J	262	1	31.7	5.9	21050
288	J	241	1	31.6	6.2	19540
289	J	66	1	31.4	5.6	19140
291	J	127	1	30.3	6.2	20610
292	J	196	1	30.2	7.2	20700
293	J	199	1	30.1	6.2	18820
294	J	193	1	29.9	4.4	21280
295	J	89	1	29.6	4.8	20370
296	J	97	1	29.3	5.1	19260
297	J	70	1	29.2	4.9	15960
298	J	45	1	29.2	5.3	19190
299	J	171	1	29.1	5.7	19180
300	J	84	1	29	6.3	16800
301	J	94	1	29	3.4	14870
305	J	136	1	28.5	4.2	22900
306	J	24	1	28.5		
307	J	115	1	28.3	5.1	23710
309	J	106	1	28	5.5	22210
310	J	159	1	27.7	4.4	19950
311	J	8	1	27.7		
312	J	104	1	27.5	5.2	19590
314	J	60	1	27.2	5.6	17970
316	J	43	1	27	4.6	17260
317	J	110	1	26.9	5.4	22770
321	J	109	1	26.3	5.3	21950
322	J	26	1	26.3		
323	J	65	1	26.2	4.4	13210
324	J	75	1	26.2	4.4	18000
325	J	18	1	26.1		
326	J	117	1	26	4.8	14430
327	J	52	1	25.8	5	15790
328	J	28	1	25.8		
332	J	47	1	25.4	4.3	18880
334	J	52	1	25.1	4.6	18700
337	J	31	1	24.9	4.4	18760
338	J				4.1	21210

Cow	Breed	Dsf	Lactation	Age (mo)	Last % Fat	305 Milk
339	J	65	1	24.6	5.4	21150
342	J	26	1	23.3		
343	J	5	1	23.1		
775	J	142	7	101.3	5.9	21680
803	J	200	5	90.8	3.4	15790
807	J	276	5	89.2	3.9	22540
814	J	248	4	88	4.6	20750
836	J	121	6	85	5.6	19060
844	J	405	4	83.5	5.4	21150
852	J	71	5	81.4	3.8	20530
870	J	52	5	77	4	19080
880	J	284	4	75.3	4.7	21950
886	J	43	5	74.3	5.1	17050
890	J	427	4	73.6	3.4	26370
891	J	374	4	73.5	4.2	25320
903	J	208	4	71.9	4.2	24940
923	J	187	4	67.9	4.8	24410
939	J	107	4	65.4	4.1	18540
1983	H	218	5	101.9	5.5	20670
2033	H	251	5	87.9	3.4	27410
2060	H	65	5	81.4	5.2	17750
2066	H	291	5	80.1	3.9	32410
2068	H	254	5	79.5	4.8	28630
2078	H	449	4	76.8	3.2	32350
2083	H	352	4	75.5	3.6	36760
2119	H	230	4	67.7	3.5	14370
2123	H	96	4	67.4	4.2	27270
2129	H	306	3	66.4	3.9	33430
2136	H	121	4	64.8	3.2	27460
2201	H	166	3	63.3	4.4	26140
2206	H	342	3	62.3	4.3	38860
2212						
2214	H	71	3	59	4.3	20560
2217	H	217	3	57.6	3.4	29990
2220						
2232	H	128	3	54	3.9	30120
2237	H	353	2	52.3	4.1	32220
2242	H	40	3	50.7	3.7	28160
2245	H	342	2	50.3	4.2	36870
2247	H	153	2	50.1	4.3	22800
2248	H	464	2	50.1	4.3	31700
2252	H	447	2	49.2	3.4	38820
2253						
2257	H	275	2	48.6	3.9	34890
2259	H	303	2	47.4	3.4	40010
2260	H	225	2	47.4	3.9	27810

Cow	Breed	Dsf	Lactation	Age (mo)	Last % Fat	305 Milk
2264	H	131	2	47.2	4.2	24550
2265	H	353	2	47.1	4.2	33420
2266	H	287	2	46.5	3.4	37330
2268	H	246	2	46.1	3.6	31790
2269	H	267	2	46	2.5	25840
2273	H	306	2	45.3	4.7	28270
2275	H	218	2	45.2	4.1	28170
2278	H	243	2	44.8	3.9	21770
2281	H	167	2	44.3	3.8	23950
2284	H	318	2	43.8	4.7	34380
2288	H	163	2	43.5	2.8	28220
2289	H	241	2	43.1	3.5	30280
2290	H	194	2	43.2	3.4	29960
2296	H	454	1	42.5	3.5	38230
2297						
2299	H	194	2	41.8	4.1	31550
2300	H	228	2	41.6	4.2	26160
2302						
2303	H	141	2	39.9	4.4	24770
2305	H	82	2	39.3	4	25250
2307						
2308	H	114	2	39	3.6	31130
2309						
2310	H	80	2	38.2	4.3	21180
2316	H	23	2	36.9		
2317	H	427	1	36.9	4.3	30520
2318	H	206	1	36.9	5.7	22080
2324	H	71	2	35.9	3.5	20110
2326	H	266	1	34.3	4.7	22360
2328	H	348	1	34.1	3.6	34790
2329	H	371	1	33.9	4.6	28070
2331	H	160	1	33.4	3.2	29770
2337	H	164	1	32.5	3.9	21840
2338	H	285	1	32.4	4.1	27580
2339	H	205	1	31.6	4.3	25650
2340	H	94	1	31.6	3.4	23550
2341	H	110	1	31.3	4	25770
2342	H	291	1	31.3	3.7	23130
2343	H	137	1	31.3	4	27410
2346						
2347	H	233	1	30.2	4	35900
2348	H	189	1	30.1	4.4	22640
2349	H	196	1	29.9	3.8	22980
2350	H	60	1	29.3	3.8	23750
2352	H	213	1	28.8	3.9	29540
2354	H	115	1	28.4	3.5	26330

Cow	Breed	Dsf	Lactation	Age (mo)	Last % Fat	305 Milk
2356	H	83	1	28	3.9	21730
2358	H	18	1	27.7		
2361	H	77	1	26.9	4.3	18910
2362	H	97	1	26.8	3.9	23640
2363	H	83	1	26.8	4.2	21680
2364	H	109	1	26.5	4	25890
2365	H	40	1	26.5		
2366	H	141	1	26.5	3.5	30820
2367	H	90	1	26	3.9	18100
2369	H	79	1	25.6	3.5	24770
2372	H	66	1	25.1	3.6	24800
2377	H	5	1	23.2		
2380	H	10	1	22.7		
3030	H	5	4	65.1		
3045	H	137	2	45.1	4.3	24450
6860	H	193	2		3.7	
20194	H	181	2	42	3.9	29300

APPENDIX G. Data from the DPTC Foss Milkoscan FT2 software in April 2010

Cow ID	#Sub	Fat %	Protein %	Casein %	Citric acid %	Lactose %	TS %	SNF %	Acidity °TH	Urea mg/dl	Density SG	FPD mC	FFA mekv/l
CP-105	1	4.805	3.64	2.73	0.2	4.72	14.3	9.31	18.58	293	1029.3	513	0.206
CP-105	2	4.795	3.65	2.73	0.2	4.72	14.29	9.32	18.69	332	1029.1	513	0.219
CP-105	SD	0.0066	0.006	0.001	0.001	0.002	0.006	0.007	0.078	27	0.13	0	0.0088
CP-105	Mean	4.8	3.64	2.73	0.2	4.72	14.3	9.32	18.63	312	1029.2	513	0.212
CP-108	1	1.616	3.82	3.04	0.17	5.03	11.3	9.65	18.17	464	1031.9	507	0.581
CP-108	2	1.277	3.84	3.05	0.17	5.06	10.95	9.69	18.1	439	1032	507	0.593
CP-108	SD	0.2394	0.012	0.005	0	0.021	0.245	0.028	0.047	18	0.08	0	0.0086
CP-108	Mean	1.447	3.83	3.05	0.17	5.05	11.12	9.67	18.14	452	1031.9	507	0.587
CP-110	1	1.481	3.71	2.95	0.17	4.91	10.89	9.4	16.12	390	1030.8	504	0.294
CP-110	2	1.561	3.71	2.87	0.18	4.9	10.99	9.41	15.65	380	1030.8	505	0.342
CP-110	SD	0.0563	0	0.055	0.001	0.008	0.067	0.008	0.329	6	0.03	0	0.0342
CP-110	Mean	1.521	3.71	2.91	0.18	4.9	10.94	9.41	15.88	385	1030.8	504	0.318
CP-111	1	3.925	3.63	2.42	0.13	4.06	12.67	8.52	15.3	351	1026.6	512	0.388
CP-111	2	3.92	3.64	2.4	0.13	4.06	12.67	8.54	15.42	338	1026.6	513	0.411
CP-111	SD	0.0035	0.001	0.011	0	0.006	0.001	0.014	0.085	10	0.02	1	0.0163
CP-111	Mean	3.922	3.63	2.41	0.13	4.06	12.67	8.53	15.36	345	1026.6	513	0.4
CP-118	1	3.402	3.59	2.77	0.12	4.85	12.75	9.23	18.63	446	1030.4	504	0.551
CP-118	2	2.279	3.62	2.8	0.12	4.93	11.63	9.31	18.92	503	1031.2	504	0.57
CP-118	SD	0.7941	0.022	0.025	0.002	0.057	0.791	0.058	0.206	40	0.51	0	0.0131
CP-118	Mean	2.84	3.61	2.79	0.12	4.89	12.19	9.27	18.78	475	1030.8	504	0.56
CP-128	1	0.909	3.41	2.67	0.22	4.92	10.01	9.2	19.2	526	1029.2	523	0.207
CP-128	2	0.814	3.4	2.68	0.22	4.94	9.93	9.23	19.16	437	1029.3	524	0.204
CP-128	SD	0.0672	0.007	0.008	0	0.012	0.055	0.021	0.027	63	0.05	1	0.0021
CP-128	Mean	0.861	3.41	2.67	0.22	4.93	9.97	9.22	19.18	482	1029.3	523	0.205
CP-130	1	4.328	3.41	2.58	0.2	4.76	13.59	9.1	15.19	355	1027.8	511	0.745
CP-130	2	4.29	3.41	2.59	0.2	4.77	13.59	9.12	15.23	325	1027.8	511	0.723
CP-130	SD	0.027	0.001	0.009	0	0.005	0.002	0.015	0.029	21	0	0	0.0158
CP-130	Mean	4.309	3.41	2.59	0.2	4.77	13.59	9.11	15.21	340	1027.8	511	0.734
CP-135	1	4.367	3.3	2.64	0.2	4.81	13.5	9.03	15.16	450	1027.6	521	0.122
CP-135	2	4.312	3.31	2.65	0.21	4.82	13.44	9.06	15.08	447	1027.5	521	0.107
CP-135	SD	0.039	0.003	0.005	0.001	0.007	0.044	0.023	0.06	2	0.06	0	0.0107
CP-135	Mean	4.34	3.3	2.64	0.21	4.82	13.47	9.04	15.12	449	1027.6	521	0.114
CP-145	1	4.192	3.58	2.81	0.22	4.68	13.47	9.24	15.7	504	1029.3	508	0
CP-145	2	4.125	3.59	2.85	0.22	4.69	13.4	9.24	15.85	531	1029.3	508	0
CP-145	SD	0.0474	0.005	0.028	0.001	0.009	0.052	0.003	0.112	19	0	0	0
CP-145	Mean	4.159	3.58	2.83	0.22	4.69	13.43	9.24	15.78	518	1029.3	508	0
CP-1532	1	3.887	3.74	2.75	0.15	4.57	13.3	9.2	17.58	498	1029.2	518	0.799
CP-1532	2	3.32	3.76	2.79	0.15	4.6	12.75	9.25	17.63	448	1029.8	519	0.739
CP-1532	SD	0.4011	0.011	0.023	0	0.023	0.386	0.04	0.036	35	0.41	1	0.0424
CP-1532	Mean	3.603	3.75	2.77	0.15	4.58	13.02	9.22	17.6	473	1029.5	518	0.769
CP-158	1	3.793	3.96	3.1	0.18	5.09	13.76	9.9	18.16	403	1033.5	510	0.142
CP-158	2	3.829	3.96	3.1	0.18	5.1	13.81	9.91	18.22	381	1033.5	509	0.074
CP-158	SD	0.025	0.003	0.002	0.001	0.004	0.039	0.006	0.044	16	0.01	0	0.0482
CP-158	Mean	3.811	3.96	3.1	0.18	5.1	13.78	9.91	18.19	392	1033.5	509	0.108
CP-166	1	3.4	3.99	3.07	0.16	4.81	13.21	9.67	18.97	420	1031.3	517	0.59
CP-166	2	2.258	4.04	3.08	0.16	4.89	12.11	9.76	18.74	401	1032.3	519	0.558
CP-166	SD	0.8074	0.03	0.006	0	0.05	0.784	0.066	0.163	14	0.72	1	0.0223
CP-166	Mean	2.829	4.02	3.07	0.16	4.85	12.66	9.71	18.85	411	1031.8	518	0.574
CP-167	1	2.477	3.28	2.49	0.17	4.99	11.64	9.15	18.25	363	1030.1	519	0.506
CP-167	2	2.075	3.29	2.54	0.17	5.02	11.24	9.19	18.46	411	1030.3	520	0.505
CP-167	SD	0.2839	0.012	0.034	0.002	0.023	0.285	0.033	0.146	34	0.19	1	0.0005
CP-167	Mean	2.276	3.29	2.52	0.17	5.01	11.44	9.17	18.35	387	1030.2	519	0.505
CP-170	1	5.15	3.96	2.98	0.13	4.76	14.9	9.49	17.88	382	1030.4	508	0.371
CP-170	2	4.755	3.98	2.94	0.13	4.78	14.53	9.53	18	408	1030.5	507	0.386
CP-170	SD	0.2794	0.013	0.026	0.001	0.016	0.264	0.03	0.085	18	0.11	1	0.0109
CP-170	Mean	4.953	3.97	2.96	0.13	4.77	14.72	9.51	17.94	395	1030.5	508	0.379
CP-174	1	4.402	4.3	3.29	0.16	4.62	14.38	9.74	18.96	452	1031.3	511	0.491
CP-174	2	4.612	4.3	3.32	0.16	4.6	14.59	9.74	19.16	421	1031.1	511	0.488
CP-174	SD	0.1485	0.004	0.017	0	0.01	0.155	0.001	0.14	22	0.14	0	0.0018
CP-174	Mean	4.507	4.3	3.3	0.16	4.61	14.49	9.74	19.06	437	1031.2	511	0.49
CP-179	1	2.521	3.92	3.03	0.19	4.95	12.31	9.73	17.45	424	1032.1	506	0.311
CP-179	2	2.221	3.93	3.09	0.19	4.98	12.01	9.74	17.44	436	1032.2	506	0.316
CP-179	SD	0.212	0.007	0.039	0	0.019	0.215	0.012	0.011	8	0.06	0	0.004
CP-179	Mean	2.371	3.92	3.06	0.19	4.97	12.16	9.73	17.44	430	1032.1	506	0.314
CP-187	1	4.204	3.44	2.6	0.15	4.65	13.35	8.97	17.54	363	1028.4	501	0.477
CP-187	2	3.964	3.46	2.61	0.15	4.68	13.11	8.99	16.75	378	1028.5	504	0.401
CP-187	SD	0.1696	0.013	0.006	0.002	0.022	0.168	0.016	0.562	10	0.1	2	0.0538
CP-187	Mean	4.084	3.45	2.6	0.15	4.67	13.23	8.98	17.14	370	1028.5	502	0.439

Cow ID	#Sub	Fat %	Protein %	Casein %	Citric acid %	Lactose %	TS %	SNF %	Acidity °TH	Urea mg/dl	Density SG	FPD mC	FFA mekv/l
CP-192	1	3.004	4.28	3.27	0.16	4.6	12.84	9.68	17.91	522	1031.9	508	0.404
CP-192	2	1.983	4.32	3.32	0.16	4.67	11.84	9.76	18.29	531	1032.5	510	0.331
CP-192	SD	0.7214	0.027	0.034	0.002	0.049	0.711	0.059	0.271	6	0.46	1	0.052
CP-192	Mean	2.494	4.3	3.3	0.16	4.64	12.34	9.72	18.1	526	1032.2	509	0.368
CP-193	1	2.421	4.17	3.11	0.13	4.39	11.9	9.33	17.51	451	1030.7	503	0.373
CP-193	2	1.896	4.19	3.09	0.13	4.43	11.4	9.38	17.2	393	1031.1	504	0.322
CP-193	SD	0.371	0.016	0.009	0.001	0.027	0.35	0.036	0.219	41	0.29	0	0.0364
CP-193	Mean	2.159	4.18	3.1	0.13	4.41	11.65	9.35	17.35	422	1030.9	503	0.347
CP-1983	1	3.142	2.86	2.18	0.12	4.87	11.71	8.51	14.33	350	1027.4	510	0.351
CP-1983	2	3.031	2.86	2.21	0.12	4.87	11.59	8.5	14.5	362	1027.4	510	0.372
CP-1983	SD	0.0785	0	0.017	0	0.001	0.084	0.012	0.121	8	0.02	0	0.0146
CP-1983	Mean	3.086	2.86	2.2	0.12	4.87	11.65	8.5	14.42	356	1027.4	510	0.362
CP-1985	1	2.935	3.25	2.19	0.18	4.62	11.84	8.75	13.27	373	1027	514	0.609
CP-1985	2	2.862	3.26	2.2	0.18	4.62	11.76	8.76	12.91	372	1027	513	0.595
CP-1985	SD	0.0513	0.004	0.003	0.001	0.002	0.057	0.008	0.254	1	0.01	0	0.0102
CP-1985	Mean	2.899	3.26	2.2	0.18	4.62	11.8	8.76	13.09	372	1027	514	0.602
CP-201	1	5.295	3.79	3.08	0.11	4.38	14.78	9.32	31.86	422	1030.2	523	1.644
CP-201	2	5.277	3.79	3.07	0.11	4.38	14.76	9.34	32.05	420	1030.4	523	1.542
CP-201	SD	0.0132	0	0.004	0	0.003	0.008	0.009	0.134	2	0.1	0	0.0719
CP-201	Mean	5.286	3.79	3.08	0.11	4.38	14.77	9.33	31.95	421	1030.3	523	1.593
CP-203	1	3.586	3.94	3.02	0.16	4.82	13.34	9.59	19.19	415	1031.3	515	0.532
CP-203	2	3.088	3.95	3.01	0.16	4.85	12.86	9.62	19	369	1031.8	515	0.508
CP-203	SD	0.3523	0.013	0.006	0.002	0.023	0.337	0.023	0.135	32	0.39	0	0.0169
CP-203	Mean	3.337	3.94	3.01	0.16	4.84	13.1	9.61	19.09	392	1031.6	515	0.52
CP-2033	1	2.678	2.81	2.1	0.18	4.63	11.03	8.33	14.74	407	1025.1	519	0.203
CP-2033	2	2.678	2.82	2.12	0.18	4.63	11.01	8.31	14.88	468	1025.2	519	0.188
CP-2033	SD	0.0004	0.001	0.009	0.001	0.004	0.013	0.012	0.096	43	0.07	0	0.0104
CP-2033	Mean	2.678	2.82	2.11	0.18	4.63	11.02	8.32	14.81	437	1025.2	519	0.196
CP-2038	1	3.297	3.69	2.81	0.17	4.72	12.75	9.26	16.59	523	1028.8	520	0.638
CP-2038	2	2.998	3.7	2.85	0.17	4.73	12.44	9.3	16.92	549	1028.9	520	0.573
CP-2038	SD	0.2115	0.008	0.022	0.001	0.008	0.221	0.028	0.235	18	0.08	0	0.0457
CP-2038	Mean	3.147	3.69	2.83	0.17	4.73	12.6	9.28	16.76	536	1028.9	520	0.605
CP-205	1	3.072	3.57	2.77	0.17	4.85	12.42	9.25	19.3	470	1030.5	506	0.443
CP-205	2	2.653	3.58	2.79	0.17	4.88	11.99	9.29	19.31	472	1030.6	506	0.469
CP-205	SD	0.2962	0.007	0.015	0.002	0.025	0.303	0.029	0.004	1	0.07	0	0.0179
CP-205	Mean	2.863	3.57	2.78	0.17	4.87	12.2	9.27	19.3	471	1030.6	506	0.456
CP-2054	1	2.614	2.89	2.06	0.19	4.47	10.93	8.28	14.46	369	1025	506	0.303
CP-2054	2	2.511	2.89	2.04	0.19	4.47	10.81	8.26	14.36	349	1025.2	507	0.313
CP-2054	SD	0.0733	0.005	0.012	0	0.002	0.085	0.013	0.067	14	0.12	1	0.0073
CP-2054	Mean	2.562	2.89	2.05	0.19	4.47	10.87	8.27	14.41	359	1025.1	506	0.308
CP-2066	1	2.71	3.16	2.36	0.19	4.98	11.8	9.02	15.59	440	1028.7	523	0.451
CP-2066	2	2.715	3.16	2.38	0.19	4.98	11.79	9.01	15.58	443	1028.5	522	0.405
CP-2066	SD	0.0035	0.001	0.012	0.001	0	0.007	0.008	0.008	2	0.11	1	0.0326
CP-2066	Mean	2.713	3.16	2.37	0.19	4.98	11.8	9.02	15.59	442	1028.6	523	0.428
CP-2068	1	3.809	2.94	2.21	0.18	4.85	12.65	8.71	15.3	436	1027.2	513	0.288
CP-2068	2	3.815	2.95	2.25	0.18	4.86	12.64	8.74	15.58	483	1027.2	513	0.31
CP-2068	SD	0.0044	0.001	0.024	0	0.006	0.001	0.022	0.2	33	0.03	0	0.0154
CP-2068	Mean	3.812	2.94	2.23	0.18	4.86	12.65	8.73	15.44	460	1027.2	513	0.299
CP-2070	1	3.941	3.73	2.79	0.17	4.67	13.41	9.23	15.17	407	1028.3	515	0.578
CP-2070	2	3.947	3.73	2.79	0.17	4.66	13.4	9.23	15.29	411	1028.2	515	0.549
CP-2070	SD	0.0042	0.004	0.001	0	0.004	0.005	0	0.084	3	0.06	0	0.0201
CP-2070	Mean	3.944	3.73	2.79	0.17	4.67	13.41	9.23	15.23	409	1028.2	515	0.564
CP-2078	1	3.62	3.16	2.27	0.17	4.54	12.34	8.55	14.67	444	1025.9	511	0.448
CP-2078	2	3.62	3.15	2.26	0.17	4.55	12.36	8.57	14.79	403	1026.1	511	0.483
CP-2078	SD	0.0002	0.001	0.005	0	0.003	0.016	0.012	0.085	29	0.12	0	0.0243
CP-2078	Mean	3.62	3.16	2.26	0.17	4.55	12.35	8.56	14.73	424	1026	511	0.466
CP-2082	1	2.746	3.59	2.68	0.21	4.58	12	9.12	16.22	450	1028.3	513	0.562
CP-2082	2	2.983	3.58	2.65	0.21	4.57	12.24	9.11	16.36	413	1028.2	513	0.614
CP-2082	SD	0.1671	0.004	0.015	0.002	0.01	0.171	0.009	0.101	27	0.11	0	0.0373
CP-2082	Mean	2.864	3.58	2.67	0.21	4.58	12.12	9.12	16.29	431	1028.2	513	0.588

Cow ID	#Sub	Fat %	Protein %	Casein %	Citric acid %	Lactose %	TS %	SNF %	Acidity °TH	Urea mg/dl	Density SG	FPD mC	FFA mekv/l
CP-2083	1	2.764	3.07	2.25	0.17	4.82	11.56	8.74	15.56	501	1027.5	513	0.579
CP-2083	2	1.978	3.09	2.24	0.17	4.9	10.83	8.82	15.41	489	1027.9	514	0.473
CP-2083	SD	0.556	0.009	0.002	0	0.053	0.518	0.058	0.108	9	0.27	0	0.0749
CP-2083	Mean	2.371	3.08	2.24	0.17	4.86	11.19	8.78	15.48	495	1027.7	514	0.526
CP-2087	1	1.553	3.23	2.41	0.16	5.13	10.73	9.18	15.06	419	1029.8	512	0.419
CP-2087	2	1.12	3.24	2.42	0.16	5.17	10.29	9.19	14.78	432	1030.1	513	0.322
CP-2087	SD	0.3062	0.004	0.004	0	0.033	0.311	0.007	0.198	10	0.22	1	0.0686
CP-2087	Mean	1.336	3.24	2.41	0.16	5.15	10.51	9.18	14.92	425	1029.9	513	0.37
CP-209	1	3.921	3.19	2.39	0.2	4.5	12.65	8.63	16.4	449	1026.2	500	0.547
CP-209	2	3.769	3.19	2.38	0.2	4.5	12.49	8.63	16.22	492	1026.2	499	0.525
CP-209	SD	0.1072	0.002	0.009	0	0	0.112	0.001	0.127	30	0.06	1	0.0154
CP-209	Mean	3.845	3.19	2.38	0.2	4.5	12.57	8.63	16.31	470	1026.2	500	0.536
CP-210	1	5.046	3.56	2.74	0.17	4.81	14.5	9.25	17.51	454	1028.6	517	0.72
CP-210	2	5.035	3.56	2.76	0.17	4.82	14.49	9.25	17.71	450	1028.6	517	0.723
CP-210	SD	0.0075	0.001	0.017	0	0.005	0.007	0.001	0.138	3	0.02	0	0.0022
CP-210	Mean	5.04	3.56	2.75	0.17	4.81	14.5	9.25	17.61	452	1028.6	517	0.722
CP-2116	1	1.435	3.21	2.43	0.17	4.98	10.41	8.99	14.8	464	1029	513	0.376
CP-2116	2	1.508	3.2	2.4	0.16	4.97	10.48	8.97	14.78	466	1029	513	0.409
CP-2116	SD	0.0521	0.007	0.022	0.002	0.002	0.05	0.013	0.011	1	0.04	0	0.0234
CP-2116	Mean	1.472	3.21	2.42	0.16	4.98	10.45	8.98	14.79	465	1029	513	0.393
CP-2119	1	3.196	2.87	2.11	0.17	4.72	11.78	8.51	14.17	391	1026.6	515	0.32
CP-2119	2	2.601	2.88	2.11	0.17	4.76	11.15	8.55	14.01	389	1027.1	516	0.253
CP-2119	SD	0.4205	0.007	0.005	0.001	0.028	0.445	0.029	0.111	1	0.33	1	0.0473
CP-2119	Mean	2.899	2.88	2.11	0.17	4.74	11.47	8.53	14.09	390	1026.9	515	0.286
CP-212	1	3.91	3.24	2.56	0.2	4.8	12.89	8.91	15.97	412	1028.2	510	0
CP-212	2	3.423	3.25	2.56	0.2	4.84	12.41	8.94	15.85	457	1028.4	510	0.007
CP-212	SD	0.3445	0.003	0.001	0.001	0.023	0.334	0.019	0.086	32	0.21	0	0.0048
CP-212	Mean	3.666	3.24	2.56	0.2	4.82	12.65	8.93	15.91	435	1028.3	510	0.003
CP-2129	1	0.962	2.6	1.93	0.24	4.32	8.68	7.84	15.01	465	1022.8	508	0.121
CP-2129	2	1.053	2.58	1.92	0.24	4.3	8.75	7.79	15.06	466	1022.7	507	0.12
CP-2129	SD	0.0645	0.015	0.006	0	0.013	0.051	0.032	0.033	1	0.04	0	0.0009
CP-2129	Mean	1.007	2.59	1.92	0.24	4.31	8.71	7.81	15.03	466	1022.8	508	0.12
CP-213	1	3.65	3.66	2.79	0.18	4.94	13.29	9.49	17.84	380	1030.2	506	0.476
CP-213	2	3.513	3.67	2.79	0.18	4.94	13.14	9.5	17.59	380	1030.4	506	0.452
CP-213	SD	0.0971	0.007	0.005	0	0.001	0.106	0.008	0.173	0	0.1	0	0.0171
CP-213	Mean	3.582	3.67	2.79	0.18	4.94	13.22	9.5	17.72	380	1030.3	506	0.464
CP-2206	1	4.051	3.17	2.23	0.15	4.69	12.94	8.73	16.31	515	1027.3	522	0.966
CP-2206	2	3.618	3.19	2.28	0.16	4.73	12.52	8.79	16.17	482	1027.6	524	0.877
CP-2206	SD	0.3066	0.014	0.031	0.001	0.026	0.292	0.044	0.098	24	0.22	1	0.0624
CP-2206	Mean	3.835	3.18	2.26	0.16	4.71	12.73	8.76	16.24	499	1027.4	523	0.922
CP-2217	1	2.258	2.92	2.19	0.18	4.89	10.92	8.7	16.91	539	1028.1	520	0.383
CP-2217	2	2.369	2.9	2.23	0.18	4.88	11.03	8.68	17.25	448	1028	520	0.42
CP-2217	SD	0.0786	0.01	0.021	0	0.012	0.078	0.012	0.242	64	0.04	0	0.0262
CP-2217	Mean	2.313	2.91	2.21	0.18	4.88	10.97	8.69	17.08	493	1028	520	0.401
CP-2220	1	2.628	3.58	2.62	0.15	4.78	11.93	9.19	18.43	445	1029.7	522	0.642
CP-2220	2	2.869	3.57	2.65	0.15	4.75	12.16	9.15	18.56	432	1029.6	523	0.661
CP-2220	SD	0.17	0.003	0.018	0.001	0.018	0.164	0.022	0.094	9	0.07	1	0.0134
CP-2220	Mean	2.749	3.57	2.63	0.15	4.77	12.04	9.17	18.5	439	1029.7	523	0.652
CP-2235	1	3.058	4.09	2.69	0.08	3.94	12.13	8.82	17.42	448	1028.5	500	0.559
CP-2235	2	2.618	4.11	2.66	0.08	3.96	11.69	8.84	17.02	411	1028.8	500	0.6
CP-2235	SD	0.311	0.013	0.015	0.002	0.012	0.311	0.013	0.28	26	0.28	0	0.029
CP-2235	Mean	2.838	4.1	2.68	0.08	3.95	11.91	8.83	17.22	429	1028.7	500	0.579
CP-2237	1	4.834	3.36	2.33	0.16	4.65	13.95	8.94	16.89	457	1027.9	520	0.768
CP-2237	2	4.834	3.37	2.32	0.16	4.64	13.93	8.93	16.73	413	1028	520	0.797
CP-2237	SD	0.0003	0.003	0	0.001	0.006	0.012	0.006	0.109	32	0.13	0	0.0206
CP-2237	Mean	4.834	3.37	2.32	0.16	4.65	13.94	8.94	16.81	435	1027.9	520	0.783
CP-2238	1	4.106	3.3	2.35	0.15	5.02	13.44	9.16	17.95	400	1029.5	514	1.029
CP-2238	2	4.11	3.31	2.33	0.15	5.02	13.45	9.14	17.67	445	1029.4	514	1.05
CP-2238	SD	0.0029	0.003	0.015	0.001	0.001	0.004	0.012	0.196	32	0.08	0	0.0148
CP-2238	Mean	4.108	3.3	2.34	0.15	5.02	13.44	9.15	17.81	423	1029.5	514	1.039
CP-2242	1	2.006	3.65	2.74	0.17	4.95	11.53	9.44	17.96	409	1030.8	509	0.698
CP-2242	2	2.702	3.63	2.74	0.17	4.9	12.21	9.39	17.92	399	1030.4	509	0.828
CP-2242	SD	0.4919	0.011	0.004	0.001	0.038	0.477	0.038	0.03	7	0.29	0	0.0924
CP-2242	Mean	2.354	3.64	2.74	0.17	4.93	11.87	9.42	17.94	404	1030.6	509	0.763

Cow ID	#Sub	Fat %	Protein %	Casein %	Citric acid %	Lactose %	TS %	SNF %	Acidity °TH	Urea mg/dl	Density SG	FPD mC	FFA mekv/l
CP-2245	1	2.225	3.09	2.13	0.15	4.49	10.68	8.39	15.66	436	1026.1	502	0.35
CP-2245	2	2.138	3.09	2.16	0.16	4.49	10.59	8.41	15.81	385	1026.1	502	0.321
CP-2245	SD	0.0613	0.001	0.022	0	0.003	0.06	0.011	0.107	36	0.02	0	0.0202
CP-2245	Mean	2.181	3.09	2.15	0.16	4.49	10.64	8.4	15.73	410	1026.1	502	0.335
CP-2248	1	2.667	3.63	2.46	0.12	4.43	11.73	8.86	15.12	418	1027.9	507	0.557
CP-2248	2	2.657	3.62	2.45	0.12	4.44	11.72	8.86	15.14	435	1027.9	507	0.568
CP-2248	SD	0.0075	0.003	0.003	0	0.002	0.006	0.002	0.018	11	0.02	0	0.008
CP-2248	Mean	2.662	3.62	2.46	0.12	4.44	11.72	8.86	15.13	427	1027.9	507	0.563
CP-2251	1	1.516	3.4	2.49	0.13	4.93	10.63	9.1	15.89	431	1030.3	507	0.525
CP-2251	2	1.201	3.41	2.5	0.13	4.96	10.31	9.11	15.92	386	1030.4	507	0.441
CP-2251	SD	0.2227	0.004	0.012	0.001	0.026	0.22	0.012	0.017	32	0.07	0	0.0596
CP-2251	Mean	1.359	3.4	2.5	0.13	4.94	10.47	9.11	15.91	408	1030.3	507	0.483
CP-2253	1	4.038	3.35	2.41	0.16	4.82	13.28	9.06	18	381	1028.6	518	0.84
CP-2253	2	4.054	3.35	2.36	0.16	4.82	13.29	9.06	17.63	358	1028.7	518	0.79
CP-2253	SD	0.0111	0.001	0.035	0.001	0.004	0.011	0	0.263	17	0.01	1	0.0353
CP-2253	Mean	4.046	3.35	2.39	0.16	4.82	13.28	9.06	17.81	370	1028.7	518	0.815
CP-2257	1	0.394	2.94	2.13	0.16	5.03	9	8.72	16.76	411	1028.7	511	0.259
CP-2257	2	0.674	2.94	2.17	0.16	5	9.26	8.7	16.98	448	1028.5	510	0.304
CP-2257	SD	0.1979	0.005	0.029	0.002	0.028	0.189	0.015	0.154	26	0.12	1	0.0318
CP-2257	Mean	0.534	2.94	2.15	0.16	5.01	9.13	8.71	16.87	429	1028.6	511	0.281
CP-2259	1	3.122	2.68	1.94	0.23	4.69	11.47	8.35	15.26	446	1025.6	515	0.324
CP-2259	2	3.119	2.68	1.96	0.23	4.69	11.48	8.36	15.32	440	1025.5	515	0.327
CP-2259	SD	0.0017	0.002	0.012	0.001	0.002	0.007	0.004	0.04	5	0.01	0	0.0021
CP-2259	Mean	3.121	2.68	1.95	0.23	4.69	11.47	8.35	15.29	443	1025.6	515	0.326
CP-2260	1	2.463	2.96	2.13	0.16	4.78	11.1	8.63	14.77	325	1027.7	523	0.25
CP-2260	2	2.499	2.96	2.12	0.16	4.76	11.09	8.59	14.63	342	1027.6	522	0.206
CP-2260	SD	0.0252	0.003	0.005	0	0.009	0.008	0.03	0.095	12	0.06	1	0.0316
CP-2260	Mean	2.481	2.96	2.13	0.16	4.77	11.1	8.61	14.7	333	1027.6	522	0.228
CP-2261	1	3.962	2.85	2.13	0.13	4.78	12.57	8.49	15.5	361	1027.2	513	0.423
CP-2261	2	3.962	2.86	2.11	0.13	4.77	12.56	8.5	15.34	381	1027.2	513	0.421
CP-2261	SD	0.0002	0.002	0.017	0.001	0.003	0.006	0.007	0.113	14	0.05	0	0.0014
CP-2261	Mean	3.962	2.86	2.12	0.13	4.77	12.56	8.49	15.42	371	1027.2	513	0.422
CP-2264	1	3.255	3.93	3.08	0.17	4.86	13.09	9.67	19.32	520	1031.2	517	0.61
CP-2264	2	2.513	3.97	3.08	0.17	4.92	12.37	9.72	18.83	510	1031.8	517	0.567
CP-2264	SD	0.5245	0.026	0.001	0.001	0.039	0.511	0.04	0.344	8	0.37	0	0.0299
CP-2264	Mean	2.884	3.95	3.08	0.17	4.89	12.73	9.7	19.07	515	1031.5	517	0.588
CP-2265	1	3.303	3.31	2.56	0.16	5	12.32	9.18	15.43	537	1029.1	519	0.619
CP-2265	2	2.768	3.32	2.56	0.17	5.02	12.09	9.21	15.49	477	1029.4	520	0.626
CP-2265	SD	0.1852	0.004	0.001	0	0.016	0.165	0.025	0.045	42	0.19	0	0.0051
CP-2265	Mean	2.899	3.31	2.56	0.16	5.01	12.21	9.19	15.46	507	1029.3	520	0.622
CP-2266	1	4.225	3.12	2.27	0.2	4.73	13.16	8.81	17.12	492	1027	528	0.58
CP-2266	2	4.223	3.12	2.31	0.2	4.74	13.15	8.79	17.36	444	1027	527	0.644
CP-2266	SD	0.0011	0	0.026	0.002	0.008	0.006	0.012	0.168	34	0	1	0.0452
CP-2266	Mean	4.224	3.12	2.29	0.2	4.73	13.16	8.8	17.24	468	1027	527	0.612
CP-2268	1	1.707	2.97	2.27	0.21	4.93	10.39	8.75	15.17	556	1028	512	0.313
CP-2268	2	1.791	2.97	2.27	0.21	4.93	10.47	8.73	15.1	555	1028	512	0.316
CP-2268	SD	0.0597	0.001	0.005	0.001	0.001	0.056	0.01	0.05	1	0.01	0	0.0025
CP-2268	Mean	1.749	2.97	2.27	0.21	4.93	10.43	8.74	15.13	556	1028	512	0.315
CP-2269	1	2.735	2.98	2.24	0.15	4.84	11.52	8.71	16.6	404	1027.5	510	0.543
CP-2269	2	3.192	2.98	2.22	0.15	4.79	11.96	8.67	16.68	436	1027.2	510	0.609
CP-2269	SD	0.3235	0.006	0.015	0.001	0.035	0.311	0.027	0.057	23	0.21	0	0.0471
CP-2269	Mean	2.963	2.98	2.23	0.15	4.81	11.74	8.69	16.64	420	1027.4	510	0.576
CP-2273	1	5.051	3.46	2.44	0.15	4.81	14.41	9.12	16.26	401	1028.6	516	0.746
CP-2273	2	5.05	3.46	2.45	0.15	4.82	14.4	9.11	16.16	380	1028.6	515	0.788
CP-2273	SD	0.0013	0.001	0.008	0.002	0.002	0.005	0.011	0.067	15	0.03	1	0.0299
CP-2273	Mean	5.051	3.46	2.45	0.15	4.81	14.4	9.12	16.21	391	1028.6	516	0.767
CP-2275	1	1.828	3.21	2.4	0.17	4.93	10.8	9.02	16.75	460	1029.9	510	0.268
CP-2275	2	1.558	3.21	2.44	0.17	4.96	10.54	9.06	16.82	409	1030.2	511	0.227
CP-2275	SD	0.1904	0.004	0.027	0	0.02	0.182	0.03	0.05	36	0.17	0	0.0293
CP-2275	Mean	1.693	3.21	2.42	0.17	4.95	10.67	9.04	16.79	434	1030	511	0.247
CP-2276	1	2.843	3.8	2.83	0.14	4.79	12.42	9.41	17.96	419	1030.9	506	0.675
CP-2276	2	2.413	3.82	2.89	0.14	4.82	11.97	9.46	18.14	434	1031.3	505	0.629
CP-2276	SD	0.3047	0.011	0.043	0.001	0.017	0.318	0.034	0.132	11	0.25	0	0.0323
CP-2276	Mean	2.628	3.81	2.86	0.14	4.8	12.19	9.44	18.05	426	1031.1	506	0.652

Cow ID	#Sub	Fat %	Protein %	Casein %	Citric acid %	Lactose %	TS %	SNF %	Acidity °TH	Urea mg/dl	Density SG	FPD mC	FFA mekv/l
CP-2278	1	2.025	3.12	2.25	0.14	4.8	10.81	8.7	16.22	324	1028.1	517	0.759
CP-2278	2	2.147	3.12	2.23	0.14	4.79	10.94	8.7	16.24	304	1028.1	516	0.753
CP-2278	SD	0.0862	0.001	0.015	0	0.006	0.097	0.003	0.012	14	0.05	0	0.0045
CP-2278	Mean	2.086	3.12	2.24	0.14	4.79	10.88	8.7	16.23	314	1028.1	517	0.756
CP-2284	1	3.939	3.29	2.44	0.15	4.79	13.1	8.97	16.42	413	1028.1	509	0.713
CP-2284	2	3.887	3.29	2.43	0.15	4.8	13.05	8.97	16.76	391	1028.1	509	0.727
CP-2284	SD	0.0368	0.004	0.008	0	0.002	0.035	0.003	0.239	15	0.02	0	0.01
CP-2284	Mean	3.913	3.29	2.44	0.15	4.79	13.07	8.97	16.59	402	1028.1	509	0.72
CP-2289	1	1.884	2.89	2.14	0.17	4.74	10.33	8.49	16.86	393	1027.2	508	0.36
CP-2289	2	1.58	2.89	2.18	0.17	4.78	10.02	8.5	16.9	403	1027.3	508	0.345
CP-2289	SD	0.2155	0.001	0.026	0	0.023	0.219	0.005	0.028	7	0.1	0	0.0106
CP-2289	Mean	1.732	2.89	2.16	0.17	4.76	10.18	8.5	16.88	398	1027.3	508	0.353
CP-229	1	5.465	4.34	3.17	0.13	4.8	15.77	9.99	23.34	427	1032.2	508	1.105
CP-229	2	5.405	4.34	3.19	0.13	4.8	15.69	9.97	23.43	469	1032.3	508	1.106
CP-229	SD	0.0426	0.001	0.013	0.002	0.002	0.059	0.01	0.063	30	0.08	0	0.0004
CP-229	Mean	5.435	4.34	3.18	0.13	4.8	15.73	9.98	23.38	448	1032.2	508	1.105
CP-2290	1	4.374	3.37	2.5	0.19	4.72	13.62	9.09	18.64	477	1028.9	521	0.634
CP-2290	2	4.403	3.37	2.45	0.19	4.71	13.64	9.08	18.31	482	1028.9	520	0.584
CP-2290	SD	0.02	0.001	0.038	0.001	0.005	0.02	0.01	0.236	3	0.02	0	0.0351
CP-2290	Mean	4.388	3.37	2.48	0.19	4.72	13.63	9.08	18.48	479	1028.9	520	0.609
CP-2293	1	2.292	2.87	2.12	0.18	4.77	10.79	8.52	16.89	407	1027.4	506	0.297
CP-2293	2	2.138	2.88	2.14	0.17	4.79	10.64	8.53	16.93	451	1027.4	505	0.336
CP-2293	SD	0.1084	0.002	0.01	0.001	0.016	0.104	0.006	0.025	31	0.02	0	0.0274
CP-2293	Mean	2.215	2.87	2.13	0.17	4.78	10.71	8.52	16.91	429	1027.4	506	0.316
CP-2296	1	3.241	3.46	2.57	0.2	4.8	12.52	9.17	17	479	1028.7	509	0.921
CP-2296	2	2.399	3.47	2.6	0.2	4.87	11.69	9.22	17.4	484	1029.4	509	0.819
CP-2296	SD	0.5959	0.013	0.02	0.001	0.049	0.586	0.034	0.283	3	0.55	0	0.0724
CP-2296	Mean	2.82	3.47	2.58	0.2	4.83	12.11	9.2	17.2	481	1029.1	509	0.87
CP-2299	1	0.224	4.88	4.31	0.23	7.21	12.94	12.91	27.8	648	1048.1	770	0
CP-2299	2	0.437	3.31	2.43	0.16	5.07	9.4	9.12	15.21	428	1031.5	499	0.112
CP-2299	SD	0.1509	1.112	1.332	0.048	1.51	2.502	2.677	8.902	156	11.75	191	0.0789
CP-2299	Mean	0.33	4.09	3.37	0.2	6.14	11.17	11.02	21.5	538	1039.8	635	0.056
CP-2300	1	1.792	3.05	2.27	0.16	5.03	10.67	8.91	15.76	413	1029.2	507	0.308
CP-2300	2	1.165	3.05	2.29	0.16	5.09	10.05	8.95	15.75	440	1029.6	508	0.298
CP-2300	SD	0.4434	0.004	0.014	0.002	0.042	0.438	0.026	0.011	19	0.28	1	0.007
CP-2300	Mean	1.479	3.05	2.28	0.16	5.06	10.36	8.93	15.76	426	1029.4	507	0.303
CP-2302	1	3.885	3.36	2.39	0.18	4.71	13.1	9.03	18.24	323	1027.7	510	0.723
CP-2302	2	3.893	3.37	2.41	0.18	4.71	13.12	9.02	18.15	343	1027.9	511	0.719
CP-2302	SD	0.005	0.001	0.009	0	0.001	0.013	0.004	0.058	14	0.12	0	0.0029
CP-2302	Mean	3.889	3.36	2.4	0.18	4.71	13.11	9.03	18.19	333	1027.8	511	0.721
CP-2305	1	4.419	3.43	2.46	0.15	4.86	13.78	9.13	17.96	384	1029.2	511	1.088
CP-2305	2	4.421	3.43	2.46	0.14	4.86	13.8	9.12	18.15	366	1029.1	510	1.077
CP-2305	SD	0.0014	0.002	0	0	0.001	0.016	0.006	0.139	13	0.1	1	0.0082
CP-2305	Mean	4.42	3.43	2.46	0.14	4.86	13.79	9.13	18.05	375	1029.1	511	1.083
CP-2307	1	4.246	3.54	2.49	0.17	4.88	13.78	9.32	18.65	379	1029.1	517	1.169
CP-2307	2	4.247	3.54	2.47	0.16	4.89	13.79	9.32	18.6	376	1029.1	517	1.163
CP-2307	SD	0.0006	0.002	0.016	0.001	0.005	0.007	0	0.039	2	0.03	0	0.0044
CP-2307	Mean	4.246	3.54	2.48	0.17	4.88	13.78	9.32	18.63	377	1029.1	517	1.166
CP-2309	1	3.136	3.06	2.18	0.13	4.45	11.62	8.33	14.9	407	1025.2	518	0.548
CP-2309	2	3.116	3.06	2.22	0.13	4.46	11.58	8.32	15.48	450	1025.3	518	0.517
CP-2309	SD	0.0138	0.003	0.031	0	0.006	0.033	0.007	0.41	30	0.05	0	0.0223
CP-2309	Mean	3.126	3.06	2.2	0.13	4.45	11.6	8.32	15.19	429	1025.2	518	0.532
CP-2313	1	2.954	3.73	2.92	0.21	5.1	12.87	9.78	17.41	440	1031	580	0.586
CP-2313	2	3.227	3.45	2.62	0.19	4.78	12.53	9.16	16.97	435	1028.3	540	0.666
CP-2313	SD	0.1928	0.2	0.213	0.009	0.226	0.243	0.435	0.31	3	1.93	28	0.0565
CP-2313	Mean	3.091	3.59	2.77	0.2	4.94	12.7	9.47	17.19	438	1029.7	560	0.626
CP-2316	1	3.429	3.62	2.79	0.17	5	13.05	9.46	17.78	544	1030.4	518	0.579
CP-2316	2	3.075	3.63	2.79	0.17	5.02	12.68	9.49	18.11	551	1030.8	518	0.575
CP-2316	SD	0.2504	0.004	0.005	0.001	0.015	0.259	0.015	0.23	5	0.28	0	0.0029
CP-2316	Mean	3.252	3.63	2.79	0.17	5.01	12.86	9.48	17.94	548	1030.6	518	0.577
CP-2317	1	3.466	3.6	2.63	0.17	5	13.14	9.47	15.24	377	1029.8	507	0.809
CP-2317	2	3.1	3.6	2.64	0.17	5.02	12.77	9.5	15.55	423	1030.2	507	0.757
CP-2317	SD	0.2584	0.003	0.009	0	0.018	0.259	0.023	0.219	32	0.25	0	0.0369
CP-2317	Mean	3.283	3.6	2.63	0.17	5.01	12.96	9.48	15.39	400	1030	507	0.783

Cow ID	#Sub	Fat %	Protein %	Casein %	Citric acid %	Lactose %	TS %	SNF %	Acidity °TH	Urea mg/dl	Density SG	FPD mC	FFA mekv/l
CP-2318	1	4.549	3.06	2.32	0.14	4.99	13.56	8.91	18.2	432	1029.5	516	0.592
CP-2318	2	4.482	3.06	2.29	0.14	4.99	13.49	8.92	17.75	457	1029.7	516	0.598
CP-2318	SD	0.0478	0.005	0.015	0	0.005	0.055	0.01	0.319	18	0.13	0	0.0044
CP-2318	Mean	4.516	3.06	2.31	0.14	4.99	13.53	8.91	17.97	445	1029.6	516	0.595
CP-2318	1	4.131	3.57	2.53	0.19	4.54	13.4	9.03	17.18	448	1026.8	517	1.03
CP-2318	2	4.122	3.58	2.5	0.19	4.54	13.38	9.02	16.95	459	1026.9	517	1.061
CP-2318	SD	0.0065	0.003	0.026	0.001	0.004	0.018	0.003	0.163	8	0.05	0	0.022
CP-2318	Mean	4.126	3.57	2.51	0.19	4.54	13.39	9.03	17.06	453	1026.8	517	1.046
CP-232	1	4.974	4.26	3.3	0.24	4.66	15.03	9.83	16.48	611	1029.8	511	0.503
CP-232	2	4.945	4.26	3.36	0.24	4.65	14.97	9.82	16.88	630	1029.8	511	0.537
CP-232	SD	0.0204	0.001	0.042	0	0.005	0.036	0.002	0.283	13	0.01	0	0.0241
CP-232	Mean	4.959	4.26	3.33	0.24	4.66	15	9.82	16.68	621	1029.8	511	0.52
CP-2326	1	4.662	3.2	2.37	0.16	4.89	13.79	8.97	16.72	399	1028.6	515	0.58
CP-2326	2	4.647	3.19	2.37	0.16	4.88	13.78	8.95	16.8	422	1028.6	515	0.535
CP-2326	SD	0.0105	0.003	0.001	0.001	0.004	0.01	0.018	0.053	16	0.05	0	0.0317
CP-2326	Mean	4.655	3.2	2.37	0.16	4.89	13.78	8.96	16.76	411	1028.6	515	0.557
CP-2328	1	3.57	2.9	2.13	0.2	4.85	12.32	8.68	16.17	452	1026.9	509	0.603
CP-2328	2	3.567	2.9	2.15	0.2	4.84	12.31	8.67	16.27	473	1026.9	510	0.605
CP-2328	SD	0.0018	0	0.013	0	0.001	0.004	0.01	0.072	15	0.05	0	0.0015
CP-2328	Mean	3.569	2.9	2.14	0.2	4.85	12.31	8.67	16.22	463	1026.9	509	0.604
CP-2329	1	4.389	3.19	2.09	0.2	4.7	13.51	8.9	17.31	420	1027.2	513	1.185
CP-2329	2	4.386	3.19	2.13	0.2	4.7	13.51	8.9	17.54	456	1027.2	514	1.21
CP-2329	SD	0.0021	0.002	0.028	0.001	0.004	0	0.004	0.161	26	0.02	0	0.0176
CP-2329	Mean	4.388	3.19	2.11	0.2	4.7	13.51	8.9	17.42	438	1027.2	513	1.197
CP-2339	1	4.413	3.25	2.5	0.15	5.03	13.68	9.14	18.46	509	1030.2	531	0.575
CP-2339	2	4.394	3.25	2.51	0.15	5.03	13.66	9.16	18.61	504	1030.2	531	0.533
CP-2339	SD	0.0138	0.001	0.007	0	0	0.016	0.015	0.104	4	0.01	0	0.0298
CP-2339	Mean	4.403	3.25	2.51	0.15	5.03	13.67	9.15	18.54	507	1030.2	531	0.554
CP-2342	1	3.711	3.5	2.54	0.16	5	13.29	9.38	19.15	399	1030.5	513	1.161
CP-2342	2	3.676	3.49	2.57	0.16	5.01	13.27	9.39	19.21	332	1030.6	513	1.175
CP-2342	SD	0.0251	0.004	0.023	0	0.008	0.018	0.003	0.041	47	0.06	0	0.0099
CP-2342	Mean	3.693	3.49	2.55	0.16	5.01	13.28	9.38	19.18	365	1030.5	513	1.168
CP-2347	1	0.123	3.78	3.61	0.25	7.21	11.76	11.86	25.26	839	1041.5	862	0
CP-2347	2	0.441	2.86	2.31	0.19	5.5	9.42	9.17	15.99	596	1030.1	628	0
CP-2347	SD	0.2242	0.653	0.921	0.041	1.209	1.659	1.901	6.561	172	8.06	166	0
CP-2347	Mean	0.282	3.32	2.96	0.22	6.36	10.59	10.52	20.62	718	1035.8	745	0
CP-2348	1	1.504	4.03	3.14	0.2	4.91	11.3	9.86	19.24	416	1034	522	0.114
CP-2348	2	1.313	4.04	3.18	0.2	4.93	11.09	9.86	19.37	466	1034.1	521	0.089
CP-2348	SD	0.1353	0.007	0.03	0	0.011	0.15	0.001	0.094	36	0.07	0	0.0176
CP-2348	Mean	1.409	4.03	3.16	0.2	4.92	11.2	9.86	19.31	441	1034	522	0.102
CP-2349	1	1.178	3.25	2.46	0.18	5.24	10.45	9.38	17.63	416	1031.9	513	0.271
CP-2349	2	0.896	3.25	2.47	0.19	5.27	10.17	9.41	17.22	399	1032.1	514	0.231
CP-2349	SD	0.1998	0	0.006	0.001	0.022	0.197	0.015	0.287	12	0.13	1	0.0286
CP-2349	Mean	1.037	3.25	2.46	0.19	5.26	10.31	9.4	17.42	407	1032	513	0.251
CP-2352	1	2.913	2.85	2.18	0.14	4.98	11.64	8.71	18.91	354	1028.7	512	0.627
CP-2352	2	2.595	2.86	2.15	0.14	5.02	11.33	8.73	18.5	415	1029	512	0.56
CP-2352	SD	0.2254	0.007	0.018	0.001	0.023	0.216	0.014	0.287	43	0.17	0	0.0468
CP-2352	Mean	2.754	2.86	2.17	0.14	5	11.48	8.72	18.71	384	1028.9	512	0.593
CP-236	1	3.143	3.97	3.15	0.14	4.91	12.9	9.64	19.79	482	1032.4	508	0.637
CP-236	2	2.607	3.97	3.12	0.14	4.96	12.38	9.68	20.01	483	1032.7	507	0.732
CP-236	SD	0.3788	0.003	0.022	0.001	0.031	0.367	0.032	0.157	1	0.18	0	0.0669
CP-236	Mean	2.875	3.97	3.13	0.14	4.94	12.64	9.66	19.9	482	1032.5	507	0.685
CP-237	1	3.986	3.95	3.1	0.18	5.16	14.1	9.95	18.68	503	1032.3	534	0.365
CP-237	2	4.51	3.8	2.92	0.18	4.98	14.34	9.64	18.15	470	1031	517	0.43
CP-237	SD	0.3709	0.101	0.132	0.004	0.129	0.173	0.221	0.376	23	0.95	12	0.0461
CP-237	Mean	4.248	3.87	3.01	0.18	5.07	14.22	9.8	18.41	486	1031.7	525	0.398
CP-238	1	4.671	3.7	2.72	0.18	4.76	14.2	9.34	19.25	413	1029.3	512	0.892
CP-238	2	4.657	3.7	2.75	0.17	4.77	14.18	9.34	19.82	384	1029.4	511	0.903
CP-238	SD	0.0098	0.001	0.019	0.002	0.004	0.013	0.003	0.397	20	0.03	0	0.0072
CP-238	Mean	4.664	3.7	2.73	0.18	4.76	14.19	9.34	19.53	398	1029.4	512	0.898
CP-2380	1	3.918	3.42	2.42	0.18	4.79	13.23	9.11	18.61	379	1028.4	516	0.838
CP-2380	2	3.926	3.42	2.44	0.18	4.79	13.24	9.13	18.42	388	1028.3	517	0.877
CP-2380	SD	0.0058	0.002	0.013	0	0	0.006	0.015	0.134	6	0.04	0	0.0273
CP-2380	Mean	3.922	3.42	2.43	0.18	4.79	13.23	9.12	18.51	384	1028.3	517	0.858

Cow ID	#Sub	Fat %	Protein %	Casein %	Citric acid %	Lactose %	TS %	SNF %	Acidity °TH	Urea mg/dl	Density SG	FPD mC	FFA mekv/l
CP-240	1	3.836	3.8	2.89	0.17	4.8	13.39	9.44	20.68	356	1031.2	510	0.627
CP-240	2	3.52	3.81	2.91	0.16	4.83	13.1	9.48	20.93	398	1031.4	509	0.605
CP-240	SD	0.2232	0.009	0.012	0	0.022	0.205	0.026	0.173	30	0.2	0	0.0151
CP-240	Mean	3.678	3.8	2.9	0.17	4.82	13.25	9.46	20.8	377	1031.3	510	0.616
CP-241	1	0.953	3.86	3.08	0.2	5.07	10.68	9.78	19.96	477	1032.4	498	0.581
CP-241	2	0.926	3.86	3.07	0.2	5.06	10.66	9.78	19.91	426	1032.4	499	0.587
CP-241	SD	0.0192	0.001	0.009	0.001	0.006	0.012	0.001	0.036	36	0.04	0	0.004
CP-241	Mean	0.939	3.86	3.07	0.2	5.07	10.67	9.78	19.93	452	1032.4	498	0.584
CP-243	1	5.884	3.71	2.78	0.2	4.71	15.53	9.35	18.97	394	1028.4	514	0.805
CP-243	2	5.871	3.71	2.75	0.2	4.72	15.53	9.37	18.75	402	1028.3	514	0.752
CP-243	SD	0.0092	0.003	0.022	0	0.007	0	0.015	0.156	5	0.07	0	0.0374
CP-243	Mean	5.877	3.71	2.77	0.2	4.72	15.53	9.36	18.86	398	1028.3	514	0.779
CP-247	1	4.141	3.21	2.43	0.17	4.78	13.07	8.85	17.75	450	1028.2	494	0.374
CP-247	2	4.01	3.21	2.45	0.17	4.79	12.95	8.85	17.86	423	1028.2	494	0.35
CP-247	SD	0.0932	0	0.014	0.001	0.011	0.09	0.004	0.079	19	0	0	0.0167
CP-247	Mean	4.076	3.21	2.44	0.17	4.79	13.01	8.85	17.81	436	1028.2	494	0.362
CP-248	1	3.446	3.84	2.94	0.17	4.94	13.18	9.62	18.64	481	1031.4	519	0.727
CP-248	2	3.011	3.85	2.98	0.17	4.97	12.77	9.67	18.87	443	1031.8	520	0.699
CP-248	SD	0.3074	0.007	0.023	0	0.023	0.295	0.029	0.166	27	0.3	0	0.0197
CP-248	Mean	3.228	3.85	2.96	0.17	4.96	12.98	9.64	18.75	462	1031.6	519	0.713
CP-251	1	4.122	3.81	3.02	0.19	4.74	13.74	9.46	15.95	522	1029.4	518	0.438
CP-251	2	4.022	3.8	3.04	0.2	4.75	13.64	9.46	15.57	462	1029.6	518	0.452
CP-251	SD	0.0708	0.006	0.015	0.001	0.007	0.073	0.001	0.271	43	0.16	0	0.0101
CP-251	Mean	4.072	3.8	3.03	0.19	4.74	13.69	9.46	15.76	492	1029.5	518	0.445
CP-253	1	2.768	3.94	3.16	0.15	4.8	12.42	9.54	19.83	540	1031.2	517	0.638
CP-253	2	2.166	3.96	3.18	0.15	4.84	11.82	9.59	19.67	589	1031.6	518	0.642
CP-253	SD	0.4252	0.014	0.012	0	0.032	0.422	0.038	0.112	35	0.31	1	0.0028
CP-253	Mean	2.467	3.95	3.17	0.15	4.82	12.12	9.56	19.75	565	1031.4	517	0.64
CP-255	1	3.092	3.43	2.47	0.17	4.52	12.04	8.82	16.63	372	1028.1	505	0.656
CP-255	2	2.461	3.46	2.5	0.17	4.58	11.44	8.9	16.66	353	1028.6	506	0.583
CP-255	SD	0.4467	0.025	0.023	0.002	0.039	0.422	0.056	0.016	13	0.34	1	0.052
CP-255	Mean	2.776	3.44	2.49	0.17	4.55	11.74	8.86	16.65	363	1028.3	506	0.619
CP-256	1	6	3.93	2.99	0.15	4.8	15.84	9.54	20.77	400	1030.3	508	0.843
CP-256	2	5.988	3.93	2.93	0.15	4.81	15.83	9.57	20.68	399	1030.3	508	0.867
CP-256	SD	0.0081	0.004	0.04	0.001	0.003	0.006	0.02	0.064	1	0.01	0	0.0169
CP-256	Mean	5.994	3.93	2.96	0.15	4.8	15.84	9.56	20.72	400	1030.3	508	0.855
CP-258	1	1.398	3.66	2.68	0.19	4.95	10.85	9.45	18.64	436	1031.2	504	0.589
CP-258	2	1.036	3.66	2.7	0.19	4.98	10.48	9.46	18.93	445	1031.4	505	0.573
CP-258	SD	0.2561	0.001	0.014	0.001	0.025	0.259	0.012	0.206	7	0.14	1	0.011
CP-258	Mean	1.217	3.66	2.69	0.19	4.96	10.67	9.46	18.79	441	1031.3	505	0.581
CP-262	1	3.318	3.95	3.08	0.19	4.96	13.25	9.81	19	383	1032	511	0.459
CP-262	2	2.213	3.98	3.11	0.18	5.04	12.16	9.89	19.1	423	1032.9	511	0.433
CP-262	SD	0.7812	0.024	0.023	0	0.057	0.771	0.057	0.072	29	0.64	1	0.0182
CP-262	Mean	2.766	3.96	3.09	0.19	5	12.71	9.85	19.05	403	1032.5	511	0.446
CP-267	1	3.753	3.53	2.7	0.17	4.84	13.07	9.22	18.8	372	1030	506	0.551
CP-267	2	2.612	3.58	2.77	0.17	4.92	11.97	9.34	18.89	426	1031	508	0.546
CP-267	SD	0.8069	0.034	0.046	0.001	0.059	0.777	0.079	0.062	38	0.7	1	0.0035
CP-267	Mean	3.182	3.55	2.73	0.17	4.88	12.52	9.28	18.84	399	1030.5	507	0.548
CP-268	1	5.076	3.66	2.69	0.15	4.88	14.71	9.39	20.12	393	1030.1	509	0.964
CP-268	2	5.09	3.67	2.69	0.15	4.87	14.72	9.4	20.09	363	1030.1	509	0.986
CP-268	SD	0.0096	0.005	0.001	0	0.002	0.006	0.007	0.016	21	0.03	0	0.0152
CP-268	Mean	5.083	3.67	2.69	0.15	4.87	14.72	9.39	20.11	378	1030.1	509	0.975
CP-271	1	1.085	3.33	2.62	0.2	5.18	10.36	9.35	18.05	445	1030.9	509	0.651
CP-271	2	0.959	3.33	2.61	0.2	5.2	10.24	9.36	17.74	440	1031	509	0.647
CP-271	SD	0.0892	0.001	0.008	0	0.013	0.086	0.002	0.224	4	0.06	0	0.0023
CP-271	Mean	1.022	3.33	2.62	0.2	5.19	10.3	9.36	17.9	443	1030.9	509	0.649
CP-273	1	3.449	3.87	2.95	0.17	5.01	13.39	9.78	19.6	473	1031.8	508	0.951
CP-273	2	2.146	3.92	3	0.18	5.13	12.13	9.91	19.15	462	1032.9	510	0.855
CP-273	SD	0.9214	0.035	0.035	0.001	0.083	0.893	0.094	0.312	8	0.73	2	0.0685
CP-273	Mean	2.797	3.9	2.98	0.18	5.07	12.76	9.84	19.38	467	1032.4	509	0.903
CP-275	1	3.408	3.54	2.68	0.17	4.86	12.75	9.25	20	467	1030.3	508	0.589
CP-275	2	2.789	3.55	2.7	0.17	4.91	12.16	9.29	20.16	396	1030.7	508	0.6
CP-275	SD	0.4376	0.01	0.016	0	0.035	0.423	0.032	0.119	50	0.3	0	0.0073
CP-275	Mean	3.098	3.54	2.69	0.17	4.88	12.45	9.27	20.08	431	1030.5	508	0.594

Cow ID	#Sub	Fat %	Protein %	Casein %	Citric acid %	Lactose %	TS %	SNF %	Acidity °TH	Urea mg/dl	Density SG	FPD mC	FFA mekv/l
CP-276	1	1.93	3.53	2.82	0.16	5.05	11.33	9.4	18.87	471	1030.9	510	0.432
CP-276	2	1.599	3.53	2.83	0.16	5.07	10.98	9.39	18.86	460	1031.1	510	0.448
CP-276	SD	0.2339	0.001	0.005	0.001	0.018	0.245	0.005	0.01	8	0.14	0	0.0113
CP-276	Mean	1.764	3.53	2.82	0.16	5.06	11.16	9.39	18.87	466	1031	510	0.44
CP-2782	1	4.951	4.06	2.87	0.17	4.37	14.58	9.31	17.65	435	1028.7	517	1.002
CP-2782	2	4.461	4.08	2.92	0.17	4.39	14.12	9.36	17.81	413	1029.1	515	0.942
CP-2782	SD	0.3466	0.014	0.037	0.002	0.016	0.325	0.037	0.112	16	0.29	1	0.0422
CP-2782	Mean	4.706	4.07	2.89	0.17	4.38	14.35	9.33	17.73	424	1028.9	516	0.972
CP-280	1	5.199	3.29	2.61	0.18	4.49	14.24	8.86	22.6	456	1026.7	516	1.191
CP-280	2	4.974	3.31	2.56	0.18	4.53	14.04	8.89	21.17	438	1026.8	516	0.982
CP-280	SD	0.1591	0.018	0.035	0.006	0.029	0.142	0.017	1.006	13	0.07	0	0.1481
CP-280	Mean	5.087	3.3	2.58	0.18	4.51	14.14	8.88	21.88	447	1026.8	516	1.087
CP-282	1	1.712	3.92	3.07	0.13	5.13	11.53	9.8	20.26	492	1033.6	510	0.548
CP-282	2	1.493	3.94	3.07	0.13	5.16	11.31	9.82	20	468	1033.8	511	0.468
CP-282	SD	0.1552	0.012	0	0.001	0.017	0.153	0.017	0.185	17	0.13	0	0.0563
CP-282	Mean	1.603	3.93	3.07	0.13	5.15	11.42	9.81	20.13	480	1033.7	510	0.508
CP-284	1	1.046	3.09	2.45	0.2	5.02	9.93	9.02	17.48	456	1029.4	515	0.316
CP-284	2	0.982	3.09	2.44	0.2	5.04	9.86	9.01	17.14	428	1029.4	516	0.322
CP-284	SD	0.0457	0.003	0.005	0.001	0.011	0.049	0.004	0.245	20	0.03	0	0.0039
CP-284	Mean	1.014	3.09	2.45	0.2	5.03	9.89	9.02	17.31	442	1029.4	515	0.319
CP-285	1	1.017	3.41	2.66	0.17	4.98	10.09	9.16	17.94	568	1030.4	510	0.43
CP-285	2	0.919	3.41	2.67	0.17	4.99	9.98	9.17	18.28	557	1030.4	510	0.41
CP-285	SD	0.0695	0.002	0.013	0.001	0.005	0.076	0.011	0.245	8	0.02	0	0.0143
CP-285	Mean	0.968	3.41	2.66	0.17	4.99	10.04	9.17	18.11	563	1030.4	510	0.42
CP-286	1	4.441	3.42	2.54	0.17	4.86	13.84	9.21	18.15	398	1028.8	514	0.837
CP-286	2	4.346	3.43	2.61	0.17	4.87	13.74	9.21	18.52	450	1028.6	513	0.902
CP-286	SD	0.0671	0.001	0.046	0	0.005	0.068	0.002	0.267	37	0.13	0	0.0455
CP-286	Mean	4.394	3.43	2.58	0.17	4.87	13.79	9.21	18.34	424	1028.7	513	0.87
CP-287	1	5.625	3.36	2.9	0.12	4.67	15.24	9.45	29.08	377	1028.5	513	2.51
CP-287	2	5.394	3.38	2.85	0.12	4.71	15	9.47	27.87	382	1029.1	513	2.333
CP-287	SD	0.1635	0.017	0.033	0.005	0.032	0.167	0.016	0.852	4	0.4	0	0.1251
CP-287	Mean	5.509	3.37	2.88	0.12	4.69	15.12	9.46	28.48	379	1028.8	513	2.421
CP-288	1	5.317	3.51	2.72	0.15	4.99	14.8	9.32	17.89	530	1030.5	510	0.385
CP-288	2	5.276	3.52	2.75	0.15	5.01	14.77	9.34	17.89	479	1030.4	510	0.404
CP-288	SD	0.0296	0.006	0.02	0	0.009	0.019	0.011	0.006	37	0.03	0	0.0132
CP-288	Mean	5.297	3.51	2.73	0.15	5	14.79	9.33	17.89	504	1030.5	510	0.395
CP-292	1	4.119	3.6	2.87	0.23	4.5	13.34	9.17	19.91	505	1029.1	515	0.397
CP-292	2	4.054	3.61	2.87	0.23	4.51	13.3	9.19	19.89	506	1029.2	515	0.403
CP-292	SD	0.0462	0.007	0.002	0	0.01	0.029	0.007	0.015	1	0.05	0	0.004
CP-292	Mean	4.087	3.61	2.87	0.23	4.5	13.32	9.18	19.9	505	1029.2	515	0.4
CP-293	1	0.626	3.87	2.98	0.18	5.01	10.18	9.69	19.82	380	1033.6	518	0.185
CP-294	1	3.641	3.93	3.08	0.21	4.76	13.43	9.7	19.83	427	1031.9	515	0.585
CP-294	2	3.569	3.93	3.06	0.21	4.76	13.37	9.71	19.56	422	1032	515	0.578
CP-294	SD	0.0507	0	0.016	0.001	0.004	0.041	0.012	0.19	4	0.08	0	0.005
CP-294	Mean	3.605	3.93	3.07	0.21	4.76	13.4	9.7	19.7	424	1031.9	515	0.581
CP-303	1	4.16	3.95	3.07	0.24	4.43	13.7	9.47	23.63	580	1031.3	537	0.714
CP-303	2	4.133	3.95	3.13	0.25	4.44	13.67	9.48	23.72	602	1031.3	538	0.644
CP-303	SD	0.0192	0.005	0.039	0.002	0.006	0.023	0.005	0.064	15	0.04	0	0.0499
CP-303	Mean	4.147	3.95	3.1	0.24	4.44	13.69	9.48	23.67	591	1031.3	537	0.679
CP-3030	1	4.469	3.22	2.35	0.2	4.64	13.46	8.81	15.18	446	1026.2	521	0.454
CP-3030	2	4.469	3.22	2.36	0.2	4.64	13.44	8.79	15.04	435	1026.3	522	0.408
CP-3030	SD	0.0002	0	0.007	0.001	0.006	0.016	0.015	0.1	8	0.06	1	0.0322
CP-3030	Mean	4.469	3.22	2.35	0.2	4.64	13.45	8.8	15.11	441	1026.2	521	0.431
CP-636	1	4.403	3.86	2.79	0.17	4.29	13.64	8.99	15.06	394	1027.3	515	0.557
CP-636	2	4.355	3.86	2.84	0.17	4.29	13.6	8.99	15.27	382	1027.3	515	0.502
CP-636	SD	0.0341	0.001	0.036	0.001	0.002	0.029	0.003	0.152	8	0	0	0.0389
CP-636	Mean	4.379	3.86	2.81	0.17	4.29	13.62	8.99	15.17	388	1027.3	515	0.529
CP-643	1	5.033	3.77	2.66	0.12	4.24	14.06	8.74	16.62	408	1028	514	0.514
CP-643	2	4.993	3.77	2.63	0.12	4.25	14.04	8.77	16.33	389	1028	514	0.47
CP-643	SD	0.0286	0.001	0.02	0.001	0.006	0.013	0.022	0.205	13	0.01	0	0.0307
CP-643	Mean	5.013	3.77	2.65	0.12	4.24	14.05	8.75	16.48	399	1028	514	0.492
CP-807	1	4.499	3.22	2.41	0.19	4.62	13.34	8.71	14.97	501	1026.7	514	0.119
CP-807	2	4.434	3.21	2.44	0.19	4.62	13.29	8.73	14.99	421	1026.9	514	0.13
CP-807	SD	0.0459	0.001	0.021	0.001	0.003	0.034	0.01	0.011	56	0.12	0	0.0075
CP-807	Mean	4.467	3.21	2.43	0.19	4.62	13.31	8.72	14.98	461	1026.8	514	0.124

Cow ID	#Sub	Fat %	Protein %	Casein %	Citric acid %	Lactose %	TS %	SNF %	Acidity °TH	Urea mg/dl	Density SG	FPD mC	FFA mekv/l
CP-809	1	3.114	3.73	2.67	0.13	4.64	12.5	9.2	19.71	367	1030.2	508	0.593
CP-809	2	2.734	3.74	2.66	0.13	4.67	12.12	9.25	19.38	348	1030.4	508	0.608
CP-809	SD	0.2686	0.01	0.002	0.001	0.02	0.268	0.031	0.232	13	0.15	0	0.0104
CP-809	Mean	2.924	3.74	2.67	0.13	4.66	12.31	9.22	19.55	358	1030.3	508	0.6
CP-814	1	3.412	2.95	2.13	0.18	4.48	11.72	8.28	16.36	409	1026.4	474	0.244
CP-814	2	3.007	2.96	2.15	0.18	4.5	11.32	8.3	16.19	381	1026.6	474	0.27
CP-814	SD	0.2868	0.007	0.015	0.001	0.015	0.287	0.015	0.117	20	0.17	0	0.0189
CP-814	Mean	3.21	2.96	2.14	0.18	4.49	11.52	8.29	16.28	395	1026.5	474	0.257
CP-852	1	4.235	3.66	2.76	0.16	4.74	13.66	9.22	16.5	460	1028.9	509	0.674
CP-852	2	3.957	3.67	2.8	0.16	4.76	13.39	9.25	16.81	457	1029.2	509	0.764
CP-852	SD	0.1961	0.006	0.027	0	0.011	0.193	0.025	0.219	2	0.24	0	0.0635
CP-852	Mean	4.096	3.67	2.78	0.16	4.75	13.52	9.23	16.65	459	1029.1	509	0.719
CP-870	1	2.058	3.72	2.83	0.17	4.88	11.55	9.45	19.43	393	1030.8	520	0.564
CP-870	2	1.872	3.73	2.82	0.17	4.9	11.39	9.47	19.38	380	1031	521	0.567
CP-870	SD	0.1316	0.003	0.007	0	0.014	0.115	0.017	0.037	9	0.13	1	0.0022
CP-870	Mean	1.965	3.72	2.83	0.17	4.89	11.47	9.46	19.4	386	1030.9	520	0.565
CP-876	1	0.567	4.84	3.92	0.19	5.35	11.61	10.99	17.81	305	1036.8	629	0
CP-876	2	2.272	3.14	2.03	0.13	3.7	10.03	7.62	17.53	271	1022.6	403	0.399
CP-876	SD	1.2059	1.204	1.336	0.04	1.169	1.115	2.385	0.194	24	10.07	160	0.2819
CP-876	Mean	1.42	3.99	2.98	0.16	4.53	10.82	9.3	17.67	288	1029.7	516	0.199
CP-880	1	1.356	3.7	2.98	0.22	4.84	10.74	9.41	17.32	368	1030.3	520	0.129
CP-880	2	1.227	3.71	2.97	0.22	4.84	10.61	9.41	17.22	387	1030.3	520	0.115
CP-880	SD	0.0916	0.004	0.013	0.001	0.003	0.095	0	0.071	14	0.01	0	0.0101
CP-880	Mean	1.292	3.7	2.97	0.22	4.84	10.68	9.41	17.27	378	1030.3	520	0.122
CP-886	1	1.423	3.61	2.84	0.17	4.82	10.71	9.26	19.15	387	1030.1	511	0.407
CP-886	2	1.233	3.61	2.82	0.17	4.84	10.52	9.28	19.14	384	1030.2	512	0.38
CP-886	SD	0.1344	0	0.011	0.001	0.013	0.137	0.019	0.008	2	0.08	1	0.0195
CP-886	Mean	1.328	3.61	2.83	0.17	4.83	10.62	9.27	19.15	385	1030.1	511	0.394
CP-891	1	2.72	3.33	2.47	0.16	4.77	11.73	8.95	18.17	452	1028.8	512	0.449
CP-891	2	2.383	3.34	2.49	0.15	4.78	11.36	8.94	18.12	464	1029	512	0.389
CP-891	SD	0.2386	0.008	0.013	0	0.012	0.26	0.005	0.032	9	0.16	0	0.0425
CP-891	Mean	2.551	3.33	2.48	0.16	4.78	11.54	8.94	18.14	458	1028.9	512	0.419
CP-897	1	4.014	3.8	2.79	0.15	4.5	13.34	9.13	18.14	358	1029.1	520	0.603
CP-897	2	4.002	3.8	2.76	0.15	4.5	13.35	9.13	18.19	317	1029.2	520	0.617
CP-897	SD	0.0088	0.002	0.019	0	0.002	0	0.001	0.035	30	0.05	1	0.0104
CP-897	Mean	4.008	3.8	2.78	0.15	4.5	13.34	9.13	18.16	338	1029.2	520	0.61
CP-903	1	1.497	3.11	2.45	0.22	4.94	10.34	8.95	14.95	409	1029	515	0.245
CP-903	2	1.376	3.11	2.47	0.22	4.95	10.24	8.96	15.17	364	1029	515	0.296
CP-903	SD	0.0862	0.002	0.01	0	0.009	0.069	0.003	0.153	31	0.01	0	0.036
CP-903	Mean	1.437	3.11	2.46	0.22	4.94	10.29	8.95	15.06	387	1029	515	0.271
CP-923	1	2.817	4.23	3.38	0.25	4.47	12.63	9.79	21.61	562	1032.4	524	0.593
CP-923	2	2.042	4.26	3.41	0.26	4.54	11.89	9.84	21.34	537	1032.8	525	0.506
CP-923	SD	0.548	0.019	0.026	0.003	0.049	0.522	0.038	0.191	18	0.27	1	0.0614
CP-923	Mean	2.43	4.25	3.39	0.25	4.51	12.26	9.82	21.47	549	1032.6	524	0.549
CP-939	1	1.207	4.06	3.11	0.12	4.77	10.83	9.54	20.23	513	1032.4	515	0.548
CP-939	2	1.164	4.06	3.13	0.12	4.77	10.76	9.56	20.18	536	1032.4	514	0.53
CP-939	SD	0.0304	0.001	0.019	0	0.001	0.043	0.013	0.033	17	0.03	0	0.0125
CP-939	Mean	1.185	4.06	3.12	0.12	4.77	10.79	9.55	20.2	525	1032.4	514	0.539

APPENDIX H. Data from the DPTC Foss Milkoscan FT2 software in September 2010

Cow ID	#Sub	Fat %	Protein %	Casein %	Citric acid %	Lactose %	TS %	SNF %	Acidity °TH	Urea mg/dl	Density SG	FPD mC	FFA mekv/l
CP-139	1	10.925	4.07	2.92	0.2	4.02	20.53	9.08	17.38	462	1021.2	550	0.336
CP-15707	1	2.398	3.57	2.46	0.09	4.19	11.08	8.53	15.94	273	1026.5	523	0.064
CP-15707	2	2.399	3.57	2.45	0.09	4.2	11.09	8.54	15.79	295	1026.5	524	0.042
CP-172	1	13.412	2.62	2.54	0.03	3.61	21.77	8.42	35.18	362	1018.6	567	3.606
CP-172	2	13.398	2.63	2.66	0.03	3.6	21.78	8.46	35.22	343	1019.2	569	3.622
CP-1833	1	1.108	3.68	2.79	0.16	5	10.53	9.46	18.96	406	1032.2	521	0.348
CP-1864	1	2.47	3.25	1.65	0.07	2.55	9.38	6.72	22.88	351	1015.7	529	0
CP-1864	2	2.463	3.25	1.68	0.07	2.54	9.36	6.69	22.92	312	1015.7	529	0
CP-190	1	9.677	3.92	2.84	0.15	4.51	19.5	9.48	24.02	233	1027.2	542	0.593
CP-190	2	9.704	3.92	2.85	0.15	4.52	19.54	9.48	23.92	287	1026.8	542	0.541
CP-1983	1	5.948	3.27	2.41	0.1	4.74	14.97	8.76	14.41	310	1026.9	522	0.592
CP-1983	2	5.955	3.27	2.42	0.1	4.74	14.98	8.75	14.61	276	1027	523	0.576
CP-20194	1	3.857	3.3	2.48	0.17	5.12	13.31	9.3	15.5	342	1029.1	515	0.489
CP-20194	2	3.865	3.3	2.47	0.17	5.12	13.36	9.32	15.51	306	1029.1	515	0.505
CP-2033	1	2.901	3.52	2.61	0.15	4.68	12.08	9.06	15.29	341	1028.5	531	0.365
CP-2033	2	2.908	3.52	2.64	0.15	4.69	12.07	9.06	15.42	332	1028.4	531	0.436
CP-2060	1	5.647	2.74	1.97	0.25	4.34	14.06	8.22	15.94	474	1023.5	532	0.698
CP-2060	2	5.651	2.75	2.03	0.25	4.33	14.07	8.23	15.94	445	1023.6	532	0.71
CP-2066	1	4.119	3.38	2.37	0.16	4.64	13.27	8.93	14.14	326	1027.2	512	0.683
CP-2066	2	4.121	3.38	2.32	0.16	4.64	13.29	8.94	13.99	297	1027.4	512	0.685
CP-2068	1	4.945	3.43	2.61	0.15	4.79	14.27	9.13	17.82	413	1028.5	527	0.369
CP-2068	2	4.942	3.44	2.61	0.15	4.79	14.28	9.13	17.78	409	1028.5	528	0.398
CP-2083	1	3.358	3.44	2.37	0.14	4.49	12.32	8.79	15.2	349	1027.1	526	0.5
CP-2083	2	3.356	3.45	2.35	0.14	4.49	12.28	8.77	15.11	366	1027.2	526	0.544
CP-2087	1	5.013	3.92	2.88	0.14	4.95	14.99	9.72	16.63	327	1031.3	519	0.517
CP-2087	2	5.013	3.92	2.87	0.14	4.96	14.98	9.74	16.52	310	1031.3	520	0.526
CP-2119	1	3.42	3.34	2.09	0.12	3.64	11.55	7.88	15.72	288	1022.7	531	0.413
CP-2119	2	3.416	3.35	2.08	0.12	3.65	11.54	7.87	15.35	248	1022.8	532	0.425
CP-2123	1	3.7	2.89	2.2	0.14	4.75	12.25	8.51	15.48	487	1027.2	530	0.231
CP-2123	2	3.692	2.89	2.15	0.14	4.75	12.23	8.51	14.94	468	1027.3	530	0.231
CP-2129	1	3.694	3.06	2.21	0.18	4.33	12.15	8.33	16.66	357	1025.1	531	0.346
CP-2129	2	3.701	3.06	2.22	0.19	4.34	12.15	8.34	16.67	376	1025	532	0.39
CP-2136	1	3.134	3.23	2.36	0.16	4.81	12.15	8.92	15.39	358	1028.1	521	0.392
CP-2136	2	3.135	3.23	2.4	0.16	4.8	12.15	8.93	15.69	368	1028.3	522	0.371
CP-2201	1	3.937	3.55	2.56	0.14	4.79	13.33	9.22	19.28	347	1029.7	523	0.728
CP-2201	2	3.937	3.55	2.55	0.14	4.8	13.31	9.21	18.93	362	1029.6	524	0.687
CP-2206	1	4.709	3.81	2.65	0.13	4.59	14.17	9.21	15.27	444	1028.6	529	0.921
CP-2206	2	4.719	3.8	2.67	0.13	4.58	14.19	9.21	15.36	463	1028.5	530	0.966
CP-221	1	2.064	4.09	3.15	0.14	5.02	12.07	9.92	18.96	331	1033.1	523	0.248
CP-221	2	2.06	4.09	3.16	0.14	5.03	12.09	9.9	19.16	344	1033.2	523	0.243
CP-2214	1	4.596	3.44	2.58	0.16	4.98	14.02	9.26	14.89	357	1029.5	521	0.443
CP-2214	2	4.592	3.44	2.56	0.16	4.98	14.03	9.29	14.87	318	1029.6	521	0.492
CP-2214	1	1.474	3.97	2.86	0.12	4.47	10.77	9.2	16.44	262	1029.7	521	0.115
CP-2214	2	1.471	3.97	2.86	0.12	4.47	10.75	9.17	16.51	322	1029.8	521	0.088
CP-2217	1	3.618	3.1	2.32	0.17	4.91	12.66	8.91	15.8	386	1027.5	520	0.518
CP-2217	2	3.622	3.1	2.29	0.17	4.89	12.64	8.92	15.56	403	1027.6	520	0.545
CP-2220	1	3.756	4.08	2.94	0.12	4.9	13.68	9.74	16.04	351	1032.5	525	0.618
CP-2220	2	3.749	4.08	2.98	0.12	4.9	13.68	9.74	16.39	327	1032.5	525	0.627
CP-2232	1	4.005	3.34	2.33	0.15	4.65	13.02	8.88	16.73	301	1028.2	525	0.599
CP-2232	2	3.998	3.33	2.39	0.15	4.66	13.03	8.89	17.01	327	1028.3	526	0.599
CP-2237	1	4.295	3.78	2.61	0.15	4.61	13.77	9.32	17.52	310	1030.2	517	0.677
CP-2237	2	4.292	3.78	2.61	0.15	4.61	13.78	9.32	17.15	358	1030.2	517	0.727
CP-2242	1	3.922	3.51	2.7	0.13	5	13.38	9.35	16.64	368	1031.4	527	0.196
CP-2242	2	3.916	3.51	2.68	0.13	4.99	13.36	9.33	16.68	376	1031.3	526	0.223
CP-2245	1	4.27	3.67	2.6	0.15	4.59	13.63	9.15	16.62	337	1028.7	509	0.535
CP-2245	2	4.268	3.66	2.6	0.15	4.59	13.61	9.14	17.05	304	1028.6	510	0.598
CP-2247	1	4.716	3.43	2.58	0.17	5.09	14.36	9.46	16.26	344	1030	526	0.657
CP-2247	2	4.703	3.42	2.55	0.17	5.11	14.35	9.46	16.51	330	1030	526	0.721
CP-2248	1	4.322	3.55	2.34	0.12	4.41	13.44	8.83	15.52	330	1027.8	508	1.09
CP-2252	1	3.4	3.35	2.1	0.12	3.88	11.64	8.07	14.62	344	1024.6	507	0.345
CP-2252	2	3.405	3.35	2.09	0.12	3.87	11.66	8.07	14.75	336	1024.6	507	0.378
CP-2257	1	3.992	3.5	2.47	0.13	4.57	13.11	8.91	17.05	412	1028.2	524	0.67
CP-2257	2	3.993	3.5	2.48	0.13	4.58	13.12	8.92	17.17	416	1028.3	524	0.654

Cow ID	#Sub	Fat %	Protein %	Casein %	Citric acid %	Lactose %	TS %	SNF %	Acidity °TH	Urea mg/dl	Density SG	FPD mC	FFA mekv/l
CP-2259	1	3.475	3.1	2.21	0.21	4.63	12.21	8.69	14.07	377	1027.2	519	0.308
CP-2259	2	3.479	3.09	2.18	0.22	4.63	12.24	8.71	13.78	350	1027.1	519	0.336
CP-2260	1	3.899	3.45	2.47	0.14	4.56	13.01	8.94	15.48	271	1027.8	519	0.481
CP-2260	2	3.897	3.46	2.4	0.14	4.56	12.99	8.93	14.93	262	1027.9	519	0.525
CP-2264	1	4.449	3.82	2.91	0.17	4.92	14.28	9.64	17.25	393	1031	520	0.309
CP-2264	2	4.45	3.81	2.9	0.17	4.92	14.29	9.66	17.28	367	1030.9	521	0.328
CP-2265	1	4.425	3.6	2.77	0.17	4.87	13.98	9.35	15.46	411	1029.6	525	0.473
CP-2265	2	4.428	3.61	2.71	0.17	4.88	14.01	9.38	15.41	440	1029.6	525	0.471
CP-2266	1	2.948	3.79	2.73	0.17	4.47	12.27	9.16	16.47	343	1028.3	516	0.336
CP-2266	2	2.953	3.8	2.73	0.17	4.47	12.28	9.16	16.35	351	1028.5	515	0.403
CP-2268	1	3.592	3.48	2.56	0.17	5.03	13.12	9.38	15.67	380	1029.6	520	0.672
CP-2268	2	3.589	3.47	2.58	0.17	5.01	13.11	9.36	15.71	382	1029.6	521	0.692
CP-227	1	7.025	3.22	2.4	0.21	4.25	15.8	8.56	14.36	415	1023.8	534	0.041
CP-2273	1	5.033	3.93	2.75	0.13	4.69	14.75	9.45	16.76	307	1030.3	515	0.677
CP-2273	2	5.03	3.93	2.78	0.13	4.68	14.75	9.45	16.99	319	1030.2	514	0.691
CP-2275	1	4.131	3.47	2.47	0.13	4.87	13.51	9.22	18.38	324	1030	525	0.711
CP-2275	2	4.127	3.47	2.48	0.13	4.87	13.51	9.24	18.45	314	1030	525	0.715
CP-2278	1	3.947	3.77	2.63	0.13	4.7	13.52	9.31	16.1	355	1029.9	518	0.986
CP-2278	2	3.97	3.78	2.64	0.13	4.71	13.57	9.35	16.23	314	1030	521	0.953
CP-2281	1	3.761	3.25	2.51	0.2	4.9	12.98	9.12	17.54	430	1028.6	541	0.473
CP-2281	2	3.755	3.24	2.5	0.2	4.91	12.98	9.11	17.54	408	1028.5	540	0.502
CP-2284	1	4.841	3.68	2.71	0.17	4.8	14.54	9.45	15.13	303	1028.5	519	0.652
CP-2284	2	4.849	3.67	2.69	0.17	4.81	14.53	9.41	14.95	295	1028.4	519	0.606
CP-2288	1	2.582	3.39	2.58	0.21	4.87	11.84	9.18	15.71	342	1028.6	520	0.493
CP-2288	2	2.583	3.39	2.61	0.21	4.87	11.86	9.18	15.89	346	1028.5	520	0.511
CP-2289	1	3.294	3.64	2.63	0.18	4.66	12.63	9.2	16.58	296	1029	510	0.636
CP-2289	2	3.294	3.64	2.6	0.18	4.66	12.63	9.2	16.48	297	1028.9	511	0.748
CP-2290	1	3.431	3.33	2.54	0.14	4.93	12.7	9.13	16.07	345	1028.8	526	0.408
CP-2290	2	3.421	3.33	2.59	0.14	4.93	12.69	9.13	16.01	328	1028.9	527	0.407
CP-2296	1	3.434	3.75	2.73	0.16	4.81	13.05	9.42	15.99	369	1029.8	522	0.765
CP-2296	2	3.432	3.76	2.76	0.16	4.8	13.05	9.42	16.38	309	1029.6	522	0.823
CP-2297	1	5.104	3.84	2.96	0.16	4.91	14.93	9.54	13.78	478	1029.8	541	0.524
CP-2297	2	4.963	3.27	4.98	0.24	4.93	15.2	10.61	40.07	0	1031.5	565	0
CP-2299	1	3.935	3.42	2.51	0.15	5.03	13.36	9.28	16.42	389	1030.1	511	0.695
CP-2299	2	3.937	3.41	2.48	0.15	5.03	13.35	9.26	16.28	372	1030.2	512	0.722
CP-2300	1	4.139	3.43	2.4	0.15	4.84	13.5	9.17	15.58	361	1029.1	510	0.722
CP-2300	2	4.138	3.43	2.37	0.14	4.86	13.51	9.18	15.24	288	1029.1	510	0.776
CP-2302	1	3.376	3.63	2.69	0.19	4.86	12.99	9.46	17.53	276	1029.9	521	0.575
CP-2303	1	4.398	3.21	2.41	0.16	4.92	13.56	9.07	15.17	409	1029.2	524	0.185
CP-2303	2	4.391	3.21	2.36	0.15	4.92	13.53	9.06	14.86	408	1029.4	525	0.272
CP-2305	1	3.956	3	2.3	0.14	4.97	12.94	8.86	16.03	300	1028.4	516	0.504
CP-2305	2	3.963	3	2.34	0.14	4.97	12.91	8.84	15.99	317	1028.4	517	0.503
CP-2308	1	3.615	3.06	2.3	0.19	4.93	12.64	8.93	15.5	378	1027.8	519	0.326
CP-2308	2	3.61	3.06	2.34	0.18	4.94	12.62	8.92	15.35	377	1027.9	520	0.354
CP-2308	1	2.902	3.68	2.48	0.11	4.03	11.59	8.53	17.15	294	1026.5	510	0
CP-2308	2	2.902	3.69	2.44	0.11	4.03	11.62	8.56	16.93	284	1026.5	509	0.031
CP-2310	1	4.549	3.01	2.23	0.16	4.67	13.31	8.64	15.7	348	1026.6	525	0.371
CP-2310	2	4.545	3.01	2.23	0.16	4.67	13.29	8.65	15.67	338	1026.6	526	0.377
CP-2317	1	4.423	3.55	2.49	0.16	4.75	13.91	9.23	15.21	373	1028.6	519	0.931
CP-2317	2	4.424	3.55	2.53	0.16	4.75	13.88	9.2	15.33	386	1028.6	520	0.935
CP-2318	1	6.134	3.79	2.79	0.12	4.95	15.93	9.53	17.76	318	1030.9	517	0.763
CP-2318	2	6.13	3.79	2.76	0.11	4.95	15.92	9.52	17.61	329	1030.9	518	0.783
CP-2324	1	3.48	3.18	2.43	0.18	4.72	12.36	8.81	16.05	314	1028.1	515	0.186
CP-2324	2	3.468	3.18	2.4	0.18	4.71	12.34	8.81	16.26	313	1028.1	515	0.257
CP-2326	1	4.827	3.65	2.75	0.14	4.98	14.54	9.51	16.57	302	1030.6	533	0.55
CP-2326	2	4.82	3.65	2.66	0.14	4.97	14.53	9.51	16.18	326	1030.6	532	0.569
CP-2328	1	3.603	3.13	2.35	0.21	5	12.79	9.1	15.83	381	1028.4	522	0.421
CP-2328	2	3.601	3.13	2.37	0.22	5	12.8	9.11	16.08	369	1028.3	522	0.459
CP-2329	1	4.747	3.3	2.2	0.18	4.71	14.01	9.01	17.26	308	1027.2	518	1.178
CP-2329	2	4.747	3.3	2.22	0.18	4.72	14.02	9.02	17.54	347	1027.4	518	1.175
CP-2331	1	2.903	2.98	2.25	0.2	4.96	11.84	8.92	17.91	386	1027.7	526	0.583
CP-2331	2	2.904	2.98	2.24	0.2	4.95	11.85	8.93	17.83	367	1027.7	525	0.53
CP-2337	1	4.193	3.81	2.72	0.19	4.9	14.09	9.68	18.34	243	1030.7	516	1.099
CP-2337	2	4.189	3.82	2.73	0.19	4.89	14.08	9.7	18.53	261	1030.7	516	1.086

Cow ID	#Sub	Fat %	Protein %	Casein %	Citric acid %	Lactose %	TS %	SNF %	Acidity °TH	Urea mg/dl	Density SG	FPD mC	FFA mekv/l
CP-2338	1	4.001	3.37	2.33	0.15	4.88	13.34	9.15	16.83	303	1029.1	510	1.072
CP-2338	2	3.994	3.37	2.3	0.15	4.89	13.34	9.13	16.53	363	1029.1	509	1.032
CP-2339	1	4.539	3.64	2.87	0.16	5.05	14.27	9.54	16.47	468	1030.2	533	0.441
CP-2339	2	4.529	3.63	2.86	0.16	5.05	14.27	9.55	16.66	433	1030.2	532	0.439
CP-234	1	1.224	4.44	3.47	0.15	5.2	11.71	10.43	20.43	343	1035.2	513	0.152
CP-234	2	1.236	4.44	3.45	0.15	5.2	11.69	10.4	20.08	390	1035.3	513	0.19
CP-2340	1	3.334	3.43	2.59	0.16	4.94	12.7	9.26	17.22	364	1029.8	531	0.413
CP-2340	2	3.332	3.44	2.57	0.16	4.94	12.69	9.26	16.99	377	1029.8	530	0.474
CP-2341	1	3.925	3.14	2.38	0.21	4.91	13.07	9.04	16.19	355	1028.1	521	0.369
CP-2341	2	3.93	3.15	2.36	0.21	4.91	13.07	9.06	16.06	335	1028.2	522	0.355
CP-2342	1	3.738	3.77	2.79	0.14	4.95	13.54	9.56	17.38	340	1030.8	509	0.969
CP-2342	2	3.741	3.8	2.71	0.14	4.97	13.56	9.6	16.74	295	1031	511	0.985
CP-2343	1	3.917	3.21	2.46	0.24	4.89	13.21	9.17	16.73	322	1027.7	533	0.384
CP-2343	2	3.926	3.21	2.41	0.24	4.89	13.23	9.17	16.37	294	1027.5	531	0.371
CP-2346	1	3.995	3.51	2.57	0.15	5.25	13.83	9.64	16.76	320	1031.3	522	1.117
CP-2346	2	3.99	3.51	2.55	0.15	5.25	13.84	9.65	16.62	329	1031.2	522	1.171
CP-2347	1	4.164	3.05	2.23	0.14	4.71	12.88	8.59	15.96	423	1026.7	530	0.601
CP-2347	2	4.165	3.05	2.2	0.14	4.71	12.9	8.61	15.99	376	1026.8	530	0.592
CP-2348	1	4.605	4.05	3.01	0.19	4.91	14.72	9.9	18.08	417	1031.2	524	0.717
CP-2348	2	4.61	4.05	3	0.19	4.91	14.74	9.91	18.12	373	1031.3	524	0.758
CP-2349	1	3.829	3.45	2.56	0.12	5	13.26	9.29	16.66	320	1030.2	516	0.587
CP-2349	2	3.822	3.46	2.58	0.12	4.99	13.25	9.27	17	355	1030.2	517	0.601
CP-2350	1	4.003	3.42	2.54	0.16	5	13.43	9.33	18.37	318	1031.1	517	0.57
CP-2350	2	3.998	3.43	2.55	0.16	5	13.45	9.33	18.35	324	1031.1	518	0.521
CP-2352	1	3.908	3.29	2.5	0.19	5.01	13.27	9.25	17.54	375	1029.2	522	0.551
CP-2352	2	3.91	3.29	2.58	0.19	5.01	13.28	9.27	17.99	382	1029.2	522	0.549
CP-2354	1	3.542	3.08	2.26	0.16	4.9	12.52	8.87	16.25	320	1028.1	513	0.605
CP-2354	2	3.539	3.08	2.28	0.17	4.91	12.53	8.9	16.19	289	1028.3	514	0.576
CP-2356	1	4.215	2.99	2.28	0.15	4.89	13.13	8.84	17.17	383	1028.7	518	0.596
CP-2356	2	4.215	3	2.29	0.15	4.89	13.12	8.83	17.48	438	1028.8	517	0.626
CP-2361	1	4.378	3.18	2.44	0.15	5	13.55	9.08	17.89	319	1029.3	521	0.554
CP-2361	2	4.378	3.18	2.39	0.15	5	13.54	9.06	17.73	322	1029.4	521	0.506
CP-2362	1	4.034	3.13	2.4	0.17	5.04	13.3	9.15	17.81	348	1028.9	511	0.614
CP-2362	2	4.023	3.13	2.4	0.18	5.03	13.3	9.15	17.87	325	1028.8	511	0.601
CP-2363	1	4.435	3.4	2.56	0.15	5.08	13.98	9.35	17.68	388	1030.3	512	0.576
CP-2363	2	4.433	3.4	2.59	0.15	5.07	13.98	9.38	17.77	397	1030.3	512	0.636
CP-2364	1	4.158	3.35	2.47	0.17	5.11	13.68	9.35	15.08	354	1029.5	523	0.605
CP-2364	2	4.154	3.34	2.48	0.17	5.11	13.68	9.35	15.1	349	1029.4	523	0.593
CP-2365	1	3.513	3.28	2.43	0.18	4.77	12.59	9.06	22.05	323	1030.7	516	0.554
CP-2365	2	3.488	3.28	2.41	0.18	4.78	12.57	9.07	22.02	305	1030.6	516	0.578
CP-2366	1	2.931	3.07	2.26	0.2	4.95	11.95	8.96	15.8	303	1028.1	519	0.525
CP-2366	2	2.926	3.07	2.29	0.2	4.95	11.96	8.98	15.94	334	1028	519	0.569
CP-2367	1	3.812	3.3	2.4	0.14	4.93	13.06	9.12	16.87	288	1030.2	516	0.609
CP-2367	2	3.803	3.3	2.42	0.14	4.93	13.02	9.1	17.16	304	1030.3	516	0.61
CP-2369	1	3.451	3.12	2.31	0.18	5.03	12.63	9.08	16.74	343	1029.2	520	0.726
CP-2372	1	3.514	3.28	2.49	0.16	4.98	12.7	9.14	17.54	371	1030	527	0.446
CP-2372	2	3.5	3.27	2.47	0.16	4.98	12.69	9.13	17.82	424	1030.1	526	0.503
CP-246	1	15.734	3.52	3.04	0.14	4.26	25.22	9.38	35.08	136	1022.9	598	1.237
CP-246	2	15.898	3.55	3.16	0.13	4.25	25.39	9.41	35.49	207	1023.3	601	1.364
CP-250	1	1.772	3.94	3.14	0.16	4.93	11.49	9.65	17.85	366	1031.3	537	0.072
CP-250	2	1.767	3.95	3.14	0.16	4.93	11.51	9.66	17.88	359	1031.4	537	0.099
CP-2768	1	3.7	4.12	3.05	0.17	4.52	13.43	9.5	16.2	308	1029.4	515	0.21
CP-2768	2	3.692	4.12	3.06	0.17	4.52	13.44	9.52	16.28	306	1029.4	515	0.263
CP-2938	1	5.898	3.84	2.78	0.14	4.49	15.39	9.19	18.03	312	1028.2	520	0.296
CP-2938	2	5.894	3.84	2.76	0.14	4.5	15.37	9.19	18.33	330	1028.1	520	0.301
CP-3021	1	1.869	4.71	3.59	0.16	4.75	12.31	10.27	19.55	360	1034	522	0.178
CP-3021	2	1.877	4.71	3.59	0.16	4.75	12.33	10.3	19.56	356	1033.9	522	0.096
CP-3045	1	4.65	3.08	2.17	0.16	4.73	13.56	8.74	14.71	234	1026.8	522	0.726
CP-3045	2	4.648	3.08	2.16	0.15	4.73	13.56	8.7	14.82	276	1026.9	521	0.721
CP-6860	1	1.277	3.7	2.87	0.2	5.2	10.98	9.76	20.34	334	1033	526	0.423
CP-6860	2	1.573	3.7	2.81	0.2	5.16	11.27	9.73	19.8	379	1032.9	526	0.442
CP-803	1	8.104	3.51	2.7	0.16	4.56	17.45	9.08	18.08	418	1025.9	538	0.581
CP-844	1	2.408	3.69	2.58	0.08	4.18	11.19	8.62	17.53	308	1027.5	522	0.077
CP-844	2	2.403	3.7	2.58	0.08	4.18	11.18	8.61	17.39	272	1027.6	523	0.061
CP-890	1	2.581	3.88	2.85	0.12	4.76	12.21	9.48	20.18	300	1031.7	526	0.35
CP-890	2	2.584	3.88	2.82	0.12	4.75	12.2	9.47	19.91	277	1031.7	525	0.345

APPENDIX I. Statistical results of citrate content in milk based on the DGAT1 genetic variants using Minitab software version 16.

General Linear Model: Citrate versus Breed, Genotype

Factor	Type	Levels	Values
Breed	fixed	2	H, J
Genotype	fixed	3	AA, AG, GG

Analysis of Variance for Citrate, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Breed	1	0.000248	0.000379	0.000379	0.35	0.555
Genotype	2	0.003166	0.001774	0.000887	0.82	0.442
Breed*Genotype	2	0.000391	0.000391	0.000195	0.18	0.835
Error	133	0.143735	0.143735	0.001081		
Total	138	0.147541				

S = 0.0328743 R-Sq = 2.58% R-Sq(adj) = 0.00%

Unusual Observations for Citrate

Obs	Citrate	Fit	SE Fit	Residual	St Resid
10	0.030000	0.175781	0.005811	-0.145781	-4.51 R
54	0.080000	0.175781	0.005811	-0.095781	-2.96 R
62	0.250000	0.175781	0.005811	0.074219	2.29 R
66	0.250000	0.173704	0.004474	0.076296	2.34 R
73	0.240000	0.173704	0.004474	0.066296	2.04 R
96	0.150000	0.163333	0.018980	-0.013333	-0.50 X
116	0.190000	0.163333	0.018980	0.026667	0.99 X
128	0.240000	0.173704	0.004474	0.066296	2.04 R
135	0.150000	0.163333	0.018980	-0.013333	-0.50 X

R denotes an observation with a large standardized residual.

X denotes an observation whose X value gives it large leverage.

Least Squares Means for Citrate

Breed	Mean	SE Mean
H	0.1673	0.006907
J	0.1723	0.004582
Genotype	Mean	SE Mean
AA	0.1628	0.010528
AG	0.1704	0.004552
GG	0.1762	0.004797
Breed*Genotype	Mean	SE Mean
H AA	0.1633	0.018980
H AG	0.1650	0.007009
H GG	0.1737	0.004474
J AA	0.1623	0.009118
J AG	0.1758	0.005811
J GG	0.1787	0.008488

Residual Plots for Citrate

Time Series Plot of COOK1

Descriptive Statistics: Citrate

Variable	N	N*	Mean	StDev	Minimum	Q1	Median	Q3
Citrate	148	1	0.17193	0.03205	0.03000	0.15000	0.17000	0.19750

Variable	Maximum	IQR
Citrate	0.25000	0.04750

Tally for Discrete Variables: Genotype

Genotype	Count
AA	16
AG	55
GG	69
N=	140
*=	9

General Linear Model: Citrate versus Breed, Genotype

Factor	Type	Levels	Values
Breed	fixed	2	H, J
Genotype	fixed	3	AA, AG, GG

Analysis of Variance for Citrate, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Breed	1	0.000248	0.000379	0.000379	0.35	0.555
Genotype	2	0.003166	0.001774	0.000887	0.82	0.442
Breed*Genotype	2	0.000391	0.000391	0.000195	0.18	0.835
Error	133	0.143735	0.143735	0.001081		
Total	138	0.147541				

S = 0.0328743 R-Sq = 2.58% R-Sq(adj) = 0.00%

Unusual Observations for Citrate

Obs	Citrate	Fit	SE Fit	Residual	St Resid
10	0.030000	0.175781	0.005811	-0.145781	-4.51 R
54	0.080000	0.175781	0.005811	-0.095781	-2.96 R
62	0.250000	0.175781	0.005811	0.074219	2.29 R
66	0.250000	0.173704	0.004474	0.076296	2.34 R
73	0.240000	0.173704	0.004474	0.066296	2.04 R

96	0.150000	0.163333	0.018980	-0.013333	-0.50 X
116	0.190000	0.163333	0.018980	0.026667	0.99 X
128	0.240000	0.173704	0.004474	0.066296	2.04 R
135	0.150000	0.163333	0.018980	-0.013333	-0.50 X

R denotes an observation with a large standardized residual.
X denotes an observation whose X value gives it large leverage.

Least Squares Means for Citrate

Breed		Mean	SE Mean
H		0.1673	0.006907
J		0.1723	0.004582
Genotype			
AA		0.1628	0.010528
AG		0.1704	0.004552
GG		0.1762	0.004797
Breed*Genotype			
H	AA	0.1633	0.018980
H	AG	0.1650	0.007009
H	GG	0.1737	0.004474
J	AA	0.1623	0.009118
J	AG	0.1758	0.005811
J	GG	0.1787	0.008488

Residual Plots for Citrate

Main Effects Plot for Citrate

Interaction Plot for Citrate

Boxplot of Citrate

APPENDIX J. Statistical results of citrate content in milk holding DIM and lactation number constant using Minitab software version 16.

General Linear Model: Citrate versus Breed, Genotype

Factor	Type	Levels	Values
Breed	fixed	2	H, J
Genotype	fixed	3	AA, AG, GG

Analysis of Variance for Citrate, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Breed	1	0.0031130	0.0031130	0.0031130	5.63	0.027
Genotype	2	0.0026963	0.0014778	0.0007389	1.34	0.284
Breed*Genotype	2	0.0012704	0.0012704	0.0006352	1.15	0.336
Error	21	0.0116167	0.0116167	0.0005532		
Total	26	0.0186963				

S = 0.0235197 R-Sq = 37.87% R-Sq(adj) = 23.07%

Unusual Observations for Citrate

Obs	Citrate	Fit	SE Fit	Residual	St Resid
1	0.210000	0.166667	0.009602	0.043333	2.02 R
14	0.200000	0.160000	0.013579	0.040000	2.08 R

R denotes an observation with a large standardized residual.

Least Squares Means for Citrate

Breed		Mean	SE Mean
H		0.1644	0.007840
J		0.1872	0.005544
Genotype			
AA		0.1650	0.008315
AG		0.1833	0.008315
GG		0.1792	0.008315
Breed*Genotype			
H	AA	0.1633	0.013579
H	AG	0.1700	0.013579
H	GG	0.1600	0.013579
J	AA	0.1667	0.009602
J	AG	0.1967	0.009602
J	GG	0.1983	0.009602

Residual Plots for Citrate

Main Effects Plot for Citrate

Interaction Plot for Citrate

APPENDIX K. Statistical results of citrate content in milk obtained during two different time points from Holstein cows using Minitab software version 16.

General Linear Model: April, September versus Genotype

MANOVA for Genotype

s = 2 m = -0.5 n = 18.0

Criterion	Test		DF		P
	Statistic	F	Num	Denom	
Wilks'	0.79151	2.356	4	76	0.061
Lawley-Hotelling	0.25692	2.377	4	74	0.060
Pillai's	0.21362	2.332	4	78	0.063
Roy's	0.22855				

MTB > GLM 'April' 'September' = Genotype;
T

ANOVA: April, September versus Genotype

Factor Type Levels Values
Genotype random 3 AA, AG, GG

Analysis of Variance for April

Source	DF	SS	MS	F	P
Genotype	2	0.0009050	0.0004525	0.59	0.559
Error	39	0.0298718	0.0007659		
Total	41	0.0307768			

S = 0.0276757 R-Sq = 2.94% R-Sq(adj) = 0.00%

Analysis of Variance for September

Source	DF	SS	MS	F	P
Genotype	2	0.0050078	0.0025039	3.51	0.040
Error	39	0.0278118	0.0007131		
Total	41	0.0328196			

S = 0.0267044 R-Sq = 15.26% R-Sq(adj) = 10.91%

Boxplot of April, September