How Genetic and Nutritional Factors affect New Zealand Goat Milk Composition

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Abstract

Demand for goat milk produced in New Zealand (NZ) is rapidly growing, particularly in expanding Asian markets. The aim of this study was to investigate how genetic and nutritional factors influence milk composition of NZ dairy goats. Little is known about these two factors in dairy goats, especially when compared to extensive research conducted in the area on dairy cows. Therefore in an attempt to improve goat milk production and composition, one important gene and three common nutritional supplements were investigated as part of this study.

The gene *CSN1S1* was chosen for genetic analysis as it is highly polymorphic and can produce a range of effects on milk composition. 126 dairy goats were genotyped and 100 of these were aligned to herd-test and fatty acid data. Key findings include (a) 'medium' and 'low' *CSN1S1* variants are the most common in the NZ dairy goat population, (b) *CSN1S1* genotype significantly influences milk protein content, (c) in some circumstances *CSN1S1* genotype can affect milk yield and fat content (d) *CSN1S1* genotype has no effect on somatic cell count or the kilograms yielded of fat and protein or milk solids and (e) *CSN1S1* genotypes produce small differences in two fatty acids (C10:0 and C18:3n3).

Due to their increasing popularity as alternative animal feeds, palm kernel extract (PKE), biscuit waste (BW) and yeast nutritional factors were investigated. PKE significantly increased C12:0 and C14:0 fatty acids which were reduced to control-farm levels following removal of the supplement in the next season. BW had no clear effects on milk fatty acid composition while yeast supplementation had no effect on any aspect of milk composition. Significant seasonal effects were observed for some fatty acids.

Overall this research has shown that milk produced from NZ dairy goats has the potential to be modified through genetic and dietary means. Genetic factors such as *CSN1S1* and nutritional supplements, especially PKE can alter milk composition. The healthfulness of goat milk can therefore be optimised to better suit the nutritional needs of the consumer.

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List of Abbreviations

ACC	Acetyl-CoA carboxylase
ADF	Acid detergent fibre
AS-PCR	Allele-specific PCR
ALA	Alpha-linolenic acid
ANOVA	Analysis of variance
BLAST	Basic local alignment search tool
BW	Biscuit waste
CTAB	Cetyl trimethylammonium bromide
CM	Chylomicron
CLA	Conjugated linoleic acid
DGC	Dairy Goat Co-operative
DEPC	Diethylpyrocarbonate
DM	Dry matter
EDTA	Ethylenediaminetetraacetic acid
FASTA	Fast-all (sequence alignment program and format)
FA	Fatty acid
FAS	Fatty acid synthase
G3P	Glyceraldehyde 3-phosphate
GITC	Guanidinium thiocyanate
HWE	Hardy–Weinberg equilibrium
IEF	Isoelectric focussing
IPTG	Isopropyl β-D-1-thiogalactopyranoside
LPL	Lipoprotein lipase
LIC	Livestock Improvement
LCFA	Long chain fatty acid
LC PUFA	Long chain polyunsaturated fatty acid
LINE	Long Interspersed Elements
LB	Luria Base
MAS	Marker assisted selection
MCFA	Medium chain fatty acid
MFG	Milk fat globule
MUFA	Mono-unsaturated fatty acid
NDF	Neutral detergent fibre
PKE	Palm kernel extract
PKM	Palm kernel meal
PKO	Palm kernel oil
PBS	Phosphate buffered saline
PAGE	Polyacrylamide gel electrophoresis
PEG	Polyethelene glycol
PCR	Polymerase chain reaction
PUFA	Poly-unsaturated fatty acid
QTL	Quantitative trait loci

RP-HPLC	Reverse phase high-performance liquid chromatography
SCFA	Short chain fatty acid
SNP	Single nucleotide polymorphism
SDS	Sodium dodecyl sulfate
SCC	Somatic cell count
SCD	Stearoyl-CoA desaturase
TVA	*Trans*-vaccenic acid
TG	Triglyceride
TAE	Tris-acetate-EDTA
TE	Tris-EDTA
UTR	Untranslated
VLDL	Very low density lipoprotein
VFA	Volatile fatty acid

Chapter One: Introduction

Goats (*Capra hircus*) were one of the first domesticated animals and have been raised by humans for meat, fibre, hides and dairy products since around 10,500 ca. BP (Naderi et al., 2008). At last count there were over 800 million goats worldwide (FAOSTAT, 2009), with over half of those stocks in Asia (including India). Goat milk and meat remain key commodities, particularly in Asian and African continents where the goat's climatic tolerance and efficient production makes them a highly valuable resource. Given that over three-quarters of the world's population live in these continents (Population Reference Bureau, 2011), goat products are one of the most widely consumed of any species.

By contrast, New Zealand (NZ) has a comparatively small number of goats (111,981 according to Statistics NZ, 2007). In NZ goats are raised predominantly for the production of dairy products in a highly organised commercial operation (Dubeuf et al., 2004) . There is one major dairy factory for goat milk in NZ, Dairy Goat Co-operative (DGC). DGC processes on average 17,250,000 litres of milk each year from a total of 56 goat farms. Virtually all of this milk is processed into goat milk powder, infant formula and specialty nutritional products which are exported to growing Asian and African markets.

Milk is designed to meet the entire nutritional needs of new-borns, making it a highly valuable nutritional source. Fat and protein are the most demanded fractions of goat milk, with recent emphasis being on the types of fat and protein. 'Good fats' such as omega 3, omega 6 and conjugated linoleic acid (CLA) have received much attention given their benefits over and above their regular nutritive value. The different types of proteins are of importance as well, particularly regarding milk allergies (such as alpha-s1 and alpha-s2 caseins) and different protein functions (for example immunoglobulins and whey proteins). These beneficial components in goat milk have the potential to be enhanced by both diet and genetics**.**

New Zealand goat farms have undertaken a number of nutritional changes in the last five years due to two factors. Firstly, in order to simplify animal management and reduce parasite problems, the majority of farmers have moved to an indoor farming system. Indoor farming combined with an increased range of alternative feedstuffs available has meant that the diet of dairy goats has changed from what was fed in traditional farming systems. Secondly, at the start of 2011 DGC removed Palm Kernel Extract (PKE) and Biscuit Waste (BW, leftovers from confectionary production) as allowable diets for DGC dairy goats. These highly utilised feed sources were removed due to the unknown effect of foreign (PKE) and human-food (BW) as animal feed supplements.

Protein levels in goat milk are typically very difficult to modify via diet, however genetics can play a significant role in the synthesis of milk protein. One of the most studied genes in the goat is *CSN1S1*. *CSN1S1* encodes the protein alpha-s1-casein (αs1-casein) which forms around 80% of total casein in goat milk. In addition to influencing the level of milk protein, *CSN1S1* can also influence fatty acid composition and milk allergenicity (discussed later in Chapter 2). Given that this gene is highly polymorphic in the goat (so far 18 variants have been identified), breeding programmes can select for different forms of this gene to alter the level of protein in goat milk.

From the current information available, no studies on the effect of nutritional or *CSN1S1* genetic factors on milk composition have been conducted in New Zealand dairy goats. Therefore, in order to address these problems, this thesis will investigate how *CSN1S1* genotype as well as PKE, BW and yeast diets influence goat milk composition in New Zealand dairy goats. Such findings could then be used to better understand dairy goat nutrition and genetics to ultimately improve the quality of goat milk produced in New Zealand.

Chapter Two: Literature Review

2.1 Milk

Milk is a specialised, exceptionally complex and nutrient rich fluid produced by mammals designed specifically to meet the needs of the young. Key components include protein, fat, lactose, and various vitamins and minerals which exist in different phases in milk (Table 1).

Table 1: Major constituents of milk. Adapted from Jensen *et al,* (1991).

Compartment	Major constituents
Aqueous phase	Solution ash, Ca, Mg, PO_4, Na, K, Cl, CO_2, whey proteins (α-lactalbumin, β-lactoglobulin), lactoferrin, immunoglobulin, lysozyme, serum albumin, lactose, oligosaccharides, amino acids, urea, B-vitamins, ascorbic acid.
Colloidal dispersion	Caseins (α, β, κ), Ca, PO_4
Emulsion	Fat globules, triacylglycerols, fat soluble vitamins, cholesterol esters
Fat globule membrane	Milk fat globule membrane proteins, phospholipids, enzymes, trace minerals
Cells	Macrophages, neutrophils, lymphocytes, epithelial cells, leukocytes

The composition of ruminant milk is to be affected by diet, stage of lactation, environment, breed, nutrition, energy balance, health status of the udder, diet and genetic factors (Goetsch et al., 2011, Chilliard and Ferlay, 2004, Chilliard et al., 2000, Chilliard et al., 2003, Jensen et al., 1991, Park et al., 2007b, Walker et al., 2004). Although milk is the common feature among all mammals, each species differs in composition (Table 2).

Table 2: Gross milk composition across different species. Adapted from Park *et al,* (2007) and USDA National Nutrient Database for Standard Reference. g and kJ are per 100g milk.

Nutrient	Unit	Goat	Sheep	Cow	Human
Water	g	87.03	80.7	88.13	87.5
Energy	kJ	288	451	255	291
Fat	g	4.14	7	3.25	4.38
Protein	g	3.56	5.98	3.15	1.03
Carbohydrate	g	4.45	5.36	4.8	6.89
Casein	%	2.4	4.2	2.6	0.4
Lactose	%	4.1	4.9	4.7	6.9

2.2 Desired characteristics of milk for human consumption

Early nutritional studies identified milk as a healthy, complete food source to provide a versatile combination of fat, protein, vitamins and minerals. However milk and dairy products began to receive negative attention from around 1950 when saturated fats were identified as a contributor to coronary heart disease (Segall, 1977, Keys, 1953). More recent evidence now highlights the fact that not all saturated fats have negative effects (Woodside and Kromhout, 2005) and that some saturated fats can have positive health effects (Aro et al., 1997).

Most of the nutritive value of goat milk (both regular and functional) is derived from fat and protein components. Goat milk in NZ is primarily produced for infant formula products which have strict compositional requirements. Infant formula is designed to match human breast milk and it must meet the normal growth needs of the infant (Prosser et al., 2010). Recommendations for infant formula specific to protein and fat composition are outlined below in Table 3.

Table 3: Recommended standards for the composition of infant formula. Adapted from (Koletzko et al., 2005, MacLean Jr et al., 2010). NS = Not specified. LC-PUFA = Long Chain-Polyunsaturated Fatty Acids. LA = Linoleic acid. ALA = α-linoleic acid.

Component	Unit	Min	Max	Comments
Protein	g/100 kcal	1.8	3	Required to provide essential and 'conditionally essential' amino acids.
Total Fat	g/100 kcal	4.4	6.0	40-54% of energy intake similar to human milk
C18:2n6 (LA)	g/100 kcal	0.3	1.2	-
C18:3n3 (ALA)	mg/100 kcal	50	NS	Considered a dietary indispensable fatty acid
LA:ALA	ratio	5:1	15:1	Ensures proper balance of these fatty acids and LC-PUFA resulting from their metabolism.
C12:0 + C14:0	% of fat	NS	20	Due to potential negative effects of these fatty acids on cholesterol and lipoprotein concentration
Trans fatty acids	% of fat	NS	3	No known nutritional benefit for infants and a number of less desirable biological effects

Of particular importance is the maximum allowable level of C12:0 and C14:0 fatty acids due to their contribution to cholesterol and lipoprotein elevation in humans (reviewed later in section 2.3.6). *Trans*-fatty acids and long chain polyunsaturated fatty acids (LC-PUFAs) such as linoleic acid (LA) and alpha-linolenic acid (ALA) are also important components when considering goat milk composition for infant formula applications. A more detailed description of these fatty acids is presented in the following section.

2.3 Milk Fat

Milk fat contains approximately 400 different fatty acids, making it the most complex of natural fats (Mansson, 2008). These fatty acids exist in a milk fat globule predominantly as triglycerides (97.5%), diacyglycerides (0.48%), monoacylglycerides (0.04%) or free fatty acids (0.4%). Also in the milk fat globule are phospholipids (1.0%), cholesterol (0.4%) and glycolipids in trace amounts (Jensen et al., 1991).

2.3.1 Fatty acid structure and nomenclature

Fatty acids are classified based on the number of carbons and their level of saturation (number of double bonds between the carbon atoms of the fatty acid chain). Most fatty acids in biological systems have an even number of carbons in the carbon chain. Short chain fatty acids (SCFA) are typically four to ten carbons in length, medium chain fatty acids (MCFA) twelve to 16 carbons and long chain fatty acids (LCFA) 17 carbons or more.

Saturated fats have no double bonds (for example C18:0 is an eighteen-carbon chain with no double bonds), while unsaturated fats can be monounsaturated (one double bond e.g. C18:1, referred to as MUFAs) or polyunsaturated (more than one double bond e.g. C18:3, known as PUFAs).

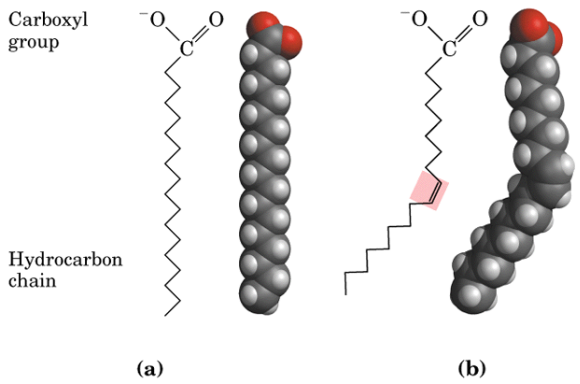

Figure 1: Structural differences between a saturated fatty acid (a) and an unsaturated fatty acid (b) with one double bond (monounsaturated). Adapted from Nelson and Cox, (2008).

Where different fatty acid isomers exist, the conformation and position of that isomer is noted (e.g. C18:1 *cis*-9). Omega 3 or omega 6 fatty acids are commonly referred to as n-3 or n-6 respectively. These omega fatty acids have a double bond starting after the third or sixth carbon atom from the methyl end of the carbon chain (Voet and Voet, 2004). Fatty acids also have a systematic name derived from the parent hydrocarbon and a common name (outlined below in section 2.3.2). For the purpose of this thesis fatty acid symbols and common names will be used.

2.3.2 Fatty acids in goat milk

Common fatty acids in goat milk are outlined below in Table 4. As with many ruminant and animal products, palmitic acid (C16:0) is the most prevalent fatty acid in goat milk (Månsson, 2008). Caproic, capryllic and capric acids (6:0 – C10:0) are named after the goat species (*Capra hircus*) due to the higher proportion of these SCFA in goat milk compared with other milks such as bovine (Tomotake et al., 2006).

Table 4: Common fatty acids in milk. Table adapted from (Park et al., 2007b). * value for all C18:1 isomers combined. ** sytematic name for the most common CLA isomer (*cis*9, *trans*11).

Symbol	Common name	Systematic Name	% in goat milk
C4:0	Butanoic	Butyric	2.18
C6:0	Caproic	Hexanoic	2.39
C8:0	Caprylic	Octanoic	2.73
C10:0	Capric	Decanoic	9.97
C12:0	Lauric	Dodecanoic	4.99
C14:0	Myristic	Tetradecanoic	9.81
C14:1	Myristoleic	*cis*-9-tetradecenoic	0.18
C15:0	Pentadecylic	Pentadecanoic	0.71
C15:1	-	Pentadecenoic	
C16:0	Palmitic	Hexadecanoic	28.2
C16:1	Palmitoleic	*cis*-9-hexadecenoic	1.59
C17:0	Margaric	Heptadecanoic	0.72
C18:0	Stearic	Octadecanoic	8.88
C18:1 *trans* 11	*trans*-Vaccenic (TVA)	*trans*-11- octadecenoic	
C18:1 *cis 9*	Oleic	*cis*-9-octadecenoic	19.3*
C18:1 *cis* 11	cis-Vaccenic (CVA)	*cis*-11-octadecenoic	
C18:2n6	Linoleic (LA)	*cis*-9,*cis*12-octadecadienoic	3.19
C18:2n7	Conjugated linoleic (CLA)	*cis*-9, *trans*-11-octadecadienoic**	
C18:3n3	Alpha-linolenic (ALA)	all-*cis*-9,12,15-octadecatrienoic	0.70

2.3.3 Fatty acid formation

Fatty acids in milk fat globules are primarily formed from the microbial degradation and fermentation of dietary carbohydrates, protein and fat in the rumen. The rumen is effectively a fermentation vat consisting of bacteria, protozoa and fungi (Ishler et al., 1996) . The environment of the rumen dictates the presence and activity of various microorganisms which break-down dietary components. Such breakdown produces fatty acids and fatty acid precursors which are used for the animal's energy needs and the production of milk fat. These fatty acids are absorbed directly through the rumen or large intestine and circulated in the blood as chylomicrons (CM), very-low-density lipoproteins (VLDL) or free fatty acids.

Approximately 50% of triglycerides are formed through the 'diet pathway' from fatty acids in CMs or VLDLs (Figure 2). The remaining 40-45% are synthesised from free fatty acids via the '*de novo* pathway' in the mammary gland through the actions of acetyl-CoA carboxylase (ACC) and fatty acid synthase (FAS). Around 5% may be mobilised from the lipid reserves of the animal depending on the energy balance of the animal (Chilliard et al., 2003) .

Virtually all LCFA and some MCFA (C12-C22) are pre-formed in the rumen and follow the 'diet pathway' to be subsequently modified in the mammary gland by SCD. C16:0 in milk is derived half from the diet pathway and half via *de novo* fatty acid synthesis from VFA precursors and the actions of ACC and FAS. Almost all SCFA (C4:0-C10:0) are synthesised via the *de novo* pathway.

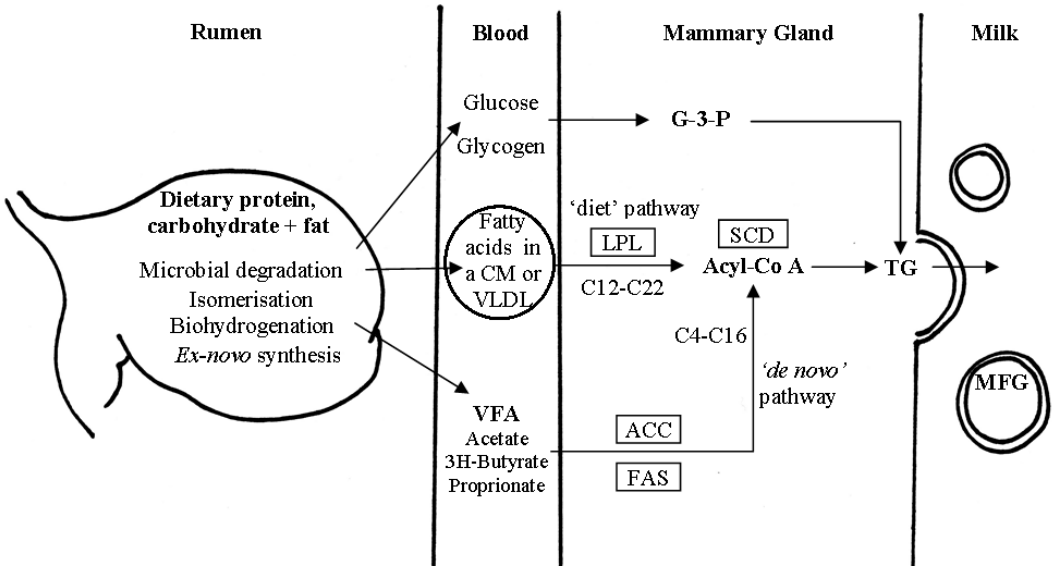

Figure 2: Milk fat synthesis and secretion in the lactating ruminant. Shown in the rumen are the key processes contributing to fatty acid production. Enzymes associated with each process are shown in rectangular boxes. ACC= Acetyl-CoA carboxylase. CM = chylomicron. FAS= Fatty acid synthase. G-3-P= Glyceraldehyde 3-phosphate. LPL= Lipoprotein lipase. MFG= Milk Fat Globule. SCD = Stearoyl-CoA Desaturase, also known as Δ9-desaturase. TG= Triglyceride. VFA = Volatile fatty acids. VLDL = Very low-density lipoprotein. Figure adapted from (Chilliard et al., 2000, Mele et al., 2008, Harvatine et al., 2009a) and not drawn to scale.

2.3.4 Biohydrogenation and isomerisation

Biohydrogenation and isomerisation are two key processes affecting the conversion of diet into milk fatty acids, particularly LCFA (Figure 3). Microbial biohydrogenation is the process of converting unsaturated fatty acids to more saturated end products by gut microbes (Mosley et al., 2002). The main bacterium involved in biohydrogenation is *Butyrivibrio fibrisolves*, first described by Kepler and Tove (1967).

Isomerisation by rumen microbes on the other hand gives rise to several geometric and positional fatty acid isomers (Laverroux et al., 2011). Therefore although linoleic (LA, C18:2n6) and alpha-linolenic (ALA, C18:3n3) acids are the main unsaturated fatty acids in the diet of ruminants, these processes within the rumen mean that the major fatty acid leaving the rumen is C18:0 (Woods and Fearon, 2009).

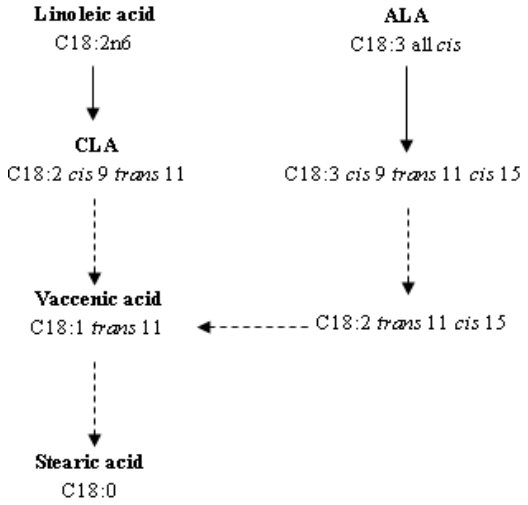

Figure 3: Biohydrogenation and isomerisation pathways of linoleic and alpha-linolenic acids in the rumen. Solid arrows represent isomerisation processes while dashed arrows symbolise hydrogenation (reduction). Adapted from (Mele et al., 2008, Harvatine et al., 2009a).

2.3.5 Stearoyl-Co A desaturase (SCD) activity

The activity of SCD in the mammary gland has an important effect on the level of saturated fats in the milk. SCD (alternatively known as Δ9 desaturase) introduces a double bond in position 9 of the carbon chain, thereby partially reversing any hydrogenation that occurred in the rumen. In this way much of the stearic acid (C18:0) is desaturated back to oleic acid (C18:1*cis*9) while approximately 30% of vaccenic acid (C18:1 *trans* 11 (TVA)) can be desaturated to the C18:2 *cis*9 *trans*11 CLA isomer (Chilliard and Ferlay, 2004, Griinari et al., 2000).

2.3.6 Fatty acid benefits for human health

Following human consumption of milk, fatty acids are released in the upper intestine, immediately absorbed, processed, and released into the blood stream, where they are rapidly taken up by the liver (Smith et al., 1998). These fatty acids can then have a variety of effects on human health

Of the saturated fats in milk, lauric (C12:0) and myristic (C14:0) remain the most concerning for their hypercholesterimic effects. Other SFAs such as C4:0-C10:0 are less strongly implicated and can exert a range of antimicrobial, antiviral and anticancer effects (Table 5). Similarly stearic acid (C18:0) appears to have more positive health benefits than negative.

LC-PUFAs are deemed to be the most beneficial for human health, with anticarcionogenic, antioxidant, antimicrobial and hypocholestericmic effects (Table 5). Special note should be mentioned of CLA. There are 2 isomers of CLA, however the *cis*9 *trans*11 isomer represents about 75-90% of total CLA in milk fat (Tsiplakou et al., 2006). This fatty acid is considered one of the most beneficial, with antiadipogenic (Corino et al., 2005, Park et al., 2007a) anticarcinogenic (Ip et al., 1999, Ou et al., 2007), antiatherogenic (Kritchevsky et al., 2000, Lee et al., 1994), antidiabetogenic (Ryder et al., 1999, Choi et al., 2007) and having antinflammatory properties (Lee et al., 2009, Yu et al., 2002).

Lastly, goat milk is often seen as an alternative to cow milk because of the higher proportion of MCFAs. These are more easily broken down by salivary, gastric and pancreatic lipases than LCFA (Greenberger et al., 1966), enabling more rapid digestion. MCFA are also absorbed more simply than LCFA (Guillot et al., 1993) which is valuable for infants.

Table 5: Main health effects associated with common fatty acids found in goat milk.

Fatty acid	Health effect	References
C4:0	Gene expression, cancer prevention - especially colonic cancers. Anti-inflammatory.	(Hague et al., 1995, Kolar et al., 2007, Emenaker et al., 2001)
C6:0	Antimicrobial agent.	(Huang et al., 2011)
C8:0	Antimicrobial and antiviral agent	(Huang et al., 2011, Thormar et al., 1994)
C10:0	Antimicrobial and antiviral. Vasorelaxant.	(Huang et al., 2011, Thormar et al., 1994, White et al., 1991)
C12:0	Antiviral, antibacterial and anti-plaque but increases blood cholesterol	(Mensink et al., 2003, Thormar et al., 1994, Schuster et al., 1980)
C14:0	Can be antiviral, however increases blood cholesterol	(Mensink et al., 2003, Parang et al., 1997)
C14:1	Largely unknown. May be anti-arthritic.	(Diehl and May, 1994)
C15:0	Unknown	
C15:1	Unknown	
C16:0	Conflicting reports on blood cholesterol. Can improve intestinal and Ca^{2+} absorption and have antimicrobial effects	(Mensink et al., 2003, Huang et al., 2011, Carnielli et al., 1996)
C16:1	Antitumor.	(Ito et al., 1982)
C17:0	Unknown	
C18:0	Generally regarded as neutral for CHD. Some studies show reducing plasma LDL cholesterol and cardiovascular disease contributors.	(Crupkin and Zambelli, 2008, Hegsted et al., 1965, Keys and Parlin, 1966, Woollett and Dietschy, 1994)
C18:1 *trans*	The only dietary precursor for beneficial CLA.	(Field et al., 2009, Santora et al., 2000)
C18:1 *cis9*	Hypocholestoremic. Anticancer.	(Martin-Moreno et al., 1994, Kris-Etherton et al., 1999, Woollett and Dietschy, 1994)
C18:1 *cis*11	Unknown for this C18:1 isomer	
C18:2n6	Hypocholestoremic. Anti-atherosclerotic. Precursor for beneficial EPA and DHA.	(Penumetcha et al., 2011, Haug et al., 2007)
C18:2n7	Main CLA isomer (c*is* 9 *trans* 11) anticarcinogenic, antioxidant and decreases body fat mass	(Delany et al., 1999, Tsuboyama-Kasaoka et al., 2000, Thom et al., 2002, O'Shea et al., 2000, Nakamura and Omaye, 2009)
C18:3n3	Reduced cardiovascular risk, anticarcinogenic. Precursor for beneficial EPA and DHA.	(Dawczynski et al., 2010, Bartoli et al., 1993, Haug et al., 2007)

2.4 Milk protein

Protein is a highly important aspect of milk composition. Major proteins in milk are the caseins (α_{s1}, α_{s2}, β and κ) and the whey proteins (α-lactglobulin, β-lactalbumin). For many of these protein fractions, goat milk is more similar to human milk than cow's milk (Table 6).

Table 6: Milk protein composition across species. * % of total casein ** g per 100g milk. Total casein in goat milk represents around 80% of total milk protein. Table adapted from (Akers, 2002, Inglingstad et al., 2010, Farrell Jr et al., 2006)

Protein component	Goat	Cow	Human
Casein *			
αs1-casein	5-17	38	Trace
αs2-casein	6-20	10	Trace
β-casein	50	40	70
κ-casein	15	12	27
Whey protein **			
α-lactalbumin	0.12	0.12	0.18
β-lactoglobulin	0.22	0.33	Absent
Total Protein	2.7	3.3	0.9

2.4.1 Milk protein synthesis

Milk specific proteins are either synthesised from amino acids in the bloodstream or from amino acids synthesised by the mammary secretory cells (Akers, 2002). Synthesis of the amino acids within mammary secretory cells is dependent on the transcription and translation of nuclear DNA encoding each protein. α-lactalbumin and β-lactoglobulin are each encoded by a relatively small, single-copy gene, while the caseins are encoded by a cluster of genes (Rosen et al., 1999).

Because milk proteins are destined for secretion, they are synthesised by ribosomes attached to the endoplasmic reticulum and then translocated to the Golgi (Akers, 2002). Due to their hydrophobicity, caseins are formed into micelles in the Golgi by incorporating calcium and inorganic phosphate (Mepham, 1987). Milk proteins are then transported via vesicles to be secreted from mammary epithelial cells into the alveolar lumen and thus into milk.

2.4.2 Alpha-s1-casein (αs1-casein)

Polymorphisms of the gene encoding αs1-casein (*CSN1S1*) show that αs1-casein variants influence milk pH (Devold et al., 2011), fatty acid composition (Chilliard et al., 2006, Valenti et al., 2009), protein and fat content (Bevilacqua et al., 2001). Goat milk contains lower levels of αs1-casein than cow milk and as such, the αs1-casein content serves as a distinguishing factor between milk produced by these two species.

The αs-caseins have been implicated in a number of health effects, most notably in milk allergies. In most cases, if an individual is allergic to cow milk they will also be allergic to goat milk due to the immunological cross-reactivity of the proteins (Shimojo et al., 1997, Bellioni-Businco et al., 1999). However where an individual is sensitive to cow milk, goat milk can be considered an alternative (Park, 1994). This can be attributed to the lower levels of αs1- and αs2- caseins in goat milk, thereby reducing the allergenic burden. Other factors also contribute to this lower allergenicity, such as the reduced level of αs1-casein in goat milk assisting with the digestion of β-lactoglobulin (Almaas et al., 2006, Bevilacqua et al., 2001) which itself can also have allergenic effects (Gall et al., 1996).

2.5 Nutrition and milk composition

Diet affects the milk composition of ruminants, primarily by altering rumen pH and the precursors available for rumen microbes. Nutritional factors that raise pH favour cellulolytic microorganisms that increase acetic (C2:0) and butyric acids (C4:0), while feeds that lower the pH favour amylolytic microorganisms that increase propionic acid (C3:0) in the rumen (Lu et al., 2005). Additional factors such as the size of feed particles and the degree of protection (e.g. lipid encapsulation or calcium salts) can influence the bioavailability of fatty acid precursors for microbial transformation and thus milk fatty acid composition.

Traditionally, goats have been raised solely on native pasture; the composition of which varies based on the geographical location. For smaller operations this remains the case, however with commercial production systems as in New Zealand, goats are often raised on cultivated pastures. These pastures are typically ryegrass and clover, however goats tend to discriminate against clover (Langer, 1990). The FA content of pasture is highly unsaturated (70–90%), with a large amount of linoleic (C18:2) and linolenic (C18:3) acids (Chilliard et al., 2003, Schroeder et al., 2004). Therefore, the consumption and digestion of pasture can influence milk composition by increasing the level of C18:3n-3 (ALA) and CLA in milk.

When pasture or forage is limited, concentrates are used to provide a high energy source. Concentrates can include any combination of barley, corn grain, beet pulp, soyabean cake, chickpeas, cereal, maize, wheat middlings, mustard cake, expeller, oats, vitamins and minerals (Atti et al., 2006, Chilliard et al., 2006, Matsushita et al., 2007, Pizzoferrato et al., 2007, Ryhanen et al., 2005, Tyagi et al., 2009). Typically a concentrate diet increases milk yield, milk fat and alters fatty acid composition depending on the type of concentrate. Fat supplements (vegetable oils, marine oils, oilseeds) also tend to increase milk LCFA. A summary of major studies of forage, concentrates and fat supplementation on goat milk composition are outlined below in Table 7.

Table 7: Summary of major studies investigating diet and milk composition in dairy goats. LCFA = Long chain fatty acids. CLA = conjugated linoleic acid. FA= fatty acid. SO= sunflower oil. LO= Linseed oil. MCFA = medium chain fatty acids. MUFA = monounsaturated fatty acids. TVA = trans vaccennic acid.

Diet	Country	Effect	Reference
Forage or pasture			
Grass or hay vs non-forage	Italy	Grass and hay increased LCFA	(Rapetti et al., 2005)
Forage: concentrate ratio	Italy	Increased concentrate, increased milk production	(Tufarelli et al., 2009)
Lucerne hay vs native pasture	Hungary	Native pasture increased total fat, protein solids and CLA	(Pajor et al., 2009)
Concentrates			
Concentrate	America	Increased milk yield, milk fat, and milk protein (slightly)	(Min et al., 2005)
Concentrate + canola seeds	France	Increased milk yield and milk fat. Altered FA composition.	(Andrade and Schmidely, 2006)
Fat or oil supplements			
Canola and soybean oilseed (protected)	Australia	Altered FA composition	(Gulati et al., 1997)
Calcium salts of CLA	France	Reduced milk fat yield (inhibits *de novo* synthesis)	(Shingfield et al., 2009)
Sunflower or linseed oil	France	SO or LO increased milk fat and LCFA, decreased odd chain FA	(Bernard et al., 2009)
Forage + rapeseeds or sunflower oil	France	Altered fatty acid composition	(Ollier et al., 2009)
Castor or Licuri oil	Brazil	Decreased fat content, altered flavour and odour	(Pereira et al., 2010)
Canola oil	Canada	Increased milk fat, C18:1 and CLA, decreased MCFA	(Mir et al., 1999)
Palm oil	Nigeria	Increased milk yield and fat %	(Otaru et al., 2011)
Soybean, canola and sunflower oils	Brazil	Sunflower oil produced highest CLA in milk, while soybean had highest MUFA and PUFA.	(Matsushita et al., 2007)
Soybean oil	Spain	Increases milk fat, CLA, and TVA	(Bouattour et al., 2008)

2.5.1 New Zealand feed supplements

Pasture remains the main diet for all of New Zealand's DGC dairy goats. However, with many DGC farms shifting to an indoor farming system most of the pasture is 'cut and carried' to the goats. Indoor farming has made it easier to supplement with various feed additives such as palm kernel extract (PKE) and biscuit waste (BW). These were two popular supplements fed to NZ dairy goats up until their removal in 2011. Yeast supplementation is an additional feed supplement suggested to be beneficial for NZ dairy goats. These three feedstuffs are outlined below.

2.5.1.1 Palm Kernel Extract

NZ imports over one-million tonnes of PKE each year for animal nutrition purposes (MAF, 2011). PKE is a by-product of palm oil production and can vary considerably in chemical composition depending on the extent and methodology of oil removal and the proportion of endocarp remaining (Hindle et al., 1995). The remaining residue of 'screw process' extraction is the expeller (PKE), while by-products of solvent extraction are termed palm kernel meal (PKM) or palm kernel cake (PKC) (O'Mara et al., 1999). PKM and PKC are used commonly overseas as feed supplements, while PKE is fed extensively to NZ dairy cows and prior to 2011, dairy goats.

PKE is used as an animal feed as it is cost-effective, high in protein and high in fibre (Carvalho et al., 2006, Salama et al., 2002). Very little is published on the effect of PKE (or PKM and PKC) on milk composition, even in dairy cows. Carvalho *et al,* found this surprising given that PKM is a common raw material used in diets for lactating dairy cows in the UK. Most studies feeding goats palm kernel have focussed on digestibility and nutrient utilisation effects (O'Mara et al., 1999, Hindle et al., 1995, Chanjula et al., 2011, Dahlan et al., 2000), however no studies could be found with regard to PKE and milk fatty acid composition in any species.

2.5.1.2 Biscuit Waste

Biscuit waste (BW) is an animal feed supplement derived as a by-product of confectionary production. A study on BW fed to pigs showed that it could be used as an animal supplement for weight gain and cost-effectiveness if additional protein was added (Narayanan et al., 2009). However other than this paper, very little scientific literature exists with regard to any aspect of animal consumption of confectionary wastes.

Commercial information sheets from NZ suppliers of BW state that it is very high in energy and highly palatable, with high sugar, starch and oil levels (GP Feeds Ltd, 2011). However the lack of scientific research on this feed means this alternative animal feed has a largely unknown effect on milk composition.

2.5.1.3 Yeast

Yeast (*Saccharomyces ceresvisiae*) can be added to animal diets in a dried or liquid form. El Ghani (2004) studied the influence of diet supplementation with yeast culture on performance of Zaraibi goats and found that adding yeast to a mix of concentrate and roughage stimulated milk yield. Similarly live yeast supplementation significantly increased milk production in early lactation Saanen goats (Stella et al., 2007).

However Hadjipanayiotou *et al*, (1997) found that the inclusion of yeast did not improve the performance of dairy ewes or goats. Similarly Salama *et al*, (2002) found that yeast had no effect on milk parameters but did increase body condition of the goats. The only study on fatty acid composition (Giger-Reverdin et al., 2004) found no effect of yeast on fatty acid constituents in nitrogen deficient goats. Thus the effect of yeast supplementation on milk composition is not clear in dairy goats.

2.6 Genetics and milk composition

In many instances, individual animals of the same breed and fed the same diet have different milk compositions, suggesting other factors such as genetics play a role. Genes and single nucleotide polymorphisms (SNPs) that influence milk composition have long been studied in numerous ruminant species. Research from NZ dairy cows has shown that gains of 1 - 2.5% can be achieved each year by incorporating genetic information (quantitative trait loci (QTL) or marker-assisted selection) in selective breeding programs (Spelman and Garrick, 1997).

The genetics of dairy goats however remains a relatively new area, with very little artificial insemination (AI) and only a handful of milk genes sequenced and characterised. A reference sequence for the goat genome is yet to be established, although some regions have been sequenced. The gene encoding αs1-casein (*CSN1S1*) has been the most widely studied, however others such as αs2-casein (*CSN1S2*), β-casein (*CSN2*) and growth hormone (*GH*) have been shown to milk composition in dairy goats (see reviews by Ibeagha-Awemu *et al,* (2008) and Moioli *et al,* (2007)) .

2.6.1 Casein genes

The casein genes (*CSN1S1, CSN1S2*, *CSN2* and *CSN3*) are found only in mammalian genomes and encode αs1-casein, αs2-casein, β-casein and κ-casein proteins. Evolution of the caseins are suggested to have emerged from an ancestral gene before mammalian radiation (>300 Myr) (Kawasaki et al., 2011, Lefevre et al., 2010) and involved extensive exon shuffling and gene duplications (Rijnkels, 2002, Yu-Lee et al., 1986). In ruminants, these genes are clustered together over a 250kb stretch (Figure 4) and are closely linked.

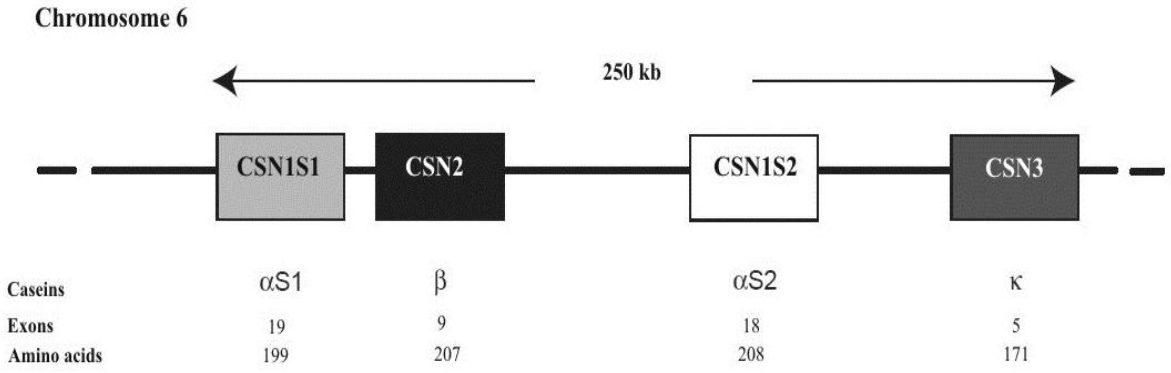

Figure 4: Casein gene cluster in ruminant species. Amino acid length of each transcribed casein gene in the goat is indicated. Adapted from Martin and Leroux (2000) and Marletta *et al*, (2007).

2.6.2 *CSN1S1*, gene encoding αs1-casein

Although a minor protein, *CSN1S1* has been the most extensively studied in the goat due to its remarkable genetic polymorphism and subsequent variation in milk composition. *CSN1S1* is 17.5kb long and consists of 19 exons varying in length from 24-358bp (Ramunno, Cosenza, Rando *et al* 1995). Comparative analysis of the first 200bp of the *CSN1S1* promoter regions shows a homology between goat and other ruminants (~96% with cattle, sheep and yak) and less with non-ruminants (88% rabbit, 80.5% human and 77% rat) (Ramunno et al., 2004). The main differences between the bovine and caprine species are clustered in the central part of the gene between introns 2 and intron 12 (Ramunno et al 2004).

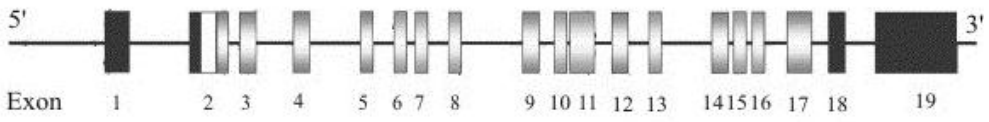

Figure 5: Structural organisation of the gene encoding *α*s1-casein (*CSN1S1*). Dark boxes at 5' and 3' ends represent un-translated exons. The white box as part of exon 2 highlights the region encoding the signal peptide which directs the protein to be secreted from the cell following translation. Figure adapted from Grosclaude *et al*, (1994).

CSN1S1 is the most variable of all the casein genes and the goat is the most polymorphic species studied at the *CSN1S1* locus to date. The first 7 protein variants were described in the early 1980's by Boulanger *et al* (1984) on the basis of their different electrophoretic motility. Now, with the expansion of molecular biology, over 18 *CSN1S1* variants have been identified at the genomic level in the goat. These variants can be grouped into those producing 'high' (3.6g), 'intermediate' (1.6g), 'low' (0.6g) and 'null' (trace amounts) of αs1-casein per kg of milk .

B1 is considered to be the original allele, from which A-type (A,G,I,H,01, and 02) and B-type (B2,B3,B4,C,E,F,L, and D) originated (Martin *et al*, 1999). The M and N alleles are thought to be the result of inter-allelic recombination events between A-type and B-type alleles (Bevilacqua et al., 2002, Ramunno et al., 2005). The proposed evolution of these alleles is illustrated below (Figure 6) and specific details regarding the mutation events leading to each variant outlined in Table 8.

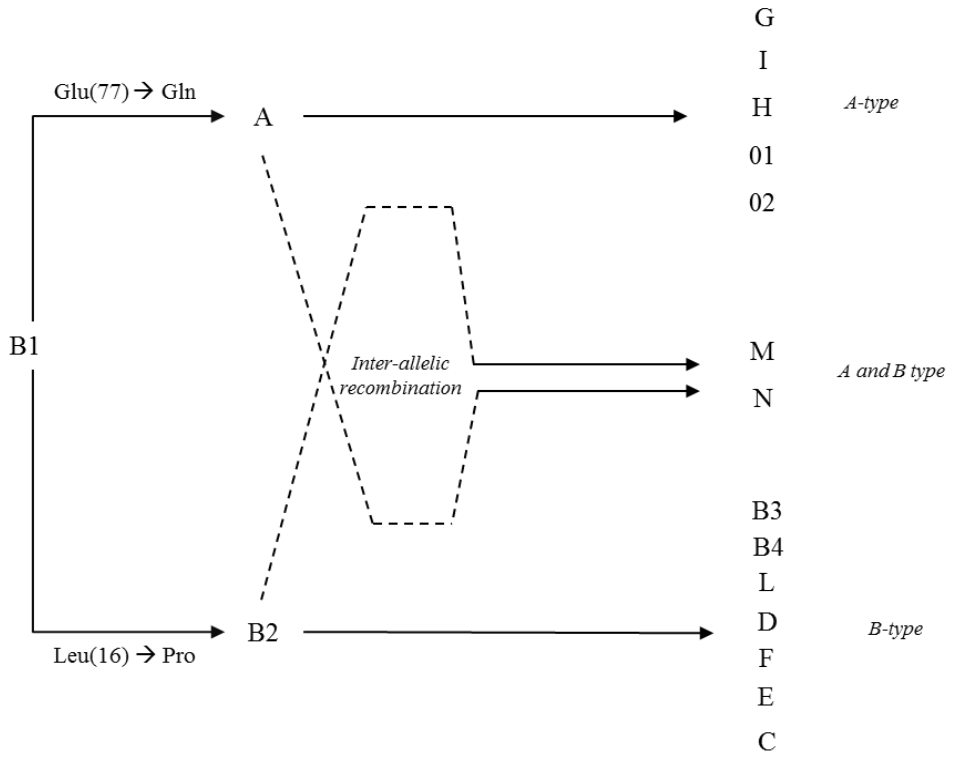

Figure 6: Schematic of proposed evolution of CSN1S1 alleles. Major amino acid changes between the A-type and B- type lineages are indicated. Adapted from Martin and Leroux (2000).

Table 8: *CSN1S1* allelic variants with the mutational event, functional change and predicted αs1-casein in the milk associated with each allele. Table continued over.

Allele	Mutational event	Amino acid / functional change	Predicted milk αs1-casein	Reference
A	Exon 10, nt 22: C→G	Glu(77)→Gln	High	(Boulanger et al., 1984, Brignon et al., 1990a, Ramunno et al., 2005)
B1	Original	'Reference' 199aa residues	High	(Grosclaude et al., 1997)
B2	Exon 4, nt 8: T→C	Leu(16)→Pro	High	(Boulanger et al., 1984, Brignon et al., 1990b, Grosclaude et al., 1997, Ramunno et al., 2005)
B3	Exon 4, 8th nt: T→C	Leu(16)→Pro. Arg(100)→Lys	High	(Grosclaude et al., 1997, Ramunno et al., 2005)
B4	Exon 4, 8th nt: T→C	Leu(16)→Pro. Arg(100)→Lys. Thr(195)→Ala	High	(Grosclaude et al., 1997, Ramunno et al., 2005)
C	Exon 4, 8th nt: T→C	Leu(16)→Pro. Arg(100)→Lys. Thr(195)→Ala. His(8)→Ile	High	(Boulanger et al., 1984, Brignon et al., 1990b, Ramunno et al., 2005)
H	unknown	Glu(77)→Gln. Arg(1)→Lys	High	(Chianese et al., 1997)
L	Exon 4, 8th nt: T→C	Leu(16)→Pro. Arg(90)→His	High	(Chianese et al., 1997, Ramunno et al., 2005)
M	Exon 9, 23rd nt C→T	Ser(66)→Leu. Loss of two phosphate groups within the major phosphorylation site.	High	(Bevilacqua et al., 2001, Chianese et al., 1997, Bevilacqua et al., 2002)
I	Unknown	Uncharacterised.	Intermediate	(Chianese et al., 1997)
E	Exon 4, 8th nt: T→C Exon 19, 124th nt: 457nt LINE insertion	Leu(16)→Pro. Arg(100)→Lys, Thr(195)→Ala. Instability and 3-fold reduction of mRNA	Intermediate	(Grosclaude et al., 1987, Perez et al., 1994, Ramunno et al., 2005)
D	Exon 4, 8th nt: T→C Unknown	Leu(16)→Pro. Deletion of 10 a.a (**59**-69). Abnormal processing of primary transcript, loss of multiple phosphorylation sites	Low	(Brignon et al., 1990b, Ramunno et al., 2005)

Allele	Mutational event	Amino acid / functional change	Predicted milk αs1-casein	Reference
F	Promoter region: nt 1319 mutation Exon 4, 8th nt: T→C Exon 4, 24th nt: C→G 24th Exon 9, 23rd nt:11bp insertion Intron 9: 3bp insertion Exon 10, 22nd nt: C→G Intron 14: 7bp deletion Exon 19 (3'UTR),132nd nt: T→C	Leu(16)→Pro. Premature stop codon in exon 12. Multiple alternatively spliced transcripts, mostly alternative skipping of exons 9-11. Deletion of 37 aa (59-95). Loss of multiple phosphorylation sites. Mutation in promoter region suggested creates an extra putative activator protein (AP-1) binding motif . 6x less mRNA transcribed than A allele.	Low	(Brignon et al., 1990b, Grosclaude et al., 1987, Leroux et al., 1992, Ramunno et al., 2005)
G	Transition G→A in 5' splice site consensus sequence of intron 4.	Glu(77)→Gln. Deletion of 13 aa (14→26). Alternatively spliced mRNA →outsplicing of exon4. Affects the proteins middle or N-terminal region. Same expression level as F.	Low	(Picariello et al., 2009, Martin and Leroux, 1994)
N	Exon 9, 23rd nt: deletion Promoter: G→A transition	1 nt frameshift → premature stop codon in exon 12. mRNA 1/3 of F despite same mutation. Alternatively spliced. AP-1 of F suggested responsible for different expression of F and N.	Null	(Ramunno et al., 2005)
01	Intron 12: last 7 exons deleted (8.5kb) Promoter: G→A transition	Non-functional protein.	Null	(Cosenza et al., 2003)
02	Large insertion, uncharacterised		Null	(Martin et al., 1999a, Leroux et al., 1990)

The most commonly studied *CSN1S1* variants are A, B, E, F, N and 01 due to their effect on milk composition and relative ease of identification. *CSN1S1* A and B alleles differ by just one amino acid substitution and are both associated with a high level of αs1-casein expression. *CSN1S1* allele E is characterised by a 457nt LINE insertion in the 3' UTR of the gene (Perez et al., 1994). Although un-translated, this AT-rich insertion is presumed to cause an instability of the mRNA and thus result in less αs1-casein than high *CSN1S1* alleles (Perez et al., 1994). The *CSN1S1* F variant is interesting due to multiple insertions that cause exon skipping and the production of alternatively spliced transcripts (Leroux et al., 1992, Ferranti et al., 1997, Ferranti et al., 1999). This results in a low level of αs1-casein expression and the production of only 0.45g/L of αs1-casein per allele (Martin et al., 1999a). N and 01 are null variants which produce no functional protein, resulting in no detectable levels of αs1-casein. This is due to a single-nucleotide deletion causing a premature stop codon in the N allele and an extensive 8.5kb deletion in the 01 allele (Cosenza et al., 2003, Ramunno et al., 2005).

With regard to fatty acid composition, significant differences between *CSN1S1* genotypes have been found in two studies. Pierre *et al,* (1998) compared A and O variants and found more short and medium-chain SFAs in A milk, but lower C16:0 content. Chilliard *et al.,* (2006) found that 'high' *CSN1S1* goats had more C8:0-C12:0 SFA, more stearic acid (C18:0), less palmitic (C16:0), oleic (C18:1 *cis*9), linoleic (C18:2n6) and CLA (C18:2n7) acids than 'low' goats. These authors also found that SCD desaturation ratios were higher in 'low' *CSN1S1* goats, suggesting these animals had higher mammary activity.

2.6.3 *CSN1S1* allele frequencies

NZ dairy goats are bred from a fairly limited stock, with very little use of artificial insemination or foreign bucks. Over 80% of DGC dairy goats are Saanens with the remaining breeds mostly Toggenburg, British Alpine and Nubian. Saanens are the predominant breed due to their higher days in milk and milk yield, making them better performers for dairy goat industry (Serradilla, 2001, Singireddy Sr, 1997). Toggenburgs are similarly strong dairy

goats, as are British Alpines which are renowned for their consistency and long lactations (Coleby, 2001).

CSN1S1 allele frequencies for any NZ dairy goat breed could not be found in the scientific literature. Based on overseas studies Saanens have a high frequency of 'intermediate' E and 'low' F *CSN1S1* alleles (Maga et al., 2009, Soares et al., 2009, Torres-Vázquez et al., 2008). Toggenburgs show a high average frequency of allele F (0.69) (Clark and Sherbon, 2000, Torres-Vázquez et al., 2008) while data on *CSN1S1* allele frequencies of the British Alpine breed (which is different to French Alpine) could not be found.

2.6.4 Methods for *CSN1S1* genotyping

Early analysis of *CSN1S1* was at the protein level, using isoelectric focussing (IEF), two dimensional electrophoresis, SDS-PAGE, immunoelectrophoresis, reverse-phase high-pressure liquid chromatography (RP-HPLC) and/or mass spectrometry (MS) to identify the different protein variants (Grosclaude et al., 1987, Jordana et al., 1996, Martin et al., 1999a, Leroux et al., 1990). Today, molecular techniques are used to genotype based on SNPs and mutations at the nucleotide level that lead to various αs1-casein phenotypes.

The restriction fragment length polymorphism (RFLP) technique was first used for genotyping goat caseins by Leroux *et al,* (1990). The most common RFLP is the use of restriction enzyme XmnI on exon 9 amplified products, originally used for *CSN1S1* genotyping by Ramunno *et al.*, (2000). This method allows simultaneous identification of A*, B*, N and F alleles. However it does not distinguish between A and G, H, I, 01 and 02 or B and B1, B2, B3, B4, B', E, C and L alleles as these have SNPs in other regions of *CSN1S1*. Further allele-specific PCRs (AS-PCR) are then needed to distinguish between variants of interest.

A key limitation in the genotyping of goats at the *CSN1S1* locus is that although the complete sequence of the gene encoding the goat αs1-casein has been determined by Rammuno et al (2004), only alleles A, F and N have been entirely sequenced (Table 9). Partial sequences exist for *CSN1S1* 01 (intron 12),

and E (exon 19), however full sequences for these and the other 12 un-sequenced variants have not been reported on public databases.

Table 9: Sequenced *CSN1S1* variants submitted to NCBI BLAST

Allele	Region sequenced	Length of sequence (bp)	Accession #
A	Entire (exons1-19)	19,408	AJ504710.2
F	Entire (exons1-19)	19,414	AJ504711.2
N	Entire (exons1-19)	19,406	AJ504712.2
E	Exon 19	437	FJ164044.1
O1	Intron 12	283	AJ252126.1

2.7 Purpose and Scope

This study will investigate how genetic factors (*CSN1S1* genotype) and nutritional factors (PKE, BW and yeast) affect NZ goat milk composition. The overall purpose is to enhance our understanding of these genetic and nutritional aspects of goat milk production, with specific reference to how they influence key components in goat milk.

Given the relevance of this research for the dairy goat industry, the study will take place a commercial setting involving DGC goat farms which are carrying out their everyday farming practice. The *CSN1S1* locus is chosen as the genetic factor of interest due to little knowledge of this gene in the NZ dairy goat population and its importance for milk parameters. For these reasons, alleles A, B, E, F, N and 01 will be the focus of *CSN1S1* genotyping.

In light of dietary changes recently implicated on DGC dairy goats, the effect of alternative supplements (PKE, BW and yeast) will be the focus of nutritional factor analysis. Milk volume, protein, fat and fatty acid composition parameters are the key milk components of interest due to their commercial implications and importance for human health.

Chapter 3: Genetic Methods

This genetic methods chapter outlines the processes used to select goats for genetic analysis, collect and extract DNA samples, genotype at the *CSN1S1* locus, correlate to milk composition data and determine statistical significance.

3.1 Sampling

Ethical approval for the collection of milk and hair samples was obtained from The University of Waikato Animal Ethics Committee (Protocol # 805). All provisos made by the ethics committee were adhered to throughout the course of this research.

DNA samples were taken from male (buck) and female (doe) goats from two Waikato farms; Farm A and Farm B. Both farms were feeding a combination of pasture, forage and grain and had similar breed compositions. 55 does and 9 bucks from Farm A and 68 does and 5 bucks from Farm B were selected for genotyping. Buck samples were taken from current season bucks used for breeding, while doe samples were chosen randomly from the order they walked into the milking shed. Only multiparous does between 2 and 8 years of age were used in the alignment of *CSN1S1* genotype to milk composition data.

Milk from each individual doe was collected by hand milking into a 15mL falcon tube (Greiner Bio-One). Hair samples were plucked from the rump of bucks (or does if not in milk) and placed into individual envelopes. All milk and hair samples were labelled with the doe or buck's farm identification number. Samples were transported to the laboratory and kept at 4°C until further analysis. DNA was extracted from all samples within 24hrs of collection.

3.2 DNA extraction from milk

Milk samples were centrifuged at 3000xg (Heraeus mμLtifuge 1_{S-R}) for 10 minutes, the fat rimmed off using a 200μL pipette tip and all supernatant discarded. Pellets were rinsed in PBS (NaCl 137mmol/L, KCl 2.7 mmol/L, Na_2HPO_4 10.0 mmol/L, KH_2PO_4, 2.0 mmol/L pH 7.4) to remove any residual protein and fat. Cells were re-suspended in 750μL 5M GITC (20mM sarkosyl, 30mM tri-sodium citrate, 0.7% mercaptoethanol, pH 7) by flushing up and down with a transfer pipette.

Suspended cells were transferred to a 1.7mL microtube (Axygen) and mixed on a rotator wheel (Global Science) for 10 minutes. 500μL of pH5.2 phenol (Sigma) was added to each sample, mixed vigorously by hand and placed back on the rotator wheel for 10 minutes. Following this 250μL of chloroform (Ajax Chemicals) was added, shaken vigorously and then placed on to the rotator wheel for another 5 minutes. These were then centrifuged at 16100rcf for 15 minutes using a bench-top centrifuge (Eppendorf 5415D). The aqueous layer was removed using a transfer pipette, placed into a new 1.7mL microtube along with 100μL of 3M NaAc (pH 5.2, Ajax Chemicals) and an equal volume of isopropanol (Ajax Chemicals). These were then inverted several times to mix and placed at -20°C for at least 30 minutes to allow the DNA to precipitate.

Samples were centrifuged at 16100rcf for 10 minutes. The supernatant was removed and pellets rinsed with 1mL of 70% ethanol (Ajax Chemicals, diluted with DEPC water). All ethanol was removed using an autopipette (Eppendorf) and sample tubes left open in fume cabinet for 20 minutes to evaporate. Pellets were re-suspended in 50μL of TE (10mM Tris, 1mM EDTA, pH 8) and vortexed to mix. The concentration (ng/μL) and purity (260/280 absorbance ratio) of the DNA was then detected using a ND-1000 spectrophotometer (Nanodrop).

3.3 DNA extraction from hair

Hair samples were trimmed using sterile scissors and placed into a 1.7mL microtube, bulb down. 350µL of lysis solution (100mM Tris pH9, 0.5% SDS, 50mM EDTA, 142mM NaCl) and 20µL of Proteinase K (20mg/mL, Invitrogen) were added to each tube and incubated at 56°C for 2-3 hours using an Eppendorf thermomixer.

Following incubation tubes were centrifuged briefly (10 seconds) to draw hair to bottom and enable easy removal of hair from liquid. Hair was discarded and an equal volume of 5M LiCl (Ajax Chemicals) was added to the remaining solution. This was inverted and an equal volume of chloroform added, vigorously mixed by hand and placed on the rotator mixer for 15 minutes.

Samples were then centrifuged for 10 minutes at 16100rcf. The top aqueous layer was removed using a transfer pipette and placed into a new 1.7mL microtube. An equal volume of isopropanol was added, samples were inverted and put at -20°C for at least 30 minutes to allow DNA to precipitate.

Following precipitation samples were centrifuged for 10 minutes and the supernatant carefully removed using a transfer pipette. The pellet was washed with 70% ethanol and tubes left open to air-dry in fume-cupboard for approximately 30 minutes. DNA was then re-suspended in 20µL of TE and measured using the Nanodrop spectrophotometer as per the quantification of DNA extracted from milk.

3.4 DNA purification

Any samples extracted from milk or hair which did not render sufficient DNA quality (a 260/280 ratio of less than 1.5 or an excessive peak at 230nm) underwent CTAB clean up. Any pellets which were large, bright white and difficult to dissolve were cleaned up by the SDS method.

3.4.1 CTAB DNA clean-up

470µL of TE and 30µL of 10% w/v SDS (Roche) was added to 100µL of DNA. This was incubated at 65°C and mixed at 800rpm for 10 minutes. 100µL of 5M NaCl (Ajax) was added along with 80µL of pre-warmed CTAB (10% w/v CTAB in 0.7M NaCl) and further incubated at 65°C, 800rpm for 10 minutes. An equal volume of chloroform was added, mixed vigorously by hand and then placed on the rotator mixer for 10 minutes. Samples were then centrifuged at 16.1 rcf for 15 minutes and the top aqueous layer collected using a transfer pipette. An equal volume of isopropanol was added and the same final precipitation methods described for previous extractions were applied. Samples were re-suspended in 50µL of TE.

3.4.2 SDS DNA clean-up

470µL of TE and 30µL of 10% SDS were added to 100µL of DNA. This was incubated at 65°C and mixed at 800rpm for 10 minutes. An equal volume of 5M LiCl was added and inverted to mix, followed by the addition of double the volume of chloroform. The solution was vigorously mixed by hand and placed on the rotator mixer for 15 minutes. Samples were then centrifuged for 15 minutes, the aqueous layer removed and an equal volume of isopropanol added. The same final precipitation methods described for previous extractions were applied. Samples were re-suspended in 20µL of TE.

3.5 General *CSN1S1* genotyping methods

The following sections outline the general methods used to genotype at the *CSN1S1* locus. Methods specific to each exon are outlined in later in section 3.6.

3.5.1 Primers

Primers were synthesised by Integrated DNA Technologies Ltd and diluted to 200pmol/µL in TE (10mM Tris, 1mM EDTA, pH8). Working primer solutions were made by diluting the 200pmol stock in TE to make a final concentration of 20pmol/µL. For most reactions, all primers (forward and reverse) were mixed in the one 20pmol/µL working solution. Individual primer sequences for each exon are outlined in section 3.6.

3.5.2 PCR

PCR reactions (ranging from 20µL-50µL) were carried out in 200µL PCR tubes (Axygen). Each reaction mix contained 250µM dNTPs (Invitrogen), 2.5 mM $MgCl_2$ (Ajax Chemicals), 0.6U Hot-fire Pol® Taq DNA polymerase (Solis BioDyne), 1x HotFirePol B1 PCR Buffer (Solis BioDyne), 1 ng template DNA and 5pmol of each forward and reverse primer. The reactants were put in a thermal cycler (Bio Rad), which carried out the appropriate PCR program.

3.5.3 Agarose Gel Electrophoresis

PCR products were electrophoresed on TAE (0.04M Tris-Acetate, 0.001M EDTA) agarose (SeaKem®) gels, stained with 0.5µg/mL EtBr (USB), ranging from 2-4% (w/v) depending on the exon amplified. 5-10µL of PCR product was mixed with 2µL of loading dye (0.05% Bromophenol Blue, 0.05% Xylene Cyanol, 6% Glycerol) and loaded into the gel. Gels tanks were filled with TAE buffer and gels electrophoresed at 90-110V. Product base-pair lengths were compared to a 100bp ladder (2-3% gels) or 20bp ladder (4% gels). Both ladders were supplied by Solis BioDyne. All gels were visualised using UV light (Life Technologies) and images captured using COHU High Performance CCD camera.

3.5.4 Product removal from agarose gels

Gel-punching was used to remove bands separated by gel electrophoresis in cases where unknown PCR products resulted. PCR products were electrophoresed on a 4% agarose gel stained with 0.05µL/mL of 20,000x Red-safe™ dye (Intron Biotechnology). Gels were visualised using Safe Imager™ (Invitrogen) and bands punched using an X-Tracta Gel Extraction Tool (Sigma-Aldrich). Punched bands were cleaned up using the following freeze-thaw method.

For each sample a 0.6mL microtube was punctured at the base by inserting a flamed 19_G1 needle (Becton Dickinson). A 3mm glass bead (Ajax Chemicals) was placed inside the 0.6mL tube which was then put inside a 1.7mL microtube. The punched gel fragment was inserted into the 0.6mL tube and frozen at -80°C for 30 minutes. Tubes were removed and thawed at room temperature for 30 minutes and then centrifuged at 16100rcf for 15 minutes. The 0.6mL tube and its contents were discarded and the liquid captured in the 1.7mL tube was cleaned up using the following Polyethelene Glycol (PEG) precipitation method.

An equal volume of PEG solution containing 20% PEG 8000 (AppliChem) in 2.5M NaCl was added to the liquid, vortexed to mix and left to stand at room temperature for 10 minutes. Samples were centrifuged at 16100rcf for 10 minutes, the supernatant removed and the pellet rinsed in 70% ethanol. Tubes were left open to air-dry and product was re-suspended in 15µL of MilliQ-H_20 (Barnstead). Samples were then re-amplified using the same primers and PCR they were originally extracted from to ensure they yielded a single band and then sent for sequencing.

3.5.5 Cloning

Where direct sequencing was unsuccessful, cloning was used prior to DNA sequencing in order to attain cleaner, full length sequences.

One *E. coli* (DH5α) colony was inoculated into Luria Base (LB) broth (Invitrogen) and incubated at 37°C, 200rpm overnight. For each sample a ligation mix was made containing 5.5µLwater, 1µL 10x T4 DNA Ligase Buffer (Fermentas), 1µL KS/SK+ vector (Bluescript), 2.5U T4 DNA Ligase (Fermentas) and 2µL of PCR product. The ligation solution was vortexed and incubated at 22°C for 10 minutes then kept at 4°C overnight.

The following day 1.5mL of DH5α culture was removed, placed into a 1.7mL microtube and centrifuged at 16.1rcf for 1 minute. The supernatant was discarded and 100µL of transformation solution (0.1M CaCl2, 1% PEG 8000) was added. Cells were re-suspended by flicking the tube and placed on ice for 5 minutes. All 10µL of ligation mix was then added to the microtube, flicked to mix and placed on ice for 20 minutes. This was followed by precisely 1.5 minutes at 42°C then on ice for another 5 minutes.

1mL of sterile LB broth was added and incubated at 37°C for 1hr, mixing at 9rpm. Samples were centrifuged for 1 minute at 16100rcf and the supernatant removed. 100µL of sterile LB broth was added to re-suspend the pellet and flicked to mix. All of the solution was spread onto an LB+ agar plate (2.5% w/v Luria Base Broth, 1.5% w/v agarose, 100µg/mL ampicillin) which 3 hours prior to transformation was incubated with 40µL of Xgal (20mg/mL, Invitrogen), and 4µL IPTG (8uM, Fermentas). Each plate was incubated at 37°C overnight.

Next day plates were checked for blue and white colonies. If not visible, plates were incubated for a further 4 hours and placed at 4°C for 30 minutes to better visualise colonies. Using a sterile pipette tip single white colonies were selected and streaked onto LB+ plates which were incubated at 37°C for 24hrs.

The following day individual colonies were removed from the LB+ plate using a sterile loop (Raylab) and placed in a 1.7mL microtube with 100µL of PEG-KOH lysis solution (60% PEG200, 18.6 mM KOH, pH 13.5). The solution was mixed and incubated at 95°C for 5 minutes.

2µL of lysed cells were added to a 50 µL PCR reaction mix containing 250µM dNTPs, 2.5 mM $MgCl_2$, 1x HotFirePol B1 PCR Buffer, 1.25U HOT FIREPol® DNA Polymerase and 5pmol of each T7 / T3 primer (IDT). PCR conditions were 95°C for 15 minutes, followed by 39 cycles of 95°C for 20 seconds, 55°C for 20 seconds, 72°C for 30 seconds, and then a final extension of 68°C for 5 minutes. Products were run on a 1.5% agarose gel stained with EtBr and run in TAE buffer at 120V for 30 minutes.

3.5.6 DNA sequencing

One sample of each genotype was sent for DNA sequencing to check the correct region was amplified and to compare sequences to previously sequenced variants. PCR products were purified by adding 10U of ExonucleaseI (Fermentas) and 1U of Alkaline Phosphatase (Roche) to 20µL of PCR product. This was incubated at 37°C and mixed at 750rpm for 30minutes. Deactivation was initiated by incubating at 85°C for 10 minutes.

Sequencing was conducted by the Waikato DNA sequencing facility (Hamilton, New Zealand) using an Applied Biosystems 3130xl Genetic Analyzer. Both forward and reverse sequencing reactions were conducted. Sequences were analysed using Applied Biosystems software, FASTA sequence comparison and BLAST online tools.

3.6 *CSN1S1* exon-specific genotyping methods

All DNA from hair and milk samples was PCR amplified over the exon 9 region and digested with XmnI for preliminary genotyping. Depending on the exon 9 product, subsequent PCRs were carried out at exon 12 and / or exon 19 regions to determine the final *CSN1S1* genotype.

3.6.1 Exon 9

Primers for the amplification of exon 9 were the same as those used in other goat *CSN1S1* genotyping studies designed by Ramunno *et al.,* (2000). Primer sequences are outlined below in Table 10 and the PCR reaction protocol in Table 11. Product lengths were expected to be between 212 - 224bp depending on the genotype.

Table 10: Primers used in the amplification of *CSN1S1* exon 9.

Primer	Primer Sequence (5'-3')
Exon9 F	TTCTAAAAGTCTCAGAGGCAG
Exon9 R	GGGTTGATAGCCTTGTATGT

Table 11: Touch-down PCR cycling conditions for amplification of *CSN1S1* exon 9. Reactions were carried out in 25µL volumes.

Step		Conditions	No. Cycles
1.	Activation	95°C for 15minutes	1
2.	TD-PCR	95°C for 20sec, 65°C for 20sec (decreasing by 1°C per cycle), 72°C for 30sec	10
3.	PCR	95°C for 20sec, 55°C for 30sec, 72°C for 30sec	35
4.	Final extension	68°C for 5 minutes.	1

5µL of PCR product was electrophoresed on a 2% TAE agarose gel, with the remaining 20µL digested with 2U of XmnI (New England Biolabs). The digestion was carried out in a thermomixer (Eppendorf) for 4 hours at 37°C followed by deactivation at 65°C for 20minutes.

The XmnI recognition site occurs at 5'GAANN≠NNTTC 3' (NEB). The cytosine at the 3' end is deleted in F and N variants, meaning these alleles do not to cut under XmnI. Alleles A (including G, H, I, 01 and 02) and E (including B,C and L) do have the cytosine in position 23 of exon 9, creating the restriction site.

Digested samples were electrophoresed on a 4% (w/v) agarose TAE gel stained with EtBr at 90V for 2-3 hours. An additional 10µL (0.1mg) of EtBr was added to gel running buffer (TAE) to better visualise products. Product base-pair lengths were compared to a 20bp ladder (Invitrogen).

3.6.2 Intron 12

Samples which produced a band at 150bp following exon 9 PCR-RFLP were subsequently amplified at intron 12 to distinguish between A* and 01 variants. The 8.5kb deletion characterising the 01 allele begins within intron 12 and can be detected using primers designed by Sztankòová *et al.,* (2006) outlined in Table 12.

Table 12: Primers used in the amplification of exon 12 for A/01 variants. Primer A is at the beginning of intron 12 and amplifies both A and 01 alleles. Primer B is specific to the 01 allele and amplifies in the reverse direction Primer C is the reverse primer for allele A.

Primer	Primer Sequence (5'-3')
Intron12 A	CCCCAGCTGGTAATGTTTTA
Intron12 B	GGTCCATCAATTCCCTGTGT
Intron12 C	TGTATGGATCCCTGATTCCTT

PCR conditions were as follows: An initial denaturation of 95°C for 15 minutes, followed by 35 cycles of 95°C for 20 seconds, 62°C for 35 seconds, 72°C for 30 seconds and a final extension of 68°C for 5 minutes. Products were electrophoresed on a 3% TAE agarose gel at 100V for 30 minutes. Base-pair lengths were expected to be 249-281 depending on the *CSN1S1* genotype.

3.6.3 Exon 19

Genotyping at exon 19 was essential for samples which produced a band at 161bp following PCR-RFLP of exon 9 to distinguish between B*(B1-B4, B',C, L) and E variants. *CSN1S1* E alleles have a 457nt AT rich insertion which is not present in the B* allele and can be identified through standard PCR or real-time PCR.

Standard PCR of exon 19 was conducted using primers designed by Dettori *et al.*, (2009) (Table 13). PCR conditions were altered from the original paper by extending initial activation and lowering the annealing temperature. This was done to suit the DNA polymerase and produce better quality product. Refined conditions were 95°C for 15 minutes, followed by 30 cycles of 95 °C for 20 seconds, 55°C for 30 seconds, 72°C for 40 seconds, with a final extension step at 72°C for 5 minutes.

Table 13: Primers used for the amplification of Exon 19. The F primer is at the beginning of exon 19 and amplifies both A and E variants. The reverse primer occurs after the LINE insertion while the LINE primer is internal to the LINE insertion characterising the E-allele, but not in an AT-rich region.

Primer	Primer Sequence (5'-3')
Ex19 F	TCAGGAGCAGTGGGTATGTG
Ex19 R	CCTCCCAATGGAATAATGACA
Ex19 LINE	TGTTTGGGAACGCATGTAAG

PCR products were electrophoresed on a 2% TAE agarose gel, as described in the general methods (section 3.5). Product lengths were expected at 437-583bp depending on the genotype.

Real-time PCR and melt curve analyses were used as an alternative method for distinguishing between E and B* variants. The PCR reaction mix remained the same as described in general genotyping methods, however less primers (0.01pmol), more DNA Polymerase (0.625U) and 0.025mM of Syto82 fluorescent dye (Invitrogen) were added to each reaction.

Real-time primers were different to those used in exon 19 standard PCR to improve the efficiency of real-time PCR by reducing product lengths. Two primer sets (designed by Feligini *et al.*,(2005)) were used in separate reactions: F1/R and F2/R. The F1 primer occurs in the region of consensus between B* and E alleles before the LINE insertion and thus amplifies both alleles while the F2 primer is internal to the LINE insertion and specific to the E-allele.

Table 14: Real-time PCR primers used in the amplification of exon 19.

Primer Name	Primer Sequence
RT F1	CAACCTCAAATTGAAGGCACT
RT F2	TGGTGTTTTTCTTTCTGGCTTA
RT R	CAAGCTCTTAGGACAATTTCA

Real-time PCR was conducted using a Corbett Rotor Gene 6000 Real-time PCR machine. Cycling parameters were: 95°C for 15 minutes, followed by 45 cycles of 95°C for 15 seconds, 51°C for 15 seconds, 72°C for 30 seconds (acquiring on cycling A, yellow) and 80°C for 10 seconds (acquiring on cycling B, yellow). Following PCR, products were melted from 60°C to 95°C, rising by 0.5°C each step.

The cycle threshold (Ct) value was set at the steepest part of the curve with values before approximately cycle 15 eliminated. Melt curve dF/dT thresholds were set below the main peak of the lowest negative sample while the temperature threshold was set just prior to the first main peak. The method used for genotyping *CSN1S1* E variants based on Exon 9 PCR-RFLP results and real-time PCR results is outlined in (Table 15).

Table 15: Method for genotyping using exon 19 Real-time PCR. Average Ct and melt temperatures (°C) values for each genotype are given. 'E' alleles in the exon 9 PCR-RFLP g/t column are either E or B*. B* include alleles B1-B4, B', C or L. Any sample with a Ct higher than the average negative Ct was withdrawn from Real-time analysis. G/t= genotype. N/r = not required for genotyping.

Exon 9 PCR-RFLP g/t	F2/R primers		F1/R average melt temp	Final Real-time g/t
	Average melt temp	Possible g/ts		
AE	77.5	AE	n/r	AE
AE	81.5	AB	n/r	AB*
EE	77.5	EE or BE	> 77	B*E
			74-76.5	EE
EF	81.5	BF	n/r	B*F
EF	77.5	EF	n/r	EF

Following real-time amplification, products were electrophoresed a 2% agarose gel and selected samples sent for sequencing to verify that the product of interest was reproduced. *CSN1S1* E allele products using F2/R were expected at 90bp while A alleles do not produce any discernible product using this primer set. F1/R primers amplify allele A at 90bp and allele E at 549bp.

3.7 Milk composition analysis

Whole herd tests were carried out by Livestock Improvement Corporation (LIC) in accordance with standard herd testing protocols. Milk from individual goats was automatically siphoned off using wide-bore milk meters provided by LIC. The quantity partitioned into sample cups depended on the volume from each goat to the main vat milk. Sample cups were lined with bronopol preservative to prevent bacterial contamination influencing component analysis results. Samples were labelled with the goat identification number and sent to LIC for milk volume, protein, fat, solids and somatic cell count (SCC) analysis using an automatic milk analyser.

Samples for fatty acid analysis were collected by hand-milking. The first secretions of milk were directed to waste and then approximately 50mL was collected in a 50mL tube (Sterlin) labelled with the individual goat number. Samples were immediately frozen and then sent to AgResearch (Hamilton, New Zealand) for fatty acid analysis once the dietary samples were also collected and ready for fatty acid analysis.

3.8 Data analysis

CSN1S1 genotypes from both doe and buck samples were used for allele and genotype frequency data. Hardy Weinberg calculations were conducted for genotype frequencies on each farm using the conventional test for HWE based on chi-squared statistic. Farm A's p-values for chi-square analysis were calculated with 4 degrees of freedom (9 genotypes – 5 alleles) while Farm B had 3 degrees of freedom (8 genotypes – 5 alleles).

Alignment to milk composition data was done using STATISTICA. Farm A had four herd tests from November-March for feed trial purposes, however for genotype analysis only November data was used as this was when all goats were on the same dietary treatment (no yeast supplementation) and at a similar stage of lactation to Farm B. Herd test data from farm B was collected early in lactation (October). For these and other varying factors between farms (such as

quantity and quality of pasture, farm management and location), data for compositional analysis was not pooled between the two farms.

Data was checked for normality using the Shapiro–Wilk test using STATISTICA software. ANOVA was used to determine significant differences between genotypes for each milk parameter. A p-value <0.05 was considered significant, with a p-value <0.01 highly significant.

Chapter 4: Genetic Results

The following chapter outlines the results of goat DNA extraction, *CSN1S1* genotyping and the effect of genotype on goat milk composition.

4.1 DNA extraction

Genomic DNA was isolated from 24 hair and 129 milk samples sourced from two Waikato dairy goat farms. 91% of samples were taken from Saanen goats, the breed which made up the majority of both herds. A small number of British Alpine x Saanen, Toggenburg x Saanen, Nubian x Saanen and Nubian were also sampled (Appendix B).

DNA extracted from milk and hair ranged in concentration from 1.5ng/µL to over 3000 ng/µL and with a variety of purities. 40 samples required CTAB clean-up while 13 required SDS. 25 were removed due to low DNA yields (<5ng/µL), lab error or insufficient purity for PCR amplification. A summary can be found below in Table 16, with full extraction records in Appendix B.

Table 16: Summary table of DNA extraction from goat milk and hair.

Farm	Sample type	No. extracted	No. withdrawn	Final no. available for genotyping
A	Milk	66	13	53
A	Hair	9	4	5
B	Milk	63	4	59
B	Hair	15	5	10
Total		**153**	**26**	**127**

4.2 Identification of *CSN1S1* alleles

The identification of *CSN1S1* genotype was carried out by investigating polymorphisms in exon 9, intron 12 and exon 19.

4.2.1 Exon 9

PCR amplification of exon 9 produced fragments at 224, 223, 213 and 212 bp (Figure 7). Based on other studies these fragments can be identified as *CSN1S1* allele F (223bp) and either E,B,C or L at 224bp. *CSN1S1* allele A (including G H, I, 01 and 02) produced one band at 213bp while the *CSN1S1* N allele attained product at 212bp. All exon 9 PCR products were sequenced to confirm these variants (section 4.2.4).

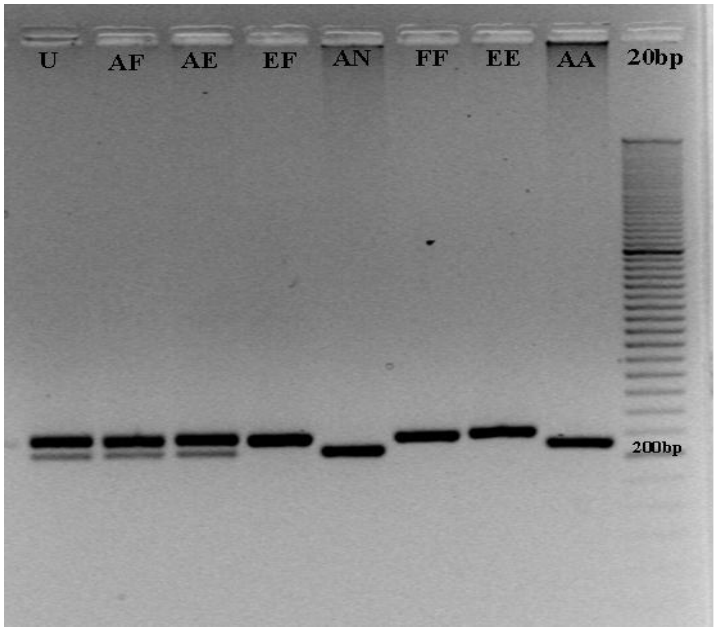

Figure 7: Exon 9 prior to digestion. PCR products were electrophoresed on a 4% agarose gel, stained with EtBr, run at 90V in TAE buffer for three hours. A-variants include A,G,H,I,01 and 02 while E variants include B1-B4,C,E and L. U= unknown genotype.

CSN1S1 F and N alleles did not digest under XmnI and produced fragments at the same bp lengths as their undigested products (223 for allele F and 212 for allele N). *CSN1S1* E(B,C,L) variants cut to produce two bands, 161 + 63bp in length. A(G,H,I,01,02) variants also cut with bands at 150 + 63bp (Figure 8).

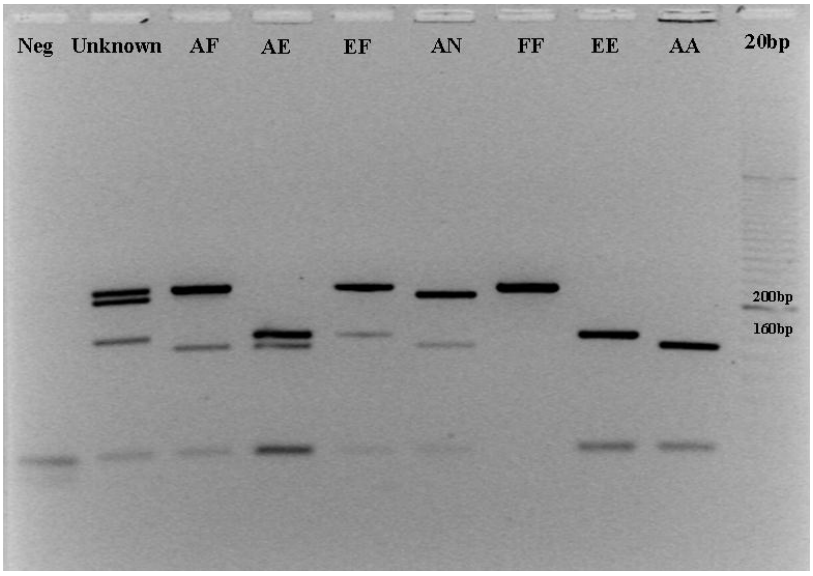

Figure 8: Exon 9 following digestion with XmnI. Products were electrophoresed on a 4% agarose gel, stained with EtBr, run at 90V in TAE buffer for 3.5 hours. A-variants include A, G H, I, 01 and 02 while E-variants include B1-B4, C, E and L. The band present in the negative sample represents primer-dimers which are similar in size to the 63bp products of cut samples.

Therefore amplification of exon 9 allowed the identification of A(G,H,I,01,02), E(B,C,L), F and N alleles. A full list of all genotypes resulting from exon 9 amplification and digestion can be found in Appendix C. Further PCRs were required to distinguish between important alleles grouped together at exon 9. Amplification at intron 12 was necessary to distinguish between A*(G,H,I,02) and the null 01 variant. PCR of exon 19 was required to separate E (medium αs1-casein) from B* alleles (B1-4, B', C and L) which produce high αs1-casein.

4.2.2 Intron 12

All 52 samples which carried an A* variant were screened for 01. *CSN1S1* A* alleles were successfully amplified over intron 12, as identified by product at 281bp. No *CSN1S1* 01 variants (expected to be 249bp in length) were detected.

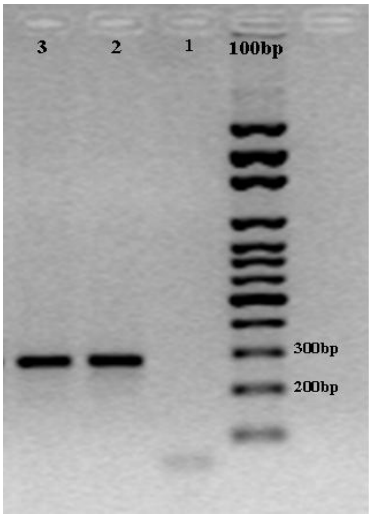

Figure 9: Intron 12 PCR products. Electrophoresed on a 2% agarose gel, stained with EtBr, run at 100V in TAE buffer for 45 minutes. Lane 1, negative. Lanes 2 and 3, A*-allele positive samples (281bp).

4.2.3 Exon 19

77 samples required E versus B* genotyping. The presence of the 457nt AT-rich LINE insertion in the E-variant made it difficult to discriminate between B* and E variants using ordinary PCR. Amplification using primers specified by Dettori *et al,* (2009) did produce B*allele product at 583bp and product from the E allele at 437bp. However the PCR was precarious as carriers of the E allele amplified variably. Conducting gradient PCRs on B*B* and EE variants showed that reducing annealing temperatures improved E allele amplification, however E variants still amplified poorly using this method (Figure 10).

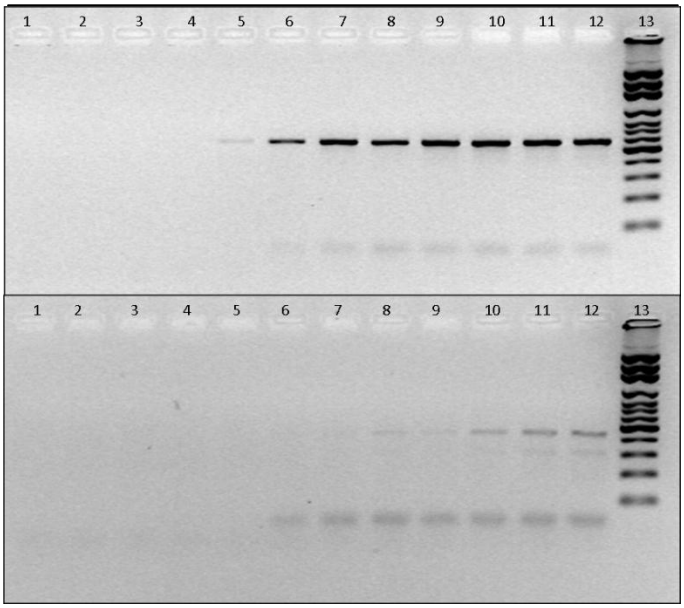

Figure 10: Gradient PCRs of B*B* (top) and EE (bottom) samples at identical temperatures showing the E variant is poorly amplified at temperatures suited for A variants. Lane 1 = 65.0°C, 2= 64.8°C, 3=64.3°C, 4=63.5°C, 5=62.4°C, 6=61.0°C, 7=59.3°C, 8=57.8°C, 9=56.7°C, 10=55.9°C, 11=55.3°C, 12=55.0°C 13= 100bp ladder. Products were electrophoresed on a 2% TAE agarose gel for 30 minutes.

Employing real-time PCR and melt-curve analysis allowed more reliable and efficient differentiation E and B* variants. For 80% of samples, only the F2/R primer set was required to complete genotyping. *CSN1S1* genotypes that carried an E allele produced a distinct peak at approximately 77.5°C whereas A, B, F and N homozygotes produced a melt curve at around 81.5°C (Figure 11). Negative controls melted at approximately 75.5°C with a low peak and high ct value.

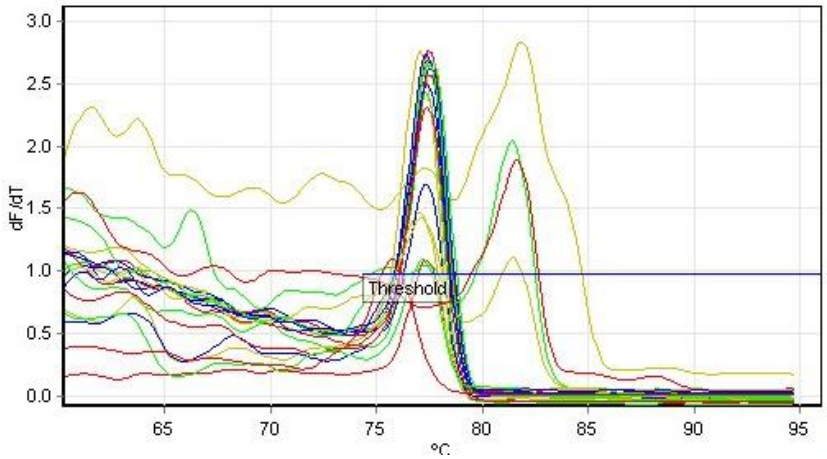

Figure 11: Real-time PCR melt curves using F2/R primer set. Samples carrying an E-allele had a peak at 77°C while those without an E had a peak at approximately 81.5°C. Threshold is set below the negative samples, which melt at around 75°C.

14 samples required amplification with the F1/R primer set as EE and BE genotypes could not be discriminated using the F2/R primer set and exon 9 PCR-RFLP alone. The F2/R primers produced less distinct results (only one degree difference) however was sufficient to allow genotyping (Figure 12).

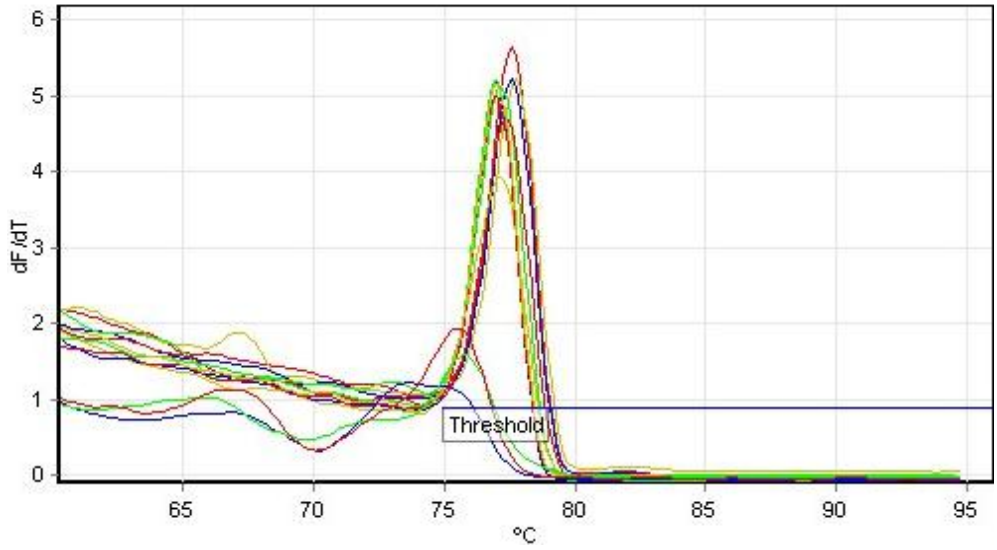

Figure 12: Real-time melt curves resulting from amplification with F1/R primer set.
Negative samples melted at around 75°C, EE at 76°C and BE >77°C

Selected 'unknown' genotypes were also analysed through real-time to aid in their characterisation. Unknown genotype samples were positively identified as carrying an E-allele using real-time PCR.

One sample from non-E genotypes (AA, AF, AN) were run as positive controls for method validation. None of these three genotypes produced E-allele real-time profiles.

Multiplexing all primers could be used for B* E discrimination, however it was more difficult to determine genotypes as the melt curves for each genotype were similar. Moreover both primers produced product at 90bp for E (using F1/R) and non-E alleles (using F2/R). All gel images, melt temperatures and Ct values used to genotype via real-time PCR can be found in Appendix D.

4.2.4 Verification of CSN1S1 genotypes via sequencing

All PCR reactions amplified the correct region, as shown through the alignment of sequenced PCR products to documented sequences of the goat *CSN1S1* gene. These positive identifications and unique sequence findings are outlined in sections 4.2.4.1- 4.2.4.7 below.

4.2.4.1 Exon 9, A allele sequences

Sequencing of AA genotyped samples returned a 100% alignment with the documented *Capra hircus CSN1S1* gene for alpha s1 casein, allele A (AJ504710.2). The region which matched documented sequences was from 9879-10031 which extended from the end of intron 8, over exon 9 (9864-9896) and to the beginning of intron 9. The exon 9 primers allowed the detection of the cytosine at position 9886 of the A allele which causess genotypes carrying the A allele to cut with XmnI digestion. The alignment can be found in Appendix E.

4.2.4.2 Exon 9, F allele sequences

Sequencing of selected FF samples yielded a 100% alignment to *Capra hircus CSN1S1* gene for alpha s1 casein, allele F (AJ504711.2). The alignment was from 9881-10040 of the F variant, which similar to the *CSN1S1* A-allele covered intron 8 through to intron 9. Importantly this sequence detected the 11bp insertion, as well as the deletion of the cytosine at position 9886 characterising the F-allele. The sequence alignment can be found in Appendix E.

4.2.4.3 Exon 9, E allele sequences

As outlined in Chapter 2, no sequences have been submitted to BLAST characterising the E allele at exon 9. Sequencing results of EE homozygotes aligned best to *Capra hircus CSN1S1* gene for alpha s1 casein, allele F (AJ504711.2). Like *CSN1S1* allele F, the 11bp insertion was present, however unlike the F-allele the cytosine at position 9888 was not deleted (highlighted in Figure 13 alignment below). The presence of this cytosine will cause genotypes carrying the E allele to cut under XmnI digestion.

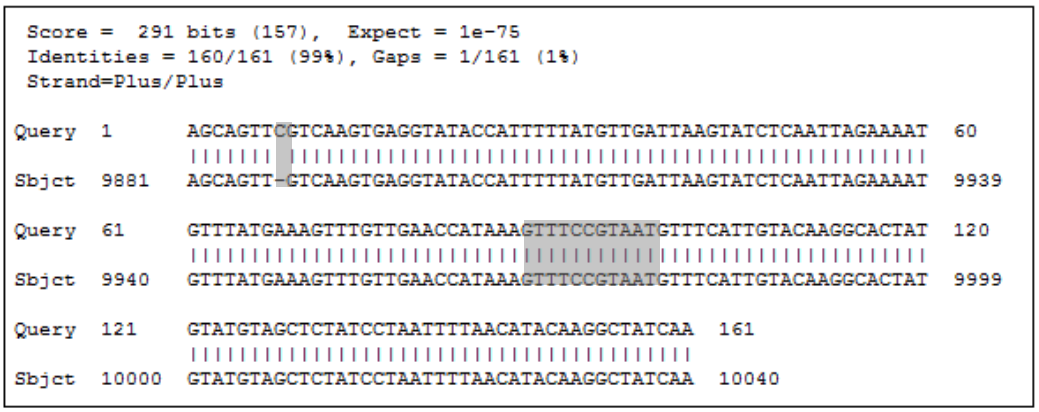

Figure 13: Sequence alignment of EE sample (Query) to F allele (Subject) using BLAST. Note the presence of the 11bp insertion typically characterising the F allele (highlighted**)** as well as the cytosine present in the E-allele but absent in the F.

4.2.4.4 Exon 9, N allele sequences

Only heterozygotes for the N allele were found in the samples genotyped and AN heterozygotes only sequenced successfully in the reverse direction. The sequence partially aligned with both A and N variants (9973-9987 allele A) and (9971-9885 allele N), meaning the correct region was amplified. However the second half of the reverse sequence returned data which did not match any BLAST sequence (Figure 15). This was due to the reverse sequence of the N-allele having the guanidine deleted (which would be the cytosine at position 9886 on the forward strand), meaning that all of the sequence after the deletion was shifted by one nucleotide (Figure 15).

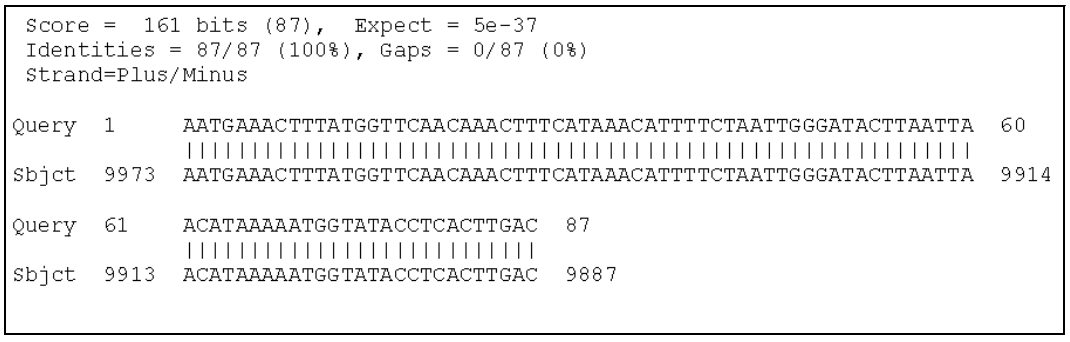

Figure 14: BLAST sequence alignment of AN heterozygote 'clean' sequence in the reverse direction (Query), aligned to documented allele A sequence (Sbjct). This sequence covers intron 10 and half of exon 9.

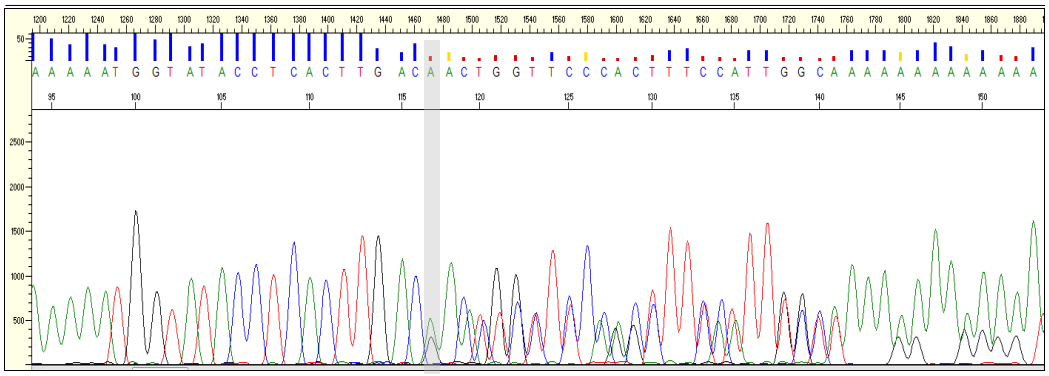

Figure 15: Electropherogram results showing the transition from clean sequence to 'double peaks' following the deletion of guanidine from the reverse strand). Note the sequence is in the reverse direction (left-right intron 10, exon 9, intron 9). Image from sequence data analysed in Applied Biosystems sequence scanner software.

4.2.4.5 Intron 12 sequences

PCR of intron 12 did amplify the correct region, as shown through the 100% identity with *CSN1S1* allele A (AJ504710.2) from position 12253-12448. Intron 12 starts at 12193-12835 in the A allele, and the deletion characterising the 01 variant begins at 181st nucleotide (12374) of 12th intron extending nearly 8.5kb (Cosensa *et al.*, 2003). The sequence data covered this region and therefore PCR of intron 12 should detect the deletion. No samples were found containing the 01 variant and therefore could not be sequenced.

```
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 Identities = 196/196 (100%), Gaps = 0/196 (0%)
 Strand=Plus/Plus

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Sbjct  12253  TTTACTTTGACTAAATTCTACATCAAATCATGTTAGAGCTTCCCCAATGATTCATTATAT  12312

Query  61     TCTGGAATTTATTCTGTATTTATTTTCCCAAATTCAGTTCTGAGTGGTGATTCCATACAT  120
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  12313  TCTGGAATTTATTCTGTATTTATTTTCCCAAATTCAGTTCTGAGTGGTGATTCCATACAT  12372

Query  121    ATGCCTCCTTGTGAAAGCACAGATCATTGCCAAATAAACATTCCATTTAAAAAACAATGT  180
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  12373  ATGCCTCCTTGTGAAAGCACAGATCATTGCCAAATAAACATTCCATTTAAAAAACAATGT  12432

Query  181    AGACTCTGTAGAAGGA  196
              ||||||||||||||||
Sbjct  12433  AGACTCTGTAGAAGGA  12448
```

Figure 16: BLAST sequence alignment of intron 12 sequence of A genotyped samples (query) to the documented CSN1S1-A-allele (subject). The sequenced region covers intron 12 and thereby intron 12 primers have amplified the correct region.

4.2.4.6 Exon 19, sequences

The B* allele attained quality sequences which aligned with exon 19 of the *Capra hircus CSN1S1* gene for alpha s1 casein, allele A (AJ504710.2). At exon 19, all non-E variants are the same, hence why B* variants matched *CSN1S1* A allele sequences on BLAST (no B allele sequences have been submitted to this database). Using sequences from forward and reverse primers, the sequenced region extended from intron 18 to the very end of the *CSN1S1* gene (end of exon 19). The sequence alignment can be found in Appendix E.

Exon 19 of the E-allele proved difficult to sequence due to the presence of an AT-rich LINE insertion. Despite cloning EE samples prior to sequencing, poor sequence quality remained. Only the forward primer returned useable sequences, which were 'clean' up until the LINE insertion (Figure 17 and Figure 18). The clean region aligned to the documented *CSN1S1* E-allele exon 19 sequence (AJ504712.2). The position of sequence quality change matched where the LINE insertion begins in the documented sequence (position 287). A and T signals dominated the region of poor sequence quality. For these reason this sequence was deemed sufficient to confirm the presence of the *CSN1S1* E-allele in such samples.

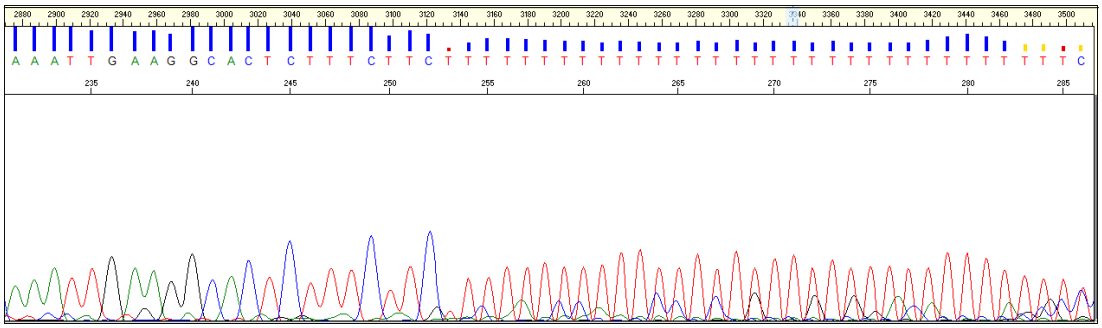


Figure 17: Electropherogram of E-allele sequence data. Image from sequence data viewed in Applied Biosystems sequence scanner software.

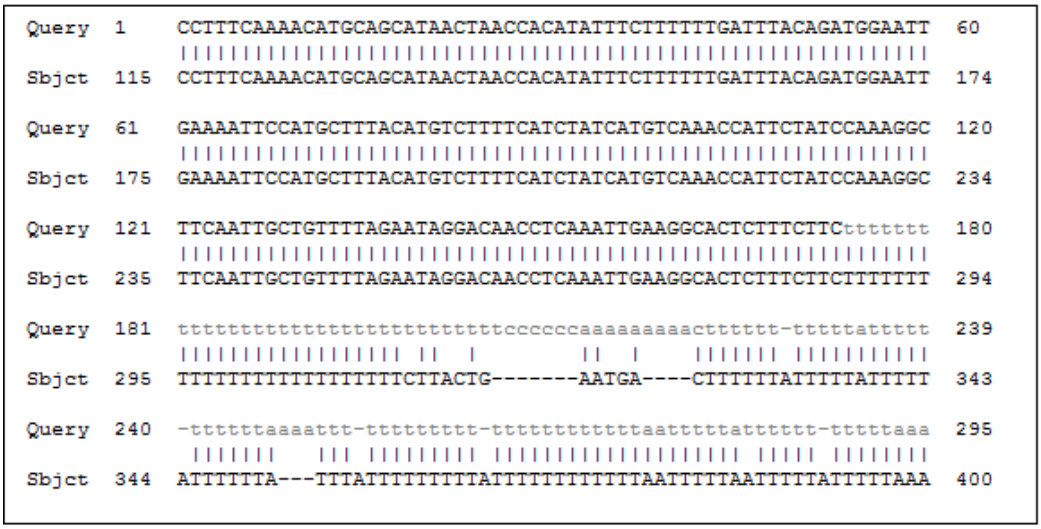


Figure 18: Sequence alignment of EE sample sequence (query) to documented exon 19 E allele (Subject). The LINE insertion part of the sequence shows partial alignment to that of the E variant despite low quality sequence data for this region.

4.2.4.7 Exon 19 Real-time PCR sequences

Only short lengths of sequences could be obtained using primers used for real-time PCR analysis. Despite this, the correct region was amplified as partial alignments were made to exon 19 of the A-allele (AJ504710.2). The sequence alignment can be found in Appendix E.

4.2.5 'Unknown' *CSN1S1* genotype

Nine 'unknown' variants were found at reasonably high frequency on Farm A (0.161) and were present in three of the new-season bucks. The unknown variants were characterised by three bands following the amplification of exon 9 (Figure 8). One product appears between 200 and 220bp, while two products are produced between 220-240bp (Figure 19). This was observed in both hair and milk samples and was not a reflection of DNA quality or purity. Digestion of exon 9 products with XmnI produced three bands, one at ~160bp, one between 200 and 220bp and another between 220 and 240bp. Direct sequencing could not be used as the presence of three products meant no quality sequence data could be produced. Samples were also re-collected and re-extracted in case it was contamination however none of these methods changed the 'three-product' outcome.

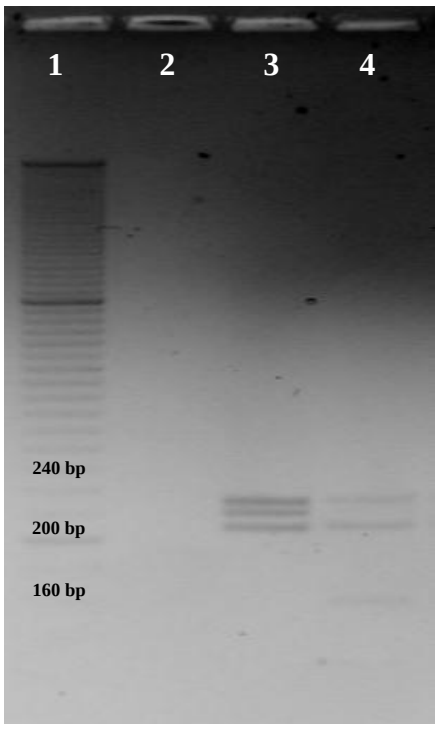
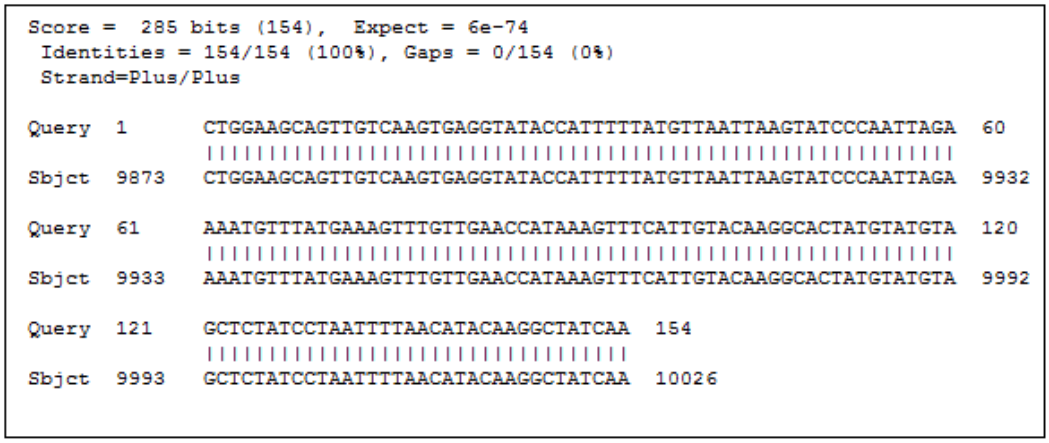


Figure 19: Image of 4% agarose gel with 'unknown' genotype products. Lane 1, 20bp ladder. Lane 2, negative control. Lane 3, undigested unknown genotype sample. Lane 4, XmnI digested unknown genotype sample. Gel was electrophoresed in TAE buffer with additional EtBr for 3 hours.

To overcome direct sequencing issues, multiple gel-extraction methods were tried to extract individual bands for sequencing. Gel-punching proved the most successful, particularly for the bottom and middle undigested fragments.

The bottom band of the undigested sample (lane 3 of Figure 19 above) extracted with 32ng/µL of DNA and sequence data showed a 100% alignment to Allele N. This product had the cytosine deletion in exon 9, and thus would not cut under XmnI (Figure 20). This is evidenced in the gel image which shows the bottom band remaining following digestion (lane 4).



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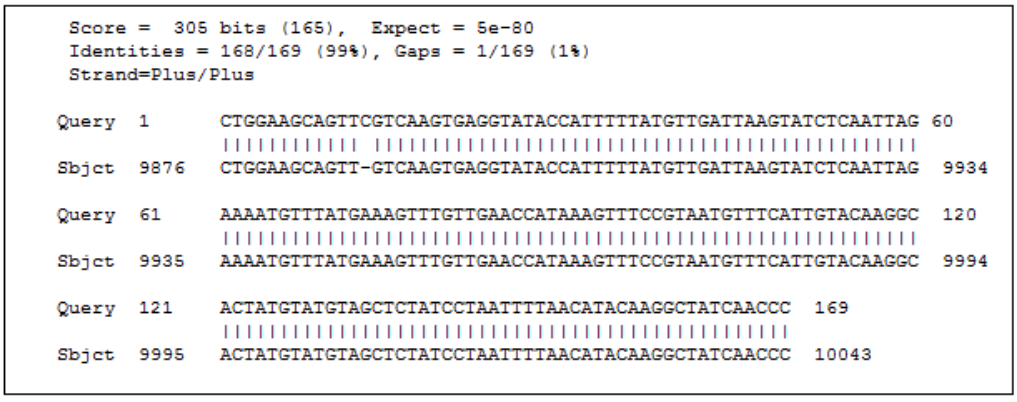
Query  61     AAATGTTTATGAAAGTTTGTTGAACCATAAAGTTTCATTGTACAAGGCACTATGTATGTA  120
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  9933   AAATGTTTATGAAAGTTTGTTGAACCATAAAGTTTCATTGTACAAGGCACTATGTATGTA  9992

Query  121    GCTCTATCCTAATTTTAACATACAAGGCTATCAA  154
              |||||||||||||||||||||||||||||||||
Sbjct  9993   GCTCTATCCTAATTTTAACATACAAGGCTATCAA  10026
```

Figure 20: Sequence alignment of the bottom band extracted from gel of the unknown genotype, amplified at exon 9 (query) to the documented exon 9 sequence of the *CSN1S1* allele N (sbjct).

Gel extraction of the undigested middle band produced sufficient DNA (18.4ng/µL) and sequence data showed a similar sequence to the exon 9 E allele found in this study (no exon 9 E allele sequences have been published to BLAST). The sequence aligned to allele F (had the 11bp insertion) but had the cytosine insertion which would allow it to cut under XmnI. The middle band disappears following digestion as it is cut into 161bp and 63bp fragments (lane 4). The 63bp fragment is too faint to see in the gel image following three hours of electrophoresis, despite the addition of extra EtBr. Identifying this product as *CSN1S1* allele E is supported exon 19 real-time PCR results which showed the presence of the E allele in these unknown samples (Appendix D).



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 Strand=Plus/Plus

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Sbjct  9876   CTGGAAGCAGTT-GTCAAGTGAGGTATACCATTTTTATGTTGATTAAGTATCTCAATTAG  9934

Query  61     AAAATGTTTATGAAAGTTTGTTGAACCATAAAGTTTCCGTAATGTTTCATTGTACAAGGC  120
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  9935   AAAATGTTTATGAAAGTTTGTTGAACCATAAAGTTTCCGTAATGTTTCATTGTACAAGGC  9994

Query  121    ACTATGTATGTAGCTCTATCCTAATTTTAACATACAAGGCTATCAACCC  169
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Sbjct  9995   ACTATGTATGTAGCTCTATCCTAATTTTAACATACAAGGCTATCAACCC  10043
```

Figure 21: Sequence alignment of the middle band extracted from the gel of undigested unknown variant amplified at exon 9 (query) to the documented sequence for *CSN1S1* allele F (subject). Note the presence of the cytosine at the 13th nucleotide of the query (unknown) sequence which creates the XmnI restriction site and is not present in the *CSN1S1* F-allele.

DNA of sufficient quantity and quality could not be obtained from the top band (5.4ng/µL), despite multiple methods (gel-stab, gel capture and gel-punch). The top band was very close to the middle band and required at least three hours of electrophoresis to separate on a 4% agarose gel. By which time, the products had faded significantly and were difficult to sequence. Attempts at cloning the top band also failed, presumably due to low DNA concentration and / or contamination remaining from 4% agarose gels. A sample of top-band sequence data is outlined below (Figure 22).

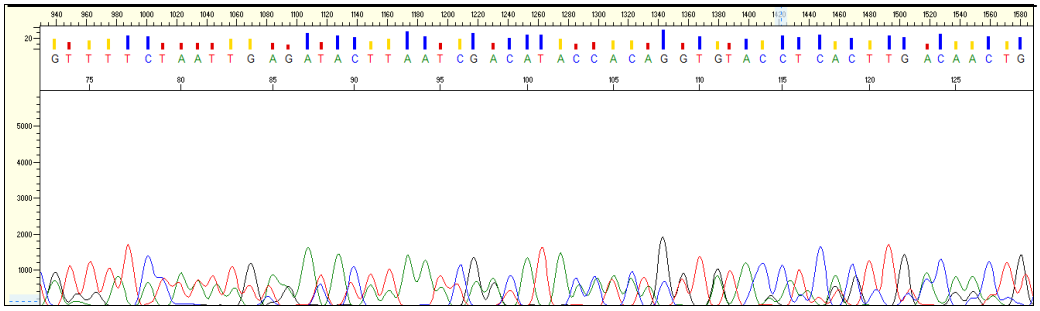

Figure 22: Sequencing of the unknown genotype 'top band' extracted from 4% gels produced poor quality sequence results. Image from sequence data analysed in Applied Biosystems Sequence Scanner software.

Therefore these 'unknown' variants do possess *CSN1S1* E and N alleles in the middle and bottom bands respectively however the top band remains unidentified. As a result these samples retain the 'unknown' genotype status.

4.3 *CSN1S1* allele and genotype frequencies

A total of 126 bucks and does were genotyped at the *CSN1S1* locus for A*, B*, E, F, N and 01 variants. Allele frequencies showed that *CSN1S1* E,F and A* alleles were the most common at the *CSN1S1* locus in both herds. Only two *CSN1S1* N alleles were found on Farm A and no 01 variants were found on either farm.

Table 17: Allele frequencies of *CSN1S1* between two DGC farms. The 9 unknown variants were excluded from allele frequency analysis. A* include G,H,I, and 02 variants. B* include B1-B4, B', C and L.

Allele	Farm A		Farm B	
	No.	**Frequency**	**No.**	**Frequency**
A*	22	0.229	44	0.310
B*	5	0.052	4	0.035
E	37	0.385	44	0.327
F	30	0.313	48	0.342
N	2	0.020	0	0.000
01	0	0.000	0	0.000
Total	**96**		**140**	

Genotype frequencies showed that EF and AE genotypes were the most frequent on both farms, with A*F and FF genotypes more frequent on Farm B than Farm A (Table 18). Hardy Weinberg analysis showed that neither population was in Hardy Weinberg Equilibrium (HWE), with Farm B trending further away from HWE than Farm A (Table 19 and Table 20).

Table 18: Genotype frequencies of *CSN1S1* between two DGC farms. A* include G,H,I, and 02 variants. B* include B1-B4, B', C and L.

Genotype	Farm A		Farm B	
	No.	**Frequency**	**No.**	**Frequency**
A*A*	4	0.071	8	0.104
A*E	10	0.179	18	0.254
A*F	2	0.036	10	0.149
A*N	2	0.036	0	0.000
B*E	3	0.054	1	0.015
B*F	2	0.036	3	0.045
EE	5	0.089	5	0.075
EF	13	0.232	15	0.209
FF	6	0.107	10	0.149
Unknown	9	0.161	0	0.000
Total	**56**		**70**	

Table 19: Hardy Weinberg Analysis, Farm A. P-value is chi-square with 4 degrees of freedom (9 genotypes – 5 alleles). *P*-value is > 0.05 and therefore reject the null hypothesis that the Farm A population is in HWE. A* include G,H,I, and 02 variants. B* include B1-B4, B', C and L. Unknown genotypes were excluded from HWE analysis.

	Observed								HW - Expected		p-value
	no	**A**	**B**	**E**	**F**	**N**	**%**	**Freq**	**%**	**Chi-sq.**	
A*A*	4	8	0	0	0	0	8.51	2.57	6.45	0.789	
A*E	10	10	0	10	0	0	21.28	8.43	21.09	0.294	
A*F	2	2	0	0	2	0	4.26	1.85	4.63	0.012	
A*N	2	2	0	0	0	2	4.26	0.47	1.17	5.014	
B*E	3	0	3	3	0	0	6.38	2.30	5.75	0.215	
B*F	2	0	3	0	2	0	4.26	1.85	4.63	0.012	
EE	5	0	0	10	0	0	10.64	6.89	17.26	0.520	
EF	13	0	0	13	13	0	27.66	11.11	27.81	0.323	
FF	6	0	0	0	12	0	12.77	4.47	11.20	0.521	
Total	**47**	**22**	**6**	**36**	**29**	**2**	**100**	**39.94**	**100**	**7.700**	**0.1032**

Table 20: Hardy Weinberg Analysis, Farm B. *P*-value is chi-square with 3 degrees of freedom (9 genotypes – 5 alleles). *P*-value is > 0.05 and therefore reject the null hypothesis that the Farm A population is in HWE. A* include G,H,I, and 02 variants. B* include B1-B4, B', C and L. Unknown genotypes were excluded from HWE analysis.

	Observed								HW - Expected		p-value
	no	**A**	**B**	**E**	**F**	**N**	**%**	**Freq**	**%**	**Chi-sq.**	
A*A*	7	14	0	0	0	0	10.45	6.27	15.70	0.084	
A*E	17	17	0	17	0	0	25.37	12.85	32.17	1.340	
A*F	10	10	0	0	10	0	14.93	14.38	36.00	1.334	
B*E	1	0	1	1	0	0	1.49	1.25	3.14	0.051	
B*F	3	0	3	0	3	0	4.48	1.40	3.51	1.818	
EE	5	0	0	10	0	0	7.46	6.58	16.48	0.380	
EF	14	0	0	14	14	0	20.90	14.73	36.88	0.036	
FF	10	0	0	0	20	0	14.93	8.24	20.64	0.375	
Total	**67**	**41**	**4**	**42**	**47**	**0**	**100**	**65.72**	**100**	**5.419**	**0.1436**

4.4 *CSN1S1* genotype and milk composition

Only genotyped does aged 2-8 years with milk composition data were used to correlate the effect of *CSN1S1* on milk parameters. Due to low numbers of some genotypes, does were grouped into 5 categories based on the predicted level of αs1-casein produced by each genotype (Table 21).

Table 21: Genotype and number of does with composition data in each *CSN1S1* genotype category. The placement of genotypes in each category is based on the predicted level of αs1-casein gene-expression associated with each variant.

***CSN1S1* category**	***CSN1S1* genotypes**	**Farm A**	**Farm B**
Low	FF	5	9
Medium-low	EF	13	11
Medium	EE, AF, BF, AN	9	15
Medium-high	AE, BE	12	11
High	AA, BB	3	6
Total		**48**	**52**

A significant effect of *CSN1S1* genotype was found on one milk parameter on Farm A, and three milk parameters on Farm B (Table 22). Age and breed did not have any effect on milk composition ($p>0.05$).

Table 22: ANOVA for significant effects for *CSN1S1* genotype on milk composition. * significant at $p < 0.05$, ** highly significant at $p < 0.01$ based on ANOVA analysis.

Milk composition	***p*-value**	
	Farm A	**Farm B**
Milk Volume	0.279	0.017 *
Protein (%)	0.048 *	0.001 **
Protein (kg)	0.349	0.493
Fat (%)	0.103	0.003 **
Fat (kg)	0.241	0.456
Milk solids (kg)	0.480	0.447
SCC (000)	0.508	0.121

4.4.1 Milk Volume

Milk volume was significantly affected by *CSN1S1* genotype on Farm B,but not Farm A (Table 23). On Farm B goats with low *CSN1S1* genotypes produced on average 1.6L per day more than those with high genotypes. The differences between *CSN1S1* genotypes on each farm can be visualised below in Figure 23.

Table 23: Average milk volume and standard deviations for both farms across the five genotype categories. SD = standard deviation. Genotypes within each category were determined by the predicted level of αs1-casein gene-expression associated with each variant. P-values were determined by ANOVA analysis.

Genotype category	Farm A		Farm B	
	Mean (L)	SD	Mean (L)	SD
Low	2.08	1.22	4.16	1.22
Medium-low	1.97	0.83	3.68	0.83
Medium	2.04	1.03	3.56	1.03
Medium-high	2.07	0.86	3.11	0.86
High	1.40	0.47	2.48	0.47
***p*-value**	**0.279**		**0.017**	

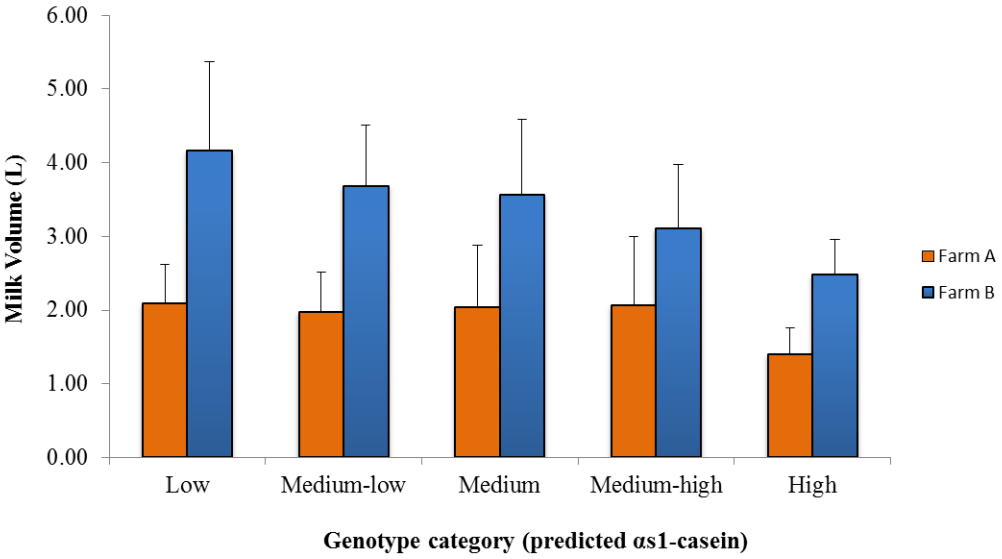

Figure 23: Mean milk volume (L) across the five CSN1S1 genotype categories on Farm A (orange) and Farm B (blue). Error bars represent the standard deviation of the mean. Significant differences were found on Farm B only ($p<0.05$).

4.4.2 Milk Protein

Milk protein content (%) was significantly affected by genotype on both farms, particularly when comparing high *CSN1S1* genotypes with low. High αs1-casein genotype goats produced around 0.5% more protein on Farm A and 1% on Farm B compared to low genotypes (Table 24). This effect was significant on both Farm A (p=0.048) and Farm B (p=0.0012).

Table 24: Milk protein % averages and standard deviations for both farms across the five genotype categories. SD = standard deviation. Genotypes within each category was determined by the predicted level of αs1-casein gene-expression associated with each variant. P-values were determined by ANOVA analysis.

Genotype category	Farm A		Farm B	
	Mean (%)	SD	Mean (%)	SD
Low	3.05	0.16	2.98	0.25
Medium-low	3.30	0.21	3.31	0.58
Medium	3.31	0.24	3.36	0.51
Medium-high	3.22	0.23	3.86	0.59
High	3.57	0.30	4.09	0.13
***p*-value**	**0.048**		**0.0012**	

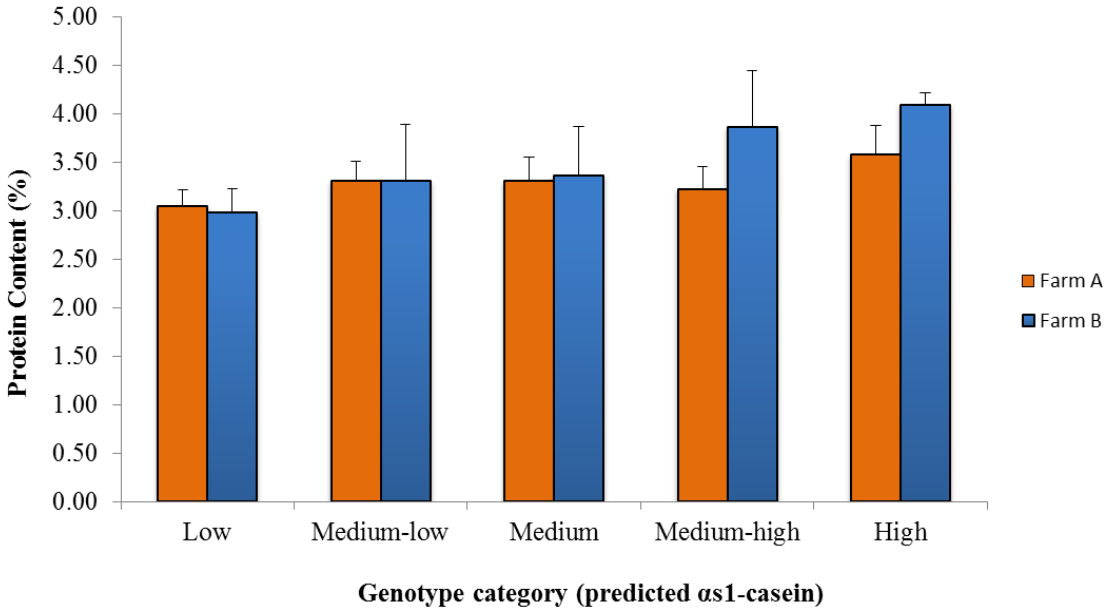

Figure 24: Mean protein content (%) across the five *CSN1S1* genotype categories on Farm A (orange) and Farm B (blue). Error bars represent the standard deviation of the mean. Significant differences were found on Farm A (p<0.05) and Farm B (p<0.01).

Despite a significant difference in protein content between genotypes, no differences in protein yield (kg) were observed between *CSN1S1* genotype categories on either farm (Table 25).

Table 25: Milk protein yield (kg) average and standard deviation for both farms across the five genotype categories. SD = standard deviation. Genotypes within each category was determined by the predicted level of αs1-casein gene-expression associated with each variant. P-values were determined by ANOVA analysis.

Genotype category	Farm A		Farm B	
	Mean (kg)	SD	Mean (kg)	SD
Low	0.05	0.01	0.12	0.03
Medium-low	0.07	0.02	0.12	0.03
Medium	0.07	0.02	0.11	0.02
Medium-high	0.07	0.03	0.11	0.03
High	0.06	0.02	0.09	0.02
***p*-value**	**0.349**		**0.493**	

4.4.3 Milk Fat

Milk fat content was significantly influenced *CSN1S1* genotype on Farm B only (Table 26). High αs1-casein genotypes had greater fat % than low genotype samples on Farm B, however this effect was not observed on Farm A. The average kilograms of fat produced however was not significant between *CSN1S1* genotype categories (Table 27).

Table 26: Milk fat % averages and standard deviations for both farms across the five genotype categories. SD = standard deviation. Genotypes within each category was determined by the predicted level of αs1-casein gene-expression associated with each variant. P-values were determined by ANOVA analysis.

Genotype category	Farm A		Farm B	
	Mean (%)	SD	Mean (%)	SD
Low	3.26	0.14	3.97	0.47
Medium-low	3.30	0.33	4.02	0.49
Medium	3.25	0.43	4.33	0.88
Medium-high	3.34	0.32	4.70	0.93
High	3.53	0.51	4.94	0.44
***p*-value**	**0.103**		**0.033**	

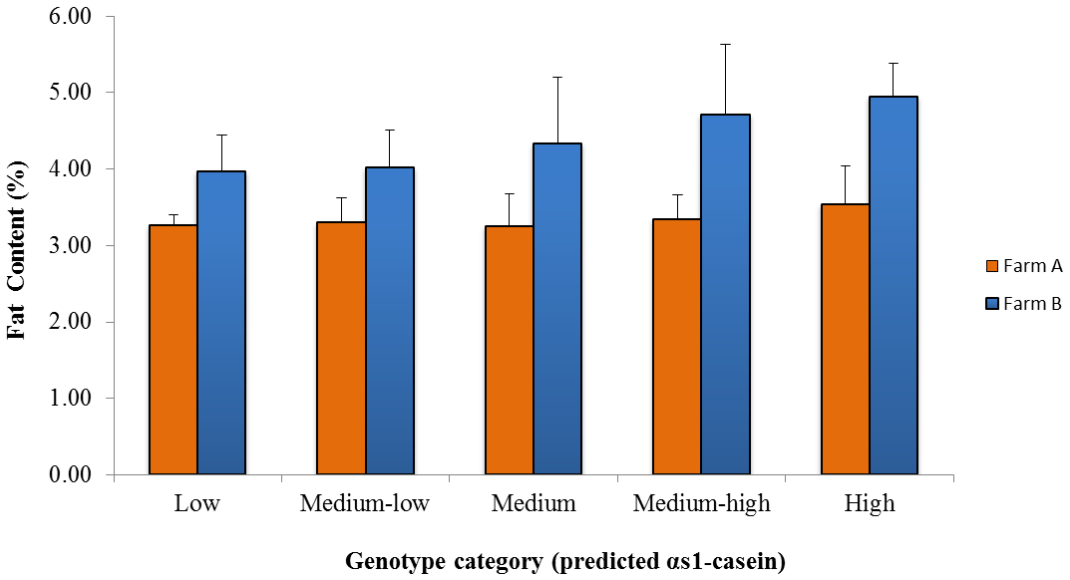

Figure 25: Mean fat content (%) across the five CSN1S1 genotype categories on Farm A (orange) and Farm B (blue). Error bars represent the standard deviation of the mean.

Table 27: Average Milk fat yield (kg) between farms across the five genotype categories. SD = standard deviation. Genotypes within each category was determined by the predicted level of αs1-casein gene-expression associated with each variant. P-values were determined by ANOVA analysis.

Genotype category	Farm A		Farm B	
	Mean (kg)	SD	Mean (kg)	SD
Low	0.06	0.01	0.16	0.05
Medium-low	0.07	0.02	0.15	0.04
Medium	0.07	0.02	0.15	0.05
Medium-high	0.07	0.03	0.14	0.04
High	0.07	0.02	0.12	0.03
***p*-value**	**0.241**		**0.456**	

4.4.4 Milk Solids

Milk solids takes into account the protein and fat content (%) and volume (milk L) to give an overall yield of fat and protein. Although on average high *CSN1S1* genotypes produced lower milk solids on Farm B, no significant effects of genotype category were found on either farm ($p>0.05$) (Table 28).

Table 28: Average milk solids (kg) between farms across the five genotype categories. SD = standard deviation. Genotypes within each category was determined by the predicted level of αs1-casein gene-expression associated with each variant.

Genotype category	Farm A		Farm B	
	Mean (kg)	SD	Mean (kg)	SD
Low	0.16	0.04	0.28	0.09
Medium-low	0.20	0.06	0.26	0.06
Medium	0.14	0.05	0.26	0.07
Medium-high	0.13	0.06	0.25	0.07
High	0.10	0.03	0.21	0.05
***p*-value**	**0.480**		**0.447**	

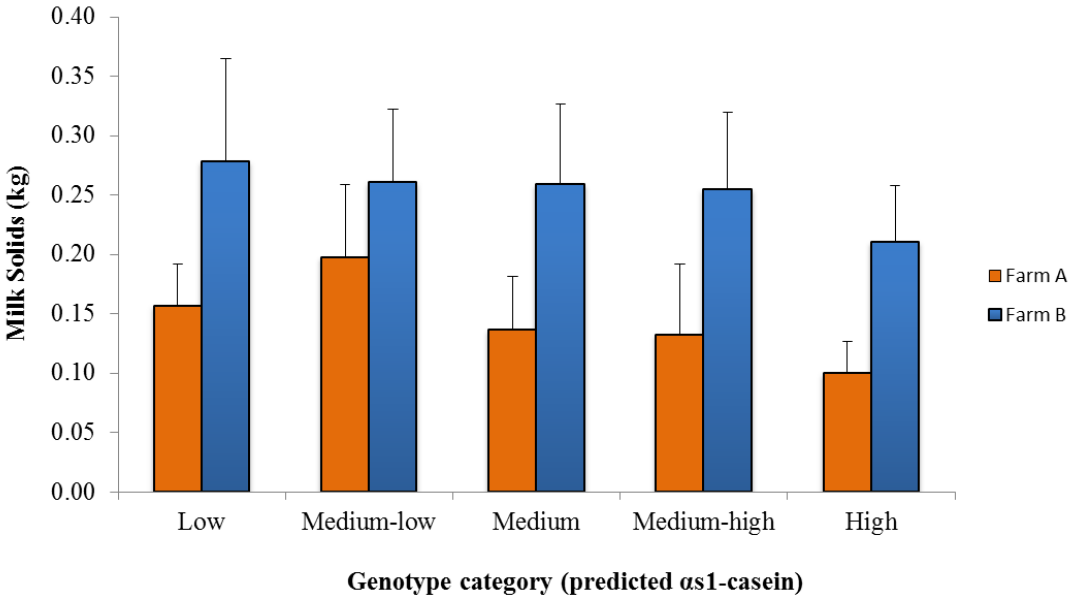

Figure 26: Mean milk solids (kg) across the five *CSN1S1* genotype categories on Farm A (orange) and Farm B (blue). Error bars represent the standard deviation of the mean.

4.4.5 Somatic Cell Count

Although on average it appears high casein genotypes produce higher SCCs, no significant differences were found on either farm ($p>0.05$) with regard to *CSN1S1* genotype and SCC (Table 29). Note the high variability of SCC in the standard deviations of each mean.

Table 29: Average somatic cell count (SCC) between farms across the five genotype categories. SD = Standard deviation Genotypes within each category was determined by the predicted level of αs1-casein gene-expression associated with each variant. P-values were determined by ANOVA analysis.

Genotype category	Farm A		Farm B	
	Mean (000)	SD	Mean (000)	SD
Low	743.4	771.3	178.1	102.6
Medium-low	779.3	646.6	322.1	211.6
Medium	1295.6	1439.2	525.7	866.1
Medium-high	1038.3	874.8	906.9	1194.8
High	1385.2	3083.4	1133.8	1152.8
***p*-value**	**0.508**		**0.121**	

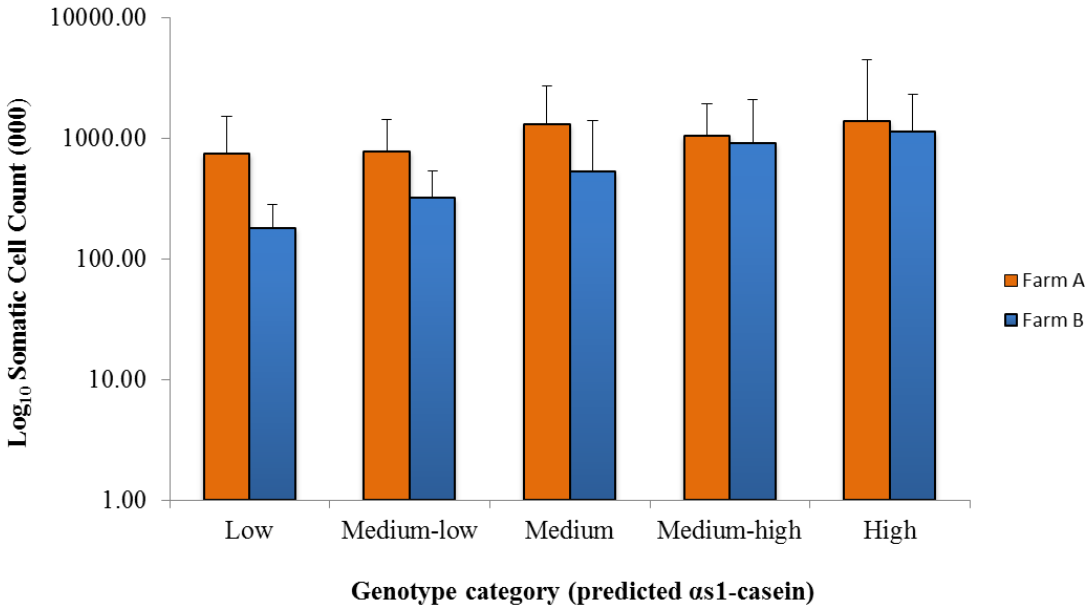

Figure 27: Mean somatic cell count (000) across the five CSN1S1 genotype categories on Farm A (orange) and Farm B (blue). Error bars represent the standard deviation of the mean. SCCs are plotted on a $\log_{10}$ scale due to the exponential nature of SCC.

4.4.6 Farm A seasonal correlation

Given that herd test data was available for each month during mid-late lactation for Farm A, the correlation of genotype to milk composition was tried with herd test data collected in December and January. This analysis yielded no significant effects of *CSN1S1* genotype ($p<0.05$) on any milk composition parameter in the later months.

Table 30: Farm A p-values for *CSN1S1* genotype and milk composition. * = statistically significant ($p<0.05$). P-values were determined by ANOVA analysis.

Milk composition	November	December	January
Milk Volume	0.280	0.067	0.199
Protein (%)	0.048 *	0.926	0.893
Protein (kg)	0.349	0.254	0.630
Fat (%)	0.103	0.123	0.244
Fat (kg)	0.241	0.060	0.272
Milk solids (kg)	0.481	0.082	0.362
SCC	0.909	0.252	0.387

4.4.7 *CSN1S1* genotype and fatty acid composition

With a limited number of genotyped samples tested for fatty acids, medium and medium-low *CSN1S1* genotypes used for previous analyses were grouped into one 'low' group, while high and medium-high genotypes were grouped as 'high'. The numbers in each category are outlined below in Table 31.

Table 31: Genotype categories and genotypes of samples used for fatty acid analysis

Genotype category	Genotypes	No. samples
High	AA, AE	5
Low	EF, FF	9

Although on average 'high' *CSN1S1* genotypes had a greater percentage of all saturated fatty acids and LA, significant differences between genotypes could only be found in two fatty acids; C10:0 (Capric acid) and C18:3n3 (ALA) (Figure 28 and Table 32). C10:0 and ALA levels were both significantly ($p<0.05$) greater in high *CSN1S1* samples compared to low *CSN1S1* genotypes. The difference in C10:0 and C18:3n3 between genotypes however reflected only a small percentage of total fatty acids (1.1% and 0.17% respectively).

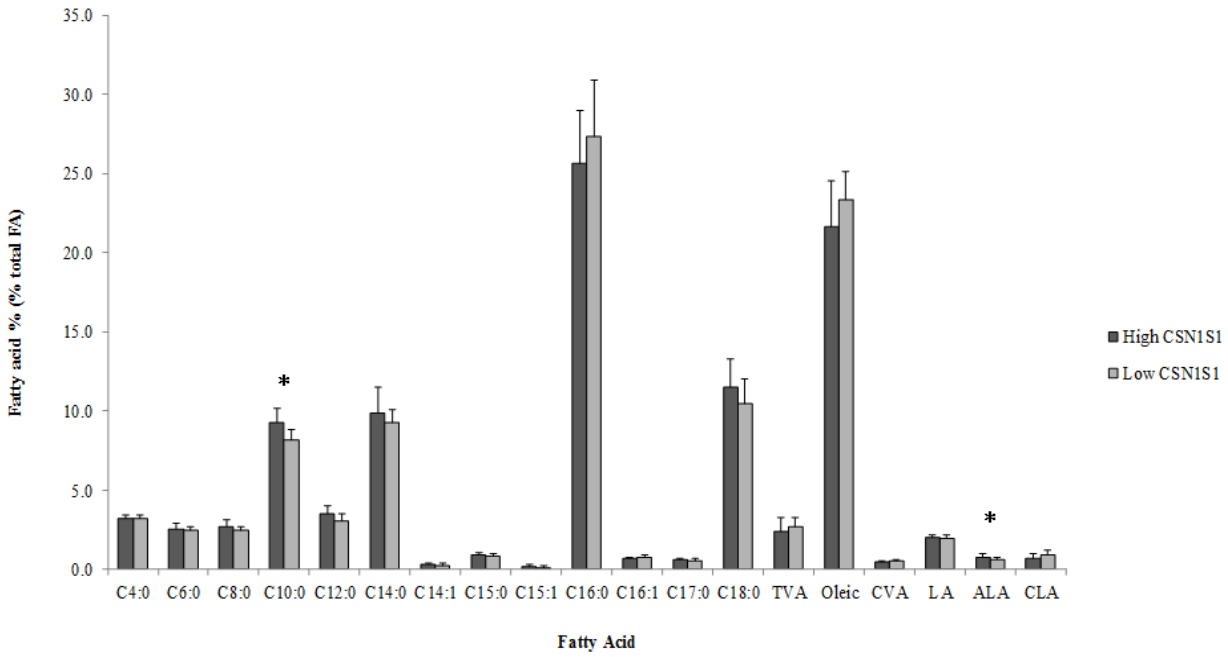

Figure 28: Fatty acid composition of 'High' *CSN1S1* and 'Low' *CSN1S1* genotypes. Significant differences ($p<0.05$) are indicated with a *. Common names are used for LCFA with lengthy symbols. Error bars are the standard deviation of the mean.

Table 32: Average fatty acid composition for high and low *CSN1S1* samples. *p*-values were determined by ANOVA. * = $p < 0.05$. NS = not significant.

Fatty Acid	High *CSN1S1*		Low *CSN1S1*		*p*-value	Significance
	Mean	SD	Mean	SD		
C4:0	3.18	0.20	3.15	0.29	0.887	NS
C6:0	2.53	0.33	2.41	0.26	0.452	NS
C8:0	2.69	0.40	2.41	0.23	0.116	NS
C10:0	9.24	0.94	8.14	0.70	0.029	*
C12:0	3.49	0.47	3.04	0.46	0.106	NS
C14:0	9.88	1.64	9.29	0.83	0.384	NS
C14:1	0.30	0.04	0.22	0.17	0.322	NS
C15:0	0.89	0.12	0.83	0.16	0.449	NS
C15:1	0.14	0.13	0.08	0.12	0.351	NS
C16:0	25.62	3.34	27.36	3.52	0.385	NS
C16:1	0.69	0.06	0.75	0.10	0.280	NS
C17:0	0.61	0.06	0.54	0.11	0.252	NS
C18:0	11.47	1.79	10.47	1.51	0.286	NS
TVA	2.38	0.90	2.67	0.56	0.473	NS
Oleic	21.62	2.91	23.33	1.78	0.195	NS
CVA	0.48	0.05	0.53	0.09	0.331	NS
LA	2.02	0.14	1.93	0.24	0.451	NS
ALA	0.77	0.17	0.60	0.12	0.048	*
CLA	0.66	0.29	0.87	0.34	0.272	NS

4.5 Conclusions

Analysis of exon 9, intron 12 and exon 19 allowed the genotyping of A (G,H,I,02) B(C,L), E, F, N and 01 alleles at the *CSN1S1* locus. Sequence results verified the correct amplification of each variant. One unique genotype, which remains uncharacterised, was found at a reasonably high frequency on one farm. Of the 126 goats genotyped for the major alleles of the *CSN1S1* locus, E and F variants were the most common. Neither farm population was in Hardy-Weinberg equilibrium. *CSN1S1* yielded a significant difference on protein content on both farms, while milk volume and fat percent were only significant on Farm B. Although on average there were trends with fat, protein and milk solid yields, statistically these were indifferent between genotypes. *CSN1S1* had a significant effect on two fatty acids, C10:0 and C18:3n3, although the relative changes were small.

Chapter 5: Genetics Discussion

Genetic factors play a pivotal role in milk composition, and the goat species is renowned for wide variations in milk trait production. The *CSN1S1* locus was chosen for analysis in the New Zealand (NZ) population due to reported effects of *CSN1S1* alleles on milk composition. Of particular interest was role of *CSN1S1* in the production of fat, protein and fatty acid compositions which are highly important for infant formula and specialty nutritionals. This research has shown that NZ dairy goats are polymorphic at the *CSN1S1* locus and that the *CSN1S1* genotype can impact milk composition. The following chapter evaluates the methods used for genotyping at this locus and discusses genotype frequency and milk composition findings. In doing so, this research outlines the first genetic evaluation of the *CSN1S1* locus in NZ dairy goats.

5.1 Evaluation of methods for *CSN1S1* genotyping

Goat genomic DNA is often extracted from blood samples, however this research showed DNA can be successfully extracted from milk and hair to allow the genotyping of 126 bucks and does. DNA extraction proved more successful from milk (87%) than hair (63%), most likely due to differences in extraction methods and the number of nucleated cells present in each sample. Compared to other goat milk extraction methods, such as d'Angelo (2007) and Tokarska (2001b), the phenol-chloroform method used in this study produced better results for *CSN1S1* genotyping purposes.

No single method has been described for the simultaneous identification of all 18 known *CSN1S1* alleles. Alleles A, B1, B2, B3, B4, B', C, H, L and M are high expressing or 'strong' alleles which produce around 3.5g/L of αs1-casein per allele (Bevilacqua et al., 2002, Brignon et al., 1990a, Chianese et al., 1997, Martin et al., 1999a). Intermediate alleles (E and I) produce 1.1g/L of αs1-casein each while low expressing 'weak' alleles (F, D and G) produce just 0.45g/L (Martin et al., 1999b). There are also three null alleles which produce no αs1-casein, 01, 02 and N (Cosenza et al., 2003, Ramunno et al., 2005).

Six *CSN1S1* alleles (A*, B*, E, F, N and 01) were chosen for genotyping based on their expected frequency in the NZ population and their proposed effect on milk production. F and N alleles could be determined solely through the amplification and digestion of exon 9. A* and 01 required exon 9 and intron 12 analyses while B* and E required exon 9 and real-time PCR of exon 19. A* included G, H, I and 02 while B* included all B-subtypes, C and L as these could not be differentiated using SNPs in exon 9, intron 12 and exon 19. Grouping such alleles was not considered to have a detrimental effect on analyses as these variants either produce a similar amount of casein or have been found in very low frequencies by other authors.

The success of genotyping using PCR-RFLP of exon 9 highlights the effectiveness of this method for the initial screening of *CSN1S1* variants. This is most likely why the majority of *CSN1S1* genotyping studies today employ this method (Caravaca et al., 2008, Cosenza et al., 2008, Gigli et al., 2008, Maga et al., 2009, Soares et al., 2009, Torres-Vázquez et al., 2008, Vacca et al., 2009). However few use real-time PCR for identifying E alleles at exon 19, despite its effectiveness found in this study and reported by Feligini *et al.,* (2005). This is surprising given the more accurate genotyping of the E variant, improved efficiency and avoidance of gels found in this research by using real-time PCR.

Sequencing proved necessary to verify PCR reactions amplified the correct regions of interest. Importantly it identified the cytosine in exon 9 required for restriction digests with XmnI. Sequencing also clarified PCR uncertainties, especially for AN heterozygotes and samples carrying the *CSN1S1* E allele.

However sequence data did not always align to sequences documented in BLAST. This highlights the lack of complete sequences of *CSN1S1* in public databases and that new variants may not yet be characterised. The exon 9 sequence of the E allele was determined in this study, which was previously unknown. This sequence data confirmed the presence of an 11bp insertion which explains why PCR-RFLP products of exon 9 E alleles are 161bp, not 150bp like the A allele. The sequence of this 11bp insertion is identical to the one characterising the F-allele which is thought to have arisen from a homologous

sequence duplication in the F allele (Leroux et al., 1992). Given E and F alleles both arise from the B2 lineage (Grosclaude et al., 1997), the presence of this 11bp insertion could represent a commonality in the evolution of these two variants which differentiate them from other B2-lineage variants that do not have this insertion. Further sequencing of the entire *CSN1S1* E allele would verify this suggested phylogeny.

5.2 The 'unknown' genotype

A relatively high frequency of bucks and does from Farm A were found with 'unknown' genotypes. Amplification of exon 9 produced three bands over 200bp that could not be attributed to contamination as samples were re-collected and DNA re-isolated with the same result. Sequencing and real-time PCR revealed *CSN1S1* N and E alleles are present in these samples. The top band (>220bp) however could not be identified despite multiple methods. Failure to identify this band could be due to low DNA concentration, DNA purity or the presence contaminants remaining from gel-extraction. Alternatively the top band could be a new variant that is difficult to sequence due to a LINE insertion similar to exon 19. However this theory does not explain why three variants (E, N and 'new') would occur in one sample.

Interestingly Tokarska *et al.*, (2001a) found two goats in Poland with the presence of three alleles ('High', F and E) in cDNA sequencing results. They could not explain the presence of these 'three transcript variants' which were not alternatively spliced F-transcripts and remained even after re-collection and re-isolation of the samples.

Overall, the presence of three products in the current study is unusual and the cause unknown. This suggests some anomalies still exist with methods for identifying *CSN1S1* variants and there is the potential for new variants to be found. Importantly however, goats carrying unknown genotypes showed no detrimental effects on milk composition, producing average milk protein, fat and yield. As such, their characterisation may only be of interest from a genetic and evolutionary point of view.

5.3 *CSN1S1* allele and genotype frequencies

Genotyping of two representative dairy goat farms show that *CSN1S1* genotype EF was the most prevalent in the NZ dairy goat population (0.23 and 0.17 for Farm A and 0.21 and 0.25 for Farm B respectively). This most likely represents the dominance of the Saanen breed in DGC herds which show EF genotypes in other populations at a similar frequency (Clark and Sherbon, 2000, Grosclaude et al., 1987, Maga et al., 2009, Martin and Leroux, 2000, Ouafi et al., 2002, Soares et al., 2009, Torres-Vázquez et al., 2008). Slight differences were found between farms; in particular Farm B had a greater frequency of AE genotypes (0.254) while Farm had a higher frequency of unknown (0.16) and AN (0.036) genotypes. This could be attributed to different founder populations and subsequent breeding strategies on each farm. For example Farm A typically keeps kids based on birth order and the mother's general health status while Farm B selects on milk production using extensive herd-testing records. With virtually no interbreeding with other populations, use of foreign bucks or AI, alleles become fixed within each herd. This is reflected in the HWE results in which both farms rejected HWE, especially Farm B.

5.4 Effect of genotype on milk composition

CSN1S1 genotype had a significant influence on milk composition, however the effect was not observed equally on each farm. One reason for this effect is that although the pasture and grain diets were similar, Farm B was an indoor system, whereas Farm A was outdoor. Outdoor diets are likely to be more heterogeneous as the goats can select and browse pasture, a factor which reportedly influences milk composition (Bonanno et al., 2008). Pasture for indoor diets is mown and carried to the goats and therefore is more homogenous. In this respect, individual animals were more consistent with dietary factors on the indoor farm and perhaps why more of an effect was observed on Farm B. Another alternative is that although both farms were feeding the same diet, Farm B produced more milk volume (on average 1-2L more per doe) than Farm A, suggesting the energy status of does on Farm B was greater. This aspect is discussed further in Chapter 9 regarding nutritional and genetic factors combined.

Another cause of different effects of *CSN1S1* genotype between farms is that herd-test dates were 21 days apart. Therefore the herds may have been at a different stage of lactation. α and β caseins have been shown to decrease with lactation progression (Brown et al., 1995) and αs1-casein mRNA is known to be seasonally expressed (Tokarska et al., 2001b, Szumska et al., 2001). Pierre *et al.*, (1998) found a maximum difference between A and 01 variants in fat content early in lactation which slowly decreased to similar levels in late lactation. Therefore the difference in herd-test date may have been significant enough to affect the influence of *CSN1S1* genotype on milk composition. Certainly the monthly herd-test data results from Farm A highlight this with only the first month (mid-lactation) producing a statistically significant results. To confirm whether this is the cause an investigation into the seasonal expression of αs1-casein mRNA or the alignment of *CSN1S1* genotypes to more regular herd-test data would be required.

Lastly, the difference could simply be due to the number of genotypes at the extremes of predicted-casein. Farm A had approximately half the number of 'high' and 'low' αs1-casein genotypes as Farm B. Therefore, statistically this may not have been enough to determine an effect of genotype on milk parameters other than strongly implicated effects.

5.4.1 Protein

In line with other studies on *CSN1S1* genotype and milk composition, *CSN1S1* has a strong effect ($p<0.01$ Farm B, <0.05 Farm A) on milk protein content. The protein percentage in goat milk supplied to DGC typically only varies by ~2%. Therefore the ~1% difference between high and low genotypes could account for half approximately half of the variation in milk protein. Given that αs1-casein represents a small fraction of total protein in goat milk, around 8% (Park et al., 2007b), *CSN1S1* must have wider effects on protein content than just the production of αs1-casein.

Electron microscopy, cell culture and protein labelling experiments in goat mammary epithelial cells (MECs) suggest αs1-casein is required for the optimal transport of all other caseins by promoting their transport to the Golgi apparatus (Chanat et al., 1999, Neveu et al., 2002). More recently knock-out trials in mice Kolb *et al.,* (2011) have confirmed this observation and the chaperone mechanisms of both αs1- and αs2-caseins have been characterised in bovine milk (Treweek et al., 2011). Therefore the significantly higher protein content of high *CSN1S1* genotypes could be due to an improved efficiency of total protein secretion as a result of higher αs1-casein present in MECs of high genotype goats.

5.4.2 Milk Fat

The significantly greater fat content produced by high *CSN1S1* genotypes has been reported as a surprising result by many authors (Barbieri et al., 1995, Barlowska et al., 2007, Grosclaude et al., 1994, Manfredi et al., 1993, Vassal et al., 1994, Zullo et al., 2005). This current research found a significant effect on fat, although only on one farm. The reasons for the association between fat % and high *CSN1S1* genotypes are still not known but could include differences in secretory mechanisms as outlined for protein, linkage between fat and protein genes or differences in milk lipase activity.

Investigations into milk fat gene expression profiles of high and low *CSN1S1* goats have produced mixed results. Leroux *et al.*, (2003) found no association between genotype and the expression of genes encoding key lipogenic enzymes (ACC, FAS, LPL and SCD). However Ollier *et al.,* (2008) found 41 differentially expressed genes from high and low *CSN1S1* goats using a bovine oligonucleotide microarray. In particular, they showed a down-regulation of FAS and G3P are in agreement with the low fat content associated with low *CSN1S1* does. Thus although the mechanism is not fully understood, it appears that *CSN1S1* genotype could be associated with the expression of some genes involved in fat synthesis.

5.4.3 Milk Volume

CSN1S1 genotype had a significant effect on milk volume on one farm only. The reduced volume associated with high *CSN1S1* genotypes on Farm B meant that the increase in fat and protein content did not translate into any greater yield of fat, protein or total milk solids from these genotypes. Grosclaude (1994) also found an effect of *CSN1S1* genotype on fat percentage but not fat yield suggesting *CSN1S1* is less influential on milk volume than other milk parameters. Similar to Chilliard *et al* (2006), Farm A showed no effect of *CSN1S1* genotype on milk volume.

However like the results found in this study on Farm B, Barbieri *et al.*, (1995) found AA goats produced less milk yield than low genotypes. Thus the *CSN1S1* genotype can have an effect on milk volume in certain circumstances, which based on these results may be due to differences between farms such as stage of lactation or energy balance of the animal.

5.4.4 Somatic Cell Count

A large number of factors influence SCC in ruminants, many of which are non-pathological. For these reasons, the significant individual variation and lack of effect of *CSN1S1* genotype on SCC found in the current study is not surprising. However a genetic basis for SCC has been found by others. A highly negative effect ($p<0.001$) of αs1-casein on SCC has been observed with polymorphisms of the αs1-casein 5′ flanking region (*CSN1S1*-5') in German Holstein cows (Prinzenberg et al., 2005). Interestingly this polymorphism was also associated with QTL for udder shape, suggesting an association between casein and udder morphology genes, and providing a possible mechanism for its effect on SCC. *CSN1S1* may also influence SCC through its association with the gene encoding β-casein (CSN2). These two casein genes are known to be tightly linked (discussed later in section 5.4.6) and in dairy cows *CSN2* genotypes have a significant effect on SCC (Morris et al., 2005). Thus the casein cluster haplotype, particularly with *CSN1S1* and *CSN2* genotypes or the association of *CSN1S1* with other traits may generate more significant effects on SCC than *CSN1S1* alone.

5.4.5 Fatty acid composition

A number of investigations have noticed more 'goaty' flavours in milk from 'high' *CSN1S1* genotypes versus milk from low genotypes (Grosclaude et al., 1994, Martin and Leroux, 2000, Vassal et al., 1994) suggesting an impact on fatty acid composition. In this research a significant ($p<0.05$) effect of high (AA, AE) versus low (EE, FF) *CSN1S1* genotypes was found on two fatty acids, capric acid (C10:0) and ALA (C18:3n3). However only 14 genotyped goats had fatty acid data and therefore it was difficult to elucidate a trend. On the other hand Chilliard *et al.,* (2006), using 71 does found significant differences in at least 17 fatty acids, including C10:0. In both studies C10:0 was significantly reduced in 'low' genotype goats. It could be that low *CSN1S1* genotypes are linked with lower activities of genes encoding, FAS and ACC activity, reducing the *de novo* synthesis of C10:0. However as outlined in section 5.4.2 the effect of *CSN1S1* on genes involved in fat and fatty acid synthesis remains undetermined. Therefore why C10:0 is reduced in low genotypes is not yet known.

The effect on ALA is not in agreement with Chilliard *et al.,* (2006) who found no significant difference between high (AA) and low (FF) *CSN1S1* genotypes. This could be explained by nutritional differences, whereby these authors used goats fed concentrates, versus this study in which goats were raised predominantly on pasture which is high in ALA. Interestingly *CSN1S1* genotype has been shown to influence diet selection in a free choice trial in dairy goats (Avondo et al., 2009). Therefore, in an outdoor system, high and low *CSN1S1* genotype goats could select pasture differently, thereby influencing ALA concentrations in the milk. However this speculation needs to be substantiated with further research. Moreover the changes to C10:0 and ALA were small as a proportion of total fatty acids and therefore the overall effect of *CSN1S1* genotype on fatty acid composition was small. Thus more genotyped goats need to be analysed for fatty acid composition to more fully understand the effect of *CSN1S1* on fatty acid composition.

5.4.6 Other considerations

While conducting this research it was noted that some AF genotype does produced milk with high fat and protein content, while others produced low fat and protein content. It is possible that this could be attributed to alternatively spliced transcripts derived from exon skipping. Exon skipping is not rare in the caseins, with events characterised in ovine (Passey et al., 1996, Giambra et al., 2010, Boisnard et al., 1991), equine (Lenasi et al., 2003, Mateos et al., 2009) bovine (Mohr et al., 1994, Bouniol et al., 1993, Mahe et al., 1999) and human caseins (Johnsen et al., 1995, Martin and Leroux, 1992, Menon et al., 1992). Leroux *et al.,* (1992) found 9 different transcripts of the *CSN1S1* F allele, most of which were 'aberrantly spliced' and lacked three exons, however the properly spliced messengers were also produced. These were attributed to various SNPs and insertions occurring in the introns downstream of exon 9, reducing the efficiency and accuracy of splicing machinery. It is therefore possible that at the time of herd-testing, some AF individuals produced more of the correctly transcribed *CSN1S1* allele F mRNA, while others produced more short-form, exon-deprived transcripts. This could have resulted in high and low total protein respectively of AF variants.

Another factor to consider is that *CSN1S1* is not the only polymorphic casein gene and that whole casein haplotypes exist. At present there are 5 β-casein (*CSN2*), 16 κ-casein (*CSN3*) and 5 αs2-casein (*CSN1S2*) variants identified to date, in addition to the 18 *CSN1S1* alleles. Studies at the casein haplotype level have been conducted in Italian (Albenzio et al., 2009, Caroli et al., 2006, Gigli et al., 2008, Sacchi et al., 2005), Norwegian (Hayes et al., 2006) and African (Caroli et al., 2007, Vacca et al., 2009) dairy goat populations. These all suggest strong linkage disequilibrium between the casein genes, in particular between SNPs in *CSN1S1* and *CSN2* and SNPs in *CSN1S2* and *CSN3* (Hayes et al., 2006). Such haplotypes have been shown to have a significant effect on milk protein and fat content (Albenzio et al., 2009, Hayes et al., 2006). One hypothesis is that when expression of one casein gene is down-regulated, the others can be up-regulated to compensate (Leroux et al., 2003). Thus the lack of correlation of *CSN1S1* genotype to some aspects of milk production may be explained by the unknown genotypes of other casein variants and highlights the value of whole haplotype analysis rather than each gene in isolation.

5.5 Implications for the dairy goat industry

As a result of this initial evaluation of *CSN1S1* in the NZ dairy goat population, there are two key impacts for the NZ dairy goat industry. Firstly, *CSN1S1* showed no significant effect on milk solids which translates into no economic gain for farmers based on the current pay-out scheme. Recent moves within the industry were to adjust the pay-out structure and reward for additional protein content. Similar schemes exist in Norway where farmers are paid for kilograms of milk, with a bonus for dry-matter content (Hayes et al., 2006). Encouraging a similar pay-out here could be worthwhile given the economic efficiency of producing, transporting and processing large volumes when nearly all goat milk is spray-dried into powders. Therefore rewarding farmers for fat and protein content may make more sense from an efficiency perspective. In this instance, selecting for high *CSN1S1* variants would be beneficial by significantly improving protein and fat percentages.

However care must be taken when selecting based on a single trait due to the polygenic nature of milk production and possible implications on other functional traits. For example Barillet *et al.,* (2007) modelled a system where selection was on milk traits only and found that in the long term such selection will lead to baggy udders that would be more difficult to milk by machine and more susceptible to mastitis. Therefore care must be taken if recommending a selection scheme, particularly when we do not fully understand the genetic structure of other linked casein genes and functional traits such as udder morphology and disease susceptibility in the NZ population.

Lastly, if DGC were to encourage selection based on *CSN1S1* for high protein and high fat percent, consideration must be given to the impact on milk allergenicity. Selecting for low or null αs1-casein genotypes has been shown to significantly reduce the allergenic burden (Ballabio et al., 2011, Park, 1994). Both αs1- and αs2-casein have been implicated in producing the allergenic response (Marletta et al., 2004, Bidat, 2010, Restani et al., 2009) and therefore a more effective strategy would be to select on the basis of a haplotypes that carry low *CSN1S1* and *CSN1S2* alleles. However, because low *CSN1S1* genotypes decrease fat and

protein content in some situations, the production of low allergenicity milk would be best as a 'niche' production system, separate from the mainstream spray-dry powder business unit.

5.6 Areas for future research

Firstly better characterisation of the 'unknown' genotype would be of value for (a) improving methods used to genotype at the *CSN1S1* locus and (b) to better understand polymorphisms of the *CSN1S1* gene. Improving sequence data would be the best strategy for achieving this characterisation.

There is the potential to improve milk composition using genetic factors such as *CSN1S1*. Although this is one gene, it has clearly demonstrated a significant effect in some circumstances in a NZ dairy farming system. Further clarifying the situations which allow *CSN1S1* to influence milk production could be useful. Based on the results of this study, diet and stage of lactation would be two worthy candidates.

Additional research would also prove valuable with regard to the genetics of fatty acid composition and SCC in milk. Both are of great significance to the human health aspect of goat milk and understanding their genetic basis would add value to a proposed selection scheme. Similarly a better understanding on whole casein haplotypes and milk composition could improve milk quality. In particular, the polymorphism in β-casein would be of value given its contribution to total milk protein, as well as *CSN1S2* for its effects on milk allergenicity.

Overall, a stronger focus on dairy goat genetics would extend the findings from this initial study, and allow better breeding practices for the production of goat milk in NZ.

Chapter 6: Nutrition Methods

The nutritional component of this study sought to determine the effect of key dietary supplements on milk composition. To achieve this, three feed trials were conducted. Feed Trial 1 compared fatty acid composition between farms under palm kernel extract (PKE) and biscuit waste (BW) feeding regimes. Feed Trial 2 investigated milk composition of those same farms in the next season following the removal of PKE and BW supplements. Feed Trial 3 investigated the effect of yeast-supplementation on fatty acid profiles, milk volume, protein, fat, solids and somatic cell count from control and yeast-treated goats. The methods used to carry out these three feed trials are outlined below.

6.1 General methods

This section outlines the general methods employed for farm selection, milk composition analysis and data analysis.

6.1.1 Farm selection

A detailed record of every Dairy Goat Co-operative (DGC) farm is conducted annually by the DGC veterinarian. These cover all aspects of farm practice including animal feeding, animal health, farm management, breeding and housing. From these records, DGC farms were chosen for feed trial analyses. Key factors in farm selection were:

- The type of primary feed supplement in addition to pasture / forage
- Location of the farm (North Island, New Zealand).
- Type of farm (indoor, outdoor or indoor plus loafing barn)
- Similarity of other factors such as minor feed supplements, pasture composition or mineral supplementation.
- No recent major animal health issues
- No major changes in farm management, nutrition, herd size or breeding planned between 2009 and 2011.

6.1.2 Sample collection – fatty acid samples

Milk samples for Feed Trial 1 were collected December-January 2009-2010 while Feed Trial 2 samples were collected through the same period in 2010-2011. These periods were chosen as they are mid-lactation and typically the most stable between farms in terms of milk composition. For both of these trials a total of four vat samples were taken from each farm; two in December and two in January. Milk was taken from the vat following the evening milking. Vat samples were collected and stored in a 50mL tube (Sterlin) and labelled with the farm identification number and date. Samples for Feed Trial 3 were collected in December. One milk sample was collected from each control or yeast-treated goat by hand-milking into a 50mL tube, as per genetic sample collection described in Chapter 3. All samples were stored at -20°C until they could be sent to AgResearch for fatty acid profile analysis using GC-MS.

6.1.3 Sample collection – herd test samples

Herd testing was used to measure animal performance and milk composition of individual goats in Feed Trial 3. Farm A's herd tests were conducted once each month from November 2010-March 2011. Farm B had one herd test in October. All herd test sampling and analysis were conducted as per LIC herd-testing protocols outlined in Chapter 3.

6.1.4 Data Analysis

All milk composition data from AgResearch and LIC was directly imported into Microsoft Excel and analysed using STATISTICA software. Significant differences were calculated using one-way ANOVA. ANOVA determined whether the differences between groups (farms in Feed Trial 1, seasons in Feed Trial 2 and treatment in Feed Trial 3) were more significant than the internal variation within each group. A *p*-value of less than 0.05 was considered significant. Graphs were plotted in Microsoft Excel using means and standard deviation as the estimate of error.

6.2 Feed Trial 1: PKE, Biscuit Waste and Control Farms

Suitable farms based on the criteria outlined in section 6.1.1 were categorised by whether they were supplementing with PKE, BW or a control diet of forage and grain. For all farms pasture remained the main diet. Farms that were selected and used in Feed Trial 1 are outlined below in Table 33. Herd sizes ranged from 270 to 1,300 does, with the average farm having 640 milking does. The total number of does from all 15 farms used in Feed Trial 1 was 9,602.

Table 33: Farms used in Feed Trial 1, selected based on the predominant feed supplement in 2009-2010. Each farm is given an identification (ID) letter and the number of farms in each category is outlined.

Feed Supplement	Farm ID	no. farms
Control	E,F,G,H,J,K,M	7
Biscuit Meal	A, B, D	3
PKE	C,I,L,N,O	5
Total		**15**

All farms were managed as per normal commercial dairy goat farming operations. All supplements were sourced from NZ suppliers and the provision of PKE and BW supplements were as recommended by feed suppliers and the DGC veterinarian. Typically PKE was supplemented at 0.3kg/doe/day and BW at 0.2kg/doe/day which was fed to the goats in the shed during milking. Compositions of the two supplements are outlined below in Table 34.

Table 34: Typical compositions of PKE and BW commercial animal feed supplements. n/a no data available. NDF = Neutral Detergent Fibre. ADF = Acid Detergent Fibre. Data sourced from RD1 (2011) and SourceNZ (2011).

Composition	PKE	BW
Energy (MJ/kg)	11.5	14.1
Crude Protein %	17	10.7
Dry matter %	92	92
NDF %	67	n/a
ADF %	42	n/a
Fat %	8	6.1

6.3 Feed Trial 2: Removal of PKE and BW Supplements

Feed Trial 2 investigated fatty acid composition in the same period as Feed Trial 1 (December and January) but in the next season (2010-2011). In the 2010-2011 season PKE and BW farms from Feed Trial 1 were no longer feeding the supplements. The effect of these supplements was determined by comparing fatty acid composition in 2009-2010 season with 2010-2011 data. The differences between seasons was analysed relative to seasonal changes in control farms which did not change diets. All farms were checked using updated farm record data to ensure they still met the initial farm selection criteria.

6.4 Feed Trial 3: Yeast Supplement versus Control

One Waikato outdoor farm was used for the investigation of yeast supplementation on milk composition. 20 randomly selected does were fed a yeast supplement mixed with a concentrate (Yeast Group) with another 20 randomly selected goats fed concentrate (Control Group). This was in addition to normal outdoor feeding conditions applied to both groups. The yeast was an active dry yeast (*Saccharomyces cerevisiae*) supplied by Coultar Grain. The concentrate was a cereal mix of maize (74%), peas (17%), soybean oil (3.8%) and molasses (2.8%) supplied by The Straw Warehouse (Cambridge, New Zealand). The nutritional characteristics of concentrate blended with yeast is outlined in Table 35 and quantity fed to each treatment group in Table 36.

Table 35: Nutritional characteristics of active dry yeast + concentrate supplement. The yeast had a minimum of 15 billion cells / gram. DM = Dry matter. NDF = Neutral detergent fibre. ADF= Acid detergent fibre.

Nutrient	Unit	Quantity
ME	MJ/kg	12.7
Protein	% DM	9.9
Oil	% DM	6.9
Fibre	% DM	2.4
NDF	% DM	9.2
ADF	% DM	3.0

Table 36: Quantities of each supplement fed to Control and Yeast treated goats.

Group	Treatment
Control	1kg concentrate / doe
Yeast	1kg concentrate + 20g yeast / doe

All yeast and control treatment goats were the same breed, multiparous, of good health and similar in age (2-5 years). Yeast and control goats were fed the supplement after morning milking once a day for four months. Supplementation began in November 2010 and remained until February. The whole herd (n=320) went onto the yeast treatment from February to the end of lactation in March.

The effect of yeast supplementation with the whole herd in March 2011 was unable to be compared to March 2010 when no yeast was supplemented as the herd milked for three weeks longer in 2011. This meant the herd was at a different stage of lactation in March of each year and thus milk composition data was incomparable.

Chapter 7: Nutrition Results

This chapter outlines results from the three feed trials investigating palm kernel extract (PKE), biscuit waste (BW) and yeast supplementation.

7.1 Feed Trial 1 – PKE, BW and Control Farms

Significant differences between PKE, BW and Control farms were found in 14 fatty acids. Most of these differences were highly significant, with *p*-values less than 0.01 (Table 37). Although significant, some differences were only small in terms of proportion of fatty acids.

Table 37: Differences in fatty acid composition between farms based on supplementation category in the 2009-2010 season. p-values were determined by ANOVA analysis using STATISTICA. Significant results were denoted by a * ($p<0.05$), ** ($p<0.01$) or NS for non-significant data ($p>0.05$).

Fatty Acid	Control		PKE		BW		*p*-value	Significance
	Mean	SD	Mean	SD	Mean	SD		
C4:0	3.29	0.02	3.28	0.05	3.25	0.02	0.70	NS
C6:0	2.88	0.03	2.74	0.04	2.91	0.05	<0.01	**
C8:0	2.85	0.04	2.58	0.05	2.92	0.08	<0.01	**
C10:0	8.98	0.14	8.03	0.17	9.12	0.25	<0.01	**
C12:0	4.50	0.24	7.52	0.61	4.03	0.14	<0.01	**
C14:0	10.32	0.14	11.87	0.44	9.32	0.25	<0.01	**
C14:1	0.36	0.02	0.36	0.02	0.28	0.03	0.050	Borderline
C15:0	1.06	0.03	0.97	0.02	0.79	0.02	<0.01	**
C15:1	0.26	0.01	0.19	0.00	0.24	0.02	<0.01	**
C16:0	25.98	0.13	24.76	0.37	28.96	0.98	<0.01	**
C16:1	0.61	0.01	0.72	0.03	0.76	0.04	<0.01	**
C17:0	0.60	0.02	0.50	0.02	0.47	0.02	<0.01	**
C18:0	9.13	0.12	8.43	0.49	8.20	0.19	0.08	NS
TVA	0.33	0.05	0.43	0.05	0.75	0.11	<0.01	**
C18:1 *cis*9	20.07	0.27	19.68	0.50	20.29	0.29	0.56	NS
CVA	0.74	0.01	0.92	0.04	0.82	0.04	<0.01	**
LA	2.21	0.09	1.49	0.18	2.03	0.06	<0.01	**
CLA	0.83	0.03	0.83	0.04	0.74	0.05	<0.01	**
ALA	0.99	0.04	0.74	0.06	0.69	0.06	0.25	NS

PKE had a significant effect ($p<0.01$) on MCFAs C12:0 and C14:0, with a higher percentage of these fatty acids relative to control farms (Figure 33 and Figure 34). BW farms produced milk with significantly more C16:0 (Figure 31) and *trans*-vaccenic acid (TVA) (Figure 32).

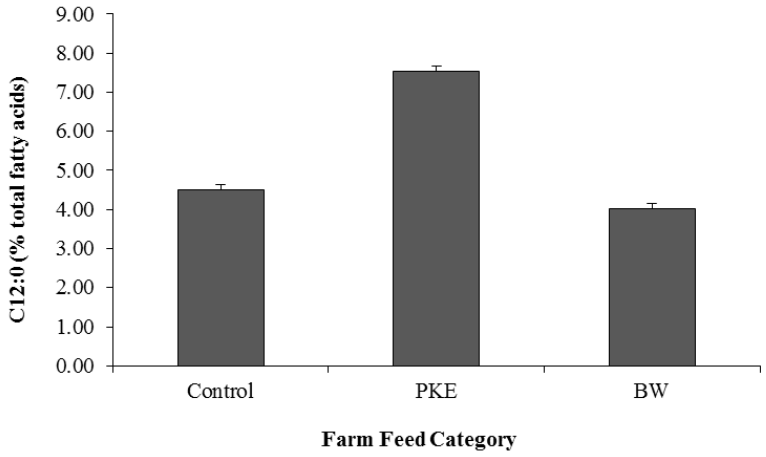

Figure 29: PKE farms had significantly higher C12:0 % ($p<0.01$) compared to Control and BW farms. Error bars denote the standard deviation.

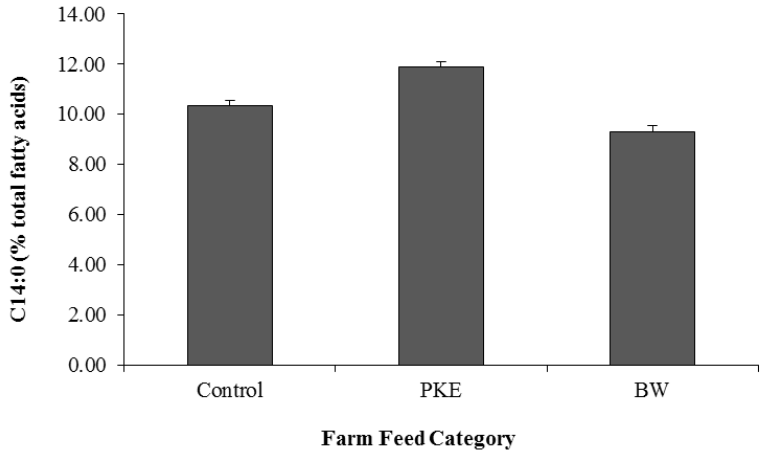

Figure 30: PKE farms had significantly higher C14:0 % ($p<0.01$) compared to Control and BW farms. Error bars denote the standard deviation.

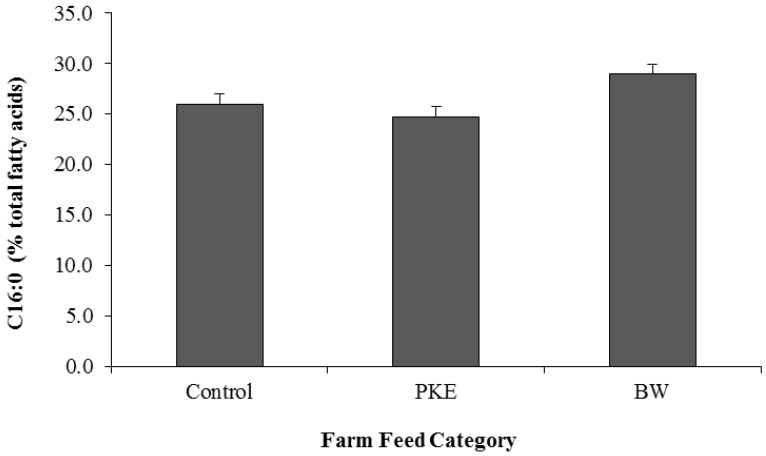

Figure 31: BW farms had significantly ($p<0.01$) higher C16:0 % compared to Control and PKE farms. Error bars denote the standard deviation.

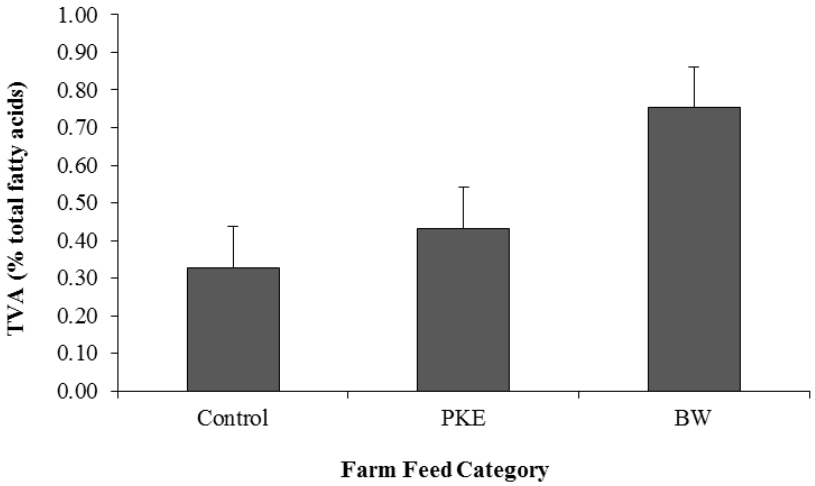

Figure 32: BW-supplemented farms had significantly more TVA % in milk compared to control and PKE farms (p<0.01). Error bars denote the standard deviation.

Butyric (C4:0), stearic (C18:0), oleic (C18:1 *cis*9) and ALA (C18:3n3) showed no significant difference between farm supplementation categories. Myristoleic acid (C14:1) showed borderline effects (p=0.05).

Therefore most farms showed a statistically significant difference from each other based on their respective feeding regimes. The most notable effects were on MCFAs for PKE and C16:0 / TVA for BW supplementing farms.

7.2 Feed Trial 2: Removal of PKE and BW nutritional factors

Most fatty acids exhibited significant seasonal variation, as shown by significant *p*-values generated from differences between 2009-2010 and 2010-2011 seasons on control farms (Table 38). Therefore, to factor in seasonal variations in fatty acid composition, significant effects of PKE and BW removal were determined relative to control-farm changes.

The most notable effect was the removal of PKE on C12:0, C14:0. The percentages of C12:0 (Figure 33) and C14:0 (Figure 34) were reduced to control farm levels following the removal of PKE relative to control farms.

Table 38: Fatty acid composition between 2009-2010 and 2010-2011 seasons for control, PKE and BW farms. 2010-2011 season no PKE or BW was supplemented on 'PKE' and 'BW' farms. *P*-value highly significant at ** <0.01, * p=<0.05 or NS (not significant, p>0.05).

Fatty Acid	Control Farms					PKE Farms					BW Farms				
	2009-2010		2010-2011		*p*-value	2009-2010 (PKE)		2010-2011 (no PKE)		p-value	2009-2010 (BW)		2010-2011 (no BW)		*p*-value
	Mean	SD	Mean	SD		Mean	SD	Mean	SD		Mean	SD	Mean	SD	
C4:0	3.29	0.02	2.78	0.04	**	3.28	0.05	2.78	0.03	**	3.25	0.02	2.75	0.03	**
C6:0	2.88	0.03	2.29	0.03	**	2.74	0.04	2.40	0.04	**	2.91	0.05	2.39	0.03	**
C8:0	2.85	0.04	2.36	0.05	**	2.58	0.05	2.56	0.06	NS	2.92	0.08	2.59	0.06	**
C10:0	8.98	0.14	8.45	0.23	NS	8.03	0.17	8.91	0.29	**	9.12	0.25	9.56	0.38	NS
C12:0	4.50	0.24	3.31	0.10	**	7.52	0.61	3.23	0.13	**	4.03	0.14	3.68	0.17	NS
C14:0	10.32	0.14	9.67	0.28	*	11.87	0.44	8.43	0.25	**	9.32	0.25	9.73	0.41	NS
C14:1	0.36	0.02	0.33	0.01	NS	0.36	0.02	0.29	0.01	**	0.28	0.03	0.28	0.01	NS
C15:0	1.06	0.03	0.96	0.02	**	0.97	0.02	0.85	0.05	*	0.79	0.02	0.84	0.03	NS
C15:1	0.26	0.01	0.25	0.01	NS	0.19	0.00	0.23	0.01	**	0.24	0.02	0.24	0.01	NS
C16:0	25.98	0.13	28.63	0.31	**	24.76	0.37	27.32	0.39	**	28.96	0.98	28.70	0.55	NS
C16:1	0.61	0.01	0.79	0.02	**	0.72	0.03	0.68	0.02	NS	0.76	0.04	0.77	0.01	NS
C17:0	0.60	0.02	0.67	0.01	*	0.50	0.02	0.61	0.02	**	0.47	0.02	0.61	0.02	**
C18:0	9.13	0.12	10.30	0.27	**	8.43	0.49	10.36	0.33	**	8.20	0.19	9.51	0.34	**
TVA	0.33	0.05	1.79	0.11	**	0.43	0.05	2.44	0.15	**	0.75	0.11	2.07	0.24	**
C18:1*c*9	20.07	0.27	21.55	0.39	**	19.68	0.50	20.99	0.52	NS	20.29	0.29	20.58	0.73	NS
CVA	0.74	0.01	0.35	0.01	**	0.92	0.04	0.39	0.02	**	0.82	0.04	0.39	0.02	**
LA	2.21	0.09	2.63	0.10	**	1.49	0.18	4.55	0.35	**	2.03	0.06	2.41	0.20	**
CLA	0.83	0.03	0.67	0.04	**	0.83	0.04	0.92	0.04	NS	0.74	0.05	0.72	0.07	NS
ALA	0.99	0.04	0.67	0.04	**	0.74	0.06	0.73	0.04	NS	0.69	0.06	0.72	0.08	NS

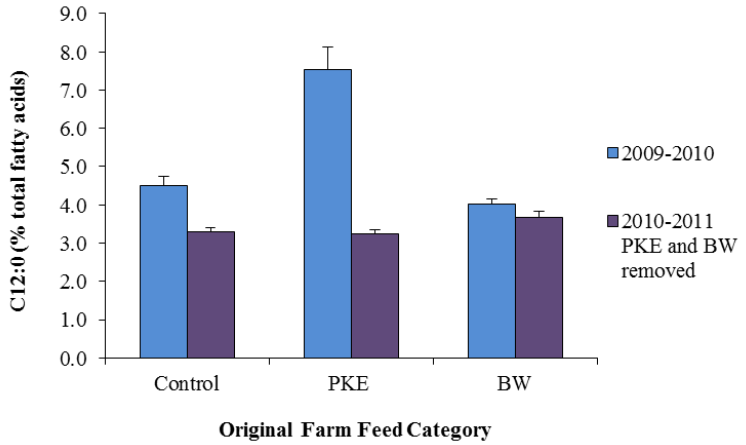

Figure 33: A significant reduction ($p<0.01$) in C12:0 % following the removal of PKE in 2010-2011. Although control farms also decreased ($p<0.01$) between these two periods, the relative change of PKE farms was almost double that of controls. Removal of PKE reduced C12:0 down to non-PKE farm levels. Error bars represent the standard deviation of the mean.

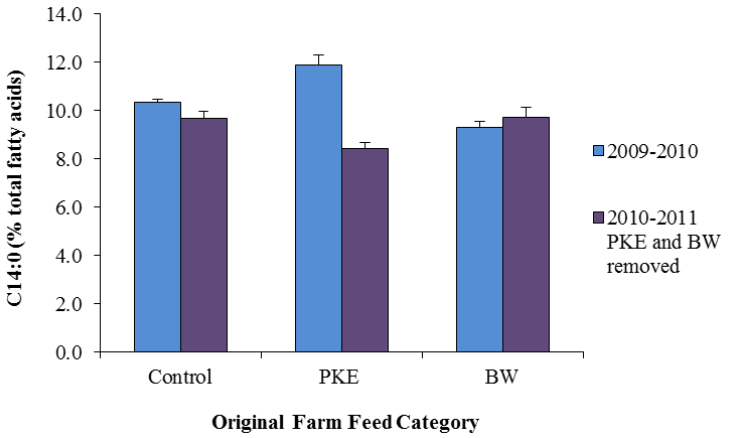

Figure 34: A significant reduction ($p<0.01$) in C14:0 following the removal of PKE in 2010-2011. Although less pronounced than C12:0, relative to control farm reduction PKE removal caused a significant decrease in C14:0. Error bars represent the standard deviation of the mean.

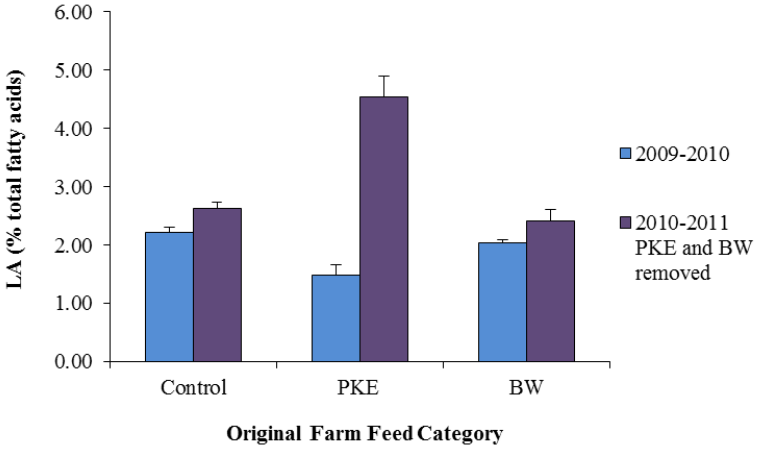

Figure 35: A significant increase in Linoleic Acid (LA) following the removal of PKE in 2010-2011 season. Relative to control farms, PKE significantly increased LA, while BW followed the same trend as Control farms.

Although BW elevated C16:0 and TVA in Feed Trial 1 relative to control farms, removal of these supplements had no effect on C16:0 (Figure 36). In the case of TVA, removal of BW increased this fatty acid even further (Figure 37).

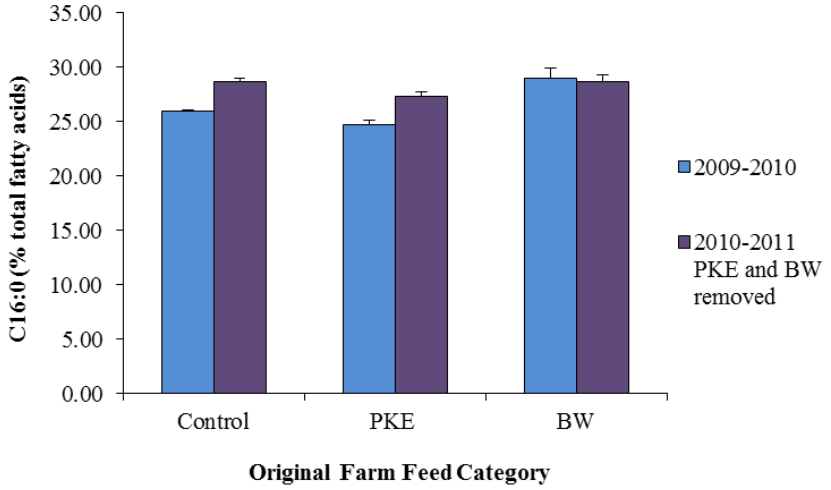

Figure 36: Removal of BW in 2010-2011 season had no effect on C16:0 %. Error bars represent the standard deviation of the mean.

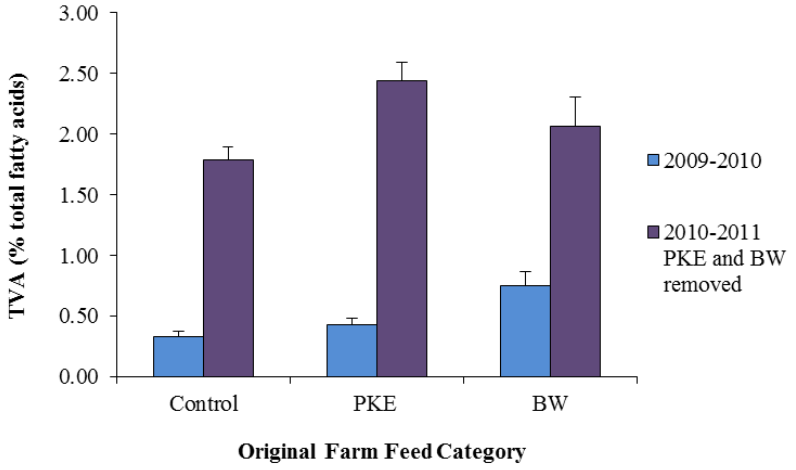

Figure 37: Removal of BW did not decrease TVA to control farm levels. Instead these farms showed significant seasonal increases in TVA ($p<0.01$). Error bars represent the standard deviation.

Variable effects of supplement removal were observed in C14:1, C15:0, C15:1, C16:1, C17:0 and CLA (Appendix G). These fatty acids were present in milk at less than 1% and thus the relative changes to fatty acid composition were small.

C4:0, C6:0, C8:0, C16:0, C18:0, C18:1 *cis*9 and TVA showed significant seasonal effects. As such they were unaffected by the removal of PKE and BW supplements (Appendix G).

7.3 Feed Trial 3: Yeast supplementation

Sufficient fat for fatty acid analysis could be recovered from milk samples of 17 control and 12 yeast-treated goats. No significant effect of yeast supplementation was found for any fatty acid (Table 39).

Table 39: Fatty acid comparison of control and yeast supplemented goats. *p*-values were determined by ANOVA analysis. Significant effect determined by a *p*-value <0.05. SD = standard deviation. NS = not-significant (*p* >0.05).

Fatty Acid	Control		Yeast		p value	Significance
	Mean	SD	Mean	SD		
C4:0	3.20	0.28	3.33	0.25	0.20	NS
C6:0	2.55	0.32	2.65	0.22	0.39	NS
C8:0	2.62	0.29	2.68	0.36	0.63	NS
C10:0	8.92	1.14	8.87	0.97	0.90	NS
C12:0	3.31	0.57	3.23	0.53	0.71	NS
C14:0	9.65	1.28	9.20	1.00	0.32	NS
C14:1	0.25	0.13	0.16	0.15	0.11	NS
C15:0	0.84	0.12	0.80	0.12	0.41	NS
C15:1	0.14	0.15	0.09	0.15	0.40	NS
C16:0	26.49	2.74	26.21	3.83	0.82	NS
C16:1	0.70	0.10	0.74	0.11	0.25	NS
C17:0	0.57	0.09	0.54	0.05	0.33	NS
C18:0	10.36	1.27	10.97	1.48	0.24	NS
TVA	2.32	0.65	2.82	0.82	0.08	NS
C18:1 *cis* 9	22.90	2.64	22.35	1.78	0.54	NS
CVA	0.46	0.09	0.50	0.07	0.32	NS
LA	1.91	0.22	2.04	0.24	0.15	NS
CLA	0.74	0.31	0.83	0.27	0.44	NS
ALA	0.66	0.14	0.66	0.14	0.99	NS

Results from herd test data showed no significant effect of yeast supplement on milk volume, fat, protein, solids or somatic cell count. Herd tests from mid-lactation (November) to the end of lactation (March) show that the supplementation of yeast had no effect throughout this period.

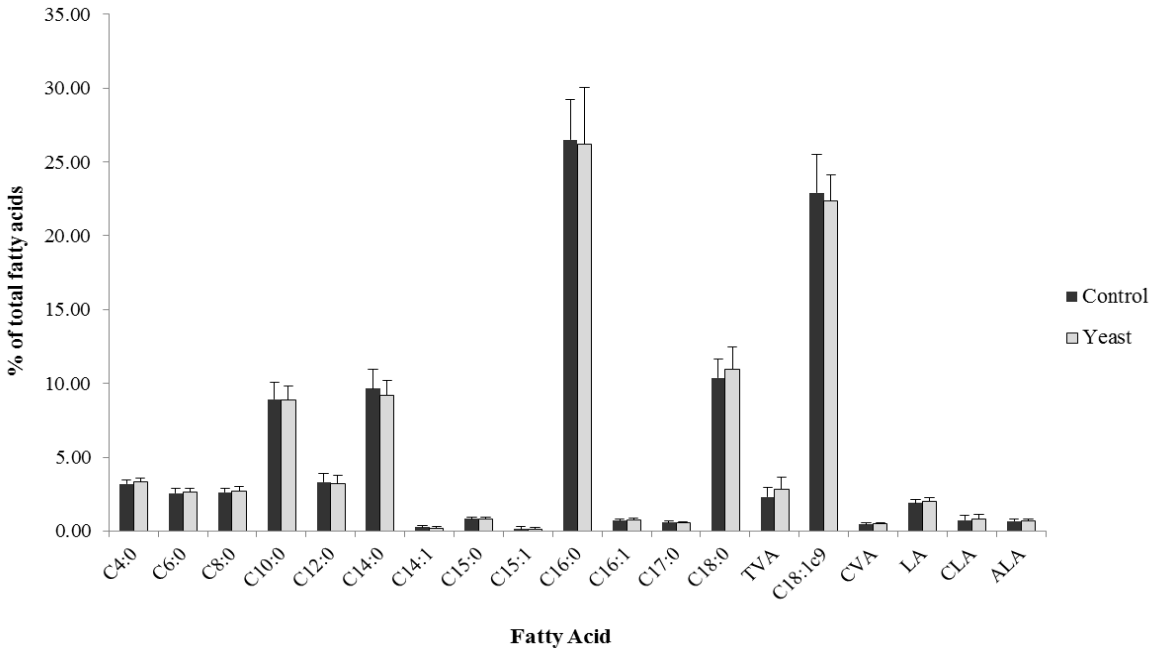

Figure 38: No effect of yeast was found on any fatty acid in milk of control and yeast treated does (p>0.05). Error bars represent the standard deviation of the mean.

Table 40: No effect of yeast supplementation was found on herd-test parameters in any month. A *p*-value of <0.05 was considered significant. SD= standard deviation.

Milk composition	Herd Test	Control		Yeast		*p*-value	Significance
		Mean	SD	Mean	SD		
Milk vol (L)	Nov	1.92	0.77	1.91	0.77	0.96	NS
	Dec	1.99	0.63	2.01	0.66	0.86	NS
	Jan	2.10	0.71	1.98	0.74	0.42	NS
	Mar	1.38	0.48	1.38	0.46	0.93	NS
Milk Fat %	Nov	3.55	0.50	3.39	0.60	0.15	NS
	Dec	3.39	0.57	3.29	0.52	0.37	NS
	Jan	3.33	0.57	3.21	0.51	0.28	NS
	Mar	4.02	0.77	3.83	0.70	0.23	NS
Milk Fat kg	Nov	0.07	0.03	0.07	0.02	0.85	NS
	Dec	0.07	0.02	0.07	0.02	0.85	NS
	Jan	0.07	0.02	0.07	0.03	0.78	NS
	Mar	0.06	0.02	0.05	0.02	0.41	NS
Protein %	Nov	3.23	0.30	3.29	0.36	0.44	NS
	Dec	3.19	0.30	3.19	0.31	0.95	NS
	Jan	3.17	0.31	3.15	0.36	0.75	NS
	Mar	3.36	0.31	3.39	0.33	0.72	NS
Protein kg	Nov	0.06	0.02	0.06	0.03	0.74	NS
	Dec	0.06	0.02	0.06	0.02	0.99	NS
	Jan	0.07	0.02	0.07	0.02	0.35	NS
	Mar	0.05	0.01	0.05	0.01	0.93	NS
Milk Solids (kg)	Nov	0.13	0.05	0.14	0.12	0.44	NS
	Dec	0.13	0.03	0.13	0.04	0.89	NS
	Jan	0.14	0.04	0.13	0.04	0.41	NS
	Mar	0.15	0.05	0.15	0.05	1.00	NS
SCC (000)	Nov	1623.4	2846.4	1401.4	1910.9	0.65	NS
	Dec	1791.3	3155.2	1749.3	4250.8	0.96	NS
	Jan	1160.7	1593.3	1287.1	1477.4	0.69	NS
	Mar	1513.3	2082.1	1447.8	1284.5	0.87	NS

7.4 Conclusions

The supplementation of PKE resulted in milk with significantly higher levels of C12:0 and C14:0 fatty acids. Removal of PKE from these farms reduced C12:0 and C14:0 down to control farm levels. Farms feeding BW had higher levels of C16:0 and TVA in Feed Trial 1, however the removal of these supplements had variable and inconclusive effects. Yeast supplementation had no effect on any fatty acid, nor on milk volume, protein, fat, solids or SCC. Significant seasonal effects on fatty acid composition were observed, particularly in SCFAs and TVA. Therefore supplementing NZ dairy goats with PKE is the only diet which had a discernible effect on milk composition.

Chapter 8: Nutrition Discussion

The successful marketing of goat milk produced in New Zealand (NZ) relies on a positive perception of milk quality in overseas markets. Nutrition is one of the fastest and most effective ways to modulate milk composition and can be used to improve the healthfulness and quality of goat milk. The aim of this project was to determine the effect of some alternative supplements, namely palm kernel extract (PKE), biscuit waste (BW) and yeast on goat milk composition. This chapter presents a discussion of key results and findings from this research, with specific reference to the implications for the NZ dairy goat industry. An evaluation of methods and areas for future research are also outlined later in the chapter.

8.1 Seasonal effects of Feed Trials 1 and 2

Regardless of feed category, all farms showed a similar decrease in C4:0, C6:0, and CVA and increase in C17:0, C18:0 and TVA content between Feed Trials 1 and 2, indicating a significant seasonal effect on these fatty acids. The 2009-2010 season was characterised by a significant drought throughout the North Island, the peak of which occurred during the Feed trial 1 sampling period. Such events are known to alter the quantity and type of pasture, as well as the fatty acid composition of forages (Langer, 1990, Mel'uchova et al., 2008), ryegrass in particular (Khan et al., 2011, Boufaied et al., 2003). Moreover, during times of pasture shortages more concentrated energy feeds are supplemented which are also known to impact milk fatty acid composition (Samkova et al., 2009, Slots et al., 2009).

Such seasonal effects on goat diets could explain the significantly higher levels of TVA and C18:0 in milk collected from all farms during the 2010-2011 season compared to 2009-2010 samples. Fresh green pasture is very high in ALA (C18:3n3) and LA (C18:2n6) which are isomerised and hydrogenated in the rumen to TVA and C18:0. Thus the TVA and C18:0 data suggest more fresh pasture was available in the 2010-2011 season compared to the drier 2009-2010 season. Additionally, all farms experienced a significant reduction in CVA in milk collected during the 2010-2011 season. CVA is predominantly

formed by the desaturation of C18:0 in the mammary gland by SCD, which is known to be inhibited by C18 isomers, particularly the *trans*-fatty acids. Thus the increased TVA (arising from high ALA in pasture) could have down-regulated SCD activity to reduce CVA content in 2010-2011 season milk. An analysis of SCD gene expression would be required to verify this, however similar observations have been noted in goats and sheep (Addis et al., 2005, Cabiddu et al., 2005). In particular the CVA decrease and C18:0 increase relate strongly to the effect on stearic acid desaturation ratios (CVA:C18:0) following pasture consumption described by Sans Sampelayo *et al.*, (2007).

The physiology of ruminants during dry periods also needs consideration. Although goats are renowned for their climatic tolerance, high yielding dairy goats can suffer from heat-stress which can affect milk production and composition (Ishler et al., 1996). The significantly ($p<0.01$) elevated levels of C4:0 and C6:0 during the dry 2009-2010 season could be due to increased rates of lipolysis arising from the mobilisation of body reserves during periods of low feed (Chilliard *et al.* 2003). Such effects have been observed in milk of heat-stressed dairy ewes which also showed higher proportions of these short-chain fatty acids (Sevi et al., 2001) and thus could explain the seasonal effect on these fatty acids in goat milk.

Therefore changes associated with dry weather, pasture composition and heat-stress on dairy goats are likely to have had more of an effect on some fatty acids (particularly C4:0, C6:0, C18:0, CVA and TVA) than PKE or BW diets alone. Climatic variations are a natural part of dairy goat farming in NZ and as shown in this research, can have a significant impact on milk composition.

8.2 Palm kernel extract

New Zealand is one of the largest importers of PKE for stock-feed purposes and up until late 2010 over 80% of Dairy Goat Co-operative farms were feeding the supplement. As outlined in Chapter 7, this research has shown that PKE significantly increased C12:0 and C14:0 fatty acids in goat milk. Removal of this supplement reduced C12:0 and C14:0 back to non-PKE levels and thus

indicates a very clear effect of PKE on these medium-chain fatty acids (MCFA).

Fatty acid data for PKE is scarce, however palm kernel oil (PKO) is known to be rich in C12:0 and C14:0 (53.2 and 19.3% of total fatty acids respectively) (Bora et al., 2003). Thus assuming a similar composition of PKE to PKO, the C12:0 and C14:0 consumed in PKE diets would appear to be transferred directly to milk. This is a likely scenario as C12:0 and C14:0 are predominantly formed via the 'diet' pathway, rather than by *de novo* synthesis (refer Chapter 2) and therefore are strongly impacted by dietary factors. Given these fatty acids are already saturated, no biohydrogenation or isomerisation processes can modify C12:0 or C14:0 in the rumen. Moreover, this study showed no effect of PKE supplementation on C14:1 which suggests minimal Stearoyl-CoA-desaturase (SCD) modification in the mammary gland, at least for C14:0. Therefore C12:0 and C14:0 consumed in PKE diets do not appear to be modified by rumen bacteria or mammary enzymes and are thereby transferred directly from this diet into the milk.

8.3 Biscuit waste

Biscuit waste is a unique supplement in which little is known of its effects on milk composition. Results from this research showed no clear effects of BW supplementation on milk composition. Although significant differences were observed in Feed Trial 1, removal of BW did not reverse these effects. The majority of fatty acids either showed no significant difference between seasons or mirrored control farm changes.

This is surprising given that biscuits and confectionery are typically high in saturated and *trans*-monounsaturated fats (USDA, 2011). Based on other studies it would be expected that increasing such fats in the diet would result in higher levels of C18:1, C18:0, C16:0 and C14:0 due to microbial hydrogenation and isomerisation processes acting on long-chain saturated and unsaturated fats in the rumen. A high level of conjugated and *trans*-fats are known to reduce SCD activity in sheep, cows and goats (Perfield et al., 2006,

Baumgard et al., 2000, Harvatine and Bauman, 2011, Peterson et al., 2002, Loor and Herbein, 1998, Bernard et al., 2005) and therefore result in higher levels of saturated fats in ruminant milk. However without fatty acid compositional data for BW supplements it is difficult to predict likely outcomes for the effect of BW in the diet.

A factor that may have affected BW non-significant results is that following the elimination of BW supplements not all farms reverted to a control-type diet. In most cases alternative supplements were introduced, namely dried distillers grain (DDG) or brewers grain (BG). In dairy cows both distillers and brewers grains have been shown to alter fatty acid composition (Sasikala-Appukuttan *et al.*, 2008, Schingoethe et al., 1999, Miyazawa et al., 2007). Thus fatty acid changes following the removal of BW may have been masked by the effects of DDG / BG supplements. This could also apply to fatty acids that did not show any effects with PKE supplementation and removal.

8.4 Yeast

Yeast supplementation has been used as a non-hormonal feed additive to promote growth by improving digestion and increasing feed intake in ruminants (Higginbotham et al., 1994, Enjalbert et al., 1999, Castro et al., 2002, Alshaikh et al., 2002, Harrison et al., 1988, Sune and Muhlbach, 1998). There are however mixed reports on yeast and milk composition in dairy goats (Desnoyers et al., 2009). Results from this research show no significant effect of 20g/yeast/doe/day on fatty acid composition, milk volume, protein, fat, solids or somatic cell count from mid-late lactation.

Other yeast experiments in dairy cows and goats have similarly shown yeast addition did not produce any difference in milk composition. For example 10g of yeast did not affect feed intake, milk yield, milk fat, milk protein or milk casein in dairy goats (Salama et al., 2002). Hadjipanayiotou *et al.*, (1997) also found no effect on ewe or goat milk composition with 12.5g yeast/kg of concentrate while multiple studies found no of effect of 3-90g/day of yeast in dairy cows (Arambel and Kent, 1990, Chiquette, 1995, Kamalamma et al., 1996, Yalcin et al., 2011). No published results were found for yeast and fatty

acid composition in dairy goats, however no effect was found in with ewes (Masek et al., 2008) or cows (Castillo et al., 2006).

On the contrary research by El Ghani (2004) *et al.,* found that supplementing early-mid lactation Zairaibi does with 6g yeast mixed with 60% concentrate and 40% roughage (straw) did increase milk volume, protein content and milk solids. Stella *et al.,* (2007) also found an effect using just 0.2g/day with early lactation Saanen does on milk production. Thus lower quantities of yeast than used in the current study yielded significant effects in some circumstances.

It could be that yeast only impacts milk composition during pre-kidding or early lactation periods. However this would need to be verified with another trial during these periods as such a conclusion is not consistent with successful yeast feed trials in the literature. Other influences such as the basal diet, strains of yeast, yeast activity, rumen microbe populations, level of drenching or climate may also be important and could warrant further investigation.

8.5 Implications for the dairy goat industry

The increase in C12:0 and C14:0 following PKE supplementation could have important implications for powdered nutritional products produced from NZ goat milk. Infant formula has a strict maximum requirement of 20% for C12:0 and C14:0 combined. Farms which supplemented PKE in the 2009-2010 season produced milk with an average C12:0+C14:0 total of 19.3% ± 0.53, versus non PKE farms at 14.8% ±0.19. Therefore farms supplementing with PKE were producing milk with less suitable fatty acid profiles for infant formula production. Consequently, if all farms produced fatty acid profiles with more than 20% C12:0+C14:0, extensive modifications would be needed to alter the fatty acid composition of powdered products. Doing so would require additional processing costs and be of detriment to the quality perception of naturally produced goat milk products from NZ.

Additionally, C12:0 and C14:0 are the two fatty acids with the least beneficial health effects for humans due to their contribution in raising blood plasma levels, low density lipoprotein, cholesterol and the risk of heart disease. However a key driver for the consumption of goat milk instead of cow milk is the higher proportion of MCFAs which enhance digestibility and nutrient absorption (Greenberger et al., 1966, Guillot et al., 1993). Although PKE removal reduced C12:0 and C14:0 MCFAs, goat milk from these farms still had more small and medium chain fatty acids (28.3%) than the average cow milk (25.1% according to Mansson (2008)). Therefore goat milk without PKE supplementation markedly reduced the atherogenicity index while retaining a digestive advantage over cow milk. As such, DGC's decision to remove PKE as an allowable diet is likely to have improved milk composition for human consumption by being better suited to infant formula needs as well as general health.

Regarding BW it could be suggested that the removal of BW did not produce any significant alterations to milk composition and therefore could be used again as an allowable feed. This is purely from a nutritional view, as other factors such as milk production, rumen function or animal health were not investigated as part of this study. Moreover the perception from overseas buyers of NZ dairy goats consuming high fat and sugar would also need to be taken into consideration.

CLA content has been gradually decreasing in spray-dry powders produced by DGC over the last ten years. It was thought this may be due to an increase in supplements like PKE and BW in conjunction with a reduction of pasture consumption. From these results it is unclear whether PKE and BW supplementation were the cause of decreasing CLA. Variable effects were found on CLA content following the removal of PKE and BW, while control farms showed a significant seasonal effect on this fatty acid. Replacement of PKE/BW diets with DDG/BG supplements could explain the inconclusive effects of PKE/BW removal, while seasonal variations in pasture composition may be the cause of decreased CLA in 2010-2011 on control farms.

However fresh pasture is thought to increase CLA content (Chilliard and Ferlay, 2004, Pajor et al., 2009, Bargo et al., 2006), not decrease as observed on control farms in the 2010-2011 season. TVA, the precursor of CLA was significantly increased in the 2010-2011 season, however this did not translate into higher levels of CLA. As discussed previously, this could be due to high levels of TVA inhibiting the conversion of TVA to CLA by SCD in the mammary gland. Overall, it cannot be concluded from these results whether PKE and / or BW supplementation has caused the gradual decline in CLA content of DGC goat milk and requires further investigation.

With regard to yeast supplementation it is clear from the current research that under NZ goat farming conditions, yeast (20g/doe with 1kg of maize concentrate) has no effect on milk composition parameters tested in this study. Effects on body condition score, animal health, digestion or rumen function were not investigated as part of this research however from a milk fatty acid composition, yield, protein and fat viewpoint the supplementation of yeast is not effective for this purpose. Thus for the dairy goat industry it would be suggested that, at least during mid-late lactation, the supplementation of yeast is not an effective feed additive to increase production or alter composition of goat milk.

8.6 Evaluation of methods and areas for future research

This research has allowed the investigation of nutritional factors using three different feed trial methods. The use of commercial dairy goat farms in such research has a number of benefits including large sample numbers, ease of animal management and the production of results which are commercially relevant and applicable to 'normal' farming situations. However the downside to such experiments is the inherent variability associated with multiple, independently managed farms, sampled across two seasons.

In this respect results from Feed Trial 1 detected between-farm differences, however additional studies such as supplement removal (conducted in Feed Trial 2) or isolated feed trials are required to verify that a particular diet caused

that effect and not some other factor of farm management. Alternatively, extending PKE and BW supplementation through a number of seasons and increasing the number of samples collected could assist in determining such dietary effects. Similarly Feed Trial 2 could be improved by more focussed feed trials (e.g. control versus treatment goats on the same farm, during the same period) to ensure supplement removal effects are not associated with seasonal variations such as climate and pasture composition.

Feed trial 3 had a more controlled experimental design in that it reduced many of the varying factors present in Feed Trials 1 and 2. In saying this, the results may only be applicable to that particular farm's basal diet, climate and farm management and may not represent the potential effect of yeast supplementation on NZ dairy goat farms as a whole. As such, extending the yeast trial to other NZ farming systems (different base diets, indoor farming) and periods of lactation could add value to the results. Similarly trialling different concentrations and types of yeast may produce significant effects of this supplement on milk composition.

A further investigation into why previous PKE farms showed elevated LA content in the 2010-2011 season may also be of interest given the health benefits regularly promoted for this fatty acid. It may be that newer DDG and BG supplements are promoting increased LA milk content. This would need to be verified using controlled feed trials similar to Feed Trial 3. Since many farmers are now feeding these two supplements it may be worth investigating the effect of these diets on milk composition.

Lastly, in light of the fact that PKE is the major supplement fed to New Zealand's expansive dairy cow industry, it would be interesting to see if PKE supplementation also increases C12:0 and C14:0 milk content from dairy cows. Cow milk produced in NZ is also used for a similar infant formula and spray-dry powder purposes and predominantly exported to overseas markets, however no milk compositional studies of PKE supplementation have been reported to date.

Chapter 9: Interaction of Nutritional and Genetic Factors

The formation of milk constituents is a complex process, one which involves many interacting elements. Nutritional and genetic factors are no exception and can have combined effects on milk composition. Diet is known to influence gene expression, as reflected in the growing field of nutrigenomics. One of the most noted 'nutrigenomic' effects observed in ruminants is the regulation of milk fat synthesis by long-chain *trans* and conjugated fatty acids such as TVA and CLA which can result in milk fat depression, particularly in dairy cows (Bernard et al., 2008, Bauman et al., 2008, Harvatine et al., 2009b, Shingfield and Griinari, 2007). In this way, diet can influence the expression of genetic factors to have a significant impact on milk composition.

The reverse can also occur, where genetic factors influence the effectiveness of ruminant diets. This has been shown for *CSN1S1*, where Pagano *et al.*, (2010) trialled 27 goats of three genotypes (AA, AF and FF) with three different diets at 100%, 65% and 30% energy levels (hay concentrate). They found a significant energy x genotype effect ($p<0.05$), where AA goats only showed effects on milk yield increase when fed 100% concentrate. The authors concluded that strong alleles are associated with a greater efficiency of feed utilisation and seem to show that a high energy level of the diet can further improve this efficiency.

The same group (Valenti et al., 2010) also analysed the effect on fatty acid composition of AA and FF goats at 100% and 65% concentrate. The genotype x diet interaction was significant for 11 different milk fatty acids. In particular, C8:0, C10:0, C12:0, C14:0 increased when FF animals shifted from 100% to 65% concentrate, while the same fatty acids did not significantly change in AA animals. Additionally LCFAs increased in AA and decreased in FF goats, leading the authors to conclude that *CSN1S1* causes goats to respond in a different way when fed diets with different energy levels.

Although not specifically studied as part of this research, the interaction of dietary and genetic factors can be considered in some instances where unexpected results have occurred. For example where Farm A had no effect of genotype while Farm B did. Although both were on the same basal diet of pasture and forage, the dietary energy is likely to have been more on Farm B which regularly fed more concentrated forages such as maize and lucerne silage. This higher energy level may be why Farm B produced significant differences in milk yield, protein and fat as a result of *CSN1S1* genotype. Although stage of lactation and farming system differences may also have had an effect, the significantly higher milk production on Farm B compared to Farm A is in agreement with the higher energy theory for diet x genetic interaction effects.

In a commercial situation it is necessary to understand the circumstances in which a particular genotype or diet is effective. Thus where interactions are known to occur, such as *CSN1S1* genotype and dietary energy level, this may influence selective breeding or supplementation strategies. The benefits of doing so have long been exploited in the NZ dairy cow industry. Extensive breeding records, progeny testing and artificial insemination have all allowed the genetic gain to be optimised in combination with significant nutritional research. Although it is common knowledge that well-fed animals produce more milk, understanding the mechanisms and effects of genetic and nutritional factors in dairy goats is under-studied compared to dairy cows. As such, further research into these interactions would be useful in the application of genotype, nutrition and milk composition effects identified in this research.

Chapter 10: Conclusions

Overall this research has highlighted the complexity of genetic and nutritional factors in the synthesis of milk components. This study is the first of its kind in New Zealand dairy goats and shows that milk produced by NZ dairy goats has the potential to be modified through genetic and dietary means.

From this research we can conclude that PCR-RFLP, AS-PCR and real-time PCR methods allow the successful identification of A* (G,H,I,02) B*(C,L), E, F, N and 01 alleles at the *CSN1S1* locus. EF and A*E genotypes are the most common. One unique genotype, which remains uncharacterised, was found at a reasonably high frequency on one farm. This genotype contains *CSN1S1* E and N variants, however peculiarities remain with the additional PCR product which could not be identified. This genotype had a medium effect on milk composition so is unlikely to be a detrimental variant for milk composition. New Zealand goat farms are not in Hardy-Weinberg equilibrium at the *CSN1S1* locus, as would be expected of populations with significant selection pressure, founder effects and in-breeding.

CSN1S1 genotypes can have a significant effect on milk composition, but the effects are not necessarily standard between farms. Protein content is the most significantly affected by *CSN1S1*, however this gene may also affect fat content and milk yield under certain circumstances. The exact situations in which *CSN1S1* has an effect on milk composition requires further investigation, but may include high energy status of the goats and early lactation.

Fatty acid composition was significantly affected by 'high' versus 'low' *CSN1S1* genotypes, although the relative changes were small. Fatty acid composition was most affected by nutritional factors, particularly palm kernel extract (PKE). Farms feeding PKE produced milk with significantly elevated levels of C12:0 and C14:0. It is likely these fatty acids were transferred into milk directly from PKE diets, with little modification by rumen microbes or mammary enzymes. The increase of C12:0 and C14:0 fatty acids in the milk of PKE-supplemented goats decreases the healthfulness of goat milk.

Biscuit waste (BW) and yeast had no significant effects on milk composition. Farms feeding BW had higher levels of C16:0 and TVA in Feed Trial 1, however the removal of these supplements had variable and inconclusive effects. Yeast supplementation had no effect on any fatty acid, nor milk volume, protein, fat, solids or SCC.

The effect of drought and other seasonal variations were inadvertently included in the study through the experimental design of Feed Trial 2. Seasonal differences appeared to have a significant effect on the milk fatty acid profile, especially TVA and SCFA.

Overall, this study has found significant effects of both genetic and nutritional factors on milk composition. Milk quality could be improved on the basis of these results by altering breeding strategies and feeding practices based on the desired milk composition. This study has provided the foundation for future research into NZ dairy goats, particularly regarding *CSN1S1* genotypes, PKE, BW and yeast. Ultimately the findings from this research will allow better decisions to be made which will improve the healthfulness and quality of goat milk produced in NZ.

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Appendices

Appendix A: Genotype Frequencies

Table 41: Overview of allele frequencies from other caprine CSN1S1 studies. •= unable to be distinguished from other alleles or not determined. Note most A alleles are used as 'all other'. Some alleles may be grouped together with the A, B, N, E, F and O alleles – see original paper(s) for specific details. PCR = Polymerase chain reaction. AS-PCR = Allele specific PCR. RFLP = Restriction fragment length polymorphism. SDS-PAGE = Sodium dodecyl sulphate polyacrylamide gel electrophoresis. IEF = Isoelectric focussing. MALDI-TOF-MS= Matrix-assisted laser desorption/ionization- time-of-flight mass spectrometry. RP-HPLC = Reversed phase high performance liquid chromatography. PCR-SSCP = PCR-single strand conformation polymorphism.

Country	Breed	No.	CSN1S1 allele						Methods	Reference
			A	B	N	E	F	O		
Brazil	Alpine	83	0.20	•	•	0.48	0.28	0.01	AS-PCR, PCR-RFLP	(Soares et al., 2009)
Brazil	Saanen	62	0.30	•	•	0.35	0.30	0.02	AS-PCR, PCR-RFLP	(Soares et al., 2009)
Czech Republic	Brown Shorthair	45	0.98	•	•	•	•	0.02	AS-PCR	(Sztankoova et al., 2006)
Czech Republic	White Shorthair	123	0.95	•	•	•	•	0.05	AS-PCR	(Sztankoova et al., 2006)
Czech Republic	Brown Shorthair	165	0.30	•	•	0.09	0.06	0.02	PCR-RFLP, AS-PCR	(Sztankoova et al., 2007)
Czech Republic	White Shorthair	333	0.27	•	•	0.05	0.66	•	PCR-RFLP, AS-PCR	(Sztankoova et al., 2007)
France	Alpine	213	0.15	0.05	•	0.34	0.41	0.05	SDS-PAGE	(Grosclaude et al., 1987)
France	Saanen	159	0.07	0.06	•	0.41	0.43	0.03	SDS-PAGE	(Grosclaude et al., 1987)
France	Corse	106	0.06	0.13	•	0.14	0.59	0.08	PCR-RFLP	(Martin and Leroux, 2000)
France	Poitevine	209	0.05	0.35	•	0.45	0.14	0.00	PCR-RFLP	(Martin and Leroux, 2000)
France	Rove	147	0.12	0.05	•	0.62	0.10	0.11	PCR-RFLP	(Martin and Leroux, 2000)
France	Alpine (1990)	400	0.52	0.33	0.02	0.27	0.17	•	AS-PCR, PCR-RFLP	(Ouafi et al., 2002)
France	Alpine (2000)	312	0.71	0.11	0.01	0.12	0.05	•	AS-PCR, PCR-RFLP	(Ouafi et al., 2002)
France	Poitevine	243	0.08	0.48	0.01	0.34	0.08	•	AS-PCR, PCR-RFLP	(Ouafi et al., 2002)
France	Pyreneenne	213	0.09	0.15	0.03	0.60	0.13	•	AS-PCR, PCR-RFLP	(Ouafi et al., 2002)
France	Saanen (1990)	312	0.17	0.04	0.01	0.59	0.19	•	AS-PCR, PCR-RFLP	(Ouafi et al., 2002)
France	Saanen (2000)	261	0.27	0.07	0.01	0.58	0.08	•	AS-PCR, PCR-RFLP	(Ouafi et al., 2002)
Hungary	Hungarian milking	103	0.09	0.29	•	0.08	0.46	0.00	AS-PCR, PCR-RFLP	(Kusza et al., 2007)
Hungary	Hungarian milking	109	0.61	•	•	0.08	0.31	•	AS-PCR, PCR-RFLP	(Veress et al., 2004)
India	Barbari	475	0.77	0.01	•	0.00	0.12	•	PCR-RFLP, SDS PAGE	(Kumar et al., 2007)
India	Beetal	45	0.72	0.00	•	0.00	0.17	•	PCR-RFLP, SDS PAGE	(Kumar et al., 2007)
India	Jakhrana	68	0.68	0.00	•	0.00	0.18	•	PCR-RFLP, SDS PAGE	(Kumar et al., 2007)
India	Jamunapari	179	0.72	0.01	•	0.00	0.15	•	PCR-RFLP, SDS PAGE	(Kumar et al., 2007)
India	Local MP	42	0.52	0.00	•	0.00	0.08	•	PCR-RFLP, SDS PAGE	(Kumar et al., 2007)

Table continued over

Country	Breed	No.	CSN1S1 allele						Methods	Reference
			A	B	N	E	F	O		
India	Local UP	110	0.59	0.00	•	0.00	0.19	•	PCR-RFLP, SDS PAGE	(Kumar et al., 2007)
India	Marwari	70	0.56	0.01	•	0.00	0.41	•	PCR-RFLP, SDS PAGE	(Kumar et al., 2007)
India	Sirohi	69	0.77	0.07	•	0.00	0.04	•	PCR-RFLP, SDS PAGE	(Kumar et al., 2007)
India	Barbari	475	0.74	0.01	•	•	0.12	0.09	SDS-PAGE	(Rout et al., 2010)
India	Beetal	45	0.72	0.00	•	•	0.17	0.11	SDS-PAGE	(Rout et al., 2010)
India	Ganjam	40	0.45	0.00	•	•	0.10	0.45	SDS-PAGE	(Rout et al., 2010)
India	Jakhrana	68	0.68	0.00	•	•	0.18	0.15	SDS-PAGE	(Rout et al., 2010)
India	Jamunapari	179	0.72	0.01	•	•	0.15	0.13	SDS-PAGE	(Rout et al., 2010)
India	Marwari	70	0.56	0.01	•	•	0.41	0.01	SDS-PAGE	(Rout et al., 2010)
India	Sirohi	69	0.77	0.07	•	•	0.04	0.13	SDS-PAGE	(Rout et al., 2010)
Italy	Garganica	71	0.51	0.20	0.02	0.01	0.24	0.01	AS-PCR, PCR-RFLP	(Albenzio et al., 2009)
Italy	Camosciata	88	0.44	0.20	0.01	0.11	0.23	0.01	IEF, PCR RFLP, AS-PCR	(Caroli et al., 2006)
Italy	Frisa	70	0.13	0.01	0.00	0.20	0.56	0.12	IEF, PCR RFLP, AS-PCR	(Caroli et al., 2006)
Italy	Orobica	66	0.00	0.01	0.00	0.01	0.96	0.02	IEF, PCR RFLP, AS-PCR	(Caroli et al., 2006)
Italy	Verzasca	67	0.00	0.04	0.00	0.20	0.75	0.01	IEF, PCR RFLP, AS-PCR	(Caroli et al., 2006)
Italy	Neopolitan Goat	285	0.14	0.17	0.23	0.08	0.37	0.00	AS-PCR, PCR-RFLP	(Cosenza et al., 2008)
Italy	dell'Etna	42	0.25	0.27	0.00	0.00	0.17	•	AS-PCR, PCR-RFLP	(Gigli et al., 2008)
Italy	Derivata di Siria	47	0.30	0.10	0.00	0.00	0.33	•	AS-PCR, PCR-RFLP	(Gigli et al., 2008)
Italy	Girgentana	263	0.35	0.13	0.04	0.00	0.19	•	AS-PCR, PCR-RFLP	(Gigli et al., 2008)
Italy	Maltese	139	0.25	0.11	0.01	0.01	0.33	•	AS-PCR, PCR-RFLP	(Gigli et al., 2008)
Italy	Messinese	49	0.24	0.23	0.00	0.00	0.23	•	AS-PCR, PCR-RFLP	(Gigli et al., 2008)
Italy	dell'Etna	183	0.63	•	•	•	0.37		AS-PCR, PCR-RFLP	(Marletta et al., 2007)
Italy	Girgentana	341	0.60	•	•	•	0.40	•	AS-PCR, PCR-RFLP	(Marletta et al., 2007)
Italy	Garganica	54	0.61	0.37	•	0.00	0.02	0.00	PCR-RFLP	(Martin and Leroux, 2000)
Italy	Maltese	81	0.33	0.28	•	0.11	0.27	0.01	PCR-RFLP	(Martin and Leroux, 2000)
Italy	Saanen	70	0.03	0.03	•	0.49	0.46	0.00	PCR-RFLP	(Martin and Leroux, 2000)
Italy	Garganica	38	0.27	0.41	0.00	0.00	0.22	0.00	AS-PCR, PCR-RFLP	(Sacchi et al., 2005)
Italy	Jonica	110	0.35	0.31	0.00	0.06	0.28	0.00	AS-PCR, PCR-RFLP	(Sacchi et al., 2005)
Italy	Maltese	70	0.41	0.16	0.00	0.06	0.37	0.00	AS-PCR, PCR-RFLP	(Sacchi et al., 2005)
Italy	Roccaverano	77	0.23	0.12	0.02	0.21	0.38	0.05	AS-PCR, PCR-RFLP	(Sacchi et al., 2005)
Italy	Vallesana	83	0.03	0.13	0.00	0.28	0.39	0.18	AS-PCR, PCR-RFLP	(Sacchi et al., 2005)
Italy	Cilentana Milking	86	0.16	0.30	•	0.21	0.33	•	disc-PAGE, IEF	(Zullo et al., 2005)

Table continued over

Country	Breed	No.	CSN1S1 allele						Methods	Reference
			A	B	N	E	F	O		
Mexico	Saanen	97	0.03	0.11	0.07	0.42	0.37	•	AS-PCR, PCR-RFLP	(Torres-Vázquez et al., 2008)
Mexico	Alpine	81	0.19	0.14	0.15	0.24	0.28	•	AS-PCR, PCR-RFLP	(Torres-Vázquez et al., 2008)
Mexico	Mosaico Lagunero	30	0.18	0.35	0.18	0.05	0.23	•	AS-PCR, PCR-RFLP	(Torres-Vázquez et al., 2008)
Mexico	Murciano-Granadina	26	0.25	0.14	0.06	0.44	0.12	•	AS-PCR, PCR-RFLP	(Torres-Vázquez et al., 2008)
Mexico	Toggenburg	92	0.14	0.21	0.18	0.15	0.32	•	AS-PCR, PCR-RFLP	(Torres-Vázquez et al., 2008)
Montenegro	Balkan	196	0.63	•	•	•	0.37	•	AS-PCR, Sequencing	(Markovic et al., 2009)
Morocco	Draa	132	0.24	0.51	0.04	0.02	0.20	•	AS-PCR, PCR-RFLP	(Ouafi et al., 2002)
Morocco	Noire-Rahalli	102	0.27	0.67	0.01	0.03	0.02	•	AS-PCR, PCR-RFLP	(Ouafi et al., 2002)
Norway	Norwegian Dairy	254	0.17	•	0.70	0.02	0.11	•	AS-PCR, IEF, MALDI-TOF	(Devold et al., 2011)
Spain	Canaria	74	0.28	0.32	•	0.20	•	0.20	SDS-PAGE, IEF, AS-PCR	(Jordana et al., 1996)
Spain	Majorera	21	0.07	0.38	•	0.24	•	0.31	SDS-PAGE, IEF, AS-PCR	(Jordana et al., 1996)
Spain	Malaguena	373	0.09	0.09	•	0.65	0.04	0.13	SDS-PAGE, IEF, AS-PCR	(Jordana et al., 1996)
Spain	Murciano-Granadina	109	0.08	0.23	•	0.59	0.08	0.02	SDS-PAGE, IEF, AS-PCR	(Jordana et al., 1996)
Spain	Palmera	22	0.68	0.23	•	0.09	•	•	SDS-PAGE, IEF, AS-PCR	(Jordana et al., 1996)
Spain	Payoya	111	0.05	0.19	•	0.76	•	•	SDS-PAGE, IEF, AS-PCR	(Jordana et al., 1996)
Spain	Tinerfena	31	0.15	0.35	•	0.32	•	0.18	SDS-PAGE, IEF, AS-PCR	(Jordana et al., 1996)
Tunisia	Arbi	111	0.27	0.34	0.14	0.06	0.19	0.01	AS-PCR, PCR-RFLP	(Vacca et al., 2009)
USA	Alpine	42	0.12	0.07	0.00	0.36	0.46	•	multiplex PCR	(Maga et al., 2009)
USA	Dwarf Nigeria	23	0.48	0.50	0.00	0.00	0.02	•	multiplex PCR	(Maga et al., 2009)
USA	La Mancha	48	0.58	0.08	0.00	0.19	0.15	•	multiplex PCR	(Maga et al., 2009)
USA	Nubian	26	0.37	0.52	0.12	0.00	0.00	•	multiplex PCR	(Maga et al., 2009)
USA	Oberhasli	24	0.25	0.00	0.00	0.54	0.21	•	multiplex PCR	(Maga et al., 2009)
USA	Saanen	39	0.00	0.00	0.00	0.71	0.30	•	multiplex PCR	(Maga et al., 2009)
USA	Toggenburg	55	0.03	0.00	0.02	0.03	0.93	•	multiplex PCR	(Maga et al., 2009)
USA	Alpine	37	0.04	0.08	•	0.20	0.62	0.05	RP-HPLC	(Clark and Sherbon, 2000)
USA	La Mancha	17	0.23	0.06	•	0.18	0.53	0.00	RP-HPLC	(Clark and Sherbon, 2000)
USA	Nubian	6	0.33	0.08	•	0.00	0.50	0.08	RP-HPLC	(Clark and Sherbon, 2000)
USA	Oberhasli	2	0.00	0.00	•	0.50	0.50	0.00	RP-HPLC	(Clark and Sherbon, 2000)
USA	Saanen	11	0.09	0.14	•	0.32	0.46	0.00	RP-HPLC	(Clark and Sherbon, 2000)
USA	Toggenburg	9	0.06	0.06	•	0.06	0.83	0.00	RP-HPLC	(Clark and Sherbon, 2000)
West Africa	Borno	37	0.19	0.38	•	0.00	0.03	•	RCR-RFLP, PCR-SSCP	(Caroli et al., 2006)
West Africa	Dwarf Cameroon	39	0.08	0.62	•	0.00	0.01	•	RCR-RFLP, PCR-SSCP	(Caroli et al., 2006)
West Africa	Dwarf Nigeria	27	0.26	0.44	•	0.00	0.00	•	RCR-RFLP, PCR-SSCP	(Caroli et al., 2006)
West Africa	Red Sokoto	57	0.18	0.48	•	0.02	0.01	•	RCR-RFLP, PCR-SSCP	(Caroli et al., 2006)

Appendix B: DNA Extraction Results

Initial extraction results and clean-up methods required for each sample. IS= insufficient DNA or sample. LE= Lab error. NHT= No herd test data. Breeds: S= Saanen, BA= British Alpine, T= Toggenberg, N=Nubian.

Goat ID	Breed	Age	Date sampled	Farm	Sample Type	Status	DNA (ng/µL)	260/230	260/280	CTAB	SDS	Notes
7	S	2	17-Jan-11	A	Milk	Genotyped	221.72	0.89	1.86	Yes		
10	S	5	13-Feb-11	A	Milk	Genotyped	44.70	0.26	1.79	Yes		
15	S	3	17-Jan-11	A	Milk	Genotyped	42.04	0.49	1.96			
16	S	3	17-Dec-10	A	Milk	Genotyped	115.34	0.24	1.10	Yes		
19	S	3	17-Dec-10	A	Milk	Withdrawn	100.90	0.21	1.24	Yes		LE, sample mix-up
24	S	8	4-Nov-10	A	Milk	Genotyped	3337.60	1.98	1.89			
38	S	3	17-Dec-10	A	Milk	Genotyped	429.43	0.51	1.48	Yes		
47	S	3	13-Feb-11	A	Milk	Genotyped	411.33	1.15	1.99			
49	S	6	13-Feb-11	A	Milk	Withdrawn	0.89	0.66	1.16			IS DNA
50	BA/S	8	4-Nov-10	A	Milk	Genotyped	328.09	1.09	2.09			
59	S	7	13-Feb-11	A	Milk	Genotyped	183.30	1.11	1.85			
68	S	4	13-Feb-11	A	Milk	Genotyped	198.98	0.92	1.27	Yes		
83	S	5	4-Nov-10	A	Milk	Withdrawn	35.26	2.28	1.80			IS to complete g/t
84	S	2	17-Dec-10	A	Milk	Genotyped	812.81	0.63	1.24	Yes		
88	S	7	17-Dec-10	A	Milk	Withdrawn	60.21	0.21	1.08	Yes		Low DNA post CTAB
89	S	2	20-Feb-11	A	Hair (buck)	Genotyped	27.27	0.24	1.92			
90	S	2	20-Feb-11	A	Hair (buck)	Withdrawn	23.82	2.00	1.87			PCR fail
91	S	3	20-Feb-11	A	Hair (buck)	Withdrawn	47.60	2.17	1.93			PCR fail
92	S	2	20-Feb-11	A	Hair (buck)	Genotyped	43.11	2.13	1.90			
93	S	3	20-Feb-11	A	Hair (buck)	Withdrawn	40.64	2.17	1.96			PCR fail
94	S	4	20-Feb-11	A	Hair (buck)	Genotyped	43.37	2.12	1.89			

Table continued over

Goat ID	Breed	Age	Date sampled	Farm	Sample Type	Status	DNA (ng/µL)	260/230	260/280	CTAB	SDS	Notes
96	S	3	20-Feb-11	A	Hair (buck)	Genotyped	16.46	0.53	1.49	Yes		
98	S	3	20-Feb-11	A	Hair (buck)	Genotyped	44.40	1.96	1.79			
99	S	5	20-Feb-11	A	Hair (buck)	Withdrawn	13.20	2.10	1.79			PCR fail
110	S	2	17-Jan-11	A	Milk	Genotyped	304.97	1.06	1.85			
119	S	4	13-Feb-11	A	Milk	Genotyped	185.02	1.21	1.87			
125	S	3	17-Jan-11	A	Milk	Genotyped	164.84	0.50	1.81			
130	S	7	13-Feb-11	A	Milk	Genotyped	149.20	0.79	1.89			
135	S	4	17-Jan-11	A	Milk	Genotyped	196.10	0.90	1.89			
149	S	2	17-Jan-11	A	Milk	Genotyped	82.85	0.56	1.70			
150	S	2	13-Feb-11	A	Milk	Genotyped	97.23	0.32	1.99		Yes	
151	S	6	17-Dec-10	A	Milk	Genotyped	74.34	0.11	1.52	Yes		
153	T/S	7	13-Feb-11	A	Milk	Genotyped	84.10	0.73	1.74			
170	S	6	17-Dec-10	A	Milk	Withdrawn	56.17	0.16	1.09	Yes		Low 260/230 post CTAB
187	S	2	4-Nov-10	A	Milk	Genotyped	126.16	0.33	1.63			
195	S	6	13-Feb-11	A	Milk	Genotyped	34.93	0.45	1.64			
208	S	9	17-Dec-10	A	Milk	Genotyped	338.35	0.84	1.39	Yes		
219	S	5	13-Feb-11	A	Milk	Genotyped	562.36	0.67	1.66			
219	S	2	13-Feb-11	A	Milk	Withdrawn	1.55	1.66	1.58			IS DNA
222	S	6	13-Feb-11	A	Milk	Genotyped	454.02	1.34	1.57			
225	S	9	17-Dec-10	A	Milk	Genotyped	82.76	0.24	1.10	Yes		
234	S	5	17-Jan-11	A	Milk	Genotyped	67.54	0.04	1.25	Yes		
236	S	8	17-Jan-11	A	Milk	Genotyped	166.09	0.98	1.85			
239	S	6	17-Dec-10	A	Milk	Genotyped	66.59	0.24	1.52			
242	S	4	17-Dec-10	A	Milk	Genotyped	248.44	0.33	1.41	Yes		
243	S	4	13-Feb-11	A	Milk	Genotyped	204.57	1.05	1.48	Yes		

Table continued over

Goat ID	Breed	Age	Date sampled	Farm	Sample Type	Status	DNA (ng/µL)	260/230	260/280	CTAB	SDS	Notes
251	S	2	17-Jan-11	A	Milk	Genotyped	101.55	1.83	1.73			
262	S	3	17-Jan-11	A	Milk	Genotyped	582.27	1.34	1.95			
293	S	2	13-Feb-11	A	Milk	Genotyped	293.00	1.07	1.99			
296	S	2	17-Dec-10	A	Milk	Genotyped	225.33	0.48	1.33	Yes		
300	S	8	17-Dec-10	A	Milk	Genotyped	102.37	0.26	1.26	Yes		
302	S	2	17-Dec-10	A	Milk	Genotyped	89.47	0.49	1.02	Yes		
303	S	4	17-Jan-11	A	Milk	Genotyped	103.29	0.37	1.89			
307	S	3	13-Feb-11	A	Milk	Withdrawn	456.42	0.90	1.46			LE, Extraction
320	S	6	17-Dec-10	A	Milk	Genotyped	101.25	0.16	1.06	Yes		
324	T/S	8	13-Feb-11	A	Milk	Genotyped	61.82	0.85	1.51			
326	S	3	13-Feb-11	A	Milk	Genotyped	621.26	0.42	1.55	Yes		
330	S	8	17-Jan-11	A	Milk	Genotyped	189.84	0.46	1.60			
332	S	6	17-Jan-11	A	Milk	Genotyped	341.33	1.09	1.91			
334	S	2	17-Dec-10	A	Milk	Genotyped	116.55	0.28	1.09	Yes		
334	S	7	17-Dec-10	A	Milk	Withdrawn	102.37	0.79	1.62			LE, Extraction
349	S	6	4-Nov-10	A	Milk	Genotyped	96.70	1.89	1.79			
354	S	5	13-Feb-11	A	Milk	Genotyped	55.23	0.61	1.44	Yes		
355	S	7	13-Feb-11	A	Milk	Genotyped	244.60	0.33	1.68	Yes		
357	S	4	4-Nov-10	A	Milk	Withdrawn	72.56	0.55	1.64			LE, Extraction
358	S	2	17-Dec-10	A	Milk	Genotyped	105.16	0.19	1.00		Yes	White pellet
364	S	6	13-Feb-11	A	Milk	Genotyped	498.88	0.66	1.99		Yes	
370	S	8	17-Dec-10	A	Milk	Genotyped	150.00	0.27	1.17	Yes		
376	S	2	13-Feb-11	A	Milk	Genotyped	126.89	0.64	1.26			
376	S	3	17-Dec-10	A	Milk	Withdrawn	23.56	0.23	1.90		Yes	
395	S	5	17-Dec-10	A	Milk	Withdrawn	114.32	0.15	0.99	Yes		Low 260/230 post CTAB

Table continued over

Goat ID	Breed	Age	Date sampled	Farm	Sample Type	Status	DNA (ng/µL)	260/230	260/280	CTAB	SDS	Notes
396	S	3	17-Dec-10	A	Milk	Genotyped	118.32	0.17	1.50	Yes		
262a	S	9	17-Dec-10	A	Milk	Withdrawn	135.40	0.21	1.04			triplicate
262b	S	9	17-Dec-10	A	Milk	Withdrawn	64.95	0.40	1.98			triplicate
vat	S	7	4-Nov-10	A	Milk	Genotyped	23.40	0.97	1.93			Frozen sample, low DNA
2	S	8	2-Jul-11	B	Hair (doe)	Genotyped	13.44	1.00	1.63			
8	S	5	3-Jul-11	B	Milk	Genotyped	456.61	0.81	1.53			
9	S	4	1-Oct-11	B	Milk	Genotyped	331.90	1.30	1.90			
17	S	2	1-Oct-11	B	Milk	Genotyped	483.63	0.56	1.85			
22	S	6	2-Jul-11	B	Hair (doe)	Genotyped	8.66	1.18	1.78			
25	S	4	1-Oct-11	B	Milk	Genotyped	72.96	0.54	1.50			
42	S	2	1-Oct-11	B	Milk	Genotyped	18.12	0.03	2.61			
49	S	5	3-Jul-11	B	Milk	Genotyped	327.01	0.89	1.56			NHT
55	S	4	3-Jul-11	B	Milk	Genotyped	281.29	1.36	1.82			NHT
71	S	2	1-Oct-11	B	Milk	Genotyped	1057.60	1.40	1.79			
72	S	6	1-Oct-11	B	Milk	Genotyped	122.67	0.46	1.44			
73	S	8	1-Oct-11	B	Milk	Genotyped	480.31	1.24	1.84			
77	S	5	1-Oct-11	B	Milk	Genotyped	448.03	1.70	1.56			
79	S	4	11-Jul-11	B	Milk	Genotyped	285.28	0.32	1.50	Yes		PCR fail
80	S	6	1-Oct-11	B	Milk	Genotyped	159.36	0.27	1.90			
82	S	2	1-Oct-11	B	Milk	Genotyped	36.09	1.45	1.31			
83	S	3	3-Jul-11	B	Milk	Withdrawn	56.27	0.96	0.45	Yes		Low DNA post CTAB
91	S/N	2	3-Jul-11	B	Milk	Genotyped	2341.50	0.36	1.48	Yes		NHT
103	S	4	11-Jul-11	B	Milk	Genotyped	498.36	0.34	1.53			NHT
111	S	8	1-Oct-11	B	Milk	Genotyped	71.82	0.33	1.48	Yes		
124	S	3	3-Jul-11	B	Milk	Genotyped	59.31	0.34	1.41	Yes		
127	S	5	1-Oct-11	B	Milk	Genotyped	101.17	0.26	1.59			

Table continued over

Goat ID	Breed	Age	Date sampled	Farm	Sample Type	Status	DNA (ng/µL)	260/230	260/280	CTAB	SDS	Notes
137	S	2	1-Oct-11	B	Milk	Genotyped	2101.40	2.08	1.84			
140	S	6	1-Oct-11	B	Milk	Genotyped	115.60	0.76	1.75			
151	S	4	2-Jul-11	B	Hair (doe)	Genotyped	317.58	0.69	1.84			
153	S	2	1-Oct-11	B	Milk	Genotyped	87.99	0.72	2.25		Yes	
156	S	4	1-Oct-11	B	Milk	Genotyped	380.73	1.33	1.73			
160	S	2	1-Oct-11	B	Milk	Genotyped	107.04	0.85	1.55			
162	S	5	1-Oct-11	B	Milk	Genotyped	2869.50	2.22	1.77			
163	S	6	1-Oct-11	B	Milk	Genotyped	84.15	2.29	1.64			
165	S	4	1-Oct-11	B	Milk	Genotyped	155.80	1.32	1.75			
182	S	4	3-Jul-11	B	Milk	Genotyped	196.96	2.12	1.85			
186	S	6	1-Oct-11	B	Milk	Genotyped	71.90	4.54	1.60		Yes	White pellet
209	S	4	11-Jul-11	B	Milk	Genotyped	410.13	0.50	1.68			
210	S	4	2-Jul-11	B	Hair (doe)	Genotyped	14.78	0.12	1.83			NHT
224	S	8	11-Jul-11	B	Milk	Genotyped	124.92	0.32	1.44			NHT
226	S	6	1-Oct-11	B	Milk	Genotyped	74.33	1.71	1.66			
232	S	4	11-Jul-11	B	Milk	Withdrawn	5.60	0.12	1.23			NHT
234	S/N	7	1-Oct-11	B	Milk	Genotyped	17.63	1.66	3.11			
236	S	4	3-Jul-11	B	Milk	Genotyped	92.24	0.24	1.35	Yes		NHT
254	S	5	1-Oct-11	B	Milk	Genotyped	208.49	0.10	1.79		Yes	Un-dissolvable pellet
257	S	4	1-Oct-11	B	Milk	Withdrawn	8.95	0.02	2.84			PCR fail
263	S	6	1-Oct-11	B	Milk	Genotyped	37.33	0.11	1.39	Yes		
275	S	3	11-Jul-11	B	Milk	Genotyped	288.90	0.26	1.50	Yes		
284	S	4	11-Jul-11	B	Milk	Genotyped	239.44	0.50	1.74			NHT
298	S	3	1-Oct-11	B	Milk	Genotyped	36.42	-10.90	1.57		Yes	White pellet
316	S	4	3-Jul-11	B	Milk	Genotyped	149.96	2.11	1.90			
321	S	7	1-Oct-11	B	Milk	Genotyped	284.62	0.99	1.68			
327	S	2	1-Oct-11	B	Milk	Genotyped	772.82	1.13	1.85			

Table continued over

Goat ID	Breed	Age	Date sampled	Farm	Sample Type	Status	DNA (ng/µL)	260/230	260/280	CTAB	SDS	Notes
332	S	2	1-Oct-11	B	Milk	Genotyped	256.07	0.32	1.91	Yes		
333	S/N	2	11-Jul-11	B	Milk	Genotyped	301.01	0.38	1.49	Yes		
338	S/N	2	11-Jul-11	B	Milk	Genotyped	280.51	0.38	1.43	Yes		NHT
339	S	4	11-Jul-11	B	Milk	Genotyped	93.70	0.22	1.56	Yes		
343	S/N	2	1-Oct-11	B	Milk	Genotyped	36.65	0.71	2.22			
348	S	2	1-Oct-11	B	Milk	Genotyped	108.46	0.15	3.26		Yes	White pellet
350	S	3	1-Oct-11	B	Milk	Genotyped	62.52	0.36	1.93	Yes		
355	S	5	3-Jul-11	B	Milk	Genotyped	164.43	0.22	1.33		Yes	NHT
359	S	9	1-Oct-11	B	Milk	Genotyped	1588.80	1.43	1.55		Yes	
376	S	3	1-Oct-11	B	Milk	Genotyped	73.74	1.54	1.88			
380	S	4	3-Jul-11	B	Milk	Genotyped	237.15	1.28	1.70			
388	S	5	3-Jul-11	B	Milk	Genotyped	56.54	0.96	1.92			
398	S	5	1-Oct-11	B	Milk	Genotyped	11.02	0.20	5.11		Yes	White pellet.
407	S	3	2-Jul-11	B	Hair (doe)	Withdrawn	8.14	0.09	1.91			
416	S	7	1-Oct-11	B	Milk	Genotyped	12.75	0.23	2.02			
418	S/N	2	1-Oct-11	B	Milk	Genotyped	116.94	0.79	1.75			
427	S	5	1-Oct-11	B	Milk	Genotyped	1716.70	1.52	1.86			
1073	S	3	3-Jul-11	B	Hair (buck)	Withdrawn	7.35	0.92	1.24			IS
9302	S	5	2-Jul-11	B	Hair (buck)	Genotyped	50.40	0.52	1.59			
10101	S	3	3-Jul-11	B	Milk	Withdrawn	13.20	0.69	1.55			IS
BKM	S	5	2-Jul-11	B	Hair (doe)	Withdrawn	13.40	0.86	1.67			IS
BKSF	S/N	4	2-Jul-11	B	Hair (buck)	Genotyped	22.32	0.32	1.77			
DOLLY	S	6	3-Jul-11	B	Milk	Genotyped	624.30	2.08	1.66		Yes	Small while pellet
MACK	N	8	2-Jul-11	B	Hair (buck)	Genotyped	43.18	1.00	1.75			
NICK	S/N	6	2-Jul-11	B	Hair (buck)	Withdrawn	13.25	0.02	1.10			PCR fail
Nub Big	N	4	2-Jul-11	B	Hair (doe)	Genotyped	35.26	0.19	1.28			PCR fail
Nub Med	N	4	2-Jul-11	B	Milk	Genotyped	172.19	0.82	1.77			

Table continued over

Goat ID	Breed	Age	Date sampled	Farm	Sample Type	Status	DNA (ng/µL)	260/230	260/280	CTAB	SDS	Notes
Nub Small	N	4	2-Jul-11	B	Hair (doe)	Withdrawn	7.54	0.32	1.35			IS
PFB	S	6	2-Jul-11	B	Hair (buck)	Genotyped	34.65	0.29	1.80			
RASTUS	N	8	2-Jul-11	B	Hair (buck)	Genotyped	28.54	0.19	1.80			

Appendix C: Individual Genotyping Results

Genotyping table for each goat sample. Genotyping at Intron 12 and exon 19 depended on the exon 9 genotype. Some samples were characterised at exon 19 twice (both regular PCR and Real-time PCR) as Exon 19 PCR results were often ambiguous. UTD= unable to determine. E-pos = E-allele positive.

Goat #	Farm	Exon 9	Intron 12	Exon 19 PCR	Exon 19 Real-time		Final g/t
					F2/R	F1/R	
7	A	EF		AE	EF		EF
10	A	EF			EF		EF
15	A	EF		AE	EF		EF
16	A	Unknown		UTD	E-pos		Unknown
24	A	Unknown		UTD	E-pos		Unknown
38	A	AE	A	UTD	AE		AE
47	A	EE		UTD	EE	AE	BE
50	A	EF		UTD	EF		EF
59	A	AE	A	UTD	AE		AE
84	A	EE		EE	EE	EE	EE
89	A	EF			EF		EF
92	A	Unknown		AE	E-pos		Unknown
94	A	Unknown		AE	E-pos		Unknown
96	A	FF					FF
98	A	Unknown		UTD	E-pos		Unknown
110	A	Unknown		AE	E-pos		Unknown
119	A	Unknown		UTD	E-pos		Unknown
125	A	FF					FF
130	A	AE	A	AE	AE		AE
135	A	AA	A	AA			AA
149	A	Unknown		AE	E-pos		Unknown
150	A	AE	A		AE		AE
151	A	EF		AE	EF		EF
153	A	AN	A	AA	AN		AN
187	A	AE	A	AE	AE		AE
195	A	AE	A	AE	AE		AE
208	A	AE	A	AE	AE		AE
219	A	EE			EE	EE	EE
222	A	EE		UTD	EE	EE	EE
225	A	EE			EE	AE	BE
234	A	EF			EF		EF
236	A	EF			EF		EF
239	A	EF			EF		EF
242	A	EF			EF		EF
243	A	EE		EE	EE	EE	EE
251	A	AN	A	AA	AN		AN
262	A	Unknown		AE	E-pos		Unknown
293	A	EE			EE	EE	EE
296	A	AA	A	AA			AA
300	A	FF					FF
302	A	FF					FF
303	A	AA	A	AA			AA
320	A	EF		AE	EF		EF
324	A	FF					FF
326	A	AA	A	AA			AA
330	A	EF			EF		EF
332	A	EF		UTD	EF		EF

Table continued over

Goat #	Farm	Exon 9	Intron 12	Exon 19 PCR	Exon 19 Real-time		Final g/t
					F2/R	F1/R	
334	A	AE	A	UTD	AE		AE
349	A	EE			EE	BE	BE
354	A	FF					FF
355	A	EF		AE	EF		EF
358	A	EF			EF		EF
364	A	AF	A				AF
370	A	AE	A	UTD	AE		AE
376	A	AF	A				AF
396	A	EF			EF		EF
vat	A	AFEN	A	AE	IS		AFEN
2	B	EF		UTD	EF		EF
8	B	EE		EE	EE	EE	EE
9	B	EF			EF		EF
17	B	AE	A		AE		AE
22	B	EF		AE	EF		EF
25	B	EE			EE	EE	EE
42	B	AA	A				AA
49	B	EF			EF		EF
55	B	AA	A				AA
71	B	AE	A		AE		AE
72	B	EE			EE	EE	EE
73	B	AE	A		AE		AE
77	B	AA	A				AA
79	B	AE	A		AE		AE
80	B	EF			BF	BF	BF
82	B	AF	A				AF
91	B	EF			BF	BF	BF
103	B	AE	A		AE		AE
111	B	EE			EE	EE	EE
124	B	FF					FF
127	B	AE	A		AE		AE
137	B	AE	A	AE	AE		AE
140	B	FF					FF
151	B	EE			EE	EE	EE
153	B	FF					FF
156	B	AE	A		AE		AE
160	B	FF					FF
162	B	AA	A				AA
163	B	AF	A				AF
165	B	AA	A				AA
182	B	AF	A				AF
186	B	AF	A				AF
209	B	AA	A				AA
210	B	EF		AE	EF		EF
224	B	AA	A				AA
226	B	AE	A	UTD	AE		AE
234	B	FF					FF
236	B	EF			EF		EF
254	B	EF			EF		EF
263	B	AE	A	UTD	AE		AE
275	B	EF			EF		EF
284	B	FF					FF
298	B	FF					FF
316	B	AE	A		AE		AE
321	B	AE	A	AE	AE		AE

Table continued over

Goat #	Farm	Exon 9	Intron 12	Exon 19 PCR	Exon 19 Real-time		Final g/t
					F2/R	F1/R	
327	B	EF			EF		EF
332	B	AF	A				AF
333	B	AE	A		AE		AE
338	B	EF			EF		EF
339	B	EF			EF		EF
343	B	AF	A				AF
348	B	EF			EF		EF
350	B	EF			BF	BF	BF
355	B	AF	A				AF
359	B	EE			AE	AE	BE
376	B	EF			EF		EF
380	B	AF	A				AF
388	B	FF					FF
398	B	EF			EF		EF
416	B	AA	A				AA
418	B	AF	A				AF
427	B	FF					FF
9302	B	EF			EF		EF
BKSF	B	AE	A		AE		AE
DOLLY	B	AE	A		AE		AE
MACK	B	AE	A		AE		AE
Nub Big	B	AE	A		AE		AE
Nub Med	B	AF	A				AF
PFB	B	AF	A				AF
RASTUS	B	AE	A		AE		AE

Appendix D: Real-Time PCR Genotyping Results

Real-time genotyping results. G/t = genotype. Req = required. IS = insufficient DNA or sample

Goat #	Farm	Exon 9 g/t	F2/R primers			F1/R primers			Notes
			Ct	Melt	F2 g/t	Ct	Melt	F1 g/t	
7	A	EF	22.3	77.15	EF				
10	A	EF	22.3	77.40	EF				
15	A	EF	23.4	77.40	EF				
16	A	Unknown							
24	A	Unknown							
38	A	AE	22.0	77.50	AE				
47	A	EE	22.3	77.50	EE or BE	20.9	76.20	BE	F1 req
50	A	EF	22.4	77.50	EF				
59	A	AE	24.9	77.40	AE				
84	A	EE	23.7	77.10	EE or BE	26.5	76.60	EE	F1 req
89	A	EF	22.0	77.50	EF				
92	A	Unknown							
94	A	Unknown							
96	A	FF							
98	A	Unknown							
110	A	Unknown	21.9	77.25	E-positive	26.8	77.50	E - het	Unknown +ve control
119	A	Unknown							
125	A	FF							
130	A	AE	24.0	77.50	AE	25.3	77.20	AE	AE +ve control
135	A	AA							
149	A	Unknown							
150	A	AE	24.3	77.40	AE				
151	A	EF	22.7	77.50	EF				
153	A	AN							
187	A	AE	24.3	77.30	AE				
195	A	AE	22.4	77.15	AE				
208	A	AE	20.0	77.25	AE				
219	A	EE	24.5	77.30	EE or BE	22.3	76.00	EE	F1 req
222	A	EE	21.9	77.50	EE or BE	21.0	74.10	EE	F1 req
225	A	EE	25.3	77.30	EE or BE	22.0	77.50	BE	F1 req
234	A	EF	23.5	77.50	EF				
236	A	EF	23.8	77.40	EF				
239	A	EF	22.4	77.50	EF				
242	A	EF	23.5	77.25	EF				
243	A	EE	22.1	77.50	EE or BE	28.2	76.50	EE	F1 req
251	A	AN	29.2	73.0/75.3/ 81.0	AN	20.4	77.50	AN	AN +ve control
262	A	Unknown							
293	A	EE	25.6	77.60	EE or BE	26.2	76.30	EE	F1 req
296	A	AA	32.0	81.40	AA	23.6	77.30	AA	AA +ve control sample
300	A	FF							
302	A	FF							
303	A	AA							
320	A	EF	25.6	77.70	EF				
324	A	FF							

Table continued over

Goat #	Farm	Exon 9 g/t	F2/R primers			F1/R primers			Notes
			Ct	Melt	F2 g/t	Ct	Melt	F1 g/t	
326	A	AA							
330	A	EF	22.3	77.50	EF				
332	A	EF	22.1	77.40	EF				
334	A	AE	21.9	77.25	AE				
349	A	EE	22.3	77.35	EE or BE	21.1	75.90	EE	F1 req
354	A	FF							
355	A	EF	23.1	77.25	EF				
358	A	EF	29.3	77 / 81.5	BF	21.3	77.00	BF	F1 to check
364	A	AF							
370	A	AE	24.6	77.50	AE				
376	A	AF							
396	A	EF	22.0	77.10	EF				
vat	A	AFEN			IS			IS	
2	B	EF	26.5	77.40	EF				
8	B	EE	24.9	77.25	EE or BE	30.7	76.50	EE	F1 req
9	B	EF	26.3	77.60	EF				
17	B	AE	22.1	77.40	AE				
22	B	EF	23.2	77.80	EF				
25	B	EE	22.1	77.25	EE or BE	31.1	76.11	EE	F1 req
42	B	AA							
49	B	EF	25.3	77.30	EF				
55	B	AA							
71	B	AE	22.0	77.50	AE				
72	B	EE	22.0	77.50	EE or BE	26.3	75.80	EE	F1 req
73	B	AE	20.0	77.50	AE				
77	B	AA							
79	B	AE	22.3	77.50	AE				
80	B	EF	32.1	75/77/81.3	BF	20.2	76.20	BF	F1 to check
82	B	AF							
91	B	EF	28.3	72/75/81.5	BF	22.0	76.60	BF	F1 to check
103	B	AE	23.6	77.40	AE				
111	B	EE	24.6	77.25	EE or BE	31.4	76.10	EE	F1 req
124	B	FF							
127	B	AE	22.8	77.35	AE				
137	B	AE	22.3	77.40	AE				
140	B	FF							
151	B	EE	19.3	77.50	EE or BE	29.6	75.80	EE	F1 req
153	B	FF							
156	B	AE	20.6	77.50	AE				
160	B	FF	32.5	75/81.52	FF	23.4	77.40	FF	F +ve control.
162	B	AA							
163	B	AF							
165	B	AA							
182	B	AF							
186	B	AF	25.6	81.30	AF	22.8	77.30	AF	AF +ve control
209	B	AA							
210	B	EF	24.2	77.10	EF				
224	B	AA							
226	B	AE	26.5	77.40	AE				

Table continued over

Goat #	Farm	Exon 9 XmnI	F2/R			F1/R			Notes
			Ct	Melt	F2 g/t	Ct	Melt	F1 g/t	
234	B	FF							
236	B	EF	21.2	77.35	EF				
254	B	EF	28.9	77.02	EF				
263	B	AE	25.2	77.25	AE				
275	B	EF	21.0	77.35	EF				
284	B	FF							
298	B	FF							
316	B	AE	27.3	77.50	AE				
321	B	AE	24.4	77.50	AE				
327	B	EF	21.0	77.35	EF				
332	B	AF							
333	B	AE	21.1	77.35	AE				
338	B	EF	24.2	77.20	EF				
339	B	EF	22.5	77.40	EF				
343	B	AF							
348	B	EF	22.8	77.60	EF				
350	B	EF	28.9	77/ 81.6	BF	22.0	78.00	BF	F1 to check
355	B	AF							
359	B	EE	22.2	77.40	EE or BE	21.5	76.20	BE	F1 required
376	B	EF	25.8	77.25	EF				
380	B	AF							
388	B	FF							
398	B	EF	32.0	77.10	EF				
416	B	AA							
418	B	AF							
427	B	FF							
9302	B	EF	26.8	77.35	EF				
BKSF	B	AE	28.9	77.75	AE				
DOLLY	B	AE	26.5	77.50	AE				
MACK	B	AE	30.2	77.52	AE				
Nub Big	B	AE	29.3	77.90	AE				
Nub Med	B	AF							
PFB	B	AF							
RASTUS	B	AE	28.5	77.25	AE				

Appendix E: Sequence Alignment Results

Exon 9 Allele A

Forward sequence of AA product amplified at exon 9 aligned using BLAST to *Capra hircus CSN1S1* gene for alpha s1 casein, allele A, exons 1-19 (AJ504710.2). The cytosine insertion (required for XmnI digestion) is highlighted. Query = AA genotype sample sequence. Sbjct= documented BLAST sequence for *CSN1S1* allele A.

```
Score = 283 bits (153),  Expect = 2e-73
 Identities = 153/153 (100%), Gaps = 0/153 (0%)
 Strand=Plus/Plus

Query  1      AGCAGTTCGTCAAGTGAGGTATACCATTTTTATGTTAATTAAGTATCCCAATTAGAAAAT  60
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  9879   AGCAGTTCGTCAAGTGAGGTATACCATTTTTATGTTAATTAAGTATCCCAATTAGAAAAT  9938

Query  61     GTTTATGAAAGTTTGTTGAACCATAAAGTTTCATTGTACAAGGCACTATGTATGTAGCTC  120
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  9939   GTTTATGAAAGTTTGTTGAACCATAAAGTTTCATTGTACAAGGCACTATGTATGTAGCTC  9998

Query  121    TATCCTAATTTTAACATACAAGGCTATCAACCC  153
              |||||||||||||||||||||||||||||||
Sbjct  9999   TATCCTAATTTTAACATACAAGGCTATCAACCC  10031
```

Exon 9 allele F

Forward sequence of FF sample amplified using exon 9 primers aligned using BLAST to *Capra hircus CSN1S1* gene for alpha s1 casein, allele F (AJ504711.2). The 11bp insertion characterising the F allele is highlighted. Query = FF genotype sample sequence. Sbjct= documented BLAST sequence for *CSN1S1* allele F.

```
Score =  296 bits (160),  Expect = 3e-77
 Identities = 160/160 (100%), Gaps = 0/160 (0%)
 Strand=Plus/Plus

Query  1      AGCAGTTGTCAAGTGAGGTATACCATTTTTATGTTGATTAAGTATCTCAATTAGAAAATG  60
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  9881   AGCAGTTGTCAAGTGAGGTATACCATTTTTATGTTGATTAAGTATCTCAATTAGAAAATG  9940

Query  61     TTTATGAAAGTTTGTTGAACCATAAAGTTTCCGTAATGTTTCATTGTACAAGGCACTATG  120
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  9941   TTTATGAAAGTTTGTTGAACCATAAAGTTTCCGTAATGTTTCATTGTACAAGGCACTATG  10000

Query  121    TATGTAGCTCTATCCTAATTTTAACATACAAGGCTATCAA  160
              ||||||||||||||||||||||||||||||||||||||||
Sbjct  10001  TATGTAGCTCTATCCTAATTTTAACATACAAGGCTATCAA  10040
```

Exon 9, A*N heterozygote

Clean sequence of the AN heterozygote amplified at exon 9 occurred up until position 9886 of the A-allele, at which point the N-allele has the G deleted (C deleted in the forward strand sequence).

```
Query  1     AATGAAACTTTATGGTTCAACAAACTTTCATAAACATTTTCTAATTGGGATACTTAATTA  60
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  9973  AATGAAACTTTATGGTTCAACAAACTTTCATAAACATTTTCTAATTGGGATACTTAATTA  9914

Query  61    ACATAAAAATGGTATACCTCACTTGAC  87
            |||||||||||||||||||||||||||
Sbjct  9913  ACATAAAAATGGTATACCTCACTTGAC  9887
```

Aligning the known *CSN1S1* A allele forward sequence with the N allele reverse-complement sequence (manually derived off the electropherogram) shows the region of sequence alignment (underlined) and the point where the 1-nt shift occurred following the C deletion (G in reverse strand sequence data).

```
9864  CAAATGAAAGCTGGAAGCAGTTCGTCAAGTGAG  9896  A-allele sequence
      |||||||||||||||||||||| ||||||||||
9862  CAAATGAAAGCTGGAAGCAGTT-GTCAAGTGAG  9894  N-allele RC sequence
```

Exon 19, Allele A*

Forward sequence of A*A* sample amplified using exon 19 primers (standard PCR). Alignment shows correct amplification of exon 19 and aligns with previously documented exon 19 of the A allele (AJ504710.2). Query = A* homozygote sample sequence. Sbjct= documented BLAST sequence for *CSN1S1* allele A.

```
 Score =  894 bits (484),  Expect = 0.0
 Identities = 487/487 (100%), Gaps = 0/487 (0%)
 Strand=Plus/Plus

Query  1      TAAAGTGTATCTTATCATAACAGTAGCTTCTCCTTTCAAAACATGCAGCATAACTAACCA  60
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  18291  TAAAGTGTATCTTATCATAACAGTAGCTTCTCCTTTCAAAACATGCAGCATAACTAACCA  18350

Query  61     CATATTTCTTTTTTGATTTACAGATGGAATTGAAAATTCCATGCTTTACATGTCTTTTCA  120
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  18351  CATATTTCTTTTTTGATTTACAGATGGAATTGAAAATTCCATGCTTTACATGTCTTTTCA  18410

Query  121    TCTATCATGTCAAACCATTCTATCCAAAGGCTTCAATTGCTGTTTTAGAATAGGACAACC  180
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  18411  TCTATCATGTCAAACCATTCTATCCAAAGGCTTCAATTGCTGTTTTAGAATAGGACAACC  18470

Query  181    TCAAATTGAAGGCACTCTTTCTTCTTGAGTTCTCTACTGTATTTTAGATTGTGTAACATC  240
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  18471  TCAAATTGAAGGCACTCTTTCTTCTTGAGTTCTCTACTGTATTTTAGATTGTGTAACATC  18530

Query  241    CTTAAGTGAAATTGTCCTAAGAGCTTGTTACCTAAATTCCAGTAGTATCACGCTGGTATA  300
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  18531  CTTAAGTGAAATTGTCCTAAGAGCTTGTTACCTAAATTCCAGTAGTATCACGCTGGTATA  18590

Query  301    AAGGCCACTGACTCAAAGGGAATTACAGTCTTCATTAAATTTCTATATGGAAAATGTTTT  360
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  18591  AAGGCCACTGACTCAAAGGGAATTACAGTCTTCATTAAATTTCTATATGGAAAATGTTTT  18650

Query  361    TAAAGCCTTTGAATCACCTCTCCTGTAAGTGCCATCATTTCAAATAACTGTGTGCAGTAA  420
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  18651  TAAAGCCTTTGAATCACCTCTCCTGTAAGTGCCATCATTTCAAATAACTGTGTGCAGTAA  18710

Query  421    CTGAGATTTTGTCTTTCTTCTTTTCAATAAATTACATTTTAAGGCACTATTCCTATTTTT  480
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  18711  CTGAGATTTTGTCTTTCTTCTTTTCAATAAATTACATTTTAAGGCACTATTCCTATTTTT  18770

Query  481    GTCATTA  487
              |||||||
Sbjct  18771  GTCATTA  18777
```

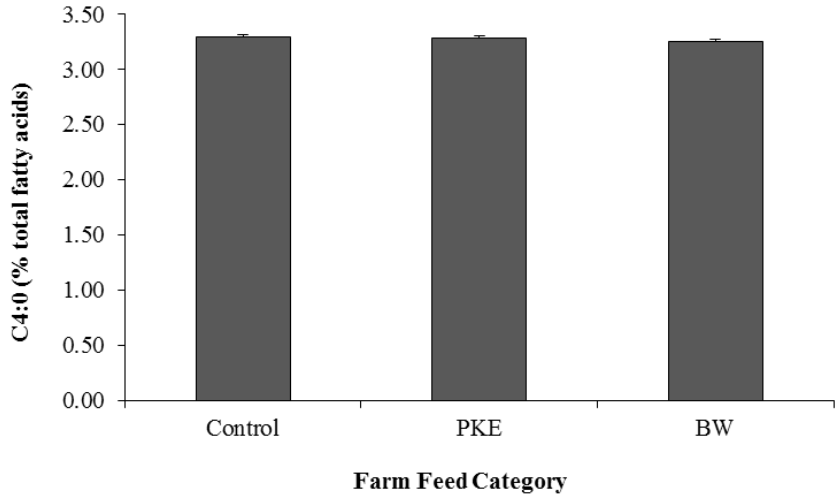
Real time – Exon 19

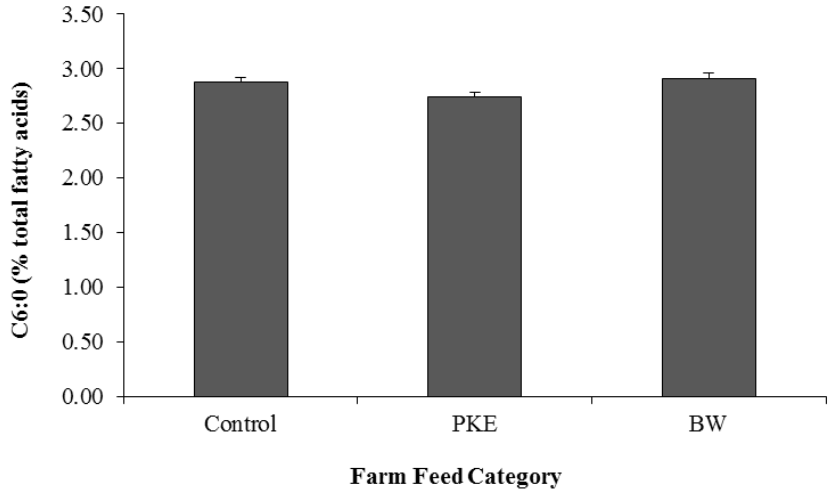
Only sequences from the A-variant produced useable sequence results with the F2/R primer, although the quality was poor. Despite this, the alignment shows that exon 19 was correctly amplified. Query = A* homozygote sequence. Sbject = Exon 19 for the A allele (AJ504710.2).

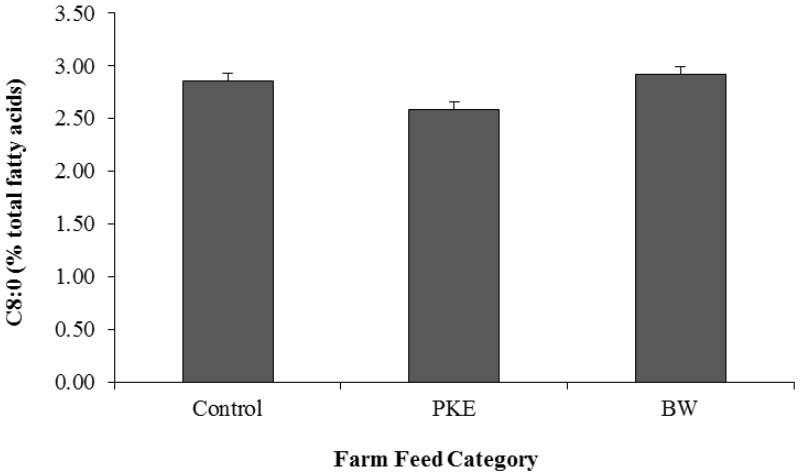
```
 Score = 75.8 bits (38),  Expect = 1e-11
 Identities = 38/38 (100%), Gaps = 0/38 (0%)
 Strand=Plus/Plus

Query  1      TGTGTAACATCCTTAAGTGAAATTGTCCTAAGAGCTTG  38
              ||||||||||||||||||||||||||||||||||||||
Sbjct          18520          TGTGTAACATCCTTAAGTGAAATTGTCCTAAGAGCTTG          18557
```

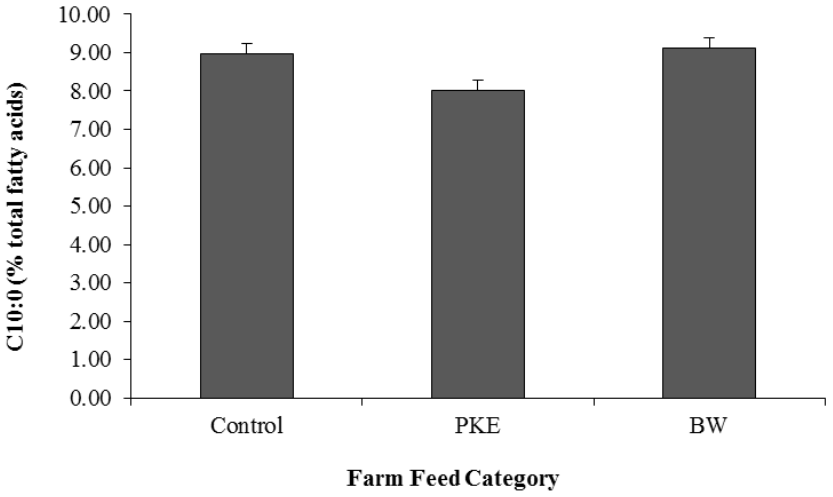
Appendix F: Feed Trial 1 Graphs

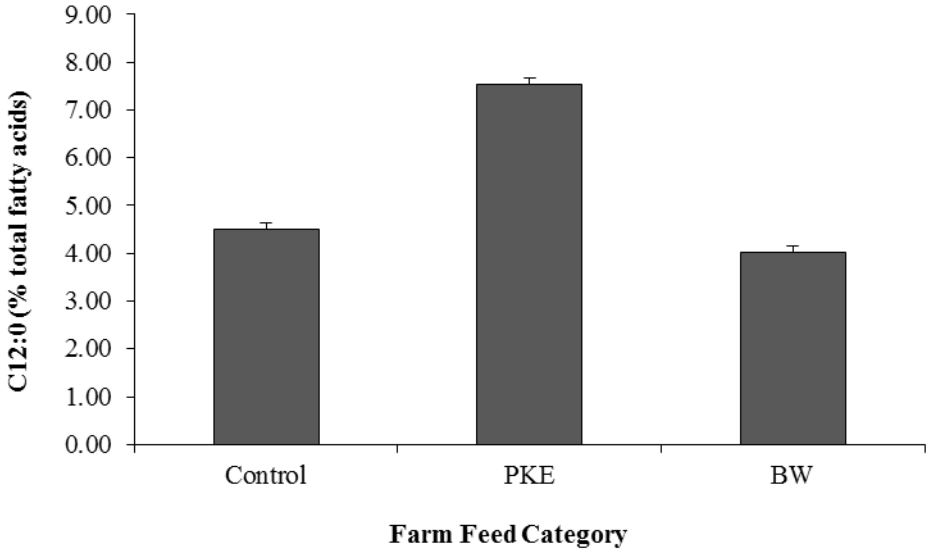
Presented below are the mean fatty acid (% of total fatty acids) values of control, PKE and BW farms for each fatty acid. Error bars are the standard deviation of the mean. Significant differences are outlined in Table 37 of section 7.1.

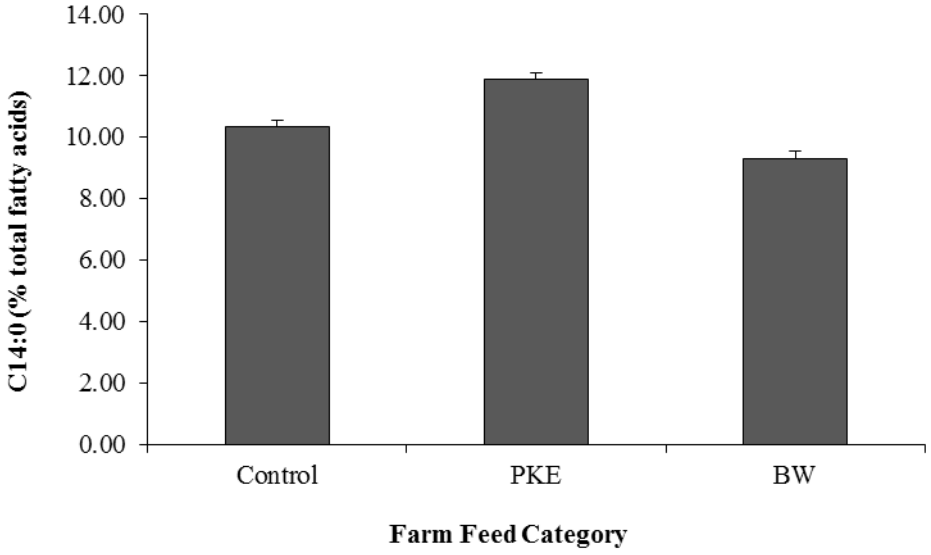


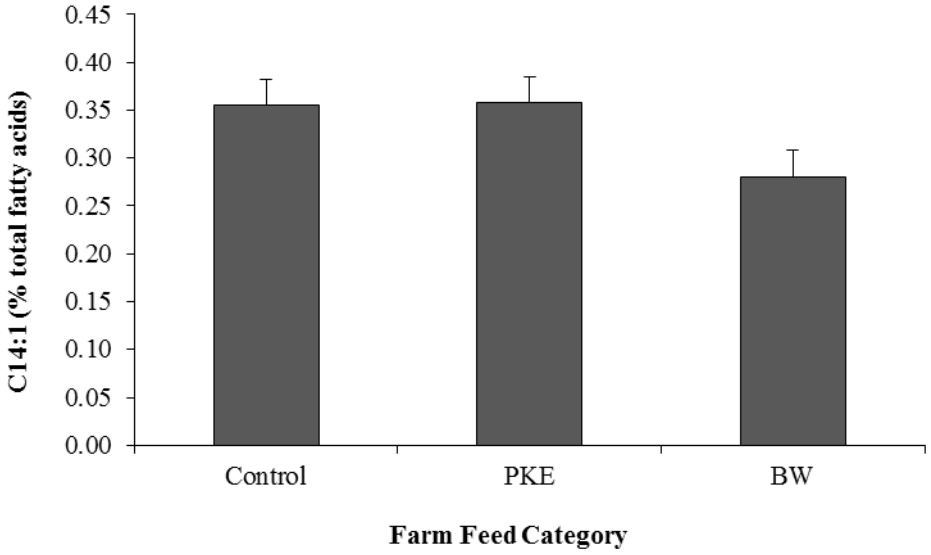


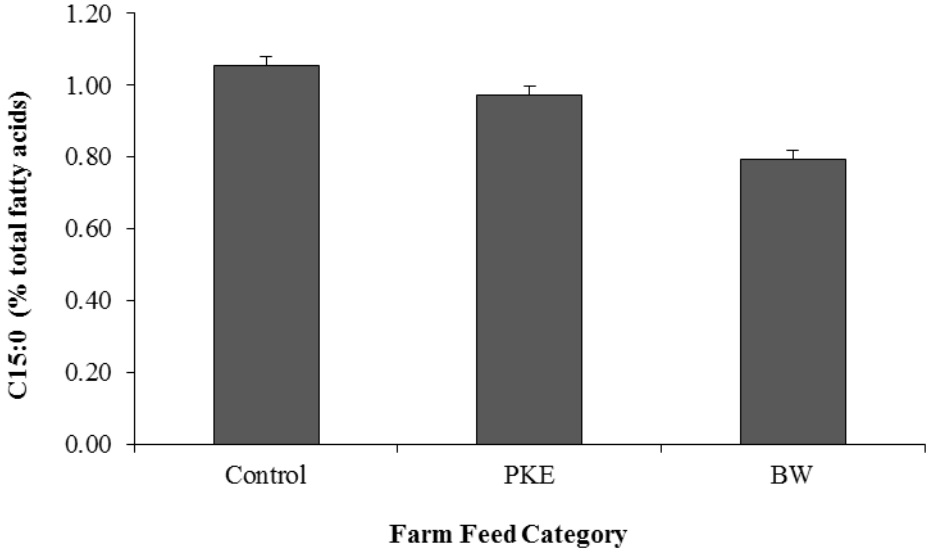

3.50
3.00
2.50
2.00
1.50
1.00
0.50
0.00
C8:0 (% total fatty acids)
Control
PKE
BW
Farm Feed Category

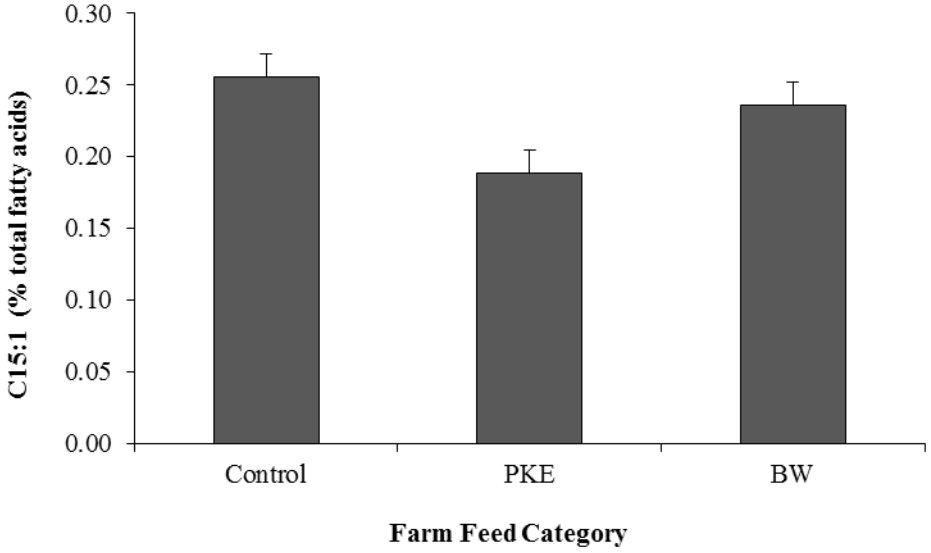

10.00
9.00
8.00
7.00
6.00
5.00
4.00
3.00
2.00
1.00
0.00
C10:0 (% total fatty acids)
Control
PKE
BW
Farm Feed Category

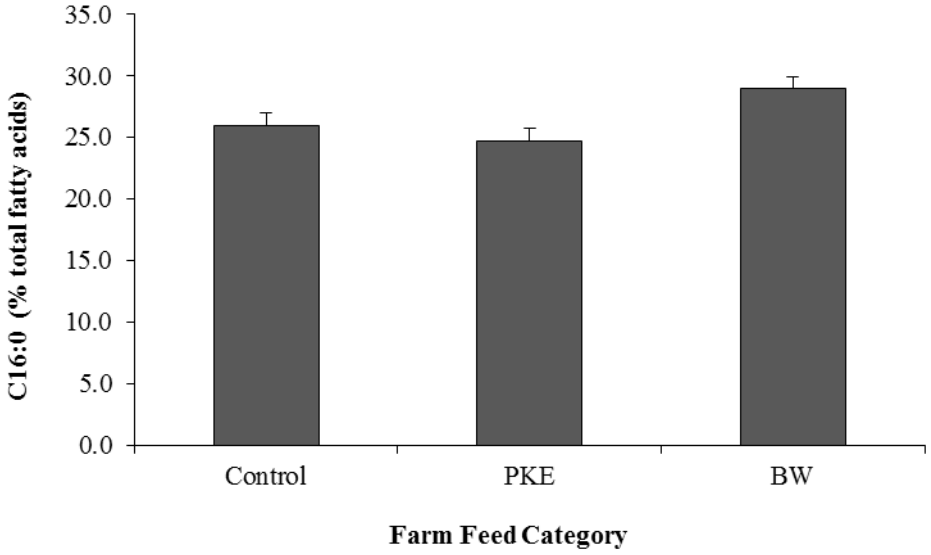

9.00
8.00
7.00
6.00
5.00
4.00
3.00
2.00
1.00
0.00
C12:0 (% total fatty acids)
Control
PKE
BW
Farm Feed Category

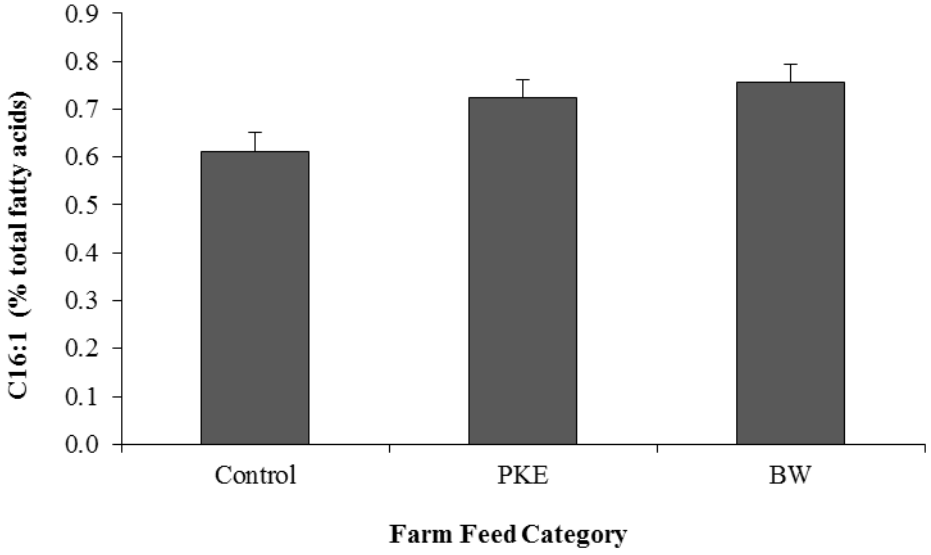

14.00
12.00
10.00
8.00
6.00
4.00
2.00
0.00
C14:0 (% total fatty acids)
Control
PKE
BW
Farm Feed Category

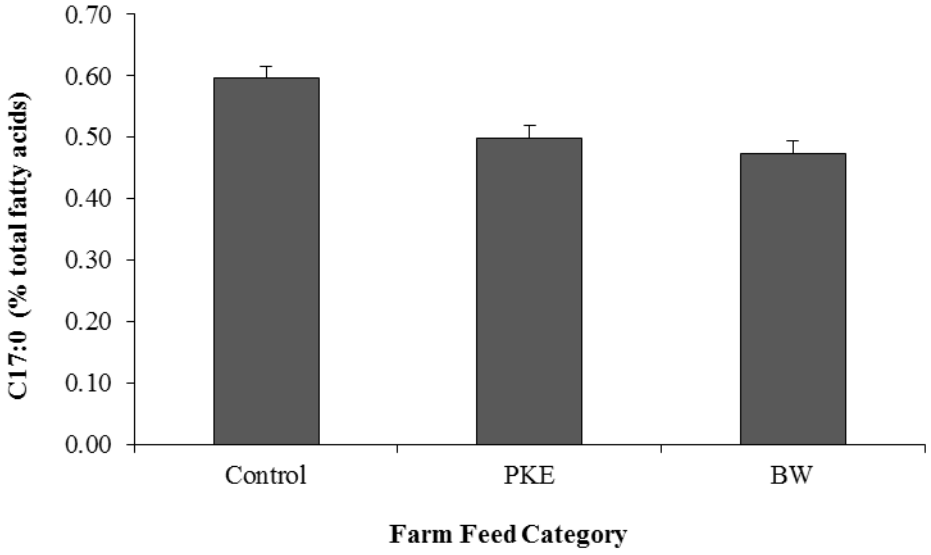

0.45
0.40
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0.00
C14:1 (% total fatty acids)
Control
PKE
BW
Farm Feed Category

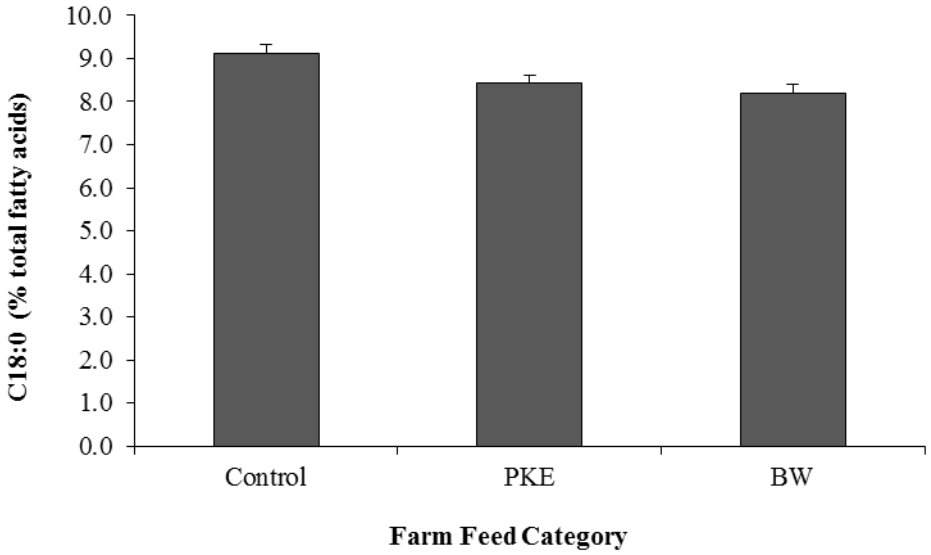

1.20
1.00
0.80
0.60
0.40
0.20
0.00
C15:0 (% total fatty acids)
Control
PKE
BW
Farm Feed Category

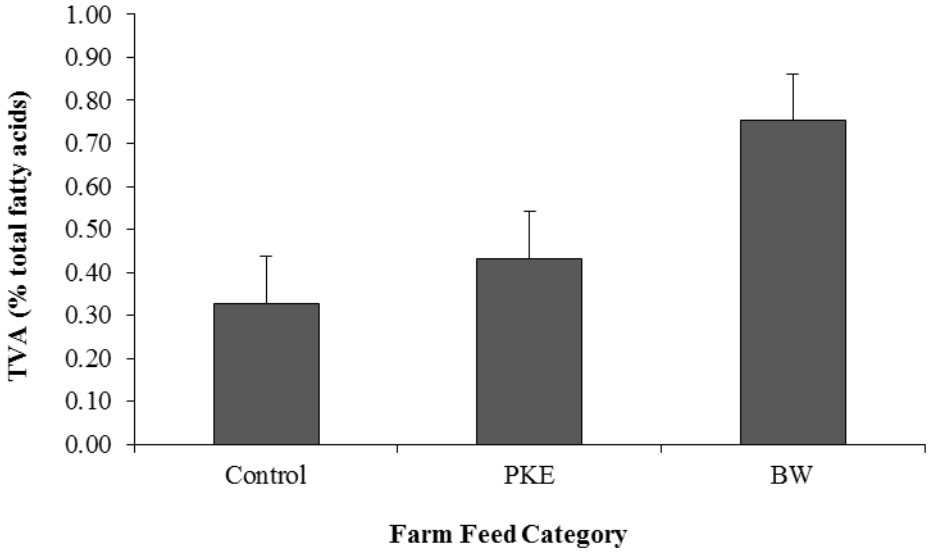

0.30
0.25
0.20
0.15
0.10
0.05
0.00
C15:1 (% total fatty acids)
Control
PKE
BW
Farm Feed Category

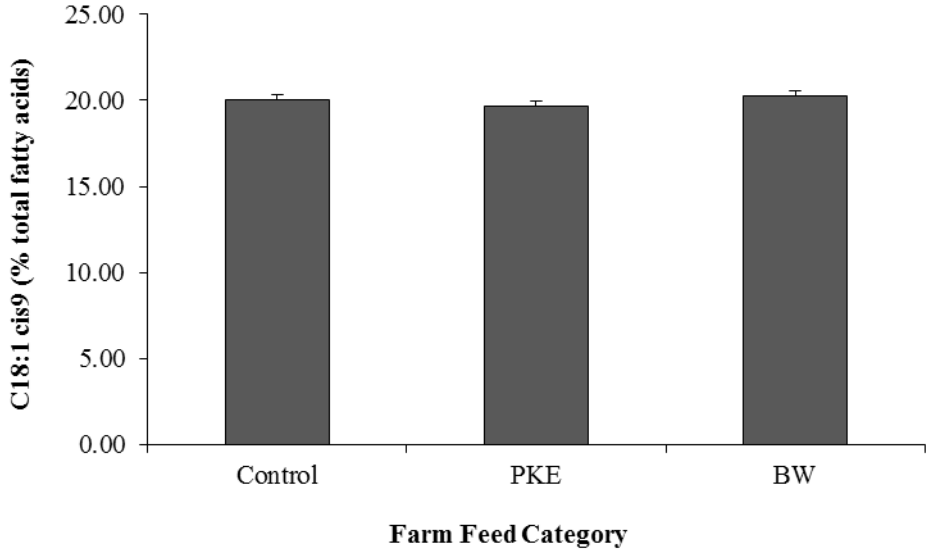

35.0
30.0
25.0
20.0
15.0
10.0
5.0
0.0
C16:0 (% total fatty acids)
Control
PKE
BW
Farm Feed Category

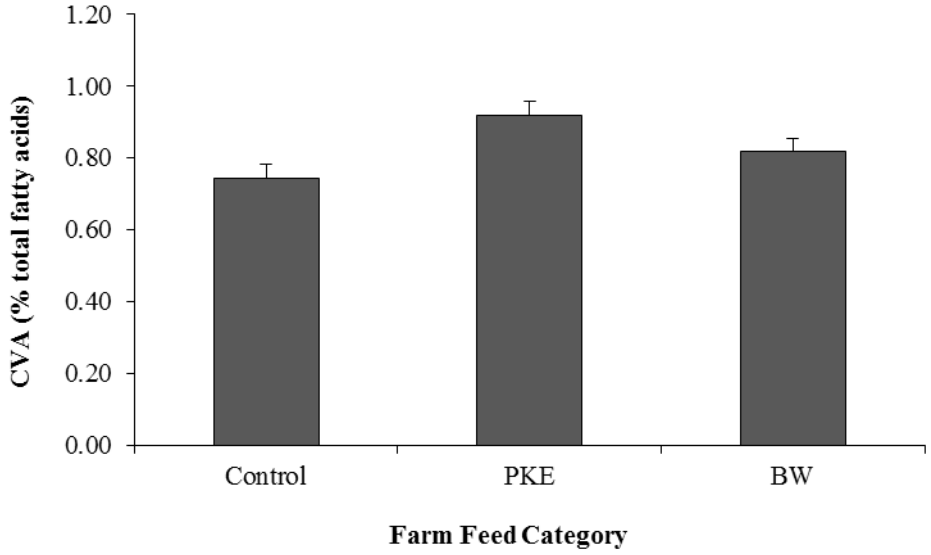

0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
C16:1 (% total fatty acids)
Control
PKE
BW
Farm Feed Category

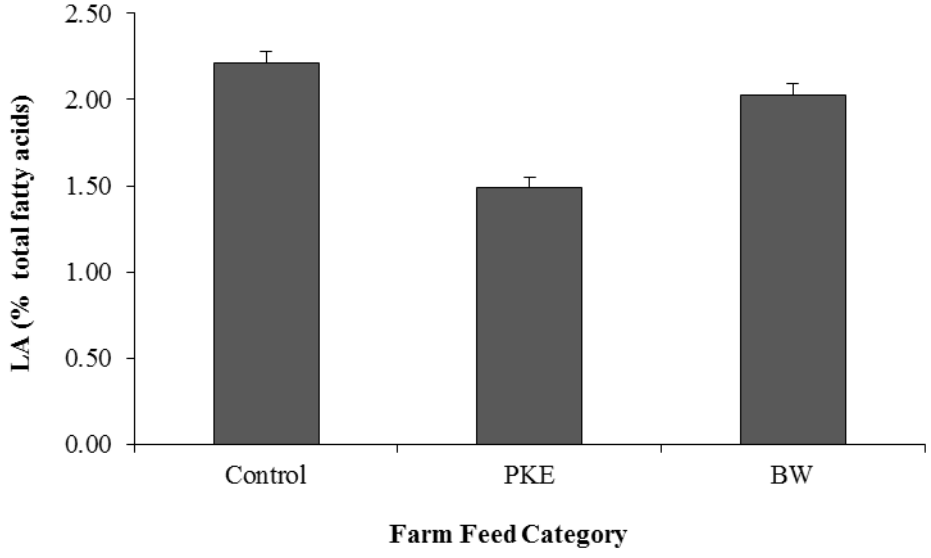

0.70
0.60
0.50
0.40
0.30
0.20
0.10
0.00
C17:0 (% total fatty acids)
Control
PKE
BW
Farm Feed Category

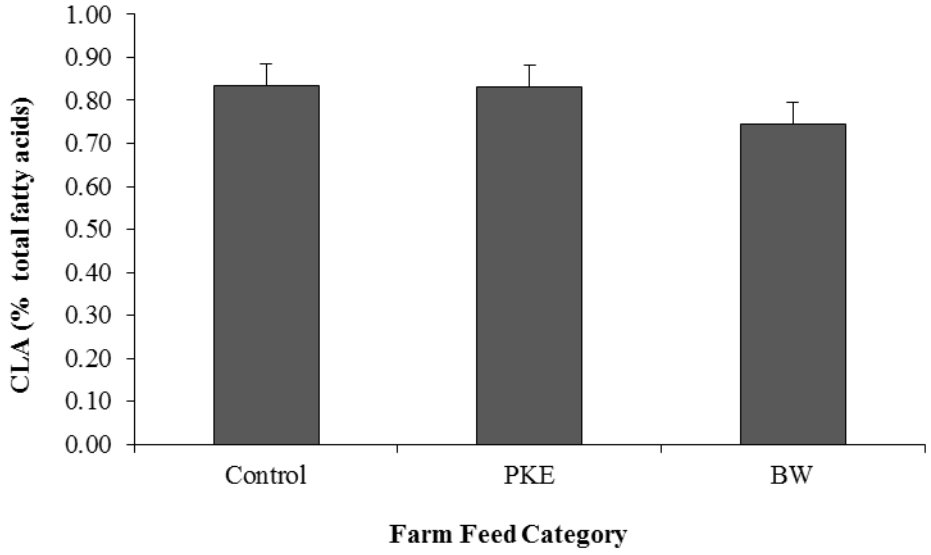

10.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
C18:0 (% total fatty acids)
Control
PKE
BW
Farm Feed Category

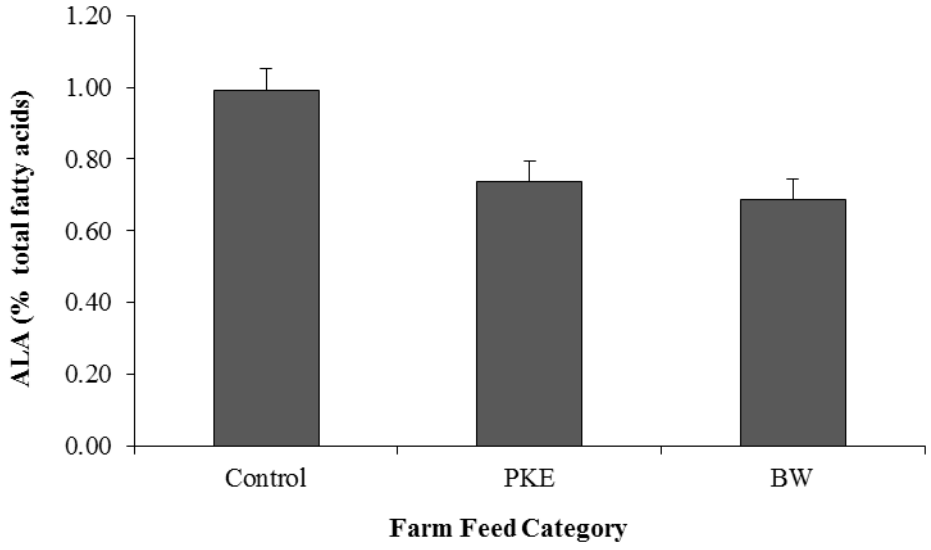

1.00
0.90
0.80
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0.00
TVA (% total fatty acids)
Control
PKE
BW
Farm Feed Category


25.00
20.00
15.00
10.00
5.00
0.00
C18:1 cis9 (% total fatty acids)
Control
PKE
BW
Farm Feed Category


1.20
1.00
0.80
0.60
0.40
0.20
0.00
CVA (% total fatty acids)
Control
PKE
BW
Farm Feed Category

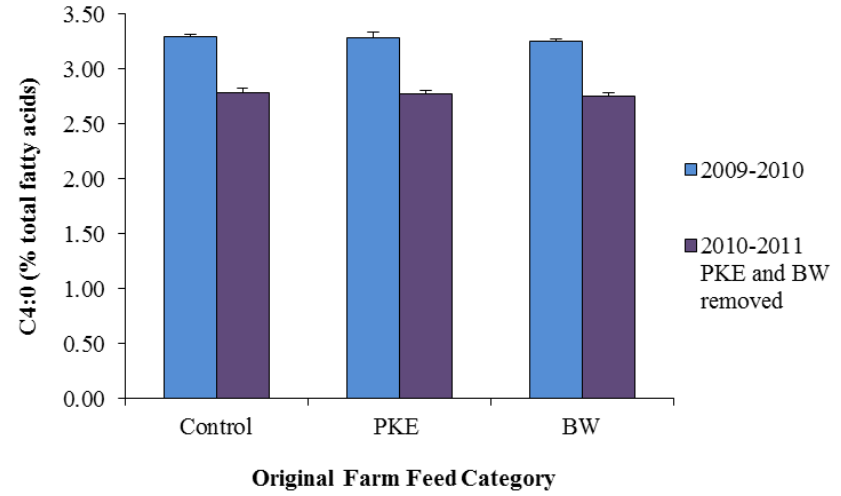

2.50
2.00
1.50
1.00
0.50
0.00
LA (% total fatty acids)
Control
PKE
BW
Farm Feed Category

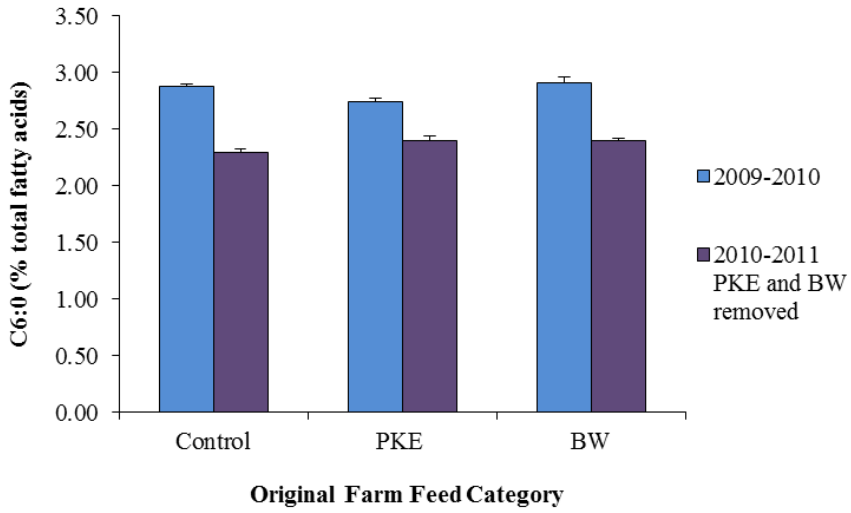

1.00
0.90
0.80
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0.00
CLA (% total fatty acids)
Control
PKE
BW
Farm Feed Category

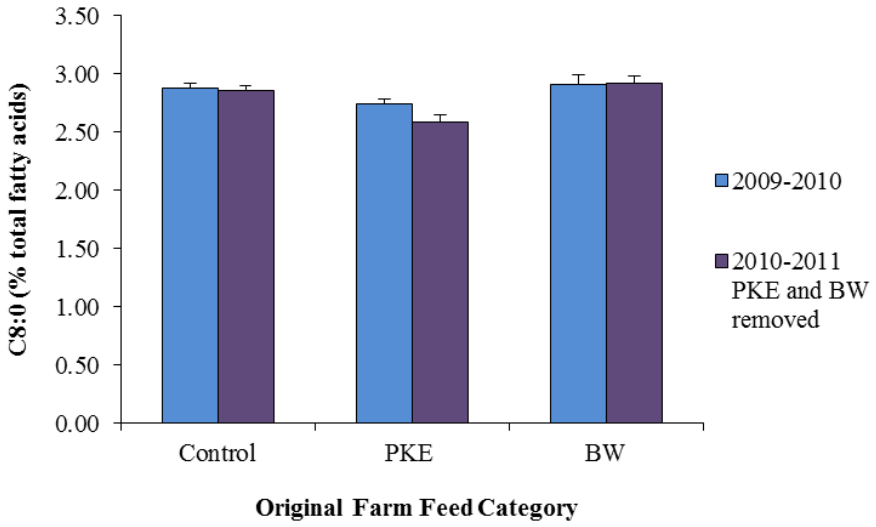

1.20
1.00
0.80
0.60
0.40
0.20
0.00
ALA (% total fatty acids)
Control
PKE
BW
Farm Feed Category

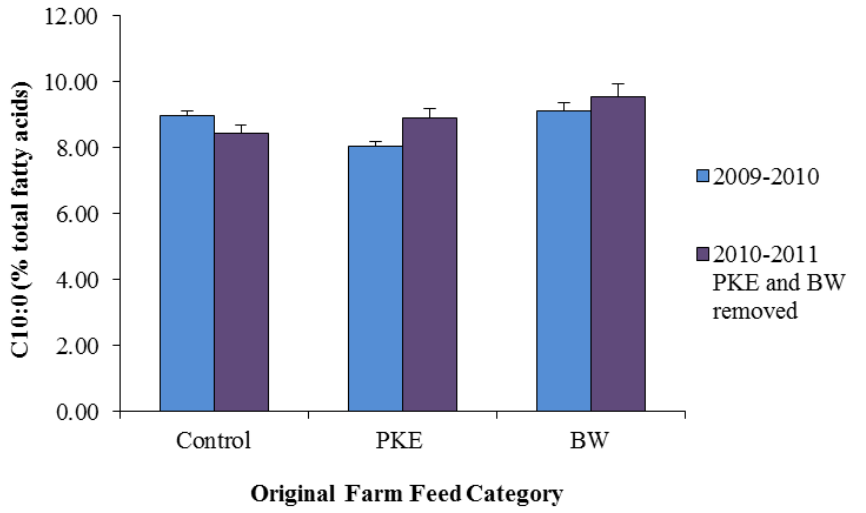
Appendix G: Feed Trial 2 Graphs

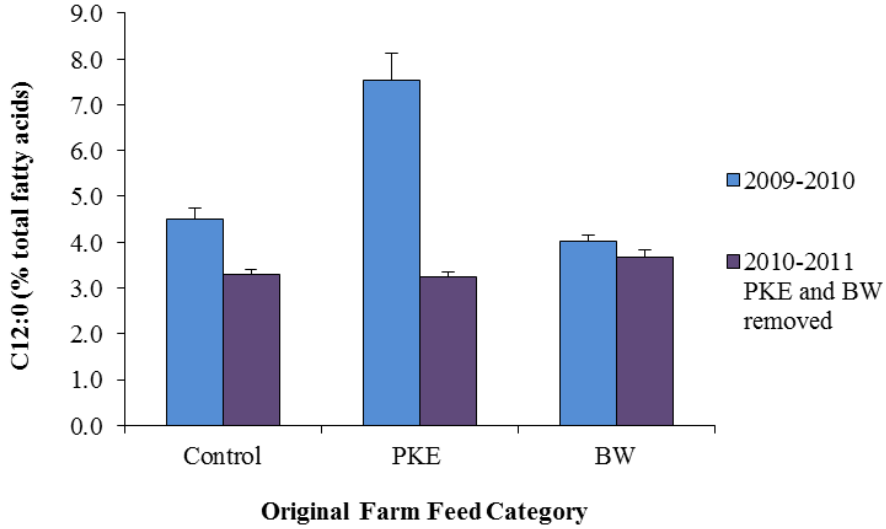
Presented below are the mean fatty acid (% of total fatty acids) values of control, PKE and BW farms for each fatty acid between 2009-2010 and 2010-2011 seasons. Error bars are the standard deviation of the mean. Significant differences are outlined in Table 38 of section 7.2.

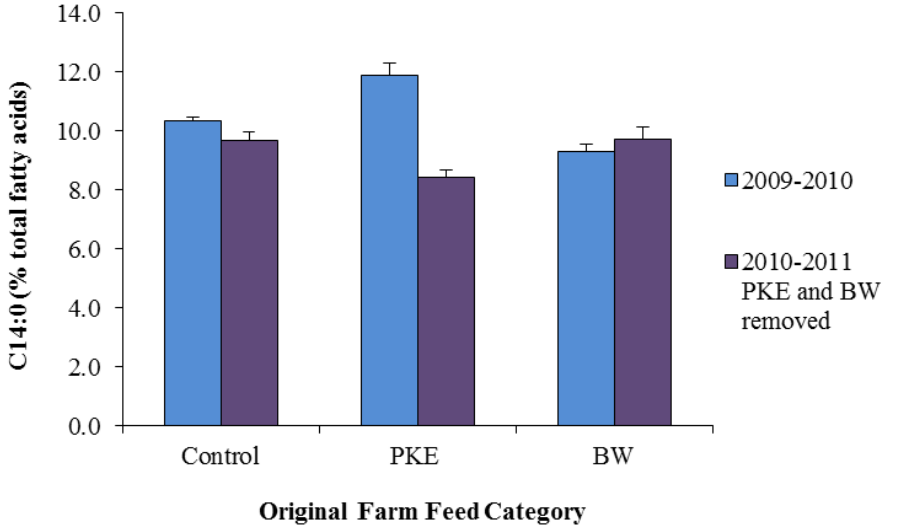


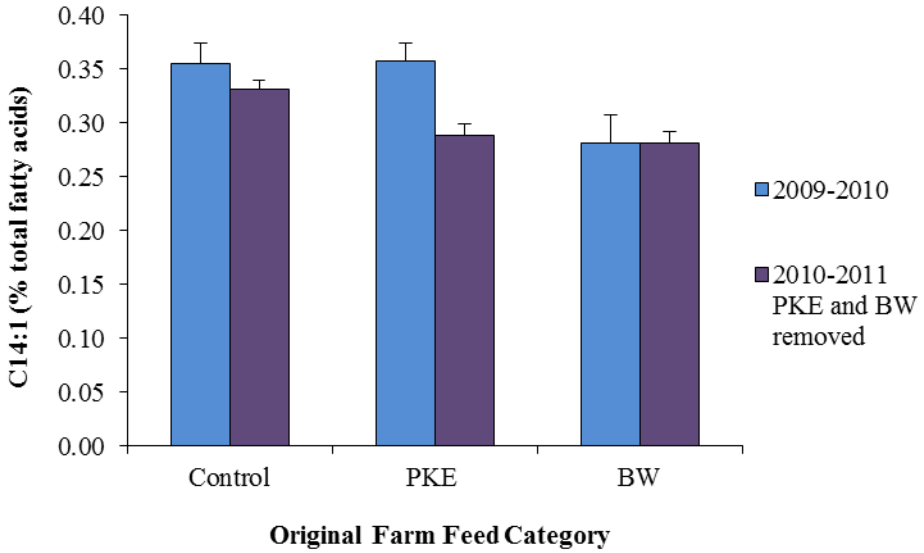


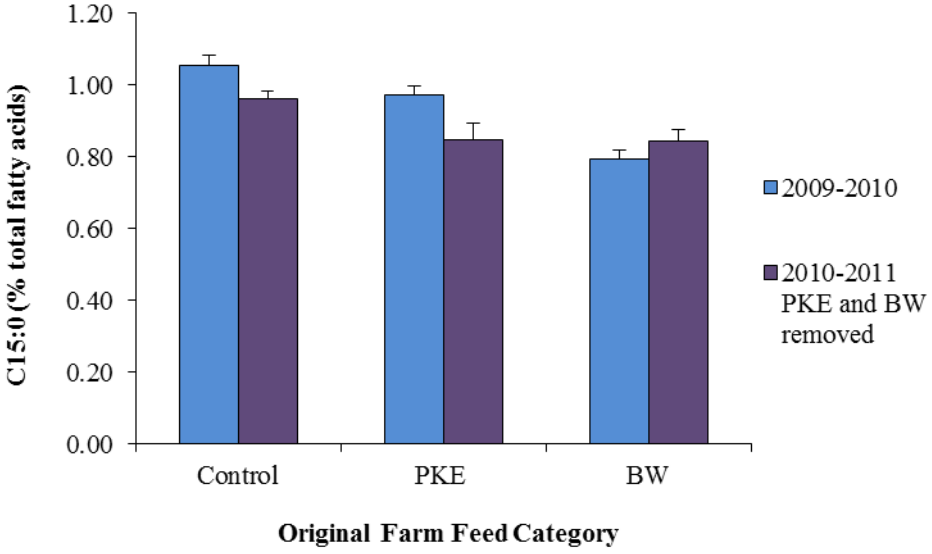

3.50
3.00
2.50
2.00
1.50
1.00
0.50
0.00
C8:0 (% total fatty acids)
Control
PKE
BW
Original Farm Feed Category
2009-2010
2010-2011 PKE and BW removed

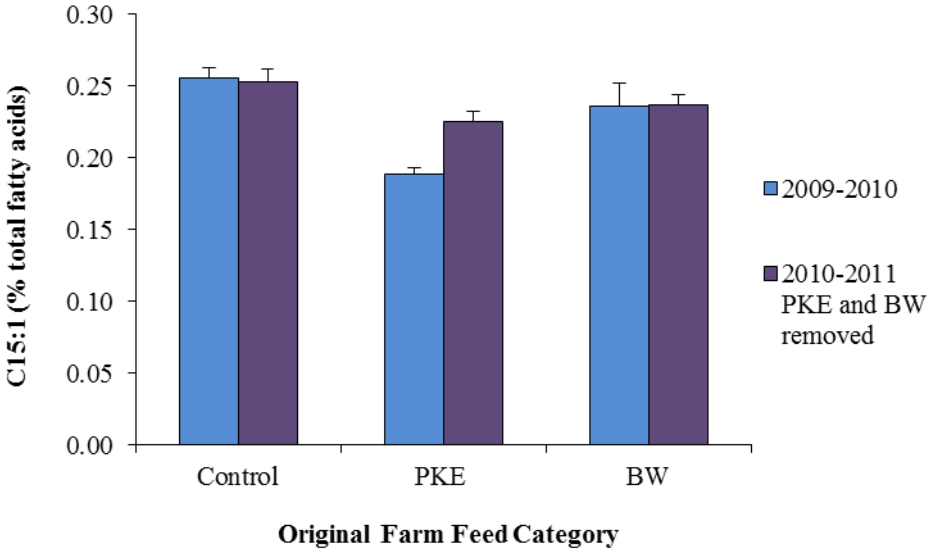

12.00
10.00
8.00
6.00
4.00
2.00
0.00
C10:0 (% total fatty acids)
Control
PKE
BW
Original Farm Feed Category
2009-2010
2010-2011 PKE and BW removed

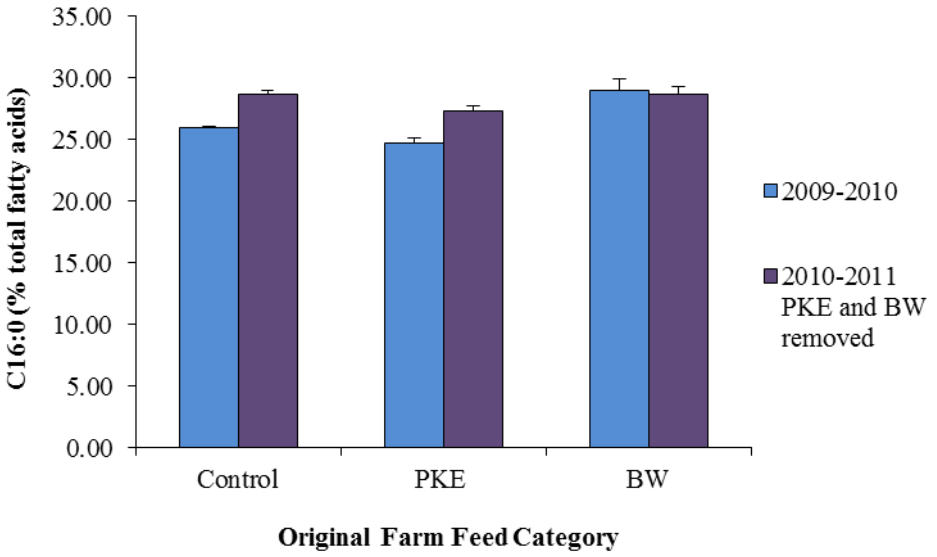

9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
C12:0 (% total fatty acids)
Control
PKE
BW
Original Farm Feed Category
2009-2010
2010-2011 PKE and BW removed

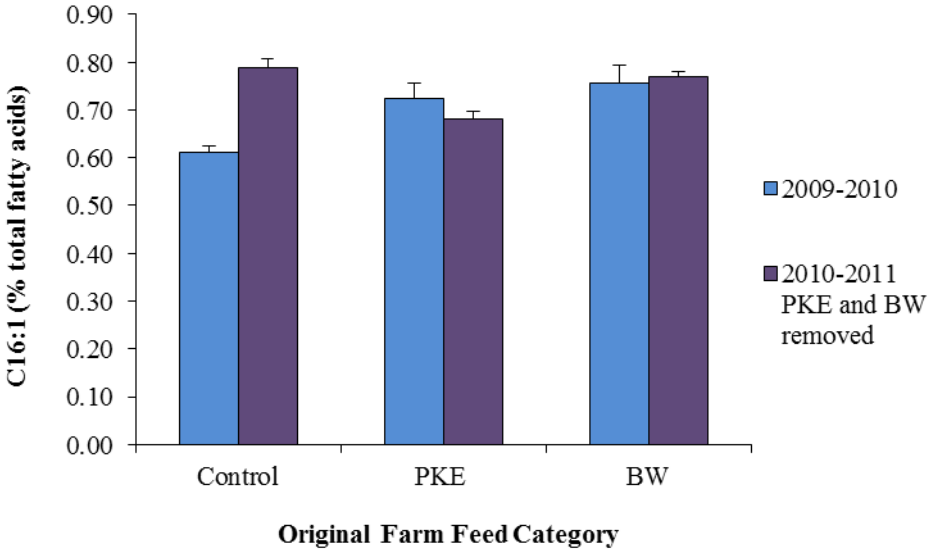

14.0
12.0
10.0
8.0
6.0
4.0
2.0
0.0
C14:0 (% total fatty acids)
Control
PKE
BW
Original Farm Feed Category
2009-2010
2010-2011 PKE and BW removed

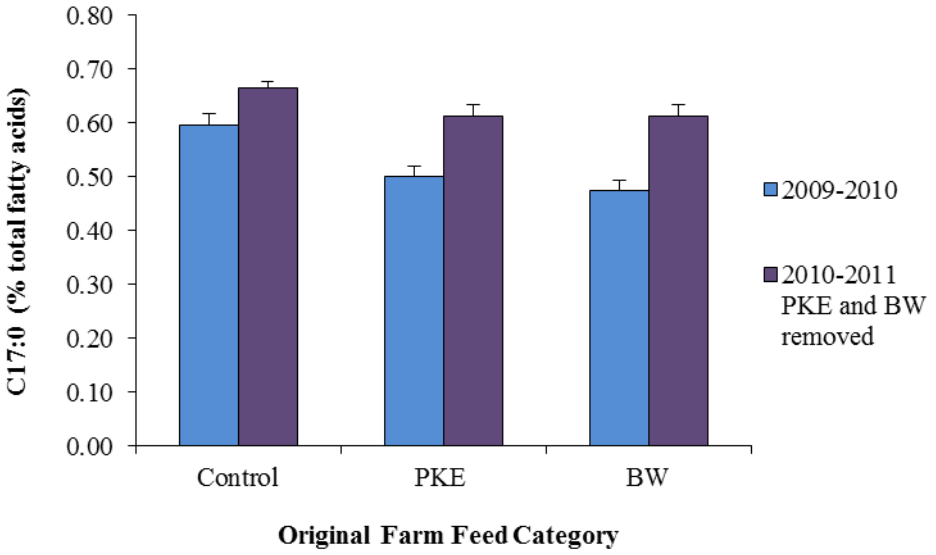

0.40
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0.00
C14:1 (% total fatty acids)
Control
PKE
BW
Original Farm Feed Category
2009-2010
2010-2011 PKE and BW removed

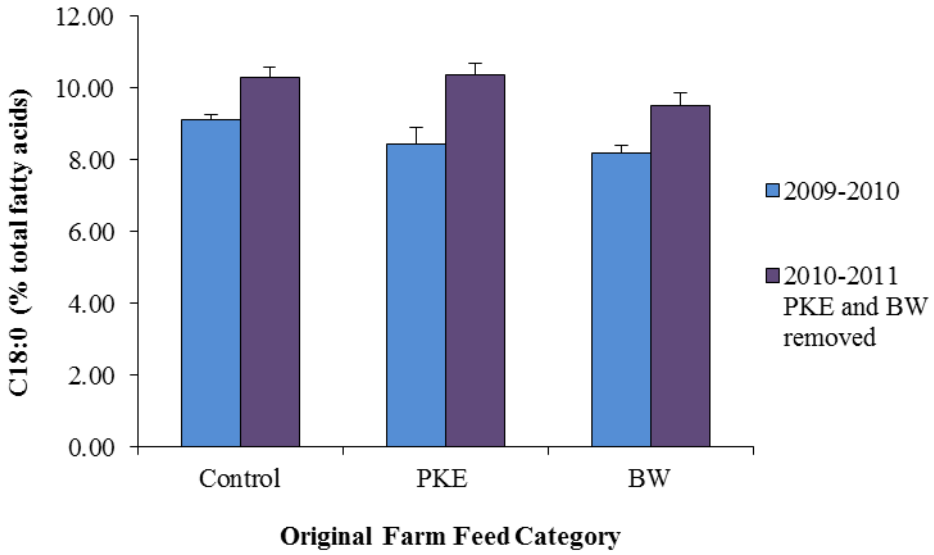

1.20
1.00
0.80
0.60
0.40
0.20
0.00
C15:0 (% total fatty acids)
Control
PKE
BW
Original Farm Feed Category
2009-2010
2010-2011 PKE and BW removed

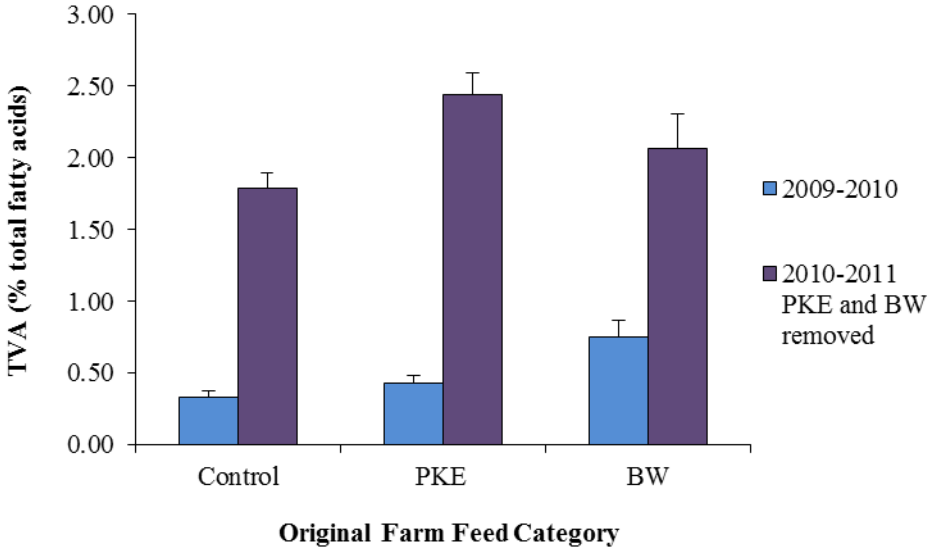

0.30
0.25
0.20
0.15
0.10
0.05
0.00
C15:1 (% total fatty acids)
Control
PKE
BW
Original Farm Feed Category
2009-2010
2010-2011 PKE and BW removed

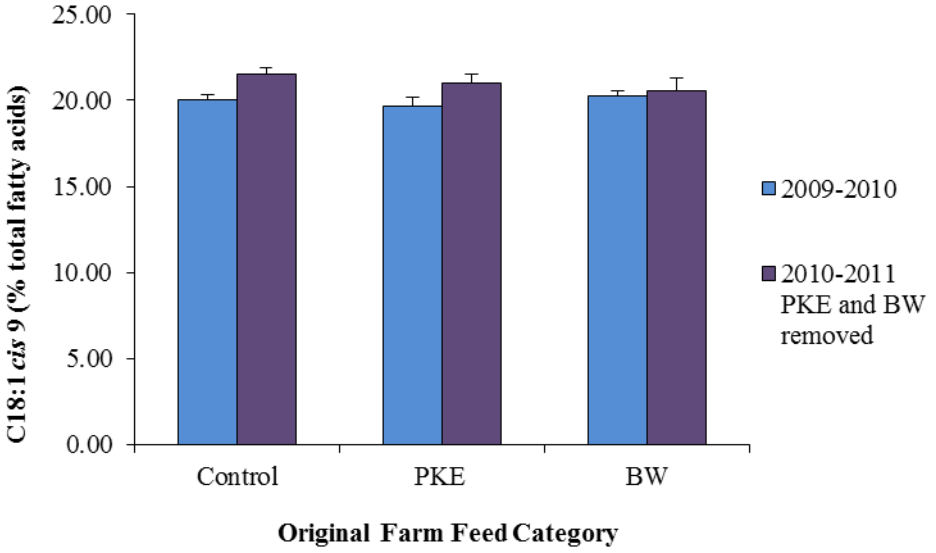

35.00
30.00
25.00
20.00
15.00
10.00
5.00
0.00
C16:0 (% total fatty acids)
Control
PKE
BW
Original Farm Feed Category
2009-2010
2010-2011 PKE and BW removed

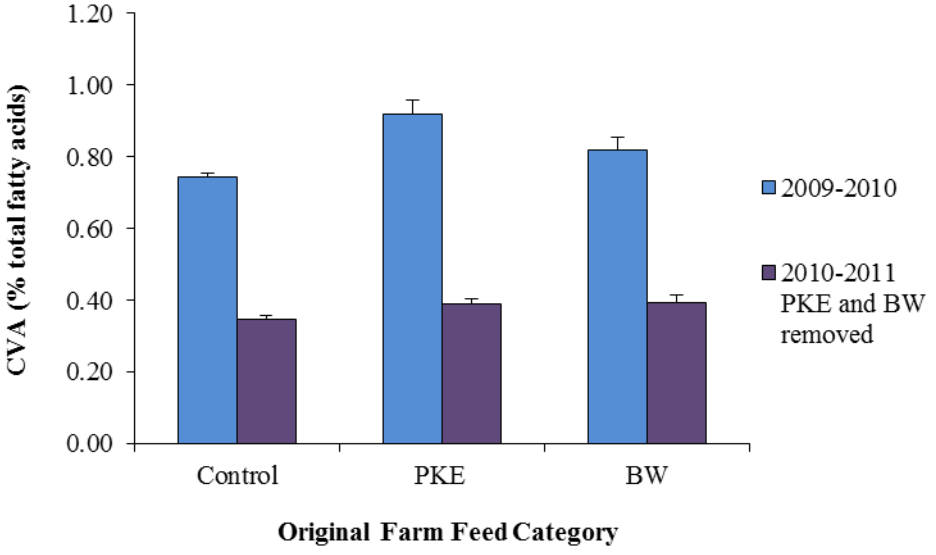

0.90
0.80
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0.00
C16:1 (% total fatty acids)
Control
PKE
BW
Original Farm Feed Category
2009-2010
2010-2011 PKE and BW removed

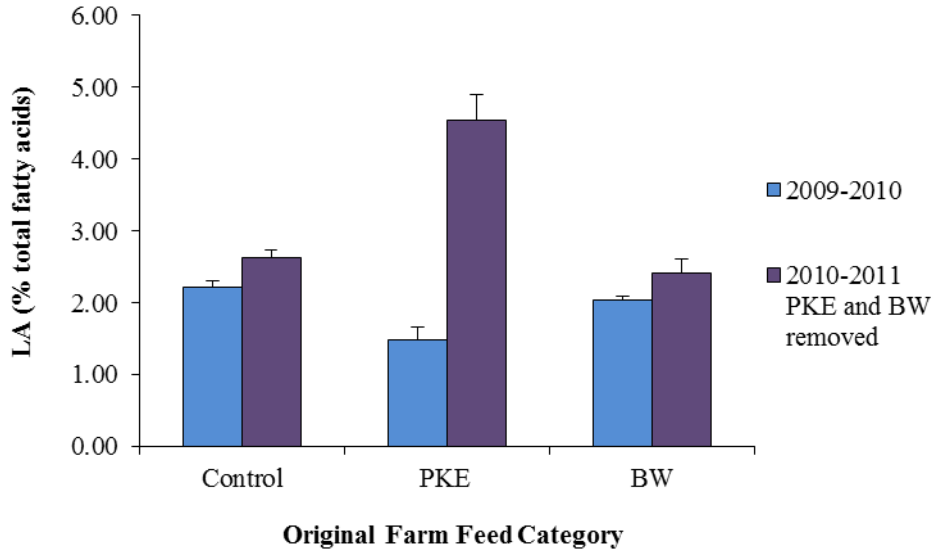

0.80
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0.00
C17:0 (% total fatty acids)
Control
PKE
BW
Original Farm Feed Category
2009-2010
2010-2011 PKE and BW removed

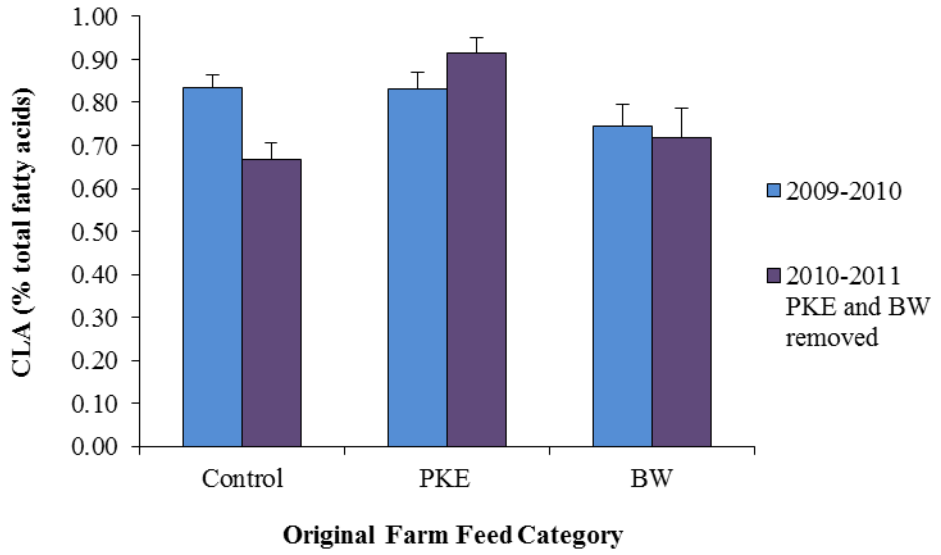

12.00
10.00
8.00
6.00
4.00
2.00
0.00
C18:0 (% total fatty acids)
Control
PKE
BW
Original Farm Feed Category
2009-2010
2010-2011 PKE and BW removed

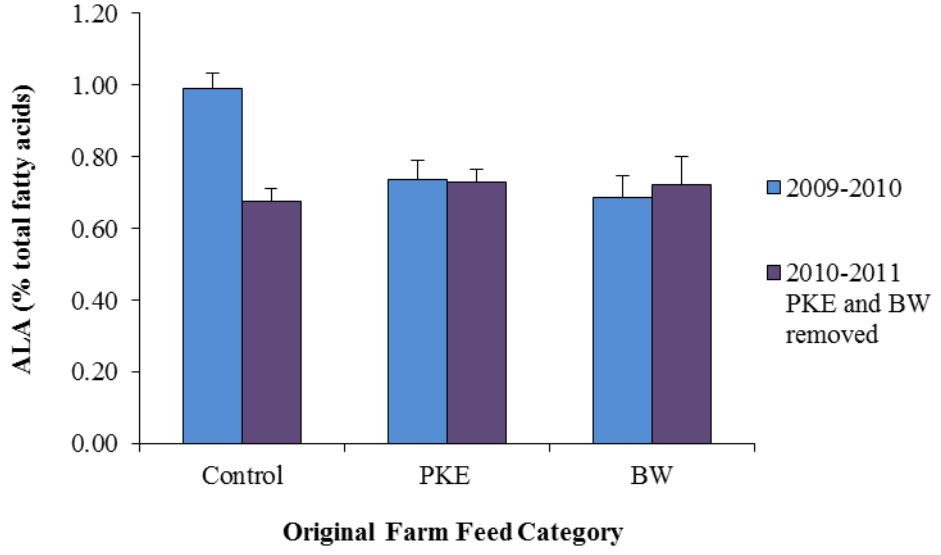

3.00
2.50
2.00
1.50
1.00
0.50
0.00
TVA (% total fatty acids)
Control
PKE
BW
Original Farm Feed Category
2009-2010
2010-2011 PKE and BW removed


25.00
20.00
15.00
10.00
5.00
0.00
C18:1 *cis* 9 (% total fatty acids)
Control
PKE
BW
Original Farm Feed Category
2009-2010
2010-2011 PKE and BW removed


1.20
1.00
0.80
0.60
0.40
0.20
0.00
CVA (% total fatty acids)
Control
PKE
BW
Original Farm Feed Category
2009-2010
2010-2011 PKE and BW removed


6.00
5.00
4.00
3.00
2.00
1.00
0.00
LA (% total fatty acids)
Control
PKE
BW
Original Farm Feed Category
2009-2010
2010-2011 PKE and BW removed


1.00
0.90
0.80
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0.00
CLA (% total fatty acids)
Control
PKE
BW
Original Farm Feed Category
2009-2010
2010-2011 PKE and BW removed


1.20
1.00
0.80
0.60
0.40
0.20
0.00
ALA (% total fatty acids)
Control
PKE
BW
Original Farm Feed Category
2009-2010
2010-2011 PKE and BW removed