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` **Investigation of Ovine *DGAT1* intron 1 variation and its association with variation in carcass traits**

A Dissertation

submitted in partial fulfilment

of the requirements for the Degree of

Bachelor of Science with Honours

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by

Sarah Barr

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Abstract of a Dissertation submitted in partial fulfilment of the requirements for the Degree of Bachelor of Science with Honours.

Investigation of Ovine *DGAT1* intron 1 variation and its association with variation in carcass traits

by

Sarah Barr

The economic importance of a carcass is determined by the quantity of saleable meat. The quality of a carcass is determined by the lean meat yield and the ratio of lean meat to fat. Therefore, the heightened quality is portrayed by a carcass that is low in subcutaneous fat, high in lean meat yield with a minimum of 3% of intramuscular fat to give the meat marbling, enhancing eating attributes such as, juiciness and tenderness. Diacylglycerol acyltransferase 1 or *DGAT1* has shown to have a strong association with fat production as the last committed step to triglyceride synthesis. In this trial, 338 NZ Romney lambs were genotyped using PCR-SSCP to see if variation occurred in *DGAT1* intron 1. It was determined that a variation occurred, and following sequencing, the variation was identified to be a C to T substitution at position 171 between primer sites and equated to three genotypes; AA, AB and BB. 190 lambs were further investigated for association between their genotypes and carcass traits. Three data sets were used, 2015, Glenleith progeny 2014 and Doughboy progeny 2014, of these, two (2015 and Glenleith 2014) showed a strong correlation, P=0.040, P=0.027 respectively, between an increase in leg yield and the presence of allele B. With further analysis it showed that the presence of B increased the leg yield by between 670-706g. It may be assumed that this increase is due to heightened intramuscular fat and therefore the identification of the presence of allele B may be incorporated into a selective breeding system to enhance the intramuscular fat in a leg cut in NZ Romney lambs.

Keywords: Diacylglycerol acyltransferase 1, DGAT1, PCR-SSCP, genetic variation, ovine, leg yield, selective breeding, NZ Romney

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And lastly to all my friends and family, for laughing at me most of the time when crying from tiredness, stress or just because Friends wasn't on when I thought it was. Massive thank you to Mum and Dad for the endless support and providing free board and food when I was poor.

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Chapter 1

Literature Review and Introduction

The financial value of a lamb carcass is largely determined by the quantity of saleable meat on the carcass, and therefore producing higher lean meat yielding lambs for slaughter can be beneficial to producers, processors, retailers and consumers. It improves the efficiency of the lamb supply chain (Pearce, 2016).

With higher yielding carcasses producers can produce more weight of meat per animal, and possibly without facing penalties for having too much carcass fat. These lambs can be slaughtered earlier leading to increased efficiency in feed use. High yielding lambs are more efficient to butcher as they require less time and effort to trim unwanted fat and remove bone, and this can mean less wastage. The consumer may perceive health benefits with leaner, meatier carcasses and therefore prefer purchasing meat that displays a high quantity of meat with less bone and subcutaneous fat (Pearce, 2016).

Carcasses muscle and fat traits do however show phenotypic variability, suggesting either a polygenic basis to inheritance and/or that the genetic effects are small and phenotype is strongly influenced by environment. As our understanding of key carcass traits increases, including increased understanding of specific genes and associated biochemical pathways, we will be in a better position to control carcass variability and add value to lamb carcasses.

Diacylglycerol acyltransferase 1 (DGAT1) has been revealed to influence triglyceride synthesis in animals and the gene is expressed ubiquitously with the highest mRNA levels being found in organs that make large amounts of triglycerides. This includes the small intestines, liver, mammary gland and adipose tissue (Yen et al, 2008). DGAT1 is used to catalyse the last step in triacylglycerol synthesis which is the esterification of a fatty acyl-CoA to the sn-3 position of a diacylglycerol (Cases *et al.*, 1998).

Scientific studies in cattle have allowed the identification of specific polymorphisms in the gene encoding DGAT1 (*DGAT1*) and related this genetic variation to the expression of components of milk in dairy cows. However, little research has occurred in sheep and especially whether DGAT1 plays any role in determining variation in carcass traits, especially carcass fat traits. This will therefore be the focus of this research.

DGAT1 Protein Structure and Function

DGATs are involved in the joining of diacylglycerol to fatty acyl CoA. The diacylglycerol is a product of either the hydrolysis of phosphatidic acid in the glycerol phosphate pathway, or alternatively, by acylation of monoacylglycerol in the monoacylglycerol pathway.

Cases *et al.* (1998) identified two DGAT proteins: DGAT1 and DGAT2. Initially, using homology searches, they identified an expressed sequence tag (EST) in a clone, while looking for an EST for the known coding sequences of acyl-CoA: cholesterol acyltransferase (ACAT). ACAT is an enzyme that also uses fatty acyl-CoA as a substrate. The identified EST clone shared homologous DNA sequences with the ACAT C-terminus.

Analysis of the EST containing clone sequence revealed an open reading frame for a 498 amino acid peptide, which was 20% similar to a mouse ACAT. Because of this similarity, experiments were then designed to test whether the cDNA clone encoded an enzyme which catalysed cholesterol esterification. The cDNA was expressed in insect cells using a baculovirus expression system and following thin layer chromatography (TLC), it was revealed that the membranes from cells that contained the expression construct had increased triacylglycerol mass and had incorporated more oleoyl-CoA into triacylglycerols, when compared to membranes from wild-type virus infected cells. The DGAT-like activity was five-fold higher in the membranes of the tagged insect cells and this gave a ten-fold increase in triacylglycerol mass in the cells expressing the putative DGAT cDNA than the wild-type cells. DGAT-like activity also increased directly proportional to the amount of cDNA clone

It was predicted that the structure of DGAT would be similar to that of ACAT, and include 7-9 transmembrane domains and the conserved serine residue that is required for the activity of ACAT (Lin *et al.*, 1999). The substrate binding sites of DGAT1 were predicted (Buhman et al. 2001) to appear in its large luminal extra-membranous loop. This loop contains a conserved motif, FYxDWWN, which plays a pivotal role in the acyl Co-A binding, as well as a common motif found in protein kinases that have a diacylglycerol binding domain (Lopes *et al.*, 2014). Lin *et al.* (1999), also highlight that the DGAT1 protein is held in a homo-tetramer. Like DGAT, Yu et al. (1999) revealed that highly purified ACAT1 also forms a homo-tetramer, and this demonstrates that no other bridge protein is required for its subunits to interact.

Siloto *et al.* (2010) conducted a study that assessed DGAT protein alignments from 30 different organisms. The study indicated that there were a total of 7% identical sequences between plants and animals with several conserved regions. Animal N-terminus regions were on average 85 residues long, as opposed to plants where the N-terminus averaged 115 amino acid residues. The N-terminus

was thought to be the least conserved region, although McFie *et al.* (2010) have described a potential Acyl Co-A binding site in a recombinant N-terminal segment. The exception to the N-terminus variability is a region of 20 amino acid residues following the hydrophobic domain that is conserved across plants and animals.

Multiple studies have concluded that acyl-CoA binding coincides with the hydrophilic N-terminus of DGAT1 and that this allosterically regulates the protein. For example Cheng *et al.* (2001) revealed that the DGAT1 N-terminus assists in the formation of dimers and tetramers. However, in addition to this work, it has also been shown that when the N-terminus is removed, the DGAT1 enzyme still worked at full capacity, suggesting the N-terminus sequence motif is not the primary binding site for acyl-CoA (McFie *et al.* 2010).

The functionality of DGAT1 lies within the protein structure, and critical amino acid residues appear to have been conserved over evolution because they are essential for enzymatic activity (Cao, 2011). These conserved amino acid residues are clustered at the active centres of the enzymes, and Cao (2011) describes 41 completely conserved residues. The majority of the conservation is within the carboxyl terminal regions, suggesting the catalytic domains of DGATs are located here. Further evidence for this comes from the observation that mutations in the C-terminus regions result in the loss of DGAT1 enzymatic function (Cao, 2011).

Cao (2011) identified a catalytic histidine at position 426 (H426), that when mutated to alanine resulted in an impaired ability for DGAT1 to synthesis triglycerides, retinyl and wax esters in mice. Further evidence to confirm the importance of H426, came from analysis of the MBOAT family (membrane bound O-acyltransferases). Other members of the family catalyse O-acylation reactions by transferring acyl chains onto hydroxyl, or thiol groups of lipids and protein, such as ACAT and ski protein. The MBOAT family can be characterised by a highly conserved region containing an arginine and histidine and thus Cao (2011) concluded that these residues were directly involved in DGAT1's catalytic activity as previously identified in the human ACAT.

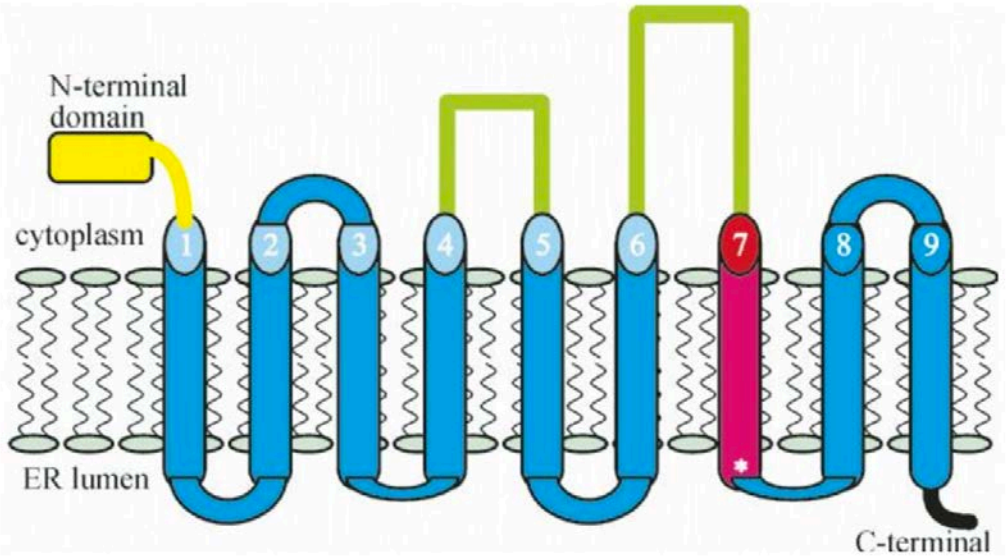


Figure 3.1 A general endoplasmic reticulum membrane topology model for the ACAT family of enzymes. The putative cholesterol/diacyglycerol-binding transmembrane domain region (TMD7) is shown in red, and other TMDs are shown in blue. The possible acyl-coenzyme A-binding regions (the loop between TMD6 and TMD7 and the loop between TMD4 and TMD5) are shown in green. The position of the active site HIS residue in TMD7 is indicated by a white star. Derived from; Liu *et al.* (2009).

DGAT1 Gene (*DGAT1*) Structure

DGAT1 has been mapped to human chromosome 8q24 and contains 18 exons (Genbank, Gene ID: 8694). The gene has been located and identified in mouse, cow, rat, pig, sheep, monkey, dog, water buffalo, chimpanzee, ferret and Chinese hamster. Grisart *et al.* (2002) determined the structure of bovine *DGAT1* using DNA sequence analysis. Primers were designed based on available bovine, murine and human cDNA sequences and these were either used for direct sequencing of the BAC clone or to generate PCR products which were then cycle-sequenced. A computer program called Phred/Phrap was used to merge established sequences to produce a finalised sequence. Reverse transcription polymerase chain reaction (RT-PCR) followed by RACE and PCR were then used to establish the exact gene measurements (Grisart *et al.*, 2002) and it was concluded that the gene spanned 8.6Kb and included 17 exons (Figure 3.1).

Scata *et al.* (2009) used this bovine sequence as a basis for sequencing the ovine gene. They identified 200bp of 5'-UTR, 17 exons and their corresponding introns and 128 bp of 3'-UTR. The coding region of 498-amino acid residues shared nucleotide identity (97%) with the bovine orthologous sequence with only five non-synonymous mutations (Scata *et al.* 2009). The non-coding region of ovine *DGAT1* had 90% nucleotide identity with the bovine sequence. The sequences of the small introns: 6, 10, 14 and 16 differed from the corresponding bovine sequence by over 20%. They

were also highlighted as the location for 20% of the total insertions and deletions ovserved in the characterised sequence.

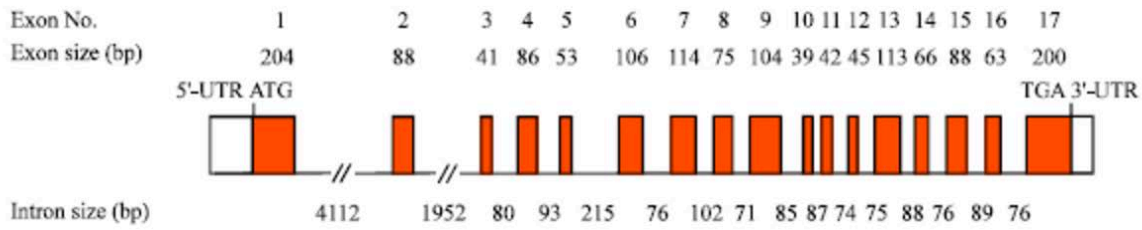


Figure 3. 2 Predicted structure of ovine *DGAT1.* Red rectangles represent Exons. Derived from; Breeds, (2011)

DGAT1 Function and overexpression/knockout affect in mice

DGAT1 has also been hypothesised to be directly involved in intestinal fat absorption, lipoprotein assembly and the regulation of plasma triacylglycerol concentrations, fat storage in adipocytes and energy metabolism in muscle production (Cases *et al.,* 1998). It is expressed in a number of tissues, with the highest expression levels present in adipose tissue and the small intestine (Chen and Farese, 2000; Buhman *et al.*, 2002).

Chen *et al.* (2002) revealed that over-expression of *DGAT1* in white adipose tissue of transgenic mice increases adipocyte size and mass, and that the level of overexpression was directly proportional to the degree of adiposity. In a different *DGAT1* "knock-out" mouse model, Smith *et al.* (2000) revealed that although deficient in DGAT1 activity, mice were still viable and capable of synthesising triglycerides with normal fasting serum triglyceride levels and normal adipose tissue conformation. Buhman *et al.* (2002) also explored the knock-out effect in mice and revealed that DGAT1 was not essential for quantitative dietary triacylglycerol absorption, even in mice fed a high fat diet, or for chylomicron formation.

Smith *et al.* (2000) also demonstrated that female mice with knocked-out DGAT1 genes were unable to produce any milk as a consequence of the impairment of triglyceride synthesis in the mammary gland. It was hypothesised that although the DGAT1 deficient knock-out mice could synthesise triglycerides through non-DGAT pathways, they were still not producing equivalent amounts of triglycerides compared to wild-type mice (Smith et al, 2000). Chen *et al.* (2002) suggested that the DGAT1 deficiency changed fatty acid composition in adipose tissue and skeletal muscle, such that saturated fatty acid levels are increased and monounsaturated fatty acids are consequently decreased. This in turn would affect triglyceride metabolism in tissues such as the mammary gland and thus prevent milk production (Chen *et al.*, 2002).

DGAT1 deficient mice also have reduced post-absorptive chylomicronaemia one hour after a high-fat challenge (Buhman *et al.*, 2002). When chronically fed a high-fat diet, they accumulated neutral lipid droplets in the cytoplasm of enterocytes, suggesting reduced triacylglycerol absorption (Buhman *et al.*, 2002).

Associations between variation in *DGAT1* and variation in phenotypic traits

Grisart *et al.* (2002) identified 13 bulls who were predicted to be heterozygous for the QTL on chromosome 14 known as Qq. Allele Q, when passed on to offspring, significantly increases milk fat content compared to q. Furthermore, the Q alleles are in strong linkage disequilibrium with two specific microsatellite haplotypes known as $\mu HQ^{H\text{-}D}$ and $\mu HQ^{H\text{-}NZ}$ that occur in Dutch and New Zealand Holstein-Friesian populations respectively. The alternative, q alleles appeared in each populations and were revealed to be associated with multiple microsatellite haplotypes referred to as µhq (Grisart *et al.*, 2002). Grisart *et al.* (2002) predicted that the Q and q alleles would directly correspond to functionally distinct *DGAT1* alleles and hence differ by one or more mutations that caused them to be functionally different. Following sequencing of the DGAT1 gene from both the Dutch and New Zealand groups and their offspring, the K232A amino acid substitution was identified resulting from an ApA to GpC substitution in exon 8. Other polymorphisms were also found including an A to G substitution in intron 12, a C to T substitution in intron 12 and a C to T transition in the 3-UTR region.

With the K232A mutation, the lysine (K) residue is conserved in mammals and Grisart *et al.* (2002) took this to represent its functional importance. Phenotypic variation associated with K232A includes increased milk fat production being associated with the presence of the lysine residue and vice versus with the alanine residue. Winter *et al.* (2002) also identified and confirmed the K232A variation and described other gene regions that were variable too (See Table 2.1).

Since this time, various other cattle breeds have been shown to have variation in milk traits associated with the K232A variation in *DGAT1*. For example, Kuhn *et al.* (2004), revealed associations in German Holstein cattle, while Rahmatella *et al*. (2015), described how the *DGAT1* K232A alanine variant had an effect on milk yield, fat content and protein yield when compared with the lysine variant. Weller et al. (2003) also reported similar results when studying Israeli Holstein cattle. Overall this suggests the K232A variation is widely found in different cattle breeds.

Table 2.1 Nucleotide Variation in Bovine DGAT1. (From Winter et al., 2002)

Position*	Variation	Position*	Variation
1465	$(AGGCCCCGCCCTCCCCGG)_n$†	10515	$(G)_n$‡
3343	C → G/T	≈10800	+ → −§
3399	T → G	11030	G → A
7233	A → G	11048	C → T
8567	A → G	11993	T → C
8607	G → A	12005	A → C
9284	T → C	12036	T → C
10147	A → C	12056	A → G
10433	G → A	12136	G → A
10434	C → A	13309	G → C

*Numbering is according to the deposited sequence (GenBank accession no. AJ318490).
† $n = 3, 4, 5, 6, 7$.
‡ $n = 6, 7$.
§ +, PCR product present; −, no or very weak PCR product.

Thaller *et al.* (2003) explored the relationship between intramuscular fat (IMF) variation and *DGAT1* variation in cattle because of the relationship between adiposity and DGAT1. There was a positive relationship between increased IMF levels in the semi-tendinosus muscle and the presence of the alanine K232A mutation in cattle. IMF is highly valued in the beef cattle industry, hence Yuan *et al.* (2013), furthered the understanding of these interactions based around a study on commercial Chinese cattle. They used 17 known SNPs in the exon region of *DGAT*, of which, three of these were then genotyped using CRS-PCR (c.572A>G in exon 6, c.1241C>T in exon 15 and c.1416T>G in exon 17). These SNP's were then screened over 483 cattle from eight breeds for their effects on the following traits; live weight (LW), average daily gain (ADG), carcass weight (CW), dressing percentage (DP), meat weight (MW), backfat thickness (BFT), longissimus muscle area (LMA), marbling score (MS), meat colour (MC), fat colour (FC) and Warner-Bratzler shear force (WBSF). The results revealed that two of the three SNPs typed (c.572A>G and the c.1416T>G) were associated with carcass quality traits, with c.572A>G affecting BFT, MS, FC and WBSF, while c.1416T>G was associated with BFT, LMA, MS, FC and WBSF (Yuan *et al.*, 2013). In contrast, Casas *et al.*, (1998) found no associations between K232A polymorphism and carcass composition traits in the Brahman breed, while Souza *et al.*, (2010) identified a significant interaction between the K232A polymorphism, but only with rump height.

There is no evidence to date of variation in ovine *DGAT* exon 8. Xu *et al.* (2009) have reported a SNP in exon 17 in three Chinese sheep breeds. The variation GCT (Ala) - GCC (Ala) was a silent substitution at nucleotide 1461. PCR-RFLP and electrophoresis confirmed three genotypes, TT, TC and CC (Xu *et al.*, 2009). The allele distribution was not in Hardy-Weinburg equilibrium and allele frequency analysis suggested there was no difference among between the three breeds. The T allele was revealed to have a positive effect on IMF content, tenderness, drip loss rate and marbling score, but there was no obvious effect on colour, pH value, hot carcass weight, loin-eye area, GR value, fat thickness and net meat percentage (Xu *et al.,* 2009). Similar results have also been obtained in Lori sheep (Nanekarani *et al.*, 2015) and Moghani sheep (Noshahr and Rafat, 2014). Mohammedi *et al.*, (2013) also explored the exon 17 variation and reported that CC sheep had fat-tail weights and backfat thickness. All these ovine studies suggested that the specific mechanism of action of the DGAT1 gene polymorphism need further investigation, but that the results could be used in marker assisted selection and breeding programs for carcass weight (Xu *et al.*, 2009, Nanekarani *et al.*, 2015, Noshahr and Rafat, 2014 and Mohammedi *et al.*, 2013).

Scata et al. (2009) investigated the complete coding region of ovine *DGAT1* in three Italian sheep breeds: the Altamaurana, Gentile di Pugila and Sarda. They identified five novel SNPs (see Table 2.2). One of the SNP's identified was located in intron 2 (g. 5553C>T) and was reported to occur at a similar frequency in the three breeds. It was negatively correlated with milk fat content. The SNP identified in the 5'UTR region (g. 127C>A), appeared to have a lower frequency in the higher milk-fat breeds (the Altamurana and Gentile di Puglia) and had negative association with milk fat content in the Sarda sheep. Scata *et al.* (2009) concluded that due to DGAT1 playing a fundamental role in triacylglcerol synthesis, the novel SNP in the 5'UTR of the DGAT1 gene might partially explain the variation of fat content in the milk of the Sarda sheep.

Table 2. 2 DGAT1 variation in three breeds of sheep: the Altamurana, Gentile di Pugila and Sarda (from Scata *et al.*, 2009)

SNP	Position in the gene	Frequency of the mutated allele		
		Altamurana	Gentile di Puglia	Sarda
g.127C>A	5' UTR	0.05	0.02	0.19
g.1655C>T	Intron 1	0.02	0	0
g.5553C>T	Intron 2	0.25	0.32	0.41
g.7492C>T	Intron 10	0	0.07	0
g.8539C>T	Exon 17	0.06	0.07	0

Evidence of the associations between variations in intronic regions and phenotypic variation

An area for further investigation of *DGAT1* variation is the introns. Introns were once thought of as junk DNA that must be removed to create a translatable mRNA, but they are now thought to be facilitators of molecular evolution and regulators of gene expression (Nott *et al.*, 2003). Introns have been shown to promote the creation of new genes by enabling exon-duplication and allow the synthesis of multiple proteins from a single gene, by alternate splicing. Research has also revealed that for optimal gene expression, constitutively spliced introns are necessary (Nott *et al.*, 2003).

Callus *et al.* (1987) and Bourdon *et al.* (2001) have demonstrated in maize plants that optimal expression of many endogenous genes requires the presence of one or more intron. Bourdon *et al.* (2001) highlighted that the presence of an intron can alter gene expression levels with great variability, and between having no effect to having more than a 400-fold increase in mRNA levels. Elements that may influence the magnitude of alteration are intron identity, exon sequence context and intron position within the gene (Nott *et al.*, 2003). The variability is likely explained by the introns ability to influence gene expression at multiple steps, with the individual introns contributing differentially to each step (Nott *et al.*, 2003). The steps shown to be influenced by introns are; transcription, polyadenylation, mRNA export, translation efficiency, and mRNA decay (Nott *et al.*, 2003).

He *et al.* (2011) studied the mechanism of transcription and expression of the myostatin gene by cloning and analysing the sequence of the bovine myostatin gene promoter and first intron from Qinchuan and Red Angus cattle. Then they constructed eukaryotic expression vectors encoding the Green fluroscent protein (GFP) vector by replacing the CMV promoter with the bovine myostatin promotor using PCR and hence obtaining an expression vector coding GFP report gene with first intron. By transfecting C1C12 cells, they then compared the effect on GFP expression of the promotor and normal first intron of Qinchian and Red Angus cattle with that from the promotor and a Qinchuan allele with a 16 base pair insertion. They found that myostatin gene first intron increased the expression of GFP C2C12 cells. As discussed earlier, intron regulated gene expression has been shown to occur in precursor RNA splicing, transcription, RNA editing, mRNA of the nuclear transport, mRNA translation and nonsense decay processes in eukaryotic cells. The study concludes that it may be possible to increase muscle growth in meat producing animals by knocking out the first intron to suppress the expression of the myostatin gene (He *et al.*, 2011). Thus, a key example of the role introns can specifically play in gene expression.

Objectives of this study

Although there is evidence describing exonic variation in *DGAT1* in both cattle and sheep, there has been little study of intron regions. Variation in introns can affect animal traits, through their effect on gene expression and this suggests that variation in *DGAT1* introns is worthy of further study. Accordingly in this study, intron 1 of ovine *DGAT1* will be investigated using PCR-SSCP and if sequence variation is found, then the association of that variation with carcass traits will also be investigated.

Chapter 2

Materials and Methods

2.1 Sheep Investigated and DNA Purification

New Zealand (NZ) Romney rams were investigated to determine whether variation exists in the ovine DGAT1 gene. For the association analysis, 338 lambs that were the progeny of eight rams that were produced over two seasons were investigated. Phenotypic data for growth and carcass traits was available for these lambs and they were obtained from the NZ Romney Progeny Test.

All lambs were ear-tagged with a unique identification number within 12 h of birth, and birth date, birth weight, tailing weight, birth rank (i.e. whether they were a single, twin or triplet), rearing rank, gender and dam number were recorded. All of the ewes and lambs were brought together at tailing (lambs were aged between 2–6 weeks old) and remained together until weaning. At weaning each lamb was weighed and their pre-weaning growth rate was calculated as the difference between weaning weight and birth weight divided by age in days (expressed as grams/day).

At weaning, lambs were separated according to gender. Only male lambs were used in carcass trait analyses, as female lambs were retained as flock replacements. At weaning, those lambs weighing 36 kg and over were drafted and sent to the Alliance Group meat processing plant at Pukeuri, NZ. Two subsequent drafts occurred at four weekly intervals. At the second draft, male lambs were drafted at weights over 30 kg and draft three consisted of all the remaining male lambs regardless of weight. Draft age and weight were recorded for each male lamb.

Hot carcass weights (H-W) were measured directly on the processing chain. H-W is the weight in kilograms of the carcass components minus the pelt, head and gut. Video imaging analysis (VIASCAN^{TM} Sastek), developed by Meat and Livestock Australia and described in Hopkins *et al.* (2004), was used to estimate the following carcass traits: lean meat yield (expressed as a percentage of H-W) in the leg (leg yield), loin (loin yield) and shoulder (shoulder yield) expressed as a percentage of H-W), total yield (the sum of the leg, loin and shoulder yields for any given carcass), the proportion leg yield, the proportion loin yield and the proportion shoulder yield. The proportion yield of leg, loin or shoulder is the yield of the specific area divided by the total yield expressed as a percentage.

Samples of blood from these sheep were collected directly onto FTA cards (Whatman BioScience, Middlesex, UK) and DNA for analysis was purified from 1.2 mm punches from the cards, using a procedure described by Zhou *et al.* (2006).

2.2 DNA Purification

Genomic DNA was purified from sheep studied using a two-step method described by Zhou *et al.*, 2006. In this method, a 1.2-mm blood disc was punched from each blood spot and transferred to a 200 µL PCR tube. Solution of 200 µL of 20 mM NaOH was added and incubated for 30 min at room temperature. The solution was then discarded and the disc was equilibrated in 200 µL of 1 x TE buffer (10 mM Tris-HCl, 0.1 mM EDTA, pH 8.0). After removal of the TE buffer, the disk was air dried, prior to its use in PCR.

2.3 PCR Primers and PCR Amplification

PCR primers (Intron 1 up-5'-CTCAACTTCTAGACGCCC TC-3' and dn-5'-CATCAGTCCTTCAGCTAAGC-3) were designed based on ovine *DGAT1* sequences, to amplify an intron 1 fragment of *DGAT1*. The primers were synthesized by Integrated DNA Technologies (Coralville, IA, USA).

PCR amplification was undertaken using the purified genomic DNA on one punch of the FTA paper, 0.25 µM of each primer, 150 µM of each dNTP (Bioline, London, UK), 2.5 mM of Mg^{2+}, 0.5 U of *Taq* DNA polymerase (Qiagen, Hilden, Germany) and 1× reaction buffer supplied in a 15-µL reaction. The thermal profile for amplification consisted of 2 min at 94°C, followed by 35 cycles of 30 s at 94°C, 30 s at 62°C and 30 s at 72°C, with a final extension of 5 min at 72°C. This was done in S1000 thermal cyclers (Bio-Rad, Hercules, CA, USA).

Amplicons were visualized by electrophoresis in 1% agarose (Quantum Scientific, Queensland, Australia) gels, using 1 x TBE buffer (89 mM Tris, 89 mM boric acid, 2 mM Na2EDTA) containing 200 ng/mL of ethidium bromide.

2.3.1 Polymorphism screening and sequencing of allelic variants

PCR amplicons were subject to SSCP analysis. A 0.7-µL aliquot of each amplicon was mixed with 7 µL of loading dye (98% formamide, 10 mM EDTA, 0.025% bromophenol blue, 0.025% xylene-cyanol) and after denaturation at 95°C for 5 min, the samples were cooled rapidly on wet ice and loaded on 16 cm × 18 cm, 14% acrylamide: bisacrylamide (37.5:1) (Bio-Rad) gels. Electrophoresis was performed using Protean II xi cells (Bio-Rad), at 200 V for 20 h at 25-30°C in 0.5 × TBE buffer. The gels were silver-stained by the method of Byun *et al.* (2009).

2.4 Sequencing

Amplicons representative of different PCR-SSCP patterns from sheep that were homozygous for the each region of the ovine ADRB3 were directly sequenced at the Lincoln University DNA Sequencing Facility. For rare variants, only found in heterozygous sheep, a band corresponding to the rare variant

was excised as a gel slice from the first PCR-SSCP gel. This gel slice was macerated, then used as a template for re-amplification with the original primers to produce a PCR-SSCP gel pattern equivalent to a sheep homozygous for that rare variant. This second amplicon was then directly sequenced. Sequence alignments, translations and comparisons were carried out using DNAMAN (version 5.2.10, Lynnon Biosoft, Vaudreuil, Canada). The BLAST algorithm was used to search the NCBI GenBank databases (http://blast.ncbi.nlm.nih.gov/) for homologous sequences

2.5 Statistical Analysis

General Linear Mixed-effects Models (GLMMs) were used to explore the effect of the presence of a particular intron variant in a sheep's genotype on body composition traits. For each of the data sets (2015, Glenleith 2014 and Doughboy 2014), GLMMs were used to assess the effects of the presence/absence of the A and B alleles. The 2015 data set used the model to depict effects on carcass traits such as; V-Gr, V-HW, weaning weight, leg yield, loin yield, shoulder yield and total yield., Rearing rank and draft age were fitted as fixed factors, while sire was fitted as a random factor. In each of the 2014 data sets (Glenleith and Doughboy), V-GR, leg yield, loin yield and shoulder yield were assesed for the effect of the presence and absense of each allele. The model included draft age as it's random factor and rearing rank as a fixed factor. However, due to all of the samples holding the A allele in Glenleiths progeny, the presence and absence of A was not calculated. All models were held in normal distribution and fitted a bell shaped curve.

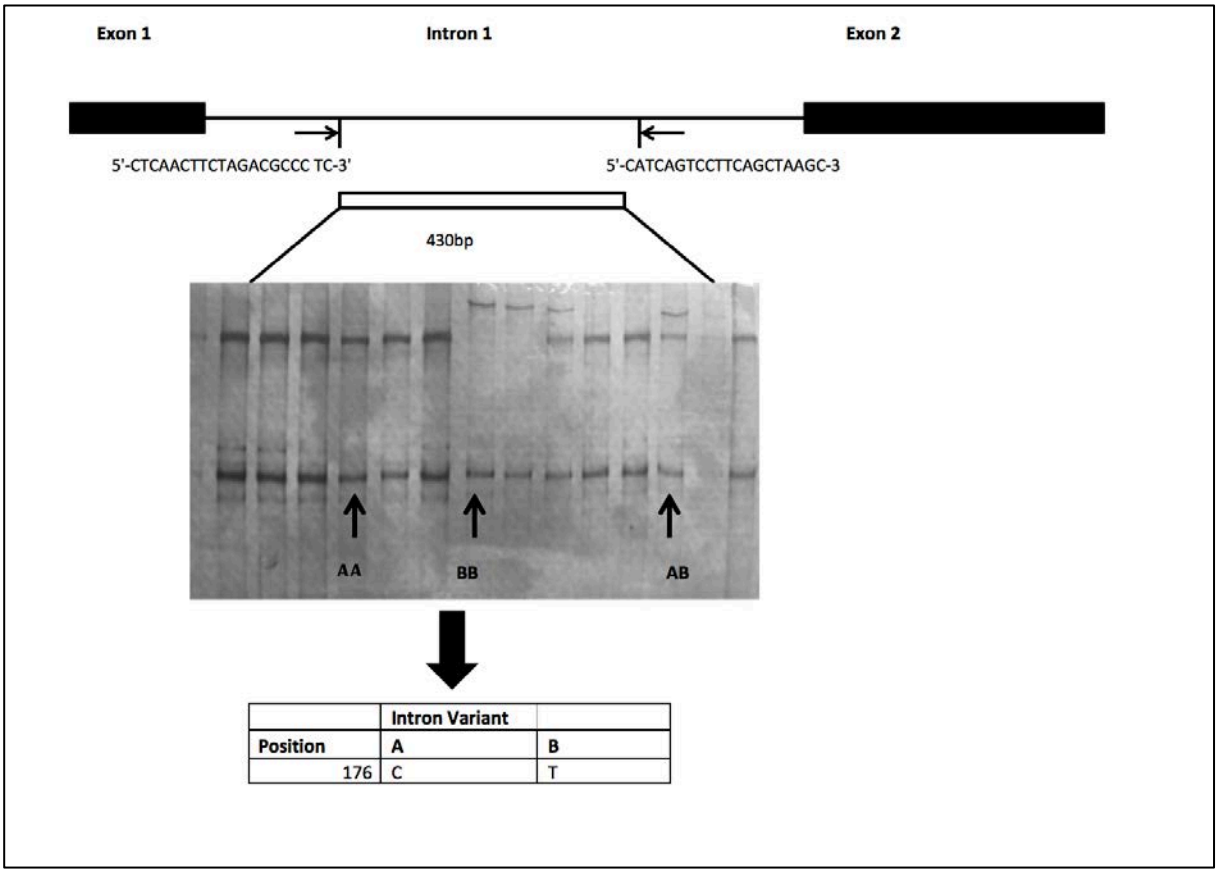
If a significant result was found ($P<0.05$), a multipile pair-wise comparison with sidak corrections was applied to ascertain the association between the genotype and body composition

Chapter 3

Results

Variation within intron 1 of ovine *DGAT1*

Three unique PCR-SSCP banding patterns were observed for the approximately 430 bp intron 1 amplimers of ovine *DGAT1* (Figure 3.1 and see Appendix A).



	Intron Variant	
Position	A	B
176	C	T

Figure 3. 3 The PCR-SSCP patterns and the single nucleotide variations detected in intron 1 of ovine *DGAT1*

Frequencies of the ovine *DGAT1* variants in the NZ Romney Sheep

PCR-SSCP analysis of the 338 Romney sheep revealed two variant sequences for intron 1. These were named variant **A** and *B*. The variants were found at the following frequencies: A (66%) and B (34%) in the lambs. The frequency of genotypes in the progeny of the sire "Doughboy", which was heterozygous, was AA (47%), AB (45%) and BB (8%). Sire "Glenleith", which was homozygous AA, was AA (75%), AB (25%) and BB (0%).

Correlations between lamb body composition traits in the NZ Romney sheep

Pearson correlation coefficients between the lamb carcass traits have been evaluated and are listed in Tables 2.2, 2.3 and 2.4.

Table 2.2 shows Pearson correlation coefficients for the 2015 data. VIAScan V-HW was correlated with V-GR (P<0.001) and shoulder yield (P<0.05). Leg yield was correlated with V-GR (P<0.05), loin yield (P<0.001), shoulder yield (P<0.001) and total yield (P<0.001). Loin yield was correlated with shoulder yield (P<0.001) and total yield (P<0.001). Lastly, shoulder yield was correlated with total yield (P<0.001).

Table 2.3 shows Pearson correlation coefficients for the 2014 data for the "Glenleith" ram progeny. V-GR was correlated with leg yield (P<0.05). Leg yield was correlated with loin yield (P<0.001) and total yield (P<0.001). Loin yield was correlated with shoulder yield (P<0.001) and total yield (P<0.001). Lastly, shoulder yield was correlated with total yield (P<0.001).

Table 2.4 shows Pearson correlation coefficients for the 2014 data the "Doughboy" ram progeny. V-GR was correlated with leg yield (P<0.001). Leg yield was correlated with loin yield (P<0.001), shoulder yield (P<0.05) and total yield (P<0.001). Loin yield was correlated with should yield (P<0.05) and total yield (P<0.001). Lastly, shoulder yield was correlated with total yield (P<0.001).

Table 2.3 Correlation coefficients for comparisons between lamb carcass traits, 2015

	V-HW	V-GR	LEG YLD	LOIN YL	SHLD YLD
V-GR	0.525 0.000*				
LEG YLD	-0.182 0.073*	-0.268 0.008*			
LOIN YLD	0.144 0.158*	0.095 0.352*	0.731 0.000*		
SHLD YLD	0.242 0.016*	0.078 0.446*	0.457 0.000*	0.667 0.000*	
TOTAL YIELD	0.038 0.710*	-0.079 0.438*	0.886 0.000 *	0.905 0.000*	0.789 0.000*

*P<0.05

Table 2.4 Correlation coefficients for comparisons between lamb carcass traits Glenleith progeny 2014

	V-GR	LEG YLD	LOIN YLD	SHLD YLD
LEG YLD	-0.300 0.038*			
LOIN YLD	-0.051 0.731*	0.769 0.000*		
SHLD YLD	-0.081 0.586*	0.235 0.108*	0.410 0.004*	
TOTAL YIELD	-0.205 0.161*	0.892 0.000*	0.907 0.000*	0.596 0.000*

*P<0.05

Table 2.5 Correlation coefficients for comparisons between lamb carcass traits, Doughboy progeny 2014

	V-GR	LEG YLD	LOIN YLD	SHLD YLD
LEG YLD	-0.475 0.001*			
LOIN YLD	-0.047 0.766*	0.701 0.000*		
SHLD YLD	-0.033 0.836*	0.375 0.013*	0.365 0.016*	
TOTAL YIELD	-0.291 0.058*	0.906 0.000*	0.828 0.000*	0.676 0.000*

*P<0.05

Association between presence/absense of ovine DGAT1 intron 1 variants and various carcass trait measures in the NZ Romney Sheep

As a result of the generalised linear model, the 2015 data set showed that the presence of B had a higher leg yield (P=0.040) associated. Adversely, the presence of A had no significant effects on the leg yield (P=0.316) (Table 2.5). In a sidak multiple pairwise comparison, highlighted that in the presence of B (n=50) the mean leg yield was; 22.8633kg, comparing to the absence of B (N=48) which showed a 709.1g difference at 22.1542kg (Table 2.8). No significant associations were made with the presence or absence of A and B and the V-GR, V-HW, weaning weight, loin yield, shoulder yield and total yield (Table 2.5).

2014 Glenleith data set also showed that the presence of B had a higher leg yield (P=0.027) (Table 2.6). In a sidak multiple pairwise comparison, the presence of B equated to a total mean of 21.7125kg, whereas the absence of B had a mean of 21.0419kg, a 670.6g difference (Table 2.9). The

presence and absence of A had no significant effect on leg yield. No other significant associations were made with the presence and absence of A and B for the V-GR, loin yield, shoulder yield and total yield.

The doughboy 2014 data set showed no significant correlations across any of the traits; V-GR, leg yield, loin yield, shoulder yield and total yield (Table 2.7). The presence of the B allele did have a minor effect on leg yield (P=0.073).

Table 2.6 Analysis of association between ovine *DGAT1* intron 1 variants and various lamb carcass traits in NZ Romney Sheep, 2015

Trait[2]	Variant being assessed	Presence	n	Absence	n	P-value[1]
		Mean [1] ± Std Error				
VGR	A	3.93±0.23	90	4.0 ± 0.66	9	0.896
	B	3.88± 0.28	51	4.0± 0.34	48	0.688
V-HW	A	15.96± 0.13	90	16.2± 0.35	9	0.523
	B	16.05± 0.15	51	15.9± 0.19	48	0.390
Weaning Weight (kg)	A	33.34± 0.37	88	34.6± 0.88	9	0.624
	B	33.01± 0.43	49	33.9± 0.55	48	0.201
Leg Yield	A	22.14± 0.17	90	22.4± 0.49	9	0.316
	B	22.41± 0.22	51	21.9± 0.24	48	**0.040**
Loin Yield	A	14.45± 0.10	90	14.6± 0.34	9	0.517
	B	14.41± 0.11	51	14.5± 0.16	48	0.681
Shoulder Yield	A	16.87± 0.12	90	17.7± 0.27	9	0.782
	B	16.98± 0.14	51	16.9± 0.19	48	0.655
Total Yield	A	53.46± 0.34	90	54.8± 0.99	9	0.522
	B	53.79± 0.38	51	53.3± 0.53	48	0.142

[1]P-value associated with the variant that is being assessed. Means and P values were derived from generalised linear mixed models with variant presence/absence and rearing rank and draft days as fixed factors and sires fitted as a random factor. (P< 0.050 in **bold**)

[2]V-GR: Fat depth; V-HW: carcass hot weight

Table 2.7 Analysis of association between ovine *DGAT1* intron 1 variants and various lamb carcass traits in NZ Romney Sheep, Glenleith progeny, 2014

Trait[2]	Variant being assessed	Presence	n	Absence	n	P-value[1]
		Mean ± Std Error				
VGR	A	6.6± 0.49	48	nil	0	-
	B	6.0± 0.97	12	6.8± 0.57	36	0.771
Leg Yield	A	21.3± 0.13	48	nil	0	-
	B	21.8± 0.30	12	21.1± 0.14	36	**0.027**
Loin Yield	A	14.5± 0.09	48	nil	0	-
	B	14.7±0.22	12	14.4± 0.10	36	0.12
Shoulder Yield	A	16.9± 0.08	48	nil	0	-
	B	16.9± 0.08	12	16.9± 0.10	36	0.997
Total Yield	A	52.6± 0.25	48	nil	0	-
	B	53.4± 0.55	12	52.4± 0.27	36	0.085

[1]P-value associated with the variant that is being assessed. Means and P values were derived from generalised linear mixed models with variant presence/absence and rearing rank and draft days as fixed factors and sires fitted as a random factor. (P< 0.050 in **bold**)

[2]V-GR: Fat depth; V-HW: carcass hot weight

Table 2.8 Analysis of association between ovine *DGAT1* intron 1 variants and various lamb carcass traits in NZ Romney Sheep, Doughboy progeny, 2014

Trait[2]	Variant being assessed	Presence	n	Absence	n	P-value[1]
		Mean ± Std Error				
VGR	A	6.5± 0.51	39	8.2± 1.0	4	0.268
	B	7.6± 0.66	23	5.6±0.61	20	0.183
Leg Yield	A	21.8± 0.18	39	21.4± 0.95	4	0.803
	B	21.4± 0.21	23	22.2± 0.29	20	0.073
Loin Yield	A	14.8± 0.10	39	14.8± 0.17	4	0.26
	B	14.8± 0.12	23	14.8± 0.17	20	0.544
Shoulder Yield	A	17.0± 0.12	39	16.4± 0.50	4	0.414
	B	16.8± 0.17	23	17.0± 0.16	20	0.337
Total Yield	A	53.6± 0.32	39	53.0± 1.90	4	0.933
	B	53.0± 0.38	23	54.1±0.54	20	0.133

[1]P-value associated with the variant that is being assessed. Means and P values were derived from generalised linear mixed models with variant presence/absence and rearing rank and draft days as fixed factors and sires fitted as a random factor. (P< 0.050 in **bold**)

[2]V-GR: Fat depth; V-HW: carcass hot weight

Table 2.9 Sidak Multiple Pairwise comparison of the presence and absence of the B allele on leg yield of the 2015 data set

Allele B	n	Mean	Grouping[1]	
Present	50	22.8633	A	
Absent	48	22.1542		B

[1]Means that do not share a letter are significantly different.

Table 2.10 Sidak Mulitple Pairwise comparison of the presence and absence of the B allele on leg yield of Glenleith's progeny data set, 2014

Allele B	n	Mean	Grouping[1]	
Present	12	21.7125	A	
Absent	36	21.0419		B

[1]Means that do not share a letter are significantly different.

Chapter 4

Discussion

Identified variation in ovine *DGAT1* intron 1

One single nucleotide substitution in intron 1 of ovine *DGAT1* was identified (Figure 3.3). The substitution occurred at position 171 of the segment amplified by PCR-SSCP which detected the C to T substitution. This substitution was also identified by Scata *et al*, 2009 who demonstrated the variation in the Altamurana sheep breed (Table 2.2). Association of the presence of this SNP was not further investigated by Scata et al, 2009. There has been no evidence to suggest this variation also occurs in cattle.

Association between variant ovine *DGAT1* and carcass traits

An association between the presence and absence of the B allele and leg yield was confirmed in the GLMM's and multiple pairwise comparison across the 2015 and Glenleith 2014 data sets. The result showed between 670-709g increase in the presence of B in the leg yield (Table 2.8, Table 2.9). Thus we may confer that this apparent growth in yield may either be put down to an increase in muscle content or an increase in intramuscular fat. Due to the nature of *DGAT1* and the role it plays in fat deposition and synthesis it would be likely to hyposthesise the latter. Previoulsy, Thaller *et al*, 2003 has described the impact of the K232A substitution on the intramuscular fat of two cattle breeds; Holstein and Charolais. Interestingly, the result of their study showed that the *DGAT1* lysine variant had the greatest impact on the intramuscular fat located in the m. semitendiosus area. Thus, making it more evident that the variation in our study has the greatest impact on the intramuscular fat content of the leg yield rather than the total muscle. Other evidence is portrayed by Roorda et al, 2002 who states that in rats overexpressing *DGAT1* increases intramyocellular lipid content and hence indicates the importance *DGAT1* may play in incorporating fat into the muscle. Another study by Xu *et al*, 2009 identified a silent allele, GCT (Ala)- GCC (Ala) mutation in exon 17 at the 1461st base pair which had a significant effect on the intramuscular fat content of the sheep.

Economic Importance

The contribution fat plays to meat palatability and quality traits must be balanced between the negative effect it may have on reducing the lean meat yield percent and the positive improvements it may make to tenderness, taste and juciness. Intramuscular fat can be difficult to trim and therefore may risk fatty meat being presented to consumers. Macfarlane *et al*, 2009, states that there should, however, be a minimum of 3% intramuscular fat for nutritional value, juciness, flavour and tenderness. Intramuscular fat may also have an effect on the visual appearance of the meat, as fat is

lighter in colour than muscle, and may contribute to a lighter meat. To select for lower intramsucular fat therefore would be a hindrance of all these given characteristics and hence diminish the eating quality. However, to select for a higher fat would also negatively correlate with consumer demands, therefore a balance of lean muscle and intramuscular fat must be met.

Affect of Introns on gene expression – Direct effect?

Lehir *et al,* 2003 have demonstrated that introns are able to regulate and affect the gene expression and mRNA splicing. It has also been widely pronounced that introns impact mRNA stability and optimise transcriptional efficieny of their host gene. This evidence, therefore, may explain variation in the phenotype, due the existance of these mutations in the non-translated regions of the gene that effect regulatory expression elements from transcription to translation (LeHir *et al*, 2003). The genetic architecture of complex traits and principally the number of loci affecting the trait and distirbution of their effects are still unknown. Hence, further studies into assessing the effects of the polymorphism in the non-coding region of the candidate gene and their association to biochemical pathways related to the traits would be imperative. It may confer, that the variation found in the intron is held in linkage disequilibrium with another causal mutation that directly effects the variation of quantitative traits. Further studies would be needed to confirm this. Recent evidence, has suggested this hypothesis in many intron regions in *DGAT1* associating with given traits. Yu *et al*, 2002 outlined that the regulation of *DGAT1* expression in adipocytes occurs mainly at transcriptional and post-transcriptional levels. And following this, evidence from Scata *et al*, 2009, identified a significant negative correlation between a variant held in intron 2 of *DGAT1* and milk fat content in three Italian sheep breeds. An *et al,* 2013, identified a variant in *DGAT1*, intron 14 that had a strong association and milk fat and milk yield in dairy goats.

Frequency of alleles

Due to the higher frequency of allele A (66%) we may confer that historically, this allele has been more highly selected for due to more desirable charactersitics. As we do not have exact measures for the amount of intramuscular fat in the leg in this trial, we may not completely determine which allele is more desirable. As we note the B allele is much rarer (34%) and has shown a large increase in leg yield we may assume this is due to an increase in intramuscular fat due to the previous literature (Thaller *et al,* 2003, Xu *et al*, 2009). However, as the A allele is more prominent in the population we may assume that this is because sheep containing the A allele have a more desired intramuscular fat level due to higher selection and the B allele may be overproducing intramuscular fat. Nevertheless, the study provides insight to what may contribute to increasing the intramuscular fat and could be important in breeds or populations that need to increase intramuscular fat in the leg

Incorporation to selective breeding

Given the ability to alter carcass traits as means of increasing the economic value has been proven to be efficient when enabling the product to suit different market demands. Not only must the producer focus on meeting the optimal carcass composition, they must also balance this with the importance held in meeting demands of desired eating attributes. With recent improvements on technological advances, genetically, we are able to more easily select for these traits which will persist given the right environmental conditions are met. With the knowledge gained in this study, we are then able to hypothesise therefore, that with the presence of the SNP in intron 1, we can utilise this in marker assisted breeding. We can confirm that in the presenece of the B allele, an increase in intramuscular fat may be gained in the leg, improving the marbling score and desired eating characteristics.

Further Studies

Many further studies can be based on this trial to further confirm the outcomes of the increased leg yield. It might be noteworthy to compare the differences in other sheep breeds, for example, a known fattier sheep breed may have a higher allelic frequency for the B allele which could explain some differences. A further direct study on the exact intramuscular fat content would be highly advantageous to specifically associate this variant with the intramuscular fat content and hence ruling out the possibility of it's associaiton with muscle content. Investigations looking into the actual effect this variant is causing on directly associating with intramuscular fat on a biochemical scale, whether it is direct or indirect in association with another causal mutation would also be beneficial.

Chapter 5

Conclusion

Overall conclusions from this study have shown an association between a variation in intron one of *DGAT1*, and increased leg yield. Previous studies have led to the assumption that the increase is a result of an higher deposition of intramuscular fat which consequently increased the overall yield. The SNP may be utilised in marker assisted breeding for selection to enable to the producer to increase marbling in the leg cuts if desired. Further studies, would be needed to directly relate this polymorphism to intramuscular fat and associated biochemical pathways. Nevertheless, the trial provides for an advancement and baseline for breeders looking to increase intramuscular fat in leg cuts of NZ Romney sheep.

Appendix A

Sequence of A and B variants of DGAT1 intron 1

A.1 Sequence

A -

CTCAACTTCTAGACGCCCTCCCTCTGCTTTCCTTAGTTGGGTTCTGAAGCTTTCCCAGGGTGATCCCACCACGCA
CAGTGTCCTCTACCAGGAAGGAGATACGGGGGTCCCTCCTGAGGGCTATGAGGGGTGCCTTGTGGGTTGATA
AAGCTCCCGGAGGAGGAGGGTGGACTGGCGGAGAATAGGGTCAGGGGCAGTGCGAGATTTCTCATCCCTTG
CAGACCCTCCAGAGAATGGTCTTCACAAAAGTCCCTCATCCGTCACCCGGCGATTGACTGGCCTAGGGTCCTGC
TTATTACCAGCACAAATACCTGCTCTAGGGTCAAAGTGGGTCCTGTAATGGGACCCTCACCCCTGGTTGAGGTG
CAGGGGAGGAGTTGCAAGCGTGCACACCCACAGGTGGGCACCCTGCTTAGCTGAAGGACTGATG

B –

CTCAACTTCTAGACGCCCTCCCTCTGCTTTCCTTAGTTGGGTTCTGAAGCTTTCCCAGGGTGATCCCACCACGCA
CAGTGTCCTCTACCAGGAAGGAGATACGGGGGTCCCTCCTGAGGGCTATGAGGGGTGCCTTGTGGGTTGATA
AAGCTCCCGGAGGAGGAGGGTGGACTGGTGGAGAATAGGGTCAGGGGCAGTGCGAGATTTCTCATCCCTTG
CAGACCCTCCAGAGAATGGTCTTCACAAAAGTCCCTCATCCGTCACCCGGCGATTGACTGGCCTAGGGTCCTGC
TTATTACCAGCACAAATACCTGCTCTAGGGTCAAAGTGGGTCCTGTAATGGGACCCTCACCCCTGGTTGAGGTG
CAGGGGAGGAGT TGCAAGCGTGCACACCCACAGGTGGGCACCCTGCTTAGCTGAAGGACTGATG

References

An XP, Hou JX, Zhao HB (2013) Polymorphism identification in goat DGAT1 and STAT5A genes and association with milk production traits. Czech J Anim Sci; 58(7): 321–327

Bourdon, V., Harvey, A., and Lonsdale, D.M. 2001. Introns and their positions affect the translational activity of mRNA in plant cells. EMBO Rep. 2: 394–398.

Breeds, I. S. (2011). Polymorphism of a mutation of DGAT1 gene in four Chinese indigenous sheep breeds. Asian Journal of Animal and Veterinary Advances, 6(5), 460-468.

Buhman, K. K., Chen, H. C., & Farese, R. V. (2001). The enzymes of neutral lipid synthesis.Journal of Biological Chemistry, 276(44), 40369-40372.

Buhman, K. K., Smith, S. J., Stone, S. J., Repa, J. J., Wong, J. S., Knapp, F. F., Burri, B.J., Hamilton, R. L., Abumrad, N. A & Farese, R. V. (2002). DGAT1 is not essential for intestinal triacylglycerol absorption or chylomicron synthesis. *Journal of Biological Chemistry*, *277*(28), 25474-25479.

Callis, J., Fromm, M., and Walbot, V. 1987. Introns increase gene expression in cultured maize cells. Genes & Dev. 1: 1183–1200

Cao, H. (2011). Structure-function analysis of diacylglycerol acyltransferase sequences from 70 organisms. *BMC research notes*, *4*(1), 1.

Cases S., Smith S.J & Zheng Y.W. (1998) Identification of a gene encoding an acyl CoA: diacylglycerol acyltransferase, a key enzyme in triacylglycerol synthesis. *Proceedings of the National Academy of Sciences of the United States of America* 95, 13018–23.

Cases, S., S. J. Stone, P. Zhou, E. Yen, B. Tow, K. D. Lardizabal, T. Voelker, R. V. Farese, Jr. (2001). Cloning of DGAT2, a second mammalian diacylglycerol acyltransferase, and related family members. *J. Biol. Chem.* 19:276(42):38870–38876.

Chang, C. C. Y., Lee, C.-Y. G., Chang, E. T., Cruz, J. C., Levesque, M. C. and Chang, T.-Y. (1998) Recombinant acyl-CoA : cholesterol acyltransferase-1 (ACAT-1) purified to essential homogeneity utilizes cholesterol in mixed micelles or in vesicles in a highly cooperative manner. J. Biol. Chem. 273, 35132–35141

Cheng D., Meegalla, R. L., Bokang, H. E., Cromley, D. A., Billheimer, J. T., & Young, P. R. (2001). Human acyl-CoA: diacylglycerol acyltransferase is a tetrameric protein.*Biochemical Journal*, *359*(3), 707-714.

Coppieters, W., Riquet, J., Arranz, J. J., Berzi, P., Cambisano, N, Grisart, B., Karim, L., Marcq, F (1998) A QTL with major effect on milk yield and composition maps to bovine Chromosome 14. *Mammalian Genome, 9:* 540-544

Grisart, B., W. Coppieters, F. Farnir, L. Karim, C. Ford, N. Cambisano, M. Mni, S. Reid, R. Spelman, M. Georges, and R. Snell. (2002). Positional candidate cloning of a QTL in dairy cattle:identi- fication of a missense mutation in the bovine DGAT1 gene with major effect on milk yield and composition. *Genome Research 12*: 222–231

Jones, D. T., Taylor, W. R & Thornton, J. M (1994) A Model Recognition Approach to the Prediction of all-helical Membrane Protein Structure and Topology, *Biochemistry*, 33: 3038-3049

Kühn, C., Thaller, G., Winter, A., Bininda-Emonds, O. R., Kaupe, B., Erhardt, G., Bennewitz, J., Schwerin, M & Fries, R. (2004). Evidence for multiple alleles at the DGAT1 locus better explains a quantitative trait locus with major effect on milk fat content in cattle. *Genetics*,*167*(4), 1873-1881.

LeHir H, Nott A, Moore M. (2003) How introns influence and enhance eukaryotic gene expression. Trends Biochem Sci; 28(4): 215–220

Lin, S., Cheng, D., Liu, M. S., Chen, J., & Chang, T. Y. (1999). Human acyl-CoA: cholesterol acyltransferase-1 in the endoplasmic reticulum contains seven transmembrane domains. *Journal of Biological Chemistry*, *274*(33), 23276-23285.

Liu, Y., & Guo, Z. (2009). Acyl-coenzyme A: cholesterol acyltransferase family. *Frontiers of Biology in China*, *4*(2), 129-136.

Lopes, J. L., Nobre, T. M., Cilli, E. M., Beltramini, L. M., Araujo, A. P., & Wallace, B. A. (2014). Deconstructing the DGAT1 enzyme: binding sites and substrate interactions. *Biochimica et Biophysica Acta (BBA)-Biomembranes*, *1838*(12), 3145-3152.

Macfarlane, J. M., Lewis, R. M., Emmans, G. C., Young, M. J & Simm, G (2009) Predicting tissue distribution and partitioning in terminal sire sheep using x-ray computed tomography, Journal of Animal Science, 87: 107-118

McFie, P. J., Stone, S. L., Banman, S. L., & Stone, S. J. (2010). Topological orientation of acyl-CoA: diacylglycerol acyltransferase-1 (DGAT1) and identification of a putative active site histidine and the

role of the n terminus in dimer/tetramer formation. *Journal of Biological Chemistry*,*285*(48), 37377-37387

Mohammed, S. A, Rahamtalla. S. A, Ahmed, S. S, Elhafiz, A, Dousa, B. M, Elamin, K. M & Ahmed, M. K. A (2015) DGAT1 Gene in Dairy Cattle, *Global Journal of Animal Scientific Research 3(1):*191-198.

Pearce, K (2016) Improving Lamb Lean Meat Yield, *CRC for Sheep Industry Innovation and Meat & Livestock Australia*

Rahmatella, S. A., ReiBmann, M., Mueller, U & Brockmann, G. A (2015) Identification of Genetic Variants Influencing Milk Production Traits in Sudanese Dairy Cattle. *Research Journal of Animal Sciences* ,9, (2-4):12-22

Riquet J, Coppieters W, Cambisano N, Arranz JJ, Berzi P et al. (1999) Fine mapping of quantitative trait loci by identity by descent in outbred populations: application to milk production in dairy cattle. *Proc Natl Acad Sci USA 96*, 9252–9257

Roorda B. D., Hesselink, M. K., Schaart, G., Moonen, E., Martinez, P., Losen, M., Baets, M. H., Mensink, R. P & Schrauwen, P (2005) DGAT1 overexpression in muscle by in vivo DNA electroporation increases intramyocellular lipid content. Journal of Lipid Research, 46(2): 230-236

Scata, M. C., Napolitano, F., Casu, S., Carta, A., De Matteis, G., Signorelli, F., ... & Moioli, B. (2009). Ovine acyl CoA: diacylglycerol acyltransferase 1–molecular characterization, polymorphisms and association with milk traits. *Animal genetics*, *40*(5), 737-742.

Siloto, R. M. P., Liu, Q., Weselake, R. J., He, X & McKeon, T (2010) Insights into the Structure and Function of Acyl-CoA: Diacylglycerol Acyltransferase, Biocatalysis and Biomolecular Engineering, 1:3-24

Smith, S. J., S. Case, D. R. Jensen, H. C. Chen, E. Sande, B. Tow, D. A. Sanan, J. Raber, R. H. Eckel, and R. V. Farese, Jr. (2000). Obesity resistance and multiple mechanisms of triglyceride synthesis in mice lacking Dgat. *Nature Genetics.* 25(1):87–90.

Souza FR, Mercadante ME, Fonseca LF, Ferreira LM, Regatieri IC, Ayres DR, Tonhati H, Silva SL, Razook AG, Albuquerque LG (2010) Assessment of DGAT1 and LEP gene polymorphisms in three Nelore (Bos indicus) lines selected for growth and their relationship with growth and carcass traits. J Anim Sci 88:435–441

Thaller, G., Kühn, C., Winter, A., Ewald, G., Bellmann, O., Wegner, J., ... & Fries, R. (2003). DGAT1, a new positional and functional candidate gene for intramuscular fat deposition in cattle.*Animal genetics*, *34*(5), 354-357.

Weller, J. I., Golik, M., Seroussi, E., Ezra, E., & Ron, M. (2003). Population-wide analysis of a QTL affecting milk-fat production in the Israeli Holstein population. *Journal of dairy science*, *86*(6), 2219-2227.

Xu, Q. L., Chen, Y. L., Ma, R. X., & Xue, P. (2009). Polymorphism of DGAT1 associated with intramuscular fat‐mediated tenderness in sheep. *Journal of the Science of Food and Agriculture*, *89*(2), 232-237.

Yen, C. L. E., Stone, S. J., Koliwad, S., Harris, C., & Farese, R. V. (2008). Thematic review series: glycerolipids. DGAT enzymes and triacylglycerol biosynthesis. *Journal of lipid research*, *49*(11), 2283-2301.

Yu YH, Zhang YY, & Oelkers P (2002) Posttranscriptional control of the expression and function of diacylglycerol acyltransferase-1 in mouse adipocytes. J Biol Chem; 277(52): 50876–50884.

Yu, C., Chen, J., Lin, S., Liu, J., Chang, C. C. Y. and Chang, T. Y. (1999) Human acyl-CoA : cholesterol acyltransferase-1 is a homotetrameric enzyme in intact cells and in vitro. J. Biol. Chem. 274, 36139–36145

Yuan, Z., Li, J., Li, J., Gao, X., Gao, H., & Xu, S. (2013). Effects of DGAT1 gene on meat and carcass fatness quality in Chinese commercial cattle. *Molecular biology reports*, *40*(2), 1947-1954.

Zhou, H., Hickford, J. G., and Fang, Q. (2006). A two-step procedure for extracting genomic DNA from dried blood spots on filter paper for polymerase chain reaction amplification. *Anal Biochem* 354, 159-61