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The Use of Quantitative Trait Loci (QTL) Affecting Muscling in Sheep for Breeding

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Declaration of Originality

I hereby declare that this thesis is my own composition and the research described in this thesis was originated by myself, except where otherwise stated.

Amer Yassin Masri

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Abstract

Breeding programmes that use elite sires with the best estimated breeding values for muscling traits have achieved significant improvement in lamb production in the UK. Further acceleration of the rate of genetic gain for the desirable production traits could be achieved using DNA marker-assisted selection (MAS) breeding strategies. The underlying causal genetic variants associated with improved muscling may be unknown and lying between a cluster of genes known as quantitative trait loci (QTL) or could be single nucleotide polymorphisms (SNP). LoinMAXTM, Texel muscling QTL (TM-QTL) and c.*1232G > A myostatin mutation were genetic variants that reported to be associated with improved muscling characteristics and hence subjected to further analysis in this project.

It is essential before incorporating segregating genetic variants in any breeding scheme to comprehensively evaluate their effects on carcass traits. *In-vivo* scanning (ultrasound scanning (US) and computed tomography scanning (CT)), and carcass video image analyses (VIA) were used in the current studies. Objective VIA-prediction weights of the carcass primal cuts could be the backbone of a value-based marketing system that is suggested to replace the current Meat and Livestock Commission (MLC) carcass grades for conformation scores (MLC-C) and fat class (MLC-F).

The effect of a single copy of LoinMAXTM QTL (LM-QTL) compared to non-carriers was evaluated in UK crossbred lambs out of Scottish Mule ewes. *M. longissimus lumborum* (MLL) width, depth and area, as measured by CT scanning, were significantly greater in lambs heterozygous for LM-QTL compared to non-carriers. VIA detected a significant effect of the LM-QTL on the predicted weight of saleable meat yield in the loin primal cut (+2.2%; $P < 0.05$).

The effects of the ovine c.*1232G > A myostatin mutation (MM), found on sheep chromosome 2, on carcass traits in heterozygous crossbred lambs sired by Texel and Poll Dorset rams were studied. Texel crossbred lambs carrying MM had increased loin depth and area. In both crossbred lambs, MM-carriers had significantly higher CT-estimated lean weight and proportion (2 to 4%) and muscle to bone ratios (by ~3%). Poll Dorset heterozygous crossbred animals had higher muscle to fat ratio (28%) and significantly lower fat-related measurements.

The c.*1232G > A (MM) mutation as well as TM-QTL effects were evaluated in a different genetic background of Texel x Welsh Mountain crossbreed lambs. Carrying two copies of MM was associated with a significant positive effect on 8 week weight, a negative effect on ultrasound fat depth, a substantial decrease in MLC-fat score, positive impact on VIA-estimated weight of the hind leg, chump and loin primal cuts, as well as the muscularity of the hind leg and loin regions with greater loin muscle width, depth and area. Two copies of MM altered lambs' morphological traits with significantly wider carcasses across the shoulders, breast and hind legs and greater areas of the back view of the carcass when measured by VIA. TM-QTL significantly increased US-muscle depth and TM-QTL carriers had significantly greater loin muscle width and area measurements.

Comparing TM-QTL genetic groups (homozygote allele carriers (TM/TM), heterozygote carriers of paternal and maternal origin of allele (TM/+ and +/TM, respectively) and homozygote non-carriers (+/+)) and TM-QTL mode of action were then studied. TM/TM carcasses were significantly heavier than non-carriers by 1.6 kg and scored higher conformation values when compared to heterozygote groups only. TM/+ lambs had significantly higher VIA-predicted weight and muscularity in the hind leg and loin, and higher loin dimensions relative to some other genotypic groups. The effect of TM-QTL on some carcass shape measurements was significant. TM-QTL mode of action results on the loin muscling traits supports the earlier reports of polar over dominance.

In the light of growing calls to replace the current subjective carcass payment system with the objective VIA system that values the carcass according to the superiority of its cuts, I investigated the ability of US and CT measurements to predict the VIA-estimated weights of the carcass primal cuts. Several prediction equations were examined but the best could be achieved when ultrasound measurement, CT linear measurements and live weight were fitted in the model.

Since CT scanning information of elite sires is now being used for genetic selection for carcass merit, genetic parameters and genetic relationships between CT scanning measurements and post mortem traits (VIA and MLC-FC) were estimated. However, results were not sufficiently accurate to be of practical use due to lack of data.

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List of Abbreviations

A	Estimate of the direct additive effect
A1	Back area of the carcass from shoulder to buttocks
A2	Back area of the whole carcass
A3	Side area from loin to hock
A4	Side area of the saddle and hind legs
A5	Side area of the whole carcass
AHDB	Agriculture and Horticulture Development Board
Adj-R ²	Adjusted coefficient of determination
Av. s.e.d.	Averaged standard error of difference
BA	Bone area
BLUP	Best linear unbiased prediction
BONE_Prop	Bone proportion
BONEwt	Bone weight of the whole carcass
Bp	Basepair (a unit used as a measure of length of a DNA segment)
BW	Body weight
c.*1232G > A	Mutation in myostatin gene
Carc_COMP	Carcass compactness as W5 / TL
CCWT	Cold carcass weight
Cm	Centimetre
cM	Centimorgan (the distance equal to 1% recombination between

	two loci)
CONF	Conformation
CT	Computer tomography
CT-Age	Age at computed tomography scanning
CT_L1	Length from the last cervical vertebra to the W4 measured by CT
CT_L2	Length from W4 to the bottom point of the hock measured by CT
CT_L3	Length from the last cervical vertebra to the start of the femur measured by CT
CT_L4	Length from the last cervical vertebra to the 1 st sacral vertebra measured by CT
CT_W1	Width between left shoulder muscle to right shoulder muscle, in line with the top of the chest measured by CT
CT_W2	Width of body at the 5 th thoracic vertebra (rib) excluding the skin folds on both sides measured by CT
CT_W3	Body width between the 3 rd and the 4 th lumbar vertebrae measured by CT
CT_W4	Body width of the junction point between the top of the muscle and the lower abdomen (the flank) measured by CT
CT_W5	Body width between the joint of the femur and tibia measured by CT
CT_W6	Body depth between the 1 st thoracic vertebra and the widest point of the chest measured by CT
CT_W7	Body depth between the 1 st sacral vertebra straight the lower abdomen measured by CT
CTLWT	Computed tomography live weight

CV	Coefficient of variation
D	Estimate of the dominance effect
DEFRA	Department for Environment, Food and Rural Affairs
2D	Two dimensions
2D MLL_Musc	2 dimensional muscularity of the m. longissimus lumborum
2D Leg_Musc	2 dimensional leg muscularity
3D	Three dimensions
EBLEX	English Beef and Lamb Executive
EBVs	Estimated breeding values
E,U,R,O,P	Conformation grades: excellent, good, average, poor, very poor
e.g.	For example
Exp 1 or 2	Experiment 1 or 2
FA	Fat area
FAO	Food and Agriculture Organisation
FAT	Fat class
FAT_Prop	Fat proportion
FATwt	Fat weight of the whole carcass
F1	First crossing generation
F2	Second crossing generation
g	Grams
GDF	Growth differentiation factor
Allele / +	Heterozygous genotype of the allele

Allele / Allele	Homozygous genotype of the allele
GEBV	Genomic estimated breeding value
GLM	General linear model
GR	Grade rule
h	Hour
h^2	Heritability
HLMV	Hind leg muscle volume
HLCMI	Hind leg muscle index
IBD	Identical-by-descent
IBERS	Aberystwyth University
IMF	Intramuscular fat
ISC	Ischium bone
Kb	Kilobase (unit of length for DNA)
Kg	Kilograms
KO%	Killing out percentage
L1	Length buttocks to shoulder [dorsal view] estimated by VIA
L2	Length hock to buttocks [dorsal view] estimated by VIA
L3	Length between the tibia and the end of the leg [side view] estimated by VIA
L4	Length from the hock to the front of the shoulder [side view] estimated by VIA
L5	Length from tibia to the front of shoulder estimated by VIA
LD	Musculus longissimus dorsi

Leg_COMP	Leg compactness as $[W5+W8]^{1/2} / L3$
Leg_W	Leg width
Leg_D	Leg depth
LoinMAX TM or LM-QTL	Loin muscling quantitative trait locus
LRMV	Loin region muscle volume
LRMI	Loin region muscularity index
LSMs	Least square means
LV5	5 th lumbar vertebrae
LWT	Live weight
MA	Muscle area
MAS	Marker-assisted selection
Max	Maximum
Mean s.e.d	Mean standard error of difference
mHz	Megahertz
Min	Minimum
MLC	Meat and Livestock Commission
MLC-CF	Meat and Livestock Commission's carcass classification scores for conformation and fatness
MLL	Musculus longissimus lumborum
MLL-A	Area of the m. longissimus lumborum estimated by CT
MLL-W	Width of the m. longissimus lumborum estimated by CT
MLL-D	Depth of the m. longissimus lumborum estimated by CT

MLL-VIAA	Area of the m. longissimus lumborum estimated by VIA
MLL-VIAD	Depth of the m. longissimus lumborum estimated by VIA
MLL-VIAW	Width of the m. longissimus lumborum estimated by VIA
mm	Millimetres
MD	Muscle density
MSTN	Myostatin gene
MUSC_Prop	Muscle proportion
MUSCwt	Muscle weight of the whole carcass
MyoMAX [®] or MM	c.*1232G > A mutation in myostatin gene
M : B	Ratio muscle weight / bone weight (whole carcass)
M : F	Ratio muscle weight / fat weight (whole carcass)
N	Number of animals
Ns	Not significant
OAR 2 / OAR18	Sheep chromosome 2 or 18
P	Allele frequency
P_	Primal carcass cut
PRESS	Prediction residual sum of squares
QMS	Quality Meat Scotland
QTL	Quantitative trait loci
R ²	Coefficient of determination
REML	Restricted maximum likelihood
REM	Rib eye muscle

r_g	Genetic correlation
r_p	Phenotypic correlation
RMSE	Root mean squared error
r.s.d	Residual standard deviation
SAC	Scottish Agricultural College
SCTS	Spiral computed tomography scanning
SD	Standard deviation
s.e.	Standard error
s.e.d	Standard error of difference
SL-Age	Slaughter age
SMY	Saleable meat yield
SNP	Single nucleotide polymorphism
SRS	Sire reference scheme
STAR	Sheep Tomogram Analysis Routines software
t1 or t2	Trait 1 or 2
TM-QTL	Texel muscling-quantitative trait loci
TOT_L	Total length
TP_	Trimmed primal carcass cut
TS	Terminal sire
TV8	8 th thoracic vertebrae
UFD	Ultrasonic fat depth
UK	United Kingdom

UMD	Ultrasonic muscle depth
US	Ultrasound scanning
USA	United States of America
USDA	United States Department of Agriculture
USLWT	Ultrasound live weight
UTR	Untranslated region
VBMS	Value based marketing system
VIA	Video image analysis
VSS2000	Lamb video image analysis system from E+V Technology GmbH
W1	Maximum shoulder width [dorsal view] estimated by VIA
W2	Minimum breast width [dorsal view] estimated by VIA
W3	Maximum breast width [dorsal view] estimated by VIA
W4	Minimum loins width [dorsal view] estimated by VIA
W5	Maximum rump width [dorsal view] estimated by VIA
W6	Maximum breast depth [side view] estimated by VIA
W7	Minimum loin depth [side view] estimated by VIA
W8	Maximum rump depth [side view] estimated by VIA
Wk	Week
8-WKWT	Weight at 8 weeks of age
WGS	Whole genomic selection
+/+	Wild genotype of the allele

% diff	Percentage difference
~	Approximately
%	Percentage
σ_{t_1,t_2}	Covariance between trait 1 and trait 2
$\sigma_{t_1}^2$	Variance of trait 1
£	Sterling pound

List of journal publications

Masri, A.Y., Lambe, N.R., Macfarlane, J.M., Brotherstone, S., Haresign, W., Rius-Vilarrasa, E., Bunger, L. (2010). The effects of a loin muscling quantitative trait locus (LoinMAXTM) on carcass and VIA-based traits in crossbred lambs. *Animal* 4, 407-416.

Masri, A.Y., Lambe, N.R., Macfarlane, J.M., Brotherstone, S., Haresign, W., Bunger, L. (2011a). Evaluation the effects of a single copy of a mutation in the myostatin gene (c.*1232 G>A) on carcass traits in crossbred lambs. *Meat Science* 87, 412-418.

Masri, A.Y., Macfarlane, J.M., Lambe, N.R., Haresign, W., Brotherstone, S., Bunger, L. (2011b). Evaluation the effects of the c.*1232 G>A and TM-QTL in Texel x Welsh Mountain lambs using ultrasound and video image analyses. *Small Ruminant Research* 90, 99-109.

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Masri, A.Y., Macfarlane, J.M., Lambe, N.R., Brotherstone, S., Haresign, W., Rius-Vilarrasa, E., Bunger, L. (2009). The effects of a loin muscling QTL (LoinMAXTM) in UK crossbred lambs. Proceedings of the QMS R&D conference, Perth 21st of January 2009.

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Chapter 1
General Introduction

1.1 General Background

The UK sheep industry is one of the major producers and exporters of lamb globally and to continue so, it is essential that its economic viability is improved. Therefore, providing carcasses that meet market requirements - currently only ca. 55% of UK lambs meet core target specifications (AHDB 2011) - is a principle component to achieve this improvement.

1.1.1 Sheep industry and lamb consumption

The sheep industry world-wide faces a fundamental problem in providing sufficient supply of sheep meat to the markets where sheep meat products make up less than 5% of overall meat products (FAO 2010). Lamb production in the UK is considered a very important part of UK agriculture with a contribution of over 10% of total livestock output ([http: & www.defra.gov.uk/statistics/foodfarm/](http://www.defra.gov.uk/statistics/foodfarm/) 2011). Figure 1. 1 shows the marked decrease in the total number of sheep recorded in the UK over the past decade ([http: & www.defra.gov.uk/statistics/foodfarm/](http://www.defra.gov.uk/statistics/foodfarm/) 2011). However, a slight increase of 0.4% in breeding flock and a 4.4% in total sheep numbers have been tabulated by DEFRA's report in 2012 https://www.gov.uk/government/uploads/system/uploads/attachment_data/file/183227/defra-stats-foodfarm-landuselivestock-farmstats-dec2012-130314.pdf.

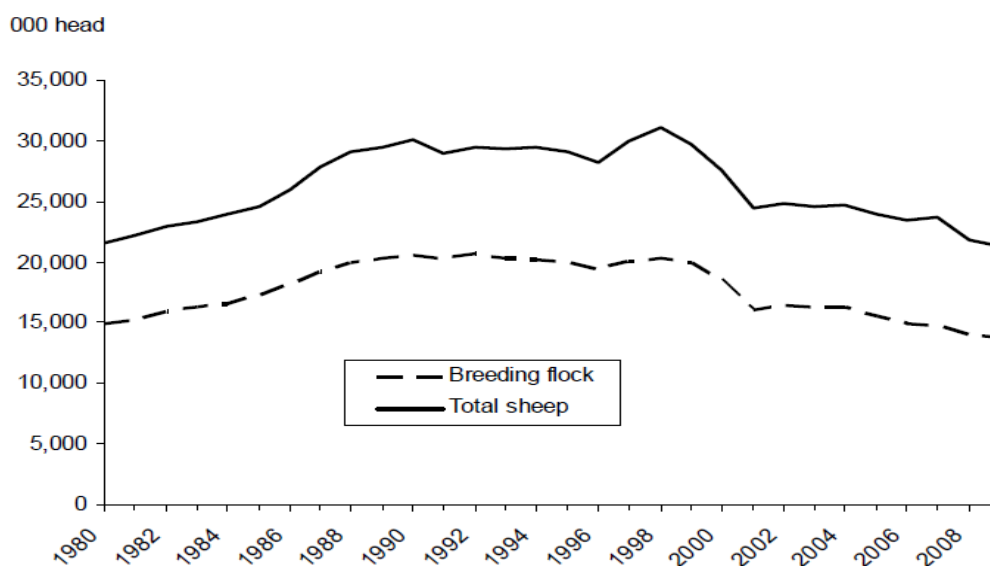


Figure 1. 1. Chart 1 UK sheep numbers from 1980 to 2009

([http: & www.defra.gov.uk/statistics/foodfarm/](http://www.defra.gov.uk/statistics/foodfarm/) 2011)

Total red meat consumption declined over the last few decades globally (FAO 2010). There are several influential factors that may contribute to the decreased demand for red meat consumption both globally and locally in the UK. Of the most important factors are the price and the consumer's satisfaction of the product portion size and quality where the consumer is always keen to spend money on a higher quality cuts (Russell et al. 2005; Troy & Kerry 2010). Another reason contributed in the decline of red meat consumption is the growing concern of the consumer over the reported linkage between red meat consumption with health risks (Pan 2012). It cannot be neglected that today's consumers are oriented and knowledgeable regarding the dietary aspects of red meat. Lamb meat consumption in the UK was not exempt from decreased demand and it has fallen by more than 10% in the last two years (AHDB 2011) (Figure 1. 2)

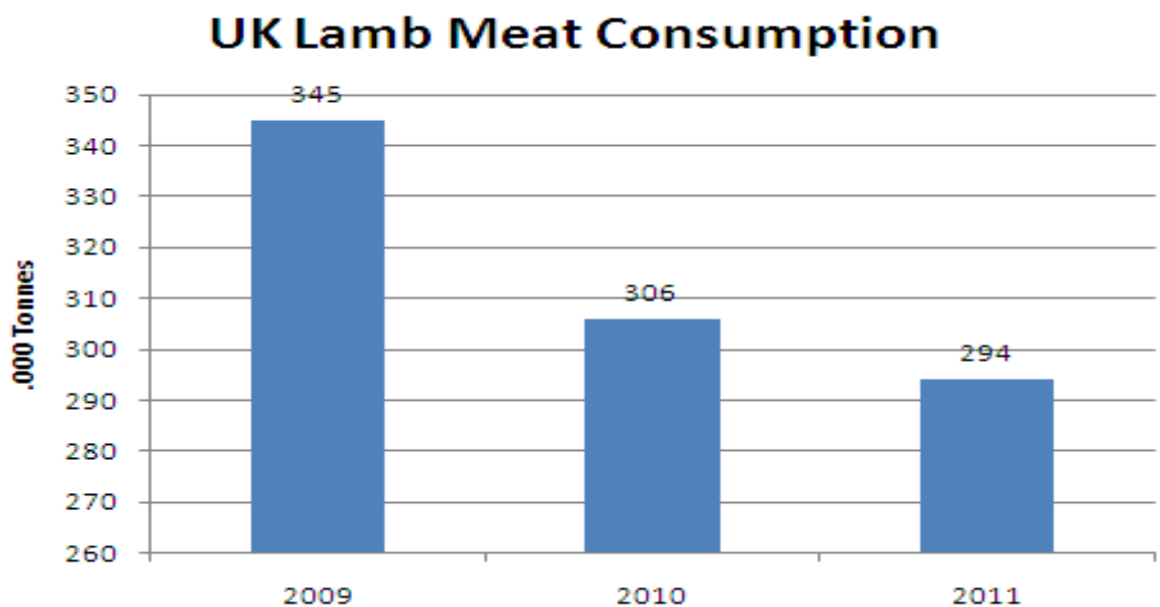


Figure 1. 2. UK lamb meat consumption (AHDB 2011)

In order to reverse the downward trend in lamb consumption both on international and local levels, the needs of the modern consumer have to be better addressed. As outlined by Ward et al. (1995), consumers require meat with more lean, optimal fat level (required to maintain juiciness and flavour), consistent quality, portions that are considered good value for money with minimal wastage and high level of choice/flexibility in available cuts.

If people are avoiding red meat for the previously mentioned reasons then a complex communication network and knowledge transfer between the consumer, researcher, producer and industry should be established. The success of this network work would probably drive the sheep sector to greater efficiency and to reverse the current trend in meat consumption.

1.1.2 Conventional breeding systems

Traditionally, genetic selection programmes in sheep and other livestock species have been efficient in increasing genetic gains, where this strong selection has resulted in the accumulation of new mutations with favourable phenotypic effects (Anderson 2001). Use of pedigree and performance information and estimated breeding values (EBV) on selection candidates have been the main basis of recent genetic improvement programmes in pure breeds and this has been very successful for traits determined by additive effects (Simm 1998). The advancements of the molecular genetic markers researches provided new opportunities to enhance breeding programmes in livestock by allowing the use of DNA markers to assign genes or QTLs that control the traits of interest (Dekkers 2004). The utilisation of identified genes/QTLs could be through marker-assisted selection (MAS) which select breeding candidate on the basis of a combination of information obtained from genetic markers associated with the genetic variant and the traditional phenotypic information (Smith & Simpson 1986). The most developed invention suggested to further increase the rate of genetic improvement is the use of high-density singular nucleotide polymorphism (SNP) genotyping for whole-genome selection (WGS) (Meuwissen et al. 2001). Genotyping of an individual for tens of thousands of SNP across the genome along with the estimation of the effect of each SNP will be used to estimate genomic estimated breeding value (GEBV). SNP-chip genotyping and GEBV will have major impacts on the production system as it will decrease the management costs since minimum recording on phenotypes and pedigree will be required as well as shortening generation intervals (Goddard & Hayes 2007).

1.1.2.1 The UK breeding structure

The UK sheep industry is unique in that it is characterised by a stratified three-tier breeding structure (Dewar-Durie 2000). The plethora of available breeds has benefited lamb production in the UK, with the sheep industry predominantly

employing systematic crossbreeding. Crossbreeding systems generally aim to utilise heterosis, and are often designed to make best use of the different attributes of sire and dam lines. If these differences are complementary, overall efficiency can be improved (Smith 1964).

The UK stratified system of sheep production enables sheep farming in varied terrains, a range of climates and environments, and management systems to be ideally suited for traditional sheep breeding management. Stratification enables hill breeds to be kept in inhospitable environments, producing replacement hill ewe and ram lambs, lightweight store or finishing lambs and cast ewes. On upland country, regular aged or cast hill ewes are crossed with longwool rams to produce hybrid ewe lambs and store or finishing wether (castrated male) lambs. Prolific, hybrid ewes such as Greyfaces, Mules and Halfbreds are crossed with terminal sire rams to produce the majority of UK finished lambs (70%) destined for human consumption (Pollott & Stone 2006). Purebred terminal sire flock are kept on some low ground farms, providing finished lambs and replacement terminal sire rams. The main advantages of stratification are: that it exploits the characteristics of different breeds, so that Greyface or Mule ewes combine longwool traits of growth rate, litter size and wool production with hill traits of hardiness and manageable size; it enables the use of terminal sires on crossbred ewes, thus producing lamb carcasses tailored to the needs of the market; and it matches the attributes of the different breeds to different areas of land use.

The sustainability of improving lamb production traits like carcass traits can be achieved by utilising genetic improvement programmes which are cumulative and cost effective (Simm & Dingwall 1989). Breeding programmes to improve sheep growth and carcass quality have been a feature of the UK sheep industry for the last few decades (Macfarlane & Simm 2008).

A major handicap to effective genetic improvement and its benefits on industry has been identified to be the limited population size of the pedigree flocks, with ewe numbers in registered flocks in most breeds in “tens”, rather than hundreds or thousands (McGuirk 2002). To overcome this problem, major schemes to performance recording and establishment of groups of breeding “nucleus flocks of elite animals” were conducted. Sheep breeder groups were established and performed intensive recording and selection procedures to produce superior animals (usually

sires) to be returned to members' flocks for mating. One of the earliest founded UK groups is the CAMDA group breeding scheme for Welsh Mountain sheep (<http://www.flockbook.org/lang-en/welsh-breeding-groups/welsh-mountain/camda>).

1.1.2.2 Sire Reference Schemes (SRSs)

Another well known type of group breeding scheme in the UK is the Sire Reference Scheme (SRS). The main TS breeds used in the United Kingdom are Charollais, Suffolk and Texel, and their TS cross progeny and together they account for approximately 71% of lambs slaughtered in the UK (Pollott & Stone 2006).

The first SRS was set up in the UK by the Meat and Livestock Commission (MLC) where Suffolk and Charollais breeding groups were first to co-operate within breed in 1989; then a Texel breeding group formed a SRS in 1991 (Simm et al. 2001). SRS is based on genetically linking flocks of members that are not sharing the same environment through artificial insemination or natural mating using common sires. This provides a common genetic baseline in each flock to calculate prediction of genetic merit and make it comparable across flocks for selecting mating candidates "which have the best breeding values". This equitable comparison increases the effective population size that can be compared adequately for selection therefore increasing selection intensity (Lewis et al. 2002). The structure of SRS aimed, besides maximising genetic gain, to provide an acceptable inbreeding level as well. The most common design of the SRS is that 30 ewes per flock are mated to two or three reference sires from a group of six. More information on the practical operation can be found in Simm et al. (2001). Briefly, a panel of elite reference sire are selected for use across members' flocks. Two or three of these reference sires are then selected for AI or naturally mating on a proportion of the ewes in each members' flock. Flocks' performances on traits of interest are then recorded to enable genetic evaluations using cross-flock, multiple-trait animal model BLUP. Estimated breeding values (EBVs) are then used to select the next generation of potential reference sires and to select sires and female replacements for each member flock.

Genetic evaluation would not be efficient without a powerful recording system. In the UK this has been mainly provided by Signet's Sheepbreeder service (<http://www.signetfbc.co.uk/>). Signet helps to identify genetically superior animals through providing a comprehensive pedigree and on farm performance record

collection, to collate the data that goes into the genetic evaluation. The introduction of new tools to measure carcass traits, economic selection indices and SRS has increased the usage of performance recording across several schemes.

The lean growth index was designed in response to consumer demand for more lean meat with minimum amount of fat content. Therefore, the selection goal was set to increase carcass lean weight while reducing or keeping carcass fat weight constant at a fixed age point (Simm & Dingwall 1989). The selection criteria were body weight (BW) usually measured at ~ 20-21 weeks old, ultrasound fat depth (UFD), and ultrasound muscle depth (UMD), all measured at 150 days of age. Relative economic values of +3 and -1 were used for lean and fat weight, respectively. These relative economic values were chosen to achieve the desired gains in the traits in the breeding goal, rather than being based on market returns and costs of production. Thus, selection on this index was expected to lead to close to maximum gains in lean weight, whilst constraining correlated increase in fat weight at about 26% of that expected from selection on live weight alone (Simm & Dingwall 1989). It was derived in this manner because of the weak relationship between carcass price and fatness in Britain at the time of its development (Simm et al. 2002). An evaluation study on experimental Suffolk flock to assess the selection process based on the lean growth index showed that selection line ram lambs were 6 kg (+10%) heavier in live weight at US scan, 1.0 mm lower in UFD (-13%), 3.00 mm higher in UMD (+13%) and 18% higher in index score to compared to control line ram lambs after 9 years of index selection (Simm et al. 2002). Annual response to the index has ranged from approximately 6 to 14 index points (Simm et al. 2001). The impact of the sustainable breeding programmes on the industry level was £2.66 per kg of carcass lean per lamb born, and £1.76 per kg of carcass fat per lamb born.

1.2 Molecular-based genetic advances

The achieved genetic improvement in sheep performance in many traits for the past decades was due to the benefit of EBVs obtained by using best linear unbiased prediction (BLUP) methodology (Villanueva et al. 2002). Enormous advances in genomics have provided powerful techniques to unravel the molecular basis of variation associated with traits of economic interest, such as muscling and meat quality.

The development of molecular biology during the past three decades created new means for studying livestock genetics and animal breeding. Selection according to genotype information has become an important tool in the breeding of farm animals (Teneva 2009).

Molecular markers, capable of detecting genetic variation at the DNA sequence level, have removed the limitations of morphological, chromosome and protein markers, but also possess unique genetic properties that make them more useful than other genetic markers. Molecular markers are numerous and distributed ubiquitously throughout the genome. These markers follow a typical Mendelian inheritance, which usually expresses in a co-dominant fashion, and are often multiallelic giving mean heterozygosity of more than 70% (Teneva 2009). Molecular markers, revealing the polymorphisms at the DNA level are now key players in animal genetics. Molecular markers serve as a useful tool for animal identification and genetic distance estimation. Parentage testing using molecular markers yields much higher exclusion probability (> 90%) than the testing with blood groups (70–90%) or other biochemical markers (40–60%) (Teneva 2009). These polymorphisms referred to as molecular markers can be used to build up genetic maps and to evaluate differences between markers in the expression of particular traits that might indicate a direct effect of these differences in terms of genetic determination on the trait (Vignal et al. 2002). Extensive genetic maps were generated in the last few decades in a variety of animal species and the latest of the sheep was published in March 2011 (<http://www.livestockgenomics.csiro.au/sheep/>).

1.2.1 The Definition of QTL

The members of Complex Traits Consortium (2003) have agreed that “the quantitative trait locus is the region of a genome that harbours one or more genes affecting a quantitative trait”. There was intensive research work in livestock species to identify genetic variants that are associated with traits of economic interest, such as growth and fatness (Dekkers & Hospital 2002). The identified QTLs, genes and markers that are associated with favourable phenotypic effects provided the opportunity to accelerate genetic improvement programmes via MAS. This can be done, firstly, by improving estimates of breeding values for a given individuals by incorporating DNA-based and phenotypic information (Dekkers 2004; Dekkers et al. 2002; Dekkers & Hospital 2002). The main use of MAS was for within-breed

selection purposes, using markers for which there is within-breed variability (Dekkers 2004). The development of DNA-based genetic markers could have a revolutionary impact on farm animal breeding as it could enable breeders to observe and exploit genetic variation in the entire genome at the molecular level. Further advances in molecular based genetics are proposed methods of predicting whole genomic breeding values with high accuracy without the need of high number of phenotypic records. This is achievable through using high-density SNP chip to genotype individual animals (Dekkers 2012; Meuwissen & Goddard 2001).

1.2.2 Genes and QTLs associated with carcass attributes

Although most production traits are quantitative in nature, meaning determined by many genes each having a small effect, there are some genes/QTLs with a visible effect on the phenotype. To date, many genes and QTLs affecting growth, muscling, carcass composition, meat and carcass quality have been reported in farm animals, several in sheep (Boman et al. 2009; Broad et al. 2000; Clop et al. 2006; Cockett et al. 1994; Cockett et al. 2005; Kijas et al. 2007; Laville et al. 2004; Marcq et al. 2002; Nicoll et al. 1998; Walling et al. 2004). QTLs affecting muscle growth in sheep will be reviewed in the following paragraphs.

1.2.2.1 The Callipyge gene

Lambs with a major increase in the hindquarter muscling and manifested as muscle hypertrophy were originally discovered in USA flocks (Jackson et al. 1993) and this was identified as the effect of the Callipyge gene (Cockett et al. 1994). The phenotype is mainly pronounced in the pelvic limb, torso and thoracic limbs along with desirable higher dressing percentage, reduced fatness (in all depots), larger longissimus area and higher leg scores (Cockett et al. 1994; Koohmaraie et al. 1995). However there is a concern that the muscle undergoing this pattern of hypertrophy, particularly the *longissimus dorsi*, is tougher than those of non-Callipyge lambs when measured by shear force (Duckett et al. 2000; Freking et al. 1999; Taylor & Koohmaraie 1998). Histological research of myofibers in muscles of the Callipyge carrier lambs, at 8 weeks old, showed a large increase in both the proportion and the average diameters for the fast-twitch glycolytic muscle fibers (Carpenter et al. 1996; Carpenter & Cockett 2000). Genetic characterisation of the Callipyge locus has shown a unique mode of inheritance termed “polar overdominance” (Cockett et al.

1996; Freking et al. 1998), in which only heterozygous offspring inheriting the mutation from their sire express the phenotype while the three other genotypes are normal in appearance.

The mutation was found to lie within a 4.6-cM interval between DLK1-GTL2 (32kb upstream of the GTL2) imprinted gene cluster in chromosome 18 (OAR18) (Cockett et al. 2005; Smit et al. 2003). Intensive work in attempt to find the causative SNP underlying the Callipyge mutation succeeded in identifying an A-to-G transition that segregates with the Callipyge allele (Freking et al. 2002).

The potential function of Callipyge SNP was derived from research on the expression level of the DLK1-GTL2 cluster and demonstrated that these imprinted gene expressions are altering according to the genotype, age-dependant and muscle-specific (expression levels of these genes are increased in the longissimus from 8-week old) (Charlier et al. 2001). Thus, the polar overdominance at the Callipyge locus results from a *cis*-effect of the mutation on the expression as well as from the post-transcriptional *trans* interactions between the products of the imprinted gene cluster (Georges et al. 2003). Therefore, the exhibited hypertrophy phenotype in paternal-origin Callipyge heterozygous lambs is likely to be dependent on the unique over-expression profile of the protein coding effectors of the DLK1 and PEG11 genes (Cockett et al. 2005). Further details on the involvement of DLK1 and PEG11 and their protein encoding roles in muscle growth can be found in Cockett et al. (2005) and Vuocolo et al. (2007). There was no utilisation of the Callipyge gene in industry due to its association with the substantial reduction in meat quality (Duckett et al. 2000; Freking et al. 1999; Taylor & Koohmaraie 1998).

1.2.2.2 Carwell, REM, LM-QTL, LoinMAX™

In New Zealand, a progeny test programme revealed that that some progeny and descendants of two rams from the Carwell Poll Dorset flock had a large positive deviation for eye muscle area (Banks 1997). In the early stages of discovering this mutation, a molecular investigation to identify the markers associated with this phenotype detected variation at markers in a region of ovine chromosome 18 which could explain the variations in phenotypes seen. This locus is referred to as the Rib Eye Muscling (REM) locus, with the Carwell allele associated with the improved phenotype (Jopson et al. 2001). Undoubtedly, these findings raised the concerns of

scientists as this QTL mapped to the distal end of ovine chromosome 18 where its location overlaps with Callipyge (Freking et al. 2002). Previous research indicated that one of the 4 markers for the Callipyge gene, TGLA 122 on chromosome 18, was found to exist in Australian Poll Dorset sheep exhibiting increased muscling (Hopkins & Fogarty 1998). The negative side of existence of TGLA 122 was found in the correlated increase in shear force in the *M. longissimus dorsi* (LD) by 35% in male lambs that had high pre-slaughter growth rate compared to non-carriers (Hopkins & Fogarty 1998). However, the increased muscle mass was limited to the LD with no other muscle group or fatness measure affected as reported by Nicoll et al. (1998). The Carwell allele reported to be associated with 8% increase in LD weight, and cross-sectional area by 10% (Nicoll et al. 1998). Jopson et al. (2001) found that loins, frozen at 24 hours post-mortem, from carrier progeny were unacceptably tough, based on values of 27-30 Newtons shear force (Hopkins et al. 2006), although the toughness disappeared when loin cuts were aged for 6 weeks. The effect of Carwell on meat quality is not permanent and can be modified by the production and processing procedures. As carcasses, in abattoirs, are processed under an accelerated conditioning procedure then myofibrillar protein degradation will overcome the differences in shear force (Hopkins et al. 2011). However, if appropriate production and processing strategies are not in place, then there is a need to be careful in the breeding programme to avoid detrimental effects on meat quality.

Jopson et al. (2001) found no evidence suggesting that LM-QTL exhibits any non-Mendelian pattern of inheritance such as that reported for Callipyge (polar overdominance, (Cockett et al. 1996), and they reported that Carwell appears to act as a dominant gene. Contrary to this, some evidence has now been accumulated suggesting that Carwell might be maternally imprinted (Campbell & McLaren 2007). In another words, the effect is only present in progeny that have inherited the allele from their sire, although no supporting results have been published. However, the Landcorp breeding programme in New Zealand has been using a set of markers that flanks around the putative gene in order to track its inheritance pattern (Dodds 2007). According to the reported mode of inheritance, industry use of Carwell is likely to be within terminal sire breeds, producing homozygous carrier rams, all of whose progeny (bred for slaughter) would express the phenotypic effect (Dodds 2007).

AgResearch and Landcorp Farming Ltd in New Zealand undertook further studies to narrow the location of the Carwell QTL and to provide a 3-marker haplotype test, which has been commercially available over the last few years as LoinMAX™ test (<http://www.ovita.co.nz/home/>). LoinMAX™ is estimated to occur in Poll Dorset sheep in New Zealand with frequencies of 5-10% (<http://www.ovita.co.nz/home/>). A number of strategies, including further fine-mapping of genes in the region, are being pursued in an effort to identify the gene polymorphisms responsible for the Carwell phenotype and to determine whether Carwell, TM-QTL (see below) and Callipyge are allelic to each other.

1.2.2.3 Texel Muscling QTL (TM-QTL)

Texel sheep are well recognized for presenting high muscularity and for their ability to produce progeny with favourable carcass traits in relation to mature size; higher eye muscle area; more uniform muscularity across the carcass; and higher rate of increase in muscularity with growth, which made this breed become a popular terminal sire in the UK (Jones et al. 2002b; Wolf & Jones 1996).

Many research groups have attempted to identify the genetic causes underpinning this increased muscle growth. Walling et al. (2004) identified a QTL in purebred UK Texel sheep. This QTL, confirmed by Matika et al. (2011), was later known as the Texel Muscling QTL (TM-QTL). The TM-QTL was mapped on ovine chromosome 18 and estimated to be ~2 cM telomeric of marker CSSM18 (Walling et al. 2004), which is close to the Carwell QTL previously reported to be mapped in a position between 2 and 6 cM telomeric of marker CSSM18 (Nicoll et al. 1998), therefore it is possible that TM-QTL is allelic to the Carwell gene. Although TM-QTL was found at the distal end of chromosome 18 in which Callipyge locates there was no evidence found of the Callipyge mutation in a trial on the Texel sires used to identify TM-QTL (Bishop, personal communication).

TM-QTL is assumed not to be widespread within the industry population of the purebred UK Texel as it has only been found to segregate in a small portion of the sire families studied by Walling et al. (2004). TM-QTL allelic frequency is unknown in the UK purebred Texel sheep flocks and is likely to remain so until a simple and reliable genotyping test is developed. The current TM-QTL genotyping test which is based on five haplotype markers and results are complicated to interpret, resulting in

frequent inaccuracy in identifying TM-QTL status. The protocol used to assign TM-QTL genotypes relies on microsatellite markers, since the underlying causal mutation has not been identified. The TM-QTL genotyping process used in the relevant experiments were described in detail in a previous study (Macfarlane et al. 2009a). Briefly, numerous microsatellite panels (haplotypes), of five microsatellite markers (CSSM18, MCMA26, OARTMR1, OY5 and OY3), were used to span the distal end of the OAR18 region. The haplotypes associated with the favourable allele of TM-QTL, within sire families, were very helpful in identifying the lambs' genotypes.

The only study that has investigated the mode of inheritance of the TM-QTL reported that it does not follow the normal Mendelian pattern of inheritance and appears to be polar over-dominant similarly to the Callipyge gene, in other words only animals receiving a single copy of the QTL from the sire demonstrate the phenotypic effect (Macfarlane et al. 2010). A later detailed study on the TM-QTL mode of inheritance, using different statistical approaches on a large data set available from commercial Texel sheep confirmed the imprinting effect of the TM-QTL with paternal parent-of-origin (Matika et al. 2010).

The first study on TM-QTL investigated its effects in commercial purebred Texels and showed that animals carrying one copy of the QTL, compared to non-carriers had an increase of 1.2-2 mm (4-8%) in loin muscle depth as measured by ultrasound scanning at the 3rd lumbar vertebra and a change in their muscle shape (Walling et al. 2004). Macfarlane et al. (2009a) verified the reported association between TM-QTL and increased loin muscling when investigating the effect of the TM-QTL in Texel cross-bred lambs out of Mule ewes at a given live weight. There were significant increases of +6.7% and +4.5% in the M. longissimus lumborum (MLL) depth using the CT scanning over the 5th lumbar vertebra and ultrasound scan the 3rd lumbar vertebra, respectively, for animals carrying one copy of the TM-QTL. In the same study, MLL width, area and volume measured by CT scan was greater in single copy carriers of TM-QTL compared to non-carriers. One of the most interesting results of this study was that of a higher dissected weight of the MLL by +7.1% in TM-QTL carriers compared to non-carriers. Using the same experimental cross-bred lambs as in Macfarlane et al. (2009a). Rius-Vilarrasa et al. (2009c) examined the ability of Video Image Analysis (VIA) to detect the effect of TM-QTL. TM-QTL carriers had

significantly deeper MLL as predicted by VIA and heavier VIA-estimated weight of the dissected MLL compared to non-carriers. The effect of TM-QTL on the meat quality of the same cross-bred lambs was studied within the same project (Lambe et al. 2010a). Results showed that male carrier lambs had significantly less intramuscular fat and higher toughness, in the loin muscle, compared to non-carriers males (Lambe et al. 2010a). No significant genotype difference in toughness was found in females when measured by shear force. However, in a follow up study by Lambe et al. (2010b) it was illustrated that longer ageing of meat (9 days vs. 3-5 days) was sufficient to overcome the increase in shear force in longissimus muscles in the TM-QTL carriers. Lower levels of IMF could negatively affect meat quality of slaughter lambs inheriting this QTL, especially given that IMF levels in loin muscle from this crossbred are already lower than recommended to guarantee consumer acceptability (Savell & Cross 1988).

1.2.2.4 Myostatin

A particular form of muscle development characterised by a large increase in muscle mass due to a polymorphism in the chromosomal 2 segment, encompassing the myostatin encoding gene (*MSTN*), has been reported in several species like mice (McPherron et al. 1997), cattle (Grobet et al. 1997), dogs (Mosher et al. 2007), horse (Dall'Olio et al. 2010), goat (An et al. 2011), chicken (Gu et al. 2002) and humans (Schuelke et al. 2004).

Similarly, in sheep, initially a QTL with a major effects on muscularity located on chromosome 2 in the region of the myostatin encoding gene was identified (Laville et al. 2004; Marcq et al. 1998; 2002). A preliminary study showed that this QTL was associated with larger muscular development in Texel × Romanov inter-cross lambs and accounted for 6-22% of the phenotypic variance in the F2 generation in muscular development traits (Marcq et al. 2002). With a confidence interval of 10cM, no clues of dissimilarity in coding sequences between hyper-muscled Belgian Texel lambs and their Romanov control contemporaries were found at that stage (Marcq et al. 2002). A higher density map was developed in sheep using the myostatin gene location in cattle as a candidate gene (McPherron & Lee 1997) and fine-mapped the QTL to a chromosome interval encompassing the mutation to the Growth-Differentiation-Factor 8 (GDF8). Different studies in Scotland (Walling et al. 2004)

and New Zealand (Broad et al. 2000) have shown associations between markers surrounding the myostatin gene and ultrasound measurements of body composition, although the evidence for a QTL was not conclusive. Marcq et al. (2002) reported stronger evidence based on carcass rather than ultrasound traits and Johnson et al. (2005a) discovered an association between this QTL and carcass yield.

Further fine-mapping of the chromosomal 2 segment in sheep (OAR2), encompassing the myostatin encoding gene (*MSTN*), has been reported to harbour 23 Single Nucleotide Polymorphisms (SNPs) (Hickford et al. 2010). Several of these SNPs (e.g. c.*1232G > A mutation in Texel (Clop et al. 2006), c.960delG in Norwegian White Sheep (Boman et al. 2009), the allelic variants in a 473-bp section of the exon 1- intron 1 region (Zhou et al. 2008), and -956 (T > C) and -41 (C > A) transitions in Beltex and Beltex×Huyang (F1) (Gan et al. 2008) have been reported to be associated with enhanced muscle growth. Two biallelic SNPs, g+6723G-A (G > A transition) and g-2449C-G in the 3' untranslated region (UTR) were found to be the underlying major causative mutations (Clop et al. 2006; Johnson et al. 2009). G > A transition in the 3'UTR that created a target site for mir1 and mir206 in the myostatin allele of Texel sheep seemed to cause translational inhibition of the myostatin gene and contributed to the muscular hypertrophy of Texel sheep.

A more recent study pointed out that this SNP was initially designated incorrectly and the correct one is the g.6223G > A SNP which should be called c.*1232G > A under the recommended nomenclature system (<http://www.genomic.unimelb.edu.au/mdi/mutnomen/>) (Hickford et al. 2010).

Although there is no formal information published on the mechanism by which myostatin controls muscle fibre proliferation in sheep, it has been demonstrated in cattle that the negative role of myostatin inhibits both the terminal differentiation of myoblast and the proliferation of the myogenic cells (Rios et al. 2002; Thomas et al. 2000). Moreover, myostatin has been shown to impact on muscle metabolism and gene expression (Hocquette et al. 1998). In mice, mutations to the protein coding region of the gene have been shown to either turn down or turn off this regulatory role, resulting in an increased muscling phenotype which primarily is due to increased protein synthesis (Varga et al. 2003).

Within British commercial Texel sheep, it has been reported that the allelic frequency of this “G > A SNP” is nearly at fixation level (Clop et al. 2006; Hadjipavlou et al. 2008). Similar high frequencies have been observed in Australian Texel sheep (Kijas et al. 2007). However, the G > A SNP was found to be segregating at intermediate frequencies ($p = 0.3$) in two distinct sheep breeds, the UK Charollais and Australian White Suffolks (Kijas et al. 2007; Hadjipavlou et al. 2008). This SNP, in the UK commercial Texel and Charollais breeds, appears to be partially recessive (Clop et al. 2006; Hadjipavlou et al. 2008).

The c.*1232G >A SNP test is now being used commercially, and replaced the initial microsatellite-based test for the corresponding QTL. The commercial name is MyoMAX[®] ([http://www.catapultsystems.co.nz/products/20_MyoMAX[®].cfm](http://www.catapultsystems.co.nz/products/20_MyoMAX[®].cfm)). MyoMAX[®] has been reported to segregate in several sheep breeds or strains, e.g. Belgian Texel, New Zealand Texel, UK Texel, Australian Texel, Poll Dorset, Charollais (Broad et al. 2000; Hadjipavlou et al. 2008; Johnson et al. 2005a; Kijas et al. 2007; Marcq et al. 2002; McRae et al. 2005; Walling et al. 2004).

Crossbred lambs, heterozygous for a QTL in the myostatin region, from Texel x Romney- or Coopworth-based dams, were compared to non-carriers, in terms of carcass and meat quality traits, as part of a New Zealand trial (Johnson et al. 2005b). The phenotypic effects associated with inheriting a single copy of the favourable haplotype were a 3% increase in leg muscle and a 10% decrease in leg fat, whilst the mutation had no detectable negative associations with objectively-assessed meat quality traits e.g. colour or pH. Similarly, analysis of meat shear force in lambs resulting from an Australian Texel x Merino cross segregating for the SNP revealed no effect on shear force but a reduction in intramuscular fat percentage and some sensory traits when carriers compared to non-carriers (Kijas et al. 2007).

1.3 In-vivo and post-mortem technologies to assess lamb carcasses

As already stated, providing carcasses that better meet market requirements is the main target for the UK to continue as a major producer and exporter of lamb. Therefore, results from new objective (rather than subjective) technologies for assessing carcass quality should be incorporated in to genetic improvement programmes in order to evaluate and accelerate the rate of genetic gain. Furthermore, with the growing interest in transferring to a value based marketing system (VBMS),

farmers should be better rewarded for the increased value of their animals through use of genetic improvement either conventionally or by using genetic markers. However, such VBMS requires objective methods to evaluate carcass value.

1.3.1 In-vivo technologies

There are several technologies available to assess body composition and they vary with respect to accuracy, reliability and cost (Speakman 2001).

1.3.1.1 Ultrasound scan

Ultrasound scanning (US) is one technology that has been frequently used in sheep to predict muscle and fat content in live animals (Berg et al. 1996; Silva et al. 2006).

In the UK, collecting US records of the muscle (UMD) and fat depth (UFD) taken across the third lumbar vertebra has substantially contributed to genetic improvement programmes since 1980s. The variation in UMD and UFD was the key element for genetic evaluations and thus to set the selection goal of increasing carcass lean weight and reducing fat content in the UK sheep breeds (Simm & Dingwall 1989). Signet's Sheepbreeder service (<http://www.signetfbc.co.uk/>), which included the collection of US records, played an important role in indentifying genetically superior animals through providing a comprehensive pedigree and on farm performance record collection.

A large numbers of factors can influence accuracy, precision and repeatability of US measurements. Of these factors are the animal itself and the scanning site (Emenheiser et al. 2010; Theriault et al. 2009), the technician (McLaren et al. 1991), and the equipment (Silva et al. 2006). The reported lack of accuracy in predicting carcass composition has been considered as one of US' shortfalls (Speakman 2001). However, in the UK sheep industry, the uptake of genetic improvement programmes over the last decades, mainly based on USD and UFD, has been substantial (Amer et al. 2007).

1.3.1.2 Computed Tomography

Consumer demands for high meat quality created the need to incorporate carcass and meat quality traits, which are often difficult to measure, in to the modern breeding programmes.

Dissection of carcasses has always been the traditional reference method to assess farm animals' carcass composition (fat adipose and muscle). However, the need of non-invasive, reliable and accurate measurement methods has introduced the in-vivo body scanning known as Computed Tomography (CT). CT is a technique for measuring carcass traits in vivo and can assess the carcass composition in lambs with high accuracy (Young et al. 2001).

The concept of the CT scan depends on two key elements: (i) the variation in tissue density; (ii) the amount of X-ray radiation transmitted through the tissues. X-ray attenuation values of the selected part of the body are then saved in a CT computer. A complex matrix transforms the attenuation values to produce an image of the targeted object as shades of gray, which relate to their density (Wegener 1993).

This technology has not only been used to predict carcass composition and tissue weights, but also to estimate muscularity traits and tissue distribution (Jones et al. 2002b; Jopson et al. 2004; Navajas et al. 2006a; Young et al. 2001). Three cross-sectional scans are taken at three particular anatomical points (Figure 1. 3) to give images from which area measurements of the bone, fat and muscle can be calculated, which are used in breed-specific previously derived prediction equations to predict weights of the carcass tissues. A suitable software procedure was developed using mathematical algorithms for image analysis and utilised to obtain and calculates the areas of the different tissues in the cross-sectional images (Glasbey & Young 2002). This consists of two main operations: 1) segmentation to remove non-carcass portions of the images; and 2) measurement of tissue areas in the segmented images. Using software (STAR: Sheep Tomogram Analysis Routines) developed at Scottish Agricultural College (SAC) and BioSS (Mann et al. 2008), both operations can be performed automatically. The complexity of removing the internal organs and to identify tissue boundaries makes the segmentation challenging and time-consuming if done manually. Automatic procedures have recently been developed (Glasbey & Young, 2002; Navajas et al. 2006a). Very high prediction accuracies across different genotypes and ages have been obtained with the highest reported in meat breeds (R^2 of 0.99, 0.98 and 0.89 for fat, muscle and bone), respectively, (Young et al. 2001).

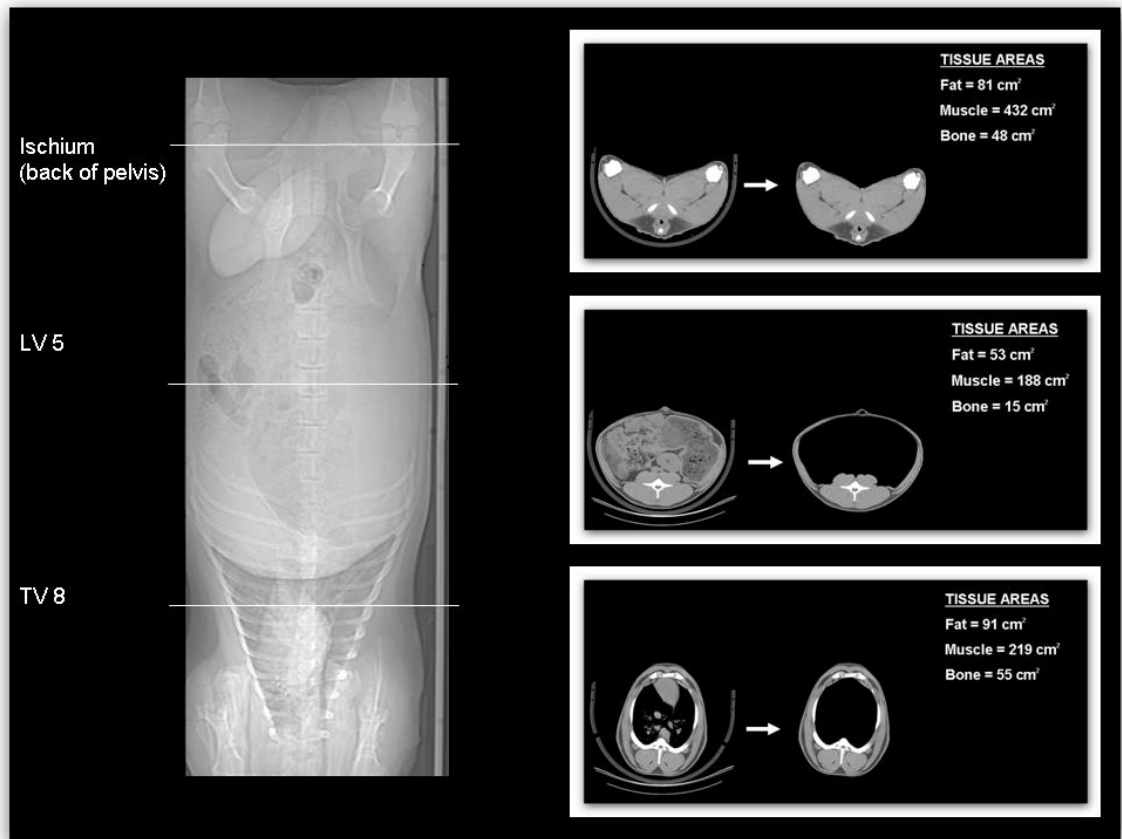


Figure 1. 3. CT scanning reference method showing a longitudinal 2D scan (topogram, left) and cross sectional images at 3 anatomical points and the corresponding tissue areas (right) (Bunger et al. 2011).

CT has been shown to hold great potential for accurate assessment, both for live animal and carcass fatness, muscularity and weight (Kvame & Vangen 2006; Lambe et al. 2003; Navajas et al. 2006a; Stanford et al. 1998). Lambe et al. (2007) acknowledged that CT scanning allows composition and muscle shape measurements to be taken in an accurate and repeatable way throughout the growth period on large numbers of animals, and can help in understanding the developmental patterns of different economically important sheep breeds. There were promising results of the ability of CT technology to evaluate meat quality through the reported correlation between CT-measured muscle density and IMF, juiciness, flavour and general palatability of the meat on Scottish Blackface lambs (Karamichou et al. 2006a).

A new generation of CT scanning technology known as spiral CT scanning (SCTS) that can take multiple slices at a single rotation plus cross-sectional images at

intervals of as little as 1 mm along the length of the body, or different body regions has now been introduced. This new CT scanning machine facilitated the creation of 3D muscularity indices of the body regions and particularly at the lumbar and hind leg regions (Navajas et al. 2007). These 3D muscularity indices may serve as alternative criteria to improve carcass conformation and lean mass at a consistent weight independently of fat deposition (Navajas et al. 2007). However, 3D-muscularity indices are not used yet commercially due to their high standard errors of the heritability estimates due to the limited size of the dataset used (Navajas et al. 2006b).

Experiments were conducted to establish the best way of incorporating CT scanning for carcass traits into terminal sire breeding programmes in the UK (Macfarlane et al. 2009b; Young et al. 2001). However, because CT scanning is an expensive tool of measurements compared to ultrasound to be incorporated in sheep breeding programmes then a two stage selection strategy in combination with ultrasound was suggested (Macfarlane & Simm 2008). The principles of this strategy are based on scanning all candidates by ultrasound and then selecting the best 15-20% for CT scanning. When data from terminal sire industry flocks was examined, results showed that response to selection is 7% (for CT-measured muscle weight), 10% (for CT-measured fat weight) and 20% (for CT-measured muscularity) higher when CT scanning is used together with ultrasound scanning compared to ultrasound alone (Moore et al. 2011).

CT scanning offers a great opportunity to detect whether identified genes/QTLs have an effect on carcass quality traits.

1.3.2 Post-mortem carcass assessment methodologies

The ultimate goal of sheep producer is to produce lambs of quality that fulfil consumer demands of carcass cuts with more lean and less fat. Therefore, a payment system is required to evaluate these factors and thus to give economic incentive to those producers who achieved the targeted carcass specifications.

1.3.2.1 Meat and livestock classification (MLC) scheme

The current commercial scheme to evaluate and value lamb carcasses in the UK is constructed upon the weight and the visual appraisal of the carcass conformation (MLC-C) and fat cover (MLC-F) (Meat and Livestock Commission's (MLC) scoring

system (Anderson 2003). This evaluation scheme is considered as an important tool for offering a fair payment to producers and as a reflector of the consumer's needs through the supply chain (Anderson 2003).

An experienced grader determines the carcass merits against a scale for conformation using the EUROP five-point scale (where “E” is for excellent and “P” is for poor conformation), and fatness, using a five-point scale from 1 (leaner) to 5 (fatter), with scores 3 and 4 sub-divided into “L” (leaner) and “H” (fatter) (Anderson 2003) (Figure 1. 4). Additional characteristics contribute in the classification judgement like the cold carcass weight, the age, the degree of maturity and dressing specifications used.

However, there are several shortfalls of the EUROP scheme: (i) no qualitative judgement such as the meat quality is attributed to the carcass value (Jones et al. 2003); (ii) shows variability between classifiers; and (iii) has been reported to be a poor predictor of percentage of lean meat yield in lamb carcass (Johansen et al. 2006).

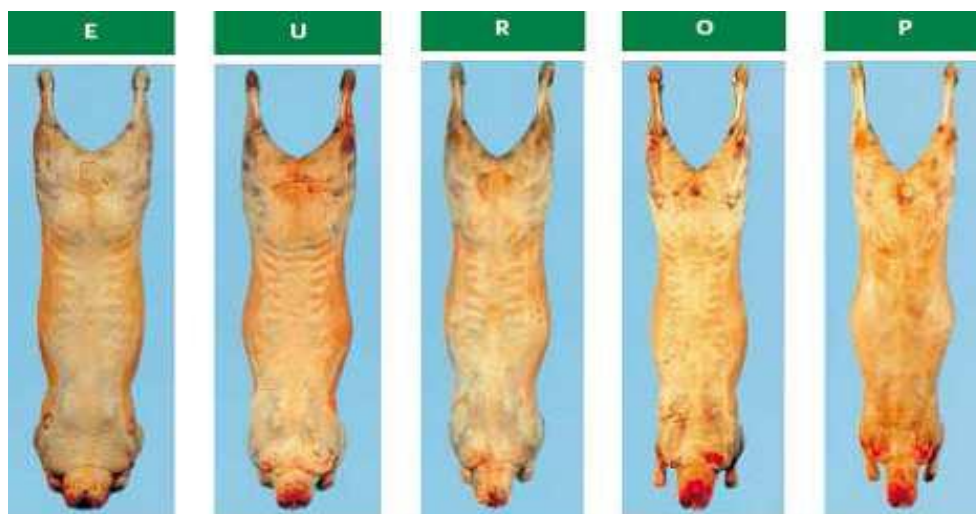


Figure 1. 4. EUROP carcass classification scheme for conformation according to MLC (Anderson 2003).

1.3.2.2 Video Image Analysis (VIA)

In order to overcome shortfalls of the EUROP based classification system (subjective classifications) and their reflections on the producer's income, a fair and objective payment system that is not biased has become an important priority world-wide. The

calls for a shift to an effective VBMS are growing, along with the demand for an objective instrumental grading system to be applied in abattoirs. The promising video image analysis (VIA) technology which is objective, automatic, non-destructive and runs at a high speed chain could form the payment benchmark in the future (Figure 1. 5). VIA was proven to predict weights of trimmed primal cuts (leg, chump, loin, breast, shoulder, and total primal meat yield) with a relatively high level of accuracy and consistency compared to the current (MLC) EUROP scoring system in sheep (Cunha et al. 2004; Hopkins et al. 2004; Stanford et al. 1998). The VIA based systems to evaluate carcasses were studied in several livestock species like beef, pig and lamb and it has been deployed on industrial abattoirs in several countries (Allen & Finnerty 2000; Horgan et al. 1995; McClure et al. 2003).

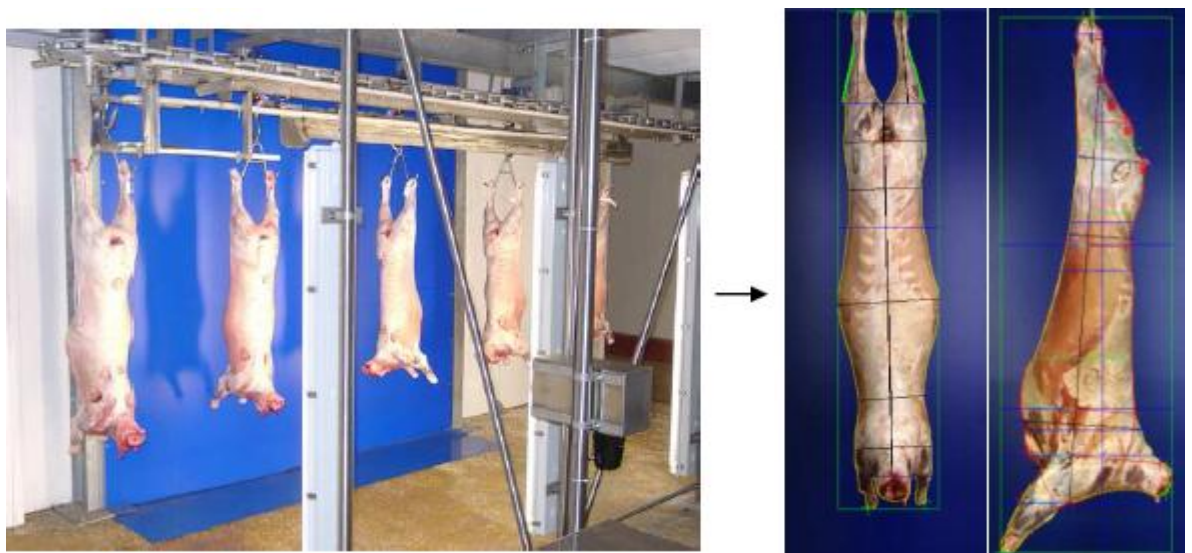


Figure1. 5. Video image analysis of lamb carcass on an abattoir chain line (VSS2000; E+V technology system).

Horgan et al. (1995) studied the potential of VIA systems for predicting commercially important characteristics of lamb carcasses compared to the current subjective inspection scores that are used in the UK. They demonstrated a great accuracy ($R^2= 0.95$, r.s.d. = 0.18 kg) in regard to predicted saleable meat yield using VIA shape variables for cold carcass alongside with carcass weight and sex. Stanford et al. (1998) highlighted that using VIA colour and shape variables for warm carcasses along with carcass weight has given a higher accuracy ($R^2= 0.71$, r.s.d. = 14g/kg) in predicting saleable meat yield compared to GR (tissue thickness over the

12th rib, 11 cm from the carcass midline) and subjective conformation scores ($R^2=0.52$, r.s.d. =18g/kg) used in the Canadian classification system.

A moderate accuracy and precision ($R^2= 0.52$, r.s.d. = 2.71%) of predicting lean meat yield in lamb carcasses have been reported by Hopkins et al. (2004) when using a similar patented technology (VIAscan[®], Meat and Livestock Australia) in comparing this approach in contrast to hot carcass weight (HCW) and tissue depth at grading rule (GR) site ($R^2= 0.41$). The American assessment of lamb carcass (USDA Yield Grades) was also tested with the outputs of the lamb vision system (LVS) (Cunha et al. 2004). A significant improvement in the ability to predict percentage yields of lamb carcasses over the traditional USDA Yield Grade was shown when variables from VIA measurements along with hot carcass weight were used explaining 67.6%, 61.9% and 73.8% of the observed variability in saleable meat, sub-primal and fat yields, respectively.

A developed payment system has been employed in New Zealand (Jopson et al, 2005) which is based on shifting towards marketing initiatives involving payment to farmers based on total meat yield in the primal cuts, directly assessed and valued on the carcass by VIA. Yet, in the UK, although such instrumental carcass grading systems have not been applied in the abattoir widely, fruitful results were published from a pioneer project conducted on a commercial population of lambs, led by MLC (Rius-Vilarrasa et al. 2009b). VIA-prediction equations, when adjusted for cold carcass weight (CCWT), of primal joints using MLC-CF scores showed high coefficients of determination (R^2) in the range of 0.82 to 0.99 and a good precision measure as root mean squared error (RMSE) (Rius-Vilarrasa et al. 2009b).

Genetic parameters of the VIA-predicted primal weights were estimated with low to moderate heritabilities (0.08 to 0.26) and a high repeatability (>0.9), suggesting that incorporating VIA information in breeding programmes would be valuable to improve carcass quality (Rius-Vilarrasa et al. 2009a). Through the estimated genetic correlations it was concluded that it is possible to increase primal meat yield without increasing overall carcass fatness (Rius-Vilarrasa et al. 2009a). Using the same dataset to estimate genetic parameters for different carcass traits, heritability estimates for objective carcass traits (VIA based linear and area measurements on the carcass) were moderate to high (0.20–0.53), suggesting that the use of these traits in

genetic improvement programmes could lead to a fast response to selection for improved carcass conformation and to a change of the primal joint tissue distribution within the carcass (Rius-Vilarrasa et al. 2010).

A substantial bank of information on carcass quality can be offered by VIA, through its automated data recording for genetic evaluation programmes. Together with electronic identification, VIA estimations would form an appropriate source of accurate and reliable information to improve EBVs and therefore rates of selection response.

The highly accurate CT estimates of carcass composition can be used as a benchmark method to overcome the difficulties of standardizing the industry practice on dressing specifications, while providing values of carcass quality based on proportions of lean, fat and bone. In this context, CT scanning measurements taken in the loin region were used to refine VIA prediction equation of all primal weights from different areas of the carcass with an average of 16% increase in prediction accuracy (Rius-Vilarrasa et al. 2009c).

1.4 PhD Objectives:

1.4.1 Background

This PhD project is an integrated part of a larger and more complex set of experiments to comprehensively evaluate direct and indirect effects (on other growth traits, welfare and reproductive traits and meat quality) of TM-QTL, LM-QTL and MM-QTL, which are all reported to affect muscle growth in lambs. Some of these aspects will be investigated in detail in the framework of this PhD project.

The magnitude of the direct and indirect effects of the identified QTLs requires investigation and validation on different genetic backgrounds to account for epistatic and pleiotropic effects. Before the recommendation to utilise or to not utilise these QTLs in the UK sheep industry, the size of their effect in the target purebred or crossbred population, typical of the UK's stratified sheep production system, has to be measured. To make optimal use of these QTLs in breeding systems, knowledge of their mode of inheritance is also required. However, for breeders to utilise the QTLs it is important to have an objective techniques to convince them that the investment

will return rewards for any increases in lean meat yield. Therefore, the ability of VIA technology, the most promising technology as a component of a future value based marketing system, to capture any increase in lean meat yield resulting from these QTLs has to be evaluated. Should there be a possibility to deploy VIA technology in the UK abattoirs, it would be of interest for the sheep farmers who get their sheep CT-scanned to have preliminary estimations of the VIA-predicted weights of the carcass primal cuts. This will help, if in future the payment system changed to VBMS, to provide an estimation of the carcass value. Furthermore, it was vital to attempt producing genetic parameter estimations of *in-vivo* traits and post mortem traits, as well as looking at the relationship between CT traits and both VIA and MLC traits.

1.4.2 The objectives

- 1- There is no study yet available on the effects of LM-QTL in commercial sheep under UK conditions. Therefore, it is essential to comprehensively evaluate the direct effects, and some indirect effects, of LM-QTL in crossbred lambs produced out of Mule ewes inseminated with semen of New Zealand Poll Dorset rams heterozygous for LM-QTL. Therefore, the objective of this chapter was to study the effects of LM-QTL on carcass composition using different objective *in-vivo* and post-mortem measurement instruments and discussed in chapter 2.
- 2- The high frequency of the myostatin gene reported in UK Texel sheep requires the validation of its effects on carcass composition in order to better plan the utilisation of this mutation in future breeding programmes. Hence, this chapter's objective was to comprehensively evaluate the direct effects of myostatin (MM-QTL) and some of the indirect effects in crossbred lambs produced out of Mule ewes inseminated with semen of Texel rams heterozygous for MM-QTL. The evaluation effects of MM-QTL used various objective *in-vivo* and post-mortem measurement techniques discussed and reported in chapter 3.
- 3- As the effects of a mutation on phenotypic performance can vary depending on the genetic background (Williams 2005), it was important to evaluate the direct effects of TM-QTL and MM-QTL in crossbred lambs out of Welsh Mountain ewes inseminated with semen from rams heterozygous for the TM-QTL and MM-QTL. The effects of TM-QTL and MM-QTL on carcass composition were

determined using several objective *in-vivo* and post-mortem measurement tools and discussed and reported in chapter 4.

- 4- It is of interest to investigate the relationship between post-mortem carcass traits obtained from VIA and *in-vivo* carcass traits like the US and CT. Also, to investigate the possibility of predicting the VIA traits through *in-vivo* US or CT-linear measurements separately or together. Thus, the available data on purebred Texel lambs enabled us to examine number of models to assess the relationship and prediction equations between *in-vivo* US and/or CT linear measurements and VIA-derived traits. These models were discussed and reported in chapter 5.
- 5- There is a need for powerful estimations of genetic correlations between CT and VIA traits and for their heritabilities in sheep. However, data for such estimations are sparse as sheep were rarely subjected to both evaluation methods. This project provided relevant initial data. Consequently, with the available performance and pedigree data on purebred Texel lambs heritabilities, phenotypic and genetic correlation estimations were produced of *in-vivo* CT scanning traits and carcass VIA-estimated traits and reported in chapter 6.

Chapter 2

The effects of a loin muscling quantitative trait locus (LoinMAX™) on carcass and VIA-based traits in crossbred lambs

Abstract

LoinMAXTM (LM) is a quantitative trait locus (QTL), which was found to segregate in Australian Poll Dorset sheep and maps to the distal end of sheep chromosome 18. LM-QTL was reported to increase *Musculus longissimus dorsi* area and weight by 11% and 8%, respectively. The aim of the present study was to comprehensively evaluate the direct effects of LM-QTL in a genetic background typical of the stratified structure of the UK sheep industry, before it can be recommended for use in the UK. Crossbred lambs, either non-carriers or carrying a single copy of LM-QTL, were produced out of Scottish Mule ewes (Bluefaced Leicester × Scottish Blackface) artificially inseminated with semen from 2 Poll Dorset rams that were heterozygous for LM-QTL. Unexpectedly, one of these rams was also heterozygous for a QTL that affects overall carcass muscling (MyoMAX[®]). This was accounted for by nesting MyoMAX[®] status (carrier or non-carrier) within sire in the statistical analysis. Lambs were weighed and scanned using X-ray computed tomography (CT) at an average age of 113 days. Ultrasound scan measurements, along with lamb weights, were taken at an average age of 140 days and lambs were then slaughtered. Carcasses were weighed and classified for fat cover and conformation scores, based on the Meat and Livestock Commission (MLC) carcass classification scheme, and then scanned using a video image analysis (VIA) system. *M. longissimus lumborum* (MLL) width, as measured by CT scanning, was greater ($P < 0.05$) in lambs heterozygous for LM-QTL compared to non-carriers. MLL in LM-QTL carrier lambs was also significantly deeper, as measured by both ultrasound (UMD) at the 3rd lumbar vertebrae (+3.7%; $P < 0.05$) and CT scanning at the 5th lumbar vertebrae (+3.4%; $P < 0.01$). Consequently, MLL area measured using CT scanning was significantly higher (+4.5%; $P < 0.01$) in lambs carrying a single copy of LM-QTL compared to non-carriers. Additional traits measured by CT, such as leg muscle dimensions, average muscle density and tissue proportions, were not significantly affected by LM-QTL. LM-QTL did not significantly affect total carcass lean or fat weights or MLC conformation and fat score classifications. Using previously-derived algorithms, VIA could detect a significant effect of the LM-QTL on the predicted weight of saleable meat yield in the loin primal cut (+2.2%; $P < 0.05$), but not in the other primal cuts, or the total carcass.

2.1 Introduction

The impressive advances that have been achieved recently by the use of molecular genetics and DNA technologies have given a valuable insight into the detection of new genes or quantitative trait loci (QTLs) associated with variation in quantitative traits of economic importance. This research field has been recently reviewed by Dodds et al. (2007). In principle this allows faster genetic progress through providing more accurate tools to select animals with high genetic merit carrying favourable genes/QTL (Campbell & McLaren 2007). This can be achieved by incorporating this information into breeding programmes through marker assisted selection schemes (MAS) (Dekkers 2004; Dekkers & Hospital 2002). To date, many genes and QTLs affecting muscle growth, carcass composition and meat quality have been reported in farm animals, several of which are found in sheep (Broad et al. 2000; Clop et al. 2006; Cockett et al. 1994; Hadjipavlou et al. 2008; Kijas et al. 2007; Laville et al. 2004; Marcq et al. 2002; Nicoll et al. et al. 1998; Walling et al. 2004). However, very few causative mutations underlying the variations associated with traits of economic importance in sheep have been successfully identified and validated (Clop et al. 2006; Cockett et al. 1994; Wilson et al. 2001).

A QTL, given different synonyms (Carwell, REM, Loin muscle QTL; here referred to as LM-QTL after the commercial haplotype test LoinMAXTM (http://www.catapultsystems.co.nz/products/30_loinmax.cfm)) on which the genotyping was based, which was originally identified in Australian Poll Dorset sheep, appeared to be responsible for an increase in eye muscle dimensions (Banks 1997). Trials in New Zealand showed that sheep carrying this QTL had an increase of approximately 11% and 8% in *M. longissimus dorsi* area and weight, respectively, and boosted the yield in this high-priced cut by 15% (McEwan et al. 2000; Nicoll et al. 1998).

This increased muscle mass was limited to the *M. longissimus dorsi*, with no other muscle group or fatness measurements affected (McEwan et al. 2000; McLaren et al. 2001; Nicoll et al. 1998). In the early stages of the discovery of this QTL, a molecular investigation detected variation at markers in a region of ovine chromosome 18 which seemed to explain the variation in phenotypes (Nicoll et al. 1998). This QTL was mapped to the distal end of ovine chromosome 18 where its

location overlaps with *Callipyge*, a hyper-muscling locus in sheep which also causes marked toughness in meat (Duckett et al. 2000; Freking et al. 2002; Koohmaraie et al. 1995). However, Jopson et al. (2001) found only a minor negative impact of the LM-QTL allele on tenderness, which could be removed by appropriate post-slaughter treatments.

No evidence has been found to suggest that LM-QTL exhibits any non-Mendelian pattern of inheritance, such as that reported for *Callipyge* (polar overdominance) (Cockett et al. 1996), and LM-QTL appears to act as a completely dominant gene (Jopson et al. 2001). In contrast, other evidence suggests that LM-QTL might be maternally imprinted (Campbell & McLaren 2007), meaning that the phenotypic effect is only present in progeny that receive the allele from their sire. However, no supporting results have been formally published on this to date.

Walling et al. (2004) reported a QTL in purebred UK Texel sheep with similar phenotypic effects on muscling and fatness to those of the LM-QTL (Nicoll et al. 1998). This QTL, later confirmed by Matika et al. (2006) and now known as TM-QTL, was mapped to the same region that encompasses the *Callipyge* gene (Freking et al. 2002) and the LM-QTL. The estimated position of the LM-QTL was mapped to 2-6cM telomeric to CSSM18 (Nicoll et al. 1998), while TM-QTL has been localised to 2-9cM telomeric to CSSM18 (Walling et al. 2004). These studies therefore suggest that TM-QTL could be either an alternative allele to LM-QTL or a closely linked locus. Further fine-mapping of genes in the region is being pursued with the aim of explicitly refining and identifying the physical position of the gene mutation responsible for the LM-QTL phenotype. McLaren et al. (2003) confirmed that the LM-QTL locus is discrete from the *Callipyge* locus, as was hypothesised by Jopson et al. (2001) and McEwan et al. (2000). The interval where the LM-QTL was localised excludes the entire cluster of imprinted genes implicated in *Callipyge*, as well as the SNP that has been proposed as the causative mutation for *Callipyge* (Freking et al. 2002). Moreover, the same study by McLaren et al. (2003) suggests that the critical interval for LM-QTL location contains 3 known genes. One of these is the *yy1* gene and one of its functions is to regulate muscle-specific gene expression, making this gene a plausible candidate for the LM-QTL effect.

Introducing the LM-QTL into the UK sheep industry may be beneficial as it has the potential to improve the yield of highly priced loin muscle in slaughter lambs. However, before being recommended for future commercial application, essential information is required about the magnitude of the direct effects of the LM-QTL in crossbred lambs, since lamb production in the UK is predominantly based on a three-way cross (Pollott & Stone 2006). Therefore, the LM-QTL effects need to be evaluated when acting in a genetic background that is relevant and typical for the stratified crossbreeding structure of the UK sheep industry (*e.g.* terminal sire-cross lambs out of Mule ewes).

The aim of this work is to evaluate the direct effects of LM-QTL on carcass traits in crossbred lambs using *in vivo* ultrasound scanning (US); *in vivo* computed tomography scanning (CT); MLC carcass classification scoring for conformation and fatness class (MLC-C&F) and video image analysis (VIA).

2.2 Material and methods

2.2.1 Experimental animals

All procedures involving animals were approved by the Scottish Agricultural College (SAC) Animal Ethics Committee and were performed under UK Home Office licence, following the regulations of the Animal (Scientific Procedures) Act 1986. Semen from 2 Poll Dorset rams heterozygous for LM-QTL was imported from New Zealand and used to inseminate 4- and 5-year old Scottish Mule (Blue-faced Leicester x Scottish Blackface) ewes ($n = 200$) that were non-carriers of LM-QTL. Of the 333 lambs born, 180 lambs were selected that were reared as twins (to minimise one source of variation due to litter size) and were recorded throughout their growth. Of these, only 167 lambs with complete records (82 male, 85 female; 106 born as twins, 61 born as triplets) were finally included in the current study. These lambs were all grazed on the same pasture with their dams to avoid any differences in nutrition between lambs of the different genotypes. Lambs were CT scanned at 16 weeks of age and then weighed and ultrasound scanned at 20 weeks of age, 7 days prior to slaughter. Lambs were weighed again immediately before transported to slaughter.

2.2.2 Genotype classes

Blood samples were collected from all the lambs studied and their dams and sent to *Catapult Genetics*, New Zealand, for genotyping. The genotypes showed, unexpectedly, that one of the rams used for the artificial insemination was also heterozygous for the MyoMAX[®] QTL (http://www.catapultsystems.co.nz/products/20_myomax.cfm), in this paper referred to as MM-QTL. The genotypes of the 167 experimental lambs, which all had complete data records, were therefore classified into four groups as shown in Table 2.1.

An initial power calculation, according to the method described by Rasch et al. (1978) and based on a minimum ‘difference of interest’ of 1mm in ultrasound muscle depth (UMD) and phenotypic standard deviation of 2.05, indicated that approximately 70 animals per genotype group (or 140 animals in total) were required to detect a significant ($P < 0.05$) difference in UMD between carrier and non-carrier lambs. However, the total number of lambs used was increased to 167 to allow for the unexpected MyoMAX[®] status of one of the sires and the unknown interactions between LM-QTL and MM-QTL. As MM-QTL segregation was totally unexpected, the numbers of lambs for this QTL were too low to permit statistical evaluation of MM-QTL, thus the MM-QTL status was nested within sire in the statistical analyses undertaken to investigate the effects of LM-QTL.

Table 2.1 Numbers of lambs in each genotype class for LoinMAXTM and MyoMAX[®] †

Genotype	LoinMAX TM		TOTAL
	+/+	LM/+	
MyoMAX [®]	+/+	77	128
	+/MM	17	39
	TOTAL	94	167

† +/+ = non-carrier, +/LM = carrier for one copy of LoinMAXTM; +/MM = carrier of one copy of MyoMAX[®]

2.2.3 Traits measured by X-ray computed tomography (CT)

All lambs were CT scanned at 16 weeks of age, at least 30 days before slaughter to ensure all lambs had the full withdrawal period required for the sedative used for CT scanning (average age = 113 days; minimum age = 110 days; maximum age = 116 days). Lamb live weight was measured at CT scanning and ranged from 22.9 kg to 37.4 kg. The method of reference CT scanning has been described in detail elsewhere (e.g. Macfarlane et al. 2006). In short, for each lamb, cross-sectional CT reference scans were taken at three positions, the end of the ischium bone (ISC), the fifth lumbar vertebra (LV5), and the eighth thoracic vertebra (TV8). Image analyses were performed on each resulting cross-sectional CT image using STAR software (Mann et al. 2003) to obtain for each reference scan carcass fat, muscle and bone areas, and average muscle density, which is likely to be an indicator of intramuscular fat content (Karamichou et al. 2006b; Navajas et al. 2007). Total weights of fat (FATwt), muscle (MUSCwt) and bone (BONEwt) in the carcass were predicted using reference scan tissue areas and live weight in relevant prediction equations developed for crossbred lambs (Macfarlane, personal communication). Very accurate predictions of carcass composition were achieved (Macfarlane et al. 2006; Young et al. 1987; 1996; 2001) when the area of tissues in cross-sectional scans and live weight were used in previous prediction equations derived in a similar way for purebred lambs of different breeds (R^2 values of 0.924, 0.978 and 0.830 for lean, fat and bone weights, respectively). Predicted total tissue weights were then used to estimate tissue proportions and ratios: muscle proportion (MUSC-Prop), fat proportion (FAT-Prop), bone proportion (BONE-Prop), the ratio of muscle to bone (M:B) and the ratio of muscle to fat (M:F). *M. longissimus lumborum* (MLL) width (mm), depth (mm) and area (mm²) were measured on both the left and the right muscles on the LV5 scan and the average of the measurements from the two sides were used in analyses. Using the approach of Jones et al. (2002a), the ratio of depth to width was multiplied by ten and averaged over both sides to provide a measure of two-dimensional (2D) loin muscularity. Linear measurements of muscle width (mm) and depth (mm) on each hind leg were taken on the ISC scan. Similarly to the loin, the ratio of depth to width measurements was multiplied by ten and the mean value over both legs was used to provide a measure of 2D shape of the hind leg, as described by Jones et al. (2002a).

2.2.4 Traits measured by ultrasound

Lambs were ultrasound scanned at 20 weeks of age, using a Dynamic Imaging Concept MLV (Caiyside Imaging Ltd) ultrasonic scanner with a 3.5 MHz transducer, to determine the fat depth (UFD) and muscle depth (UMD) over the 3rd lumbar vertebra. One muscle depth measurement was taken vertically at the deepest point of the MLL. Three fat depths were measured, one at the same lateral position as the muscle depth and the others at 1 and 2 cm lateral to the first position, and the average of these three fat depths used as the UFD measurements. Ultrasound data was not available for three lambs (2 non-carriers, 1 carrier) that were CT scanned, reducing the number of lambs for those traits to a total of 164 lambs.

2.2.5 Post mortem procedures

Lambs were slaughtered at the Welsh Country Foods abattoir in Anglesey, where they were electrically stunned, slaughtered, conventionally dressed, and then electrically stimulated. In the abattoir, carcasses were classified by an expert grader for fat cover (MLC-F) and conformation (MLC-C) scores based on the MLC classification scheme which is employed in the UK. MLC-F describes the carcass based on a 7 point scale for fat cover from 1 (very lean) to 5 (very fat), with classes 3 and 4 being subdivided into L = low and H = high (Anderson 2003). To analyse fat classification, the classes were transformed to a numerical scale based on estimated subcutaneous fat percentage, as described by Kempster et al (1986); 1 = 4, 2 = 8, 3L = 11, 3H = 13, 4L = 15, 4H = 17, 5 = 20. Conformation was visually assessed on a 5 point scale for carcass shape based on the EUROP classification scale, the common current system for specifications, pricing and monitoring used in the UK. The scale was coded from 1 to 5 for statistical analyses where E (excellent) = 5 and P (poor) = 1.

2.2.5.1 Traits measured by video image analysis (VIA)

VIA is an objective, automatic, non-invasive and reliable technology for carcass grading and predicting saleable meat yield (Allen 2005; Rius-Vilarrasa et al. 2009b) and was used here to investigate whether it can discriminate between groups of lambs with different LM-QTL genotypes. All carcasses were scanned using the VSS2000 video image analysis system (VSS2000, E+V Technology, Germany, <http://www.vision-for-you.com/start.htm>) in place at the Welsh Country Foods

abattoir. VIA data was not available from a total of six lambs that were CT scanned, due to accidental carcass damage in the slaughter line. The VIA system and procedures have been described in more detail elsewhere (Rius-Vilarrasa et al. 2009b). In short, VIA system measurements were comprised of dimensional and morphometric characteristics of the carcass at specific positions. The VSS2000 software divides the carcass image into different anatomical regions, and automatically calculates a variety of lengths, widths and areas which are combined in variables that describe carcass shape and size, and can be used to estimate the amounts of fat and lean tissue. In total, six predictor variables (function of lengths, areas and widths measured on the carcass) along with cold carcass weight (CCWT; kg), are used in prediction equations produced by E+V Technology, Germany, to provide objective predictions for weights of saleable meat yield in the carcass and weights of different primal cuts. Calibration and validation under British abattoir conditions were previously undertaken by Rius-Vilarrasa et al. (2009b). Two different prediction equations were used to estimate carcass cuts the 'standard' equations derived by E+V Technology, Germany, and the 'refined' equations later developed by E+V Technology, Germany, which were derived using calibration of the VIA system against CT measurements in the loin region (Rius-Vilarrasa et al. 2009c). Higher accuracy and precision of prediction of carcass primal and trimmed primal cuts achieved with these refined prediction equations, compared to the 'standard' VIA prediction equations, were reported by Rius-Vilarrasa et al. (2009c). The correlation between muscle weight derived by CT scanning and the sum of trimmed primal weights predicted using refined VIA prediction equations was higher in the present study ($r = 0.87$) than the corresponding correlation based on the standard prediction equations ($r = 0.85$). Although this difference was not statistically significant, the refined prediction equations were chosen for use in predicting the primal weights and trimmed primal weights in the present study. The resulting variables predicted using VIA included carcass saleable meat yield (SMY; predicted not as a sum of predicted weights of individual cuts, but by separate prediction equations), non-trimmed loin weight and loin saleable meat yield (TP_Loin), non-trimmed chump weight and chump saleable meat yield (TP_Chump), non-trimmed leg weight and leg saleable meat yield (TP_Leg), non-trimmed breast weight and

breast saleable meat yield (TP_Breast), non-trimmed shoulder weight and shoulder saleable meat yield (TP_Shoulder).

2.3 Statistical analysis

Data were analysed using the general linear model procedure (GLM) of the SAS package for Windows, Release 9.1 (SAS Institute Inc., Cary, NC, USA).

The model used was:

$$Y_{ijklmn} = a + t_i + lj + m_{ki} + s_l + w_m + \varepsilon_{ijklmn}$$

The full model tested for all traits (Y) for each lamb ($n = 1, 2, 3 \dots 167$), included a constant (a) and the fixed effects of sire t ($i = \text{sire 1 or 2}$), genotype l for LM-QTL ($j = \text{LM-QTL carrier or LM-QTL non-carrier}$), genotype m for MM-QTL ($k = \text{MM-QTL carrier or MM-QTL non-carrier}$), which was nested within sire since only one sire was segregating for this QTL, and sex s ($l = \text{male or female}$). Because all animals were sent to the abattoir as a one set and at the same day, the variation between animals was minimum and only due to the differences in birth date. Therefore, the covariate (w) was fitted, which was either live weight at the time of measurement (for the *in vivo* measurements) or carcass weight (for measurements taken on the carcass). No covariate was fitted in the model where the Y variable was live weight or carcass weight. Furthermore, animal live weight at CT was not fitted as a covariate in the model for CT tissue proportion variables. An error term (ε_{ijklmn}) was also fitted. Initially, the analyses also included dam age and the interaction term between the two QTL, however, these were consistently non-significant over all traits, so they were dropped from the final model.

Least-squares means (LSM) and their standard errors were produced and tabulated for all traits in this study. The GLM procedure also allowed for pairwise comparisons between the genotypic groups.

2.4 Results

2.4.1 Traits measured by X-ray computed tomography (CT)

Analysis revealed that live weight at CT scanning was not significantly different between LM-QTL carrier and non-carrier lambs (Table 2.2). However, LM-QTL carrier lambs had a significantly wider (+1.4%; $P < 0.05$) and deeper (+3.4%; $P < 0.01$) MLL. In consequence, the increase in both dimensions resulted in a 73 mm² larger MLL area (+4.5%; $P < 0.01$) in lambs carrying a single copy of the LM-QTL. There was no effect of LM-QTL status on muscle shape (2D MLL_Musc) ($P = 0.134$), and also leg muscle characteristics - width, depth and 2D muscularity - measured by CT scan were not significantly affected by the presence of the LM-QTL. Likewise, muscle densities (MD) in the three cross-sectional CT scans (ISC, LV5 and TV8) were not significantly affected by LM-QTL.

Weights of muscle (MUSCwt), fat (FATwt) and bone (BONEwt) in the whole carcass estimated by CT were not significantly different between carriers and non-carriers of LM-QTL (Table 2.2). Therefore, it is not unexpected that proportions of muscle (MUSC-Prop), fat (FAT-Prop) and bone (BONE-Prop) derived from total tissue weights predicted by CT measurements were also not significantly affected by LM-QTL status. LM-QTL carrier lambs and non-carrier lambs had similar MUSC-Prop and FAT-Prop. Similarly, the ratios of muscle to bone (M: B) and muscle to fat (M: F) were not significantly different between LM-QTL carrier lambs and their non-carrier contemporaries.

Table 2.2 Least-square means and standard error of the difference (s.e.d.) for LM-QTL carrier and non-carrier lambs for CT measurements (percentage difference (% diff) of LM-QTL carrier vs. non-carrier is also shown for each variable)

CT traits	LM-QTL					CTLWT
	Non-carrier	Carrier	s.e.d.	P-value	% diff [‡]	
CT LWT (kg)	31.15	31.26	0.480	0.822	0.4	-†
MLL_W (mm)	69.37	70.36	0.461	0.034	1.4	***
MLL_D (mm)	26.53	27.42	0.322	0.006	3.4	***
MLL_A (mm ²)	1604	1677	23.20	0.002	4.5	***
2D MLL_Musc	3.83	3.90	0.049	0.134	1.9	ns
Leg_W (mm)	165.66	164.24	0.887	0.111	-0.9	***
Leg_D (mm)	79.67	81.06	0.984	0.162	1.7	***
2D Leg_Musc	4.82	4.94	0.074	0.099	2.5	***
ISC_MD (g/cm ³)	1.056	1.055	0.0003	0.261	<-0.1	ns
LV5_MD (g/cm ³)	1.055	1.055	0.0003	0.854	<0.1	***
TV8_MD (g/cm ³)	1.050	1.051	0.0003	0.916	<0.1	***
FATwt (kg)	2.12	2.07	0.075	0.491	-2.4	***
MUSCwt (kg)	9.87	9.94	0.062	0.302	0.7	***
BONEwt (kg)	3.19	3.21	0.019	0.386	0.5	***
FAT-Prop	0.136	0.133	0.006	0.652	-1.9	-
MUSC-Prop	0.652	0.655	0.004	0.410	0.5	-
BONE-Prop	0.212	0.211	0.003	0.890	-0.2	-
M:B	3.1	3.1	0.024	0.931	<0.1	***
M:F	5.6	5.9	0.453	0.552	4.8	***

† (* = P < 0.05, ** = P < 0.01, *** = P < 0.001)

‡ Percentage difference is the percentage difference of tissue proportion. CTLWT is the animal live weight at CT scanning. MLL_W, MLL_D and MLL_A are the dimensional measurements of the *M. longissimus lumborum* width, depth and area; respectively measured on the CT scan through the 5th lumbar vertebra. 2D MLL_Musc is *M. longissimus lumborum* muscularity [(depth/width)*10] measured on the CT scan through the 5th lumbar vertebra. Leg_W, Leg_D are the leg width and depth respectively, measured on the CT scan through the *ischium* and 2D Leg_Musc is hind leg muscularity [(depth/width)*10] on the CT scan through the *ischium*. ISC_MD (g/cm³), LV5_MD (g/cm³) and TV8_MD (g/cm³) are the average muscle densities in the CT reference scans through the *ischium*, 5th lumbar vertebrae and 8th thoracic vertebrae, respectively. FAT-Prop, MUSC-Prop and BONE-Prop are the proportion of the fat, muscle and bone tissue weight relative to the total carcass tissue weight on the CT scan. M: B and M: F are the ratios of muscle to bone and muscle to fat, respectively.

2.4.2 Ultrasound traits

Live weight at ultrasound scanning (USLWT; 20 weeks of age) did not show a significant effect of LM-QTL (Table 2.3). However, UMD was significantly (+3.7%; $P < 0.05$) greater in LM-QTL carrier lambs. UFD was similar in LM-QTL carriers and non-carriers (Table 2.3).

Table 2.3 Least-square means and standard error of the difference (s.e.d.) for LM-QTL carrier and non-carrier lambs for ultrasound measurements (percentage difference (%diff) of LM-QTL carrier vs. non-carrier is also shown for each variable)

Ultrasound traits	LM-QTL					USLWT
	Non-carrier	Carrier	s.e.d.	P-value	% diff	
USLWT (kg)	40.23	40.23	0.759	0.989	<0.1	- [†]
UMD (mm)	24.2	25.2	0.421	0.034	3.7	***
UFD (mm)	3.31	3.27	0.089	0.633	-1.3	***

[†] (* = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$)

USLWT is the animal live weight at ultrasound. UMD and UFD are ultrasonically measured muscle and fat depths, respectively.

2.4.3 Post mortem traits (MLC-C&F and VIA traits)

The average cold carcass weight and killing out percentage (KO%), defined as cold carcass weight divided by animal live weight prior to slaughter, multiplied by 100, were not affected by the presence of one copy of the LM-QTL ($P > 0.05$; Table 2.4). There were also no significant differences between LM-QTL carriers and non-carriers in MLC conformation score and fat class (Table 2.4).

Table 2.4 Least-square means and standard error of the difference (s.e.d.) for LM-QTL carrier and non-carrier lambs for carcass weight (CCWT), killing out percentage (KO %) and MLC conformation (CONF) and fat class (FAT) scores (percentage difference (%diff) of LM-QTL carrier vs. non-carrier is also shown for each variable)

MLC traits	LM-QTL					CCWT
	Non-carrier	Carrier	s.e.d.	P-value	% diff	
CCWT (kg)	17.47	17.60	0.317	0.666	0.8	- [†]
KO%	44.96	44.66	0.249	0.234	-0.7	***
CONF	2.53	2.45	0.068	0.244	-3.2	***
FAT	10.65	10.83	0.222	0.411	1.7	***

[†] (* = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$)

CONF is MLC conformation score (EUROP) transformed to a numeric scale: E (excellent) = 5 to P (poor) = 1.

FAT is MLC fat class (1, 2, 3L, 3H, 4L, 4H, 5) transformed to a subcutaneous fat percentage as described by Kempster et al. (1986): 1 = 4, 2 = 8, 3L = 11, 3H = 13, 4L = 15, 4H = 17, 5 = 20.

The aim of using VIA in this experiment was to evaluate the potential of VIA to capture the variation between LM-QTL genotypes in carcass traits. In agreement with the results for loin muscle traits measured by CT (Table 2.2) and ultrasound scanning (Table 2.3), VIA could also detect a significant difference between carriers and non-carriers in the estimated weight of saleable meat yield in the loin primal cut (TP_Loin) (+2.2%; $P < 0.05$), but not in any other primal cut and not in the SMY of the whole carcass (Table 2.5) or any of the non-trimmed primal cuts (not presented). Only trimmed primal cut traits are presented in Table 2.5 as they are, arguably, more important traits for the producer, processor and meat retailer.

Table 2.5 Least-square means and standard error of the difference (s.e.d.) for LM-QTL carrier and non-carrier lambs for carcass traits predicted from VIA measurements (percentage difference (%diff) of LM-QTL carrier vs. non-carrier is also shown for each variable)

VIA traits	LM-QTL			P-value	% diff	CCWT
	Non-carrier	Carrier	s.e.d.			
SMY [‡] (kg)	7.691	7.695	0.020	0.814	<0.1	*** [†]
TP_Loin (kg)	1.395	1.426	0.015	0.045	2.2	***
TP_Chump (kg)	0.813	0.809	0.004	0.348	-0.5	***
TP_Leg (kg)	3.272	3.282	0.017	0.597	0.3	***
TP_Breast (kg)	1.520	1.502	0.011	0.123	-1.2	***
TP_Shoulder(kg)	3.810	3.789	0.014	0.158	-0.5	***

[†] (* = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$)

[‡] SMY is the VIA prediction of saleable meat yield in the carcass predicted by a distinct prediction equation. TP_Loin, TP_Chump, TP_Leg, TP_Breast and TP_Shoulder are the VIA predictions of saleable meat yields in the loin, chump, leg, breast and shoulder primal cut, respectively.

2.5 Discussion

This study looked at a wide range of carcass traits in crossbred lambs that were representative of the typical slaughter lamb in the UK stratified sheep industry structure. The key element of this study was to comprehensively investigate carcass traits of these lambs which had either one copy of LM-QTL or none (non-carrier lambs). The effects of another QTL, which unexpectedly also segregated in some of these lambs, were accounted for in the statistical analyses.

Only a small number of studies have so far investigated the effects of LM-QTL in lambs, and these are mainly limited to studies in Australia and New Zealand. There has been scant information on the impact of LM-QTL on lamb carcass traits in other countries/environments and other genetic backgrounds. In general, the reported effects of the LM-QTL seem to be restricted to the loin area, with no corresponding effects on other muscle areas or on fat. Nicoll et al. (1998) CT scanned Poll Dorset x

Romney lambs at approximately 5 months of age and reported an increase in CT-measured width, depth and area of the loin muscle by 2.7mm, 3mm and 3.3 cm², respectively, in LM-QTL carrier lambs compared to non-carriers. In the current study, the magnitude of the differences between carriers and non-carriers in loin muscle dimensions measured at 16 weeks is smaller (~1mm for MLL width and depth and 73 mm² for MLL area). These results are more comparable to the LM-QTL effects reported in another study of crossbred lambs out of non-carrier Romney ewes mated with LM-QTL heterozygous sires (Jopson et al. 2001), where carrier lambs at approximately ~24 weeks of age, had 1.1cm² greater ultrasound-measured muscle area compared to non-carriers. Differences in the magnitude of the effect observed between studies may be related to age, slaughter weight (although this was not specified in the previous studies), scanning method and position, or genetic background. Crucially, the current study is in agreement with those of both Nicoll et al. (1998) and Jopson et al. (2001) in the conclusion that the effects of this LM-QTL are predominantly localised in the loin muscle region.

Crossbred lambs out of Mules ewes, similar to those of our experiment, were used to quantify the effect of the Texel muscling (TM-QTL; Walling et al. 2004) on carcass traits at the same slaughter age (Macfarlane et al. 2009a). TM-QTL has been found also to affect mainly the loin region and increases the ultrasonically measured muscle depth by around 1.0-2.0mm (+4-7%) (Macfarlane et al. 2009a; Matika et al. 2006; Walling et al. 2004) or 1.9 mm (+6.7%) when measured by CT at the fifth lumbar vertebra (Macfarlane et al. 2009a). It is noticeable that both QTL effects seem to be restricted to the loin area and are relatively similar in magnitude.

Nicoll et al. (1998) suggested that LM-QTL appears to alter muscle shape since it has larger effects on the muscle depth and area of *M. Longissimus dorsi* than it has on muscle width when adjusted for live weight. Our study shows that width and depth were changed by effects of similar absolute magnitude (~1mm), corresponding to 1.4 and 3.4 %, respectively and thereby confirms the suggestion that LM-QTL results in a change in muscle shape.

Previous work by Nicoll et al. (1998) found that the *M. longissimus dorsi* weight was 8% higher in LM-QTL carrier lambs compared to non-carriers. Similar effects were found by Macfarlane et al. (2009a) for the TM-QTL, which increased MLL weight

by 7.1% in heterozygous TM-QTL carrier lambs due to significant increases in loin width and depth but no significant change in MLL length. In the current study, MLL weight was not measured and could not be predicted as this study did not involve fine dissection and no 3D CT scanning, which would have allowed calculation of muscle volume (Navajas et al. 2006a). However, if LM-QTL and TM-QTL effects can be assumed to be similar, it could be expected that LM-QTL carrier lambs would also have a higher loin muscle weight.

At present it can not be ruled out that TM-QTL and LM-QTL are alleles of the same gene (Macfarlane et al. 2009a; Walling et al. 2004). To prove this requires further molecular-genetic evidence as, to derive such a conclusion simply from the similarity of the phenotypic effects of both QTL appears too speculative.

To our knowledge, none of the previous work on LM-QTL effects in purebred or crossbred lambs has found or reported phenotypic effects other than those on the loin muscle region (Jopson et al. 2001; Nicoll et al. 1998), which is in agreement with the current findings. Macfarlane et al. (2009a) studied the TM-QTL effects on hind leg muscle volume and hind leg muscle density and analyses revealed no significant difference between carrier and non-carrier lambs. The present study did not find significant effects of the LM-QTL on the hind leg muscle characteristics of width, depth and 2D muscularity measured by CT. Muscularity describes the shape of the muscle and it has been shown that deeper muscles appeal to consumers, as they prefer rounded rather than thin chops (Laville et al. 2004). LM-QTL carrier lambs showed no significant change in muscularity in both the leg and loin regions, as measured from 2D CT scans.

Overall, this trial showed no significant effect of LM-QTL on fat traits (ultrasound fat depth, total carcass fat weight estimated by CT, carcass fat proportion derived from CT measurements or MLC fat class). The non-significance of LM-QTL effects on ultrasound live weight, CT live weight, total lean meat weight estimated by CT, M: B ratio, cold carcass weight and killing out percentage is not unexpected, as all previously-reported effects of this QTL also seem to be restricted to the loin muscle (Nicoll et al. 1998) which, in turn, represents just a small proportion of the whole carcass weight.

2.5.1 LM-QTL and meat quality

Muscle density measured by CT is considered a predictor of meat quality traits (Karamichou et al. 2006b), as it is likely to reflect differences in intramuscular fat (IMF) content. In our study there were no significant differences between LM-QTL carrier and non-carrier lambs in muscle density measured at any of the three CT scan positions. This might suggest that LM-QTL is likely to have no substantial effects on IMF. However, results reported by Jopson et al. (2001) showed that LM-QTL lambs produced lower tenderness values than non-carriers, although this effect was removed by appropriate post-slaughter treatment (chilling, ageing). It would be advisable in future to directly test tenderness in LM-QTL carrier crossbred lambs, such as those used here, to monitor this aspect of meat quality. In the future, if molecular genetic predictors of meat quality were available, these could be used to help counterbalance any negative side effect of LM-QTL on meat quality in a marker assisted selection framework (e.g. Gao et al. 2007).

2.5.2 LM-QTL effects on MLC-C&F classification and VIA

MLC carcass classification scores for conformation and fat class did not differ significantly between LM-QTL carriers and non-carrier lambs. This result is similar to that reported for TM-QTL by Rius-Vilarrasa et al. (2009c), who found that crossbred lambs from a similar genetic background to those in our study carrying one copy of TM-QTL showed no significant difference in conformation and fat class scores compared to non-carriers. The results of the present study indicate that the current industry carcass evaluation system would not be able to detect the improvement in loin muscle characteristics offered by LM-QTL.

The development of video image technology has made it possible to automatically predict some carcass characteristics such as lean meat yield, saleable meat yield and weights of the primal cuts at slaughter chain speed (Hopkins 1996; Rius-Vilarrasa et al. 2009b). Rius Vilarrasa et al. (2009b) reported that VIA achieved consistently higher accuracy and precision in predicting the carcass primal cut weights and saleable meat yield compared with the MLC classification grading for carcass conformation and fat class, which is in use in UK abattoirs.

Rius-Vilarrasa et al. (2009c) did not detect significant difference in VIA-predicted loin dimensions and primal cut weights between TM-QTL carrier and non-carrier lambs using the standard prediction equation produced by E+V Technology, Germany. By contrast, deriving refined prediction equations by calibration against CT measurements it was possible in this previous experiment to detect a significant increase in MLL depth in TM-QTL carrier lambs. Likewise, using standard algorithms, VIA could not detect a significant effect of LM-QTL on increased muscle in the loin region in the current data set (results not reported here). However, the use of the refined prediction equations did detect a significant effect of LM-QTL on trimmed loin primal cut weight predicted by VIA, but not on predictions of other carcass primal cuts or total saleable meat yield in the present study. The significant increase of +2.2% in predicted trimmed loin primal weight, the cut the market values most highly, was large enough to be reliably detected by VIA in LM-QTL carrier lambs in this data set. This indicates that introducing VIA as the basis for a value-based payment system could have the potential to financially reward producers for an increase in loin weight caused by lambs carrying one copy of the LM-QTL. Moreover, the mode of inheritance of the LM-QTL has yet to be confirmed. If this is found to be additive, larger effects in homozygous lambs might be expected, which could further increase the carcass value. This study also highlights the increased power of VIA, compared to the current grading system, to detect improvements in carcass quality, as the MLC scoring system did not detect any differences between carriers and non-carriers.

Carcass saleable meat yield, which is considered the most important trait for meat processors and producers, was not significantly different between LM-QTL carriers and non-carriers when predicted by VIA. This agrees with the effect of LM-QTL on carcass weight and on CT total muscle weight. The loin amounts only to approximately 18% of the total lean weight within the carcass. Consequently, localised changes to loin muscle traits of the magnitudes shown here would not be expected to result in significant changes in VIA-predicted total lean weight.

Comparisons of the performance of the lambs in the current study with that of other crossbred lambs sired by terminal sires more typically used in the UK industry (e.g. Texel and Suffolk) are not straightforward, due to differences between trials in sire

and dam breeds, finishing point, year and many other influencing factors. However, it is of note that the Poll Dorset-sired lambs recorded in the current study seem to be within the normal range of carcass weights and grades found for commercial slaughter lambs sired by terminal sire breeds in the UK. Results of two trials assessing carcass quality in commercially finished lambs produced by Mules ewes mated to Texel, Suffolk and Charollais rams (Ellis et al. 1997; Lewis et al. 2006) reported similar average live weights at slaughter (~40 - 42kg) as those reported here. Average cold carcass weights were slightly higher (by approximately 1 – 2 kg) than in the current trial, resulting in similar, or slightly higher, killing out percentages. Average subcutaneous fat percentage, estimated from MLC carcass fat grades, was similar to that found here in both previous trials, although average conformation scores were slightly lower in the current study (0.4 - 0.5 points on 5 point scale). From these general comparisons, it appears that the lambs in the current study do not differ substantially, in terms of slaughter weights and carcass quality traits, from crossbred lambs sired by other terminal sire breeds under UK conditions. However, tissue proportions in the carcass, as estimated by CT scanning at 16 weeks in the current study, differ for example from those reported by Ellis et al. (1997) for lambs dissected post-slaughter, where average proportion of lean was around 0.54 to 0.57 and proportion of fat around 0.23 to 0.24 (depending on sire breed). The average lean proportion in the current study agrees more closely with average dissected lean proportion reported by Lewis et al. (2006) (~0.63), although fat proportion remains lower than the 0.19 to 0.2 found in the previous study. These discrepancies may be due to the fact that the tissue proportions reported for lambs in the current study were estimated using CT scanning at 16 weeks of age (at an average live weight of 31 kg), although lambs were not slaughtered until a fixed age of 20 weeks. In the previous studies, lambs were selected for slaughter when they reached a commercial level of “finish” (estimated fat cover or condition) and tissue proportions were estimated by dissection post-slaughter. Although proportion of carcass lean is known to decrease and fat increase with maturity, studies that report dissection results from lambs of different breeds slaughtered at different ages more commonly estimate lean proportions between 0.5 and 0.6 and fat proportions between 0.2 and 0.3 (Kempster et al. 1986; McClelland et al. 1976). Nevertheless, the aim in the current study was to compare tissue composition in lambs of different

LM-QTL genotype, and the results from CT and from MLC carcass grades imply that tissue proportions across the whole carcass do not differ significantly.

The current study showed that one copy of the LM-QTL significantly improved loin muscle traits. This could potentially be exploited through Marker Assisted Selection (MAS). MAS could be a particularly useful technology in crossbreeding programmes where desirable genotypes from different backgrounds are introgressed into productive local breeds (Van der Werf 2007). However, its use should be integrated into the whole genetic evaluation system by the use of holistic approaches to devise selection rules based on MAS (Van der Werf 2007).

Incorporating LM-QTL in sheep breeding programs in the UK has the potential to result in improved meat yield of the loin cut. This improvement would also be possible through conventional sheep breeding programmes utilising ultrasound and/or CT measurements of loin muscle depth since the heritability of loin muscle measurements is moderate to high (depth 0.30 ± 0.03 , width 0.38 ± 0.10 and area 0.41 ± 0.07) and genetic correlations between live measurements on eye muscle depth, width and area were also high (0.87-0.99) (Safari et al. 2005). This study also reported that genetic correlations between loin muscle depth and width were moderate (0.28) but the correlations between loin muscle area and depth (0.86) or width (0.74) were much higher, suggesting that selection for one trait would reflect positively to the other trait and eventually improve loin muscle shape. The genetic and phenotypic correlations between loin muscle traits and other economic and welfare-related traits, like growth rate and lambing ease would also be important to consider before incorporating these traits in a breeding programme. Lambe et al. (2009a) investigated the phenotypic effects LM-QTL on growth patterns of crossbred lambs and reported no significant effects of the presence of LM-QTL. Further work is underway relating the QTL to other relevant production, behavioural and welfare-related traits.

2.6 Conclusion

This study evaluated the direct effect of LM-QTL on crossbred lambs in the UK and successfully confirmed earlier published effects of LoinMAXTM on loin muscle characteristics. LM-QTL positively increased loin muscle traits when measured by ultrasound, CT scanning and VIA. It is important before the benefits of LM-QTL can

be fully exploited to assess any possible pleiotropic or/and epistatic effects of the LM-QTL on other economically traits e.g. meat quality, lambing ease, survival, growth rate and efficiency. Additionally, the mode of inheritance is vitally important to account for optimal future breeding programmes. Assuming high linkage disequilibrium, the provision of estimated QTL EBVs would enable the use of QTLs in breeding programmes irrespective of the lack of knowledge of underlying gene. However, further attempts to fine-map the underlying gene(s) seem to be crucial to guarantee that the marker test retains its value also over future generations.

Chapter 3

Evaluation of the effects of a single copy of a mutation in the myostatin gene (c.*1232G > A) on carcass traits in crossbred lambs

Abstract

This study evaluated the effects of the ovine c.*1232G > A myostatin mutation (MM) on carcass traits in heterozygous crossbred lambs sired by Texel and Poll Dorset rams using ultrasound, CT scanning, carcass classification and VIA. In experiment 1, MM was associated with increased loin depth (+2.8 %) and area (+3.2%). MM-carriers had significantly higher CT-estimated lean weight and proportion (2 to 4%) and muscle to bone ratio (by ~3%), in both experiments, and muscle to fat ratio (28%) in experiment 2. Muscle areas in three cross-sectional CT scans, were higher (2 to 5%) in MM-carriers. In experiment 2, fat-related measurements were significantly lower in MM-carrier lambs but this was not seen in experiment 1. A significant increase in muscle density, indicative of lower intramuscular fat, in MM-carriers shows that meat quality characteristics need attention. Carrying MM significantly decreased carcass fat scores. VIA did not detect any significant MM effects.

3.1 Introduction

Meeting growing consumer demand for lean meat with low levels of fat has been one of the main targets of sheep genetic improvement programmes over the last few decades (Simm & Dingwall 1987). Use of the lean growth index, designed to increase the weight of lean while limiting any change in weight of fat in the carcass at a given age, has been successful, although its uptake in the industry has been slow (Amer et al. 2007). Substantial advances in quantitative and molecular genetics in recent years, accompanied by work to decipher the underlying genetic architecture through mapping the full ovine genome, have opened up new opportunities for the meat sector to boost the profitability of meat production and meet market requirements locally, in the UK, and globally. Marker assisted selection (MAS) could help increase rates of genetic gain through incorporating molecular genetic information into breeding programmes (Dekkers 2004; Dekkers & Hospital 2002).

Of the various quantitative trait loci (QTL) identified that influence muscle growth and carcass composition, to date only very few causative mutations have been successfully identified and verified. Polymorphisms in the myostatin (*MSTN*) gene associated with enhanced muscle growth have been reported in several species e.g. in mice (Varga et al. 1997), in cattle (Grobet et al. 1997), in chicken (Zhiliang et al. 2004), in dogs (Mosher et al. 2007) and in molluscs (Wang et al. 2010). Also in sheep, a number of *MSTN*-polymorphisms have been reported to segregate in various breeds or strains, e.g. Belgian, NZ, UK and Australian Texel, Poll Dorset, Lincoln, Charollais, NZ Romney sheep, Norwegian White sheep and East Friesian sheep (Clop et al. 2006; Johnson et al. 2009; Kijas et al. 2007; Hadjipavlou et al. 2008; Hickford et al. 2010; Boman et al. 2009; Bignell et al. 2010).

The c.*1232G > A mutation, named here according to the recommended nomenclature system (<http://www.genomic.unimelb.edu.au/mdi/mutnomen/>) is one such mutation in the ovine *MSTN* gene. This single nucleotide polymorphism (SNP), which is the subject of the current study and will be referred to here as MM, was originally identified in the Belgian Texel and known as g+6723G-A (Clop et al. 2006). It is a transition in the 3' untranslated region introducing a microRNA target site leading to translational inhibition of the myostatin gene, and contributes to the muscular hypertrophy of Texel sheep (Clop et al. 2006). The A allele is associated

with increased lean meat in Australian Texels (Kijas et al. 2007) and British Charollais sheep (Hadjipavlou et al. 2008). A recent study in New Zealand, which evaluated the effects of 0, 1 or 2 copies of the g+6723G > A in Texel-cross lambs at different ages and carcass weights, confirmed the association of the A allele with increased muscle and decreased fat using a variety of measurement techniques and on variety carcass weights (Johnson et al. 2009).

Indirect effects of the MM mutation on other traits, for example meat quality, are also important. Analysis of meat quality in crossbred (Australian Texel x Merino) lambs revealed no detectable effect on objectively assessed meat quality (including tenderness measured by shear force) in lambs carrying a single copy of MM compared to non-carriers (Kijas et al. 2007). On the other hand, meat from MM carriers had lower levels of intramuscular fat (IMF) and lower scores for flavour and overall satisfaction compared to non-carriers (Kijas et al. 2007). A New Zealand study of a QTL on chromosome 2 that is likely to harbour this SNP, although the study was conducted prior to the identification of the causative mutation, found that QTL-carrier lambs had a 3% increase in leg muscle and a 10% decrease in leg fat, compared to non-carriers, while no detectable negative associations with objectively-assessed meat quality traits were found (Johnson et al. 2005b). Provided there are no negative associations with meat quality, health or welfare traits, MM offers potential benefits to the meat industry, as lamb carcasses from carrier lambs will have more muscle and less fat at the same carcass weight.

Allelic frequency of MM in British commercial Texel sheep is approaching fixation (Hadjipavlou et al. 2008), but is intermediate ($p = 0.3$) in two other sheep breeds, the UK Charollais (Hadjipavlou et al. 2008) and Australian White Suffolks (Kijas et al. 2007). This SNP, in the UK Charollais breeds, appears to be partially recessive (Hadjipavlou et al. 2008).

The aim of this study was to evaluate the direct phenotypic effects of the presence of a single copy of the MM on a wide range of live animal and carcass traits in a genetic background that is typical for the stratified structure of the UK sheep industry (i.e. crossbred lambs out of Mule (Bluefaced Leicester x Scottish Blackface) ewes). Various evaluation techniques were used: *in vivo* ultrasound scanning (US), *in vivo*

computed tomography (CT), MLC carcass classification scoring for conformation and fat class (MLC-C&F) and carcass video image analysis (VIA).

3.2 Material and methods

3.2.1 Experimental animals

All procedures involving animals were approved by the SAC Animal Ethics Committee and were performed under UK Home Office licence, following the regulations of the Animal (Scientific Procedures) Act 1986.

3.2.1.1 Experiment 1 (exp 1)

Semen from two Texel rams, identified as having a single copy of MM, was used to inseminate Scotch Mule (Blue-faced Leicester x Scottish Blackface; $n = 87$) ewes. All ewes were between 4- and 6-years old and were allocated to be mated to one of the two rams at random. Lambs used in the experiment were pre-selected from a larger flock so that all were reared as twins, in order to eliminate litter size as a source of variation. Therefore, a total of 150 lambs (70 castrated males, 80 females), that were either non-carriers or carriers of a single copy of MM, were recorded in 2008 (Table 3.1).

3.2.1.2 Experiment 2 (exp 2)

In 2007, an experiment was conducted primarily to evaluate the effects of another muscle-enhancing QTL, the Loin Muscling QTL (LM-QTL), on crossbred lambs (Masri et al. 2010). LM-QTL segregates on chromosome 18 in the Poll Dorset breed and predominantly affects the loin muscle (Nicoll et al. 1998). In this study (exp 2), two Poll Dorset rams were mated to Scotch Mule ewes ($n = 91$) from the same flock as those used in exp 1. Unexpectedly, MM carrier and non-carrier lambs (Table 3.1) were produced from these matings, suggesting that one of the Poll Dorset rams used was also heterozygous for MM. Among the MM carrier animals, there were 22 animals carrying LM-QTL together with MM, but no significant interaction was found between the two QTLs for the traits studied. Further details of exp 2 were given by Masri et al. (2010). The data set from exp 2 on its own was not sufficiently powerful for a comprehensive evaluation of the effects of MM on crossbred lambs sired by Poll Dorset rams. However, they are of interest, alongside the results of the

main experiment (exp 1), in order to illustrate whether the presence of MM would have similar effects when segregating from a different sire background.

Table 3.1 Number of lambs of each c.*1232G > A (MM) genotype from experiment 1 and experiment 2.

MM status	Experiment 1	Experiment 2
	Texel sires	Poll Dorset sires
Non-carriers	80	128
Carriers	70	39
Total	150	167

The same management and experimental procedures were applied to lambs in both experiments. Together with their dams, all lambs within year were grazed as one flock from birth to slaughter, on the same pasture, to avoid any differences in nutrition between animals of different genotypes. Lambs were weighed and CT scanned at 16 weeks of age and then weighed and ultrasonically scanned at 20 weeks of age, directly before being sent for slaughter. Immediately following slaughter, carcasses were scanned using VIA.

3.2.2 Traits measured by X-ray computed tomography (CT)

CT scanning was performed on all lambs in both experiments at 16 weeks of age, more than 4 weeks before slaughter, to allow for the 30 day withdrawal period required for the sedative used for CT scanning. For exp 1 and exp 2, the average age at CT was 119 days (min. 114, max. 125) and 113 days (min. 110, max. 116), respectively. CT scanning of all lambs, within experiment, took place during one week. Lamb live weight was measured at CT scanning (CT-LWT) and ranged from 26.1 kg to 45.8 kg for exp 1 animals and 22.9 kg to 37.4 kg for exp 2 animals. The CT methodology has been described in detail elsewhere (e.g. Macfarlane et al. 2006). Briefly, for each lamb, cross-sectional CT reference scans were taken at three fixed anatomical positions, the end of the ischium bone (ISC), the fifth lumbar vertebra (LV5), and the eighth thoracic vertebra (TV8). Each CT image was analysed using the Sheep Tomogram Analysis Routines software STAR (Mann et al. 2003) to obtain areas (A) of muscle (M), fat (F) and bone (B) (ISC-MA, ISC-FA, ISC-BA, LV5-MA, LV5-FA, LV5-BA, TV8-MA, TV8-FA and TV8-BA, respectively). Average muscle density, measured in Hounsfield units and then transformed to true density (g/cm^3), was calculated for each of the three reference scans for each animal, as an indicator of intramuscular fat content (Karamichou et al. Bishop 2006b; Navajas et al. 2006c).

Total weights of fat (FATwt), muscle (MUSCwt) and bone (BONEwt) in the carcass were predicted using reference scan tissue areas and live weight in previously-derived prediction equations developed for crossbred lambs (accuracies of 85%, 84% and 58% for FATwt, MUSCwt and BONEwt, respectively). Proportions of fat (FAT-Prop), muscle (MUSC-Prop) and bone (BONE-Prop) in the carcass and ratios of muscle to bone (M:B) and muscle to fat (M:F) were calculated using the predicted tissue weights. Width, depth and area of the left and right *M. longissimus lumborum* (MLL) were measured on the LV5 scan and the average of the measurements from the two sides were used in analyses (MLL-W, MLL-D and MLL-A, respectively). Two-dimensional (2D) loin muscularity (2D-MLL-Muscu) was calculated using the ratio of depth to width of the MLL and averaged over both sides (Jones et al. 2002a). Muscle width and depth of each hind leg were measured on the ISC scan (Leg-W and Leg-D) and similarly to the loin, the ratio of depth to width measurements calculated and the mean value over both legs used to provide a measure of 2D shape of the hind leg (2D-Leg-Muscu) (Jones et al. 2002a).

3.2.3 Traits measured by ultrasound

All lambs in exp 1 and exp 2 were weighed at 20 weeks of age. Live weight at 20 weeks (US- LWT) averaged 38.3 kg in exp 1 and 40.2 kg in exp 2 (range 27.2 kg to 50.2 kg and 29.6 kg to 50.0 kg, respectively). Lambs were then ultrasonically scanned over the 3rd lumbar vertebra using a Dynamic Imaging Concept MLV (Caiyside Imaging Ltd) ultrasound scanner with a 3.5 MHz transducer. One vertical muscle depth measurement (UMD) was taken at the deepest point of the MLL. Three fat depth measurements were averaged to estimate ultrasound fat depth (UFD), one at the same lateral position as the muscle depth measurements, and two others at 1 and 2 cm lateral to the first position.

3.2.4 Traits measured post mortem

Lambs were transported to Welsh Country Foods abattoir in Anglesey to be slaughtered. All lambs were electrically stunned, slaughtered and eviscerated and the carcasses skinned and dressed according to normal commercial practices in UK abattoirs. Cold carcass weight (CCWT) was defined by the abattoir as hot carcass weight minus 0.5 kg. Carcasses were visually graded for conformation and fat class according to the Meat and Livestock Commission (MLC) classification scheme,

based on the EUROP grading regime. Fat class (MLC-F) characterises the carcass based on a 7 point scale for fat cover from 1 (very lean) to 5 (very fat), with classes 3 and 4 being subdivided into L = low and H = high (Anderson 2003). For statistical analyses, fat classes were converted to estimated subcutaneous fat percentage, as reported by Kempster et al. (1986), where 1 = 4%, 2 = 8%, 3L = 11%, 3H = 13%, 4L = 15%, 4H = 17%, 5 = 20%. Carcass conformation (MLC-C) was visually assessed on a 5-point scale for carcass shape based on the EUROP classification scale. The scale was coded from 1 to 5 for statistical analyses where E (excellent) = 5 and P (poor) = 1.

3.2.5 Traits measured by video image analysis (VIA)

All carcasses were scanned using the VSS2000 video image analysis system (VSS2000, E+V Technology, Germany, <http://www.vision-for-you.com/start.htm>). Further details on the VIA system functions and procedures have been described elsewhere (Rius-Vilarrasa et al. 2009b). Briefly, the VSS2000 software automatically generates a variety of lengths, widths and areas on images of the carcass taken from the back and side at slaughter line speed. These are then used to predict weights of saleable meat yield in the carcass (SMY; predicted not as a sum of predicted weights of individual cuts, but by a separate prediction equation) and the trimmed weights of different primal cuts: loin (TP-Loin), chump (TP-Chump), leg (TP-Leg), breast (TP-Breast), and shoulder (TP-Shoulder). These prediction equations were previously calibrated and validated under British abattoir conditions and further improved by calibrating them against CT measurements in the loin region (Rius-Vilarrasa et al. 2009c). VIA predictions (developed by E+V Technology) were also available for several muscularity traits in the hind leg and loin region, including muscle volumes and dimensions.

3.3 Statistical analysis

Data from the two experiments were analysed separately using the general linear model procedure (GLM) of the SAS package for Windows, Release 9.1 (SAS Inst., Inc., Cary, NC, USA). Resulting least-squares means allowed comparisons between the two genotype groups, the MM carrier lambs and non-carriers, within experimental data sets.

The model fitted to the data from exp 1 was:

$$Y_{ijkn} = \mu + t_i + m_j + s_k + w_{ijkn} + \varepsilon_{ijkn}$$

Y_{ijkn} is the performance of the n th individual lamb, of the i th sire, of the j th genotypic class (carrier or non-carrier), and of k th sex, for each trait of interest, with μ as a general mean, w_{ijkn} live weight at the time of measurement (for the *in vivo* measurements) or carcass weight (for the carcass measurements) and ε_{ijkn} an error term.

The same model was used to analyse the data from exp 2 with LM-QTL status (carrier or non-carrier) also fitted as a fixed effect. The interaction term between MM and LM-QTL was also tested in the model, but was found to be consistently not significant across all traits studied, so it was excluded from the final model.

In both experiments, weight was not fitted as a covariate in the model where the Y variable was live weight, carcass weight, a tissue proportion. For all variables from both experiments, dam age and all two-way interactions between fixed effects were also investigated in the models, but they were not significant for the traits examined and were therefore excluded from the final models used in the analyses.

3.4 Results

3.4.1 X-ray computed tomography traits

For both experiments, least-squares means for the effect of MM genotype on carcass tissue areas measured by CT are presented in Table 3.2, and all other CT-measured traits are presented in Table 3.3.

Texel-sired lambs had 4-5 kg higher mean CT-LWT compared to Poll Dorset-sired lambs. Weight differences between MM carrier and non-carrier lambs within sire breed were small and not statistically significant.

3.4.1.1 Reference scan tissue areas

MM was associated with an increase in muscle areas in both experiments across all three CT reference scanning sites (2.4 to 5.4%). All differences were significant except for LV5-MA in exp 2 ($P = 0.052$). Fat areas in all three of the CT reference scans were smaller for MM carriers compared to non-carriers in both exp 1 (4.2 to 9.4%) and exp 2 (11 to 21%), but the effects in exp 1 were not significant (Table 3.2).

Table 3.2 Least-squares means and standard error of the difference (s.e.d.) for c.*1232G > A (MM) carrier and non-carrier lambs for tissue area measurements (difference (%diff), when the MM non-carriers used as denominator, is also shown for each variable)

Trait (unit)	Description	Texel sired lambs (exp1)					Poll Dorset sired lambs (exp2)				
		NC [†]	C	s.e.d.	% diff	Sig [‡]	NC	C	s.e.d.	%diff	Sig
CT scan											
N		80	70				128	39			
ISC-MA (mm ²)	Ischium muscle area	242	248	1.809	2.4	**	227	231	1.982	3.7	**
ISC-FA (mm ²)	Ischium fat area	49.2	47.2	1.561	-4.18	ns	40.3	35.9	1.489	-10.9	**
ISC-BA (mm ²)	Ischium bone area	29.9	29.2	0.456	-2.52	ns	28.1	28.2	0.531	0.2	ns
LV5-MA (mm ²)	Muscle area at the 5 th lumbar ver [§]	79.6	83.0	0.939	4.3	**	75.8	78.2	1.129	3.2	ns
LV5-FA (mm ²)	Fat area at the 5 th lumbar ver	23.6	21.4	1.340	-9.4	ns	17.6	14.0	1.269	-20.6	**
LV5-BA (mm ²)	Bone area at the 5 th lumbar ver	9.01	8.88	0.177	-1.5	ns	8.55	8.73	0.184	2.1	ns
TV8-MA (mm ²)	Muscle area at the 8 th thoracic ver	110	113	1.316	2.8	*	102	107	1.742	5.4	**
TV8-FA (mm ²)	Fat area at the 8 th thoracic ver	52.2	48.9	2.194	-6.2	ns	40.6	34.3	2.113	-15.4	**
TV8-BA (mm ²)	Bone area at the 8 th thoracic ver	34.3	34.8	0.504	1.4	ns	29.1	29.7	0.630	2.1	ns

[†] NC and C = Non-Carriers and Carriers for MM

[‡] Sig = significant effect (ns = not significant, * = P < 0.05, ** = P < 0.01, *** = P < 0.001)

[§] ver = vertebrae.

3.4.1.2 Muscle density

Average muscle density in the MM carrier lambs was significantly higher (0.1% to 0.2%), than in non-carrier lambs, in all reference scans in each experiment, with the exception of ISC-MD in exp 2 (Table 3.3).

3.4.1.3 Predicted tissue weights and proportions

MM carriers had higher CT-predicted carcass muscle weight and proportion, and lower CT-predicted fat weight and proportion in both experiments (Table 3.3). Although the differences in muscle weights and proportions were significant across both experiments, the differences in fat weight and proportion were not significant in the Texel-sired lambs (exp 1).

M:B and M:F ratios were higher in MM carriers compared to non-carrier lambs (Table 3.3), but the difference was not significant for M:F in Texel-sired lambs.

3.4.1.4 Muscle shape characteristics

For Texel-sired lambs (exp 1), MLL-D and MLL-A were significantly greater in MM carriers compared to non-carriers (exp 1). In contrast, there were no significant differences in any of the MLL shape characteristics between carriers and non-carriers sired by Poll Dorset rams (exp 2). MM carriers were not significantly different from non-carriers in hind leg muscling characteristics (Leg-W, Leg-D, 2D-Leg-Muscu) in either experiment (Table 3.3).

Table 3.3 Least-squares means and standard error of the difference (s.e.d.) for c.*1232G > A (MM) carrier and non-carrier lambs for the main CT measurements (difference (%diff), when the MM non-carriers used as denominator, also shown for each variable)

Trait (unit)	Description	Texel sired lambs (exp1)					Poll Dorset sired lambs (exp2)				
		NC [†]	C	s.e.d.	% diff	Sig [‡]	NC	C	s.e.d.	%diff	Sig
CT scan											
N		80	70				128	39			
CT-LWT(kg)	Live weight at CT scan	35.4	35.9	0.615	1.3	ns	31.5	30.3	0.647	-3.8	ns
ISC-MD (g/cm ³)	Muscle density at the ischium	1.055	1.056	0.0003	0.15	***	1.055	1.056	0.0004	0.04	ns
LV5-MD(g/cm ³)	Muscle density at the 5 th lumbar vertebra	1.053	1.054	0.0004	0.11	**	1.055	1.056	0.0004	0.11	*
TV8-MD (g/cm ³)	Muscle density at the 8 th thoracic vertebra	1.050	1.051	0.0004	0.13	**	1.050	1.052	0.0004	0.13	**
FATwt (kg)	Total estimated fat weight	3.02	2.85	0.106	-5.5	ns	2.17	1.87	0.099	-13.8	**
MUSCwt (kg)	Total estimated muscle weight	11.0	11.3	0.069	2.6	***	9.82	10.2	0.082	3.5	**
BONEwt (kg)	Total estimated bone weight	3.52	3.51	0.024	-0.5	ns	3.19	3.22	0.025	0.9	ns
FAT-Prop	Fat proportion	0.168	0.158	0.007	-5.6	ns	0.143	0.111	0.008	-22.2	**
MUSC-Prop	Muscle proportion	0.628	0.641	0.004	1.9	**	0.647	0.672	0.005	3.7	***
BONE-Prop	Bone proportion	0.203	0.201	0.003	-1.3	ns	0.210	0.216	0.004	2.8	ns
M:B	Muscle to bone ratio	3.11	3.20	0.026	3.1	**	3.08	3.16	0.033	2.6	*
M:F	Muscle to fat ratio	4.01	4.43	0.239	10.4	ns	5.39	6.90	0.604	27.9	*
MLL-W (mm)	M. longissimus lumborum width	71.2	71.3	0.453	0.1	ns	69.8	70.1	0.611	0.5	ns
MLL-D (mm)	M. longissimus lumborum depth	26.6	27.4	0.358	2.8	*	27.0	26.9	0.426	-0.4	ns
MLL-A (mm ²)	M. longissimus lumborum area	1692	1746	25.12	3.2	*	1631	1667	30.77	2.2	ns
2D-MLL-Muscu	2 dimension MLL muscularity	3.74	3.84	0.056	2.5	ns	3.87	3.83	0.065	-1.1	ns
Leg-W (mm)	Leg muscle width	165	166	0.684	0.5	ns	164	167	1.176	1.4	ns
Leg-D (mm)	Leg muscle depth	88.7	90.4	1.279	1.9	ns	80.4	80.3	1.311	-0.1	ns
2D-Leg-Muscu	2 dimension leg muscularity	5.37	5.44	0.085	1.4	ns	4.90	4.83	0.098	-1.4	ns

[†] NC and C = Non-Carriers and Carriers for MM

[‡] Sig = significant effect (ns = not significant, * = P < 0.05, ** = P < 0.01, *** = P < 0.001)

3.4.2 Ultrasound Traits

There was no significant effect of MM on US-LWT or UMD in lambs from either sire breed (Table 3.4). For UFD, MM carrier lambs from Poll Dorset sires had significantly lower UFD (9.7%) compared to non-carriers, and carrier lambs from Texel sires showed a tendency for lower UFD compared to non-carriers although the difference was not significant ($P = 0.08$) (Table 3.4).

Table 3.4 Least-squares means and standard error of the difference (s.e.d.) for c.*1232G > A (MM) carrier and non-carrier lambs for ultrasound measurements (difference (%diff), when the MM non-carriers used as denominator, also shown for each variable)

Trait (unit)	Description	Texel sired lambs (exp1)					Poll Dorset sired lambs (exp2)				
		NC [†]	C	s.e.d.	% diff	Sig [‡]	NC	C	s.e.d.	%diff	Sig
Ultrasound scan											
N		80	70				122	39			
US-LWT (kg)	Ultrasound live weight	37.6	38.5	0.679	2.4	ns	40.4	39.8	0.748	-1.4	ns
UFD (mm)	Ultrasound fat depth	3.70	3.51	0.109	-5.3	ns	3.37	3.04	0.117	-9.7	*
UMD (mm)	Ultrasound muscle depth	22.4	22.8	0.354	1.8	ns	24.7	24.6	0.553	-0.7	ns

[†] NC and C = Non-Carriers and Carriers for MM

[‡] Sig = significant effect (ns = not significant, * = P < 0.05, ** = P < 0.01, *** = P < 0.001)

3.4.3 Carcass and VIA Traits

With the exception of MLC-F, differences between genotypes for carcass traits measured in the abattoir in both experiments were not significant (Table 3.5). MM carrier lambs were significantly less fat than non-carrier lambs when assessed for fat class using MLC classification scheme (6.6 and 6.5% in exp 1 and exp 2, respectively). MM had no significant effect, in both experiments, on any of the VIA predicted carcass traits. Muscularity traits of the hind leg or loin were not significantly different between genotype groups, so these are not presented (Table 3.5).

Table 3.5 Least-squares means and standard error of the difference (s.e.d.) for c.*1232G > A (MM) carrier and non-carrier lambs for in-plant and VIA carcass traits (difference (%diff), when the MM non-carriers used as denominator, is also shown for each variable)

Trait [§]	Description	Texel sired lambs (exp1)					Poll Dorset sired lambs (exp2)				
		NC [†]	C	s.e.d.	% diff	Sig [*]	NC	C	s.e.d.	%diff	Sig
MLC traits											
N		80	70				128	39			
CCWT (kg)	Cold carcass weight	16.8	17.4	0.397	3.3	ns	17.6	17.4	0.416	-1.4	ns
MLC-C	MLC carcass conformation	2.73	2.75	0.068	0.9	ns	2.48	2.53	0.089	1.9	ns
MLC-F	MLC carcass fat class	10.6	9.92	0.286	-6.6	*	10.9	10.2	0.290	-6.5	*
KO%	Killing out percentage	44.2	44.1	0.003	-0.2	ns	44.7	45.0	0.329	0.7	ns
VIA traits											
N		79	68				125	36			
SMY (kg)	Saleable meat yield	7.545	7.523	0.022	-0.3	ns	7.702	7.663	0.027	-0.5	ns
TP-Loin (kg)	Loin trimmed primal weight	1.381	1.389	0.017	0.6	ns	1.412	1.403	0.020	-0.6	ns
TP-Chump (kg)	Chump trimmed primal weight	0.794	0.787	0.005	-0.8	ns	0.813	0.804	0.005	-1.2	ns
TP-Leg (kg)	Leg trimmed primal weight	3.274	3.283	0.018	0.3	ns	3.268	3.301	0.023	1.0	ns
TP-Breast (kg)	Breast trimmed primal weight	1.487	1.505	0.016	1.2	ns	1.507	1.521	0.015	0.9	ns
TP-Shoulder (kg)	Shoulder trimmed primal weight	3.776	3.769	0.019	-0.2	ns	3.798	3.802	0.019	-0.1	ns

[†] NC and C = Non-Carriers and Carriers for MM

^{*} Sig = significant effect (ns = not significant, * = P < 0.05, ** = P < 0.01, *** = P < 0.001)

[§] MLC-C is the conformation score (EUROP) transformed to a numeric scale: E (excellent) = 5 to P (poor) = 1. MLC-F is the fat class (1, 2, 3L, 3H, 4L, 4H, 5) transformed to a subcutaneous fat percentage as described by Kempster et al. (1986), KO% is the carcass weight divided by the pre-slaughter live weight. SMY is predicted by a distinct prediction equation.

3.5 Discussion

This study quantified the effects of the ovine myostatin mutation (MM) c.*1232G > A, segregating in both Texel- and Poll Dorset-sired lambs, on various traits relevant to lamb meat production. Effects of MM that may be beneficial from the producer's perspective are the observed increased muscle mass and reduction in fatness. However, since the mutation is almost fixed in the UK Texel breed (Hadjipavlou et al. 2008) further characterisation of possible pleiotropic effects on other economic or functional traits, such as those associated with meat quality, reproduction, disease resistance and lamb survival, should be considered to better understand the implications of future industry use of MM in Texels or in other terminal sire breeds and help breeders to consider any unfavourable results when developing their breeding programmes. Quantification of direct and indirect effects of the mutation also enables evaluation of its economic impact, especially in comparison to other ovine myostatin mutations.

3.5.1 Muscle-related traits

The results of this study are in agreement with other results (Kijas et al. 2007; Lambe et al. 2009a) that MM had no effect on live weight or growth pattern.

The findings from the main experiment (exp 1) showed that lambs carrying one copy of MM had *M. longissimus lumborum* (MLL) that were significantly deeper with greater cross-sectional area than non-carriers, but there was no difference in MLL width. This is in agreement with the results of Kijas et al. (2007), although Johnson et al. (2009) found that MM was associated with significant wider MLL with greater area, but that MLL depth was unaffected in heterozygous lambs.

As reported by Kijas et al. (2007) using Texel-sired crossbred lambs out of Merino ewes, no significant effect of MM on UMD was found. However, a significant effect of MM on UMD was reported in 21 weeks old commercial purebred Charollais lambs in the UK by Hadjipavlou et al. (2008). Ultrasound scanning was not able to detect the phenotypic effects associated with MM, in the current study on crossbred lambs, whereas corresponding CT traits did show significant difference between genotypes. This suggest that selection for leanness and muscle growth in a

population segregating for MM using ultrasound scanning may not efficiently distinguish between heterozygous carriers and wildtype animals, at least in crossbred lambs such as those used in the current trial. This may be due to the mode of inheritance of MM, as it is partially recessive (Clöp et al. 2006; Hadjipavlou et al. 2008), and/or experimental power. Assuming that CT scanning is a more accurate assessment of carcass muscling, it might be possible that ultrasound measures in a larger population may identify a significant difference. This study did not involve lambs homozygous for MM and does not allow the assessment of whether homozygous animals could be differentiated by ultrasound scanning.

Of particular interest in the current study were the consistent findings of increased muscle mass in the MM carrier lambs relative to non-carriers, which is in agreement with other authors (e.g. Kijas et al. 2007 and Johnson et al. 2009). Despite the significantly higher muscle weights and proportions and higher muscle to bone ratio in carrier groups, no associated differences in MLC conformation grade or CCWT were identified. Producers who are paid under the current UK grading system may only benefit from the identified changes in MLC fat class.

In contrast, a different ovine *MSTN* mutation (c.960delG), identified in Norwegian White sheep was found to have a significant effect on MLC-C assessed using the EUROP classification scheme (Boman et al. 2009). It is not known whether this mutation segregates in UK Texel sheep.

3.5.2 Fat-related traits

The results presented here, from exp 2 in particular, verify the reported association of MM with reduced fat in the carcass and within different carcass regions. Studies by several groups have demonstrated an effect of MM on carcass fat measurements, with MM carrier lambs having lower fatness levels, as measured on traits such as UFD (Hadjipavlou et al. 2008; Kijas et al. 2007) and loin, hind leg and shoulder fat depths and total fat weight estimated by CT (Johnson et al. 2009). It is of note that all lambs, in both experiments, were CT scanned 4 weeks pre-slaughter, when fatness levels in these crossbreds were still relatively low. Therefore, further changes in fatness may be expected between this point and slaughter, which may not be consistent between genotypes. However, it is known that the pattern of overall live

weight gain during this period did not differ between these genotypes (Lambe et al. 2009a).

The difference in CT-predicted fat weight between carrier animals and non-carriers in exp 1 of the current study is nearly half of the -11.1% that reported by Johnson et al. (2009), and was not significant. However, the magnitude of the effect on fat weight in exp 2 (-13.8%) was similar to that found by Johnson et al. (2009). However, the experimental design does not allow us to determine whether the apparent discrepancy is due to genetic background, environment or chance.

The significant difference between MM carriers and non-carriers in subjectively-assessed MLC fat class in both experiments implies that producers may benefit from using rams carrying MM, since MM carrier lambs would be less likely to attract penalties for over-fatness or could be taken to higher carcass weights without becoming over-fat. This suggestion assumes no accompanied deterioration in carcass conformation, which is reasonable given the lack of significant effects on conformation found in this study. The lower levels of subcutaneous fat found in MM carriers may also have advantages to the meat processing industry, because less unwanted fat has to be trimmed prior to retail sale, resulting in lower costs (Gardner et al. 2006). It should be noted that the crossbred lambs studied, in both experiments, were slaughtered at a constant age, not at a constant level of finish, as is typical in commercial production, which in turn could affect the magnitude of fat class differences. To our knowledge, no formal information has been published previously on whether animals carrying MM differ from non-carriers on the basis of MLC classification scores. However, a publication in a technical newsletter presented a figure illustrating that single copy carriers of MM had lower fat class scores compared to non-carrier lambs (Thomas 2008). However, average carcass weight and killing ages were not presented to allow further comparisons.

3.5.3 Meat quality-related traits

Meat quality has not been directly assessed in this study, but average muscle density measured by CT was significantly higher in MM carriers compared to non-carriers. Although the magnitude of the difference between genotypes appears to be small, it is indicative of a reduced IMF content, which could affect meat quality. High

negative phenotypic and genetic correlations between CT muscle density and IMF were reported, for example, by Karamichou et al. (2006b) in lambs of a different breed. Lower IMF content is linked with lower tenderness, juiciness and flavour and hence palatability (Savell & Cross 1988). The higher CT muscle density in MM carrier lambs therefore implies that MM could negatively affect meat quality of the carrier lambs. Kijas et al. (2007) found that the presence of MM was associated with a significant negative effect on IMF, which resulted in a decrease in perceived eating quality and lower consumer preference. The current selection goals towards increasing lean muscle mass and decreasing fat, along with the high frequency of MM in UK Texel sheep, imply that consideration should be given to maintaining IMF through breeding programmes, to avoid any deleterious effects on meat quality.

3.5.4 VIA- related traits

There has been increased interest from retailers in objective grading systems to replace the MLC grading system, because of its inconsistency and biased evaluation of carcass quality (Allen 2005). Payment of a premium for animals with better carcass quality may help to ensure a consistent supply of high-quality product (Jones et al. 2003). VIA is one such technology and it offers significant potential for automatically predicting meat yield, fatness, shape and individual cut yield. In this study there were no significant effects of MM on any of the VIA measured traits. (Johnson et al. 2009) reported that MM significantly increased shoulder lean yield percentage and total lean yield estimated by VIA scan. That there was no effect found in the present study could be due to differences in experimental conditions, for example, age or weight of lambs at slaughter, or technical differences, such as the VIA system used, number of images captured or prediction equations used.

In Poll Dorset-sired crossbred lambs from exp 2, VIA was found to be capable of detecting the effect of LM-QTL on the predicted trimmed weight of the loin cut (+2.2%) (Masri et al. 2010) so the VIA system used in these experiments is capable of detecting quite small differences. Better estimations of carcass primal weights could be obtained in future experiments if VIA prediction equations are further refined.

3.5.5 Mode of inheritance

There is some evidence from several studies suggest that the MM mode of inheritance in sheep seems to be partially recessive (Clöp et al. 2006; Hadjipavlou et al. 2008). Other research groups reported an additive mode of action in some traits and partially recessive in the others (Bain et al. 2008; Johnson et al. 2009). This study has shown that there are differences in some traits between heterozygote carriers and non-carriers but no inference on the mode of inheritance of MM can be made from this work since the experimental population did not contain any homozygote carrier animals. Producing animals carrying 2 copies of MM within the framework of the UK sheep industry would also require introgression of MM into the maternal lines used to breed slaughter lambs (e.g. Mules), or, if it is already segregating, selection to increase its frequency.

In other species (cattle and mice), the genetic background has been shown to influence the magnitude of the phenotypic effects of myostatin mutations (Wiener et al. 2002; Short et al. 2002; Bunger et al. 2004; Varga et al. 2003). Although this study was not designed to test the interaction between the genotype and genetic background, it is possible that there is an effect of the genetic background, particularly for fatness traits, as the magnitude of the effect in some cases differed between Texel- and Poll Dorset-sired lambs.

3.6 Conclusion

This study showed that carrying a single copy of c.*1232G > A results in greater muscle mass and lower fat levels in crossbred lambs sired by Texel sires and in crossbred lambs sired by Poll Dorset rams. The current UK carcass grading and payment system, based on the subjective EUROP scoring system and carcass weight, is unlikely to reflect the magnitude of the increased muscling observed in these heterozygous lambs. VIA also could not detect differences in SMY or muscularity between MM carrier and non-carrier lambs. However, the significant effects on fat class seen here may result in MM carrier lambs being penalised less for being overfat or enable MM carrier lambs to be taken to higher carcass weight without becoming overfat which would benefit lamb producers. Other studies have suggested that this mutation is likely to be partially recessive, therefore UK breeders may not obtain

maximum benefit of the mutation if slaughter lambs produced are heterozygous for MM. In order for UK producers to benefit fully from this mutation, it is likely that introgression into, or selection for the mutation, in maternal lines, would be required. The increased CT muscle density in MM carrier lambs and its possible consequences for meat quality should be given further attention in the future breeding programmes.

Chapter 4

**Evaluation of the effects of the c.*1232G > A mutation and TM-QTL in
Texel x Welsh Mountain lambs using ultrasound and video image analyses**

Abstract

The c.*1232G > A (MM) mutation in the myostatin gene and TM-QTL are two muscle-enhancing polymorphisms found on Chr 2 and Chr18, respectively, in Texel sheep. Previous studies on purebred lambs and crossbred lambs out of Mule ewes showed that MM was associated with increased muscle mass and decreased fat content whereas heterozygous TM-QTL lambs had increased loin muscle (*M. longissimus lumborum*, MLL) depth as measured by ultrasound and increased MLL weight. Welsh Mountain ewes, like Mule ewes, contribute significantly to UK slaughter lamb production from the UK sheep industry's stratified structure. Therefore, this study aimed to evaluate the direct effects of MM and TM-QTL in the genetic background of Texel x Welsh Mountain lambs.

Four Texel sires, all MM carriers and two TM-QTL carriers, were used to inseminate Welsh Mountain ewes. Of the 175 lambs born, 130 could be classified for TM-QTL genotype [107 non-carriers (+/+), 23 heterozygous carriers (TM/+)] and 172 for MM genotype [15 non-carriers (+/+), 129 heterozygous carriers (MM/+), 28 homozygous carriers (MM/MM)]. All lambs were weighed at 8 and ~23 weeks of age and were ultrasound scanned before being slaughtered at ~24 wk of age. All carcasses were graded for conformation and fat class, based on Meat and Livestock Commission (MLC) classification scores, and then scanned using a video image analysis (VIA) system to predict weight of carcass primal cuts and trimmed primal cuts, muscularity traits and carcass shape traits.

Carrying two copies of MM was associated with a significant positive effect on 8 week weight, a negative effect on ultrasound fat depth and a substantial decrease in MLC-fat score. Two copies of MM also had a strong positive impact on VIA-estimated weight of the hind leg, chump and loin primal cuts, as well as the muscularity of the hind leg and loin regions with greater loin muscle width, depth and area. Homozygous MM carrier lambs had significantly wider carcasses across the shoulders, breast and hind legs and greater areas of the back view of the carcass when measured by VIA. Leg and carcass compactness were also significantly associated with the presence of two copies of MM. However, lambs carrying a single copy of MM were not significantly different to non-carriers in any of the traits measured. TM-QTL significantly increased ultrasound muscle depth and TM-QTL

carriers had significantly greater loin muscle width and area measurements. Carcass weight did not differ significantly between genotypic classes for MM or TM-QTL. These results agree in general with other reports of the effects of MM and TM-QTL, in both direction and magnitude, on carcass traits.

4.1 Introduction

Sheep are an important part of the global agricultural economy. However, UK lamb meat production and consumption per capita has declined over the last few decades (MLC 2009). All sectors in the lamb meat production chain need to react and try to halt this decline in consumer consumption, and need to consider that consumers have become more aware of health concerns related to fat content, by producing cuts of high quality lean meat with low fat levels. Genetic improvement schemes adopted in the UK have been effective at improving lean tissue growth rate and carcass quality among terminal sire breeds (Macfarlane & Simm 2008; Simm & Dingwall 1987), resulting in increased carcass leanness in the UK slaughter lamb crop (Simm et al. 2002). Unfortunately, the uptake of these schemes by the industry has been slow (Amer et al. 2007). In the past two decades, advances in molecular techniques have provided evidence of genes with major effects on many body growth and carcass composition traits (Cockett et al. 2005). The complementary role of integrating molecular and quantitative information would, unequivocally, enhance rates of genetic improvement in sheep by selecting animals carrying favourable genes through Marker Assisted Selection (MAS) (Campbell & McLaren 2007; Dekkers 2004).

Molecular genetic studies in sheep have led to a limited number of discoveries of quantitative trait loci (QTLs) associated with growth and muscling traits (Raadsma et al. 2009; Walling et al. 2004). Few causative mutations that influence muscle growth and carcass composition have been successfully identified in the sheep genome. This is because, to be detectable, QTLs have to have a certain magnitude of effect (moderate to strong). Most QTL detection studies are not powerful enough to detect QTLs of small effects. However, one genomic segment in *Ovis aries* chromosome 2 (OAR2), the myostatin encoding gene (*MSTN*), has been reported to harbour 23 Single Nucleotide Polymorphisms (SNPs) (Hickford et al. 2010). Several of these SNPs (e.g. c.*1232G > A mutation in Texel (Clop et al. 2006), c.960delG in Norwegian White Sheep (Boman et al. 2009) and the allelic variants in a 473-bp section of the exon 1- intron 1 region (Zhou et al. 2008) have been reported to be associated with enhanced muscle growth. Before these suggested causative mutations were identified there were reports of a QTL in a Texel-based population, where the

QTL presumably encompassed the myostatin gene and its polymorphisms. It has been found that this QTL was associated with a desirable increase in muscle mass and decrease in fat deposition (Broad et al. 2000; Johnson et al. 2005a; Laville et al. 2004; Marcq et al. 2002). At the molecular level, two biallelic SNPs g+6723G-A (G > A transition) and g-2449C-G in the 3' untranslated region (UTR) were found to be the underlying major causative mutations (Clop et al. 2006), and a more recent study pointed out that this SNP was initially designated incorrectly and the correct one is the g.6223G > A SNP which should be called c.*1232G > A under the recommended nomenclature system (<http://www.genomic.unimelb.edu.au/mdi/mutnomen/>) (Hickford et al. 2010). This SNP is considered to be the most probable causal polymorphism responsible for improved muscling characteristics (Johnson et al. 2009). Within British commercial Texel sheep, it has been reported that the allelic frequency of this “G > A SNP” is nearly at fixation level and that it appears to follow a partially recessive inheritance pattern (Clop et al. 2006; Hadjipavlou et al. 2008). Similar high frequencies have been observed in Australian Texel sheep (Kijas et al. 2007). The c.*1232G >A SNP test (abbreviated as MM in this study) is now being used commercially, and replaced the initial microsatellite-based test for the corresponding QTL. An evaluation study was carried out to quantify the effect of a single copy of MM on carcass composition in Texel- and Poll Dorset-sired crossbred lambs out of Scottish Mule ewes (Masri et al. 2011a). Using computer tomography (CT) scanning of live lambs, this earlier study revealed an increase in *M. longissimus lumborum* (MLL) depth and area, muscle to bone ratio and total muscle area in two-dimensional scans taken at three cross-sectional positions (chest, loin and leg) in MM carriers compared to non-carriers. In that study, fatness was lower in MM carrier animals, compared to non-carriers, with lower ultrasound fat depth (UFD), fat areas in CT cross-sectional scans and MLC-fat classes (Masri et al. 2011a). Crossbred lambs carrying a single copy of the QTL, comprising the MM SNP, produced from a cross between Texel sires and Romney- or Coopworth-based dams, had a 3% increase in leg muscle and a 10% decrease in leg fat compared to non-carriers (Johnson et al. 2005a).

TM-QTL is another QTL that affects muscling, which has been mapped to the distal end of chromosome 18 and is estimated to be ~2 cM telomeric of marker CSSM18 in

purebred Texel sheep (Walling et al. 2004). TM-QTL is positioned amongst a cluster of other QTL/genes (including *Callipyge* and Carwell QTL (LoinMAXTM) that have been found to affect muscle mass in sheep (Banks 1997; Cockett et al. 1994). A single copy of TM-QTL in purebred Texel lambs resulted in 4 to 7 % greater ultrasound muscle depth (UMD) compared to non-carriers (Walling et al. 2004). Similarly, crossbred Texel x Scottish Mule lambs that were heterozygous for TM-QTL were found to have 4.5 % deeper loin muscle measured by ultrasound, a difference that increased to 7% (due to the high measurement accuracy compared to US) when measured by CT scanning compared to non-carriers (Macfarlane et al. 2009a). Moreover, TM-QTL also increased the loin muscle width and area measured by CT (+3% and +5%, respectively), and the average dissected weight of the loin (7%), which indicates that the TM-QTL effect is mainly on the loin region (Macfarlane et al. 2009a). The mode of inheritance of TM-QTL has been investigated in purebred Texel lambs, and shows evidence of a polar overdominance pattern and imprinting (paternally expressed) (Macfarlane et al. 2010). This means that heterozygous animals inheriting the TM-QTL from the sire only would show the phenotype whereas maternal heterozygotes, homozygous carriers or non-carriers would not.

The existence of MM and TM-QTL in Texel sheep, which is one of the main terminal sire breeds used for slaughter lamb production in the UK (Pollott & Stone 2006), may be used to increase productivity and profitability in the lamb industry, through improving the yield of highly priced muscles in the carcass. This can be done by developing efficient breeding programmes that take account of the stratified structure of the sheep industry in the UK. Although the effects of MM and TM-QTL have been evaluated in other crosses, the importance of the cross between Texel rams and Welsh Mountain ewes to numbers of UK slaughter lambs (Pollott & Stone 2006) means that it is also necessary to evaluate the effects of MM and TM-QTL in lambs out of Texel x Welsh Mountain crosses. In the light of these results, it is also vital to assess any indirect effects on other production, behavioural and welfare-related traits.

The aim of this study was to use *in vivo* ultrasound scanning, MLC carcass classification and video image analysis (VIA) to evaluate the effects of MM and TM-QTL on carcass traits in Texel x Welsh Mountain crossbred lambs.

4.2 Materials and methods

4.2.1 Experimental animals

All procedures involving animals were approved by Aberystwyth University animal ethics committee. Welsh Mountain ewes (n = 400), on one commercial hill farm in Wales, were inseminated with semen from four rams. The total number of lambs used in this experiment for statistical analysis, with full records was 175 male castrated lambs. Due to the nature of the commercial flock there were no pedigree records available on the dams, and ewe lambs were retained as replacements for breeding and hence they were unavailable for slaughter. Lambs were tagged and identified to their dams within 24 hours of birth, and were born as singletons (n = 56), twins (n = 107), triplets (n = 11) and one as quadruplet. All lambs were grazed on pasture with their dams. Age of the ewes was not recorded, so could not be included in the statistical analyses. Lambs were weighed at 8 weeks of age (8-WKWT) and weaned at approximately 18 weeks of age, when the selected male lambs were transported to the research farm at Aberystwyth University for finishing. Lambs were then weighed and ultrasound scanned at approximately 23 weeks of age, before being transported to the Welsh Country Foods abattoir on Anglesey for slaughter in the following week.

4.2.2 Genotypic classes

Shortly after birth, all lambs studied were blood sampled using the nose-prick technique. Blood samples were spotted onto FTA[®] cards and sent to Catapult Genetics, New Zealand (now Pfizer Animal Genetics) for genotyping. The commercially available MM genotyping test (MyoMAX[®], <http://www.pfizeranimalgenetics.com/sites/PAG/aus/Pages/MyoMAX.aspx>) is based on identifying animals carrying the c.*1232G >A SNP, and so is very accurate and relatively easy to perform. Since the causal mutation for TM-QTL has not yet been identified, TM-QTL genotyping was based on microsatellite markers. A detailed

description of the TM-QTL genotyping process has been given in a previous paper (Macfarlane et al. 2009a). In brief, the sires used in this study were known to inherit TM-QTL haplotype markers, five microsatellite markers (CSSM18, MCMA26, OARTMR1, OY5 and OY3), from their own sires that showed high QTL- estimated breeding values (EBVs) for ultrasound muscle depth. The marker data from all lambs sired by the TM-QTL carriers sires used in this study were edited for marker inconsistencies and then evaluated using R-tools software to determine the haplotype of marker alleles inherited. Data from all sires, haplotypes specifically from sire families, were then subjected to variance component estimation procedures and EBVs for muscle depth were then calculated by using the phenotypic data and the pedigrees. The proportions of genes identical-by-descent (IBD) between all individuals at the QTL location were estimated using deterministic method (Pong-Wong et al. 2001). Combining the phenotypic information and marker data facilitated the partitioning of the EBVs into background (polygenic) and QTL components using MA-BLUP (Fernando & Grossman 1989). Further decomposition of the QTL-EBVs into the component inherited from the sire and the component inherited from the dam was carried out and allowed matching the sire haplotype with its QTL-EBV. Lambs carrying the exact haplotype associated with TM-QTL in that particular sire family that also had a high sire QTL-EBV were assigned carrier status. Lambs that carried the haplotype inherited from the sire that was not associated with the QTL (i.e. that inherited from paternal grand-dam) and had a low QTL-EBV were classified as non-carriers. Lambs with recombinant haplotypes that could not be identified clearly as being either carrier or non-carrier, and lambs with missing marker information, were classified as unknown. In a few instances recombinant haplotype lambs had very high sire QTL-EBVs and haplotype close to that associated with TM-QTL or a very low sire QTL-EBVs and a haplotype close to that not associated with TM-QTL. In these cases carrier or non-carrier status were assigned. Genotyping results showed that three sires used were homozygous MM carriers and one heterozygous MM carrier, and two of these sires were heterozygous for TM-QTL (sire 1 = MM MM/ + +; sire 2 = MM +/+ +; sire 3 = MM MM/TM +; sire 4 = MM MM/TM +). Moreover, unexpectedly, a number of lambs were

classified as homozygous MM carriers, which indicates that some of the Welsh Mountain dams were MM carriers.

Of the 175 lambs studied, 130 could be unequivocally classified for TM-QTL genotype: 107 non-carriers (+/+) and 23 heterozygous carriers (TM/+); with 45 lambs assigned as unknown (Table 4.1), due to a lack of informative marker information or recombination. For MM, 3 animals could not be genotyped, so classification was available only for 172 lambs: 15 non-carriers (+/+), 129 heterozygous carriers (MM/+), and 28 homozygous carriers (MM/MM).

Table 4.1 Distribution of c.*1232G > A (MM) and TM-QTL carrier status by sire.

Sire (genotype)	TM status ^a	MM status ^b			Unassigned	Total	Grand total
		+/+	MM/+	MM/MM			
1 (MM MM/+ +)	+/+	0	37	5	0	42	65
	TM/+	0	0	0	0	0	
	TM/?	0	14	6	3	20	
	Total	0	51	11	3		
2 (MM +/+ +)	+/+	12	11	2	0	25	34
	TM/+	0	0	0	0	0	
	TM/?	3	6	0	0	9	
	Total	15	17	2	0		
3 (MM MM/TM +)	+/+	0	10	5	0	15	30
	TM/+	0	10	1	0	11	
	TM/?	0	3	1	0	4	
	Total	0	23	7	0		
4 (MM +/TM +)	+/+	0	22	3	0	25	46
	TM/+	0	9	3	0	12	
	TM/?	0	7	2	0	9	
	Total	0	38	8	0		
Grand		15	129	28	3	175	

^a TM: carrier for 1 copy of TM-QTL; +: non-carrier; ?: genotype unknown

^b MM: carrier for 1 copy of c.*1232G > A; +: non-carrier

4.2.3 Traits measured by ultrasound

Live body weight and ultrasound measurements, taken over the 3rd lumbar vertebra, were recorded for all lambs at approximately 23 weeks of age. Live body weight (US-LWT) averaged 40.1 kg (range 26 kg - 58 kg). While lambs were in a standing position, the ultrasonic muscle depth (UMD) and ultrasonic fat depth (UFD) were measured using a Dynamic Imaging Concept MLV (Caiyside Imaging Ltd) ultrasound scanner with a 3.5 MHz transducer. Positioning the probe perpendicular to the spine, one UMD measurement was taken at the deepest point of the MLL. Three fat depths were measured, transverse to the spine, the first at the same position as the muscle depth measurement and the others at 1 and 2 cm intervals lateral to the first position. The average of the three measurements was used in the analysis.

4.2.4 Traits measured post mortem

At approximately 24 weeks of age lambs were electrically stunned, slaughtered and conventionally skinned according to the regular commercial procedures in UK abattoirs. Hot carcass weight was recorded following the removal of kidneys and kidney fat, and the cold carcass weight (CCWT) calculated on the abattoir electronic scale by deducting a constant 0.5 kg from the hot carcass weight, as an expected drip loss value. Each carcass was visually graded for conformation and fat class, according to the subjective MLC classification scheme based on the EUROP grading system. Conformation classes were then coded numerically as E = 5, U = 4, R = 3, O = 2, P = 1. Fat class (MLC-F) were converted to a numeric system to correspond with estimated subcutaneous fat percentage (1 = 4%, 2 = 8%, 3L = 11%, 3H = 13%, 4L = 15%, 4H = 17% and 5 = 20%) (Kempster et al. 1986).

Killing out percentage (KO%) was calculated as the CCWT as a percentage of the pre-slaughter live weight.

4.2.5 Traits measured by video image analysis (VIA)

Following MLC grading, carcasses were moved off the main slaughter line to the VIA room for scanning using the VSS2000 video image analysis system (VSS2000, E+V Technology GmbH, <http://www.vision-for-you.com/start.htm>). All carcasses, were hung with hind legs spread apart (gambrel) and shoulders un-banded, and

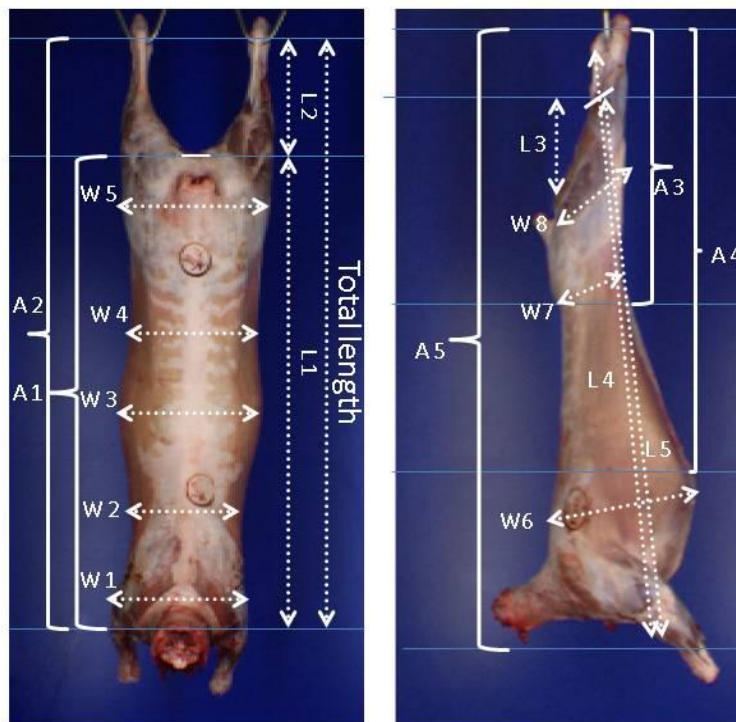
passed by, except one lamb with a damaged carcass, two cameras installed to function consistently with the slaughter line speed, to reflect the online conditions in the abattoir (800 carcasses/hour). Cameras captured two images, one for the lateral view (left side) and the other for the dorsal view of each carcass. Raw data outputs were directly transferred to a computer supported with image processing software (VSS2000). Further details on how the VIA system works can be found elsewhere (Rius-Vilarrasa et al. 2009b). In short, carcass dimensional measurements, including linear lengths and widths, as well as areas, are automatically measured in different carcass regions. Measurements obtained are used along with CCWT, in computer algorithms, to produce variables that describe carcass shape, size and yield. Subsequently, these variables are used by the VIA manufacturer (E+V Technology GmbH) to estimate objective predictions for weights of saleable meat yield (SMY) in the carcass and in different primal joints (Leg, Chump, Loin, Breast, Shoulder). Two types of predicted weights were given: for the primal joints (P-joint; with bones) and for the trimmed primal joints (TP-joint; de-boned). However, predicted trimmed weight of the breast joint was not available, because the de-boning process for this joint is very difficult and is not usually done commercially. VIA prediction equations were derived and validated under British abattoir conditions (Rius-Vilarrasa et al. 2009b). Those used in this study had been further refined by calibrating the VIA system against CT measurements in the loin region (Rius-Vilarrasa et al. 2009c).

Muscularity traits predicted by VIA, originally estimated in live lambs using spiral CT scanning, were studied in a previous study (Rius-Vilarrasa et al. 2009c). These muscularity traits include: the total muscle volume in the loin region (LRMV) and the hind legs (HLMV); muscularity indices that relate muscle volume to the length of the bone that it surrounds in the loin (LRMI) and hind leg (HLMI) (Navajas et al. 2007); MLL muscle width, depth and area (MLL-VIAW, MLL-VIAD and MLL-VIAA, respectively).

In addition to the estimated weights of the carcass primal cuts, a set of carcass dimensions comprising 8 widths (5 from the dorsal and 3 from the lateral image), 6 lengths (3 and 3), 5 areas (2 and 3) were provided by VIA (Figure 4.1). Some of those were used, among others, in the prediction equations derived by E+V

Technology, GmbH. Furthermore, functions of these measurements determining leg compactness (Leg-COMP) and carcass compactness (Carc-COMP) were also calculated from the corresponding VIA linear measurements, as explained earlier (Rius-Vilarrasa et al. 2010).

Fig 4.1: Dorsal and side carcass views and description of measurements. W1: Maximum shoulder width [dorsal view]; W2: minimum breast width [dorsal view]; W3: Maximum breast width [dorsal view]; W4: Minimum loins width [dorsal view]; W5: Maximum rump width [dorsal view]; W6: Maximum breast depth [side view]; W7: Minimum loin depth [side view]; W8: Maximum rump depth [side view]; L1: Length buttocks to shoulder [dorsal view]; L2: Length hock to buttocks [dorsal view]; L3: length between the tibia and the end of the leg [side view]; L4: length from the hock to the front of the shoulder [side view]; L5: Length from tibia to the front of shoulder; A1: Back area of the carcass from shoulder to buttocks; A2: Back area of the whole carcass; A3: Side area from loin to hock; A4: Side area of the saddle and hind legs; A5: side area of the whole carcass.



4.2.6 Statistical Analysis

Data were analysed by fitting a general linear model (PROC GLM) in the SAS package for Windows, Release 9.1 (SAS Inst., Inc., Cary, NC, USA). Analyses were performed on animals that had valid data for all the variables studied ($n = 172$ and 174 ; for MM and TM-QTL, respectively). All data, including that from lambs of unknown genotype status for TM-QTL, were used in the analyses to increase the accuracy of estimation of other fixed effects in the model, but least-squares means were only calculated for the known TM-QTL genotype classes, along with the results for the three MM genotype classes.

The statistical model used was:

$$Y_{ijkln} = \mu + s_i + m_j + t_k + r_l + w_{ijkl} + \varepsilon_{ijkln}$$

Y_{ijkln} is the performance of the n th individual lamb ($n = 1, 2, 3 \dots 174$) for each trait of interest, with μ as a general mean; s_i = fixed effect of the i th sire ($i =$ sire 1, 2, 3 or 4); m_j = fixed effect of the j th MM genotypic status ($j =$ MM non-carrier (+/+), MM heterozygous carrier (MM/+) or MM homozygous carrier (MM/MM)); t_k = fixed effect of k th TM-QTL genotypic status ($k =$ TM-QTL heterozygous carrier, TM-QTL homozygous non-carrier or unknown TM-QTL genotypic status); r_l = fixed effect of the l th litter size ($l =$ lambs born in litters of 1, 2 or 3 where the only lamb with a quadruplet litter size was included in the triplet litter size class); w_{ijk} = live weight at the time of measurement (for the *in vivo* measurements) or carcass weight (for the carcass measurements) of the n th individual. An error term (ε) was also fitted in the model for each trait.

The interaction between MM and TM-QTL was also tested in the model, but was found to be non significant ($P > 0.05$) for almost all of the traits, with the exception of P-Leg, and so was fitted in the model for this trait, but not for all other traits. While age at US scanning or at slaughter showed no significant effects when fitted as a covariate, live weight and carcass weight were found to be significant and were included in the model as a linear covariate for live and post-mortem traits, respectively. However, weight was not fitted as a covariate in the models where the Y variable was live weight (8-WKWT or US-LWT) or carcass weight (CCWT). For

VIA-dimensional measurements of carcass lengths, widths and areas, the variables reported were not adjusted for CCWT, in order to look at the true magnitude of the QTL/gene mutation effect on the traits of interest.

It was also of interest to run another set of analyses to look at the estimates of the additive effect (a) and the dominance effect (d) of MM, since all three MM genotypic classes were produced in this trial, albeit with limited numbers especially in the +/+ class. To do so, within the models fitted above, a contrast statement in SAS was used

to mimic the equations $a = \frac{1}{2}(+/+ - MM / MM)$ and

$$d = MM /+ - \left(\frac{+/+ + MM / MM}{2} \right).$$

4.3 Results

4.3.1 MM and TM-QTL effects on live weight and ultrasound tissue depths

Lambs classified as MM/MM had higher body weight (ca. 1.7kg) at 8 weeks of age than lambs classified as either MM/+ or +/+, but this difference was significant ($P < 0.05$) only for the MM/MM versus MM/+ comparison. No significant differences in US-LWT were observed between the three MM genotypic groups. While UMD did not significantly vary between MM genotype classes, MM/MM animals had significantly lower UFD than MM/+ and +/+ groups (-17% and -26%, respectively) (Table 4.2).

TM-QTL carrier lambs had a significantly deeper loin muscle than non-carriers when measured by ultrasound (+ 5.7%; Table 4.2). However, lambs carrying a single copy of TM-QTL did not differ significantly from non-carriers in UFD, or in 8-WKWT or at ultrasound scanning.

Table 4.2 Least-squares means for c.*1232G > A (MM) and TM-QTL genotypic classes for live weights and ultrasound measurements ^{†,‡}.

Trait [§]	+/+	MM/+	MM/MM	Mean s.e.d. [¥]	+/+	TM/+	s.e.d.
8-WKWT (kg)	17.65 ^{ab}	17.63 ^b	19.30 ^a	0.816	18.26	18.03	0.721
US-LWT (kg)	39.45	39.84	41.14	1.535	40.36	39.82	1.357
UMD (mm)	23.2	22.9	23.6	0.636	22.9 ^b	24.2 ^a	0.562
UFD (mm)	3.05 ^a	2.70 ^a	2.25 ^b	0.250	2.81	2.54	0.220

[†] Values, within row within gene or QTL, sharing a common character in their superscript are not significantly different ($P > 0.05$).

[‡] Pairwise contrasts between each pair of classes within each gene or QTL.

[§] 8-WKWT is the animal live weight at 8 weeks of age. US-LWT is the animal live weight at ultrasound scanning. UMD and UFD are ultrasonically measured muscle and fat depths, respectively.

[¥] Mean s.e.d.: mean standard error of the difference; averaged over the 3 pairwise comparisons.

4.3.2 MM and TM-QTL effect on post-mortem traits

CCWT, MLC-C and KO% were not significantly affected by the presence of MM and/or TM-QTL (Table 4.3). Homozygous carrier lambs of MM were classified as having significantly lower MLC-F than MM/+ and non-carrier lambs (-14% and -21%, respectively).

Table 4.3 Least-squares means for c.*1232G > A (MM) and TM-QTL genotypic classes for post mortem traits ^{†‡}.

Trait [§]	+/+	MM/+	MM/MM	Mean s.e.d. [¥]	+/+	TM/+	s.e.d.
CCWT (kg)	17.03	17.04	17.82	0.790	17.43	17.12	0.700
MLC-C	3.03	2.92	3.03	0.158	3.01	3.02	0.140
MLC-F	9.15 ^a	8.42 ^a	7.23 ^b	0.661	8.46	8.10	0.584
KO%	44.3	43.7	44.3	0.645	44.1	44.0	0.570

[†] Values, within row within gene or QTL, sharing a common character in their superscript are not significantly different ($P > 0.05$).

[‡] Pairwise contrasts between each pair of classes within each gene or QTL.

[§] CCWT is the cold carcass weight, MLC-C is the conformation score (EUROP) transformed to a numeric scale: E (excellent) = 5 to P (poor) = 1.

MLC-F is the fat class (1, 2, 3L, 3H, 4L, 4H, 5) transformed to a subcutaneous fat percentage as described by Kempster et al. (1986), KO% is the killing out percentage calculated as the carcass weight divided by the pre-slaughter live weight multiplied by 100.

[¥] Mean s.e.d.: mean standard error of the difference; averaged over the 3 pairwise comparisons.

4.3.3 MM and TM-QTL effects on VIA traits

4.3.3.1 VIA-estimated saleable meat yield, primal and trimmed primal joint weights

The VIA-estimated weights of SMY, primal (P) and trimmed primal (TP) joint weights in each genotypic class of MM and TM-QTL are given in Table 4.4. VIA-estimated weight of SMY was not significantly different between MM genotypic classes, or between TM-QTL carrier and non-carrier lambs.

Compared to MM/+ and +/+ groups, MM/MM lambs had significantly increased VIA-estimated weights of P-Leg (by ~3% for both comparisons), TP-Leg (by 4% and 6%, respectively) and TP-Chump (by 3% and 5%). VIA also estimated a significant increase in TP-Loin (4%) for MM/MM compared to MM/+. The remaining VIA-estimated weights of the primal and trimmed primal joints were not affected by MM.

P-Leg differed significantly between TM-QTL carriers and non-carriers (Table 4.4), with an increase of 2.2% in TM-QTL carrier lambs compared to non-carriers. The presence of TM-QTL did not have any substantial effect on other VIA-estimated weights of the primal or trimmed primal joints.

Table 4.4 Least-squares means for c.*1232G > A (MM) and TM-QTL genotypic classes for VIA-estimated weights of total saleable meat yield, primal and trimmed primal weights ^{†,‡}.

Trait [§]	+/+	MM/+	MM/MM	Mean s.e.d. [¥]	+/+	TM/+	s.e.d.
SMY (kg)	7.571	7.529	7.562	0.048	7.564	7.542	0.043
P-Leg (kg)	4.530 ^b	4.544 ^b	4.681 ^a	0.047	4.553 ^b	4.651 ^a	0.041
TP-Leg (kg)	3.333 ^b	3.398 ^b	3.536 ^a	0.045	3.409	3.433	0.040
P-Chump (kg)	1.384	1.376	1.396	0.022	1.383	1.392	0.019
TP-Chump (kg)	0.783 ^b	0.795 ^b	0.819 ^a	0.012	0.801	0.792	0.011
P-Loin (kg)	2.487	2.472	2.515	0.050	2.487	2.531	0.045
TP-Loin (kg)	1.428 ^{ab}	1.430 ^b	1.485 ^a	0.037	1.445	1.477	0.032
P-Breast (kg)	1.466	1.482	1.473	0.031	1.476	1.473	0.027
P-Shoulder (kg)	5.794	5.746	5.712	0.056	5.751	5.733	0.050
TP-Shoulder (kg)	3.856	3.809	3.787	0.038	3.830	3.784	0.034

[†] Values, within row within gene or QTL, sharing a common character in their superscript are not significantly different ($P > 0.05$).

[‡] Pairwise contrasts between each pair of classes within each gene or QTL.

[§] SMY is the VIA prediction of saleable meat yield in the carcass predicted by a distinct prediction equation. P-: primal (joint weight) and TP-: trimmed primal (joint saleable meat yield), both predicted by VIA.

[¥] Mean s.e.d.: mean standard error of the difference; averaged over the 3 pairwise comparisons.

4.3.3.2 VIA-estimated muscularity traits

In almost all of the muscularity traits the MM/MM lambs were superior to the other two genotype groups, whereas there was no significant difference between the heterozygous and homozygous wildtype animals (Table 4.5). The MM/MM genotypic group significantly differed from the MM/+ group in HLMI (4%). LRMI differed significantly between the MM/MM group and the other two groups, MM/+ and +/+ (by 6% and 10%, respectively), as did HLMV (by 6% for both genotype comparisons). Lambs homozygous for MM had significantly higher MLL-VIAW (increased by 2% and 3%, respectively) and MLL-VIAD (increased by 3% and 4%, respectively) compared to MM/+ and +/+. This increase in the VIA-estimated width and depth of the loin resulted in a significant increase in MLL-VIAA in MM/MM compared to MM/+ (6%). Most muscularity traits were not significantly affected by the TM-QTL; however MLL-VIAW and MLL-VIAA were significantly greater in TM-QTL carrier lambs compared to non-carriers (by 2% and 4%, respectively).

Table 4.5 Least-squares means for c.*1232G > A (MM) and TM-QTL genotypic classes for VIA-based estimation of muscularity traits ^{†,‡}.

Trait [§]	+/+	MM/+	MM/MM	Mean s.e.d. [¥]	+/+	TM/+	s.e.d.
LRMV(cm ³)	616.2	608.6	629.7	18.62	611.8	636.0	16.46
LRMI	2.71 ^b	2.81 ^b	2.97 ^a	0.067	2.85	2.81	0.059
HLMV(cm ³)	3592 ^b	3609 ^b	3810 ^a	57.52	3648	3713	50.85
HLMI	6.42 ^{ab}	6.38 ^b	6.60 ^a	0.086	6.50	6.44	0.076
MLL-VIAW (mm)	73.34 ^b	73.86 ^b	75.20 ^a	0.805	73.90 ^b	75.42 ^a	0.712
MLL-VIAD (mm)	29.37 ^b	29.73 ^b	30.66 ^a	0.541	29.87	30.25	0.479
MLL-VIAA (cm ²)	18.90 ^{ab}	18.72 ^b	19.79 ^a	0.458	19.04 ^b	19.80 ^a	0.405

[†] Values, within row within gene or QTL, sharing a common character in their superscript are not significantly different (P > 0.05).

[‡] Pairwise contrasts between each pair of classes within each gene or QTL.

[§] LRMV, LRMI, HLMV, HLMI, MLL-VIAW, MLL-VIAD and MLL-VIAA are loin region (LR) muscle volume, loin region muscularity index, hind leg (HL) muscle volume, hind leg muscularity index index, M. Longissimus lumborum width, depth and area, respectively, as predicted by VIA.

[¥] Mean s.e.d.: mean standard error of the difference; averaged over the 3 pairwise comparisons.

4.3.3.3 VIA-dimensional measurements and compactness

Whereas there were no significant TM-QTL effects on any of the VIA based anatomical carcass measurements, there were some significant effects of MM (Table 4.6). It is of note that there were no significant differences between +/+ and MM/+ animals. However, several significant differences were identified between MM/MM lambs and these two genotype groups. Regarding the anatomical measures from the back of the carcass (Figure 4.1), MM/MM carrier lambs had significantly wider measurements in the shoulder (W1) and the breast (W3) than MM/+ lambs (4% and 3%, respectively), but neither group differed significantly from the +/+ group. Width across the legs (W5) was significantly higher in MM/MM than MM/+ or +/+ (4% and 5%, respectively). MM genotypic groups did not differ significantly in length measurements from the back and side images. Two carcass areas measured on the back view (A1 and A2) were significantly greater in MM/MM lambs than MM/+ (4% and 3%, respectively), but neither of these genotypes were significantly different to +/+ lambs.

MM genotype did significantly affect the compactness of the leg and the carcass (Table 4.6). Lambs carrying two copies of MM showed a significantly higher measure of leg compactness by 6% and 8% compared to MM/+ and +/+ lambs, respectively. MM/MM carrier lambs had significantly higher overall carcass compactness relative to MM/+ and +/+ lambs (4% and 5%, respectively).

Table 4.6 Least-squares means for c.*1232G > A (MM) and TM-QTL genotypic classes for carcass shape traits measured by VIA^{†,‡}.

Trait [§]	+/+	MM/+	MM/MM	Mean s.e.d. [¥]	+/+	TM/+	s.e.d.
W1 (cm)	19.59 ^{ab}	19.49 ^b	20.18 ^a	0.437	19.76	19.44	0.387
W2 (cm)	15.47	15.52	15.84	0.303	15.64	15.48	0.268
W3 (cm)	21.05 ^{ab}	21.25 ^b	21.80 ^a	0.429	21.46	21.20	0.380
W4 (cm)	18.26	18.58	18.91	0.336	18.65	18.52	0.297
W5 (cm)	23.17 ^b	23.32 ^b	24.24 ^a	0.291	23.54	23.63	0.258
W6 (cm)	25.41	25.15	25.02	0.387	25.23	25.15	0.343
W7 (cm)	12.95	13.01	13.03	0.253	13.02	12.92	0.224
W8 (cm)	14.26	14.42	14.58	0.319	14.50	14.34	0.283
L1 (cm)	75.41	75.32	75.73	1.311	75.56	75.57	1.161
L2 (cm)	21.42	21.25	20.95	0.395	21.15	21.20	0.349
L3 (cm)	29.05	28.35	27.36	0.942	28.14	27.76	0.834
L4 (cm)	91.10	90.64	90.79	1.218	90.80	90.79	1.078
L5 (cm)	85.63	84.24	84.24	1.367	84.77	84.22	1.210
Total-L (cm)	96.59	96.27	96.42	1.484	96.50	96.45	1.313
A1 (cm ²)	1445 ^{ab}	1453 ^b	1505 ^a	402.98	1472	1460	356.63
A2 (cm ²)	1636 ^{ab}	1638 ^b	1692 ^a	431.60	1660	1647	381.97
A3 (cm ²)	4537	4551	4699	133.55	4614	4554	118.20
A4 (cm ²)	1081	1077	1091	285.60	1084	1080	252.75
A5 (cm ²)	1576	1561	1574	403.92	1571	1565	357.47
Carc-COMP	0.240 ^b	0.243 ^b	0.252 ^a	0.0031	0.244	0.245	0.0027
Leg-COMP	0.212 ^b	0.218 ^b	0.230 ^a	0.0071	0.221	0.223	0.0065

[†] Values, within row within gene or QTL, sharing a common character in their superscript are not significantly different (P > 0.05).

[‡] Pairwise contrasts between each pair of classes within each gene or QTL.

[§] Explanations for the traits can be obtained from Figure 1. Carc-COMP: W5/Total-L; Leg-COMP: [W5+W8]^{1/2}/L3.

[¥] Mean s.e.d.: mean standard error of the difference; averaged over the 3 pairwise comparisons.

4.3.4 Mode of Action

Standard errors were large due to small numbers in some of the genotype classes, therefore table 4.7 presents only those traits where MM showed a significant additive effect or dominance effect or where both were significant. In general, the estimated additive effect was significant and in the expected negative direction for the fat traits studied and in a positive direction for most traits associated with body size or muscling, particularly for the hind leg, VIA-estimated loin muscle measurements and carcass compactness (Table 4.7). UFD and MLC-F had a significant negative additive effect, whereas TP-Leg, TP-Chump, LRMI, MLL-VIAW, MLL-VIAD and leg and carcass compactness had significant positive additive effects ($P < 0.05$). It was notable that the majority of dominance effect estimations were negative with significant dominance effects for HLMI and MLL-VIAA. Other traits had significant estimates of both an additive and dominance effect, in each case these traits related to the hind leg region (P-Leg, HLMV and W5).

Table 4.7 Estimates of the additive (a) and dominance (d) effects based on the c.*1232G > A (MM) genotypic classes from *in vivo* traits and carcass traits ^a.

Trait	a ± SE ^b	d ± SE ^{c,d}
In vivo traits		
UFD	-0.40 ± 0.15**	0.054 ± 0.15
Post mortem traits		
MLC-F	-0.96 ± 0.38*	0.23 ± 0.40
VIA-estimated primal and trimmed primal weights		
P-Leg	75 ± 27.0**	-62 ± 28.5*
TP-Leg	102 ± 26.0***	-37 ± 27.4
TP-Chump	18 ± 7.2*	-6 ± 7.6
VIA-based CT traits		
LRMI	0.133 ± 0.038**	-0.023 ± 0.041
HLMV	109.1 ± 33.2**	-92.26 ± 35.0*
HLMI	0.091 ± 0.050	-0.131 ± 0.052*
MLL-VIAW	0.93 ± 0.466*	-0.41 ± 0.490
MLL-VIAD	0.64 ± 0.313*	-0.28 ± 0.330
MLL-VIAA	0.45 ± 0.265	-0.62 ± 0.280*
VIA-morphological traits		
W5	0.53 ± 0.17**	-0.39 ± 0.18*
Carc-COMP	0.006 ± 0.002**	-0.003 ± 0.002
Leg-COMP	0.009 ± 0.004*	-0.003 ± 0.005

^a Significance of estimates: *** ($P < 0.001$); ** ($P < 0.01$); * ($P < 0.05$); others (non-significant)

$$a = \frac{1}{2} (+/+ - MM/MM) \quad ; \quad d = MM/+ - \left(\frac{+ / + + MM/MM}{2} \right)$$

^d If a > 0 and d < 0, then MM is partially recessive; if a > 0 and d > 0, then MM is partially dominant; if a < 0 and d > 0, then MM is partially recessive; if a < 0 and d < 0, the, MM is partially dominant.

4.4 Discussion

The importance of this study lies in the evaluation of the effect of two known muscle enhancing polymorphisms (c.*1232G > A and TM-QTL) on carcass composition of Texel-sired crossbred lambs out of Welsh Mountain ewes. The Welsh Mountain is an important breed in the UK, not only in terms of the number of purebred animals in hill farms, but also in its relatively high contribution to the production of the UK slaughter lambs (Pollott & Stone 2006). In the literature, several experiments have been conducted to quantify the effect of c.*1232G > A and TM-QTL on carcass composition in Texel-sired crossbred lambs out of different dam breeds, but never previously using the Welsh Mountain.

The significant findings on c.*1232G > A mutation revealed in this study are in broad agreement with information published about the effects of myostatin-associated polymorphisms/QTLs in increasing muscling and decreasing fat content (Campbell & McLaren 2007; Johnson et al. 2005a; 2009; Masri et al. 2011a); and the effects of TM-QTL in increasing muscling predominantly in the loin region (Macfarlane et al. 2009a; Rius-Vilarrasa 2009c; Walling et al. 2004). It is also of note that this study revealed the existence of the c.*1232G > A mutation in the Welsh Mountain sheep, which was not reported before.

4.4.1 Live weight and in-vivo ultrasound tissue depth

4.4.1.1 MM effect

In a previous study, the effect of carrying one copy of MM did not significantly affect growth pattern in crossbred lambs out of Mule ewes compared to non-carriers (Lambe et al. 2009a). The significantly higher live weight at 8 weeks of age of MM/MM lambs compared to MM/+ lambs found in the current study was not expected, given that live weight at ultrasound (pre-slaughter) did not significantly differ between MM genotypic groups. Conflicting results for effects on live weights at different ages in cattle were reported when a myostatin mutation was studied. The suggested explanation is a possible interaction between myostatin polymorphisms and breed effects, as well as the modifying influence of the maternal effects on

weights expressed early in life (Casas et al. 2004). The absence of significant effects of TM-QTL on live weights agrees with Lambe et al. (2009a).

In disagreement with other studies, no significant effect of MM on UMD was detected in the current study. Data from commercial purebred Texel lambs revealed a significant positive association between MM and UMD when animals were ultrasonically scanned at 21 weeks old (Hadjipavlou et al. 2008). A significant increase in loin muscle depth was displayed in MM heterozygous crossbred lambs out of Mule ewes when measured using CT scanning, whereas when measured by ultrasound this difference was not significant (Masri et al. 2011a). This is likely to be due to the differences in scan position (fifth vs. third lumbar vertebrae), the higher accuracy of the CT scanning images and/or different dam genetic background used.

While UMD was not affected by MM, there was a significant difference in UFD between MM/MM lambs and MM/+ or +/+ lambs. Reducing fat content is one of the known effects associated with the presence of MM. Previous studies found lower UFD in single copy carrier animals than in non-carriers, in different genetic backgrounds (Hadjipavlou et al. 2008; Masri et al. 2011a), which was not found here. In another study to validate the effect of g+6723 G>A on Australian crossbred lambs, aged between seven to nine months, out of Merino ewes, no evidence was presented for a significant association between this mutation and UFD (Kijas et al. 2007).

4.4.1.2 TM-QTL effect

Results in this trial revealed a significant average increase of 1.3 mm in UMD in TM-QTL carrier lambs compared to non-carriers. Similar increases in UMD in single copy TM-QTL carriers were reported in crossbred lambs out of Mule ewes (Macfarlane et al. 2009a). The increased UMD in TM-QTL carrier lambs is consistent with the reported role of this QTL to predominantly positively increase loin muscle measurements (Macfarlane et al. 2009a; Walling et al. 2004).

4.4.2 Post mortem traits

There were no significant MM-effects on CCWT and KO%. These results concur with those reported by Johnson et al. (2009) in New Zealand.

Although carcasses of MM/MM lambs were less fat, as estimated using MLC-F, than MM/+ or +/+, there were no differences between MM/+ and +/+ as found in a previous study on crossbred lambs from Mule ewes (Masri et al. 2011a). Thomas (2008) also demonstrating a substantial decrease in MLC-F scores in MM/MM lambs from Welsh Mountain ewes compared to non-carriers, alongside an increase in carcass weight (~1 kg). However, average carcass weight and killing age were not reported from that study to permit comprehensive comparison.

On the basis of the results reported in the current study, the positive effect in homozygous MM carriers on some primal weights and increased muscularity combined with the negative effect on MLC-F scores could have important economic advantages for the producers of slaughter lambs. Additionally, the UK lamb processing industry may benefit from reduced levels of subcutaneous fat associated with MM, as the costs of trimming unwanted fat prior to retail sale will be lower (Gardner et al. 2006).

4.4.3 VIA traits

4.4.3.1 MM effect

In this study, SMY, predicted by VIA, did not differ significantly between different TM-QTL and MM genotypes. In contrast, total lean yield estimated by CT was significantly higher in homozygous MM carrier lambs compared to other genotypes in the New Zealand study (Johnson et al. 2009). CT scanning is more accurate in measuring lean tissue yield compared to SMY estimated by VIA. There were significant effects of MM on hind leg traits in the current study, seen in P-Leg, TP-Leg and TP-Chump in MM/MM lambs relative to other genotypes. These results agree with those shown by Johnson et al. (2009), who found that homozygous MM lambs had higher VIA-estimated lean yield in the leg, as a percentage of the total carcass weight, compared to contemporaries of other genotypes. In light of the increased demand to move towards a fairer pricing system for lamb based on weight of saleable meat in primal joints, the increased VIA-estimated weight of P-Leg and TP-Leg in MM/MM carriers is likely to provide an economic benefit to the producer, since the hind leg meat is one of the higher priced parts of the carcass. It is of note that high phenotypic and genotypic correlations were found between VIA-estimated

leg and chump weights and that of the highest priced cut which is the loin ($r_p = 0.86$ and $r_g = 0.90$; $r_p = 0.85$ and $r_g = 0.81$, respectively) in crossbred lambs in a previous UK trial (Rius-Vilarrasa et al. 2010). Therefore, this study suggested that by using the VIA-estimated weight of the leg in breeding programs as a selection criterion, which has the highest correlations with the other joints, it would be possible to improve the meat yield of all other carcass joints.

The significant increase in several muscularity traits (as predicted by VIA) in MM/MM lambs compared to MM/+ and +/+ indicates that VIA is not only capable of capturing the weight differences in the primal cuts but also has the potential to detect the changes in muscularity traits. In Texel-sired crossbred lambs in a previous study, none of the VIA predictions of CT muscularity traits in MM/+ compared to +/+ were significant whereas MLL depth and area were found to be greater in MM/+ compared to +/+ lambs when measured by CT scanning (Masri et al. 2011a).

The increase in VIA-estimated muscularity traits in the hind leg reported here in MM carriers, but this has not been reported elsewhere. Johnson et al. (2005a) presented evidence of significant positive associations between haplotype markers presumably encompassing MM and increased muscling in the hind leg. Laville et al. (2004) noted that conformation was strongly influenced by leg muscularity, although MLC-C was not affected by the genotypic classes in the current study.

Results of carcass shape traits, measured by VIA, indicate that homozygous carriers of MM were wider across the shoulder, breast and hind legs, resulting in a more compact shape of the carcass and leg region. A recent study reported moderate to high positive genetic and phenotypic correlations between some width measurements like those that found significantly different between MM genotypic groups in this study (W1, W3, and W5) and MLC-C (Rius-Vilarrasa et al. 2010). However, any potential benefits in carcass value associated with changes in carcass shape reported in the present study should be considered with caution from a welfare perspective. It is unknown how these differences due to MM might relate to lambing difficulties, which are known to be a cause of concern in more muscular breeds, especially when lambs carrying these genes are being produced by smaller ewe breeds. Similar consequences were previously studied in cattle with evidence presented on

associations between mutations in the myostatin gene and calving difficulties due to the change in the body shape of the calf (Casas et al. 1999; Short et al. 2002; Wiener et al. 2002; 2009). Although the number of animals in some of the genotype classes was small, the dimensional measurements, areas and carcass and leg compactness scores indicate that MM may have altered muscle shape in MM/MM lambs.

4.4.3.2 TM-QTL effect

Using Texel x Mule lambs, similar findings as seen in this study were reported for the effect of a single copy of TM-QTL on loin muscularity traits (as originally measured by CT in live lambs) predicted by VIA (Rius-Vilarrasa et al. 2009c). In that study, the positive effect was only detected on VIA-predicted MLL depth, whereas in the current study, TM-QTL increased the predicted MLL width (+1.5 mm; $P < 0.05$) and MLL area (+0.75 cm²; $P < 0.05$), but not MLL depth. These differences in loin muscle measurements are also associated with higher muscularity index scores in the loin region in TM-QTL carrier lambs. In the study by Rius-Vilarrasa et al. (2009c), TM-QTL carrier lambs displayed greater MLL area, width and depth when measured by CT scanning than non-carriers. It was shown that increasing MLL measurements in TM-QTL carrier lambs resulted in significantly heavier MLL dissected weight (Macfarlane et al. 2009a). In the current study, no CT or dissection data were obtained, but it is assumed that similar relationships exist on the ground of Macfarlane et al. (2009a) and Rius-Vilarrasa et al. (2009c) results.

In common with Rius-Vilarrasa et al. (2009c), VIA in the current study could not detect a significant difference in estimated weight of the loin primal joint, between TM-QTL carrier lambs and non-carriers, which is where the predominant effect of the TM-QTL would be expected. However, in the leg (for the untrimmed primal prediction only), VIA estimated a significant ~100g increase in primal weight in TM-QTL carrier lambs, relative to non-carriers. The reason for this could be due to bone weight, which possibly may have contributed to a heavier P-Leg (but not TP-leg when bone was removed), since a positive effect of TM-QTL on bone weight was shown by Macfarlane et al. (2009a).

4.4.4 MM mode of action

Our data set was not designed to fully evaluate the additive and dominance effects of MM. However, the information available from fitting the contrasts explained above suggests that the mode of action of MM, and the magnitude of additive and dominance effects, were not consistent across the traits studied. The results imply that MM is additive for fat-related traits (e.g. UFD and MLC-F) and partially recessive for some lean meat traits and muscularity, where half of the difference between the predicted means for MM/MM and +/+ was substantially larger than the difference between MM/+ and +/+ means. These findings are in agreement with those of other groups who were also not in a position to draw a clear overall conclusion about the inheritance pattern of MM (Hickford et al. 2010; Johnson et al. 2009; Kijas et al. 2007). The mode of inheritance for mutations in the myostatin gene in cattle also seems to vary (Wiener et al. 2002). Hadjipavlou et al. (2008) suggested that the genetic background of the carrier animals would probably have an important impact in determining the MM mode of inheritance, but also noted that MM appeared to be partially recessive.

4.5 Conclusion

The data in this study, from Welsh Mountain crossbred lambs reared under typical UK conditions, confirmed earlier reports that c.*1232G >A SNP is associated with greater muscle measurements and lower fat levels, and that TM-QTL increases loin muscling characteristics. Given the changes in the carcass shape measurements revealed in homozygous MM carrier lambs in this experiment, further studies are needed to investigate the possibility of any side-effects on welfare and meat quality traits, to ensure that any potential future use of MM in the industry is sustainable and not detrimental to these important groups of traits.

To achieve the full benefit from using MM in practical breeding programs, it is vital to increase the number of the Welsh Mountain ewes carrying MM through selection and mating them to MM carrier sires. This is because larger effects have been seen only in homozygous animals. On the other hand, TM-QTL would not need to be introgressed in the ewe breed as a single copy of TM-QTL inherited from the sire showed significant increase in loin muscling.

Chapter 5

Texel Muscling QTL (TM-QTL) effects on post-mortem and video image analysis traits in Texel purebred lambs

Abstract

This study quantified the effects of Texel muscling quantitative trait locus (TM-QTL), that have been previously identified to positively influence loin muscling traits in Texel crossbred and purebred lambs, on carcass and video image analysis (VIA) traits in Texel purebred lambs. All lambs (n=209) were slaughtered at around 20 weeks of age and then carcasses weighed and evaluated for conformation scores and fat classes based on the EUROP carcass classification scheme. Estimates of the primal (P-) and trimmed primal (TP-) cut weights, hind leg muscularity and loin region muscularity and dimensions, and carcass shape measurements of the width, length and surface areas were provided by VIA cameras. Four genotypic groups (homozygote TM-QTL carriers (TM/TM), heterozygote carriers with TM-QTL allele inherited from the sire (TM/+) or the dam (+/TM) and homozygote non-carriers (+/+)) were assigned from the available lambs. Homozygote TM-QTL carcasses were significantly heavier than non-carriers by 1.6 kg and scored higher conformation values when compared to heterozygote groups only. P-LEG was significantly heavier in TM/+ than +/TM and +/+ while TM/+ had significantly heavier TP-LEG estimated weight than +/+. Homozygote non-carriers had significantly less TP-LOIN (66g, 2.4%) than heterozygote animals carrying TM-QTL from the sire. Significant improvements in TM/+ in hind leg and loin muscularity traits were observed relative to some other genotypic groups. However, TM/+ carcasses had significantly wider and greater loin muscle area compared to +/TM and +/+ (4.4% and 3.2%, and 9.3% and 5.8%, respectively). The effect of TM-QTL on some carcass shape measurements was significant, particularly when TM/TM was compared to other genetic groups. Although there was no significant association between TM-QTL and leg or carcass compactness, the significant increase in carcass length, depth and surface areas suggest a possible alteration in body shape. The significantly outstanding improvement in loin muscling traits in TM/+ compared to other genotypic groups adds more evidence to the previously reported TM-QTL polar over-dominance pattern of action.

5.1 Introduction

The commercial value of a lamb carcass for meat production depends principally on the weight and on the visually appraised carcass conformation and fat cover (Meat and Livestock Commission's scoring system (MLC) (Anderson 2003). Customers' perceptions and demands of the meat have become more specific with the appearance and shape of retail cuts playing an important role in their shifting demands (Croston 2002). However, implemented breeding programmes in the UK have focused mainly on improving lean tissue growth rate and carcass quality among terminal sire breeds (Macfarlane & Simm 2008; Simm & Dingwall 1987).

Powerful statistical approaches along with huge advances in genomic research have led to the discovery of numerous quantitative trait loci (QTLs) and/or genes that are associated with growth and muscling traits (Cockett et al. 2005; Raadsma et al. 2009).

Of these discovered QTLs is the Texel Muscling-QTL (known as TM-QTL) which was found to segregate in purebred UK Texel sheep and mapped on the distal end of chromosome 18 (OAR18) (Walling et al. 2004). In a later study by Matika et al. (2011), it was confirmed that TM-QTL is segregating in commercial Texel flocks in the UK. The phenotypic effects of the TM-QTL were reported to be limited to the loin muscle with no substantial effects on muscling traits in other body regions. Studies reported increased loin muscle dimensions and weight (by ~4 to 14%) in crossbred lambs out of Scottish Mule ewes and Texel purebred lambs as measured by ultrasound (US) (Walling et al. 2004), computed tomography (CT) or dissection (Macfarlane et al. 2009a). A larger effect of TM-QTL for loin muscle depth as measured by US in commercial Texel populations in the UK was presented by Matika et al. (2010). Increased loin muscle depth measured by US was reported in TM-QTL heterozygous carrier crossbred lambs out of Welsh Mountain ewes (Masri et al. 2011b). In the latter study, the magnitude of the effects of TM-QTL on the loin muscle width and area was strong enough to be detected when video image analysis (VIA) estimation of loin muscularity traits provided (Masri et al. 2011b). VIA detected no significant differences between TM-QTL heterozygous and non-carrier animals in carcass shape in crossbred lambs out of Welsh Mountain ewes (Masri et

al. 2011b). However, VIA measurements showed that crossbred lambs out of Scottish Mule ewes carrying a single copy of TM-QTL had significantly higher carcass ‘compactness’ (defined as the ratio of maximum width at the rump to the total carcass length) of 1.2% compared to non-carriers (Rius-Vilarrasa et al. 2009c). Using the same Texel purebred lambs data that used in this study, TM-QTL was found to have an effect on live weight across a range of ages from birth to slaughter (Macfarlane et al. 2012).

The first study to investigate TM-QTL mode of inheritance reported that it does not follow the normal Mendelian pattern of inheritance and appears to be polar over-dominant, in other words only animals receiving a single copy of the QTL from the sire demonstrate the phenotypic effect (Macfarlane et al. 2010). A later detailed study on the TM-QTL mode of inheritance using different statistical approaches on data available from commercial Texel sheep confirmed the imprinting effect of the TM-QTL with paternal parent-of-origin (Matika et al. 2010).

The TM-QTL genotyping test which is a haplotype marker test is complicated and inaccurate for identifying TM-QTL status. Hence, the TM-QTL allelic frequency is currently unknown in UK purebred Texel sheep flocks. The uptake of the lamb meat industry from exploiting TM-QTL could be achieved through producing highly priced products (e.g. the loin and hind leg cuts) that meet consumer’s specification. This is approachable through breeding programmes given that most slaughter crossbred lambs in the UK are sired by Texel terminal sire (Pollott & Stone 2006).

The availability of data comprising TM-QTL homozygous and heterozygous carrier lambs and non-carrier lambs was the motivation to study the effects of TM-QTL on carcass traits in the abattoir measured on subjective Meat and Livestock Commission (MLC) classification system and the VIA.

5.2 Material and Methods

5.2.1 Experimental animals

This experiment was conducted as a part of larger experiment to evaluate the direct and indirect effects of the TM-QTL on *in-vivo* and post-mortem carcass traits as well as its effects on meat quality traits in Texel purebred lambs.

A total of 503 purebred Texel lambs were produced from two different experimental farms in Aberystwyth University in Wales and Scottish Agricultural College in Scotland. These lambs were generated by mating seven different rams carrying a single copy of TM-QTL, (3 common across sites) to 181 ewes, that also were carriers or non-carriers for TM-QTL.

Of the lambs produced, only 209 purebred Texel lambs (114 females, 95 entire males) were chosen for this study to represent the required TM-QTL genotypes. About 67% of the lambs (n=140) were reared as singletons, 27% (n= 57) were twins and 6% (n=12) were reared artificially. All lambs were grazed at a pasture where live weight and body condition score were recorded throughout their growth until slaughter in September 2009.

Lamb ages ranged from 126-155 days old (average 144 days) at slaughter day and prior to slaughter their live weight ranged from 19.7 to 50.0 kg (average 32.1 kg). The wide range in live weight is probably due to the differences in environmental husbandry and/or grazing pasture conditions between the two farms studied. However, these differences were taken into consideration in the statistical analysis.

5.2.2 Lamb genotyping

Blood samples for all lambs were spotted onto FTA[®] cards and sent to Catapult Genetics, New Zealand (now Pfizer Animal Genetics) for genotyping shortly after birth using the nose-prick technique. The TM-QTL genotyping protocol is based on microsatellite markers since the underlying causal mutation has not been identified. The TM-QTL genotyping process used in this study was described in detail in a previous study (Macfarlane et al. 2009a). Briefly, multiple microsatellite panels (haplotypes), of five microsatellite markers (CSSM18, MCMA26, OARTMR1, OY5 and OY3), were used to span the distal end of the OAR18 region. The haplotypes that were associated with the favourable allele of TM-QTL, within sire families, helped in identifying the lambs' genotypes.

The number of lambs of each genotypic class used in the study was: 40 non-carriers (+/+), 53 sire parent-of-origin heterozygous carriers (TM/+), 17 dam parent-of-origin heterozygous carriers (+/TM) and 34 TM-QTL homozygous carriers (TM/TM). TM-

QTL genotype of 65 lambs was assigned as unknown (due to shortage of pedigree information, recombinant haplotypes, or missing marker information) but used in the statistical analyses to produce better estimation of the fixed effects.

5.2.3 Post mortem traits

All lambs, from both sites, were taken to the commercial abattoir of Welsh Country Foods in Anglesey. Lambs were slaughtered after conventional electrical stunning, and then eviscerated and skinned according to the procedures applied in UK commercial abattoirs. Cold carcass weight (CCWT) which has been used in this study was electronically measured, on the chain slaughter line, after subtracting 0.5 kg, counting for the water drip loss, off the hot carcass weight. Both cold or hot carcass weight are highly correlated and therefore whichever weight will be used in the statistical analyses is of low importance. The kidney and channel fat and the kidney knobs were removed prior to carcass weighing.

All carcasses were commercially classified, by a grader, for body conformation (using EUROP scale, MLC-C) and fat classes (MLC-F). Carcass conformation was determined according to the EUROP five-points scale where E= excellent and P= poor carcass profile. Fat class categorises carcasses according to their external fat development into 7 point scale levels, where level 1= very lean and level 5= very fat, with levels 3 and 4 subcategorised into L= low and H=high (Anderson 2003; Kempster et al. 1986). Numerical transformation was applied on fat classes, for statistical analysis, to be equivalent to the estimated subcutaneous fat percentage as reported by Kempster et al. (1986), where 1=4%, 2=8%, 3L=11%, 3H=13%, 4L=15%, 4H=17% and 5=20%.

5.2.4 VIA measurements acquisition

Carcasses were steered off the main slaughter line to the VIA room for scanning. Two cameras were installed to record and operate the images taken using the VSS2000 video image analysis system (VSS2000, E+V Technology GmbH, <http://www.vision-for-you.com/start.htm>). All carcasses were hung with legs spread apart (gambrel) and shoulders un-banded, and circulated past the two cameras, in a fixed position perpendicular to the carcass, at chain slaughter line speed to reflect the

online conditions in the abattoir (800 carcasses/h). One video camera obtained an image of the exposed surface interface of the spine, and the second video camera recorded the contour of the lateral surface (left side) as the carcasses passed on the rail. Further details on how VIA system runs can be found elsewhere (Rius-Vilarrasa et al. 2009b). In short, VSS2000 software recognises all anatomical points that are required to make a set of linear carcass dimensional measurements (lengths, widths and areas) by dividing the carcass into different regions. Together with cold carcass weight (CCWT; kg), the measurements obtained were utilised to generate computer algorithms to produce variables that describe carcass shape, size and objective estimations of the saleable meat yields (SMY) in the carcass and of different primal joints (Leg, Chump, Loin, Breast, Shoulder). Besides the SMY which was predicted using its unique prediction equation, two types of predicted weights were given by the VIA manufacturer (E+V Technology GmbH), the first is the opened primal cuts (P-joint; bone-in) and the other is the trimmed primal joint (TP-joint; bone-out). The predictions of trimmed primal joints were based on the dissection protocol under specific butchery specifications to provide pre-retail level primal cut information. This was undertaken by removing the bones and no more than 6 mm of the surrounding fat at any point of the opened primal cut. A refined prediction equation, used in this study, has been developed from the initial standard one that previously calibrated and validated under British abattoir conditions (Rius-Vilarrasa et al. 2009b). This refinement of the VIA prediction equation was carried out by calibrating the VIA system against CT measurements in the loin region (Rius-Vilarrasa et al. 2009c). Calibrating the VIA against spiral CT scanner enabled VIA to produce predictions of several muscularity traits in the loin and the hind leg regions (Rius-Vilarrasa et al. 2009c). These traits are the muscle volume in the loin region (LRMV) and the hind leg (HLMV). A muscularity index, defined as the relationship of the muscle volume to the length of the bone that it surrounds, in the loin (LRMI) and hind leg (HLMI) was measured, as was MLL muscle dimensions of the width, depth and area (MLL-VIAW, MLL-VIAD and MLL-VIAA, respectively).

From the images taken, VIA could provide us not only with the estimated weights of the carcass primal cuts but also a set of carcass shape dimensions comprising 8 widths (5 from the dorsal and 3 from the lateral image), 6 lengths (3 and 3) and 5

areas (2 and 3), respectively. These linear measurements allowed us to calculate leg compactness (Leg-COMP) and carcass compactness (Carc-COMP) as defined by Rius-Vilarrasa et al. (2009c). A schematic presentation of the exact position of these dimensions is outlined in Figure 5.1.

5.2.5 Statistical Analysis

Statistical analyses were performed using the PROC MIXED procedure in SAS, Release 9.2 (SAS Inst., Inc., Cary, NC). The restricted maximum likelihood (REML) approach in mixed model was used to estimate the TM-QTL effects on the MLC and VIA traits.

The statistical model used can be described by:

$$Y_j = X_j b + Z_j u + \varepsilon_j$$

Where Y_j is the response matrix of records for lamb j , where $1 \leq j \leq n$, n is the number of lambs ($n=209$ for MLC traits and $n=205$ for VIA traits due to the loss of some carcasses during the skinning). b is the vector containing the fixed effects of TM-QTL genotype and sex in all models, farm, rearing rank and dam age where significant, u is the vector containing the random effect of the sire and ε_j is the vector of residual components. Both u and ε are assumed to be independently normally distributed with a mean of zero. Two way interactions amongst fixed effects were tested and found to be of no significance, thus were not included in the model. When analysing MLC traits, VIA-predicted primal and trimmed primal weights and VIA-predicted loin and hind leg muscularity traits, carcass weight was fitted as a linear covariate (slaughter age was tested and found not significant). VIA data of five lambs ($n=1$ (TM/TM), $n=2$ (TM/+) and 2 unknown genotypes), were missing due to a significant damage in the carcass body during the skinning process.

To enable better estimations of the fixed effects, data from all lambs, including those assigned as unknown for their genotypes, were used in the analyses. However, the results of the least-squares means and their standard errors shown will be only for the four assigned genotype classes i.e. +/+, +/TM, TM/+ and TM/TM.

Performing orthogonal contrasts along with the availability of data from all possible genotypes (+/+, +/TM, TM/+ and TM/TM) allowed us to test: the additive (-1, 0, 0, 1), the maternal dominance (-1, 2, 0, -1), the paternal dominance (-1, 0, 2, -1) and the paternal polar overdominance (-1, -1, 3, -1) mode of action of the TM-QTL after accounting for all other effects fitted in the model (Freking et al. 1999).

5.3 Results

5.3.1 Post-mortem traits (carcass weight and MLC traits)

The least-square means differences between TM-QTL genotypic groups for carcass weight and MLC conformation and fat class scores are shown in Table 5.1.

Homozygote TM/TM carcasses had the heaviest CCWT among the other genotypic groups, but the difference (10.3%) was only significant when compared to +/+ (+1.6 kg). Compared to +/TM and TM/+, homozygote TM/TM carcasses were classified as having significantly higher MLC-C scores (14% and 8% respectively). There was no significant difference in MLC-F classes between TM-QTL genotypic groups (Table 5.1).

Table 5.1 Least-squares means for TM-QTL genotypic classes for post mortem traits ^{†,‡}.

Trait [§]	+/+ (40)	+/TM (17)	TM/+ (53)	TM/TM (34)	AV s.e.d. [¥]
CCWT (kg)	13.88 ^b	14.10 ^{a,b}	14.41 ^{a,b}	15.48 ^a	1.085
MLC-C	2.82 ^{a,b}	2.56 ^b	2.74 ^b	2.98 ^a	0.224
MLC-F	8.22	7.63	7.65	8.47	0.525

[†] Values, within row within QTL classes, sharing a common character in their superscript are not significantly different ($P > 0.05$).

[‡] Pairwise contrasts between each pair of classes within each QTL class.

[§] CCWT is the cold carcass weight, MLC-C is the conformation score (EUROP) transformed to a numeric scale: E (excellent) = 5 to P (poor) = 1. MLC-F is the fat class (1, 2, 3L, 3H, 4L, 4H, 5) transformed to a subcutaneous fat percentage as described by Kempster et al. (1986).

[¥] Av. s.e.d.: mean standard error of the difference; averaged over the 4 pairwise comparisons.

5.3.2 VIA-predicted weights of the saleable meat yield, primal and trimmed primal cuts

Table 5.2 presents the pair-wise comparisons of the least-squares means for VIA-estimated weights of SMY, and primal (P) and trimmed primal (TP) joints between TM-QTL genotypic classes.

No significant difference between genotypes was found in SMY. For P-LEG, TM/+ was significantly higher than the other heterozygote genotype group +/TM and the wildtype +/+ (4.2% and 2.3%, respectively). Similarly, TP-LEG in TM/+ carcasses was significantly greater only when compared with +/+ (2.1%). While TM-QTL did not show any significant effects in P-CHUMP, TP-CHUMP in TM/+ was significantly greater when compared to +/TM (3.6%). In contrast to P-LOIN where no significant difference was observed between TM-QTL genotype classes, TM/+ carcasses showed significant difference in TP-LOIN compared to +/+ (2.4%). There were no significant differences between genotypic classes in the breast and shoulder joints.

Table 5.2 Least-squares means for TM-QTL genotypic classes for VIA-estimated weights of total saleable meat yield, primal and trimmed primal weights ^{†,‡}.

Trait [§]	+/+ (40)	+TM (17)	TM/+ (53)	TM/TM (34)	AV s.e.d. [¶]
SPMY (kg)	6.149	6.063	6.212	6.169	0.102
P-LEG (kg)	3.916 ^b	3.840 ^b	4.007 ^a	3.944 ^{a,b}	0.059
TP-LEG (kg)	2.988 ^b	2.977 ^{a,b}	3.066 ^a	3.051 ^{a,b}	0.053
P-CHUMP (kg)	1.187	1.167	1.203	1.179	0.027
TP-CHUMP (kg)	0.716 ^{a,b}	0.702 ^b	0.729 ^a	0.722 ^{a,b}	0.011
P-LOIN (kg)	1.964	1.928	2.029	2.014	0.063
TP-LOIN (kg)	1.125 ^b	1.118 ^{a,b}	1.191 ^a	1.146 ^{a,b}	0.042
P-BREAST (kg)	1.213	1.202	1.186	1.167	0.026
P-SHOULDER (kg)	4.780	4.774	4.835	4.893	0.86
TP-SHOULDER (kg)	3.247	3.239	3.241	3.261	0.046

[†] Values, within row within QTL classes, sharing a common character in their superscript are not significantly different ($P > 0.05$).

[‡] Pairwise contrasts between each pair of classes within each QTL class.

[§] SPMY is the VIA prediction of saleable meat yield in the carcass predicted by a distinct prediction equation. P-: primal (joint weight) and TP-: trimmed primal (joint saleable meat yield), both predicted by VIA.

[¶] Av. s.e.d.: mean standard error of the difference; averaged over the 4 pairwise comparisons.

5.3.3 VIA-predicted muscularity traits

Table 5.3 shows the least-squares means of the four TM-QTL genotype classes for the VIA-predicted muscularity traits in the loin region and the hind leg.

Although not significantly different from all other genotype classes, in most of VIA-predictions of the loin region muscularity and MLL dimensions TM/+ was consistently the outstanding group. TM/+ scored significantly higher estimated loin region index values compared to TM/TM and +/TM (3.8% and 4.5%, respectively). The only significant difference in the hind leg muscularity traits was observed in muscle volume (HLMV). There was a significant difference observed in the HLMV between TM/TM and TM/+ compared to +/TM (4.8% and 6.3%, respectively).

There was no significant difference in the MLL depth between genotype classes. When compared to +/TM and +/+, lambs of TM/+ had significantly wider MLL (4.4% and 3.2%, respectively) and greater MLL area (9.3% and 5.8%, respectively) estimated by VIA. The loin region volume estimated by VIA was significantly higher in homozygote TM/TM carcasses and TM/+ compared to homozygote non-carriers +/+ (11.2% and 10.3%, respectively).

Table 5.3 Least-squares means for TM-QTL genotypic classes for VIA-based estimation of muscularity traits ^{†,‡}.

Trait [§]	+/+ (40)	+/TM (17)	TM/+ (53)	TM/TM (34)	AV s.e.d. [¥]
LRMV (cm ³)	441.1 ^b	457.4 ^{a,b}	492.0 ^a	496.6 ^a	23.80
LRMI	3.02 ^{a,b}	2.95 ^b	3.09 ^a	2.98 ^b	0.050
HLMV (cm ³)	3171 ^{a,b}	3064 ^a	3270 ^a	3219 ^a	60.91
HLMI	6.67	6.70	6.65	6.62	0.116
MLL-W (mm)	72.47 ^b	71.60 ^b	74.86 ^a	73.45 ^{a,b}	1.151
MLL-D (mm)	29.22	28.81	29.84	29.65	0.581
MLL-A (mm)	17.59 ^b	16.93 ^b	18.67 ^a	18.01 ^{a,b}	0.584

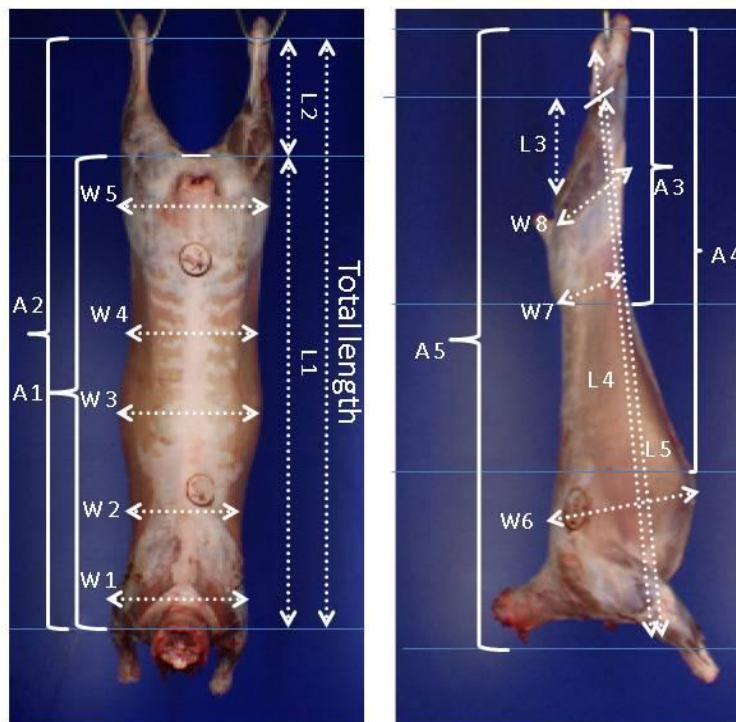
[†] Values, within row within QTL classes, sharing a common character in their superscript are not significantly different ($P > 0.05$).

[‡] Pairwise contrasts between each pair of classes within each QTL class.

[§] LRMV, LRMI, HLMV, HLMI, MLL-VIAW, MLL-VIAD and MLL-VIAA are loin region (LR) muscle volume, loin region muscularity index, hind leg (HL) muscle volume, hind leg muscularity index, M. Longissimus lumborum width, depth and area, respectively, as predicted by VIA.

[¥] Av. s.e.d.: mean standard error of the difference; averaged over the 4 pairwise comparisons.

Fig 5.1 Dorsal and side carcass views and description of measurements. W1: Maximum shoulder width [dorsal view]; W2: minimum breast width [dorsal view]; W3: Maximum breast width [dorsal view]; W4: Minimum loins width [dorsal view]; W5: Maximum rump width [dorsal view]; W6: Maximum breast depth [side view]; W7: Minimum loin depth [side view]; W8: Maximum rump depth [side view]; L1: Length buttocks to shoulder [dorsal view]; L2: Length hock to buttocks [dorsal view]; L3: length between the tibia and the end of the leg [side view]; L4: length from the hock to the front of the shoulder [side view]; L5: Length from tibia to the front of shoulder; A1: Back area of the carcass from shoulder to buttocks; A2: Back area of the whole carcass; A3: Side area from loin to hock; A4: Side area of the saddle and hind legs; A5: side area of the whole carcass.



5.3.4 VIA-measured carcass shape traits

Results of this study revealed significant changes in carcasses shape between genotype classes measured by VIA (table 5.4). VIA images from the dorsal view showed that homozygote TM/TM had significantly wider measurements in the forequarter part of the carcass (W1 and W2) compared to other genotype classes. Similarly, TM/TM carcasses were the widest in all measurements taken from the side view images, although not significantly different from some other genotype classes. W6 in TM/TM carcasses were 3.4% and 3.9% higher compared to TM/+ and +/+, respectively. Again, TM/TM carcasses were wider in W7 by +5.7% and +5.2% compared to +/TM and +/+, respectively. The difference of 5.7% in W8 was significant between TM/+ and +/TM as well as between TM/TM compared to +/TM and +/+ (8.9% and 5.4%, respectively). Contrary to the non significant differences between each genotype in most VIA measured lengths (L1, L3 and total carcass length), L2 was significantly longer (+3%) in TM/TM than in TM/+. The pattern in L4 and L5 was the same where TM/TM had significantly higher length in these traits compared only to +/+ (3.2% and 4.2%, respectively). Carcass surface areas estimated by VIA from the side view images are the only traits that showed significant differences between TM-QTL genotypes. A3 differed significantly by 10.5% and 8.7% between TM/TM carcasses and those in +/TM and +/+, respectively. The surface areas A4 and A5 in TM/TM were significantly greater from the other genotypic groups. No significant effects of TM-QTL were observed on carcass compactness and leg compactness.

Table 5.4 Least-squares means for TM-QTL genotypic classes for carcass shape traits measured by VIA^{†,‡}.

Trait [§]	+/+ (40)	+/TM (17)	TM/+ (53)	TM/TM (34)	AV s.e.d. [¥]
W1 (cm)	18.44 ^b	18.27 ^b	18.50 ^b	19.41 ^a	0.653
W2 (cm)	14.87 ^b	14.63 ^b	14.91 ^b	15.49 ^a	0.426
W3 (cm)	20.52	20.27	20.66	20.96	0.550
W4 (cm)	17.92	17.80	17.97	18.44	0.463
W5 (cm)	23.01	22.97	23.39	23.59	0.434
W6 (cm)	22.36 ^b	22.57 ^{a,b}	22.48 ^b	23.28 ^a	0.520
W7 (cm)	11.74 ^b	11.68 ^b	12.04 ^{a,b}	12.38 ^a	0.329
W8 (cm)	13.17 ^{b,c}	12.68 ^b	13.44 ^{a,c}	13.92 ^a	0.443
L1 (cm)	69.01	69.43	69.70	71.00	1.486
L2 (cm)	19.29 ^{a,b}	18.96 ^{a,b}	18.98 ^b	19.56 ^a	0.321
L3 (cm)	26.89	27.01	26.54	27.19	0.728
L4 (cm)	82.59 ^b	82.84 ^{a,b}	83.43 ^{a,b}	85.31 ^a	1.663
L5 (cm)	76.61 ^b	78.30 ^{a,b}	77.97 ^{a,b}	79.98 ^a	1.719
TOT-L (cm)	87.75	88.01	88.17	90.11	1.668
A1 (cm ²)	1293	1290	1315	1367	5660.8
A2 (cm ²)	1458	1453	1479	1539	6034.3
A3 (cm ²)	3842 ^b	3768 ^b	3990 ^{a,b}	4209 ^a	2009.7
A4 (cm ²)	8962 ^b	8945 ^b	9214 ^b	9712 ^a	4061.0
A5 (cm ²)	1283 ^b	1290 ^b	1313 ^b	1387 ^a	5745.5
Carc-COMP	0.263	0.263	0.265	0.262	0.003
Leg-COMP	0.226	0.223	0.231	0.227	0.007

[†] Values, within row within QTL classes, sharing a common character in their superscript are not significantly different ($P > 0.05$).

^{*} Pairwise contrasts between each pair of classes within each QTL class.

[§] Explanations for the traits can be obtained from Figure 5.1. Carc-COMP: W5/Total-L; Leg-COMP: $[W5+W8]^{1/2}/L3$.

[¥] Av. s.e.d.: mean standard error of the difference; averaged over the 4 pairwise comparisons.

5.3.5 TM-QTL mode of action

Results of the orthogonal contrast to test TM-QTL model of action in the traits studied are presented in table 5.5. TM-QTL did not exhibit a consistent pattern of gene action across the traits.

CCWT exhibited a significant additive effect whereas MLC-F exhibited a significant maternal dominance mode of action. Saleable meat yield estimated by VIA showed a significant additive effect. While paternal over-dominance and maternal dominance effects were significant on P-LEG, only the maternal dominance effect was significant on TP-LOIN. There was a significant maternal dominance effect on the muscularity index of the loin region. In contrast to HLMV which expressed significant polar over-dominance and paternal dominance effects, MLL-W and MLL-A showed a significant maternal dominance effect as well as a polar over-dominance mode of action. While several width measurements (W2, W3, W6 and W7) displayed significant additive modes of action, W8 was the exception that showed significant polar over-dominance, additive and paternal dominance effects. In contrast to L2 which exhibited significant maternal dominance effect, L4 and L5 were similar in showing significant additive effects. Apart from A1 and A2, all the remaining VIA-estimated carcass areas showed significant additive modes of action.

Table 5.5 Estimates of contrasts and standard errors for polar over dominance, additive, maternal and paternal dominance effects of TM-QTL for MLC and VIA traits.

Trait	Paternal polar overdominance ^a	s.e.	Additive	s.e.	Maternal dominance	s.e.	Paternal dominance	s.e.
CCWT	-1.135	2.430	-1.677*	0.782	-0.871	1.055	-1.465	0.397
MLC-C	-2.611	7.541	-0.184	2.252	5.207	3.494	-0.005	5.263
MLC-F	-1.918	1.519	-0.261	0.457	-1.706*	0.704	-1.847	1.055
SPMY	-0.342	0.265	-0.824*	0.381	-0.223	0.511	-0.796	0.820
P-LEG	-0.347*	0.161	-0.028	0.052	0.154*	0.072	-0.180	0.111
TP-LEG	-0.175	0.143	-0.063	0.046	0.092	0.063	-0.086	0.098
P-CHUMP	-0.067	0.056	0.008	0.017	0.039	0.026	-0.032	0.039
TP-CHUMP	-0.059	0.032	-0.007	0.010	0.020	0.015	-0.033	0.022
P-LOIN	-0.223	0.150	-0.050	0.049	0.079	0.065	-0.122	0.102
TP-LOIN	-0.109	0.116	-0.020	0.037	0.112*	0.052	-0.036	0.080
P-BREAST	0.039	0.079	0.046	0.024	-0.009	0.037	0.023	0.055
P-SHOU	-0.186	0.248	-0.113	0.079	-0.003	0.113	-0.125	0.171
TP-SHOU	-0.031	0.140	-0.014	0.043	-0.026	0.066	-0.029	0.097
LRMV	-52.64	72.86	-55.51	22.01	46.24	33.85	-22.73	49.85
LRMI	-0.232	0.149	0.044	0.045	0.185**	0.070	-0.093	0.103
HLMV	-458*	192.7	-47.28	60.80	150.23	83.98	-261.6*	129.2
HLMI	0.170	0.307	0.045	0.099	0.014	0.136	0.118	0.211
MLL-W	-5.97*	2.99	-0.98	0.95	3.81**	1.32	-2.71	2.05
MLL-D	-2.29	1.54	-0.43	0.48	0.81	0.69	-1.25	1.06
MLL-A	-3.46*	1.67	-0.42	0.53	1.75*	0.76	-1.73	-1.50
W1	-1.531	1.272	-0.969*	0.405	-0.848	0.539	-1.303	-1.303
W2	-1.377	0.973	-0.619*	0.310	-0.538	0.417	-1.097	0.666
W3	-1.323	1.203	-0.445	0.383	-0.163	-0.320	-0.937	0.823
W4	-0.932	1.081	-0.523	0.343	-0.421	0.465	-0.762	0.740
W5	-1.066	0.962	-0.579	0.306	0.193	0.411	-0.646	0.658
W6	-0.419	1.095	-0.916**	0.349	-0.678	0.465	-0.505	0.749
W7	-1.127	0.803	-0.64*	0.254	-0.034	0.348	-0.763	0.550
W8	-2.479*	1.049	-0.747*	0.332	-0.201	0.452	-1.719*	0.718
L1	-1.411	3.459	-1.981	1.097	-0.598	1.485	-1.140	2.368
L2	-0.788	1.011	-0.425	0.318	-0.962*	0.442	-0.846	0.694
L3	0.943	2.051	-0.347	0.631	-1.198	0.946	0.230	1.415
L4	-2.804	3.749	-2.719**	1.191	-1.033	1.603	-2.213	2.566
L5	0.351	3.867	-3.367**	1.229	-0.642	1.653	0.020	2.647
TOT-L	-1.973	3.888	-2.359	1.233	-1.520	1.670	-1.822	2.662
A1	-1040	1269	-7443	4034	-2887	5426	-7898	8689
A2	-1156	1352	-8094	4297	-3843	5778	-8991	9255
A3	-7383	4594	-3670*	1459	-706.8	1968	-5158	3145
A4	-1052	8986	-7502**	2857	-2452	3834	-7829	6148
A5	-1134	1252	-1039**	3984	-4484	5336	-9054	8569
Carc-COMP	-0.004	0.008	0.0004	0.002	0.006	0.003	0.0001	0.005
Leg-COMP	-0.018	0.018	-0.001	0.006	0.010	0.008	-0.007	0.013

^a Values with asterix in their superscript are significantly different from zero (* = P < 0.05, ** = P < 0.01)

5.4 Discussion

The novelty of this study is that it is the first to demonstrate the effects of TM-QTL on post-mortem traits in a population of purebred Texel lambs that comprises homozygote non-carriers, paternal and maternal source of origin heterozygote carriers and homozygote carriers of TM-QTL.

The data used in this study did not reveal the extent to which the variation between genotype groups was due to genetic or environmental factors, but showed that TM/TM carcasses were the heaviest among the other genotype groups with a significant difference of 1.6 kg from their homozygote non-carrier counterparts. The significant difference in CCWT implies a possibility of different rates of muscle development between these genotypes, and differences in growth rate. It may not be excluded that the differences in weights and therefore growth rate between genotypes may be due to either gross feed conversion efficiency or higher efficiency in transforming energy into muscle deposition in TM/TM compared to other genetic groups. This has important practical implications for producers in terms of the concomitant costs associated with producing meat that are likely to be closely related to age at slaughter. Furthermore, carcasses from the same group, TM/TM, graded to have the best conformation based on the EUROP scoring system, whereas +/TM and TM/+ had the poorest with +/+ being intermediate. This superiority of TM/TM in MLC-C could be due mainly to their heavier carcass weights. However, the combination of the superior results of TM/TM animals in CCWT and MLC-C is very likely to be of monetary advantage to the producers with anticipated higher returns for their premiums from meat traders. TM/+ carcasses were not significantly heavier than +/+ and were similar to them in the MLC-C grades, suggesting that they will not receive any better prices than those +/+. Previously published reports on the potential economic benefit of TM-QTL to increase the overall carcass value were evaluated in crossbred heterozygote carriers inheriting the QTL copy from the sire and were based on the significant improvements observed in loin characteristics as the most highly priced cut (Macfarlane et al. 2009a; Rius-Vilarrasa et al. 2009c). These studies found no significant differences between heterozygote carriers and non-carriers in terms of the CCWT and MLC-C.

Unlike carcass weight, SPMY predicted by VIA using its unique prediction equation did not differ significantly between genotype groups. Previous research focused on studying the TM-QTL effects on loin muscle characteristics in which they reported that its effect is only constrained on the loin (Macfarlane et al. 2009a; Rius-Vilarrasa et al. 2009c; Walling et al. 2004). It was surprising to record significant improvements in some VIA-estimated yields of the hind leg traits in TM/+ lambs

compared to some other genotype groups. The significant positive result in P-LEG found in TM/+ relative to +/+ is similar in direction and magnitude to that shown in a previous study to evaluate the effect of a single copy of TM-QTL in crossbred lambs out of Welsh Mountain ewes (Masri et al. 2011b). VIA-estimated yield of the hind leg traits failed to quantify any significant difference between heterozygote carriers and non-carriers in crossbred lambs out of Scottish Mule ewes (Rius-Vilarrasa et al. 2009c). A possible explanation of these results may be due to the differences in the dam genetic background between these studies or/and other environmental effects (e.g. year of study, farm management).

The observed significant improvement in TM/+ lambs in the VIA-estimated weights of the hind leg traits in this study was accompanied by favourable improvement in the hind leg muscle volume. Because the muscle volume is defined as the shape of the muscle, independent of fatness, this result is of commercial importance as Laville et al. (2004) described how consumers preferred plump leg joints and large round chops rather than thin chops.

This study produced results which corroborate the findings of previous work in this field with respect to the TM-QTL effects on loin muscle traits. TP-LOIN weighed significantly higher in TM/+ than in +/+ animals as predicted by VIA, however the volume of difference in the current study is just two times greater than the difference that found between heterozygote carriers and non-carriers on the dissected loin weight reported by Macfarlane et al. (2009a) (+66 g vs. 34 g, respectively). An increase in VIA-predicted loin muscle weight of 2.2 % was reported in heterozygote animals carrying the Carwell QTL (LoinMAXTM) (Masri et al. 2010). This was detected in the Poll Dorset breed on chromosome 18, and was found to have a similar effect on loin muscling in Poll Dorset-sired lambs as that reported for TM-QTL carriers (Banks 1997; Nicoll et al. 1998) compared to non-carriers (Masri et al. 2010).

The findings of this study for increased VIA-estimated loin muscle measurements (width and area) accords with earlier studies that observed a significant increase in the loin muscle characteristics in TM-QTL heterozygote carrier lambs compared to non-carriers (Laville et al. 2004; Macfarlane et al. 2009a; Masri et al. 2011b). The

absence of the TM-QTL effect on the loin depth and in contrary to earlier reports, (Macfarlane et al. 2009a; Walling et al. 2004), can be due to the instrumental measurement this trait was recorded and their accuracies (e.g. US or CT). TM-QTL may have affected the loin muscle shape with significantly greater muscle volume found in TM/+ lambs compared to +/+ and this is consistent with Macfarlane et al. (2009a). Moreover, in the present study, TM/+ carcasses had significantly higher loin muscularity index score compared to +/TM but not compared to other genotype groups. This suggests that the increase in the VIA-estimated loin muscle weight along with this favourable alteration in shape would influence consumer appeal and hence encourage the consumer to pay more for the premium cut. A longer conditioning period of the loin muscle cut (9 days) has been suggested in order to mitigate the slight increase in toughness recorded between TM/+ and +/+ crossbred lambs (Lambe et al. 2010a; 2010b). Further studies with more focus on the economic advantage for TM-QTL are recommended. The fairly high phenotypic and genotypic correlation between VIA-estimated leg and chumps weights and loin weight would be of great relevance to improve the meat yield in any future breeding programmes to utilize TM-QTL in meat industry (Rius-Vilarrasa et al. 2010).

This is the first study to report significantly wider dorsal measurements in the front quarter part of TM/TM carcasses compared to other genotypic groups. This result must be dealt with caution in any future breeding plans in order to avoid welfare problems like the lambing difficulties resulting from increased shoulder width (Dwyer & Bunger 2011; Speijers et al. 2009). As with a prior study by Rius-Vilarrasa et al. (2009c), no significant differences in VIA-measured widths were found between TM/+ and +/+ lambs. It is very important to report the outstanding VIA linear measurements in TM/TM compared to +/+ lambs taken from the side view to show the changes in the lamb morphology. The larger carcass depth, described as VIA width from the side view, across the carcass along with the higher length measurements resulted in greater carcass areas which is in common with the genetic and phenotypic correlations reported by Rius-Vilarrasa et al. (2010). The observed TM-QTL effects on increasing the side surface areas and therefore, arguably, putting more muscle in TM/TM carcasses is not in contrast with the significant difference presented on the cold carcass weight. Moreover, when adjusted

for a constant carcass weight, some linear measurements like W6, W8, L2, L4, A3 and A4 showed significant differences between TM/TM and other genotype groups (results are not presented). This result may indicate possible changes in the overall carcass shape in TM/TM animals. An earlier study by Rius-Vilarrasa et al. (2009c) reported a significant difference in the rump depth (W8) between TM-QTL heterozygote and non-carrier lambs. The increased leg length (L2) and the rump depth W8 in TM/TM may explain the improvement in the leg conformation score which was reported to have a potential influence on the overall carcass conformation (Laville et al. 2004).

5.4.1 Mode of inheritance

In agreement with previous studies on TM-QTL (Macfarlane et al. 2010; Matika et al. 2011) and similarly to the Callipyge mutation (Georges & Cockett 1996), the paternal polar over-dominance mode of action of TM-QTL in the loin muscle characteristics has been confirmed in this study. This means that the only genotype that expresses the phenotypic effects of the TM-QTL in this type of QTL action is one that inherits the QTL from the sire and a normal allele from the dam. The Carwell QTL, identified in the same chromosomal interval as TM-QTL position and Callipyge and the cluster of imprinted genes around Callipyge, has been suggested to be imprinted (Jopson et al. 2001) which means the expression of the phenotypic effects is conditioned by the paternal source in which the allele will be inherited from.

Consistent additive modes of action have been observed in this study in carcass weight and the VIA-estimated saleable carcass cut weights, although the effect of TM-QTL on the latter traits was not significant. Similarly, albeit with a different magnitude of effect, several linear measurements showed an additive pattern which is a highlight of this study. The previously unreported modes of inheritance e.g. maternal dominance and paternal dominance as well as a mix of different modes of action in single traits were reported in this study. These modes of actions could simply be due to the lack of animal numbers in some genotype groups which produces high standard errors. However, further studies with larger animal numbers

in each genotypic group would help to draw a better conclusion on TM-QTL mode of action on other carcass traits rather than the loin muscle ones.

5.5 Conclusion

The present study reveals that TM-QTL significantly affected CCWT and MLC-C with TM/TM carriers being on average 1.6 kg heavier than non-carriers, with better MLC-C scores. These results will lead to better economic returns for farmers based on the current payment system.

VIA confirmed its ability to detect differences between genotypic groups in loin muscle characteristics and supports previous findings on the effects of TM-QTL on loin muscling and the TM-QTL mode of action on loin traits. Moreover, TM-QTL effects shown in this study on the hind leg VIA-predicted weights and muscularity extends the earlier thoughts that the TM-QTL effect is restricted to the loin muscle only.

One of the novel findings of this study is the significant difference in VIA-measured carcass shape dimensions among TM-QTL genotype classes. The nature of carcass shape measurement differences implies that this issue should be addressed fully to ensure that no detrimental effects for animal welfare at lambing time occur, as a result of including the TM-QTL in future breeding schemes.

Larger numbers of animals from each genotype class are required to verify the reported results and identification of the underlying causative mutation will be very helpful before utilisation of the TM-QTL in industrial lamb production schemes in the UK.

Chapter 6

An investigation of the ability of *in-vivo* ultrasound and CT measurements in lambs to predict VIA-estimated carcass traits

Abstract

This study utilised the availability of the ultrasound (US) and computed tomography (CT) linear measurements to look at their phenotypic relationship with the video image analyses (VIA) weight predictions of the carcass primal (P-) and trimmed primal (TP-) cuts. Furthermore, it was possible by using US and CT measurements to detect the best subset regression model to predict VIA carcass P- and TP- primal cut weights. In total, six models were investigated using a forward stepwise selection to construct the best prediction model of each carcass cut. A further test was conducted to check the model's instability of the coefficient of determination (R^2) and the inflation of the standard error that may result from variables multi-collinearity.

There was a substantial positive phenotypic relationship between the predictor variables and the VIA-derived traits. The best R^2 (76 - 90) was found when the ultrasound muscle depth along with CT-linear measurements together with the live weight were combined in the model. Fitting live weight at US as a sole predictor was of a limited potential to predict VIA-derived traits although improved by ~8% when combined with US muscle depth. The results of this study might be beneficial when anticipated changes in the payment system in the UK abattoir take place from the current one which is based only on weight, conformation and fat class classification.

6.1 Introduction

The measurement of body composition and the prediction of carcass lean meat content in sheep in the past few years have become more important and intensively investigated. Accurate predictions of live sheep and carcass composition have concerned scientists as it is critical to all sections of the meat industry where the sheep carcass is valued on the basis of carcass weight and relative proportions of fat and lean.

Few new technological developments that are computerised, practicable, non-destructive and objective have been used to evaluate *in-vivo* and post mortem body composition in sheep.

Ultrasound scanning (US) is one technology that has been used frequently in sheep to predict muscle and fat content in live animal (Berg et al. 1996; Silva et al. 2006). The reported lack of accuracy in predicting carcass composition has been considered as one of the shortfalls of US technology (Speakman 2001). However, in the UK, US measurements of loin muscle and fat depth have been widely used as major components of sheep genetic improvement programmes for carcass composition that improved the rate of lean tissue growth (Simm et al. 2002).

The necessity to obtain very accurate *in-vivo* evaluation of body composition has led scientists to adapt and use computer tomography (CT), originally invented for human medical usage, on farm animal species. This non-invasive and objective technique provides very high accuracy and precision in predicting *in-vivo* and carcass composition in sheep such as lean, fat and bone contents (Johansen et al. 2007; Kongsro et al. 2009; Young et al. 2001). The high cost and restricted accessibility of CT limits its widespread commercial usage. Therefore, a two-stage selection regime combining ultrasound and CT scanning has been developed. The principle of this regime is based on selecting the top 15-20% ultrasonically scanned rams for CT scanning (Jopson et al. 2004; Lewis & Simm 2000). Collectively, live weight, ultrasound measurements and CT scan data are then used, along with some other on-farm information, in genetic evaluations to obtain estimated breeding values for lean tissue growth index scores.

Video Image Analysis (VIA) is a very promising system that is non-destructive, computerised, and objective and operates at slaughter chain-line speed to evaluate lamb carcass composition. VIA has been reported to be consistent and accurate in predicting lamb carcass quality grade (Meat and Livestock Commission grades for conformation and fat) and primal cut yields (Cunha et al. 2004; Hopkins et al. 2004; Rius-Vilarrasa et al. 2009b). As part of another study that aimed to evaluate the effects of a quantitative trait locus (QTL) for increased muscularity on carcass traits in crossbred lambs, VIA prediction equations for primal cut weights were refined. The refining of VIA predictions of primal cut weights resulted in improving the accuracy by 16% (Rius-Vilarrasa et al. 2009c).

The sheep industry is shifting closer to value-based marketing system (VBMS) in which predicted carcass composition and lean meat yield determine the carcass value. As in New Zealand and Australia, VIA will be the most likely technology to be adopted in the UK for such a payment system. It could therefore be in the breeders' interest to have preliminary predicted information on the carcass value from CT scanning the live animal. Thus, the farmer will have an approximation of the carcass's value.

Assuming VIA will be of importance in the future to obtain value measurements in the abattoir, it is vital to know how well live measurements by US and CT scan can predict the VIA measured carcass and primal cut saleable meat yield. Therefore, the objectives of this study were: (1) to report the phenotypic correlations between US measurements and CT linear measurements, and VIA-predicted carcass saleable meat yield, VIA-predicted primal and trimmed primal cut weights and (2) to investigate the best subset prediction model that includes US and/or CT linear measurements to provide acceptable predictions of the VIA-predicted carcass saleable meat yield, VIA-predicted primal and trimmed-primal cut weights. Moreover, this information has the potential to be used in future genetic improvement programmes that lead to a faster response to selection.

6.2 Materials and Methods

6.2.1 Live Animal Measurements

This experiment was conducted in 2009 at two different experimental research farms: the Scottish Agricultural College (SAC) in Scotland and Aberystwyth University in Wales. The experiment was part of a large project to evaluate the effect of the Texel muscling quantitative trait locus TM-QTL and other QTLs on lean meat yield and meat quality in sheep. A total of 164 purebred Texel lambs (91 females, 73 entire males) were selected to be used, which were produced by mating seven different rams that were carriers for TM-QTL (three common across farms) to 181 ewes, some of which were also carriers for TM-QTL. Lambs were blood sampled to classify for genotype status for TM-QTL and resulted in: 16 non-carriers (+/+), 50 sire parent-of-origin heterozygous carriers (TM/+), 17 dam parent-of-origin heterozygous carriers (+/TM) and 27 TM-QTL homozygous carriers (TM/TM). TM-QTL genotype of 54 lambs was assigned as unknown (due to shortage of pedigree information, recombinant haplotypes, or missing marker information) but data from these lambs were used in the statistical analyses to produce better estimation of the fixed effects.

Lambs at weaning were reared as 109 singletons, 44 as twins and 11 artificially reared. All lambs were grazed at pasture and regular on-farm live weight and body condition score were recorded throughout their growth until slaughter in September 2009. All procedures involving animals were approved by the Scottish Agricultural College (SAC) animal ethics committee and were performed under UK Home Office licence, following the regulations of the Animal (Scientific Procedures) Act 1986.

6.2.1.1 Ultrasound measurements

Lambs were ultrasound scanned at 20 weeks of age (average age = 139; max age = 151; min age = 119 days), using a Dynamic Imaging Concept MLV (Caiyside Imaging Ltd) ultrasonic scanner with a 3.5 MHz transducer. Average lambs weight was 33 kg (ranged from 20 to 51 kg). Ultrasound muscle depth (UMD) was determined by taking one muscle depth measurement vertically at the deepest point of the (*M. longissimus lumborum*, MLL) over the 3rd lumbar vertebra. Ultrasound fat

depth (UFD) was measured but its inclusion in the prediction models had no effects, thus not fitted in any of the studied prediction models.

6.2.1.2 CT scan measurements

All lambs were CT scanned within one week of slaughter (average age = 134; max age = 145; minimum age = 118 days). Two spiral CT scanner (SCTS) machines were used, which provide cross-sectional images along the body and generate 3 dimensional (3D) images that allow measurement of the dimension of the body and of the skeleton (Navajas et al. 2006a). Scanner 1 (Siemens, SOMATOM Esprit), the current scanner at the SAC-BioSS Unit in Edinburgh which provides cross-sectional images at intervals of 8 mm, was used for SAC animals. Scanner 2 (GE lightspeed 16-slice scanner, <http://burgessdiagnostics.com/ct-mri/ct>) is the mobile scanner that was used for Aberystwyth-animals. CT-linear dimensions were measured by using Sheep Tomogram Analysis Routines software STAR (Mann et al. 2003) after allocating anatomical landmarks on two 2D images: the back and the side view of each animal. These landmarks correspond to the linear measurements that are used by VSS2000 video image analysis system (VSS2000, E+V Technology, Germany, <http://www.vision-for-you.com/start.htm>) to produce predicted weights of the primal and trimmed primal joints (Rius-Vilarrasa et al. 2009b). It is important to note that using STAR software in SCTS 3D images enabled us to rotate the body image to have the best side view figure of the animal and thus to determine the best landmarks in the body.

As can be seen in Figures 6.1 and 6.2, from each individual lamb five width measurements (W1 to W5) and two length measurements (L1 and L2) were obtained from the back view CT image. Additionally, two width measurements (W6 and W7) and two length measurements (L3 and L4) were obtained from the side CT image. The linear measurements that were taken at fixed anatomical positions from the back CT image are: W1 = width between left shoulder muscle to right shoulder muscle, in line with the top of the chest; W2 = width of body at the 5th thoracic vertebra (rib) excluding the skin folds on both sides; W3 = body width between the 3rd and the 4th lumbar vertebrae; W4 = body width of the junction point between the top of the muscle and the lower abdomen (the flank); W5 = body width between the joint of the femur and tibia; L1 = length from the last cervical vertebra to the W4; L2 = length

from W4 to the bottom point of the hock. It is worth to note that it was defined that the vertical line for W measures with the L1 measure was set to be at perpendicular at 90 degree (Figure 6.1 and 6.2).

Similarly, the linear measurements that were taken at fixed anatomical positions from the side CT image are: L3 = length from the last cervical vertebra to the start of the femur; L4 = length from the last cervical vertebra to the 1st sacral vertebra; W6 = body depth between the 1st thoracic vertebra and the widest point of the chest; W7 = body depth between the 1st sacral vertebra straight the lower abdomen (Figure 6.1 and 6.2).

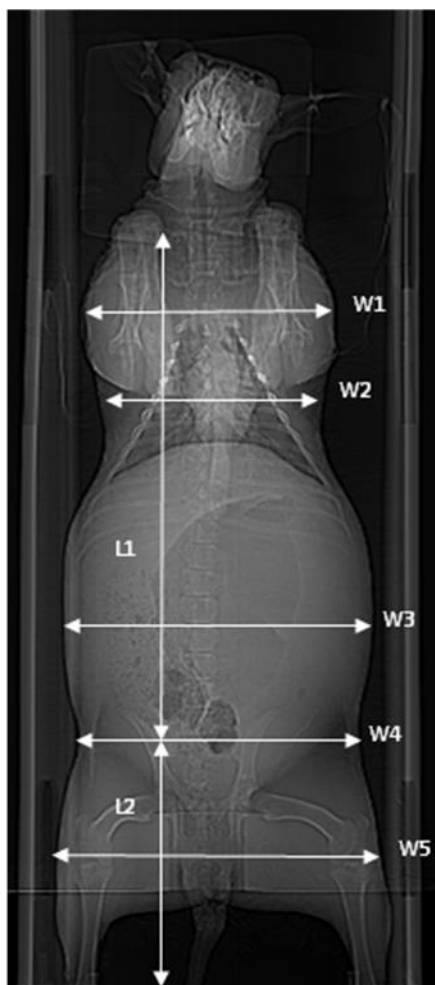


Figure 6.1 Back view image of the topogram scan

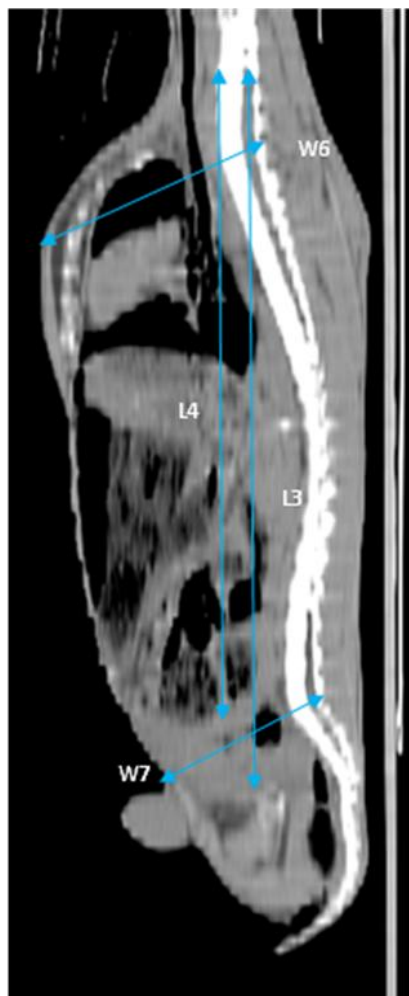


Figure 6.2 Side view image of the topogram scan

Lambs were transported to be slaughtered in the commercial abattoir of Welsh Country Foods in Anglesey. Lambs were slaughtered at a mean age of 144 days

(range 126–155 days). After applying conventional electrical stunning and then killing, carcasses were eviscerated, skinned and weighed where mean cold carcass weight (CCWT) was 14.6 kg (range 7.4-24.5 kg). CCWT was electronically measured, on the chain slaughter line, after subtracting 0.5 kg, accounting for the water drip loss, off the hot carcass weight. The kidney and channel fat and the kidney knobs were removed prior to carcass weighing.

Carcasses were then railed off the main slaughter line to the VIA room for scanning. A full description of how VIA operates can be found in previous chapters (chapters 2 to 5, and Rius-Vilarrasa et al. 2009a). In brief, two cameras are placed to take back and side view images of the passing carcass where VSS2000 software (VSS2000, E+V Technology GmbH, <http://www.vision-for-you.com/start.htm>) calculates linear carcass dimensional measurements (lengths, widths and areas). Subsequently, cold carcass weight (CCWT; kg) combined with the measurements obtained were adopted to develop computer algorithms to produce variables that characterize carcass shape and size. Additionally and most importantly, VIA produced objectively estimates of the carcass saleable meat yields (SMY) and different primal (P-; bone-in) and trimmed primal (TP-; bone-out) carcass joints (Leg, Chump, Loin, Breast, Shoulder). The predictions of trimmed primal joints were based on the dissection protocol under specific butchery specifications to provide pre-retail level primal cut information. This was undertaken by removing the bones and no more than 6 mm of the surrounding fat at any point of the opened primal cut. However, predicted trimmed weight of the breast joint was not available, because the de-boning process for this joint is very difficult and is not usually done commercially. A refined prediction equation, used in this study, has been developed from the initial standard one that previously calibrated and validated under British abattoir conditions (Rius-Vilarrasa et al. 2009b).

6.2.2 Statistical Analysis

Phenotypic correlations between all US measurements and CT-linear measurements, carcass and VIA-predicted traits were calculated. This assists in determining which predictor variables explained the greatest amount of variance in each VIA-predicted trait. All associations were checked for non-linearity and no non-linear pattern of

association was found. Restricted Maximum Likelihood (REML) analysis was performed for all predictor variables and each VIA-predicted traits, fitting a model which included fixed effects of TM-QTL genotype, sex, rearing rank (litter size) at weaning, dam age, CT scanner and farm; age (d) at US or CT for predictors and CCWT for VIA-predicted traits was fitted as a covariate; as well as sire which was fitted as a random effect. The residual values for each lamb, after accounting for the effects in this model, were then rescaled by adding the mean value to each residual. Rescaled residual values were then used in six different prediction equation models of the VIA-predicted traits (for models see below). Live weight was included or excluded as a predictor among these permutations.

Predicting VIA-traits using ultrasound scanning. VIA-predicted traits were regressed on ultrasound live weight (US_LWT) only using model 1. The effect of including UMD as a predictor in addition to US_LWT was then tested in Model 2 to investigate whether its inclusion provides a better fit.

Predicting VIA-traits using CT scanning. Two more models were tested to predict VIA-predicted traits. Model 3 investigated the best subset regression of all CT-linear measurements without fitting CT_LWT; whereas Model 4 included CT_LWT. Using the results of the best subsets regression, a subset of CT-linear dimensions was chosen for use in formulating prediction equations for all VIA-predicted traits.

Predicting VIA-traits using CT scanning and ultrasound measurements. Regression models 5 and 6 repeated models 3 and 4, respectively, but also fitting UMD as a predictor.

A forward stepwise model selection method was used to determine the optimum regression equation. The regression coefficients of all independent variables that entered the models were subjected to significance tests, at a significance level of $\alpha = 0.05$. Non-significant variables in the regression selection method were omitted from the regression analyses. Hence, regression selection methods were performed again with a small pool of independent variables, and models of predicting VIA-predicted traits were constructed.

To avoid instability of the coefficient of determination and inflation of the standard errors, the degree of multi-collinearity between final predictors that were chosen was

tested. All variables that appeared in the final prediction model had their *Variance inflation factor* (VIF) values lower than 10, and tolerance, defined as 1/VIF greater than 0.1.

The trait and trait groups included in the different models are shown below:

Model 1: US_LWT

Model2: US_LWT+ UMD

Model3: CT linear measurements

Model4: CT linear measurements + CT_LWT

Model5: CT linear measurements+ UMD

Model6: CT linear measurements+ UMD +CT_LWT

Each VIA-predicted trait had its own unique regression model with a different number of independent variables. The accuracy of each model was evaluated on the basis of their adjusted coefficient of determination R^2 values that takes the number of variables in the model into account, and the lowest root means square errors (**RMSE**) where the latter was calculated as a measure of precision. All R^2 values quoted in this chapter are adjusted coefficients of determination.

All statistical analyses were performed using SAS 9.2 (SAS Inst., Inc., Cary, NC).

6.3 Results

Descriptive statistics of the means and standard deviations (SD) of the variables studied of the 164 lambs in the sample population are summarized in Table 6.1. The overall mean weight at ultrasound was slightly higher (~2.5 kg) than at CT scan (~31 kg; Table 6.1). This may be due to the short time span between the two incidents. Cold carcass weight was over double the saleable meat yield predicted by VIA and the variation in VIA-predicted saleable meat yield was just below the half of that in cold carcass weight (Table 6.1). The heaviest primal cut predicted by VIA was the P_SHOULDER and the lightest was the TP_CHUMP. The percentage difference between the predicted weight of the primal and trimmed primal of the leg, chump,

loin and shoulder was 23, 38, 41 and 33%, respectively. Average length of CT_L3, taken from the side CT image, was the highest length measurement (54.9 cm), whereas average width CT_W5, taken from the back CT image, was the highest width measurement (31.6 cm).

Table 6.1 Statistical parameters for the raw data on live and carcass weight, ultrasound traits, CT linear measurements and VIA-predicted traits together with their means and standard deviations based on 164 lambs.

Variable	Mean	SD	Min	Max
Ultrasound traits				
US_LWT (kg)	33.41	5.92	20.0	50.6
UMD (mm)	23.34	3.44	13.1	32.3
CT traits				
CT_LWT (kg)	30.93	5.88	16.8	47.6
CT_L1 (cm)	54.8	3.57	44.2	63.0
CT_L2 (cm)	31.4	7.40	22.0	46.9
CT_L3 (cm)	54.9	3.76	44.1	67.2
CT_L4 (cm)	48.4	3.28	39.1	59.0
CT_W1 (cm)	22.9	2.92	14.2	28.4
CT_W2 (cm)	19.5	3.45	11.3	26.1
CT_W3 (cm)	27.4	3.38	19.5	33.1
CT_W4 (cm)	25.6	2.22	19.2	29.7
CT_W5 (cm)	31.6	1.50	26.7	34.5
CT_W6 (cm)	17.8	1.11	14.6	20.5
CT_W7 (cm)	14.5	1.42	11.2	18.6
Carcass and VIA traits				
CCWT (kg)	14.51	3.23	7.4	24.5
SPMY (kg)	6.14	1.60	2.60	11.20
P_LEG (kg)	3.93	0.81	1.94	6.34
TP_LEG (kg)	3.02	0.66	1.29	4.74
P_CHUMP (kg)	1.15	0.30	0.41	2.05
TP_CHUMP (kg)	0.71	0.16	0.34	1.23
P_LOIN (kg)	1.97	0.52	0.83	3.75
TP_LOIN (kg)	1.16	0.34	0.40	2.19
P_BREAST (kg)	1.19	0.32	2.39	0.60
P_SHOULDER (kg)	4.80	1.15	1.03	7.41
TP_SHOULDER (kg)	3.23	0.77	1.17	5.25

6.3.1 Phenotypic correlation between in vivo measures and carcass VIA-predicted traits

Phenotypic correlations between CCWT or VIA-predicted traits, and each *in-vivo* ultrasound and CT linear measurement trait are summarized in Table 6.2. In general, the correlations between VIA-predicted traits and UMD were significant ($P < 0.001$) and ranged between 0.62 and 0.71.

US_LWT and CT_LWT were significantly and highly correlated with all post-mortem traits and the correlation was ≥ 0.90 with CCWT and SPMY. The significant

relationships between UMD and post-mortem were 0.62 or above, and reached 0.71 with P_SHOULDER and TP_SHOULDER.

All CT-linear measurements, apart from CT_L2, were positively correlated with carcass weight and VIA-predicted traits (Table 6.2).

Animal length measurements taken by CT scan (CT_L1, CT_L3 and CT_L4), were significantly highly correlated (≥ 0.75) with VIA-predicted traits. Whilst CT_L2 correlations with all VIA-predicted traits tended to be negative and low, this relationship was only significant with respect to CCWT, TP_LEG, TP_CHUMP and P_ and TP_SHOULDER traits and was not significant for the remained VIA-predicted traits.

Amongst CT-length measurements, CT_L3, measured from the side view, had the highest values of correlations with all VIA-predicted traits (from 0.80 for TP_LOIN to 0.86 for P_LEG).

Unlike length measurements, width measurements were all moderate to lowly correlate with carcass weight and VIA-predicted traits and ranged from 0.26 to 0.67.

Table 6.2 Phenotypic correlations between CT measured in-vivo predictors and VIA-predicted traits (N=164)

Variable	CCWT	SPMY	P_LEG	TP_LEG	P_CHUMP	TP_CHUMP	P_LOIN	TP_LOIN	P_BREAST	P_SHOU	TP_SHOU
US_LWT ¹	0.90***	0.91***	0.90***	0.90***	0.90***	0.90***	0.86***	0.83***	0.87***	0.91***	0.91***
UMD	0.68***	0.70***	0.70***	0.70***	0.70***	0.70***	0.65***	0.62***	0.63***	0.71***	0.71***
CT_LWT	0.91***	0.91***	0.90***	0.90***	0.89***	0.89***	0.86***	0.84***	0.87***	0.92***	0.92***
CT_L1	0.81***	0.81***	0.81***	0.80***	0.79***	0.79***	0.77***	0.75***	0.78***	0.82***	0.81***
CT_L2	-0.16*	-0.15 ns	-0.15 ns	-0.19*	-0.14 ns	-0.15*	-0.09 ns	-0.09 ns	-0.09 ns	-0.25**	-0.23**
CT_L3	0.84***	0.84***	0.86***	0.84***	0.83***	0.83***	0.83***	0.80***	0.81***	0.85***	0.84***
CT_L4	0.83***	0.82***	0.85***	0.83***	0.82***	0.81***	0.81***	0.80***	0.79***	0.84***	0.83***
CT_W1	0.59***	0.59***	0.59***	0.61***	0.58***	0.58***	0.51***	0.52***	0.52***	0.66***	0.65***
CT_W2	0.52***	0.51***	0.50***	0.52***	0.50***	0.50***	0.43***	0.43***	0.45***	0.57***	0.56***
CT_W3	0.34***	0.32***	0.32***	0.34***	0.31***	0.32***	0.26**	0.27**	0.26**	0.41***	0.39***
CT_W4	0.36***	0.34***	0.34***	0.35***	0.33***	0.34***	0.28**	0.29**	0.29**	0.42***	0.41***
CT_W5	0.51***	0.50***	0.51***	0.52***	0.49***	0.48***	0.43***	0.42***	0.42***	0.60***	0.57***
CT_W6	0.64***	0.64***	0.64***	0.62***	0.64***	0.58***	0.62***	0.60***	0.62***	0.67***	0.65***
CT_W7	0.54***	0.53***	0.53***	0.52***	0.52***	0.52***	0.50***	0.48***	0.49***	0.57***	0.57***

¹(* P<0.05, ** P<0.01, *** P<0.001 and ns= not significant)

The relationship between width measurement predictors and VIA-predicted traits was not consistent with respect to its magnitude. CT_W6 was estimated to have the highest significant relationship with VIA-predicted traits (Table 6.2). Both width measurements CT_W3 and CT_W4 showed the lowest correlation with VIA-predicted traits in which the lowest was between CT_W3 and P_LOIN (0.26) although significant. All width measurements had their highest significant association with P_SHOULDER and TP_SHOULDER when compared to the remained VIA-predicted traits (Table 6.2).

6.3.2 Using ultrasound to predict VIA traits

As shown in Table 6.3, R^2 for VIA-predicted traits ranged between 0.50 and 0.62 when live weight at ultrasound was used solely as a predictor. However, fitting UMD in the prediction equation improved the R^2 by an average of 8% and consistently reduced RMSE.

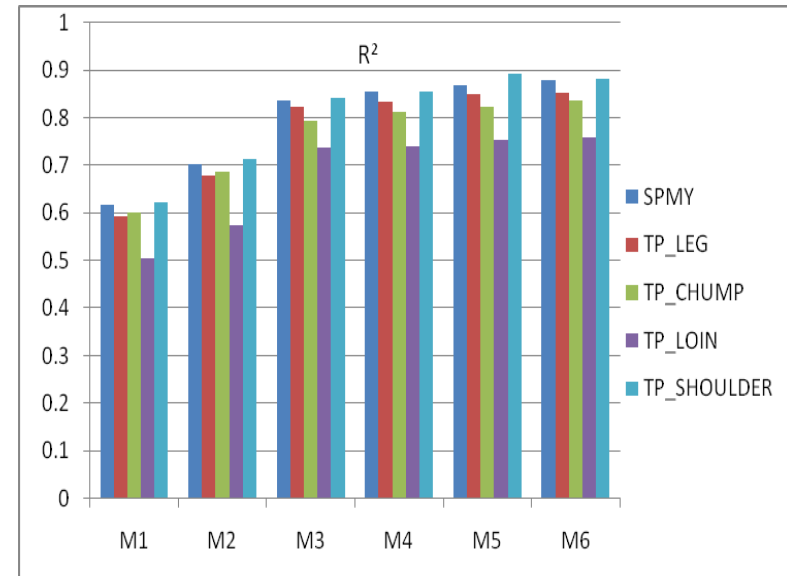
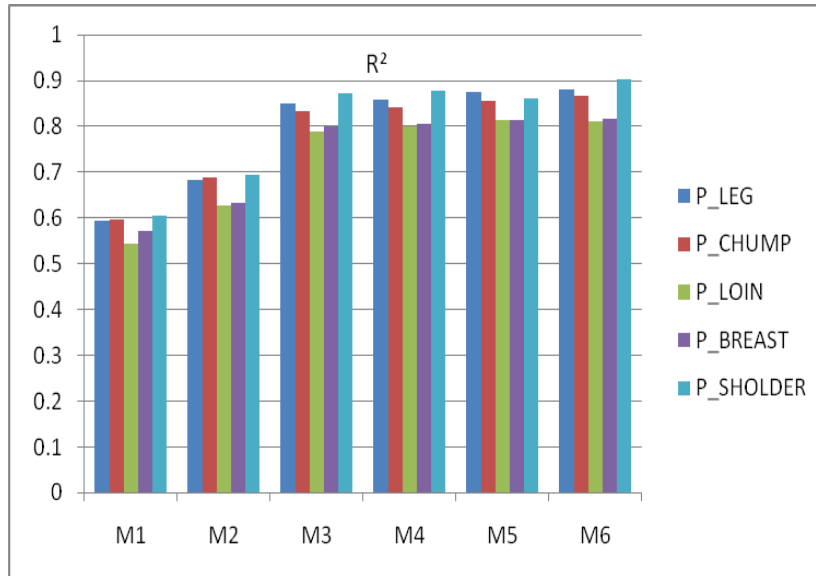
Using live weight alone to predict VIA-predicted carcass saleable meat yield gave an R^2 of 0.62 and an RMSE of 0.862. Using both live weight and mean ultrasound muscle depth improved R^2 to 0.70, and decreased RMSE 0.759 (Table 6.3, Graph 6.1). It was not unexpected that US_LWT would have the highest R^2 to predict SPMY as the correlation coefficient between these variables is very high and significant. Looking at the predictability of the highest priced cut in the carcass, adding UMD to US_LWT improved R^2 of P_LOIN and TP_LOIN by 8 to 7%, respectively, and greatly reduced RMSE.

Table 6.3. Prediction models (model 1 and 2) for the VIA-predicted traits by using ultrasound live weight alone and with ultrasound muscle depth measurement.

VIA-carcass primal cut	Model	Predictors	RMSE ²	R ²
SPMY ¹	M1	US_LWT	0.862	0.62
	M2	US_LWT+UMD	0.759	0.70
P-LEG	M1	US_LWT	0.454	0.59
	M2	US_LWT+UMD	0.402	0.68
TP_LEG	M1	US_LWT	0.367	0.59
	M2	US_LWT+UMD	0.327	0.68
P_CHUMP	M1	US_LWT	0.167	0.60
	M2	US_LWT+UMD	0.147	0.69
TP_CHUMP	M1	US_LWT	0.088	0.60
	M2	US_LWT+UMD	0.078	0.69
P_LOIN	M1	US_LWT	0.308	0.54
	M2	US_LWT+UMD	0.278	0.63
TP_LOIN	M1	US_LWT	0.210	0.50
	M2	US_LWT+UMD	0.194	0.57
P_BREAST	M1	US_LWT	0.185	0.57
	M2	US_LWT+UMD	0.171	0.63
P_SHOULDER	M1	US_LWT	0.639	0.60
	M2	US_LWT+UMD	0.562	0.69
TP_SHOULDER	M1	US_LWT	0.419	0.62
	M2	US_LWT+UMD	0.365	0.71

¹ All VIA-predicted traits defined in the text.

² R² and RMSE are adjusted R² and root mean square error.



Graph 6.1 Adjusted R^2 values using models 1-6 (M1-M6, defined in text) to estimate VIA-predicted traits.

6.3.3 Using CT to predict VIA-predicted traits

Combining all CT-linear measurements in the prediction equation, VIA-predicted traits could be predicted with a relatively high accuracy (model 3; Table 6.4) where R^2 statistic was generally greater than 0.74. The final model for each VIA-predicted trait differed in the number of predictors used in the model and ranged from 5 to 7 predicting variables. For example, the final model of the highest R^2_{adj} ($R^2_{adj} = 0.87$; P_SHOULDER) included CT_L2, CT_L4, CT_W1, CT_W2, CT_W3 and CT_W6. It was noticeable that within CT width measurements, CT_W2 and CT_W6 appeared in all of the best models predicting all VIA-predicted traits. Whilst the remaining CT width measurements were significant in other prediction models CT_W7 was the only significant predictor with regard to P_CHUMP.

Regardless of the number of predictors in the model, VIA-predicted primal cut weights were more accurately predicted (by on average of 4%) than the trimmed primal cut weight of each individual trait. There was, for instance, a 5% reduction in the prediction accuracy between P_LEG and TP_LEG (Graph 6.1). Accordingly, there was an increase in the prediction precision of the prediction of the primal cut weights compared to the trimmed primal cut weights (by 19% on average) presented as a reduction in RMSE values (Table 6.4).

Inclusion of CT_LWT (model 4) to the previous CT linear measurement prediction equation did not greatly improve R^2 (Table 6.4). The maximum improvement of 2% in R^2 was found in TP_CHUMP while TP_LOIN remained unchanged (Graph 6.1).

As noted above, adding CT_LWT led to an increase in R^2 to values ranging between 0.74 and 0.88 and that was accompanied with a consistent mean improvement in the prediction equation precision (RMSE). However, the inclusion of CT_LWT in the prediction equation resulted in changing the number of CT linear measurements as predictors by 1 predictor in most VIA-predicted traits, 4 predictors in P_BREAST or remained the same in P_LEG and TP_CHUMP. Some predictors were omitted from the prediction equations whereas others were replaced by CT_LWT.

Table 6.4 Prediction models (model 3 and 4) for the VIA-predicted traits by using CT live without and with CT linear measurements.

VIA-carcass primal cut	Model	Predictors	RMSE ²	R ²
SPMY ¹	M3	L1+L2+L4+W2+W6	0.564	0.84
	M4	L1+W1+W2+W6+CT_LWT	0.532	0.85
P-LEG	M3	L4+W1+W2+W5+W6	0.276	0.85
	M4	L4+W1+W2+W5+W6+CT_LWT	0.267	0.86
TP_LEG	M3	L4+W1+W2+W6+W7	0.242	0.82
	M4	L4+W1+W2+W6+CT_LWT	0.236	0.83
P_CHUMP	M3	L1+L2+L4+W2+W5+W6	0.108	0.83
	M4	L1+L4+W2+W5+W6+CT_LWT	0.105	0.84
TP_CHUMP	M3	L2+L3+W2+W3+W6	0.063	0.79
	M4	L3+W1+W2+W3+W6+CT_LWT	0.060	0.81
P_LOIN	M3	L2+L3+W1+W2+W3+W6	0.209	0.79
	M4	L2+L3+W1+W2+W6+CT_LWT	0.204	0.80
TP_LOIN	M3	L2+L3+W1+W2+W6	0.153	0.74
	M4	L2+L3+W1+W2+CT_LWT	0.152	0.74
P_BEAST	M3	L1+L2+L3+W2+W3+W4+W6	0.126	0.80
	M4	L1+W2+W6+CT_LWT	0.124	0.81
P_SHOULDER	M3	L2+L4+W1+W2+W3+W6	0.364	0.87
	M4	L4+W1+W2+W3+W6+CT_LWT	0.356	0.88
TP_SHOULDER	M3	L3+W1+W2+W3+W6	0.270	0.84
	M4	L3+W1+W2+W3+CT_LWT	0.259	0.86

¹ All VIA-predicted traits defined in the text.

² R² and RMSE are adjusted R² and root mean square error, respectively.

6.3.4 Using CT and UMD to predict VIA-predicted traits

Including ultrasound muscle depth along with CT linear measurements in the models increased R^2 in all VIA-predicted traits and decreased RMSE (Table 6.5). Compared to model 5 (Table 6.4), using UMD improved the overall predictability accuracy by ~3% for all VIA-predicted traits (excluding P_SHOULDER). Consistently, RMSE values fell, excluding P_SHOULDER and TP_SHOULDER, as evidence of precision improvement (Table 6.5, Graph 6.1). TP_SHOULDER showed the highest improvement in R^2 while SPMY showed the largest decrease in RMSE.

A minimum of 4 and a maximum of 5 CT linear measurements plus UMD were used in the prediction equation to produce the best prediction of the VIA traits (Table 6.5). The best R^2 value (0.89) in the present model used only four predictors and was the equation fitted to estimate TP_SHOULDER.

Adding CT_LWT to the CT linear measurement and UMD combination prediction equation explained further the variance, although slightly, in VIA-predicted traits (model 6; Table 6.5). A mean percentage improvement of ~1% in the prediction accuracy was added to model 6 when compared to model 5 (Graph 6.1).

It was found that model 6 is the model that accounted for the maximum variance in the mean values for each VIA-predicted trait as shown in Table 6.5, based on R^2 and RMSE. The best combination of predictors accounted for a very high proportion (0.76 to 0.90) of the variation in VIA-predicted traits. Different combinations of predictors were most informative for different VIA-predicted traits, with a minimum of 3 and a maximum of 6 CT linear measurements in addition to the UMD and CT_LWT traits being presented in at least one prediction equation of model 6.

Table 6.5 Prediction models (model 5 and 6) for the VIA-predicted traits by using the combination of CT linear measurements and ultrasound muscle depth together without or with CT live weight.

VIA-carcass primal cut	Model	Predictors	RMSE ²	R ²
SPMY ¹	M5	L1+L2+L4+W2+W6+UMD	0.505	0.87
	M6	L1+L2+W1+W2+W4+W6+CT_LWT+UMD	0.485	0.88
P-LEG	M5	L4+W1+W2+W5+W6+UMD	0.252	0.88
	M6	L4+ W1+W2+W5+W6+ CT_LWT+UMD	0.248	0.88
TP_LEG	M5	L4+W1+W2+W6+UMD	0.223	0.85
	M6	L4+W1+W2+ CT_LWT+UMD	0.221	0.85
P_CHUMP	M5	L1+L2+L4+W2+W5+UMD	0.100	0.86
	M6	L1+W2+W5+W6+ CT_LWT+UMD	0.096	0.87
TP_CHUMP	M5	L2+L3+W1+W2+W3+UMD	0.058	0.82
	M6	L4+W1+W2+ CT_LWT+UMD	0.056	0.84
P_LOIN	M5	L1+L2+L3+W1+W2+UMD	0.197	0.81
	M6	L2+L3+W1+W2+ CT_LWT+UMD	0.197	0.81
TP_LOIN	M5	L2+L4+W1+W2+UMD	0.148	0.75
	M6	L2+L4+W1+W2+ CT_LWT+UMD	0.146	0.76
P_BREAST	M5	L1+L2+L3+W2+W6+UMD	0.122	0.81
	M6	L1+W2+W6+ CT_LWT+UMD	0.120	0.82
P_SHOULDER	M5	L1+L2+W1+W5+W6+UMD	0.379	0.86
	M6	L4+W1+W2+W5+W6+ CT_LWT+UMD	0.319	0.90
TP_SHOULDER	M5	L4+W1+W2+W6+UMD	0.334	0.89
	M6	L4+W1+W2+W6+ CT_LWT+UMD	0.235	0.89

¹ All VIA-predicted traits defined in the text.

² R² and RMSE are adjusted R² and root mean square error.

Accuracy in predicting VIA-measured traits in model 6 was higher by 2% in comparison with model 4 which did not include UMD (Graph 6.1). Whilst TP_SHOULDER was the most notably improved in R^2_{adj} (~3%; Graph 6.1), SPMY prediction equation precision had the highest increase when model 6 compared to model 4.

6.4 Discussion

This experiment aimed at producing the best prediction model to estimate carcass VIA-predicted yields using ultrasound measurements and *in-vivo* CT-extracted body linear measurements and their phenotypic relationships. It was essential to make an appropriate comparison between prediction models to first adjust all predictors and predicted variables for fixed, random and covariant factors; hence the resulting information may be beneficial for any future breeding programmes.

6.4.1 Descriptive statistics

Generally, the values of the parameters in VIA predicted traits were within the range of variation reported when VIA was used to predict the same traits in different population of crossbred lambs that were carriers or non-carriers of different muscle enhancing QTLs (Masri et al. 2011a; 2011b; 2010).

It must also be mentioned that the present experiment used lambs from normal production systems under grazing conditions. Thus, animals were slaughtered at a fixed age rather than being finished at a fixed live weight and condition scores which may be the cause of the variation in both *in vivo* and carcass traits. Therefore, it was expected to observe variability with respect to the CT linear measurements due to the difference in growth rate, carcass weight and animal size.

6.4.2 Relationships between Ultrasonic and CT linear measurements and VIA-predicted traits

The phenotypic relationship described here has shown that there are important and significant correlations between *in-vivo* ultrasound and CT-linear measurements and VIA-predicted cut weights.

As would be expected, a highly significant association was observed between linear indices relating to the shoulder region and P_SHOULDER or TP_SHOULDER, whereas linear measurements taken in the leg played a significant role for the description of the relative VIA-predicted weight in the carcass leg and chump.

6.4.3 Prediction models using ultrasound measurements

Using animal live weight at ultrasound scan as a single predictor had limited potential to predict VIA-derived primal cut weights with sufficient accuracy and precision. The accuracy and precision of using this model in the present study is due almost entirely to the strong significant weight-to-weight relationship.

In a previous study, ultrasound live weight was reported as a reliable predictor to predict carcass lean content weights (Silva et al. 2006). In agreement with our results, fairly high improvements in the prediction accuracy were achieved when UMD was included in the regression equation alongside US live weight (Silva et al. 2006) to predict meat yields.

6.4.4 Identifying the best CT linear measurements subset to predict VIA-derived traits

Across all models where CT linear measurements were used as predictors, VIA-derived predictions in the loin cut were slightly less accurately predicted by CT than other cuts (Tables 6.3, 6.4 and 6.5). This loss of accuracy in the prediction of P_LOIN and TP_LOIN can be explained by looking at the relationships between them and the predictors that were used in their prediction models. Loin VIA-predicted traits had relatively moderate to low relationships with CT linear measurements (CT_W1, CT_W2 and CT_W3) that were included in the prediction models, compared to other VIA-predicted traits. Additional reasons why prediction accuracy of the VIA-predicted traits in the shoulder, leg and chump cuts relative to the loin cut are greater are probably due to the higher number of measurements taken and the distance between measurements which covered these parts of the body compared to those spanning the loin region.

However, using VIA technology to predict sheep meat yields in a previous study, Rius-Vilarrasa et al. (2009b) also demonstrated a better accuracy of predictions for

the shoulder and the legs in comparison to other carcass cuts. It can be concluded that the very strong correlation between extracted CT linear measurements in a specific region with its corresponding VIA-predicted primal cut weight contributes to the prediction accuracy and precision. Therefore, it could be possible, in future, to produce a region-specific simple regression models that include CT linear measurements that are most related to the corresponding VIA-predicted cut. A similar conclusion was reached in pigs when in vivo VIA shape indices were used to estimate carcass composition (Doeschl-Wilson et al. 2005).

Inclusion of CT live weight in the prediction model improved the fit of the data, although slightly, as evidenced by the lower RMSE and greater coefficient of determination, compared to just fitting CT linear measurements (model 4, Table 6.4).

6.4.5 Selection of best CT measurements subset combined with UMD to predict VIA-derived traits

The R^2 values obtained in the current study to predict the VIA-derived cut yields was not very high and may be due to the fact that CT linear measurements are predicting predicted traits. This could be explained by the accumulation of the unexplained variation from two regression equations which resulted in the loss of prediction accuracy. However, higher accuracy and precision were found when CT linear measurements were combined with UMD than with CT linear measurements alone. Previous researchers found that fitting ultrasound measurements to the external linear measurement prediction equation resulted in an increase of the prediction accuracy of carcass composition traits (Edwards et al. 1989). Overall, the ultimate highest accuracy and precision were observed when animal live weight was added to the previous combination to predict VIA-derived primal cut weights. These results are consistent with those reported in pigs where an increase of prediction powers was detected when ultrasonic backfat depth and live weight measures were augmented by VIA shape indices (Doeschl-Wilson et al. 2005).

6.4.6 Practical implication

The findings of this study are important for several reasons. Firstly, unlike the challenges when VIA measurements are taken externally in live lambs (e.g. wool

cover thickness), there is no problem in extracting data if un-shorn lambs were CT scanned. Secondly, the variation observed of VIA-derived traits in the current sample population is likely representative of purebred Texel lambs out of the sire reference scheme used in the UK. Thirdly, usefulness of using purebred Texel lambs served to decrease between-breeds variation and to maximize the benefit of the results to purebred Texel seed stock breeders, which is used as the main sire in the meat industry in the UK. The regular CT measurements of high index rams, as part of a two-stage selection programme (Jopson et al. 2004), can be enriched with CT linear measurements to predict traits from VIA that are likely to be the basis of future payment system. More importantly, a further investigation is required on the best way to benefit from CT linear dimensions on the whole body and regions to predict tissue content and meat quality at specific primal cuts using VIA. This can be achieved through combining the results obtained in this study with the highly accurate CT-derived tissue information and meat quality estimation reported elsewhere (Karamichou et al. 2006a; Lambe et al. 2009b; Young et al. 2001). Nevertheless, genetic parameters, including correlations must be assessed between CT linear measurements and VIA-predicted traits to investigate the suitability for including these traits in any breeding programme. It is worth to noting that the increased accuracy in the prediction model is not the only criteria to select the best model. Account should be taken of the difficulty and cost of taking the measurements (Hedrick 1983).

6.5 Conclusions

The purpose of this study was to detect the best subset regression model to predicted VIA-estimated saleable carcass, primal and trimmed primal weights through ultrasound, CT linear measurements and a combination of both. This study illustrated very high, positive and significant phenotypic relationships between the VIA-derived traits and these predictors. The results suggest that the highest prediction accuracy of the VIA-predicted cuts could be achieved when ultrasound measurement, CT linear measurements and live weight were fitted in the model. However, a balance between the increased prediction accuracy and the difficulty of obtaining the predictors may impose heavier economic pressure on the breeder is needed. The outcomes of the current research will be beneficial when anticipated changes in the payment system

in the UK abattoirs take place from those currently based on weight and conformation to a value based marketing system.

Chapter 7

The estimation of genetic parameters for *in-vivo* and post mortem traits and the association between computed tomography (CT) and video image analysis (VIA) and Meat and Livestock Commission (MLC) grades for conformation and fat classes

Abstract

This study was carried out to exploit the attainable data on animals that went through computed tomography (CT), video image analyses (VIA) and Meat and Livestock Commission (MLC) classification for carcass conformation and fat class in an attempt an initial estimation of genetic parameters. The design of the experiment was not helpful in estimating the genetic parameters using the animal model, therefore the sire model using ASReml was performed to make the best use of the available data.

Heritability and genetic correlation estimates using the weight or age as a covariate in the statistical model was not consistent and resulted in only indicative information.

Phenotypic correlations between CT measured traits and VIA-estimated traits showed a positive relationship between CT-measured muscle weight and VIA-derived traits. The relationship between CT-measured fat weight and VIA-derived traits was negative. The reported phenotypic correlations may be helpful for further studies with larger data set acquired to produce more robust estimation.

7.1 Introduction

The principal source of income for the meat sheep breeders in the UK is the ultimate carcass value. Breeding programme goals in the last two decades in the UK were set to improve sheep growth and carcass quality and designed to meet the consumer demand for more lean meat with less fat (Macfarlane & Simm 2008).

Significant genetic gains have been achieved by utilizing the stratified system and through the selection based on the lean growth index, focussing on the body weight and ultrasound muscle and fat depth measurements (Márquez et al. 2012; Simm et al. 2002; Simm & Dingwall 1989). The increased desire of consumers for high meat quality implied the need to develop and extend the selection indices to incorporate carcass and meat quality traits, which are often difficult to measure, in to modern breeding programmes. Therefore, CT measurements that are very accurate in quantifying carcass composition (Young et al. 2001) were the best to be included for developed selection criteria. However, due to the expensive cost of CT scanning measurements a two stage selection strategy in combination with ultrasound was recommended for terminal sire breeding programmes in the UK (Macfarlane & Simm 2008). The basis of this strategy is to scan all candidates by ultrasound and then select the best 15-20% for CT scanning. In the latest study on data from terminal sire industry flocks, inclusion of CT scanning measurements together with ultrasound scanning resulted in 7% response to selection (for CT-measured muscle weight), 10% (for CT-measured fat weight) and 20% (for CT-measured muscularity) higher compared to ultrasound alone (Moore et al. 2011).

Lamb carcasses in the UK are valued on the basis of the weight and Meat and Livestock Commission grades for conformation and fat classes MLC-CONF and MLC-FAT, respectively (MLC 2009). However, several studies reported positive genetic and phenotypic correlations between conformation and fatness (Conington et al. 1998; Karamichou et al. 2006b; Lewis et al. 1996) which is contrary to the breeding goals of meeting consumer specifications with more lean and less fat content of the carcass. Therefore, MLC carcass conformation and fat information may not be favourable in selection and breeding programme for improving carcass characteristics. A great opportunity to predict objectively the weight of individual carcass cuts and accounting for variation in carcass composition was possible with

the invention of Video Image Analysis (VIA) of carcass images (Brady et al. 2003; Cunha et al. 2004; Hopkins et al. 2004; Horgan et al. 1995; Stanford et al. 1998). VIA system (VSS2000, E+V Technology GmbH, <http://www.eplusv.de/>) was under trial in the UK lamb abattoirs and showed a relatively high accuracy in predicting primal meat yields of the lamb carcass joints (Rius-Vilarrasa et al. 2009b). The promising accurate predictions of individual carcass cuts will help greatly in the future movement to a value based marketing system (VBMS). VBMS is a payment system that will determine the actual carcass value according to the predicted composition of each carcass primal cut independently.

The knowledge of genetic parameters for the economically important production traits is essential in order to develop effective genetic improvements programmes in future. Genetic parameters of ultrasound traits were reported in different sheep breeds in the UK like hill sheep (Conington et al. 1995; Roden et al. 2003); Welsh Mountain sheep (Ap Dewi et al. 2002); Texel and Suffolk and Charollais (Matika et al. 2011) and globally (Greeff et al. 2008; Safari et al. 2005). In comparison to ultrasound scanning, few studies have reported genetic parameters for some CT scanning measurements (Jones et al. 2004; Kvame & Vangen 2007; Maximini et al. 2012). However, to our knowledge, there is only one study that published estimations of genetic parameters for VIA-predicted lamb carcass cuts (Rius-Vilarrasa et al. 2009a).

Since CT scanning information of elite sires is now being used for genetic selection for carcass merits, it was of interest to look at some genetic parameters and relationships between CT scanning measurements and post mortem traits. Therefore, the objective of this study was to quantify the genetic association between CT predicted tissue weights and both VIA-predicted weight of the primal cuts and the MLC-CF grades.

Such investigation has not been carried out before due to unavailability of data on animals that went through CT and VIA. However, the available dataset at SAC obtained in the TM-QTL project allowed us to attempt an initial estimation of genetic parameters although robust estimations demand a large performance and pedigree data. It was not clear at the outset if the dataset could provide reliable estimates and

the study attempted to make best use of these data. Therefore, results of this study will not be definitive and are preliminary estimates for any future breeding programme.

7.2 Materials and Methods

7.2.1 Data

Animals in this study were part of a larger scientific project to study the direct and indirect effects of the Texel muscling quantitative trait loci (TM-QTL) on *in-vivo* and post-mortem carcass traits as well as its effects on meat quality traits in purebred Texel lambs (e.g chapters 5 and 6 and Macfarlane et al. (2012).

The project was designed to generate homozygous TM-QTL carriers and non-carriers and heterozygous inheriting the TM-QTL from either paternal side. To do so, in 2006, one grandsire that was genotyped to be a carrier of the TM-QTL was used to produce the subsequent breeding generations of sires and ewes. There were 7 TM-QTL carrier sires used to mate 181 ewes that were TM-QTL carriers or non-carriers in two different farms Aberystwyth University in Wales (IBERS) and Scottish Agricultural College (SAC) in Scotland. Of the 7 mating sires there were 3 common across the two farms. Performance records of 210 animals with full records on CT scanning, but fewer animals for VIA and MLC data were used in this study along with a limited size of pedigree data of 3868 IDs.

All lambs (n = 210) were CT scanned at average age (CT-AGE) of 130 days (minimum age = 93 and maximum age = 145 days of age); and at average weight (CTWT) of 30.95 kg (minimum weight = 16.80 and maximum weight = 47.60) (Table. 7.1). While lambs at SAC farm were CT scanned at the CT scanning unit near Edinburgh, lambs from IBERS were scanned also by the SAC CT team but using a mobile CT scanning unit. The CT cross-sectional images were taken on three reference anatomical points: the ischium bone, the fifth lumbar vertebra and the eighth thoracic vertebra. More information on how CT cross-sectional images analysed described in details in Macfarlane et al. (2006). Total weights of fat (CT-FAT), muscle (CT-MUSC) and bone (CT-BONE) in the carcass were predicted using reference scan tissue areas and live weight in relevant prediction equations

developed for crossbred lambs (Macfarlane, personal communication). Using the former prediction equation, there were 11 lambs that had a prediction of 0.00 kg total fat weight.

On average two weeks after the CT scanning, lambs that were slaughtered in an abattoir (n = 209) at an average age (SL-AGE) of 144 days (minimum age = 126 days; maximum age 155 days). Carcasses were then weighed (CCWT) and classified according to the MLC grading system for conformation (MLC-C) and fat class (MLC-F) apart from few animals that substantially damaged during dressing and eviscerating (more details were given in previous chapters 5 and 6).

Carcasses were then taken off the main slaughter chain to the VIA room to be VIA-scanned. A visualising system of two cameras was fit to capture and process the images taken by using the VSS2000 VIA system (VSS2000, E+V Technology GmbH, <http://www.vision-for-you.com/start.htm>) (more details on how VIA runs were given in previous chapters 2,3,4,5 and 6). Only VIA-predicted weights of the saleable meat yield of the carcass primal cuts with a high economically value (loin (VIA-LOIN), leg (VIA-LEG), chump (VIA-CHUMP) and shoulder (VIA-SHOU)) were analysed using the refined VIA-prediction equations that were developed by Rius-Vilarrasa et al. (2009c) (further details in previous chapters).

7.2.2 Statistical methods

All analyses were performed using a sire model with pedigree in ASReml (Gilmour et al. 2006), applying restricted maximum likelihood procedures to fit a mixed linear model. An attempt to estimate the genetic parameters using an animal model was not successful due to singularity in matrices (implying a heritability of greater than 1) that did not allow convergence of the Log-likelihood. The reason for this failure may be due to the lack of data and possibly the fact that a very small number of sires which all traced back to one sire were used in this experiment. The impact of using offspring of a single grandsire as sires along with the lack of connectedness in the female lines was seemingly of substantial effect on the genetic variation of the base population. However, genetic parameters were estimated using a multivariate sire model including the fixed effects (when significant) of (i) sex (entire males and females), (ii) dam age, (iii) rearing rank at weaning and (iv) CT scanner or farm

(when CT scanning traits or the post mortem traits were examined, respectively). The TM-QTL genotype was tested to investigate whether it has a significant effect in the model on all traits and found to be not significant, thus removed from the analyses. Some researchers have fitted either the age or the weight as a covariate. In the current study and for comparison purposes when possible, age at CT scanning or slaughter was fitted as a linear covariate to look at the trait as an absolute weight (Model 1). When fitted as a linear covariate, live weight at measurement (CTLWT for CT traits and CCWT for VIA and MLC traits) was included in the model to look at the trait as relative to the total live weight (Model 2). The genetic effect of the sire was fitted in both models as a random factor.

Univariate analyses were performed for each trait to estimate variance components. The heritability coefficient (h^2) was calculated as the ratio of the sire variance component $\times 4$ to the phenotypic variance (the sum of all variance components).

$$h^2 = \frac{\sigma^2 A \times 4}{\sigma^2 P}$$

A set of bivariate analyses were undertaken for the combinations between CT traits, VIA-predicted traits and MLC traits to estimate phenotypic and genotypic covariances. Genetic correlations (r) between trait 1 (t_1) and trait 2 (t_2) were calculated as the ratio of the covariance between t_1 and t_2 to the square root of the t_1 and t_2 variances as

$$r_{t_1,t_2} = \frac{\sigma_{t_1,t_2}}{\sqrt{\sigma^2 t_1 \sigma^2 t_2}}$$

7.3 Results and discussion

7.3.1 Summary statistics

Means and standard deviations for *in-vivo* CT scanning measurements, VIA-predicted weights of the carcass primal cuts and the MLC-CF grades are shown in Table (7.1).

Amongst all studied traits, estimated CT-FAT showed the highest coefficient of variation of 65% due to the fact that 14 lambs were estimated to have zero content of fat. It should be noted that excluding the 14 lambs with zero estimation of fat content resulted in a reduction of CV% ($CV\% = 57.96$). The Texel breed has been reported to have the highest CV% for fat weight predicted by CT in comparison to Suffolk and Charollais breeds in an earlier study (Jones et al. 2004). The variation in CT-BONE was very small whereas the coefficient of variation of the CT-MUSC was approximately 23%.

The coefficient of variation of the VIA-predicted weights of the carcass primal was generally above 20% and ranged between 20.1% and 28.5% for VIA-LEG and VIA-LOIN respectively. The variation in the VIA-predicted traits in the current study is higher than the variation reported for the same traits in Rius-Vilarrasa et al. (2009a). This could be due to the differences in the weight ranges (minimum and the maximum weights) between the current study and the former study as well as the difference in the sample size. Another possible reason could be the effect of TM-QTL on live weight across a range of ages from birth to slaughter and on cold carcass weight particularly between TM-QTL homozygous carriers and non-carriers (Macfarlane et al. 2012). Compared to Rius-Vilarrasa et al. (2009a) the four TM-QTL genotypes and their effects on weight could be a reasonable explanation for the higher variation in VIA-traits in this study.

Table 7.1 Number of animals (N), means, standard deviation (SD), coefficient of variation (CV) and ranges of performance traits measured on live animal and post mortem.

Variable	N	Mean	SD	CV(%)	Maximum	Minimum
CT-LVWT (kg)	210	30.9	5.75	18.61	40.6	13.6
CT-AGE (day)	210	130	11.50	8.85	145	93
CT-FAT (kg)	210	1.62	1.06	65.69	4.87	0.00
CT-Muscle (kg)	210	8.95	2.05	22.95	14.48	1.52
CT-Bone (kg)	210	2.61	0.42	16.21	3.69	1.02
SL-AGE (day)	209	144	7.48	5.18	155	126
CCWT (kg)	208	14.6	3.41	23.60	24.50	7.40
VIA-LEG (kg)	203	3.94	0.79	20.06	6.34	1.94
VIA-CHUMP (kg)	203	1.15	0.29	25.25	2.05	0.41
VIA-LOIN (kg)	203	1.16	0.33	28.50	2.18	0.40
VIA-SHOULDER (kg)	203	3.25	0.74	22.89	5.25	1.17
MLC-C	207	3.03	0.81	27.11	1.00	5.00
MLC-F	207	8.00	2.65	33.59	4.00	13.0

7.3.2 Trait heritability

Heritability estimates for the CT measured traits, VIA-predicted traits and carcass MLC grades using Model 1 and Model 2 are shown in Table 7.2.

For tissue weights measured by CT scanning the heritability estimates using Model 1 were very high with respect to CT-MUSC and CT-FAT but with high standard errors (Table 7.2). On the other hand, using Model 2 h^2 estimations were low to moderate in general and ranged from 0.24 to 0.54 for CT-MUSC and CT-BONE, respectively. The standard error of the CT-traits heritability estimates was high so that the h^2 estimation was not significantly different from zero. The reason would probably be the limited number of animals available for the current study which inflates the standard error of the estimation. It is, therefore, difficult to directly compare these heritability estimates with literature values.

This study used a not very powerful sample size and therefore it seems worthwhile to compare the findings with studies based on larger sample sizes. The heritability estimates of the CT-FAT and CT-BONE in Model 2 are closer to those of the same

traits estimated and corrected for live weight on the Norwegian White Sheep (NWS) compared to the estimations resulting from adjusting for age although the standard errors of the estimates in this study is very much higher than in the NWS study (Kvame & Vangen 2007). In the NWS study, two animal models were used, one corrected for live weight and the other corrected for age, to estimate the heritability values of CT traits on a large dataset. The difference in the h^2 estimation of the CT-MUSC between Models used in this study is greater in magnitude than in the NWS study. However, the differences between h^2 estimations in Model 1 and those reported in the NWS study corrected for age appear very high with respect to CT-MUSC and CT-FAT but not very much for CT-BONE (Kvame & Vangen 2007). Some of the differences between h^2 estimations reported in the present study and the NWS study could be due to the effect of different CT procedures used. In a different study on big dataset of purebred Texel data from the SRS in the UK, an animal model at a constant age was used to estimate heritability values of the CT muscularity and tissue weight traits (Jones et al. 2004). The h^2 of CT-MUSC and CT-FAT (0.46 and 0.40, respectively) in their study appear higher than h^2 estimations of the same traits in Model 2 but lower than those in Model 1 of the current study. In Scottish Blackface sheep, h^2 estimation of the CT-MUSC and CT-FAT (0.48, 0.60, respectively) using an animal model and correcting for age was the highest found in the literature but still below the h^2 estimations using Model 1 in the current study (Karamichou et al. 2006b). In contrast, h^2 estimation of the CTBONE in the current study was very much higher in Model 2 and Model 1 than in Karamichou et al. (2006b) (0.54 and 0.30 vs. 0.14, respectively). The sample size, the model (animal or sire model) along with the covariate adjustments and the structure of the experiments are all influential factors in the differences between h^2 estimations in the reported studies.

The heritability estimates of VIA-predicted weight of the primal cuts were available with the exception of VIA-LOIN in Model 1, and the VIA-CHUMP and VIA-SHOU in Model 2 (Table 7.2). The univariate analyses of the non-obtainable h^2 estimations showed no sire variance ($\sigma_s = 0$) and as a result a zero estimation of h^2 . The h^2 estimations of the obtainable VIA-predicted traits were low to moderate in Model 1

and very low to moderate in Model 2 but both model h^2 estimations had very high standard error which makes the estimation not significantly different from zero.

The only known study that estimated genetic parameters for VIA traits in sheep and proposed the utilization of VIA measurements in the sheep breeding programmes in the UK was led by Rius-Vilarassa et al. (2010). In their study an animal model corrected for age was used to estimate the genetic parameters of the VIA-predicted traits.

Comparison between h^2 estimations in this study and Rius-Vilarrasa et al. (2009a) is for illustration only. While h^2 estimation of the VIA-LEG in Model 1 of the current study had a higher value (0.30) than in Rius-Vilarrasa et al. (2009a) Model 2 h^2 estimation of VIA-LEG was substantially lower (0.04 vs.0.20). VIA-LOIN in Model 2 in this study is very similar to that found in Rius-Vilarrasa et al. (2009a) (0.26). In contrast to VIA-SHOU which had a very close h^2 estimation between Model 1 in the current study and Rius-Vilarrasa et al. (2009a), h^2 estimation of the VIA-CHUMP in the current study was higher than in Rius-Vilarrasa et al. (2009a) (0.25 vs. 0.07, respectively).

Table 7.2 Heritability estimates and standard error (S.E[†]) of estimation obtained from univariate analyses of Model 1 and Model 2[‡] for CT, VIA and MLC traits.

Trait	Model 1 (AGE)		Model 2 (WT)	
	h^2	S.E	h^2	S.E
CTMUSC	0.70	0.46	0.24	0.25
CTFAT	0.81	0.49	0.31	0.31
CTBONE	0.30	0.29	0.54	0.40
VIALOIN	0.00	0.00	0.28	0.29
VIALEG	0.30	0.33	0.04	0.16
VIACHUMP	0.25	0.29	0.00	0.00
VIASHOU	0.10	0.18	0.00	0.00
MLC-CONF	0.00	0.00	0.01	0.11
MLC-FAT	0.00	0.00	0.17	0.24

[†]S.E was set to 0.00 when it was not obtainable.

[‡]Model 1 is corrected for age and Model 2 is corrected for weight

In addition to the differences between the current study and the other studies in the h^2 estimation model that were used, the structure of the data and the low number of animals particularly the sires used in this study made it possible to allow only a very approximate comparison. The variation in h^2 estimations between models included age or weight as a covariate was reported earlier for ultrasound traits where h^2 estimations at constant age was higher than those at constant weight (Fernandes, Wilton, & Tosh 2004). In contrast, h^2 estimations were higher when analyses included weight compared to age as a covariate (Kvame & Vangen 2007).

Heritability estimate of MLC-C was very low whereas MLC-F was moderate in Model 2 and not obtainable for Model 1 (Table 7.2). At a constant weight, Danish Texel sheep had higher h^2 estimation for carcass conformation than in the present study but a relatively close h^2 to the current study for carcass fatness (0.45 and 0.11, respectively) (Maxa et al. 2007). At a constant age, Rius-Vilarrasa et al. (2010) reported h^2 of 0.10 for MLC-C and MLC-F when an animal model was used in the analyses. In a different study on the UK hill sheep, animal model estimation on a large data set of MLC-C and MLC-F resulted in 0.09 and 0.17, respectively (Conington et al. 2001). Since these traits are subjective and are difficult to be assessed with a high level of accuracy, different h^2 estimation values can be obtained like as in crossbred lambs (Jones et al. 1999) where h^2 for MLC-C and MLC-F were 0.24 and 0.20, respectively. More heritability estimates of MLC-C and MLC-F were reported on the Scottish Blackface sheep using the animal model after adjustment for age (0.52 and 0.33, respectively) (Karamichou et al. 2006b). The design of the experiment as to whether lambs were killed at a fixed age or at a fixed degree of finishing may be a factor that determines the h^2 of the subjectively assessed MLC-C and MLC-F.

7.3.3 Phenotypic and genetic correlations

To my knowledge, there has not been any study before in the literature that looked at the relationship between the CT-measured traits and VIA-predicted weights of carcass primal cuts or with MLC-grading scores for carcass conformation and fatness classes.

This was the reason to attempt investigating these relationships in this study although we were aware of the relative low power of the available data. In other words, the weakness of the data used in this study did not restrict the attempt to investigate the genetic and phenotypic associations between *in-vivo* CT measured tissue weights and the VIA-predicted lean weights of the carcass primal cuts and the MLC classification grades for conformation and fat. Therefore, the reported results will only provide indicative information and will not be used at this stage to make any concrete recommendation for any future breeding programme.

7.3.3.1 CT measured traits and VIA-predicted traits

Genetic correlations estimated between CT-MUSC and VIA-predicted lean weights of the carcass primal cuts were positive and very high and varied from 0.83 to 0.98 in Model 1 with a good accuracy estimation of the standard error (Table 7.3). In line with that, the phenotypic correlations in Model 1 between CT-MUSC and the VIA-predicted traits were positive and as high as the genetic correlations but with a smaller standard error (Table 7.3). These results support the fact that animals with high CT-MUSC have higher VIA-predicted weights of the carcass primal cuts. It is logical to obtain such high and positive genetic and phenotypic correlations between two estimations of muscle weight but in different methods.

Table 7.3 Estimates of genetic (rg) and phenotypic (rp) correlations (S.E[†]) of Model 1 and Model 2[‡] between CT-measured traits and VIA-predicted traits.

Traits		Correlation	Model 1		Model 2	
CT-MUSC	VIA-LOIN	Rg	0.83	0.18	-0.92	0.31
		Rp	0.87	0.02	-0.02	0.09
	VIA-LEG	Rg	0.98	0.03	-0.37	0.00
		Rp	0.95	0.01	0.20	0.07
	VIA-CHUMP	Rg	0.96	0.07	-0.27	0.00
		Rp	0.94	0.01	0.18	0.07
VIA-SHOU	Rg	0.98	0.04	1.04	3.96	
	Rp	0.94	0.01	0.22	0.07	
CT-FAT	VIA-LOIN	Rg	0.99	0.00	0.98	0.29
		Rp	0.80	0.03	-0.07	0.09
	VIA-LEG	Rg	0.86	0.00	0.91	0.00
		Rp	0.87	0.02	-0.19	0.07
	VIA-CHUMP	Rg	0.99	0.00	0.29	0.00
		Rp	0.90	0.01	0.03	0.07
VIA-SHOU	Rg	0.99	0.00	-0.26	0.00	
	Rp	0.89	0.01	-0.05	0.07	
CT-BONE	VIA-LOIN	Rg	0.60	0.56	-0.90	0.00
		Rp	0.83	0.03	0.12	0.07
	VIA-LEG	Rg	0.48	0.00	-0.65	0.00
		Rp	0.89	0.02	0.12	0.07
	VIA-CHUMP	Rg	0.99	0.00	-0.61	0.00
		Rp	0.87	0.02	-0.03	0.07
VIA-SHOU	Rg	0.40	0.00	-0.12	0.00	
	Rp	0.88	0.02	0.11	0.07	

[†]S.E was set to 0.00 when it was not obtainable

[‡]Model 1 is corrected for age and Model 2 corrected for weight

In contrast to Model 1 results, genetic correlations using Model 2 between CT-MUSC and VIA-predicted traits were negative and not consistent across VIA-predicted traits. For instance, the correlation with VIA-SHOU exceeded the value of 1 with a very high standard error. It was not possible to obtain a standard error of the genetic correlation estimate between CT-MUSC and both VIA-LEG and VIA-CHUMP. However, using Model 2 to estimate phenotypic correlations between CTMUSC and VIA-predicted traits resulted in positive and moderate values $0.22 \leq rp \leq 0.18$ with a good accuracy apart from the very low negative phenotypic correlation between CT-MUSC and VIA-LOIN (Table 7.3).

The limited amount of carcass dissection data made it difficult for Jones et al. (2004) to generate comprehensive and reliable genetic parameters, thus they focused on phenotypic associations as approximation of the genetic correlations.

CT-FAT genetic and phenotypic correlations with VIA-predicted traits were positive and higher genetically than phenotypically in Model 1 with the exception of the VIA-

LEG (Table 7.3). The standard error of the genetic relationships between CT-FAT and VIA-predicted was not obtainable whereas it was very low and under the value of 0.03 with regard to the phenotypic correlations.

With the exception of the negative genetic correlation between CT-FAT and VIA-SHOU (-0.26), all remaining genetic correlations between CT-FAT and VIA-predicted traits were positive but were moderate for VIA-CHUMP and above 0.90 for VIA-LOIN and VIA-LEG in Model 2. Again, the standard errors of these genetic correlations could only be estimated for some of the traits (CT-FAT to VIA-LOIN relationship) (Table 7.3).

Contrary to Model 1, the phenotypic relationships in Model 2 between CT-FAT and VIA-predicted traits were low and negative for all traits but not for the VIA-CHUMP, which was positive and very low. Nevertheless, in Model 2 the standard errors were higher than for the phenotypic relationship estimation between CT-FAT and VIA-predicted traits but not for VIA-LEG (Table 7.3).

To further investigate the relationship between the fat and lean that appeared inconsistent as shown above, bivariate analyses between CT-FAT and CT-MUSC was examined using both models 1 and 2. Very high positive phenotypic and genetic correlations were obtained from Model 1 with good accuracy through the standard error (0.86 (0.03) and 0.98 (0.03), respectively).

Opposite to the positive signal in Model 1, high negative genetic correlation (-0.96 [no standard error available]) and moderate phenotypic correlation (-0.35 [0.06]) resulted from Model 2. In agreement to the results of this study, the negative genetic correlation between the same traits were reported when the model included live weight as covariate and was positive when age was included (Kvame & Vangen 2007).

The inconsistency between Model 1 and Model 2 with respect to the genetic and phenotypic correlations between CT-BONE and VIA-predicted traits remained unchanged as with CT-MUSC and CT-FAT. The genetic correlations between CT-BONE and VIA-predicted traits in Model 1 were positive and generally high but differed in the value of estimation and ranged between 0.48 to 0.98 for VIA-LEG

and VIA-CHUMP, respectively (Table 7.3). Nonetheless, excluding the VIA-LOIN there was no standard error acquired from the correlation estimation between the CT-BONE and the rest of the VIA-predicted traits.

Unlike to the genetic correlations between CT-BONE and VIA-predicted traits, phenotypic estimation were consistent and in the range of 0.80 ($0.89 \leq r_p \leq 0.83$) with standard errors below 0.03 (Table 7.3).

With regard to Model 2, CT-BONE genetic correlation with VIA-predicted traits shared the same negative signal but varied in the estimation value with markedly very high and low negative genetic correlation between CT-BONE and VIA-LOIN and VIA-SHOU (-0.90 and -0.12, respectively). None of the genetic correlation estimates between CT-BONE and VIA-predicted traits had standard errors available. The reason of the missing of standard error may be due to the very small (close to zero) variance between sires, small size of dataset and the structure of the experiment (Gilmour, personal communication).

The phenotypic correlations in Model 2 between CT-BONE and VIA-predicted traits were positive and very similar, with the exception of VIA-CHUMP, which was negative and very low (-0.03). The standard error of the phenotypic correlations in Model 2 between CT-BONE and VIA-predicted traits was the same (0.07) which made it higher than the estimation itself with respect to VIA-CHUMP (Table 7.3).

The large difference in the results between Model 1 and 2 could be due to the fact that live weight is more correlated to the body components than age is. This has been proved when live weight was a significant factor whereas the age was not of a significant effect when either each was used as covariates in the multivariate mixed model.

7.3.3.2 CT measured traits and MLC traits

The genetic and phenotypic correlations using Model 1 and 2 between CT measured tissue weights and MLC grades for conformation and fat classes are presented in Table (7.4).

The poor structure of the data and the low number of animals used in this study affected the results of estimating the genetic and phenotypic correlations; therefore, it was not possible to derive conclusive recommendations for their utilization in any future breeding programme.

Genetic correlations between CT-MUSC and MLC grades were different between Model 1 and Model 2. In model 1, CT-MUSC was positively and very high genetically correlated with MLC-F but low correlated with MLC-C. High positive phenotypic correlations with very low standard errors existed between the CT-MUSC and MLC-F and MLC-C (0.61 and 0.74, respectively). Dissimilar to Model 1, genetic correlations between CT-MUSC and MLC-F and MLC-C were negative in Model 2 but still moderate to high with high standard errors as well (Table 7.4). However, the phenotypic correlations between these traits were low but positive along with high standard error of estimations.

Although not directly comparable, positive and moderate genetic correlation estimations were reported between ultrasound muscle depth and carcass conformation in several breeds (Maxa et al. 2007; Olesen & HusabØ 1994).

Table 7.4 Estimates of genetic (r_g) and phenotypic (r_p) correlations (S.E[†]) of Model 1 and Model 2[‡] between CT-measured traits and MLC grading traits.

Trait		Correlation	Model 1		Model 2	
CT-MUSC	MLC-F	R _g	0.99	0.00	-0.65	0.56
		R _p	0.61	0.04	0.03	0.08
	MLC-C	R _g	0.09	0.00	-0.37	1.32
		R _p	0.74	0.03	0.10	0.07
CT-FAT	MLC-F	R _g	0.99	0.00	0.63	0.51
		R _p	0.64	0.04	0.37	0.07
	MLC-C	R _g	-0.89	0.00	0.51	0.95
		R _p	0.64	0.04	0.16	0.08
CT-BONE	MLC-F	R _g	-0.02	0.00	-0.89	0.00
		R _p	0.46	0.05	-0.26	0.07
	MLC-C	R _g	0.97	0.00	-0.92	0.00
		R _p	0.68	0.04	-0.08	0.07

[†]S.E was set to 0.00 when it was not obtainable

[‡]Model 1 is corrected for age and Model 2 corrected for weight

As expected for CT-FAT, in Model 1 the genetic correlation between CT-FAT and MLC-F was positive and very high whereas its correlation with MLC-C was highly negative with no standard errors of the estimates available. Using the same Model, phenotypic correlations between CT-FAT and MLC-F and MLC-C were positively and high and shared the same estimation value of the correlation and the standard error (0.64 and 0.04).

Model 2 generated positive and high genetic correlations between CT-FAT and MLC-F and MLC-C but accompanied with high standard errors (Table 7.4). On the other hand, phenotypic correlations between CT-FAT and MLC-F and MLC-C were positive and moderate but lower than those from Model 1 with a reliable accuracy where standard error of estimations were very low (Table 7.4).

Although not significantly different from zero, different studies reported a positive genetic relationship between ultrasonic fat depth and carcass conformation (Maxa et al. 2007; Olesen & HusabØ 1994). However, the positive genetic correlations between CT-FAT and MLC-F indicate that animals with a large amount of fat measured by CT will result in a carcass with a higher fatness score.

The favourable genetic correlations between CT-MUSC and MLC-C and CTFAT and MLC-F, show that CT measurements on live animals would be good predictors also for the final carcass classification scheme employed in the UK abattoirs. The verification of the reported results on a better designed experiment with a large number of animals is essential in order to evaluate the effect of incorporating CT measurements in breeding programmes and the expected responses on the MLC grades.

While the genetic correlation between CT-BONE and MLC-F, using Model 1, was very low and negative, the genetic correlation with MLC-C was very high and positive. The phenotypic correlations from the same Model between CT-BONE and MLC-F and MLC-C were high and positive with a small standard error.

Contrary to Model 1, correlation estimates phenotypically and genetically were negative between CT-BONE and MLC-F and MLC-C when Model 2 was fitted. The

genetic correlations were very high and negative with no standard errors of estimations available whilst the phenotypic correlations were negatively moderate to low with high standard errors of estimations (Table 7.4).

7.4 Conclusion

The purpose of this study was an attempt to quantify the genetic and phenotypic associations between *in-vivo* CT measured tissue weights and VIA-predicted lean weights of the carcass primal cuts, and the MLC classification grades for conformation and fat classes. Data for such estimations are very scarce. It is vital to estimate the genetic merits for carcass cut weights predicted by VIA and make it available early in lifetime for the potential elite parents particularly after the inclusion of CT scanning traits in the sheep breeding programmes. On the other hand, the inclusion of CT based traits in the recent sheep breeding programmes must have an assessment on the expected response on the MLC traits since it is the current carcass classification scheme according which the breeder is paid for. As shown in the results, there was no consistency in magnitude and sometimes in the direction between estimation models corrected for weight and age. The selection of the covariate will mainly depend on the breeding goal whether to it is to produce more lean meat at a fixed age, when age should be adjusted for, or to produce a higher proportion of lean meat at a fixed live or carcass weight, when live weight should be adjusted for.

The phenotypic correlations between CT measured traits and VIA-estimated traits were more sensible when corrected for age when looking at the positive relationship between CT-MUSC and VIA-traits as well as the negative relationship between CT-FAT and VIA-traits. This study, particularly the estimated phenotypic correlations, will serve as platform of further studies with larger data set acquired to produce more robust estimations.

Chapter 8
General Discussion

8.1 Introduction

The downward trend of red meat consumption both in the UK (AHDB 2011) and globally (FAO 2010) coupled with the growing preferences among consumers for leaner meats (Webb & O'Neill 2008) has had a major impact on the competitiveness of lamb production in the UK and internationally.

In the UK, there have been significant improvements in lean content of carcass through selection programmes in terminal sire sheep which focus on increasing lean mass and reducing fat (Márquez et al. 2012; Simm et al. 2002; Simm & Dingwall 1989).

Fruitful discoveries on identifying the underlying causal genetic variants that are associated with muscling traits have been achieved in the last decades. In sheep, several quantitative trait loci (QTLs) or mutations have been revealed to affect carcass composition, of which studied: (i) Carwell or LoinMAX™ (LM-QTL) at chromosome 18 of the Australian Poll Dorset breed (Banks 1997; Nicoll et al. 1998); (ii) c.*1232G > A mutation in myostatin gene (MM) at chromosome 2 of several Texel breed strains (Boman et al. 2009; Clop et al. 2006; Laville et al. 2004); (iii) Texel muscling QTL (TM-QTL) at chromosome 18 of the UK Texel breed (Walling et al. 2004).

Knowledge of the magnitude of the direct effects of the above genetic variants for muscle growth is essential before recommending their exploitation or/and introduction in the stratified structure of the lamb industry in the UK. Therefore, a detailed *in-vivo* evaluation using the commonly practiced ultrasound scanning (US) and the very informative and accurate computed tomography (CT) scanning methods were used. Furthermore, carcasses were not only evaluated by the current Meat and Livestock Commission (MLC) classification system for carcass conformation scores (MLC-C) and fat classes (MLC-F), but also were scanned by video image analysis system (VIA). VIA is a promising technology that predicts the weight of the carcass primal cuts with good accuracy and precision (Rius-Vilarrasa et al. 2009b). As VIA may be the basis of a future value based marketing system (VBMS), it was interesting to test its capability to detect the effects of the studied genetic variants on carcass primal cuts and the carcass linear measurements.

8.2 Key findings and implementations

The main findings from each of the six chapters and their implementations are given below.

8.2.1 LoinMAX™ (Chapter 2)

In this chapter, a comprehensive evaluation of the LM-QTL effects on carcass traits in crossbred lambs out of Mule ewes was performed. This was done by studying the difference between heterozygous LM-QTL carrier and non-carrier lambs using US, CT, MLC and VIA.

The importance of this trial was to investigate the magnitude of LM-QTL effects which were found to segregate in the Australian Poll Dorset breed, in a different genetic background. It has been reported that LM-QTL effect is predominantly localised on the loin muscle (Jopson et al. 2001; Nicoll et al. 1998). Our results were well in agreement with the previously mentioned studies and showed a positive effect of LM-QTL on the *M. longissimus lumborum* (MLL). An increase in MLL width, depth and area was found in LM-QTL carriers compared to non-carriers as measured by CT scanning at the 5th lumbar vertebra. Loin muscle depth measured by US (UMD) was also found to be greater in LM-QTL carrier lambs compared to non-carriers. However, the difference in the magnitude of the effect may be due to the different genetic background, different measurement method and/or different anatomical scanning point of the US between this study and previous studies. Lean meat yield in the loin region was significantly higher (+2.2%) in LM-QTL carriers compared to non-carriers as predicted by VIA.

8.2.1.1 LM-QTL utilisation in UK sheep industry

This study showed a positive impact of LM-QTL on the loin muscle, which is considered the most highly priced cut in the carcass. Despite the increase in loin muscling traits, loin accounts only for approximately 8% of the cold carcass weight, therefore and because MLC classification can not detect it, breeders will not benefit from having LM-QTL in their lambs according to the current MLC payment system. Before the introduction of LM-QTL in the UK sheep breeding programmes, it is recommended that farmers should perform a cost-benefit analysis. However, if VIA

was deployed in the commercial abattoirs along with shifting into a VBMS, breeders of LM-QTL carrier lambs may be rewarded for better rounded and heavier loin cuts. It should be noted that the intra muscular fat (IMF) content did not differ between LM-QTL carriers and non-carriers according to the muscle density measured by CT at the three scanning positions. Muscle density was found to be indicative of the IMF content (Karamichou et al. 2006b). However, results reported by Jopson et al. (2001) showed that LM-QTL lambs produced lower tenderness values than non-carriers, although this effect was removed by appropriate post-slaughter treatment (chilling, ageing). It would be advisable in future to directly test tenderness in LM-QTL carrier crossbred lambs such as those used here to monitor this aspect of meat quality.

Since LM-QTL does not segregate in UK sheep breeds and is imported from Australian Poll Dorset there will be no allele frequency estimation to build a breeding strategy to utilise this QTL. Therefore, a proposed strategy could be that of incorporating LM-QTL in crossbreeding programmes and then increasing its frequency in the target population through marker assisted selection (MAS). MAS could be a particularly useful technology in crossbreeding programmes where desirable genotypes from different backgrounds are introgressed into productive local breeds (Van der Werf 2007).

The mode of inheritance of LM-QTL is reported to appear as maternally imprinted (Campbell & McLaren 2007), meaning that the phenotypic effect is only present in progeny that receive the allele from their sire. Given so, LM-QTL incorporation in the terminal sire breeds that sire up to 70% of slaughtered crossbred lambs in the UK lamb (Pollott & Stone 2006) may be beneficial for the lamb industry.

A simple calculation of the potential economic benefits of exploiting LM-QTL in crossbred lambs out of Mule ewes can be made assuming a cold carcass weight of 17 kg, of which 8% (1.35 kg) is according to VIA-estimation in my study. LM-QTL heterozygous animals had an increased VIA-estimated loin trimmed weight of 62g across both sides, compared to non-carriers. The retail value of the lamb loin provided by EBLEX as approximately £14.00 (<http://www.eblexretail.co.uk/?page=costing+calculators>). Based on the shown VIA-estimated loin weight difference between LM-QTL and non-carriers along with the

approximate price of the loin, LM-QTL carrier lamb will return £0.87 more than non-carrier. To guarantee the production of heterozygous animals: (i) semen straws from homozygous carrier rams can be imported and used in artificial insemination; (ii) a breeding programme can be set to incorporate LM-QTL terminal sire breeds and produce lambs homozygous for LM-QTL.

8.2.2 Ovine c.*1232G > A mutation (MyoMAX[®] or MM) Chapters (3 and 4)

Ovine c.*1232G > A mutation (MM) is a polymorphism mapped on chromosome 2 in sheep (OAR2), encompassing the myostatin encoding gene (*MSTN*), and is reported to be associated with enhancing muscle growth in the Texel breed (Clop et al. 2006). The c.*1232G >A SNP test is now being used commercially and is known as MyoMAX[®] ([http://www.catapultsystems.co.nz/products/20_MyoMAX[®].cfm](http://www.catapultsystems.co.nz/products/20_MyoMAX[®].cfm)).

Unexpectedly in the TM-QTL project, it was discovered that one of the Poll Dorset rams used in the LM-QTL study was a carrier for MM (chapter 3, experiment 2). Therefore, there was a chance not only to investigate the effects of MM on Texel sired crossbred lambs (chapter 3, experiment 1) but also in Poll Dorset sired crossbred lambs.

In both experiments, comparison was made between heterozygous carrier lambs (MM/+) and non-carriers (+/+). In general, MM was associated with a favourable increase in carcass muscling traits and a significant decrease in fat measurements. In both experiments, MM carriers had higher CT-predicted carcass muscle weight, proportion and muscle area across the three scanning positions. Fat areas in all three of the CT reference scans were smaller in size for MM carriers compared to non-carriers as well as having a lower CT-predicted fat weight and proportion in both experiments. MM increased loin depth (+2.8 %) and area (+3.2%) in experiment 1 as measured by CT. In experiment 2, fat-related measurements were significantly lower in MM-carrier lambs. The MM carrier carcass had significantly lower subcutaneous fat as classified by MLC fat scores compared to non-carriers.

The MM effect on Texel sired crossbred lambs, that were from ewes of different genetic background (Welsh Mountain), was thoroughly examined since the Welsh Mountain breed contributes highly in the UK lamb industry (Pollott & Stone 2006).

Again unexpectedly, some of the ewes mated to heterozygous MM carrier rams were carriers for MM which resulted in them producing crossbred lambs that were homozygous for MM. MM genetic groups were [15 non-carriers (+/+), 129 heterozygous carriers (MM/+), 28 homozygous carriers (MM/MM)]. The availability of three genetic groups of MM allowed us to investigate the estimate of the MM additive and dominance effects on the traits of interest although the standard error of estimation was high due to the small number of animal used for this purpose.

The results of the effects of MM on fat measurements were substantial, particularly in the MM homozygous group. Carrying two copies of MM was associated with a significant negative effect on UFD and a substantial decrease in MLC-fat score compared to other genetic groups. Furthermore, homozygous carriers of MM were associated with a greater weight at 8 weeks of age and greater VIA-estimated weight of the hind leg, chump and loin primal cuts. The muscularity of the hind leg and loin regions along with loin muscle width, depth and area measured by VIA were significantly greater in homozygous MM group. VIA encountered significant changes in the carcass linear measurements in MM homozygous with wider carcasses across the shoulders, breast and hind legs and greater VIA-estimated areas of the back view.

Unlike heterozygous MM crossbred lambs out of Mule ewes, crossbred lambs out of Welsh Mountain ewes carrying a single copy of MM were not significantly different to non-carriers in any of the traits measured. These results reveal that MM effects vary according to the genetic background and they agree with previously reported interactions between MM and breed, as well as the modifying influence of the maternal effects that was found in cattle (Casas et al. 2004).

The observed mode of action of MM that varied across the traits of interest agrees with the different mode of actions on several traits reported in cattle (Wiener et al. 2002). Nevertheless, the results in this study showed that MM follows a partially recessive mode of action in some traits where half of the difference between the

predicted means for MM/MM and +/+ was substantially larger than the difference between MM/+ and +/+ means. These findings are in agreement with those of other groups who were also not in a position to draw a clear overall conclusion about the inheritance pattern of MM (Hickford et al. 2010; Johnson et al. 2009; Kijas et al. 2007).

8.2.2.1 c.*1232G > A mutation implementations in industry

The results of studying MM effects inherited from different ram breeds (Poll Dorset and Texel) in crossbred lambs of different maternal genetic background was very useful for utilising MM in lamb production in the UK. MM benefits on muscling are not restricted to a specific part of the body but were distributed across different parts of the carcass.

These studies confirmed that MM will have a consistent effect in MLC-F scores. Consequently, farmers may be rewarded by the decrease in MLC-F according to the current MLC payment system in UK abattoirs. Moreover, the MM effect in reducing subcutaneous fat level will be beneficial for the UK lamb processing industry as the costs of trimming unwanted fat prior to retail sale will be lower due to the decreased amount of the subcutaneous fat (Gardner et al. 2006). On the other hand, because there was no association between MM and both the carcass weight and MLC-C the monetary reward may be marginal.

Additionally, since MM showed a more favorable influence on hind leg weight and muscularity, selection for these traits may result in some improvement in MLC-C according to Johnson et al. (2005a) who presented a significant association between haplotype markers presumably encompassing MM and increased muscling in the hind leg. Another study by Laville et al. (2004) noted that overall carcass conformation score was strongly influenced by leg muscularity.

If industry tends to produce MM homozygous lambs, then the moderate to high genetic correlations between VIA-width measurements, found in the current study on MM homozygous, and MLC-C (Rius-Vilarrasa et al. 2009a) may be valuable for future selection programs to improve the MLC-C and therefore, the carcass value. However, it should be highlighted that any potential benefits in carcass value

associated with changes in carcass shape reported in the present studies should be considered with caution from a welfare perspective particularly if MM carrier lambs are being produced by smaller ewe breeds. Similar consequences were previously studied in cattle with evidence presented on associations between mutations in the myostatin gene and calving difficulties due to the change in the body shape of the calf (Casas et al. 1999; Short et al. 2002; Wiener et al. 2002; 2009).

Another issue that should be dealt with cautiously is that the IMF content presented by the significant increase in the average muscle density measured by CT in MM carrier lambs compared to non-carriers. Negative effects of MM on IMF were reported earlier by Kijas et al. (2007) and resulted in a decrease in the perceived eating quality and lower consumer preference. Since a high proportion of lambs produced by the lowland sector in the UK are sired by Texel rams with no known problems of meat eating quality, it seems unlikely that MM will have negative impacts on eating quality. However, to avoid deleterious effects, further investigation on meat quality traits is essential because current selection goals aim towards increasing lean muscle mass and decreasing fat regardless of the high frequency of MM in UK Texel sheep (Hadjipavlou et al. 2008).

8.2.2.2 c.*1232G > A mutation utilization in breeding strategies

Earlier reports showed that MM segregates in the UK Texel breed in a very high frequency and approached the fixation (> 95%), and the mode of action appeared to be partially recessive (Hadjipavlou et al. 2008) meaning that the effect of two copies of the mutation gave a greater increase in muscling compared with one copy. The results presented in these studies confirmed the partially recessive mode of action of MM on the muscling traits (Hadjipavlou et al. 2008). On the other hand, it was found that the MM mode of action on fat-related measurements appeared to be additive. Therefore, within the stratified structure of the UK sheep industry, it can be said that almost all Texel sired slaughtered lambs in the UK are carriers for one copy of MM. Yet, to achieve the maximum benefit from exploiting MM, according to the results presented in my studies, can be achieved with respect to lean meat related traits from producing homozygous MM lambs. This is achievable, with caution to IMF levels and meat eating quality, through the introgression of MM into hill and longwool

crossing breeds that are the parental breeds of the lowland crossbred (Mule) ewe. The potential for an adverse impact of MM on lambing difficulty as shown in cattle (Phocas 2009), fertility and meat eating quality traits still requires further detailed investigation.

8.2.3 TM-QTL, Chapters (4 and 5)

The phenomenon of increased muscularity in Texel sheep carcasses has been described and reported well since the 1980s (Jones et al. 2002b; Wolf & Jones 1996; Wood et al. 1980). The Texel breed now ranks as one of the most important terminal sire in the UK lamb production (Pollott & Stone 2006). In the last decade, researchers worked on identifying the underlying causal genetic variants associated with the increased muscle growth in the Texel breed. Known now as TM-QTL, Walling et al. (2004) identified a QTL on Ovine chromosome 18 in purebred UK Texel sheep, later confirmed by Matika et al. (2011), that is associated with favourable increase of 1.2-2.0 mm (4-8%) in UMD. Similar effects of TM-QTL in increasing MLL depth and weight were reported on the same crossbred lambs of Macfarlane et al. (2009a) but when VIA was used as an evaluation method (Rius-Vilarrasa et al. 2009c).

8.2.3.1 TM-QTL effect on crossbred lambs

The effect of TM-QTL on carcass composition on crossbred lambs from Welsh Mountain ewes was interesting to look at in order to investigate whether its action will vary in a different genetic background (chapter 4).

The results revealed in this study were in agreement with earlier reports on TM-QTL effects in increasing the muscling in the MLL where TM-QTL heterozygous carriers had +5.7% deeper UMD than non-carriers. The magnitude of the TM-QTL effect on UMD in crossbred lambs out of Welsh Mountain ewes was greater than their counterparts produced from Mule ewes (Macfarlane et al. 2009a). This is probably due to the QTL-genetic background interaction and/or environment. However, the magnitude of the effect of TM-QTL was not as big as the LM-QTL (+13.7%, Chapter 1) which is mapped very close to the chromosomal location of the TM-QTL (Walling et al. 2004). The increase in MLL width and area evaluated by VIA in TM-

QTL carrier lambs in this study was in agreement with those found on different genetic background measured by CT (Macfarlane et al. 2009a) and VIA (Rius-Vilarrasa et al. 2009c). If not confirmed in later studies, however, the new finding in this study was the significant association between TM-QTL and increased VIA-predicted weight of the untrimmed leg (P-LEG). This result could be a false positive due to the sample size and the structure of data. Nevertheless, VIA estimated increase of about 100g in the P-leg of the TM-QTL carriers compared to non-carriers but this increase was not seen in the VIA-predicted weight of the trimmed leg joint (TP-LEG). This could be due to bone weight, which possibly may have contributed to a heavier P-Leg (but not TP-leg when bone was removed), since a positive effect of TM-QTL on bone weight was shown by Macfarlane et al. (2009a).

8.2.3.2 TM-QTL effects on purebred Texel lambs

The work throughout the project was to generate a sufficient size of purebred Texel population from which lambs of all TM-QTL genotypes could be produced to be comprehensively evaluating TM-QTL effects on carcass composition (chapter 5). The TM-QTL genotyping process is based on haplotype of genetic markers that are surrounding the region of interest since the causal mutation responsible for the TM-QTL is not yet indentified. Because of the mating plan to increase the frequency of the TM-QTL in the purebred Texel population, TM-QTL carrier ewes became dams of the next generations and therefore it was possible to impute whether the source of the TM-QTL in the heterozygous genotype lambs is from the sire or the dam. However, some animals were assigned as unknown genotype due to missing marker information or recombination haplotypes.

Lambs born in 2009 were genotyped to assign TM-QTL and genotype classes were: 40 non-carriers (+/+), 53 sire parent-of-origin heterozygous carriers (TM/+), 17 dam parent-of-origin heterozygous carriers (+/TM) and 34 TM-QTL homozygous carriers (TM/TM). TM-QTL genotype of 65 lambs was assigned as unknown but still used in the statistical analyses to produce better estimation of the fixed effects.

The very rich post slaughter information on the MLC, VIA-predicted weights of carcass primal cuts and VIA carcass linear measurements traits that were available enabled the possibility to quantify how the TM-QTL affects them. Additionally, the

availability of the TM-QTL genotypes made it possible to look at the TM-QTL mode of action on the traits of interest.

TM/TM had the highest cold carcass weight (CCWT) compared to other genotypic groups and was classified to have the best MLC-C although it was not significantly different from the +/+ group. The significant difference in CCWT implies a possibility of different rates of muscle development between these genotypes, and differences in growth rate which indicates a higher efficiency in transforming energy into muscle deposition. This latter conclusion was also reported by Macfarlane et al. (2012) who found differences in live weight across different recording ages between TM-QTL genotypes. The TM-QTL was shown to affect the linear measurements of the carcass and was most significant in the TM/TM group with greater width in the shoulder from the back view and carcass depth presented as width across the side view of the carcass. The increased carcass depth with the observed significant effect of TM,-QTL on the carcass surface area measured by VIA in the TM/TM does not conflict with the significant differences between genotypes in terms of the carcass weight.

In agreement with the TM-QTL effect on loin muscle in crossbred lambs from different genetic backgrounds (Macfarlane et al. 2009a; Rius-Vilarrasa et al. 2009c), a significant increase of +2.4%, +3.2%, +5.8% and +10.3% in TM/+ compared to +/+ on purebred Texel was detected on the VIA-predicted trimmed weight of the loin joint, MLL width, MLL area and MLL volume, respectively. The effect of TM-QTL on VIA-predicted weight of leg that was found in crossbred lambs out of Welsh Mountain ewes (chapter 4) appeared in the purebred Texel lambs for trimmed and untrimmed leg weight where TM/+ were significantly different from +/+.

8.2.3.3 TM-QTL implementations in lamb industry

The results revealed in these studies are consistent with results of different research groups on the TM-QTL effects on the loin muscle and in different genetic backgrounds (Macfarlane et al. 2009a; Matika et al. 2011; Rius-Vilarrasa et al. 2009c). These results may have a beneficial implementation on the UK lamb production industry. The results of these studies go further to show that TM-QTL is having a positive effect on some hind leg characteristics.

As shown in the current studies, TM-QTL showed an additive mode of action on the carcass weight as TM/TM weighed significantly greater than other genetic groups and had an increase in the weight of +1.6 kg compared to non-carrier lambs. The improvement in weight coupled with the improvement in MLC-C would be of great financial reward to breeders according to the current MLC payment system. However, this requires further studies on the TM-QTL frequency in UK Texel sheep to identify whether TM-QTL segregates in the ewe dams. If not, then the maximum benefit would be to introduce the TM-QTL in the Texel breed females so guaranteed homozygous lambs can be produced through mating TM-QTL sires to TM-QTL dam ewes. Furthermore, fine mapping research has to further refine the chromosomal mutation location underpinning the phenotypic effects of TM-QTL. This fine mapping will help in: (i) identifying the causal genetic variant; (ii) minimizing the number of unknown TM-QTL genotype lambs. The success in discovering the SNP in the TM-QTL coupled with estimating its frequency in the UK Texel sheep will enable the estimation of TM-SNP EBV. This will help in planning the best genetic selection strategy, with consideration to the polar-overdominance mode of inheritance, to be utilised in the UK sheep industry.

The TM-QTL mode of action on the loin is reported to be polar over-dominance (Macfarlane et al. 2010; Matika et al. 2011), so heterozygous animals inheriting TM-QTL from the sire will show the greatest impact on the loin muscle. Although the improvement in the loin muscle was not of significant advantage in the current MLC payment system, VIA was able to detect such improvements in crossbred lambs out of different genetic backgrounds and in purebred Texel lambs. This supports the theory that if VIA is deployed and breeders were paid for the improvement in the premium cuts then TM/+ carriers will receive a financial reward. Nevertheless, a cost-benefit analysis must be performed before transfer of the knowledge to the farm level.

Two other issues related to TM-QTL effects on lambs must be dealt with as a precaution and require further investigation. The first is the increased linear measurements in the TM/TM group and their relationship with welfare issues like lambing difficulty or health problems. It has been reported in this project that TM-

QTL was associated with a negative impact on birth difficulty in purebred Texel lambs (Defra/BBSRC project LK0670, final report). The second precaution is that the meat quality traits which arise as a result of the presence of TM-QTL were associated with higher toughness levels in crossbred lambs out of Mule and in purebred Texel lambs (Lambe et al. 2010a; 2011). The undesirable levels of toughness in TM-QTL carrier lambs were overcome by aging the meat for 9 days (Lambe et al. 2010a; 2011).

8.3 Investigate how good in-vivo US and CT measurements in lambs are in predicting the VIA-estimated traits

In the light of growing calls to replace the current subjective carcass payment system by the objective system (VIA) that values the carcass according to the superiority of its cuts, it was important to investigate the ability of regular US measurements and CT linear measurements to predict the VIA-estimated weights of the carcass primal cuts. Several prediction equations were examined but the best could be achieved when ultrasound measurement, CT linear measurements and live weight were fitted in the model. It is essential to find a balance between the increased prediction accuracy and the difficulty plus the high cost of obtaining the predictors that may impose heavier economic pressure on the breeder.

Purebred Texel lambs (chapter 5) were used to evaluate the best model that includes US and/or CT linear measurements to predict VIA-estimated weights since VIA may be used as a benchmark for a new payment system which is VBMS. There are a good number of studies in the literature that investigated the relationship between the US measurements and the carcass composition. The new ideas of the current research were to enquire: (i) how good are US measurements to predict VIA-estimated carcass primal cut weights particularly for those breeders who will not get their animals CT-scanned; (ii) for breeders who will have their elite animals CT-scanned, how much accuracy and precision may some CT linear measurements add to the US measurements in order to predicted the VIA-estimated weight of the carcass primal cut.

Forward stepwise regression was used to evaluate five different models and a multi-collinearity test was performed to suppress any inflation of coefficient of

determination R^2 and standard error. Using UMD together with US live weight as predictors improved the coefficient of determination (R^2) and the residual mean standard error (RMSE) of the carcass saleable meat yield (SPMY) and the VIA-predicted weight of the primal and trimmed primal weight of the loin (P-LOIN and TP-LOIN) by 8%, 9% and 7% compared to UMD as a single predictor. Predicting meat yields using UMD along with US-live weight improved the prediction accuracy as reported by Silve et al. (2006).

CT-linear measurements were good predictors of the VIA-estimated weights of the carcass primal cuts with R^2 generally above 0.79. Combining the CT-live weight with the CT-linear measurements as predictors added only marginal improvements in R^2 but decreased the number of CT-linear measurements required. However, grouping CT-linear measurements, CT-live weight and UMD as predictors accounted for the maximum variance in the mean values for each VIA-predicted trait. Therefore, it was found that the latter model was the best prediction subset with R^2 ranging from 0.76 to 0.89. Previous research found that fitting ultrasound measurements to the external linear measurement prediction equation resulted in an increase of the prediction accuracy of carcass composition traits (Edwards et al. 1989).

The phenotypic relationship between measurements among the US, CT-linear measurements and VIA-predicted weights of the carcass primal cuts were reported to be preliminary information for future genetic parameters estimation studies. It can be concluded from this study that the very strong phenotypic correlation between extracted CT-linear measurements in a specific region with its corresponding VIA-predicted primal cut weight contributes to the prediction accuracy and precision. Therefore, it could be possible, in future, to produce a region-specific simple regression model that includes CT linear measurements that are highly related to the corresponding VIA-predicted cut.

8.4 The estimation of genetic parameters for in-vivo and post mortem traits

Knowledge of genetic parameters for the economically important production traits is essential in order to develop effective genetic improvement programmes in future. CT scanning information on elite sires is now being used for selection and genetic

breeding for carcass merits, thus, it was of interest to look at some genetic parameters and relationships between CT scanning measurements and post mortem traits (VIA and MLC-FC). This is because it is vital to estimate the genetic merits for carcass cut weights predicted by VIA and MLC-FC and make this information available early in the lifetime of the elite potential parents. Such an investigation has not been carried out before due to unavailability of data on animals that were scanned by both CT **and** VIA methods. However, the available dataset obtained in the TM-QTL project allowed us to attempt, making best use of the dataset, an initial estimation of genetic parameters. Knowing that robust estimations demands a large performance and pedigree data, it was decided that results of this study will not be judgmental or detrimental for any future breeding programme but qualify as first information in an area where no large datasets are available.

Performance data of the same animals in chapter 5 and 6 were used along with pedigree data to produce heritability estimations (h^2) (univariate test) and genetic correlations (bivariate test) using a sire model with pedigree in ASReml (Gilmour et al. 2006). The structure of the TM-QTL project in producing TM-QTL genetic groups by using confirmed TM-QTL carrier rams in mating and the low population size of this study resulted in singularity in matrices and sometimes did not achieve convergence of the Log-likelihood. Sire model was corrected for age or for weight (M1 and M2, respectively).

The h^2 estimates of the CT-estimated muscle weight (CT-MUSC) and CT-estimated fat weight (CT-MUSC) were very high in M1 and low to moderate in M2 with very high standard errors which made the heritability estimates not significantly different from zero. There were some unobtainable h^2 estimates (no sire variance ($\sigma_s = 0$)) of VIA-predicted traits but those estimable were moderate to low in both models with a very high standard error. Heritability estimate of MLC-C was very low whereas MLC-F was moderate in Model 2 and not obtainable for Model 1. However, such discrepancies in h^2 estimations between models correcting for age or weight was reported elsewhere (Fernandes et al. 2004; Kvame & Vangen 2007). The results in the current study are different from other studies due to the different models used,

different population size and structure of experiment design, and the model covariate factor selected in the analyses.

The relationship between CT-estimated tissue weights and the VIA-predicted traits and MLC-FC traits has been investigated in this study for the first time. Similarly to the h^2 estimation, two bivariate sire models were performed with adjustments for age (M1) and weight (M2).

Very high genetic and phenotypic correlations were found between CT-MUSC and VIA-predicted traits using M1. These results support the fact that animals with high CT-MUSC have higher VIA-predicted weights of the carcass primal cuts. Phenotypic correlations between CT-FAT and VIA-predicted traits using M2 indicate a favourable negative relationship. At a constant age, this later correlation showed a positive unfavourable relationship.

Selection for increased CT-MUSC is associated with positive phenotypically correlated MLC-C using both models. However, it seems that CT-FAT was also positively phenotypically correlated with MLC-F and MLC-C in both models which may have unfavourable consequences on the current payment system. It was reported earlier that a positive genetic relationship was observed between MLC-C and MLC-F (Conington et al. 1998; Karamichou et al. 2006b; Lewis et al. 1996) and therefore the results in the current study go in the same direction.

The phenotypic correlations between CT-measured tissue weights and VIA indicates that selection for carcass composition using CT scanning will produce the desired changes in lean meat yield predicted by VIA. Should there be a trend in the industry to breed terminal sires with specific weights or composition in the carcass primal cuts to suit the moving to the VBMS in future then the results shown here of the phenotypic correlations may be of good indicative information. This will be of maximum benefit for breeders who select the top sires according to the EBVs calculated from CT-measurements.

More investigation with a larger data set is required to accurately and reliably assess the relationship between CT, VIA and MLC traits not only in the purebred lambs but in crossbred lambs as well.

8.5 VIA ability to detect changes caused by LM-QTLTM, c.*1232G > A mutation and TM-QTL

The use of refined VIA prediction equations that were calibrated against CT (Rius-Vilarrasa et al. 2009c) made it possible to detect the changes caused by the studied genetic variants on the weight of the carcass primal cuts. The VIA confirmed the earlier reports of the LM-QTL and TM-QTL effects on MLL when LM-QTL carrier lambs were compared to non-carriers. Furthermore, beside the ability to detect the differences in weights of the carcass primal cuts, VIA was able to detect differences in muscularity traits in the loin and hind leg regions as well as the morphological changes that are caused by the genetic variants in the current studies. This confirms its ability to detect any changes in the carcass muscle distribution.

It has been reported that VIA system technology provides a fast, non-invasive, accurate and consistent method to predict weights of primal joints under UK lamb abattoir conditions (Rius-Vilarrasa et al. 2009b). Moderate to high heritabilities estimates of VIA-based traits indicate the potential of this VIA information for use in genetic programmes (Rius-Vilarrasa et al. 2010). In addition, VIA will be helpful if genetic traceability (EIDs) to link individual information of slaughter lambs with its sire and dams is needed to be used in breeding programmes.

Economic incentives placed on meat yield in carcass primal joints and increased capabilities of the VIA system to detect changes in muscle distribution could encourage the UK lamb industry to make use of this technology in the abattoirs.

8.6 Genomic selection and future efforts

This thesis concentrated on evaluating the direct effects of several genetic variants on carcass composition, muscling and primal weights using US, CT and VIA as evaluating methods.

It must be said that the studies reported in this thesis were a part of TM-QTL project that ran from 2005 to 2011. Meanwhile, enormous developments in the field of genomics and molecular genetics have been achieved.

DNA marker assisted breeding strategies in livestock are considered to substantially increase the rates of genetic gain for desirable production traits which are particularly

difficult to measure (Lande & Thompson 1990). The principle of MAS is to select individuals according to their DNA markers information that are usually in linkage disequilibrium (LD) with the causal genetic variants and the traditional phenotypic information. However, MAS has had only modest implementation in breeding programmes due to, fundamentally, difficulties associated with marker validations and the high cost of genotyping selection candidates (Misztal 2006).

Whole genome selection (WGS) that is “a form of marker assisted selection in which genetic markers covering the whole genome are used so that all QTLs are in LD with at least one marker” has become the latest development of the era of molecular genetics (Goddard & Hayes 2007).

The latest study that incorporated genotypes from whole-genome SNP assay into existing evaluation systems revealed an increase in the young animals EBV for commonly recorded traits (Wiggans et al. 2011). The accuracy of genomic selection is affected by: (i) the accuracy of marker effect estimates which in turns affected by trait heritability and the number of genotyped and phenotyped individuals (Goddard 2009); (ii) LD between SNP and unknown causal variants. The ability in selecting candidates at an early age will have a major impact on shortening generation interval and will lead to decrease in management costs like husbandry, health bills and other phenotype recording costs.

A commercially available SNP chip for sheep containing > 54000 SNPs (50K; e.g. OvineSNP50 BeadChip from Illumina Inc., San Diego, CA) is sufficiently dense to ensure most QTL will be in LD with SNP on the chip. However, the marker effects estimated is a breed specific as the example shown in cattle (Gautier et al. 2007; Hayes et al. 2009).

Dekkers (2012) stated that breeding programmes in species other than dairy cattle will have fewer opportunities to increase rates of genetic improvements due to several reasons: (i) selection is typically at an early age; and (ii) the difficulties associated with obtaining GEBV with sufficient accuracy due to the lack of training data sets of genotyped individuals with accurate phenotypic data.

In the TM-QTL project, tissue samples have been preserved and can be SNP-genotyped. If purebred Texel animals studied in this thesis had been SNP-genotyped

it will probably help in further narrowing or even identifying the causal genetic variant of the TM-QTL.

The detailed phenotypic data collected in these studies using different methods with various accuracy levels provided a platform for future correlation studies between SNP-genotypes and difficult to measure traits.

Furthermore, it is vital to utilise the genotypes and phenotypic measurements obtained in the TM-QTL project to establish such a training data set in purebred Texel sheep in the UK.

Collaboration schemes suggested by Wiggans et al. (2011) in cattle may be taken as an example to be implemented in sheep. In sire reference schemes the nucleus to establish predictor population since animals in the SRS populations are genetically related and well phenotypically recorded. Collecting enough high-density genotypes with the existing phenotypic records in SRS will be the corner-stone of improving genomic evaluation accuracy. However, low heritability traits will require a large number of records of phenotypes. Collaboration schemes may be extended to enlarge the size predictor population through adding more genotypic and phenotypic information from animals of the same breed from different countries, particularly those countries that have used common sires in recent years.

8.7 Conclusion

In this thesis, research results have contributed to the body of knowledge surrounding the effects of muscle enhancing QTLs and SNPs on carcass composition in crossbred lambs out of Mule ewes, crossbred lambs out of Welsh Mountain ewes and purebred Texel lambs. The availability of different genotypic classes of the studied QTLs and SNPs helped in further looking at the mode of inheritance which must be known for utilisation in future breeding programmes. Using CT scanning made it possible to investigate IMF content through investigating the variations between genotypic classes in terms of the muscle density.

Results of the current thesis have also provided further insight into the ability of VIA to differentiate between the estimated weight of the carcass primal cuts in QTLs and SNPs carrier and non-carrier lambs. Furthermore, VIA-linear measurements showed morphological differences between TM-QTL genotypic classes.

The comprehensive measurements obtained from US and CT linear measurements facilitated studying how well they can predict VIA-based predictions of the carcass primal cuts. Furthermore, the attempt to estimate the heritability of CT-measured tissue weight and VIA-predicted weight of the carcass primal cuts along with investigating their phenotypic and genotypic relationship traits was the first of its kind. This is due to the availability of data from animals that were CT- and then VIA-scanned. The small sample size did not give robust heritability and relationship estimation but made a platform for future studies.

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