MASACS Final Report

Executive Summary

This project was designed to meet a major policy aim of Defra to detect sheep genetic markers associated with carcass quality and productivity, within the UK sheep flock. This research was enabled by previous funding of sheep genomics in the UK, including priming money from Defra (LS2207) and then joint funded by Defra and SEERAD through LINK SLP (LK0628: QTL Identification and Utilisation in Sheep Sire Referencing Schemes; LK0656: Marker-Assisted Selection Applied to Commercial Sheep). This previous funding demonstrated the interest and enthusiasm from the sheep breeding industry in participating in genome research, as well as the feasibility of detecting and utilising QTL within commercial flocks. However, exploitation of QTL is most effective for traits which are difficult or expensive to measure, such as carcass and meat quality traits. Hence, this project aimed to deliver QTLs for carcass and meat quality traits, utilising a population of sheep previously divergently selected for carcass composition (LEAN and FAT lines of Blackface sheep). Carcass composition was assessed non-destructively by means of computerised tomograph (CT) scanning and comprehensive meat quality measurements were taken on male lambs. Additionally, this experimental design also allowed other important issues to be investigated, including the impact of altering carcass composition on meat quality traits, and the utility of *in vivo* CT measurements for predicting aspects of meat quality.

The population used in this study comprised the long-term selection lines of Blackface sheep (ca. 200 ewes) at the Roslin Institute, divergently selected for carcass composition, creating LEAN and FAT lines (approx. equal numbers of animals per line). A double backcross design created 9 half-sib families for QTL detection. Standard husbandry procedures were applied; all lambs were tagged at birth, with parentage, day of birth, sex and mortalities recorded. Computerised Tomography (CT) was used to obtain non-destructive in vivo estimates of the carcass composition of 600 lambs, at 24 weeks of age. Cross-sectional scans were taken at the ischium (ISC), the 5th lumar vertebrae (LV5) and the 8th thoracic vertebrae (TV8), and from each scan image the areas and image densities were obtained for the fat, muscle and bone components of the carcass. Comprehensive meat quality measurements were made on 100 male lambs per year that had previously been CT scanned. Meat quality traits included (i) initial and final pH of the meat, (ii) toughness (or shear force), (iii) colour, (iv) fatty acid composition of phospholipids and neutral lipids, (v) water, marbling fat and protein content of the meat, (vi) taste panel assessment of the cooked meat.

DNA was extracted from blood samples for all animals for a partial genome scan, covering chromosomes 1, 2, 3, 5, 14, 18, 20 and 21. For each chromosome heterozygous markers were chosen for each sire and all progeny were then genotyped for these markers. The informativeness of our genotyped markers was close to 0.7 across all regions genotyped. In total 139 markers were genotyped, and just less than 50000 individual genotypes.

QTL analyses were performed using regression techniques. Stringent significance thresholds were set, declaring QTL results to be significant only if they met chromosome-wide or genome-wide criteria. QTL confidence intervals were constructed by taking the region of the chromosome encompassed when reducing the largest F-ratio by the equivalent of a LOD score of either 1.0 or 2.0, to get 95% and 99% confidence intervals. For CT traits, QTL analyses were performed on measured traits rather than predicted traits (i.e. trait combinations). Heritabilities were estimated using ASREML, fitting an animal model including all 4847 known animals in the flock pedigree. Selection line differences were estimated from the pure and backcross line means. Lastly, multiple regression analyses were used to investigate the prediction of meat quality traits from CT measures, using Mallows Cp statistic to choose the best prediction model.

Six QTL reached significance at the 5% **genome-wide** level. These were for muscle density (LV5 & TV8) on chromosome 2 (LOD=6.60), Colour ‘a’ on chromosome 3 (LOD=6.20), muscle density (ISC) on chromosome 3 (LOD=6.05), bone density (ISC) on chromosome 1 (LOD=6.03), hot carcass weight on chromosome 5 (LOD=5.78) and slaughter weight on chromosome 1 (LOD=5.31). A further 12 QTL achieved significance at the 5% **chromosome-wide** level. These were for slaughter live weight (chromosome 2), hot carcass weight (chromosome 1), colour ‘l’ (chromosome 20), bone area (TV8) (chromosome 20), colour ‘l’ (chromosome 18), hot carcass weight (chromosome 21), colour ‘b’ (chromosome 1), bone density (ISC) (chromosome 20), bone area (LV5) (chromosome 20), muscle area (chromosome 5), live weight at CT scanning (chromosome 21) and bone area (LV5) (chromosome 18). Of particular interest are the QTL for muscle density, as this measure is related to intramuscular fat content as well as other meat quality attributes and can be obtained on the live animal.

Quantitative analyses revealed FAT line animals to be fatter than the LEAN line animals in all measures of fatness (from CT and slaughter data), although the differences were modest and generally less than 10%. Correspondingly, the LEAN line animals were superior in muscling measures. However, despite these relatively small line differences in primary carcass composition traits, the lines also differed in correlated traits. The FAT line had significantly greater subcutaneous fat at slaughter, significantly lower muscle density, and significant line differences for colour attributes (Colour L and Hue), with FAT line meat being significantly more reflectant and 'yellow as opposed to red'. Both colour results could be interpreted as being related in part to intra-muscular fat, a result backed by the line differences observed in muscle density.

All CT tissue areas were moderately to highly heritable as expected, with h^2 values ranging from 0.23 to 0.76. However, CT tissue densities were also strongly heritable, and for muscle and bone density they were generally more heritable than the tissue areas, indicating good possibilities for genetic change. Likewise, meat quality traits, were also moderately to highly heritable, once again indicating good possibilities for genetic change, should a means of genetically altering them be devised.

In vivo prediction of meat quality traits using CT measures was successful for colour A (correlation of observed and predicted = 0.71), juiciness (correlation = 0.58), fat class (correlation = 0.48) and ultimate pH (correlation = 0.41). In all cases where CT measures gave an adequate prediction of meat quality traits, it was muscle density that was the predominant predictor. For both colour A and juiciness, decreasing density was associated with increasing values of these traits, again implicating intramuscular fat. These results suggest that muscle density could perhaps be used as a good proxy for both of these traits, if they were to be incorporated into a breeding programme.

In summary, this project has met its primary objectives of delivering QTL for a range of meat quality and carcass traits. Even with highly stringent significance thresholds, convincing QTL have been found for various definitions of carcass and live weight, for meat colour, muscle density, muscle area, bone area and bone density. The last trait, bone density, may have a lesser relevance to meat production but it may be of particular importance as an animal model for osteoporosis. Additionally, this project has demonstrated that altering carcass fatness will simultaneously change muscle density (indicative of changes in intramuscular fatness), and aspects of muscle colour, making it lighter and more yellow as the carcass becomes fatter. The heritabilities for the meat quality traits indicate ample opportunities for altering most meat quality traits, provided that these traits can be adequately measured or predicted. Moreover, it appears that colour, juiciness and ultimate pH may be adequately predicted from measures of muscle density. Muscle density is a trait collected automatically during CT assessments of commercial animals, but currently not utilised – this represents an opportunity to be explored.

This project has produced a wealth of novel and practically useful information on the genetic control of carcass and meat quality traits in Blackface sheep. For the most part this is information that has previously not been available in the public domain. The results obtained from this project provide many potential opportunities for genetically improving meat and carcass quality. In particular, it is recommended that the observed QTL be investigated in independent populations and that the potential utility of muscle density be further explored.

1. Introduction

This project was designed to meet a major policy aim of Defra (MAFF as it was), *viz*. to detect sheep genetic markers associated with carcass quality and productivity, within the UK sheep flock. The tools to meet this objective now exist as a comprehensive sheep linkage map, with over 1000 markers (**1**), is now available and in the public domain. Utilising this linkage map, large QTL detection programmes in sheep exist in all the major sheep producing nations of the world. Unfortunately, the results of these programmes are generally (with exceptions) NOT in the public domain.

The UK now has now invested in the application of sheep genomics research, funded initially by priming money from Defra (LS2207) and then jointly funded by Defra and SEERAD through LINK SLP (LK0628: QTL Identification and Utilisation in Sheep Sire Referencing Schemes; LK0656: Marker-Assisted Selection Applied to Commercial Sheep). Project LK0628 demonstrated that (i) there is considerable interest and enthusiasm from the sheep breeding industry in participating in genome research and (ii) it is logistically feasible to undertake a research programme to detect and utilise QTL within commercial flocks. LK0656 has taken this research the next step and is seeking to utilise these detected QTL within the ongoing commercial breeding programmes. However, a critical drawback of using commercial flocks for the QTL detection phase is that it is only logistically straightforward to detect QTL for traits routinely recorded under commercial conditions. Exploitation of QTL via marker-assisted selection (MAS) is most effective for traits which are difficult or expensive to measure, expressed late in life, or sex limited. Categories of traits that fall into these categories include carcass and meat quality traits, reproductive traits, behavioural traits and disease resistance traits.

This project aimed to deliver QTLs for carcass and meat quality traits, utilising a population of sheep previously divergently selected for carcass composition (LEAN and FAT lines of Blackface sheep). Carcass composition was assessed non-destructively by means of computerised tomograph (CT) scanning. Meat quality measurements taken on male lambs included initial and final pH of the meat, (ii) toughness, (iii) colour, (iv) fatty acid composition of phospholipids and neutral lipids, (v) water, marbling fat and protein content of the meat, (vi) taste panel assessment of the cooked meat. Lastly, through additional EU funding nematode resistance was also assessed on the same animals at no additional cost to Defra.

In addition to the stated aims of the project, the experimental design also allowed other important issues to be investigated. These included the impact of altering carcass composition on meat quality traits, and the utility of *in vivo* CT measurements for predicting aspects of meat quality.

2. Methodology

2.1 Animal Population

The long-term selection lines of Blackface sheep at the Roslin Institute, divergently selected for carcass composition (**2**), afforded a unique opportunity to detect QTL for both carcass and meat quality traits. Selection for predicted carcass lean content was practised from 1988 until 1996, with random within-line selection being practised subsequently. These lines of sheep differ in carcass composition both as lambs (**3,4**) and also as mature ewes (**5,7**) (differences of 26% in internal fat depots in between lean and fat lines). Additionally, the composition of the fat depots differs between the lines, with the fat line having a greater lipid proportion within the fat tissue, a greater proportion of 18:2n-6 triacylglycerol and a lower proportion of 18:1n-9, than the lean line (**7**) Moreover, large differences are also seen in ewe fecundity (**8**) and the vitality or activity of the newborn lamb, with the lean line being superior to the fat line in both cases (**9**).

The Blackface flock consisted of ca. 200 ewes, split almost equally between the LEAN and FAT lines at the start of the project. A small proportion of LEAN x FAT line crosses were made in the Blackface selection flock at the 1999 matings, so that a cohort of F1 lambs was born in April 2000 along with a majority of purebred lean and fat line lambs. The F1 male lambs were then backcrossed to the purebred LEAN and FAT line ewes to create a population of genetically informative (LEAN x FAT) x LEAN and (LEAN x FAT) x FAT lambs from 2001 to 2003. The purebred lambs born in 2000 were used to benchmark the line differences (males and females), and to ensure continuation of the population (females). By the end of the project, the ewe flock was a more complex combination of pure line, F1 and backcross ewes.

The aim of the backcross mating strategy was to create 8 large half-sib families of approximately equal size, spread evenly across years. Due to ram deaths and a lower than expected mating success rate for two rams, a total of 9 half-sib families were created. The number of observations per half-sib family for the three categories of traits (described below): field observations, CT carcass composition and meat quality are shown in Table 1. The target number of meat quality assessments was 300, and this was not achieved due to a carcass being condemned in the final year of the project. The breakdown of observations per year is shown in Table 2, and this includes the pure line animals assessed in year 1 (2000) to help benchmark the line differences for each trait.

Table 1. *The number of observations per half-sib sire family for field observations, CT and meat quality traits.*

Family	Trait Category		
	Field Observations	**CT Carcass Composition**	**Meat Quality**
00M022	40	35	20
00M058	119	90	38
00M085	60	56	24
00M129	56	53	31
00M161	146	97	45
00M164	28	24	11
00M246	77	71	38
00M284	91	81	46
00M299	130	93	46
Total	**747**	**600**	**299**

Table 2. *The number of observations per year for the CT and meat quality traits.*

Trait Category	**Year**					**Total**
	2000		2001	2002	2003	
	LEAN	FAT				
CT	50	50	199	200	201	700
Meat Quality	25	25	99	100	100	349

2.2 Trait Measurement

2.2.1 Field Observations

All lambs were tagged at birth, with parentage, day of birth, sex, litter size and birth weight recorded. Subsequently, all lambs were weighed every 4 weeks until 24 weeks of age, and all mortalities were recorded. Weaning took place at 12 weeks. At approximately 20 weeks of age, all lambs were ultrasonically scanned, with fat and muscle depth recorded.

For assessing nematode resistance, each of the backcross lambs in 2001, 2002 and 2003 was sampled at approximately 16, 20 and 24 weeks of age (i.e. August, September and October). Each sampling consisted of a faecal sample and a blood sample. These analyses consisted of faecal egg counts, to determine the number of eggs from Strongyles and Nematodirus genera in the faeces, performed using the Modified McMaster technique. In addition to the animal samples, pasture larval counts were performed on the pastures grazed by the lambs, in order to assess the parasitic challenge faced by the lambs at each time of sampling. All lambs faced as moderate to strong parasitic challenge, and this was reflected by high average egg counts. We have in addition tested all lambs for IgA activity, using the blood samples taken in October.

2.2.2 Computerised Tomography Assessments of carcass composition

Computerised Tomography (CT) was used to obtain non-destructive *in vivo* estimates of the carcass composition of each animal. Measurements were performed in 50 LEAN and 50 FAT line lambs (split equally between sexes) in 2000 to benchmark the line differences, and subsequently our aim was to measure 200 lambs per year, again split equally between sexes.

Cross-sectional scans were taken at the ischium (ISC), the 5th lumar vertebrae (LV5) and the 8th thoracic vertebrae (TV8), and from each scan image the areas and image densities were obtained for the fat, muscle and bone components of the carcass. Additionally, live weight at scanning was recorded, and estimates of the total weights and percentages of fat, muscle and bone were made using prediction equations developed on independent populations of lambs. All measurements were performed in mid-September each year, when lambs were 24 weeks of age, on average. Withholding periods for the sedative required during the CT measurements precluded us from performing CT measures immediately prior to the meat quality measurements.

2.2.3 Meat Quality Measures

Measurements were performed on 25 LEAN and 25 FAT line male lambs in 2000 to benchmark the line differences, and subsequently our aim was to measure 100 male lambs per year. The lambs subjected to meat quality assessment had all been CT scanned, in an attempt to maximise the information and utility of the dataset. Meat quality traits includes (i) initial and final pH of the meat, (ii) toughness (or shear force), (iii) colour, (iv) fatty acid composition of phospholipids and neutral lipids, (v) water, marbling fat and protein content of the meat, (vi) taste panel assessment of the cooked meat. The **initial and final pH** measurements describe the progression of rigor mortis. There are genetic and environmental bases for variation, which are often associated with variations in water holding capacity and muscle colour. **Tenderness (toughness)** is clearly the most important aspect of meat eating quality, measured on cores of muscle removed from the *longissimus*. Meat **colour** is important to consumers. It is affected by pre-slaughter handling (via the pH effect), genetics (via fatty acids) and processing. The **lipid** within muscle (marbling fat) plays a role in meat tenderness, juiciness and flavour. The storage lipid is similar to that in other fat depots, e.g. subcutaneous fat, is comprised mainly of triacylglycerols (neutral lipid) and is a variable component, changing with animal fatness. The phospholipids are an integral part of the muscle membranes and the constituent fatty acids are much more unsaturated. The fatty acid balance in meat determines its nutritional value and the ratio of neutral lipid to phospholipid is a major factor in fatty acid percentages. Lamb is a good source of the valuable n-3 polyunsaturated fatty acids (which are preferentially deposited in phospholipids) and there may be genetic effects on their concentrations. **Water, marbling fat and protein** are the main chemical constituents of muscle. Marbling fat is important for eating quality and protein for nutritional value. **Taste panel assessment of cooked meat:** tenderness, juiciness and flavour after cooking are important in the enjoyment of meat. They are measured in loin (*longissimus*) chops taken from the carcass, conditioned for 10 days at 1C and grilled to 78C internal temperature. Potentially, these traits determine the ultimate future of the sheep meat industry.

2.3 Genotyping

DNA was extracted from blood samples for all backcross lambs, all sires and all available grandparents. This DNA was used for a partial genome scan, with chromosomes chosen on the basis of *a priori* evidence for QTL of relevance to the traits under investigation. However, DNA has been retained for additional genotyping should funding become available. Our genotyping covered chromosomes 1, 2, 3, 5, 14, 18, 20 and 21. All genotyping was subcontracted to a company in New Zealand, based at AgResearch.

For each chromosome we tested up a large number markers per sire (up to 30, depending upon the length of the chromosome) and chose a panel of markers for each sire for each chromosome that were heterozygous for that sire. In other words, each sire had panel of markers chosen per chromosome, but these marker sets differed between sires. The distribution of markers per sire per chromosome is shown in Table 3.

Table 3. *Distribution of informative markers genotype per sire family per chromosome*

Family	Chr 1	Chr 2	Chr 3	Chr 5	Chr 14	Chr 18	Chr 20	Chr 21
00M022	18	9	10	7	6	6	8	4
00M058	19	8	7	6	6	6	6	5
00M085	18	8	11	6	9	6	7	5
00M129	18	8	10	5	7	8	8	4
00M161	11	8	9	6	6	6	8	5
00M164	18	8	10	6	6	6	7	4
00M246	14	7	10	6	6	5	7	3
00M284	9	8	10	7	7	7	9	5
00M299	19	8	11	8	6	6	7	5

We then genotyped all progeny and all available grandparents for the markers we chose for that sire. The particular advantage of this approach is that we only genotype markers that are likely to be informative and we do not waste effort or resources genotyping markers that will not contribute information. This approach was successful in ensuring that our genotyping strategy was informative and cost-efficient. The informativeness of our genotyped markers, i.e. the proportion of the time we can unambiguously trace chromosomal segments back to the parent of origin, was close to 0.7 across all regions genotyped.

In total we genotyped 139 markers, and just less than 50000 individual genotypes. The actual markers genotyped were:

- **Chromosome 1:** BMS835, ILSTS44, ILSTS29, MCM58, BMS963, RM65, BM6438, BMS2321, MAF64, ILSTS004, CSSM04, BMS4000, INRA11, BMS527, DB6, BMS4001, MCM137, BM7145, BM6506, BMS4008, SOX2, TGLA415, BM8246, RM509, MCM130, BMS4045, CSSM32, BM864, LSCV105, BMS1789, BM1824, BM3205, OarHH36, URB014
- **Chromosome 2:** CSSM47, FCB226, BM3412, BMS1341, BL1080, BMS678, TGLA10, BMS1591, BM81124, CP79, TEXAN2, FCB20, BMS1126, BMS2626, ARO28, BM6444, BMS356, FCB11
- **Chromosome 3:** BMS710, BMS2569, BM827, ILSTS42, AGLA293, FCB5, ILSTS22, BMC1009, KD0103, BL4, LYZ, IFNG, CP43, MAF23, CSRD111, BM8230, BMS1248, TEXAN15, BM6433, BMS772, BM2830
- **Chromosome 5:** TGLA176, RM006, TGLA48, TGLA303, BMS2258, BMS792, BM1853, SHP1, OarAE129, MCM527, CSRD2134, BMS1247
- **Chromosome 14:** TGLA357, TEXAN10, BMS2213, MT2, ILSTS10, BM8151, MCM133, BM7109, INRA63, ILSTS002, BMS833, LSCV30, MCMA19, BM6507
- **Chromosome 18:** MCM131, ILSTS52, VH54, BP33, HH47, BMC5221, TGLA337, OY15, TGLA122, ILSTS54, MCM38, OB2, MCMA26, CSSM018, OY5, DLK
- **Chromosome 20:** INRA132, DYA, MCMA36, CP73, BM1815, DRB1, OLADRB, OMHC1, BMS468, TGLA387, CSRD226, BM1818, BP34, HH56, MCMA23

- **Chromosome 21:** BMC2228, ILSTS19, INRA175, CP20, VH110, JP15, HH22, BMC1206, BMS1948

2.4 Data Analysis

2.4.1 Quantitative Genetic Analysis

Standard descriptive analyses were performed using GENSTAT. Fixed effects included in the descriptive linear model for each trait included year.management group, litter size, age of dam, sex, slaughter day (where appropriate), the covariate day of birth, and line category. Line category was coded as follows: 1 = LEAN, 2 = FAT, 4 = F1 or F2 (from line cross), 3 = backcross to LEAN, 5 = backcross to FAT, 6 = F1 x LEAN backcross, 7 = F1 x FAT backcross. True line effects were estimated as the generalised least squares solutions to equations describing the genetic composition of the 7 line categories: $\mathbf{LINE} = (\mathbf{X'V^{-1}X})^{-1}\mathbf{X'V^{-1}Y}$, where **LINE** is a vector of genetic line solutions (LEAN and FAT) for each trait, **Y** is a vector of predicted means for the 7 line categories, **V** is the variance/covariance matrix of these solutions, and **X** is the incidence matrix relating genetic line to the 7 line categories. Standard errors of line means and differences were then constructed from the appropriate elements of $(\mathbf{X'V^{-1}X})^{-1}$.

Heritabilities for measured traits were estimated using ASREML, fitting an animal model. All known pedigree relationships, back to the foundation of the flock in 1988, were included in these analyses giving a total of 4847 animals in the pedigree. The deep and intricate relationship structure amongst animals overcomes the inherent weakness that the phenotyped animals are effectively the progeny of 9 sires (plus those born in 2000), and allows for genetic parameters to be estimated with acceptable precision (few datasets allow simultaneous satisfactory estimation of QTL and quantitative genetic parameters). Fixed effects in these analyses were the same as those described above, except that line was not fitted. For all traits the significance of a maternal litter effect was investigated (using a likelihood ratio test), but seldom was it significant.

2.4.2 QTL Analysis

Trait Definition: QTL analyses were performed for all meat quality traits, but a rationalised set of traits was chosen for the CT measures. Genetic correlations were calculated between all equivalent CT measures taken at different sites. When the genetic correlations between sites were greater than 0.8 the measures were averaged (after scaling by their standard deviations), otherwise they were treated as separate traits. Thus the traits analysed were: bone area ISC, bone area LV5, bone area TV8; bone density ISC, bone density (LV5,TV8); average fat areas; average fat densities; average muscle areas; muscle density ISC; muscle density (LV5,TV8). For the nematode faecal egg counts, (i) measures at each time point were treated as separate traits; (ii) an average egg count across the season was obtained by analysing the data using REML, fitting animal as a random effect without including genetic information. The individual animal solution was then used as the trait to be analysed.

Information Content: Information content was calculated at 1cM intervals across all the regions under investigation and across all half-sib families for each different analysis. The information content of an individual is the proportion of animals in which the allele inherited from the sire can be unambiguously identified. Information content at genome position *i* was calculated as *Var(pi)/0.25* where *pi* is the inheritance probability for each offspring included in the analysis and 0.25 is the expected variance of inheritance probabilities for a fully informative marker.

Interval Mapping: The probability of inheriting a particular sire chromosome at a particular position was calculated for each offspring from the genotype data at 1 cM intervals along each chromosome, using the method of Knott *et al*. (**10**). Each of the phenotypes was then regressed on the inheritance probabilities, at each location on each chromosome. Also included in the regression model were the same fixed effects that were used in the ASREML genetic parameter estimations. For each regression an F-ratio of the full model including the inheritance probability versus the same model without the inheritance probability was calculated. The location with the largest F-ratio was taken to be the best estimated position for a QTL for each trait.

Significance Thresholds: Although calculated as an F-ratio, the distribution of the test statistic under the H_0 of no QTL is unknown for half-sib analyses (**11**). Therefore, chromosome-wide significance thresholds were determined empirically by permutation for individual chromosome (**12**). Three significance thresholds were applied. The first level was the chromosome-wide threshold, which does take account of multiple tests on a specific chromosome but does not correct for testing on the entire genome. The second level was a suggestive linkage, where one false positive is expected in a genome scan (**13**). The suggestive level (where, by chance, we expect to obtain one significant result per genome analysis) was obtained by considering that we were analyzing 27 (independent) chromosomes, each with probability P of having a significant result. Assuming the number of significant chromosomes to follow a binomial distribution, we set the required threshold, P, such that the expected number of significant chromosomes, 27P, is equal to one. Therefore, the suggestive significance level for a specific chromosome would be P ~ 0.037. Third, the genome-wide significance levels (where, by chance, we expect 0.05 significant results per genome analysis) was obtained using the Bonferroni correction: $p_{genome\text{-}wide} = 1 - (1 - p_{chromosome\text{-}wide})^{n}$ (**14**). For example, assuming 27 chromosomes are being analysed (i.e. there are 27 independent tests), the chromosomal test significance level would be 0.001852 to give the genome-wide 0.05 level ($(1-0.001852)^{27} = 1-0.05$). All three significance levels do not take account the testing of multiple traits in the present and future studies into account. One thousand permutations were studied for each trait. In this report, results meeting only the suggestive threshold are not reported, as this is a somewhat lax threshold compared to the other two criteria.

Confidence Intervals: If the largest F-ratio indicated a QTL at the **genome wide level**, one and two LOD support intervals were produced by taking the region of the chromosome encompassed when reducing the largest F-ratio by the equivalent of a LOD score of either 1.0 or 2.0, to get 95% and 99% confidence intervals (**15**). Additionally, the bootstrap method (**16**) was empirically applied to the data, but this tended to produce conservative confidence intervals, sometimes covering the whole chromosome as expected from previous results (**17**).

2.4.3 In vivo *Prediction of Meat Quality Traits*

Meat quality traits, as described in this report, require the destruction of the animal. It would be advantageous to be able to predict these measurements on live-animal measures. It is hypothesised that some of the meat quality measures may be predictable from attributes of the CT scan image, particularly the tissue densities. Multiple regression analyses were performed on precorrected standardised data (mean = 0, standard deviation = 1) in an attempt to investigate this hypothesis. In all cases the dependent variable was a meat quality trait, and the dependent variables investigated were live weight and the areas and densities of each tissue at each site, as assessed by CT.

The criterion used to select the best prediction model among all possible models was the Mallow's Cp criterion, and this statistic was used to avoid over-parameterising the model. The Cp statistic (Mallows 1973) is: $C_p = SSR_p/s^2_\varepsilon - (n-2p)$, where SSR_p is the residual sum of squares from a model with p parameters (including $\beta 0$), and s^2_ε is the mean square error from the regression equation with the largest number of independent variables. The best-fitting model should have $C_p \approx p$.

3. Results

3.1 Quantitative Genetic Results

Summary statistics for the CT assessed traits are shown in Table 4, with significant line differences shown in bold. The lines differed in the expected direction (i.e. FAT line fatter) for predicted fat%, fat areas, fat areas scaled by live weight and predicted weight of fat. Additionally the FAT line had significantly less dense muscle, indicative of a greater **intra-**muscular fat content, and had significantly more dense bone. Counteracting the changes in fatness, the LEAN line animals had greater areas, weights and percentages of muscle and bone, and a significantly greater predicted cold carcass weight.

Inspection of individual animal values for the **predicted** traits (i.e. the weights and percentages of each tissue) indicated that the prediction equations may not have performed as well on this dataset as anticipated, as not all predictions were biologically plausible. Therefore, most subsequent analyses are performed on observed traits, rather than predictions (i.e. trait combinations).

Table 4. *Line means, trait phenotypic standard deviations, and line difference (with standard error) for CT traits.*

Trait	FAT Line	LEAN Line	S.D.	Line Difference (FAT-LEAN)	S.E. (Diff)
Live Weight at CT (kg)	32.76	33.11	4.97	-0.35	0.52
Predicted Bone %	19.87	20.16	2.11	-0.29	0.33
Predicted Fat %	20.80	19.69	5.04	**1.11**	0.51
Predicted muscle %	59.30	60.22	3.32	**-0.92**	0.42
KO%	39.14	38.77	2.63	0.37	0.40
Average Fat area† (mm^2/kg)	124	113	36.5	**10.9**	1.30
Fat area ISC† (mm^2/kg)	157	149	40.7	**8.40**	1.64
Fat area LV5† (mm^2/kg)	64	55	26.0	**8.81**	1.11
Fat area TV8† (mm^2/kg)	155	139	49.5	**16.0**	1.59
Fat area ISC (mm^2)	5021	4792	1450	**229**	8.50
Fat area LV5 (mm^2)	2140	1845	949	**295**	6.94
Fat area TV8 (mm^2)	5209	4628	1932	**581**	11.6
Fat density ISC (mm^2)	-70.1	-69.8	5.85	-0.25	0.54
Fat density LV5 (mm^2)	-65.6	-64.5	6.15	-1.13	0.63
Fat density TV8	-68.9	-68.8	6.00	-0.10	0.66
Bone area ISC (mm^2)	2451	2504	372.9	**-53.0**	5.28
Bone area LV5 (mm^2)	686	711	128.2	**-24.4**	2.40
Bone area TV8 (mm^2)	2763	2894	527.4	**-131**	5.76
Bone density ISC	326	325	39.3	1.80	1.81
Bone density LV5	369	360	52.2	**8.30**	1.68
Bone density TV8	321	315	39.2	**6.10**	1.39
Muscle area ISC (mm^2)	19971	20493	2197	**-522**	11.7
Muscle area LV5 (mm^2)	6772	6859	847.2	**-87.0**	7.54
Muscle area TV8 (mm^2)	9334	9398	1241	**-64.0**	9.86
Muscle density ISC	42.9	43.1	2.85	-0.240	0.40
Muscle density LV5	44.2	45.7	3.08	**-1.45**	0.45
Muscle density TV8	42.4	44.2	5.31	**-1.84**	0.58
Predicted fat weight (g)	2782	2611	974	**171**	7.15
Predicted bone weight (g)	2512	2564	321	**-52.0**	3.93
Predicted muscle weight (g)	7531	7717	1063	**-186**	7.75
Carcass Total weight (g)	12826	12892	2153	**-66.0**	10.8
Muscle: Bone	3.01	3.02	0.21	-0.01	0.13

Summary statistics for traits measured at slaughter and the major meat quality traits are shown in Table 5. The lines differed in the expected direction for subcutaneous fatness (i.e. FAT line fatter), but this was accompanied by an unexpected difference in live weight, with the FAT line being smaller. Non-significant trends in the same direction were also seen for hot and cold carcass weight. The other significant line differences were seen for colour attributes (Colour L and Hue), with FAT line meat being significantly more reflectant and 'yellow as opposed to red'. Both could be interpreted as being related in part to intra-muscular fat, a result backed up by the line differences observed in muscle density from the CT analyses.

Table 5. *Line means, trait phenotypic standard deviations, and line difference (with standard error) for traits measured at slaughter and meat quality assessments.*

Trait	FAT Line	LEAN Line	S.D.	Line Difference (FAT-LEAN)	S.E. (diff)
Slaughter Live Weight (kg)	37.0	39.2	5.82	**-2.22**	**1.15**
Cold Carcass Weight (kg)	17.3	17.9	3.14	-0.64	0.65
Hot Carcass Weight (kg)	17.7	18.4	3.22	-0.69	0.68
Fat class value (units)	2.19	1.97	0.48	0.224	0.29
Conformation value (units)	3.77	3.51	0.62	0.26	0.22
Subcutaneous fat (g/kg)	93.0	80.7	22.7	**12.3**	**2.34**
Texture shear	5.34	5.14	2.21	0.20	0.58
Colour a	17.0	17.3	2.43	-0.26	0.41
Colour b	8.03	7.75	1.32	0.28	0.37
Colour L	41.5	40.3	3.24	**1.11**	**0.42**
Hue	25.3	24.0	4.06	**1.30**	**0.64**
Saturation	18.9	19.0	2.45	-0.11	0.44
pH45	6.67	6.73	0.17	-0.058	0.17
pH Ultimate	5.73	5.71	0.10	0.018	0.14
pH difference	0.960	1.00	0.18	-0.040	0.18

Heritabilities for the raw CT traits are shown in Table 6. For none of these traits were maternal or litter effects significant, and the best-fit models were provided by fitting only an additive genetic effect. Almost without exception, the CT traits were moderately to highly heritable, with tissue density measurements being at least as heritable as tissue area measurements. These promising heritabilities, coupled with the large coefficients of variation for each trait (Table 4), indicate that any or all of these measures could respond rapidly to selection.

Table 6. *Heritabilities for CT assessed traits.*

Trait		**h^2**	**s.e.**
Fat Area:	ISC	0.50	0.13
	LV5	0.66	0.12
	TV8	0.76	0.08
Fat Density:	ISC	0.48	0.10
	LV5	0.46	0.11
	TV8	0.63	0.09
Muscle Area:	ISC	0.33	0.11
	LV5	0.32	0.12
	TV8	0.34	0.12
Muscle Density:	ISC	0.82	0.08
	LV5	0.45	0.10
	TV8	0.34	0.10
Bone Areas:	ISC	0.23	0.09
	LV5	0.35	0.11
	TV8	0.49	0.10
Bone Density:	ISC	0.39	0.12
	LV5	0.49	0.12
	TV8	0.48	0.09

Heritabilities for traits assessed at slaughter and meat quality traits are shown in Table 7. Again, maternal and litter effects were generally not significant. These heritabilities were less well estimated than those for the CT traits, simply because of the smaller available dataset. In general terms, a dataset of ca. 350 phenotypic observations with only 9 sires contributing most progeny would be inadequate for genetic parameter estimation (but fine for QTL analysis). However, genetic parameters are estimable from this dataset because of the large and complex pedigree available. Once again, most traits were moderately heritable and, in the case of the carcass classification measures (fat class and conformation), surprisingly heritable. However, these results indicate that genetic change, by some means, should be feasible for most traits investigated with the exception of 'pH difference'.

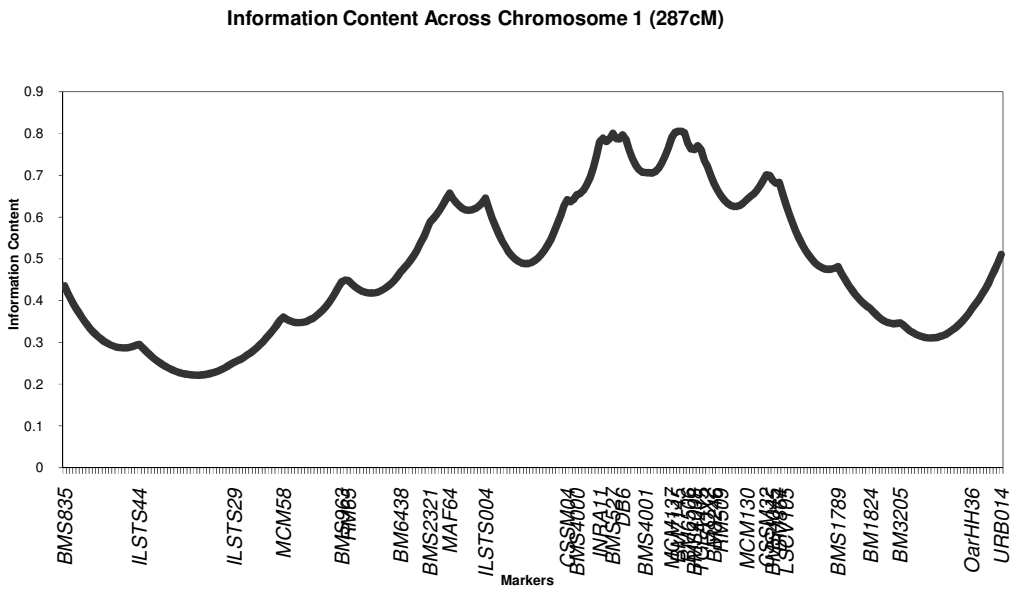
Table 7. *Heritabilities for traits assessed at slaughter and meat quality traits*

Trait	h^2	s.e.
Cold Carcass	0.47	0.19
Hot carcass	0.47	0.19
Live Weight	0.30	0.20
Fat class value	0.33	0.16
Conformation value	0.52	0.18
Subcutaneous fat	0.34	0.16
Texture shear	0.55	0.11
Colour a	0.45	0.19
Colour b	0.33	0.17
Colour L	0.15	0.12
Hue	0.30	0.15
Saturation	0.45	0.18
pH45	0.54	0.18
pH Ultimate	0.21	0.14
pH difference	0.01	0.06

3.2 QTL Results

As described above, our genotyping strategy was successful in giving us comprehensive coverage of the genomic regions that we had chosen to investigate. As an example, the average information content across the whole chromosome, for chromosome 1, is shown in Figure 1.

Figure 1. *Average information content for chromosome 1*



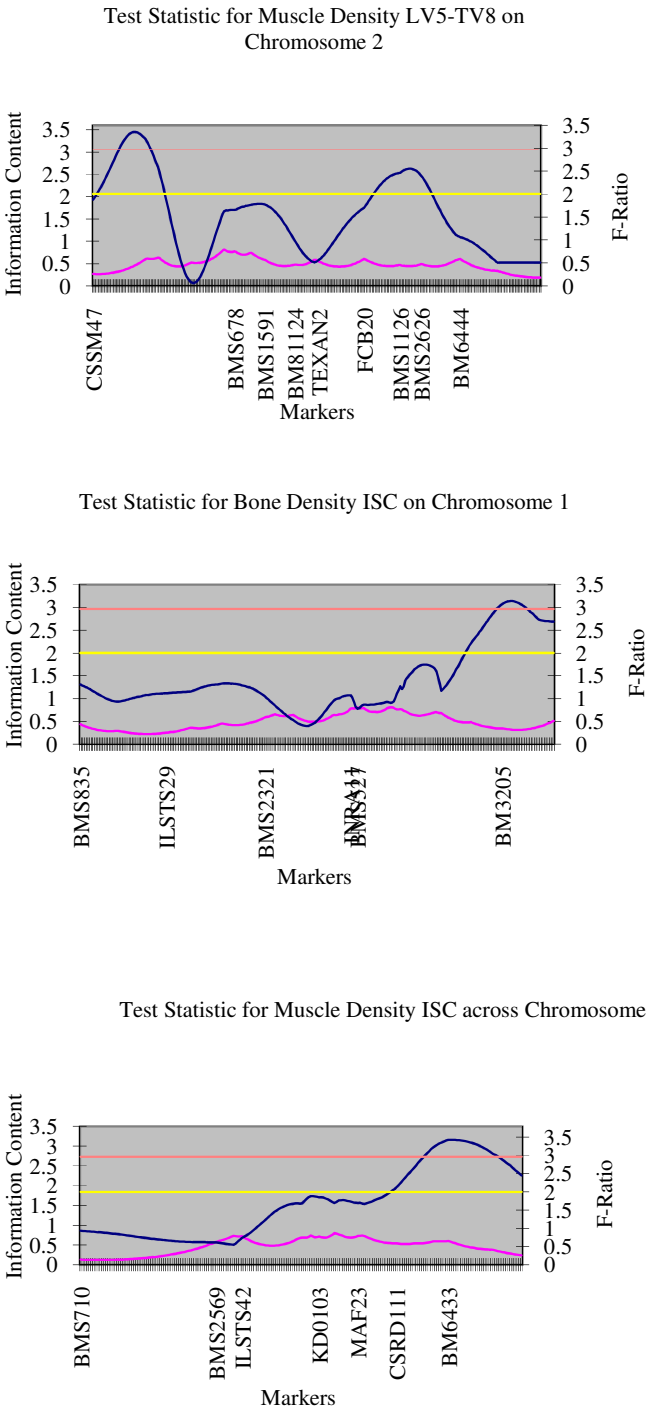
A summary of significant QTL, presented in decreasing order of significance, is presented in Table 8. Highly significant QTL have been observed for a range of traits, particularly muscle densities, live weight-related traits, and colour traits. Judging from these results, this project has been very successful and detecting QTL for traits of relevance to carcass and meat quality. Curiously, 4 out of the 18 QTL presented in this table map to the MHC region on chromosome 20.

Table 8: *Summary of significant QTL from across families analyses, presented in order of decreasing significance for CT traits and meat quality traits*

Trait	Chromosome	Position (cM)	Marker Region	F-ratio	5% Genome Wide Threshold	5% Chr.-Wide Threshold	1% Chr.-Wide Threshold
Muscle Density LV5-TV8	2	28	CSSM47-FCB226	3.45	2.97	3.45	3.95
Colour_a	3	113	KD0103-BL4	3.31	3.02	2.68	3.26
Slaughter Live Weight	1	229	LCV105-BMS1789	3.23	3.08	2.98	3.66
Muscle Density ISC	3	172	BM6433-BMS772	3.16	2.97	2.6	3.15
Bone Density ISC	1	261	BM3205-OarHH36	3.15	2.97	2.7	3.26
Hot Carcass Weight	5	0	TGLA176	3.07	3.02	2.72	3.27
Slaughter Live Weight	2	262	BM6444-BMS356	3.02	3.08	2.88	3.49
Hot Carcass Weight	1	227	LCV105-BMS1789	2.97	3.01	2.86	3.44
Colour_L	20	42	BM1815-DRB1	2.94	3.03	2.43	2.94
Bone Area TV8	20	55	OMHC1	2.90	2.96	2.5	3.08
Colour_L	18	80	ILSTS54-MCMA26	2.74	3.02	2.24	2.75
Hot Carcass Weight	21	88	HH22-BMC1948	2.72	3.01	2.48	3.14
Colour_b	1	165	INRA11-BMS527	2.55	3.02	2.55	2.95
Bone Density ISC	20	52	OLARDB-OMHC1	2.46	2.97	2.47	3.00
Bone Area LV5	20	21	MCMA36-CP73	2.45	2.97	2.46	3.24
Muscle Areas (average)	5	116	MCM527-CSRD2134	2.44	2.97	2.88	3.27
CT Live Weight	21	11		2.41	2.97	2.08	2.76
Bone Area LV5	18	83	OB2-CSSM018	2.26	2.96	2.21	2.6

QTL contour plots for the 6 QTL that achieved significance at the **genome-wide** level are shown in Figure 2, and confidence intervals for these QTL are explored in Table 9. The contour plot for the most significant QTL, muscle density on chromosome 2, is strongly indicative of multiple QTL for the same trait on this chromosome, and the LOD-drop method of bounding the confidence intervals suggests a tight interval for the strongest QTL. The LOD score for the most significant QTL on this chromosome is 6.60, far in excess of the often accepted threshold of 3.0 for significant QTL. The remaining QTL for muscle density, bone density, colour a and slaughter live weight all show a good profile definition, and reasonably tight confidence intervals as assessed by the LOD drop method. The QTL for slaughter weight on chromosome 1 corresponds to QTL for muscle depth and live weight previously detected in Suffolk and Charollais sheep. As expected, the bootstrap confidence intervals are wider, and are not an adequate description of the results for muscle density on chromosome 2, where multiple peaks were observed.

Figure 2 *Significance profiles for QTL significant at the Genome-wide threshold*



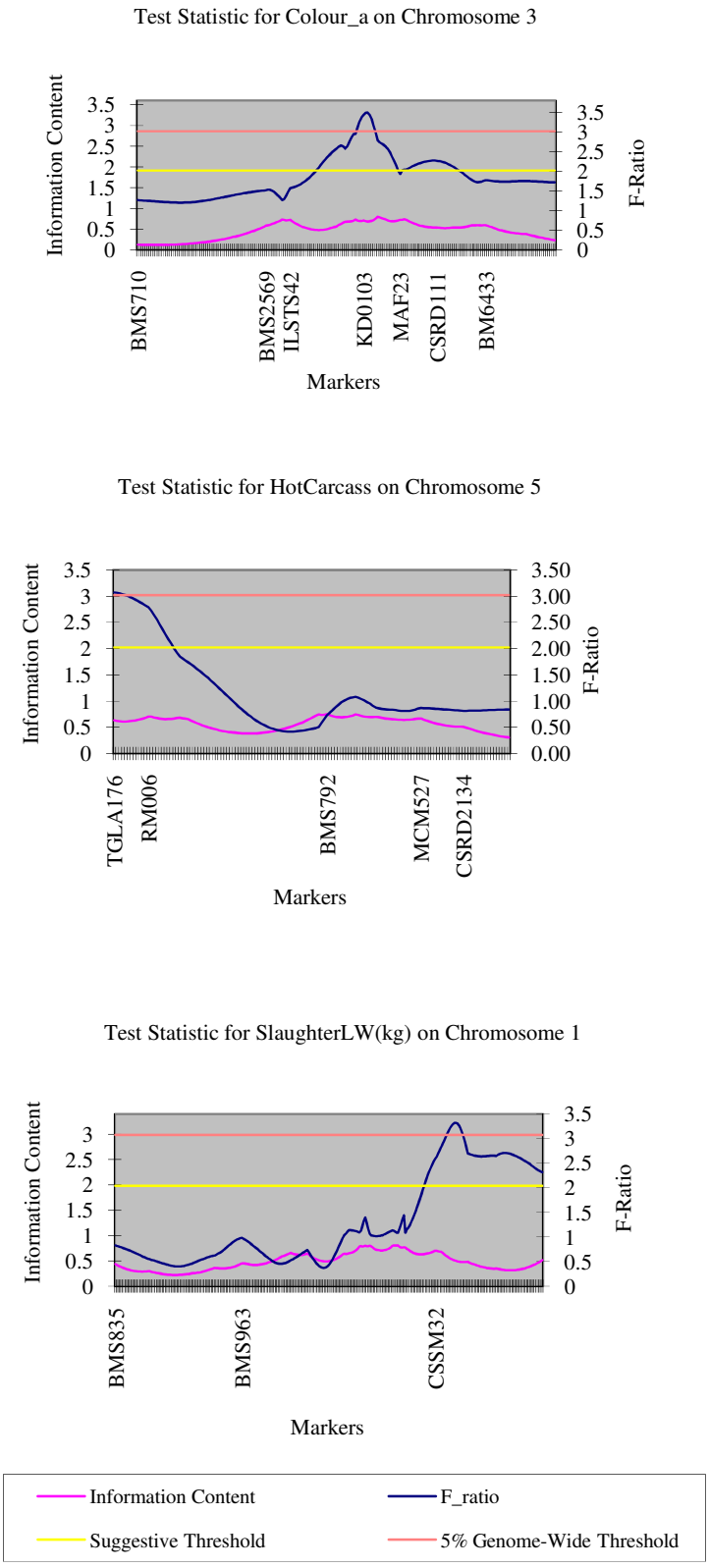


Table 9. *Confidence intervals for QTL significant at the 5% genome-wide level.*

Trait	F-Ratio	LOD Score	1 LOD CI (95%)	2 LOD CI (99%)	Position	Bootstrap (n=1000)	Chr.
Slaughter LW	3.23	5.31	218-239	209-287	229	117-287	1
Colour a	3.31	6.20	106-117	93-125	113	63-205	3
Hot Carcass	3.07	5.78	0-15	0-21	0	0-139	5
Muscle Density LV5-TV8	3.45	6.60	17-40	7-49	28	6-227	2
Muscle Density ISC	3.16	6.05	158-195	149-205	172	83-197	3
Bone Density ISC	3.15	6.03	246-279	234-287	261	0-287	1

In addition to the meat and carcass trait QTL, several significant QTL were also detected for nematode resistance traits, often being even more significant than those described above. In particular, we have strong evidence for significant QTL for Nematodirus faecal egg counts (FEC) on chromosomes 14 and 2, and for Strongyles FEC on chromosomes 2 and 3. For IgA concentrations, we detected significant QTL on chromosomes 3 and 20 (within the MHC region).

3.3 *In vivo* prediction of meat quality traits

In vivo prediction of meat quality traits using CT measures was successful for a small number of traits, as shown in Table 10. In particular, the traits colour a and juiciness were well predicted from CT measures. It is noticeable that in all case where CT measures gave an adequate prediction of meat quality traits, it was muscle density that was the predominant predictor. For both colour a and juiciness, decreasing density was associated with increasing values of these traits, again implicating intramuscular fat. In summary, both these traits are well predicted by CT measures, and muscle density can be used as a good proxy for both of these traits, if they were to be incorporated into a breeding programme.

Table 10. In vivo *prediction of meat quality traits from CT traits: correlations between observed and predicted.*

Trait	Predictor	Correlation (Observed, predicted)
Colour a	Muscle densities: TV8, ISC, LV5	0.71
Juiciness	Muscle density TV8, Fat area LV5	0.58
Fat Class	Muscle densities: LV5, ISC, TV8	0.48
Ultimate pH	Muscle densities: TV8, ISC, Fat area LV5	0.41

4. Discussion and Interpretation

4.1 General Results

This project has produced a wealth of novel and practically useful information on the genetic control of carcass and meat quality traits in Blackface sheep. For the most part this is information that has previously not been available in the public domain. As a broad summary, the results obtained from this project provide many potential opportunities for genetically improving meat and carcass quality, as well as giving insight into the implications of currently advocated breeding policies on attributes of meat quality.

In terms of QTL results, the project has met its primary objectives of delivering QTL for a range of meat quality and carcass traits. Even with highly stringent significance thresholds, convincing QTL have been found for various definitions of carcass and live weight, for meat colour, muscle density, muscle area, bone area and bone density. The last trait, bone density, possibly has a lesser relevance to meat production but it may be of particular importance as an animal model for osteoporosis. The 6 discrete traits that are significant at the genome-wide level (Table 9) all have tight confidence intervals, giving particular confidence in the results and making future exploitation simpler. In addition to these meat and carcass traits, 6 highly significant QTL for nematode resistance traits have also been observed in this population, this also being a strong of particular relevance to sustainable livestock production.

However, as well as detecting QTL, this project has also (i) quantified the impact of altering carcass composition on meat quality traits, (ii) estimated genetic parameters for meat quality traits and (iii) demonstrated how CT measures can be used to predict some meat quality traits. In particular, altering carcass fatness has simultaneously changed muscle density (indicative of changes in intramuscular fatness), and changed aspects of muscle colour, making it lighter and more yellow. These changes occur despite the rather modest observed changes in carcass fatness (somewhat smaller than observed when the sheep were still under divergent selection for fatness and before selection was relaxed (5,6,7,8)).

The heritabilities observed for the meat quality traits indicate ample opportunities for altering most (but not all) meat quality traits, provided that these traits can be adequately measured or predicted. The possibility of measuring these traits under field conditions is indicated by the predictions of colour, juiciness and ultimate pH made using CT measures, particularly aspects of muscle density. Muscle density is a trait collected automatically during CT assessments of commercial animals, but currently not utilised. Therefore, this information could feasibly be utilised at almost no extra cost for predicting these traits.

Notable in the heritability results are the small standard errors obtained, despite the fact that this population was designed for QTL detection rather than heritability estimation (optimal designs for the two types of experiments are mutually exclusive). However, relatively accurate genetic parameter estimation has been possible because of the large and complex pedigree available in this dataset, reflecting the long-term investment by Defra in this flock - investment that is paying ample dividends.

4.2 Next Steps and Recommendations

This project has shown that genetic improvement of nearly all meat quality traits is possible, and has given clues and tools for achieving this in terms of convincing QTL and strong relationships between CT traits and some meat quality traits. However, additional next steps are required to turn these tools into products that can be utilised by commercial breeders.

Firstly, commercial verification of these results in independent populations, preferably under commercial conditions, is required. This could be achieved in a relatively straightforward manner for CT predictions of meat quality, utilising commercial progeny of CT measured ram lambs.

Secondly, it is a general requirement of QTL that they should be independently verified, and QTL confidence intervals refined. There are two potential components to this. These results could be compared with those obtained by other researchers (e.g. from large QTL projects in Australia provided IP issues are resolved), with subsequent focus on those QTL regions in agreement between studies. Secondly, further experimental work could take place in relevant populations in the UK. Fortunately, some infrastructure does already exist to do this, e.g. in the LINK MASACS project, and this might provide a mechanisms to achieve this.

Thirdly, this project and the resultant dataset have proved to be unexpectedly rich, allowing further exploration of the results obtained. In recognition of this, a post-graduate student (Miss Elina Karamichou) has been assigned to this project, and she will probe the dataset in greater depth as a part of her studies. She will also attempt to evaluate and refine possible alternative breeding goals and selection strategies for meat quality traits.

Lastly, dialogue must be initiated between breeders, processors and geneticists to develop strategies to enable breeders to capture the benefits of genetically improving meat quality. Genetic improvement *per se* will be of benefit to the consumer. Unfortunately, it will not help the breeder, and therefore will not be taken on board by breeders, unless a way of rewarding breeders and producers is defined. This dialogue needs to explore ways of defining and implementing routes for capturing the benefits of genetically improving meat quality

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