Multiple Trait Analysis For Genetic Mapping Of Quantitative Trait Loci For Carcass And Beef Quality

By

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"Revision plays a very large role in writing. Sometimes it seems to be all revision. And the longer I write, the more I revise—and it's never completely right."
Ellen Hunnicutt, author of Suite for Calliope

List of Abbreviations

Abbreviation	
400Fat	Fat depth at 400 days
400Hip	Hip width at 400 days
400Ht	Body hight at 400 days
400Sti	Stifle width at 400 days
400Wt	Body weight at 400 days
400Wt	Body weight at 400 days
600Fat	Fat depth at 600 days
600Hip	Hip width at 600 days
600Ht	Body hight at 600 days
600Sti	Stifle width at 600 days
600Wt	Body weight at 600 days
600Wt	Body weight at 600 days
ANOVA	Analysis of variance
AP	Age at puberty
BC	β-carotene concentration
Birht	Body height at birth
Birlg	Body length at birth, cm
Birwd	Body width at birth
BLUE	Best linear unbiased estimation
BLUP	Best linear unbiased prediction
Bone	Estimated bone weight
Bonewt	Bone weight at side
BS	Butt shape
BTA	Bos taurus chromosome
Bwt	Birth weight
Carclg	Carcass length
Chanfat	Channel fat, kg
CIM	Composite interval mapping
Clld	Cooking loss of the *M. longissimus dorsi* muscle
Clst	Cooking loss of the *M. semitendinosus* muscle
cm	centimeter
cM	centiMorgan
Cola	Meat colour a
Colb	Meat colour b
ColL	Meat colour L
df	Degrees of freedom
DIAG	Diagonal model
DNA	Deoxyribonucleic acid
Docsco	Docility score
EMA	Eye muscle area
FA1	Factor analytic model with one factor
Fat	Estimated fat weight

Abbreviation

Fatwt	Fat weight at side
FC	Fat colour at slaughter
FCB	Fat colour on biopsy samples
Fdist	Flight distance
Gl	Gestation length
h^2	Heritability
HAS	Homo sapiens chromosome
HCW	Hot carcass weight
Heart	Heart weight as percentage of carcass weight
Imf	Intramuscular fat content
J	Jersey
Kidney	Kidney weight as percentage of carcass weight
L	Limousin
LD	*M. longissimus dorsi* muscle
Liver	Liver weight
Mar	Marbling
MAS	Marker-assisted selection
MC	Meat colour
Meat	Estimated meat weight
Meatwt	Meat weight at side
MIM	Multiple interval mapping
MP	Melting point
MQM	Multiple QTL mapping
MSTN	Myostatin
Mufa	Total mono-unsaturated fatty acids
Omenfat	Omental fat
Ossms	Ossification, score
P8	Carcass fat depth on the rump
PA	Pelvic area
pHld	pH of Longissimus dorsi muscle
pHst	pH of semitendinosus muscle
Prcarfat	Pericardial fat
QTL	Quantitative trait loci
Rbft	Fat depth at ribs 10^{th} and 11^{th}
REML	Residual maximum likelihood
REMLRT	Residual maximum likelihood ratio test
Rumeat	Rump meat weight
Rumfat	Rump fat
SF	Shear force
Silversfat	Silverside fat
SNP	Single nucleotide polymorphism

Abbreviation

SS	Silverside
ST	*M. semitendinosus* muscle
Wbld	Shear force measured on the *M. longissimus dorsi* muscle
Wbst	Shear force measured on the *M. semitendinosus* muscle
Wfat	Fat depth at weaning
Whip	Hip width at weaning
Wht	Body hight at weaning
Wsti	Stifle width at weaning
Wwt	Weaning weight
X	F1 cross between Limousin and Jersey
XJ	Back-cross between F1 (X) and Jersey
XL	Back-cross between F1 (X) and Limousin

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Declaration

I certify that this thesis contains no information which has been accepted for the award of any other degree or diploma in any University or other tertiary institution and, to the best of my knowledge and belief, the thesis contains no material previously published or written by another person, except where due reference has been made in the text. The thesis complies with the stipulations set out for the degree of Doctor of Philosophy by the University of Adelaide and I give consent to it being deposited in University library for loan and photocopy.

Ali K Esmailizadeh

Dedication

I dedicate this work to my wife, Azam and my children; Sina and Iman for their great support with love, patience and understanding during the period of the study.

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"If we knew what it was we were doing,
it would not be called research, would it?"
Albert Einstein

Abstract

The use of molecular markers to identify quantitative trait loci (QTL) affecting economically important traits has become a key approach in animal genetics, both for understanding the genetic basis of these traits and to help design novel breeding programs. The general goal of the present work was to map QTL for economically important traits in beef cattle. Because of the practical limitations of phenotypic selection for meat quality, these traits are ideal candidates for the use of marker-assisted selection. Thus, the thesis specifically focused on carcass and beef quality traits. Six half-sib families were generated by mating six Limousin × Jersey crossbred sires to purebred Jersey or Limousin cows, producing 784 backcross progeny (366 and 418 progeny in Australia and New Zealand, respectively). The six crossbred sires and all the backcross progeny were genotyped for 285 microsatellite markers (on average 189 informative loci per sire family) spread across the 29 bovine autosomes. A large number of traits were recorded on backcross progeny.

In the first phase of the research, a single-QTL model based on regression interval mapping was used to map QTL for a wide range of economically important traits in the beef industry. Chromosome-wise significant evidence for linkage was found on BTA12 ($P<0.01$) and BTA16 ($P<0.05$) for age at puberty. Thirteen QTL were found to affect calving ease related traits (birth weight, pelvic area and gestation length). BTA11, 14 and 22 were most significant linkage groups affecting calving ease traits. Several genomic regions were linked to the carcass and beef quality traits. The results revealed a major QTL on BTA2 close to the map position of myostatin gene, affecting yield, carcass fatness and beef quality traits.

In the second phase, the pleiotropic effects of a myostatin functional SNP on beef traits were studied. There was no association between this myostatin variant and birth weight and growth traits. However, the variant decreased overall fatness, increased muscle mass and improved meat tenderness, thus providing an intermediate and more useful phenotype than the more severe double-muscling phenotype caused by a major deletion in the myostatin gene described by others.

In the third phase, a multiple marker analysis approach in the framework of the mixed-effects model was developed, allowing all markers of the entire genome to be included in the analysis simultaneously. Further, exploiting a factor analytic covariance structure for modeling trait by marker or family by marker interaction terms, the approach

was extended to the multi-trait and multiple family situations. The simulation study showed that modeling multiple phenotypes and multiple families in a single linkage analysis simultaneously can markedly increase the power to detect QTL, compared to modeling each phenotype or family separately.

Finally, the multi-trait multiple QTL approach developed herein was applied to map QTL influencing carcass and meat quality traits. Several pleoitropic QTL and also trait-specific QTL affecting beef traits were mapped, resulting in a useful resource from which fine mapping can be launched for subsequent gene discovery and marker-assisted selection.

Chapter 1: Introduction and literature review

1.1 Introduction

Many characters of central importance to medicine, food production and evolutionary biology are quantitative in nature, being under the control of several genes plus the environment. Such traits include hypertension, osteoporosis and behaviour in humans, yield and quality in crop plants and farm animals and competitive ability and fitness in organisms in the wild (Kearsey and Pooni, 1996). Despite their central medical, economic and social importance, these traits are difficult to study because the phenotype does not easily provide an insight into the genotype, unlike most simple single-gene traits with major effects.

During the last century, there was an almost exponential growth in the knowledge and understanding of genetics, which led, appropriately, to the unravelling of the complete human genome sequence in February 2001 (Wolfsberg *et al*., 2001). However, despite these developments, the understanding of the genes underlying the control of quantitative, polygenic, traits is little further advanced than it was in 1918 when Fisher wrote his landmark paper providing a methodology for understanding quantitative traits. Using quantitative genetic theory it is possible to estimate the statistical effects as means, variances and covariances of groups of genes (Falconer and Mackay, 1996; Lynch and Walsh, 1998), but very little about the nature of the individual polygenes that underlie the traits has been known.

Although it is clearly not essential to understand the nature of polygenes or quantitative trait loci (QTL) to estimate heritability, select individuals, or predict selection response, it would be interesting and intellectually satisfying to have some sound understanding of the individual genes involved. Indeed, such knowledge may have a profound influence on the way that the scientists tackle theoretical and applied problems related to quantitative traits.

For many years, the application of the quantitative genetic approach allowed the identification of animals with high performance and selective breeding has contributed to the successful improvement of the animal production efficiency. The development of export markets and increased consumer demands for improved product quality has led to a changed focus in selective breeding. However, the ideal situation for selective breeding

using quantitative genetic theory is that the trait is highly heritable and that the phenotype can be observed in all individuals before the reproductive age (Lynch and Walsh, 1998). Therefore, the efficiency of this method decreases when the characteristics have a low heritability or are difficult to measure (e.g., meat tenderness). In addition, some traits are sex limited (e.g., milk production) or are expressed very late in the life (e.g., longevity). Furthermore, the traditional selection within populations has not been very efficient when the selection objective involves characteristics with unfavorable genetic correlations (for example milk production and protein content of milk) (Schwerin *et al*., 1995).

Recent developments in molecular biotechnology and statistical genomics have enabled changes in animal breeding programs. The availability of molecular markers provides an additional dimension to the use of quantitative genetics in animal breeding. Potential applications of molecular markers include marker-assisted selection, identification of the number of genes controlling quantitative traits, grouping germ-plasm into related groups, selection of parents and marker-assisted back-crossing.

The development of molecular techniques has expedited the creation of dense linkage maps across the whole genome in most livestock species. The density of the bovine microsatellite-based genetic map is quite high and it is estimated that a mean distance between two neighboring loci is 1.4 cM (Ihara *et al*., 2004). Chromosomal regions that are associated with molecular markers and with a quantitative trait are defined as quantitative trait loci (QTL). The genetic maps provide a basis for finding QTL in whole genome scans. Identifying QTL has the potential to significantly increase the rate of genetic improvement through the use of marker-assisted selection (Meuwissen and Goddard, 1996; Spelman and Bovenhuis, 1998).

1.2 Literature review

1.2.1 History

The idea of using markers associated with a trait of interest, for example, to predict the performance of individuals in the trait, is not new. The history of QTL mapping can be traced back to 1920's. Sax (1923) used the morphological markers to demonstrate an association between seed weight and seed coat colour in beans. In 1961 Neimann-Sorensen and Robertson proposed a half-sib design for QTL detection in commercial dairy cattle populations. Although the actual results were disappointing, this was the first study of marker-trait association in livestock. In addition, this was the first attempt to detect QTL in

an existing segregating population. Moreover, it was the first study to use blood groups rather than morphological markers. Furthermore, the proposed statistical analysis by Neimann-Sorensen and Robertson (1961) was unique as it attempted to estimate the power to detect QTL and to consider the problem of multiple comparisons.

Both Sax (1923) and Neimann-Sorensen and Robertson (1961)used only one marker in their studies. Thoday (1961) showed that by use of a pair of linked markers (marker bracket), it is possible to determine whether a QTL was located within the bracket or to either side. If within the bracket, the QTL could be located more exactly. Thoday (1961) noticed that the main practical limitation of the technique seems to be the lack of suitable markers.

In 1965 Law completed the first successful QTL mapping experiment and localised a QTL in wheat using substitution lines. Jayakar (1970) proposed that maximum likelihood could be used to map QTL. Two years later, Haseman and Elston (1972) proposed a sib-pair analysis method for QTL detection in human populations.

During the 1980s there was a number of QTL detection studies in agricultural plants based on isozymes using crosses between different strains or even species in order to generate sufficient electrophoretic polymorphism (Kahler and Wehrhahn, 1986; Tanksley *et al.*, 1982; Weller *et al.*, 1988). However, naturally occurring biochemical polymorphisms were insufficient for complete genome analyses in populations of interest.

Lander and Botstein (1989) introduced the maximum likelihood based interval mapping for crosses between inbred lines. This enabled the location of the QTL to be estimated separately from its effect by using information from the markers flanking a location of interest. Interval mapping became feasible because of the fast advance of molecular genetic technology, which enabled large numbers of reliable molecular markers to be identified.

1.2.2 DNA markers and genetic maps

A genetic marker is a detectable difference in DNA sequence that allows a specific point on the chromosome to be ‘tagged’ and followed through the generations of a family using a laboratory test. Genetic markers can be, but do not necessarily need to be, within the gene. However, most of the DNA markers are based on DNA sequence in between genes and simply tag an anonymous point along the chromosome.

A series of different molecular marker systems, which became available during the last two decades, can be broadly classified into three classes: a) the first generation molecular markers, including restriction fragment length polymorphism (RFLPs), random amplified polymorphic DNA (RAPDs) and their modifications; b) the second generation molecular markers, including simple sequence repeat (SSRs), amplified fragment length polymorphism (AFLPs) and their modified forms, and c) the third generation molecular markers including expressed sequence tag (ESTs) and single nucleotide polymorphism (SNPs) (Gupta *et al*., 2001).

DNA microsatellites (SSRs) are highly polymorphic and abundant, often found in non-coding regions of genes. These markers are by definition "codominant", that is the heterozygote genotype could be distinguished from either homozygote. Furthermore, microsatellites are nearly always polyallelic (Rohrer *et al*., 1994). Thus, most individuals will be heterozygous. Therefore, microsatellites markers represent a powerful way of mapping genes controlling economic traits.

A SNP is generally defined as a base pair location at which the frequency of the most common base pair is lower than 99% (Weller, 2001). The gel-based assays that are needed for most molecular markers are time consuming and expensive. However, the SNPs do not always need these gel-based assays. In addition, the SNPs are the fundamental unit of genetic variation and attractive as markers because they are abundant, both in animals and plants, genetically stable and amenable to high-throughput automated analysis (Beuzen *et al.*, 2000; Gupta *et al.*, 2001). A large number of SNPs have already been developed in the human genome (Gupta *et al.*, 2001).

A genetic map describes the chromosomal location and the relative order of known markers on each of the chromosome in the genome. Rapid advances in molecular genetics have led to the development of dense genetic maps of linked, polymorphic markers for many species. Detection and localization of QTL on the genetic map is based on co-segregation between alleles at marker loci and alleles at the QTL. A genetic map function is a mathematical relationship between recombination probabilities and map distances measured in centimorgans or Morgans. If r corresponds to the recombination frequency between a pair of markers and θ is the distance between them in Morgans, then the Haldane mapping function (Haldane, 1919) is defined by

$$\theta = -\frac{1}{2}\ln(1-2r) \qquad (1\text{-}1)$$

This mapping function assumes no crossover interference. However, the phenomenon of interference in genetic recombination is well-known. Therefore, if interference is taken into account, the Kosambi map function (1-2) (Kosambi, 1944) should be used.

$$\theta = \frac{1}{4}\ln(\frac{1+2r}{1-2r}) \qquad (1\text{-}2)$$

1.2.3 Experimental designs for QTL mapping

Studies to map QTL in humans, dairy cattle, and trees have used existing populations. However, most of the early studies performed on QTL have been based on planned crosses. Creation of a mapping population maximizes the chance to have segregating genes. It is more likely that a given QTL show segregation in a cross between two phenotypically divergent lines than within a population, which has been under strong directional selection.

The inbred lines have a high degree of homozygosity at marker loci and QTL, and their resulting offspring will have high linkage disequilibrium between alleles of all linked loci. Thus, crosses between inbred lines are highly efficient for detecting QTL. Since inbred lines do not exist in farm animals, crosses between outbred lines are common in these species (Lynch and Walsh, 1998). The drawback of the outbred line crosses is that the degree of homozygosity at marker loci is lower than in inbred lines and that it is unknown for the QTL. Since the degree of homozygosity at the QTL is unknown in the divergent breeds in the cross, the parental lines are usually assumed fixed for alternative QTL alleles (e.g., Haley *et al.*, 1994). If this is not the case in reality, there is a confounding between the allele frequency and the effect of the QTL, which decreases the power of QTL mapping.

A number of populations can be derived from a cross between divergent lines, including F2, single- or double- backcross and recombinant inbred (Weller, 2001). An F2 is more powerful than either individual backcross for detecting QTL of additive effect, and can also be used to estimate the degree of dominance for detected QTL. While the single backcross can only be used to detect the additive by additive interaction effect, the F2 or a combination of the two backcrosses can be used to detect four types of interaction between two loci (additive by additive, additive by dominance, dominance by additive and dominance by dominance). However, a relatively larger population size is needed to obtain enough power to detect these interactions.

The most popular design for QTL mapping in livestock is half sib design (daughter design). The daughter design, first proposed by Neimann-Sorensen and Robertson (1961), has been used mainly for dairy cattle in which a single sire can have hundreds or thousands of progeny with records on a number of traits, while each dam will have only a few progeny. This design essentially is a two-generation design, where offspring genotypes are used to determine the two haplotypes in the common parent. Then, for each offspring, the probability of inheriting from one of these haplotypes is calculated at locations throughout the genome using flanking informative marker information. In the regression application, the evidence for QTL comes from mean differences between the offspring inheriting the allele from one haplotype and those inheriting from the other (Knott *et al*., 1996). In the outbred situation, it is assumed that the contribution of the mates of the common parent is equal over all common parents. The population cannot be assumed to be in complete linkage disequilibrium, and so it cannot be assumed that the same marker allele is always associated with the same QTL allele in all half-sib families and, hence, when it comes to the regression analysis the QTL allele contrast nested within a half-sib family is of interest

Weller *et al.* (1990) introduced a design termed "granddaughter" design in which sons of grandsires heterozygous for the genetic markers are genotyped, and the daughters of these sons are scored for the quantitative traits. This design is similar to the F-3 design in that the residual variance is reduced because many phenotypes are scored for each individual genotyped. However, this design differs from the F-3 design in that only half of the granddaughters will receive the parental allele.

1.2.4 Methods for QTL mapping

There are four statistical methodologies for QTL analysis. Regression based methods, maximum likelihood methods, mixed model methods and Bayesian methods. The methods of QTL analysis were originally implemented using maximum likelihood (Lander and Botstein, 1989), in which information on the presence of a QTL is derived from both the mean differences between the flanking marker genotype classes and from the distribution of the trait within each marker genotype class. The disadvantage of maximum likelihood based methods for QTL analysis is their computational complexity, which makes them relatively difficult to use for the simultaneous analysis of several QTL, interactions between QTL, effects of unlinked QTL and fixed effects (e.g., year and sex) (Kim and Park, 2001). The advantage of such simultaneous analyses is their potential to

remove bias and to increase the power of the analysis via reducing the residual variance (Kim and Park, 2001). Haley and Knott (1992) showed that ordinary least squares can be used for QTL analysis and provides very similar estimates and test statistics to those obtained from maximum likelihood.

Grignola *et al*. (1996) proposed a variance component approach based on a mixed linear model to estimate the variance due to the QTL alleles, polygenic effects and residuals. This model can be fitted to any general complex pedigree and is robust to the number of QTL alleles and normality assumptions, and provides accurate estimations of QTL location and effects when family size is large.

Bayesian methods have attractive properties for QTL analysis. These methods extract additional information from phenotypes, incorporate complex pedigree relationship and provide inferences based on the joint posterior distribution of many other parameters such as QTL genotypes and their effects, number, map position and allele frequencies (Hoeschele *et al*., 1997). However, the Bayesian analyses are very demanding in terms of computing requirements particularly for a whole genome scan for multiple traits, and require operator expertise in regard to ensuring the proper mixing and convergence of the sampler (Hoeschele *et al.*, 1997).

Single marker analysis

With the advent of linkage maps, QTL mapping using single marker analysis has been reported in the literature in which potential candidate gene markers may be mapped *a priori* in the linkage group in outbred populations (Le Roy and Elsen, 1995; Weller *et al.*, 1990). However, single marker based mapping has disadvantages. Knott *et al*. (1996) summarized the drawbacks of this approach as a) heterogeneity of information content among markers biases the estimation of QTL location toward the more informative rather than the closest marker when multiple markers in the vicinity of the QTL are available, and b) there is a confounding between estimates of QTL position and effects.

Interval mapping

Interval mapping approach (Lander and Botstein, 1989) considers the intervals between pairs of flanking markers and explores the evidence of the presence of a QTL at various positions between the markers. Compared to single marker mapping, interval mapping has been shown to provide some additional power and much more accurate estimates of QTL effect and position and to be relatively robust to failure of normality assumptions (Knott and Haley, 1992; Lander and Botstein, 1989). However, the major

disadvantage of this method in outbred population as Knott and Haley (1992) and Haley *et al*. (1994) stated, is that missing genotypes and different information contents among marker intervals due to variability in marker heterozygosity cause a bias in the estimated QTL location toward the more informative marker interval. Furthermore, linked and multiple QTL on chromosome cause the bias of significant tests and estimates of QTL location and effect (Martinez and Curnow, 1992).

Multiple QTL mapping

In order to increase the reliability and accuracy of QTL mapping, the effects of possible multiple QTL on a chromosome should be adequately separated in testing and estimation. Zeng (1994) introduced a method of multiple QTL mapping, which is denoted composite interval mapping (CIM). In this method, disassociating of the linkage effects of the multiple linked QTL during the identification of individual QTL is accomplished by testing for QTL at a particular genomic region conditioned on other selected markers, known as co-factors. The purpose of using these co-factors is to minimize the effects of QTL in the remainder of the genome when attempting to identify a QTL in a particular region (Zeng *et al*., 1999). This method creates a relatively simple and systematic procedure to map multiple QTL but as stated by Zeng *et al*. (1999) there are some limitations to composite interval mapping. First, an uneven distribution of markers in the genome can affect the analysis, meaning that the test statistic in a marker-rich region may not be comparable to that in a marker-poor region. Second, the difficulty of estimating the joint contribution to the genetic variation of multiple linked QTL. Third, composite interval mapping is not directly extendable to epistasis analysis. Finally, the use of tightly linked markers as co-factor can reduce the statistical power to detect a QTL.

To address the drawbacks of composite interval mapping, Kao and Zeng (1997) and Kao *et al*. (1999) proposed and implemented a multiple interval mapping (MIM) procedure for mapping multiple QTL simultaneously. The idea behind multiple interval mapping is to fit multiple putative QTL effects and associated epistatic effects directly in model to facilitate the search, test and estimation of positions, effects and interactions of multiple QTL (Zeng *et al.*, 1999). Interaction between QTL (epistasis), has been reported as an important effect for complex phenotypes by many researchers in QTL studies (Brockmann *et al*., 2000; Cao *et al*., 2001; Nagase *et al*., 2001; Yu *et al*., 1997) and in basic biological processes in animals (Luschnig *et al*., 2000; Scanga *et al*., 2000).

Fine mapping

Genome wide scans for QTL in experimental and outbred livestock populations have revealed many QTL carrying chromosomal regions (Haley, 1999). However, the size of confidence intervals for estimated QTL position have been shown to be within 20 to 30 cM (Kim and Park, 2001) which is too large to efficiently implement technologies such as marker assisted selection, marker assisted introgression, positional cloning or positional candidate gene identification. Thus, the development and implementation of fine mapping methods is essential to provide a route toward eventually cloning QTL.

Goddard (1991) discussed exploiting linkage disequilibrium (LD) to map genes for quantitative traits. Riquet *et al.* (1999) proposed a fine mapping approach based on the utilization of historical recombinants and identity-by-descent (IBD) mapping using linkage disequilibrium (LD). Its application is suitable in young isolated populations with a relatively small number of founders (Kim and Park, 2001). As most domestic livestock populations are young and dynamic, there should be large regions of LD, as discovered by (Farnir *et al.*, 2000), where LD was found to exist over extended chromosomal interval in the Holstein-Friesian population.

Fine mapping will refine the size of the regions harbouring the QTL detected in mapping experiments, which will pave the way for more efficient implementations of schemes such as marker assisted selection and marker assisted introgression, and will allow positional candidate gene analyses to proceed with high levels of accuracy and precision.

Comparative mapping

Comparative mapping is the study of the pattern of genomic locations of known genes in different species. One way to improve the validity of detected QTL is to compare results between studies from different populations. For example, Sonstegard *et al.* (2000) reported that the bovine chromosome 27 (BTA27) contains two conserved segments corresponding to Human chromosomes 8 and 4 (HSA8 and 4), which contain QTL for fat deposition. Also Keightley *et al.* (1996) found seven QTL for body weight in the mouse; two of these QTL were located on mouse chromosome 13 and 17 (MMU13 and MMU17), which are homologous to BTA23 where Elo *et al.* (1999) found a QTL for live weight. In addition, BTA23 is homologous to the short arm of human chromosome 6 (HSA6) and to MMU13 and MMU17 (http://www.informatics.jax.org). Therefore, some potentially interesting genes might be found by mapping those genes known to be homologous

between HSA6 and MMU13 and MMU17, but which still have not been mapped in cattle (Elo *et al.*, 1999).

1.2.5 Relationships between growth, carcass and beef attributes

Understanding the relationships between economically important traits in beef cattle is of great importance in QTL mapping using multiple trait analysis, which exploits information from different traits to increase the precision and accuracy of the analysis.

Selection for growth rate leads to larger framed, heavier and later maturing animals (Mrode, 1988). This may lead to poorer carcass quality (e.g., reduced lean to bone ratio and dressing percentage) caused by a lower stage of maturity at slaughter (Andersen *et al*., 1981). Additionally, selection for growth rate seems to cause physiological changes in the muscles, which may have unfavourable consequences for meat quality traits such as colour, intramuscular fat content and tenderness (Aass and Vangen, 1997). Aass (1996) reported unfavourable genetic correlation between growth rate and carcass and meat quality traits.

Rate of growth in the period immediately pre-slaughter affects the glycogen content of muscle (Oddy *et al*., 2001), which is an important determinant of pH fall post mortem and the ultimate pH and thus colour and tenderness of muscle (Shorthose and Harris, 1991). In addition, beef colour, the first criteria consumers use to judge meat quality and acceptability (Conforth, 1994), is related to ultimate pH. Purchas and Aungsupakorn (1993) found a curvilinear relationship between ultimate pH and the tenderness of beef, a beef attribute that is closely related to the overall acceptability of beef (Chambers and Bowers, 1993), with minimum tenderness between 5.8 and 6.2 pH values.

Pitchford *et al*. (2006) reported that environmental correlation between meat (carcass weight and loin eye area) and fat traits (fat depth and intramuscular fat) were positive, but the genetic correlations were negative.

Carcass fat depositions and fatty acid composition can affect the beef quality. Although Riley *et al*. (1983) found a close association between subcutaneous fat and tenderness, Fiems *et al*. (2000) reported fat characteristics of the carcass and meat are poor predictors of meat tenderness and colour.

Fatty acids are involved in various technological aspects of meat quality. Because they have very different melting points, variation in fatty acid composition has an important effect on firmness or softness of the fat in meat, especially the subcutaneous and

intermuscular (carcass fats) but also the intramuscular (marbling) fat (Wood *et al*., 2004). Fat colour is another aspect of quality affected by mainly by β-carotene concentration and also by fatty acids. Differences in muscle fiber type between muscles are reflected in differences in fatty acid composition. Red muscles have a higher proportion of phospholipids than white muscles and therefore a higher percentage of polyunsaturated fatty acids.

1.2.6 QTL mapping in beef cattle

Importance of phenotype definition

Definition of relevant trait and collection of high quality phenotype data are the important aspects for a cattle genomic effort. By way of example, one might use a simple model in which the phenotype measured is profit per animal. Using this idea, any genetic variation, acting on average daily gain, carcass merit or a number of other variables, might be detected by using genetic markers (Smith *et al*., 2003). A more realistic example is the amount of fat on the animal, which is an important trait and impacts on efficiency of production, but the method of measurement can have significant impact on the results. An approach is to use fat depth, but accurate definition of phenotype requires a standardised system for data collection, since depth can vary from point to point along the same carcass.

The major difficulty in comparing results from QTL mapping between different studies on carcass and beef quality attributes is the lack of consistency between studies in the definition of these attributes and the use of different measurements for the same trait. This means that, in many cases, it is almost impossible to validly compare results from one experiment with those from other, very similar experiments. For example, in Australian abattoirs that use the AUS-MEAT scheme (AUS-MEAT Limited, 1998), carcasses are weighed with all internal fat sources removed and with some subcutaneous fat trimming allowed (Burrow *et al*., 2001). Consequently, yield or dressing percentages based on carcass weight data including this fat will be correlated but different traits.

Most economically important traits in beef cattle have many known components. Thus, correct identification of genetic variation requires the use of techniques that accurately measure important animal characteristics.

Definition of relevant phenotype is not the only critical step in genome research. As stated by Smith *et al*. (2003) it is essential to have a system of checks and balances to ensure that the carcass or cut of meat on which the phenotype is determined is properly

identified and, thus matched to the biological sample that was the source of DNA for genotyping.

Detected QTL in beef cattle

The basic principle underlying published beef cattle QTL experiments is the production of relatively large half-sib families using crossbred sires. The number of animals required for accurate mapping of QTL is a function of the magnitude of the effect of substituting the two sire alleles and the extent to which environmental variables affect the trait. The statistical analysis generally examines the contrast between the two alleles of the sire along each chromosome, which is why crossbred sires are employed as they increase the probability of allelic contrast.

A number of beef cattle QTL mapping experiments have been conducted, mainly at the US Meat Animal Research Centre where four resource families were developed for the identification of QTL for carcass composition and meat quality traits. The four families included Brahman × Hereford, Brahman × Angus, sire families (Keele *et al*., 1999; Stone *et al*., 1999), Piedmontese × Angus and Belgian Blue × MARC III sire families (Casas *et al*., 1998).

Kim *et al*. (2003) also conducted a genome scan for chromosomal regions influencing growth and carcass traits in an experimental population of Angus and Brahman crossbred and detected five QTL with genome wide significant evidence for linkage on bovine chromosome 2 (BTA2) and BTA6 for birth weight, BTA1 and BTA5 for yearling weight and BTA23 for hot carcass weight.

Based on the results of QTL analysis in the Piedmontese × Angus and Belgian Blue × MARC III families, it has been established that the locus causing double muscling in cattle mapped to the centromeric end of chromosome 2 (Casas *et al.*, 1998) in both Piedmontese × Angus and Belgian Blue × MARC III populations, indicating that the same locus was involved in both breeds. Further, it has been determined that double muscling was caused by mutations in the myostatin gene (Smith *et al*., 1997). Smith *et al*. (2003) reported that the loss of myostatin function caused dramatic effects on many traits in the Piedmontese × Angus and Belgian Blue × MARC III families, which were similar in nature for both Piedmontese and Belgian Blue alleles.

On a distal region from the location of myostatin, there is a QTL for carcass traits, reported in two different studies (MacNeil and Grosz, 2002; Smith *et al.*, 2003). This QTL

cannot be considered the same because of its distance from the centromeric region where myostatin resides.

Fat deposition is an economically important trait in cattle. For example, in the US the fat deposition ultimately determines carcass yield grade and quality, thus affecting profit margins (Sonstegard *et al.*, 2000). A number of identified QTL for carcass fatness have been reported in the literature (Table 1.1). Kim *et al*. (2003) identified 3 QTL influencing external fat deposition with suggestive evidence for linkage (two QTL on BTA1 and one QTL on BTA19). These researchers found two QTL with suggestive evidence for linkage on BTA2 and BTA15 affecting internal carcass fat deposition. Other suggestive QTL for fat deposition included: BTA16 (MacNeil and Grosz, 2002); BTA5 (Casas *et al*., 2000) and BTA18 (Stone *et al.*, 1999). Several detected QTL affecting other carcass traits have been reported (Table 1.1).

Chromosome 3 has been implicated in the expression of traits in beef cattle (Casas *et al.*, 2003; Kim *et al.*, 2003; Smith *et al.*, 2003). A QTL for marbling and retail product yield has been detected in two different families (Brahman × Angus and Belgian Blue × MARC III) on chromosome 3 (Smith *et al.*, 2003). In both families, the QTL for the same traits reside in a similar chromosomal region (Table 1.1). This suggests that it is the same gene, or group of genes, influencing the expression of marbling and retail product yield. It is possible that in both families comparisons are made between the Angus allele with the Belgian Blue and Brahman alleles. As stated by Smith *et al*. (2003), these results highlight the need to characterize allelic variation of QTL in several breeds and breed crosses to enable effective marker-assisted implementation.

QTL for several carcass traits including eye muscle area, birth weight, marbling, fat depth, retail product yield, dressing percentage and USDA yield grade have been found on BTA5 (Table 1.1). Casas *et al*. (2000) identified a QTL on BTA5 located at 50 to 80 cM affecting dressing percentage, yield grade, rib bone, and retail product yield. Stone *et al*. (1999) detected a QTL allele of Brahman origin on BTA5 located at 50 to 80 cM affecting rib bone and dressing percentage. MacNeil and Grosz (2002) and Mizoshita *et al*. (2004) also found QTL for carcass traits on this chromosome.

Table 1.1 Quantitative trait loci detected in beef cattle

BTA	Position (cM)[a]	Support interval (cM)		Trait[b]	Family[c]	Method[d]	References
		Lower	Upper				
1	1	-[e]	-	ABF	AB	IM-UNI	Kim et al. (2003)
1	126	-	-	ABF	AB	RA-UNI	Kim et al. (2003)
1	120	100	135	BWT	BH	IM-UNI	Casas et al. (2003)
1	50	38	74	FATYD	BA	IM-UNI	Smith et al. (2003)
1	50	38	74	FATYD	BA	IM-UNI	Smith et al. (2003)
1	53	37	72	RPYD	BA	IM-UNI	Smith et al. (2003)
1	63	41	77	YG	BA	IM-UNI	Smith et al. (2003)
1	66	-	-	YWT	AB	IM-UNI	Kim et al. (2003)
2	126	-	-	BWT	AB	RA-UNI	Kim et al. (2003)
2	4	2	6	BWT	BM, PA	IM-UNI	Casas (2002)
2	4	2	6	EMA	BM, PA	IM-UNI	Smith et al. (2003)
2	4	2	6	FAT	BM, PA	IM-UNI	Smith et al. (2003)
2	54	21	60	FAT	BH	IM-UNI	Smith et al. (2003)
2	61	-	-	KPH	AB	RA-UNI	Kim et al. (2003)
2	4	2	6	KPH	BM, PA	IM-UNI	Smith et al. (2003)
2	120	110	130	MAR	HC	IM-UNI	MacNeil and Grosz (2002)
2	4	2	6	MAR	BM, PA	IM-UNI	Smith et al. (2003)
2	54	45	70	MAR	BA	IM-UNI	Smith et al. (2003)
2	4	2	6	RPYD	BM, PA	IM-UNI	Smith et al. (2003)
2	4	2	6	YG	BM, PA	IM-UNI	Smith et al. (2003)
2	52	38	79	YG	BH	IM-UNI	Smith et al. (2003)
3	40	21	58	BWT	BH	IM-UNI	Casas et al. (2003)
3	128	-	-	BWT	AB	IM-UNI	Kim et al. (2003)
3	36	23	46	FAT	BH	IM-UNI	Smith et al. (2003)
3	77	69	85	KPH	BA	IM-UNI	Smith et al. (2003)
3	28	0	42	MAR	BH	IM-UNI	Smith et al. (2003)
3	56	9	74	MAR	BA	IM-UNI	Smith et al. (2003)
3	65	47	85	MAR	BM	IM-UNI	Casas et al. (2001)
3	68	64	85	RPYD	BM	IM-UNI	Casas et al. (2001)
3	70	55	83	RPYD	BA	IM-UNI	Smith et al. (2003)
4	60	52	67	EMA	Wagyu	IM-UNI	Mizoshita et al. (2004)
4	33	24	41	HCW	BM	IM-UNI	Casas et al. (2001)
4	55	52	62	MAR	Wagyu	IM-UNI	Mizoshita et al. (2004)
4	19	4	34	WBS3	BM	IM-UNI	Casas et al. (2001)
5	49	-	-	BWT	AB	IM-UNI	Kim et al. (2003)
5	40	-	-	DP	HC	IM-UNI	MacNeil and Grosz (2002)
5	53	38	66	EMA	BH	IM-UNI	Casas et al. (2000)
5	62	41	78	FAT	PA	IM-UNI	Casas et al. (2000)
5	64	53	71	FATYD	BH	IM-UNI	Smith et al. (2003)
5	75	62	80	MAR	BH	IM-UNI	Smith et al. (2003)
5	68	36	112	RPYD	PA	IM-UNI	Casas et al. (2000)
5	67	37	91	WBS14	PA	IM-UNI	Casas et al. (2000)
5	52	45	54	YE	Wagyu	IM-UNI	Mizoshita et al. (2004)
5	30	-	-	YG	HC	IM-UNI	MacNeil and Grosz (2002)
5	72	54	102	YG	PA	IM-UNI	Smith et al. (2003)
5	81	-	-	YWT	AB	RA-UNI	Kim et al. (2003)

Table 1.1 continued

BTA	Position (cM)	Support interval (cM)		Trait	Family	Method	References
		Lower	Upper				
6	1	-	-	BWT	AB	RA-UNI	Kim et al. (2003)
6	9	0	26	EMA	BH	IM-UNI	Casas et al. (2003)
6	52	45	67	EMA	BM	IM-UNI	Smith et al. (2003)
6	52	44	76	HCW	BM	IM-UNI	Smith et al. (2003)
7	55	44	71	FAT	BH	IM-UNI	Smith et al. (2003)
8	23	0	36	FAT	BM	IM-UNI	Casas et al. (2001)
8	30	17	43	FAT	PA	IM-UNI	Casas et al. (2001)
8	9	0	26	MAR	BM	IM-UNI	Smith et al. (2003)
9	71	46	76	MAR	BH	IM-UNI	Casas et al. (2003)
9	67	63	92	RPYD	BH	IM-UNI	Casas et al. (2003)
9	26	19	34	WBS14	BM	IM-UNI	Casas et al. (2001)
10	24	0	30	HCW	BH	IM-UNI	Smith et al. (2003)
10	4	0	28	MAR	BH	IM-UNI	Smith et al. (2003)
10	59	47	76	MAR	BM	IM-UNI	Casas et al. (2001)
11	66	27	80	YG	BH	IM-UNI	Smith et al. (2003)
13	60	43	64	RPYD	BH	IM-UNI	Stone et al. (1999)
14	33	29	39	ADG	Wagyu	IM-UNI	Mizoshita et al. (2004)
14	40	-	-	EMA	HC	IM-UNI	MacNeil and Grosz (2002)
14	14	10	25	FAT	PA	IM-UNI	Smith et al. (2003)
14	16	0	22	FAT	BH	IM-UNI	Smith et al. (2003)
14	50	45	51	HCW	Wagyu	IM-UNI	Mizoshita et al. (2004)
14	40	-	-	HCW	AB	RA-UNI	Kim et al. (2003)
14	47	30	87	MAR	BH	IM-UNI	Smith et al. (2003)
14	19	0	24	YG	BH	IM-UNI	Smith et al. (2003)
15	1	-	-	KPH	AB	IM-UNI	Kim et al. (2003)
15	28	23	32	WBS14	BH	IM-UNI	Keele et al. (1999)
16	49	32	57	HCW	BA	IM-UNI	Smith et al. (2003)
16	45	21	69	KPH	BH	IM-UNI	Smith et al. (2003)
16	62	39	73	KPH	BA	IM-UNI	Smith et al. (2003)
16	44	25	55	MAR	BA	IM-UNI	Smith et al. (2003)
17	35	0	63	FATYD	BA	IM-UNI	Smith et al. (2003)
17	50	35	75	LWT	HC	IM-UNI	MacNeil and Grosz (2002)
17	21	0	68	MAR	BM	IM-UNI	Smith et al. (2003)
18	23	11	38	HCW	BH	IM-UNI	Smith et al. (2003)
18	85	79	85	RPYD	BH	IM-UNI	Smith et al. (2003)
19	5	0	15	RPYD	BH	IM-UNI	Smith et al. (2003)
19	18	0	37	YG	BH	IM-UNI	Smith et al. (2003)
20	72	52	75	WBS14	BH	IM-UNI	Smith et al. (2003)
20	66	55	75	WBS3	BH	IM-UNI	Smith et al. (2003)
21	56	50	63	BWT	BH	IM-UNI	Casas et al. (2004b)
23	14	-	-	HCW	AB	RA-UNI	Kim et al. (2003)
23	30	21	42	MAR	BH	IM-UNI	Casas et al. (2003)
26	26	16	38	FATYD	BA	IM-UNI	Smith et al. (2003)
26	26	15	41	RPYD	BA	IM-UNI	Smith et al. (2003)
26	26	21	36	YG	BA	IM-UNI	Smith et al. (2003)
27	29	12	51	MAR	BH	IM-UNI	Smith et al. (2003)
27	60	49	64	MAR	BM	IM-UNI	Smith et al. (2003)

Table 1.1 Continued

BTA	Position (cM)	Support interval (cM)		Trait	Family	Method	References
		Lower	Upper				
29	54	45	58	HCW	BH	IM-UNI	Casas et al. (2003)
29	49	40	62	RPYD	BH	IM-UNI	Casas et al. (2003)
29	54	30	65	WBS14	BH	IM-UNI	Casas et al. (2000)
29	54	50	64	WBS14	PA	IM-UNI	Casas et al. (2000)
29	54	40	64	WBS3	PA	IM-UNI	Casas et al. (2000)
29	55	42	57	WWT	BH	IM-UNI	Casas et al. (2003)

[a] Map position (Centimorgan) based on Ihara *et al.* (2004). [b] ABF: Adjusted subcutaneous fat thickness between the 12th and 12th ribs, ADG: Average daily gain, BWT: Birth weight, DP: Dressing percentage, EMA: Eye muscle area, FAT: Fat depth at P8, FATYD: Fat yield, HCW: Hot carcass weight, KPH: Estimated kidney, heart and pelvic fat, LWT: Live weight, MAR: Marbling, RPYD: Retail product yield, WBS3: Meat tenderness measured as Wartner-Bratzler sear force at day 3 *post mortem*, WBS14: Meat tenderness measured as Wartner-Bratzler sear force at day 14 *post mortem*, WWT: Weaning weight, YE: Carcass yield estimate, YG: Yield grade, YWT: Yearling weight. [c]AB: Sired by an Angus × Brahman bull, BA: Sired by a Brahman × Angus bull, BH: Sired by a Brahman × Hereford bull, BM: Sired by a Belgian Blue × Marc III bull, HC: Sired by a Hereford × Composite gene combination bull PA: Sired by a Piedmontese × Angus bull Wagyu: Japanese black cattle. [d] IM-UNI: Interval mapping univariate analysis, RA-UNI: Univariate analysis using a random model. [e]Support interval was not reported.

One of the important criteria for meat quality is tenderness because tough meat is an important problem currently facing the beef cattle industry (Wheeler *et al*., 1994), especially for cattle containing *Bos indicus* germ plasm (Keele *et al.*, 1999). In an attempt to detect QTL for beef tenderness (defined as Warner-Bratzler shear force collected at d2 and 14 post-mortem on steaks from the longissimus muscle), (Keele *et al.*, 1999) conducted a genome scan and identified one QTL located 28 cM from the most centromeric marker on BTA15. Chromosomes 4, 5, 9 and 29 have been reported to harbour QTL for tenderness (Smith *et al.*, 2003). Casas *et al*. (2000) reported tenderness QTL on BTA29 and calpain 1 gene (CAPN1) has been considered as a functional and positional candidate gene for this QTL (Smith *et al.*, 2003). There are a number of studies showing the associations of the DNA variants in this gene with meat tenderness (Casas *et al*., 2006; Morris *et al*., 2006; Page *et al*., 2004; White *et al*., 2005).

1.3 Conclusions

The earlier studies have put a great deal of effort into the beef cattle QTL mapping. However, review of the literature revealed a significant number of gaps in knowledge that has encouraged the need for the present study. First, nearly all of the beef cattle studies have used the interval mapping (one QTL model) to locate QTL. Second, although phenotypic measurements have been available on multiple traits, only the single trait analyses have generally been performed on data from beef cattle QTL mapping experiments. Multivariate approaches can increase the power of the test and the precision of parameter estimates (Gilbert and Le Roy, 2004; Jiang and Zeng, 1995; Korol *et al*.,

1995; Meuwissen and Goddard, 2004). Third, most of the studies to detect QTL in beef cattle have focused on growth, carcass and fatness traits. A very few studies have considered beef tenderness and marbling. However, eating quality of meat depends on several important characteristics, including appearance, color, taste, fat content, texture, juiciness and tenderness. Also there is no information regarding the genes involving in the expression of beef fat. Nutritional quality of beef fat is important and knowledge about the genes underlying this characteristic can be useful to improve fat quality.

1.4 Research objectives

The overall aim of this study is to identify genomic regions affecting economic traits in beef cattle. More specifically, the present work will concentrate on carcass and meat quality traits. The traits to be studied are:

1. Reproductive traits (birth weight, pelvic area, gestation length and age at puberty)
2. Meat yield
3. Carcass fatness (internal, external and intramuscular fat deposition)
4. Meat quality (tenderness, colour and pH, cooking loss)
5. Fat quality (fat colour and fatty acids composition)

In order to bridge the gaps outlined above, the objectives of the study are:

1. To develop a multiple QTL mapping technique that can simultaneously include all of the markers of the entire genome to the analysis.
2. To develop a model for joint analysis of multiple phenotypes and families for QTL mapping
3. To apply the developed model for identifying genomic regions linked to the carcass and meat quality traits.

Some of the expected outcomes of this study include:

1. This research will provide additional insight into the actual genetic architecture of economically important traits in beef cattle.
2. This study will indicate the benefit of joint analysis of multiple traits and mapping families.
3. Results of this research will pave the way for QTL fine mapping and finally, the application of technologies such as marker-assisted selection and marker-assisted introgression.

Chapter 2: General materials and methods

2.1 Introduction

The data used for the present study were from two projects, the Davies gene-mapping project funded by the J.S. Davies Bequest to the University of Adelaide (Australian experiment) and the New Zealand AgResearch cattle gene mapping project funded by the New Zealand Foundation for Research, Science and Technology (New Zealand experiment). The two projects were designed to search for DNA markers associated with economically important traits in beef cattle. In order to avoid repetition of the common aspects of the projects in the next chapters, this chapter of the thesis describes the details of the projects.

2.2 Experimental design

The trial design involved two divergent breeds, Jersey (J) and Limousin (L), which are known to be extremely different for a number of performance traits (Cundiff *et al*., 1986). Three pairs of half-brothers were generated as first-crosses (X), and one of each pair was used for mating with both Jersey and Limousin dams in Australia and New Zealand, creating in total 784 backcross progeny (469 XJ and 315 XL) in the two countries (Table 2.1). In Australia, 366 experimental backcross calves (205 XJ and 161 XL) were born over the three years 1996-98. The XJ animals were born to Jersey dams and XL backcrosses were born to Limousin dams, at the University of Adelaide's property, located about 150 kilometres north of Adelaide, near Mintaro in the cereal zone of South Australia's mid-North. Calves were born in autumn (March-May, average 26th April), single suckled and weaned in summer (first week in February) at an average age of 250 days. After weaning, calves grazed pasture and/or hay supplemented for 430-500 days. The seasonal annual rainfall distribution patter during the experiment (1994-1998) varied, with an annual average of 586 mm, of which 34% fell in summer period (October-March) and 66% fell in winter period (April-September), a pattern typical of "Mediterranean" climates. Calves stayed with their dams on pasture and also have free access to oaten hay supplements provided to dams during the critical feed shortage period of the first year (January-June). The animals grown out on pasture in pre-allocated slaughter groups until

about two years of age, and were finished on grain concentrates for at least 180 days as part of an intensive feed efficiency trial. In New Zealand, 262 experimental backcross calves (162 XJ and 100 XL) were born in spring 1996, and another 156 were born in spring 1997 (102 XJ and 54 XL). The Jersey backcrosses were born in Jersey herds and were bucket-reared, whereas the Limousin backcrosses were born in 1996 by embryo transplant as singles or twins to Hereford x Friesian recipients on AgResearch's Whatawhata Station, and in 1997, they were born as singles in two Limousin herds. In both years, the XL calves were reared on their dams.

Table 2.1 Breeds of grand dams and grand sires and number of progeny in each sire family

Sire family	Experiment	Grand sire (breed)	Grand dam (breed)	N.of progeny
398[a]	Australia	(Jersey)	(Limousin)	113
368[b]	Australia	(Limousin)	(Jersey)	128
361[c]	Australia	(Jersey)	(Limousin)	125
417	New Zealand	(Limousin)	(Jersey)	122
402	New Zealand	(Jersey)	(Limousin)	156
394	New Zealand	(Jersey)	(Limousin)	140

[a], [b] and [c], called TOM, LOU and RYAN, respectively.

2.3 Definition of the Traits

2.3.1 Live animal measurements

Birth weights were recorded within 24 hours of birth. Body measurements were obtained from individual animals using a tape for height (measured as the distance from hip to the ground), length (measured as the distance between the first sacral bone on the shoulder and pin-bone) and girth (measured as the body circumference immediately posterior to the front leg). At weaning, calves were again weighed while full. Height was measured from the top of the hips and to the ground. The length and girth were measured similarly to that at birth. Other measurements at weaning were fat depth scanned at the P8 site on the rump as described by Arthur *et al*. (2001) using a Ezi-scan® sonic device (Amac Pty. Ltd.), plus hip width (bone) and stifle width (muscle) were measured using callipers. Hip and stifle widths were not measured on calves born in 1996. The animals' post-weaning weight, height, length, girth, fat depth at P8 site, hip and stifle widths were obtained at approximately 400 days and 600 days after birth (i.e. during winter after the

dry season and summer after the wet season, respectively) (Table 2.2).

Table 2.2 Summary statistics of the traits (Australian data)

Trait	Abbreviation	N	Mean	S.D.	Min	Max
Birth weight, kg	Bwt	366	26.4	6.1	12.8	44.7
Body height at birth, cm	Birht	366	68.7	5.0	54	84
Body length at birth, cm	Birlg	366	114.5	7.0	97	132
Body width at birth, cm	Birwd	366	53.4	3.7	38	65
Gestation length, day	Gl	363	283.7	5.0	273	293
Weaning weight, kg	Wwt	365	228.5	32.0	112	308
Body hight at weaning, cm	Wht	359	109.9	4.9	90	120
Hip width at weaning, cm	Whip	365	34.7	2.4	26	45
Stifle width at weaning, cm	Wsti	365	28.1	3.0	18	36
Fat depth at weaning, mm	Wfat	364	0.5	1.1	0	5
Body weight at 400 days, kg	400Wt	365	252.3	36.5	137	361
Body hight at 400 days, cm	400Ht	365	119.4	5.1	100	143
Hip width at 400 days, cm	400Hip	288	39.1	2.0	32	46
Stifle width at 400 days, cm	400Sti	288	28.9	2.8	22	40
Fat depth at 400 days, mm	400Fat	364	1.1	1.4	0	5
Body weight at 600 days, kg	600Wt	362	361.8	43.4	235	492
Body hight at 600 days, cm	600Ht	363	126.0	5.6	108	140
Hip width at 600 days, cm	600Hip	363	43.0	2.1	37	49
Stifle width at 600 days, cm	600Sti	363	31.9	3.5	22	45
Fat depth at 600 days, mm	600Fat	362	1.6	2.4	0	12
Age at puberty, days	AP	181	420.0	47.2	289	506
Marbling, score	Mar	355	1.5	0.8	0	4
Carcass fat depth on the rump, mm	P8	356	12.3	5.2	3	30
Meat colour, score	MC	355	1.9	0.9	1	6
Fat colour, score	FC	355	1.8	1.4	0	7
β-carotene concentration, μg/g fat	BC	363	1.2	0.6	0.1	4.2
Fat colour on biopsy samples, score	FCB	363	2.0	0.6	1	5
Eye muscle area, cm^2	EMA	355	80.7	17.0	26	166
Carcass length, cm	Carclg	356	139.0	5.8	120	155.5
Pelvic area, cm^2	PA	356	278.7	45.1	170.5	451
Channel fat, kg	Chanfat	356	12.5	3.9	4.7	22.8
Omental fat, kg	Omenfat	266	12.0	4.1	3.3	24.5
Heart weight, kg	Heart	351	1.8	0.3	1.0	5.96
Liver weight, kg	Liver	334	5.9	1.0	1.5	9.3
Kidney weight, kg	Kidney	353	1.2	0.2	0.6	1.6
Fat depth at ribs 10^{th} and 11^{th}, mm	Rbft	356	9.7	3.6	3	24
Ossification, score	Ossms	356	225.6	47.1	140	400
Butt shape, score	Butt	356	2.5	0.7	1	4
Intramuscular fat content, %	Imf	355	5.3	1.7	1.4	11.1
Melting point, ^oC	Mp	355	37.4	3.1	31	46

Table 2.2 continued

Trait	Abbreviation	N	Mean	S.D.	Min	Max
Monounsaturated fatty acids, % of triacylglyceride	MUFA	355	49.2	5.2	36.0	61.1
Hot standard carcass weight, kg	Hcw	356	334.7	61.7	168.0	479.6
Meat weight, kg[#]	Meat	330	230.4	48.5	114.5	355.2
Fat weight, kg[#]	Fat	330	45.3	11.3	11.3	82.4
Bone weight, kg[#]	Bone	330	58.9	10.0	33.5	88.7
Flight distance, meter	Fdist	357	9.7	3.8	1.5	23
Docility, score	Docsco	362	12.3	1.7	7.5	15.8
Weight of *M. longissimus dorsi*, kg	LD	351	6.3	1.5	3.1	11.5
Weight of *M. semitendinosus*, kg	ST	349	2.5	0.8	1.1	10.0
Weight of silverside, kg	SS	346	8.5	2.3	3.8	16.4
pH of *M. longissimus dorsi* muscle	pHld	355	5.6	0.1	5.4	6.7
pH of *M. semitendinosus* muscle	pHst	351	5.7	0.1	5.5	6.4
Cooking losses of LD muscle, %	Clld	355	21.8	1.9	14.5	39.1
Cooking losses of ST muscle, %	Clst	351	26.3	1.8	19.0	31.1
SF[a] of LD on day 1 post-mortem, kg	Wbld1	355	4.9	1.3	2.6	13.2
SF of LD on day 5 post-mortem, kg	Wbld2	355	4.4	1.0	2.5	10.3
SF of LD on day 12 post-mortem, kg	Wbld3	355	4.2	1.0	1.8	10.6
SF of LD on day 26 post-mortem, kg	Wbld4	355	4.0	1.0	2.1	9.9
SF of ST on day 1 post-mortem, kg	Wbst1	352	5.3	0.9	3.5	10.3
SF of ST on day 5 post-mortem, kg	Wbst2	352	5.1	0.8	2.6	7.4
SF of ST on day 12 post-mortem, kg	Wbst3	352	4.9	0.8	2.7	8.7
SF of ST on day 26 post-mortem, kg	Wbst4	351	4.7	0.8	2.7	8.4

[#] Estimated from prediction equations. [a]LD: *M. Longissimus dorsi*, [b]ST: M. semitendinosus. [c] SF= Warner-Bratzler shear force.

2.3.2 Carcass and meat quality

Australia

All animals born in 1996 were slaughtered at the Stockyard abattoir, Grantham, Queensland with those born in 1997 and 1998 slaughtered at the T&R's Murray Bridge abattoir, South Australia (age at slaughter 34-40 months). The animals were killed using a captive bolt and carcasses were electrically stimulated with a low voltage (peak 45V, 200mA) rectal-nostril stimulator for 40 seconds within five minutes of sticking. Following standard line processing, the carcasses were weighed, split and stored in a chiller (0-4°C) overnight. Approximately 18 hours after slaughter, carcasses were quartered at the 10/11th rib and carcass assessment performed by an accredited AUS-MEAT grader. Carcass traits evaluated include the cross sectional area of the *M. longissimus dorsi* (eye muscle area, EMA, cm^2), fat depth at the position 8 on the rump (P8) (mm), and surface fat between the

10^{th} and 11th ribs over the *M. longissimus dorsi* muscle (Ribfat, mm) (Table 2.2).

The left half-carcass from each animal was boned out and samples collected from the eye round (*M. semitendinosus*) and striploin (*M. longissimus dorsi*). Samples of 2.5 cm steaks were vacuum packed, randomly assigned to different ageing treatment groups (1, 5, 12, 26 days) at 0-1°C, and frozen (-20°C) after the completion of the ageing treatment. Before the tenderness measurements, the steaks were thawed overnight at 1°C and trimmed to 80-100g samples. The cooking procedure was in plastic bags in a water bath to an internal temperature of 70°C (40min) to achieve a "medium" degree of doneness as outlined by Perry *et al*. (2001a). The weight of each steak (before and after cooking) was also recorded. Cooking loss was calculated as the percentage difference between pre- and post-coked weight (Table 2.2). After storage overnight in the chiller, rectangular strips (15.0 x 6.6mm) were cut parallel to the fibres and Warner-Bratzler shear force measurements were performed on the Lloyd pressure tester according to Bouton and Harris (1972).

Ultimate pH was recorded in the *M. semitendinosus* and *M. longissimus dorsi* muscles prior to cooking using a WP-80 pH, mV, Temp-meter. pH measurements were taken after four aging treatments (1, 5, 12, 26 days after slaughter). pH was consistent across the aging treatments, so a simple average was used as the best indicator of ultimate pH of each muscle. Meat colour was assessed on the chilled carcass of the rib eye muscle area (*M. longissimus dorsi*) and scored against the AUS-MEAT beef colour reference standards (AUS-MEAT Limited, 1998). AUS-MEAT meat colour scores were 1, 1C, 2, 3, 4, 5, 6 where a high score indicates darker meat. To enable numerical analysis, score 1C was converted to a numerical value of 1.5.

Fat and meat samples were taken from the lateral part of strip-loin for determination of intramuscular fat content (IMF), fatty acid composition and melting poing. Triacylglycerol fatty acids were extracted, methylated and analysed by gas-liquid chromatography as described by Malau-Aduli *et al*. (1997). Fatty acids were classified into saturated (SFA, no double bonds), monounsaturated (MUFA, one double bond), and polyunsaturated (PUFA, two or more double bonds) and computed following Malau-Aduli *et al*. (1997). Elongation and desaturation indices were also calculated as described by Pitchford *et al*. (2002a). Elongation and desaturation indices show elongase enzyme and delta-9-desaturase enzyme activities, respectively. Melting point was recorded as the "slip point". The temperature at which the fat "slipped" was recorded as the melting point

(Pitchford *et al*., 2002a).

ß-Carotene content in the fat samples was analysed as described by Kruk *et al*. (1997b). Fat colour score (FC, Table 2.2) on carcasses was assessed in the chiller according to AUS-MEAT specifications (AUS-MEAT Limited, 1998). Subcutaneous adipose tissues were biopsied from the area between the 12th and 13th ribs in calves at 9–10 months of age. Fat colour score of adipose biopsy samples (FCB) was estimated on a 5-point scale (1-white to 5-very yellow) immediately after removing the fat from biopsy site and rinsing with water.

New Zealand

Animals were pre-allocated to slaughter groups in the springs of 1998 and 1999 over 18 and 10 kill days, respectively (once a week with approximately 15 same-sex animals per slaughter group, 18 groups in 1998 and 10 in 1999). Pre-allocation was based on breed of calf, sire, and balanced as far as possible within breed for live weight before the first slaughter day. Offspring (steers and heifers) were slaughtered off pasture in 28 year of birth by sex groups, at ages ranging from 22 to 28 months at the Ruakura Abattoir in Hamilton, New Zealand. Each animal was stunned by captive bolt and then slaughtered; no electrical stimulation was applied immediately after slaughter. After splitting the carcass and weighing the two sides (to give hot carcass weight, HCW, Table 2.3), the right striploin was removed and the right side was then stored in a chiller for 24 hours before quartering at the 10/11th rib. A butcher's dissection of the right fore- and hind-quarters was then carried out in order to record, for each joint, the weights of saleable meat plus meat trim (the combined total being referred to as "meat"), trimmed fat ('fat") leaving a fat cover of approximately 2 mm, and bone.

Initial pH was recorded within about 30 minutes of slaughter and then monitored at intervals for about 24 hours until *rigor mortis* (pH<5.5), while the striploin was held in a controlled-temperature cabinet at 15°C. Ultimate pH was the lowest pH attained in the first 24 h, defining the full development of *rigor mortis*. The rate of fall of pH was linear at constant temperature, and calculated from a linear regression of measured pH values on time during the pre-*rigor* period. Five steak portions were then cut from the striploin for cooking and shear-force tenderness measurements.

The first steak was processed upon reaching *rigor mortis*, and the four other steaks were cooked at intervals (approximately 1/3, 1.0, 1 1/3 and 4.0 days post mortem), after

continued storage at 15°C. For cooking, each steak was placed inside a plastic cooking bag, heated in a boiling water-bath to an internal temperature of 75°C, then removed and cooled rapidly in ice to an internal temperature of 2°C. Shear-force measurements were then recorded using a MIRINZ tenderometer (Fraserhust and MacFarlane, 1983), taking the average from measurements of ten 1cm by 1cm cores, aligned with the fibers running longitudinally along the core. The measurements of tenderness on the five steak samples were referred to as Cook 1-5.

Table 2.3 Summary statistics of the traits (New Zealand data)

Trait	Abbreviation	N	Mean	S.D.	Min	Max
Birth weight, kg	Bwt	306	29.5	5.7	14	50
Body weight at 400 days, kg	400Wt	241	267.7	38.2	194	371
Body weight at 600 days, kg	600Wt	413	435.7	67.1	286	642
Gestation length, day	Gl	185	284.5	5.9	258	301
Age at puberty, day	Pubert	139	371.2	48.6	199	466
Fat weight at side, kg	Fatwt	401	9.5	2.6	3.3	22.8
Bone weight, kg	Bonewt	401	23.9	3.9	15.9	35.5
Meat weight, kg	Meatwt	401	74.1	16.7	44.5	126.1
Rump meat weight, kg	Rumeat	406	5.1	1.2	3.0	8.4
Hot standard carcass weight, kg	Hscw	413	227.6	43.9	136	369
Weight of longissimus dorsi, kg	LD	405	6.0	1.4	2.9	10.8
Weight of silverside, kg	SilverS	406	8.4	2.2	4.4	16.1
Eye muscle area, cm^2	EMA	326	59.0	13.6	37.2	111.7
pH of *M. longissimus dorsi* muscle	pHld	413	5.4	0.1	5.3	6.2
SF[a] of LD[b] on *rigor mortis*, kg	Wbld1	413	14.6	2.8	5.4	2.7
SF of LD on day 1.3 post-mortem, kg	Wbld2	413	10.9	2.9	2.7	20
SF of LD on day 2.0 post-mortem, kg	Wbld3	413	7.4	1.7	3.3	17.4
SF of LD on day 2.3 post-mortem, kg	Wbld4	400	6.8	1.4	4.0	16.7
SF of LD on day 4.0 post-mortem, kg	Wbld5	413	5.8	1.2	3.6	15.6
Cooking loss of LD on *rigor mortis*, %	Clld1	413	23.4	5.0	8.6	53.9
Cooking loss of LD at day1.3, %	Clld2	413	23.6	4.0	12.4	39.3
Cooking loss of LD at day2.0, %	Clld3	413	23.1	4.0	8.8	47.8
Cooking loss of LD at day2.3, %	Clld4	400	21.7	4.0	7.7	45.2
Cooking loss of LD at day4.0, %	Clld5	413	23.8	4.2	7.7	48.8
Fat depth at ribs 12^{th} and 13^{th}, mm	Rbft	325	7.4	2.9	1	20
Meat colour L, score	ColL	411	39.8	1.9	34.1	45.8
Meat colour a, score	Cola	411	17.2	1.8	11.6	22.3
Meat colour b, score	Colb	411	8.1	1.1	4.1	10.9
Side Length, cm	Carclg	413	127.0	4.6	99.6	140.5

Table 2.3 continued

Trait	Abbreviation	N	Mean	S.D.	Min	Max
Pelvic area, cm^2	PA	413	212.6	26.3	141.5	288.4
Channel fat, kg	Chanfat	413	7.1	3.2	1.5	19.5
Omental fat, kg	Omenfat	413	4.0	2.1	0.6	12.5
Pericardial fat, kg	Prcarfat	409	0.5	0.2	0.1	1.8
Rump fat, kg	Rumfat	406	0.5	0.2	0	1
Silverside fat, kg	Silversfat	406	0.5	0.1	0.2	1.0
Intramuscular fat content, %	Imf	341	4.2	2.3	0.4	12.8
Melting point, ^oC	Melpt	405	37.2	2.6	28.5	44
Monounsaturated fatty acids, % of triacylglyceride	Mufa	402	51.2	3.0	42.4	59.3
Fat colour on biopsy samples, score	FCB	410	1.7	0.6	1	4
Heart weight, kg	Heart	413	1.6	0.2	1.1	2.5
Liver weight, kg	Liver	412	6.2	0.9	3.8	9
Kidney weight, kg	Kidney	413	0.9	0.1	0.6	1.5
Docility, score	Docsco	155	12.7	1.4	9.9	16.8

[a] SF: Shear force. [b] LD: *M. longissimus dorsi* muscle.

The correspondence between ageing time scale and cook group differed for Australia and New Zealand because of processing conditions. The half-life for the fall in shear force (SHLF) was taken by fitting an exponential decay function through the shear-force results from cook times 1 to 5 (Dransfield *et al.*, 1981), for the striploin of each animal separately. The weight of each steak (before and after cooking) was also recorded. The percentage weight loss of each steak during cooking was calculated from the steak weights, before and after cooking (Table 2.3).

2.4 DNA markers and linkage map

DNA was extracted from the New Zealand and Australian cohorts from blood samples collected in their first year of life, with the exception of the XL calves born in New Zealand in 1996 where DNA was extracted from ear cartilage (because of the possibility of blood mixing in twin-born recipient calves).

For genotyping of two experimental populations, 285 PCR-based microsatellite loci (spread across the whole genome, except for the X- and Y-chromosomes) were chosen from the Meat Animal Research Centre (MARC) map (http://www.marc.usda.gov/genome/cattle/cattle.html) to provide adequate coverage of the bovine genome (Table 2.4). To insure robustness of PCR and reliability of allele identifications, the markers were tested

and those markers that were not robust were replaced with another marker in the same region. The six sires were genotyped using the 285 markers to determine if the sires are heterozygous at each marker loci. On average the sires were informative for 189 loci (Table 2.5). The F_1 sires and their backcross progeny were genotyped based on the informative markers at AgResearch, New Zealand. The fragments were visualized by autoradiography after electrophoresis of stained polyacrylamide gels.

Table 2.4 Marker loci and their map positions (cM)[a]

BTA1																		
Locus	BMS2321	BMS711	ILSTS4	BMS4017	BM4307	TGLA57	CSSM004	INRA011	INRA049	BM6506	CSSM032	BMS1789	BM1824	BMS599	BMS2263			
Position	15.4	23.9	34.3	38.1	38.4	51.0	52.2	60.3	75.8	77.7	99.2	113.5	122.4	139.3	147.8			
BTA2																		
Locus	BY5	BULGE23	BTAFJ1	MSTN	BULGE20	ILSTS26	INRA40	TGLA431	TEXAN2	OARHH30	TGLA377	URB042	ILSTS30	OAFCB20	RM356	BMS2626	BM6444	BMS356
Position	3.9	4.5	4.6	6.0	6.5	10.8	10.9	11.9	26.0	28.6	30.7	37.6	38.9	44.0	56.9	75.3	96.9	109.0
BTA3																		
Locus	BMS871	INRA006	RM065	BMS963	BMS482	BL41	MCM58	BM4129	TGLA263	HUJ246	BM6465	BMS2145	BMS835	BMS896	BMC4214			
Position	0.0	17.1	30.6	32.9	34.0	43.3	47.9	52.5	54.0	68.0	69.2	93.8	99.1	116.5	125.8			
BTA4																		
Locus	BMS1788	BMS1172	BMS1237	MAF70	MAF50	RM067	BMS495	INRA72	BMS779	ILSTS62	OARCP26	BMS648	TGLA159	MGTG4B				
Position	12.5	30.8	34.4	46.0	51.2	51.9	59.3	63.0	68.7	72.3	76.0	91.2	102.1	112.8				
BTA5																		
Locus	BMS1095	BMS610	AGLA293	OARFCB5	BL37	MAF23	CSSM22	BMS1248	BMS772	BM2830								
Position	0.0	12.0	32.3	35.3	52.1	64.5	74.2	90.8	108.8	116.9								
BTA6																		
Locus	INRA133	BM1329	BM143	BMS483	BM4621	BM415	BM8124	BM2320										
Position	8.1	35.4	53.7	67.8	77.6	82.0	101.4	127.3										
BTA7																		
Locus	BM7160	RM006	TGLA48	TGLA303	BM741	BM6117	BMS2258	BMS792	INRA192	BMS1331	BM9065	ILSTS6	BMS522	BMS1247				
Position	0.0	25.4	38.2	39.3	48.9	62.2	77.2	79.6	82.5	90.7	101.1	116.6	120.5	133.8				
BTA8																		
Locus	RM321	RM372	BMS1591	BM4006	TGLA13	BMS1341	BMS2072	BM711	CSSM47	BMS836								
Position	19.1	21.1	31.4	50.1	54.6	55.0	66.0	92.7	118.7	122.9								
BTA9																		
Locus	BM757	ETH225	BM2504	RM216	BMS817	BMS1148	BMS1290	TGLA73	BM4208	BMS1967								
Position	5.4	12.8	30.9	37.1	42.5	50.9	64.9	77.6	90.7	109.3								
BTA10																		
Locus	CSSM38	BMS528	BMS861	BM875	BM888	BMS1620	TGLA272	BMS2614										
Position	11.0	24.0	43.0	53.9	60.0	80.4	97.2	109.4										

Table 2.4 continued

BTA11															
Locus	BM827	BMS2131	BMS2325	BM304	RM096	INRA111	BMS1716	BMS1822	RM150	BMS1048	BMS989	RM363	BMS2315	OARCP34	HEL13
Position	10.6	18.9	21.1	33.6	40.5	53.1	54.6	65.9	70.1	81.1	92.2	97.6	110	111	122.4
BTA12															
Locus	BMS410	BM6108	AGLA226	BM6404	BMS975	RM113	BMS1316								
Position	0	15.1	37.2	57.1	63.8	81.4	102								
BTA13															
Locus	TGLA23	BMS1742	ILSTS59	HUJ616	BMS1669	RM327	BL1071	AGLA232	BMS2319						
Position	9	23	41.7	51.7	59.2	73.6	81	91.4	97.3						
BTA14															
Locus	BMS1678	ILSTS11	RM011	ILSTS8	BM302	BMS740	BMS108	BM4513	BL1036						
Position	14	25.7	43.6	50.9	52.4	60.7	67.7	79.8	100						
BTA15															
Locus	BR3510	BMS2533	MAF65	HEL1	JAB4	POTCHA	BM4325	BMS812	TGLA75	BM848	BMS820	BMS429			
Position	9.4	13.9	32.7	38	39.1	76.5	76.9	84.9	89.3	97.5	98.2	109.8			
BTA16															
Locus	BMS357	HUJ614	BMS538	BM1311	TGLA53	BMS1907	BMS1207	CSSM28	BM719	BM3509	INRA13	HUJ625			
Position	2.7	14.2	26.4	32.5	38.5	43.7	53.5	54.1	77.6	84	87.4	89			
BTA17															
Locus	RM156	BMS2220	BMS941	CSSM9	OAFCB48	ILSTS23	BM8125	BL50	BM1862	BM1233					
Position	2.4	17.3	37	38.3	41.7	46.8	66.5	72.2	80.9	92.1					
BTA18															
Locus	BMS1355	TEXAN10	INRA121	BM8151	BM7109	INRA63	ILSTS2	BMS2639	BMS929	BMS2785	TGLA227				
Position	2.9	20.7	30.2	40.2	47	48	54.7	55.5	61.2	72	84.1				
BTA19															
Locus	BM9202	HEL10	BMS2142	BP20	BM17132	BMS1069	ETH3	RM388	BMS601						
Position	0	16	43.3	45.9	59.2	77.7	90	95	108						

Table 2.4 continued

BTA20									
Locus	RM106	BM1225	TGLA304	TGLA126	BM4107	BMS1120	BMS703	BM5004	BMS521
Position	2.7	8.2	20.2	31.9	57.4	58.7	60.1	71.8	80.9
BTA21									
Locus	BM8115	RM151	BM3413	AGLA233	BM103	BMC4228	ILSTS16	TGLA122	BMS743
Position	0	12.6	15	21.2	29.8	36.9	45.2	62.7	75.3
BTA22									
Locus	INRA26	BM1558	BM1303	AGLA13	BMS390	BM2613	BMS875	HMH1R	BM4102
Position	2.9	19.1	28.7	31.2	48.9	54.1	64.1	79.4	82.9
BTA23									
Locus	INRA132	UWCA1	CYP21	BM1818	BP34	BM1905	BM1443		
Position	4.7	26.5	42.4	58.2	64.4	71.6	73.8		
BTA24									
Locus	BM7151	CSSM31	BMS1743	INRA90					
Position	8.2	25.8	43.9	56.3					
BTA25									
Locus	RM074	BM4005	BP28	BM737	BMS1353				
Position	2.2	14.4	23.4	31.6	46.4				
BTA26									
Locus	BMS651	BM1314	BM4505	BM6041	MAF92	BM7237	ILSTS91		
Position	2.8	27	41.6	51.7	63.4	66.8	71.5		
BTA27									
Locus	BMS2168	BM6526	CSSM43	INRA134	BM203				
Position	0	10.1	34.5	45.3	64.1				
BTA28									
Locus	BMC6020	BL25	BM6466	BMS1714	BMC2208				
Position	8	24.8	43	49.4	59.6				
BTA29									
Locus	BMS1857	BMS764	OAVH110	OARHH22	CAPN1	BMC1206	BMS1948		
Position	1.8	11.3	24.2	41.6	59.7	62.5	65.6		

[a] The map positions relative to the most distal marker on the Meat Animal Research Centre (MARC) map, which uses the Kosambi's mapping function.

Table 2.5 Number of informative markers by sire family

	Chromosome																												
Sire	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**22**	**23**	**24**	**25**	**26**	**27**	**28**	**29**	Total
361	7	8	9	10	8	8	9	9	9	7	9	4	8	6	8	8	9	7	5	5	5	6	4	3	2	4	3	4	3	187
368	7	7	7	9	6	5	6	6	8	6	7	7	9	5	7	8	8	6	3	8	8	6	3	4	3	4	4	4	7	178
394	8	12	10	9	5	4	8	7	9	6	13	6	7	6	9	9	8	7	6	6	6	4	7	2	4	5	5	4	6	198
398	8	8	8	8	6	5	9	7	8	7	12	6	6	7	8	6	7	7	8	7	7	4	3	3	4	2	5	5	6	187
402	7	14	8	8	8	5	7	8	9	7	6	5	6	8	8	5	4	8	5	4	3	6	4	4	5	4	3	4	5	178
417	8	10	7	10	7	6	8	7	8	7	9	6	9	8	8	11	8	8	6	7	6	8	6	4	3	6	3	5	7	206
Avg	7.5	9.8	8.2	9.0	6.7	5.5	7.8	7.3	8.5	6.7	9.3	5.7	7.5	6.7	8.0	7.8	7.3	7.2	5.5	6.2	5.8	5.7	4.5	3.3	3.5	4.2	3.8	4.3	5.7	189
Min	7	7	7	8	5	4	6	6	8	6	6	4	6	5	7	5	4	6	3	4	3	4	3	2	2	2	3	4	3	178
Max	8	14	10	10	8	8	9	9	9	7	13	7	9	8	9	11	9	8	8	8	8	8	7	4	5	6	5	5	7	206

Chapter 3: Mapping QTL for economic traits in beef cattle (Interval mapping-one QTL model)

3.1 Introduction

The impact of the traditional genetic programs on beef cattle growth is well documented. However, the profitability of the beef industry depends on a number of economically important traits. For example, compared to growth traits, reproductive traits have not been successfully improved through selection. Economic impact of reproductive performance in the beef cow herd can never be overemphasized. Carcass quality is another area where opportunity for improvement through selection programs has not been exploited. The industry is striving to meet consumer demand for healthier products. Research need to be done to generate data to use for genetic evaluations. Both reproductive traits and carcass and beef quality traits are areas that also serve as excellent examples of the potential use of DNA technology.

Difficult calving or dystocia is one of the most important problems in beef production. A difficult calving, which requires assistance, can lead to increased labour and veterinarian costs. However, the further implications are reduced subsequent reproductive performance of the cow, increased calf mortality, and potential loss of the cow. Calf birth weight has been used as an indicator trait to avoid calving difficulties. Direct heritability estimates for birth weight range from 0.44 to 0.51 (Eriksson *et al*., 2004). Eriksson *et al*. (2004) reported moderate to high and positive genetic correlations between calving difficulties, stillbirth and birth weight for Hereford and Charolais breeds. This relationship is unfavourable because the estimates of the genetic correlation between direct effects on birth weight and yearling weight are approximately 0.5 across all beef cattle breeds (Koots *et al*., 1994). This genetic antagonism amongst direct effects is a situation where selection based on breeding values for yearling weight may also significantly increase birth weight, thus potentially increasing the calving difficulty. Therefore, identifying gene markers, which affect birth weight but not subsequent growth, coupled with marker-assisted selection has potential to overcome this genetic antagonism.

Gestation length has been included in some sire selection indices (Amer *et al*., 1998) in beef cattle. Gestation length shows moderate to high heritability (Andersen and Plum, 1965; Goyache and Gutiérrez, 2001), and high genetic correlations with birth weight (Bourdon and Brinks, 1982) and dystocia (Nadarajah and Burnside, 1989). In this sense,

Gestation length has been proposed as a breeding objective to reduce birth weight without affecting pre-weaning growth traits (Bourdon and Brinks, 1982; Brinks *et al*., 1991).

Disregarding malpresentations, calving difficulties are generally the result of a discrepancy between the size of the calf and the size and shape of the cow's pelvic opening (Meijering, 1984). Pelvic measurements have been thus advocated as auxiliary selection criteria to reduce the incidence of difficult calvings. (Johanson and Berger, 2003) reported that the increase of pelvic area is associated with decrease in dystocia.

Age at puberty in heifers can have a major effect on the efficiency of the beef cattle enterprise when heifers are bred to calve first as 2-yr olds, especially under a restricted breeding season. Significant genetic variation exists within and between breeds of beef cattle for age at puberty (Gregory *et al.*, 1991; Martin *et al.*, 1992). Limousin heifers reach puberty at an older age than heifers of Jersey breed (Gregory *et al.*, 1991; Martin *et al.*, 1992). The average differences for age at puberty between breeds are attributed to the additive effects of genes present in diverse frequencies within breeds Martin *et al*. (1992). Thus, there is a need to identify the genetics involved with the expression of this trait.

Beef quality comprises a set of key fresh meat quality, processing, and sensory characteristics that are important for the future profitability and competitiveness of the beef industry. These include intramuscular fat, cholesterol, ultimate pH, color, water-holding capacity or drip loss, tenderness, cooking loss, and sensory traits involving taste (Oddy *et al*., 2001). Molecular genetic techniques can overcome the well known limitations of the breeding schemes to improve meat quality traits (de Vries *et al*., 1998). Information about QTL affecting these traits is particularly interesting for its possible application in marker assisted selection schemes. However, there are very few reported QTL for beef quality, because early studies to detect QTL in beef cattle have focused on growth, carcass and fatness traits.

The use of linear regression interval mapping to detect QTL in structured outbred populations is a useful first step, providing information about important QTL. Thus, the aim of the work herein was to exploit the regression based interval mapping to locate QTL for economically important traits in the beef industry.

3.2 Methodology

An experimental cattle backcross between the Jersey and Limousin breeds was performed in Australia and New Zealand to map quantitative trait loci (QTL) for diverse

production traits. Six crossbred sires and their progeny were genotyped for 189 informative microsatellite markers covering the 29 bovine autosomes. The trait categories included in this chapter were the reproductive traits, carcass and beef quality traits from the Davies cattle gene mapping herd in Australia (Chapter 2). Pelvic area, birth weight, gestation length and age at puberty in heifers were considered as reproductive traits. Birth weight was considered as a reproductive trait because of its relationship with calving ease. QTL analysis was conducted for a wide range of the carcass and meat quality traits (Chapter 2).

3.2.1 Marker inheritance and sire haplotype reconstruction

Each marker in each sire family was considered in turn to determine marker inheritance and to reconstruct sire haplotype using the approach of Knott *et al.* (1996). Markers for which a sire was homozygous were uninformative (i.e., offspring with genotypes identical to their sire) and were removed from consideration. For markers, which were heterozygous in the sire, it was possible to determine which allele the progeny inherited. Once informative markers were identified and their inheritance determined, the haplotypes for each sire were reconstructed for the linkage group under consideration. This was done by considering, in turn, each pair of adjacent markers for which the sire was heterozygous. Progeny, in which the allele inherited from the sire were determined at both loci, were ascertained and the linkage phase was taken as that which minimised the number of recombination events in the sire. This was repeated for each pair of adjacent heterozygous markers to reconstruct the two haplotypes for each sire.

3.2.2 Information content

The information content of an individual marker is the proportion of animals in which the allele inherited from the sire can be unambiguously identified. In the first stage of the analysis, the approach described by Knott *et al.* (1996) using multiple markers was used to obtain the probabilities of the alternative genotypes for each offspring at fixed locations through the genome. These probabilities were used to investigate the information content of the markers when used together. The variance of these coefficients across the progeny gives a measure of information. Information content at genome position i was calculated as $Var(p_i)/0.25$, where p_i is the probability of receiving QTL allele i and 0.25 is the expected variance of inheritance probabilities for a fully informative marker (Knott *et al.*, 1998). When there is no information from markers, the variances are zero, and when

the genotype of all individuals is known at the location being considered, the variance is expected to be 0.5.

3.2.3 Estimation of QTL parameters

In the present chapter, the QTL analyses were performed using the QTL Express (Seaton *et al*., 2002), a web-based software at http://qtl.cap.ed.ac.uk/. The software analyses each chromosome independent of others and allows the user to fit one and two QTL. It includes tools for permutation and bootstrap analyses to calculate chromosome-wise significance thresholds and confidence intervals, respectively.

The method of Knott *et al*. (1996) assumes that there is no interference in recombination events and Haldane's mapping function (Haldane, 1919) applies. At the first stage, in this method the probabilities for each progeny inheriting the two sire haplotypes are calculated for fixed positions in the linkage group conditional upon marker genotypes. The probabilities depend only upon the alleles inherited at the two nearest informative markers flanking the position under consideration and the recombination between the markers and this position. In the second stage, these probabilities together with fixed effects are used in least squares framework to investigate the genetic model underlying the trait of interest. For each regression, an F-ratio of the model including the QTL effect compared with the model not including the QTL effect was calculated. F-ratio statistics were plotted against the location for which it was calculated to provide a curve displaying evidence for the presence of a QTL in the linkage group. The location with the largest F-ratio was taken to be the best estimated position for a QTL for each trait. QTL effects were estimated using two models, individual families and across families. First, in recognition of the fact that the samples were from outbred populations (the QTL alleles were not fixed in original breeds, Jersey and Limousin) and only three families were available, analyses were applied to each individual sire family. Second, across family analysis was applied to calculate the estimates of the putative QTL effect for each sire included in the analysis for the genomic region under investigation. This was done by nesting the regression within sires. This is because not all sires will be heterozygous for all QTL and the linkage phase between the QTL and the sire haplotype will vary from sire to sire.

A single QTL model was fitted for each trait. Models (3-1), (3-2) and (3-3) were fitted for birth weight, pelvic measurements and other traits, respectively.

$$Y_{ijlmn} = \mu_i + BP_j + CT_l + BD_m + b_i x_{ij} + e_{ijlmn} \qquad (3\text{-}1)$$

$$Y_{ijlm} = \mu_i + \beta \,\mathrm{cov}\, X\ \mathrm{cov} + CT_j + BD_l + b_i x_{ij} + e_{ijlm} \quad (3\text{-}2)$$

$$Y_{ijlm} = \mu_i + CT_j + BD_l + b_i x_{ij} + e_{ijlm} \quad (3\text{-}3)$$

Where Y_{ijlmn} is the phenotype of individual n, offspring of sire i, μ_i is the mean of sire family i, BP is birth period (j=1,…,5), CT is cohort (six year and sex combinations, l=1,…, 6), BD is breed of dam (Jersey and Limousin) b_i is the allele substitution effect of the QTL within family i, x_{ij} is the probability that animal n inherited the (arbitrarily assigned) first allele of sire i, $\beta\,\mathrm{cov}$ is regression coefficient of hot carcass weight for pelvic measurements, $X\,\mathrm{cov}$ is hot carcass weight and e_{ijlmn} is the residual effect.

3.2.4 Test statistic and significant thresholds

For a fixed position of QTL, the ratio of the regression mean square to the residual mean square provides the variance (F) ratio test statistic. In individual family analysis, the test within each family produces an F-ratio with 1 degree of freedom in the numerator and (n-2) degree of freedom in the denominator, where n is the number of offspring in the analysed family. The test across family produces an F-ratio with *s* degree of freedom in the numerator, where *s* is the number of sires analysed and ($\sum m - 2s$) degree of freedom in the denominator. An alternative approximate log-likelihood ratio (LR) test statistic is provided by

$$n\log_e(RSS_r / RSS_f) \quad (3\text{-}4)$$

where *n* is the number of observations; RSS_r and RSS_f are residual sum of squares reduced model and residual sum of squares full model, respectively. This test statistic is distributed approximately as a chi-square with degrees of freedom equal to the number of parameters included in the full model but omitted from the reduced model and dividing this test statistic by 4.605 would approximately give the log of the odds (LOD score) (Haley *et al*., 1994).

In the present study, permutation tests were performed with 1000 replicates to empirically determine P-values at the chromosome-wise significance level (Churchill and Doerge, 1994). Empirical confidence intervals for QTL location were determined using bootstrapping as described by Visscher *et al*. (1996).

3.3 Results

3.3.1 Information content

Average information content across families and all 29 autosomes was 75%. Minimum and maximum average information content (across families) were 66% and 83% for BTA6 and BTA13, respectively. The measure of information content can range from 0 (absence of information) to 1 (maximal information content). Reduced information content resulted primarily from the lack of informative markers in one or more than family and the resulting need to infer the marker genotype for the uninformative loci based on somewhat distant markers. Other factors, such as missing genotypes for individual animals and unequal segregation of alternative alleles can affect the information content. The average information content reported herein includes the values obtained for genomic positions (one centimorgan intervals) between and at the marker loci. It should be noted that information content is less between markers than at the marker loci (Weller, 2001).

3.3.2 Reproductive traits

The most likely positions, F-ratios and LOD scores and estimated effects of the detected QTL that are likely to affect calving ease age of puberty were determined (Table 3.1). The regions identified as harbouring highly significant QTL (expected number of false-positives <0.01) for calving ease related traits reside on cattle chromosomes 14, 20 and 22.

A chromosomal region with effects on birth weight (Table 3.1) was identified on BTA14. The maximum F-statistic was detected between 37 cM from the beginning of the linkage group. This QTL appears to be segregating in family 361 with the estimated allele substitution effect of 3.06 kg.

There was evidence for the presence of a QTL for birth weight and pelvic area on BTA3 (Figure 3.1). The 95% confidence interval for the birth weight QTL on BTA3 covers 0-91 cM. An overlapping, but slightly wider, confidence interval was found for PA, encompassing positions 0 through 103 cM. Birth weight and pelvic area QTL on BTA3 segregated in family 361 with the estimated allele substitution effects of 3.06 kg and 22.92 cm^2, respectively.

Table 3.1 Most likely position, F-statistic values, approximate LOD scores and allelic effects of detected QTL for calving ease traits[a]

BTA	Position (cM) [b]	Trait	Family	QTL Effect	S.E.	F-Value	LOD[c]
1	80	Birth weight, kg	361	1.56	0.81	4.6	2.9
			368	2.17	0.81		
			398	1.73	0.98		
3	58	Birth weight, kg	361	3.09	0.92	4.2	2.7
			368	0.42	0.98		
			398	1.15	0.94		
3	0	Pelvic area, cm^2	361	22.92	3.46	4	2.6
			368	5.46	3.2		
			398	3.94	3.8		
5	34	Birth weight, kg	361	0.05	0.7	4.3	2.7
			368	0.20	1.06		
			398	2.86	0.8		
6	35	Pelvic width, cm	361	5.2	1.37	4.1	2.6
			368	5.72	1.33		
			398	6.26	1.52		
6	35	Pelvic area, cm^2	361	10.54	3.24	4.4	2.8
			368	9.74	3.16		
			398	20.86	3.61		
10	102	Gestation length, day	361	1.09	0.95	4.5	2.9
			368	0.17	1.21		
			398	3.58	1.03		
14	37	Birth weight, kg	361	3.06	0.73	**7.1**	4.5
			368	1.33	0.75		
			398	0.55	0.76		
17	74	Pelvic area, cm^2	361	5.04	3.26	4.1	2.6
			368	10.04	3.72		
			398	23.52	3.78		
17	78	Pelvic hight, cm	361	1.77	2.19	4.3	2.7
			368	0.08	2.37		
			398	9.04	2.6		
18	23	Pelvic hight, cm	361	0.11	2.36	4.4	2.8
			368	2.20	2.2		
			398	8.11	2.34		
20	3	Pelvic area, cm^2	361	12.14	3.71	4	2.5
			368	5.62	3.4		
			398	22.16	3.83		
20	11	Pelvic hight, cm	361	2.73	2.32	**5.2**	5.2
			368	2.55	2.31		
			398	8.81	2.44		

Table 3.1 continued

BTA	Position (cM) [b]	Trait	Family	QTL Effect	S.E.	F-Value	LOD[c]
21	8	Birth weight (kg)	361	0.17	0.83	4.4	2.8
			368	2.40	0.74		
			398	1.38	0.82		
22	3	Pelvic area, cm^2	361	25.86	3.49	**4.8**	3.1
			368	4.34	3.11		
			398	2.50	3.55		
22	3	Pelvic width, cm	361	10.90	1.47	**4.8**	3.1
			368	1.98	1.31		
			398	0.94	1.50		
23	20	Gestation length, day	361	4.05	1.19	4.2	2.7
			368	0.56	1.47		
			398	1.44	1.45		
28	48	Pelvic width, cm	361	8.82	1.39	4.6	2.9
			368	0.04	1.32		
			398	5.80	1.50		

[a] Detected QTL at least at 5% chromosome-wise significant are reported. [b] Position (cM) based on the map of Ihara et al. (2004). [c] logarithm of odds. The highlighted F-values show 1% chromosome-wise significant.

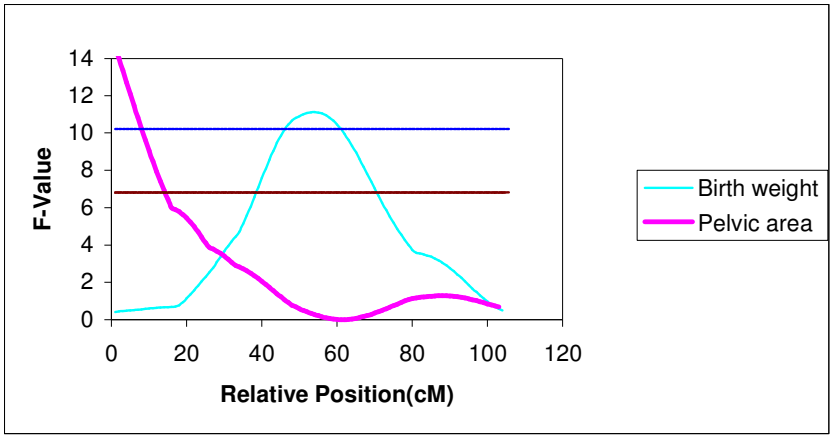

Figure 3.1 F-statistic profile from individual family analysis (sire family 361) for pelvic area and birth weight on BTA3. The lower and upper horizontal lines represent 5% and 1% chromosome-wise significant levels of linkage, respectively.

A significant F-statistic peak at the centromeric region of BTA22 indicated a QTL affecting both pelvic width and pelvic area (Figure 3.2), close to marker INRA26. The detected QTL segregating in family 361had allele substitution effects of –10.90 cm and –25.86 cm^2 for pelvic width and pelvic area, respectively.

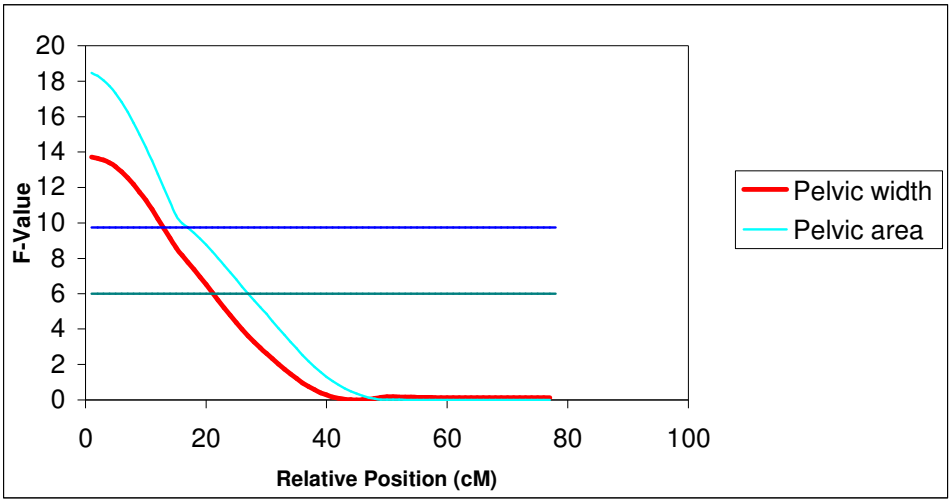

Figure 3.2 F-statistic profile from individual family analysis (sire family 361) for pelvic width and pelvic area on BTA22. The lower and upper horizontal lines represent 5% and 1% chromosome-wise significant levels of linkage, respectively.

Additional putative QTL for pelvic area with effects at or above the significant threshold (chromosome-wise significance of $P<0.05$) were detected on BTA17 and BTA20 both segregating in family 398. There was also evidence for a QTL that influenced pelvic width and pelvic area on BTA6 (Table 3.1). The highest test statistic was found on the proximal region of BTA6, close to microsatellite marker BM1329.

Linkage analysis indicated significant QTL (chromosome-wise significance of $P<0.05$) for gestation length on BTA10 and 23, segregating in families 398 and 361 with the allele substitution effects of 3.58 and 4.05 day, respectively.

BTA12 and BTA16 were linked to the age at puberty. The BTA12 QTL was segregating in family 361 (Figure 3.3) and the BTA16 QTL was segregating in family 398 (Figure 3.4).

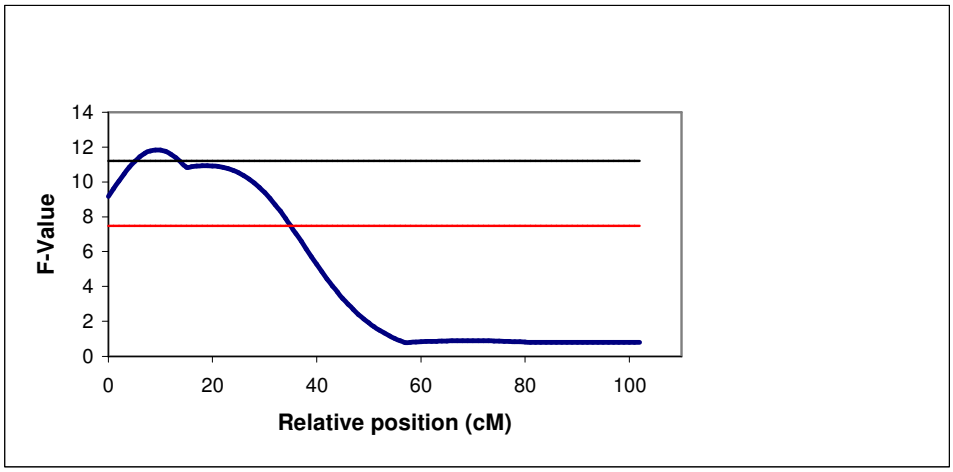

Figure 3.3 F-statistic profile from individual family analysis (sire family 361) for age at puberty on BTA12. The lower and upper horizontal lines represent 5% and 1% chromosome-wise significant levels of linkage, respectively.

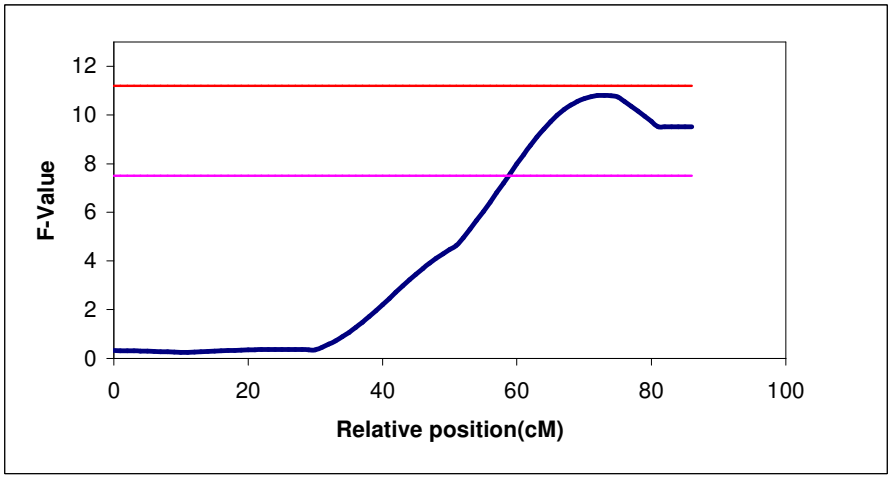

Figure 3.4 F-statistic profile from individual family analysis (sire family 398) for age at puberty on BTA16. The lower and upper horizontal lines represent 5% and 1% chromosome-wise significant levels of linkage, respectively.

3.3.2 Carcass and beef quality traits

Across family analysis revealed no evidence of linkage for carcass and meat traits on BTA1. However, two QTL affecting fatness traits were identified on BTA1 by using individual family analysis. The significant QTL (chromosome-wise significance of P<0.01) segregating in family 361 was positioned in the centromeric end of BTA1 (Figure 3.5), close to the microsatellite marker BMS2321, for back fat thickness (P8) and had allele substitution effect of 2.89 mm.

A putative QTL (chromosome-wise significance of P<0.01) segregating in family 398 was located at 91 cM on BTA1 for channel fat (Figure 3.6). The additive allele substitution effect of this QTL was 2.32 kg.

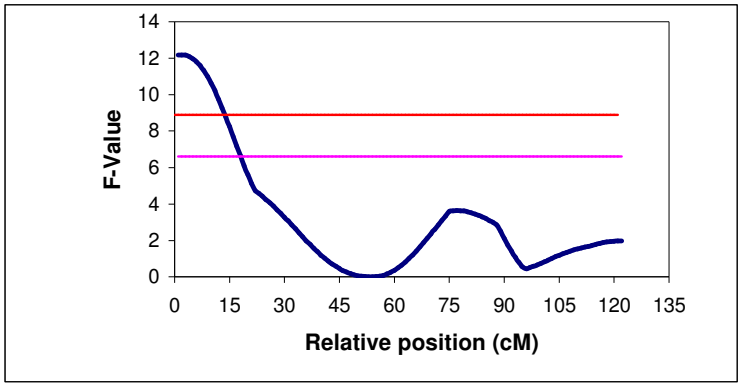

Figure 3.5 F-ratio profile from individual family analysis (sire family 361) for P8 on BTA1. The lower and upper horizontal lines represent 5% and 1% chromosome-wise significant levels of linkage, respectively

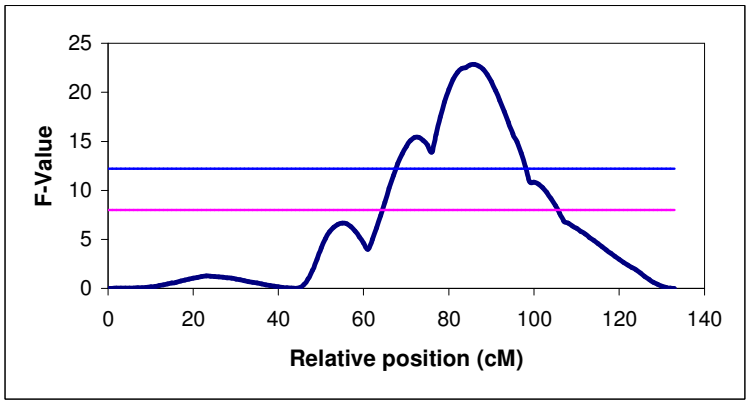

Figure 3.6 F-ratio profile from individual family analysis (sire family 398) for channel fat on BTA1. The lower and upper horizontal lines represent 5% and 1% chromosome-wise significant levels of linkage, respectively

There was highly significant evidence for the presence of QTL on BTA2 for carcass muscularity related traits (butt shape, eye muscle area, meat yield and proportion of meat to bone), meat tenderness measured as Warner-Bratzler shear force on *M. semitendinosus* muscle (peak force) and carcass fat content (Figure 3.7). The detected QTL is segregating in more than two families (Table 3.1). The maximum F-statistic obtained for all the traits, with the exception of butt shape, ranged from 10 to 18 cM from beginning of the linkage group, close to the microsatellite marker ILSTS26.

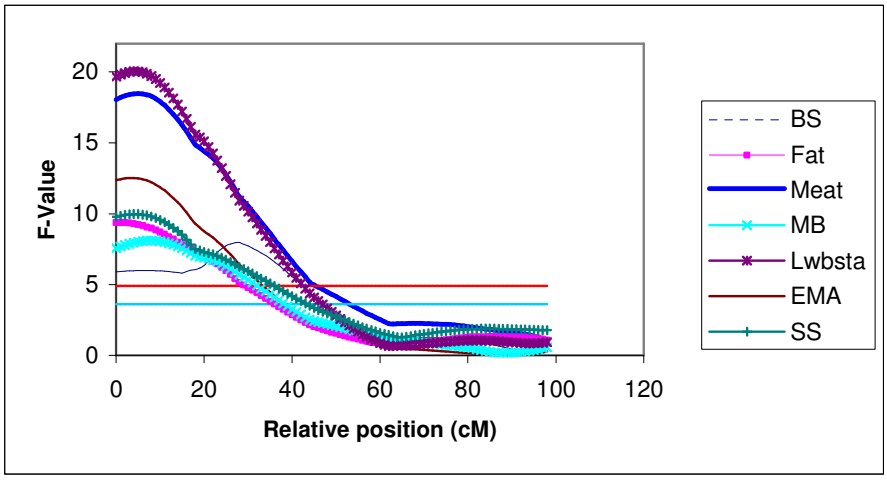

Figure 3.7 F-Ratio profile from across family analysis for butt shape (BS), carcass fat percent (Fat), meat percent (Meat), meat to bone ratio (MB), Warner-Bratzler shear force on *M. semitendinosus* muscle (peak force) (Lwbst), eye muscle area (EMA) and silverside weight (SS) on BTA2. The lower and upper horizontal lines represent 5% and 1% chromosome-wise significant evidence of linkage, respectively.

Table 3.2 Most likely position, F-statistic values, approximate LOD scores and allelic effects of detected QTL for carcass and meat quality traits (Across family analysis)[a]

BTA	Position (cM) [b]	Trait	Family	QTL ffect	S.E.	F-Value	LOD[c]
2	38	Butt shape, score	361	0.01	0.09	**8.0**	2.9
			368	0.26	0.10		
			398	0.39	0.09		
2	14	Eye muscle area, cm^2	361	8.14	2.39	**12.5**	5
			368	10.76	2.31		
			398	5.82	9.92		
2	15	Meat percent[#]	361	1.89	0.45	**18.5**	7.8
			368	2.23	0.44		
			398	1.80	0.54		
2	14	Peak shear force on ST[d], kg	361	0.10	0.02	**19.6**	11.8
			368	0.12	0.02		
			398	0.09	0.03		
2	18	Meat to bone ratio[#]	361	0.21	0.21	**8.1**	11.1
			368	0.29	0.29		
			398	0.19	0.19		
2		Fat percent[#]	361	1.47	0.45	**9.4**	5.86
			368	1.37	0.44		
			398	1.72	0.64		
2	14	Silverside weight, kg	361	0.92	0.26	**10.0**	6.2
			368	1.01	0.25		
			398	0.38	0.32		
2	16	Weight of ST, kg	361	0.40	0.12	**11.1**	6.9
			368	0.54	0.12		
			398	0.23	0.14		
3	75	Hot carcass weight, kg	361	1.40	6.97	4.6	2.9
			368	19.98	7.09		
			398	18.35	7.67		
3	16	Heart to carcass ratio, %	361	0.0200	0.0100	4.1	2.7
			368	0.0400	0.0100		
			398	0.0000	0.0100		
3	75	Aging rate on ST, kg	361	0.0001	0.0010	**5.0**	3.2
			368	0.0030	0.0009		
			398	0.0013	0.0010		
3	95	Meat percent	361	1.24	0.51	3.8	2.4
			368	0.59	0.43		
			398	0.89	0.47		

[a] Detected QTL at least at 5% chromosome-wise significant are reported. [b] Position (cM) based on the map of Ihara et al. (2004). [c] logarithm of odds. [#] predicted percentage of meat and yield. [d]ST: *M. semitendinosus muscle*. The highlighted F-values show 1% chromosome-wise significant.

Table 3.2 continued

BTA	Position (cM) [a]	Trait	Family	QTL Effect	S.E.	F-Value	LOD[b]
3	16	Fat depth at ribs 12th and 13th, mm	361	0.56	0.60	4.4	2.8
			368	0.55	0.62		
			398	2.32	0.68		
4	48	Peak shear force on LD[c], kg	361	0.1105	0.0370	4.0	2.6
			368	0.0635	0.0367		
			398	0.0049	0.0384		
4	52	Aging rate of LD, kg	361	0.0037	0.0011	4.6	2.9
			368	0.0018	0.0011		
			398	0.0005	0.0012		
4	50	Peak shear force on ST[d], kg	361	0.0415	0.0227	4.2	2.7
			368	0.0614	0.0219		
			398	0.0266	0.0234		
5	39	Hot carcass weight, kg	361	3.54	7.22	4.1	2.6
			368	30.95	10.41		
			398	14.80	8.37		
5	34	Peak shear force on ST, kg	361	0.0761	0.0217	4.2	2.7
			368	0.0025	0.0325		
			398	0.0168	0.0250		
5	90	pH of *semitendinosus* muscle	361	0.0310	0.0158	**4.8**	3.1
			368	0.0508	0.0159		
			398	0.0069	0.0175		
6	120	Heart to carcass weight ratio, %	361	0.01	0.01	4.4	2.8
			368	0.02	0.01		
			398	0.09	0.03		
8	54	Eye muscle area, cm^2	361	8.66	2.83	4.9	3.2
			368	0.70	2.14		
			398	5.64	2.41		
9	104	Intramuscular fat content, %	361	0.80	0.27	4.4	2.8
			368	0.55	0.26		
			398	0.20	0.30		
9	61	Marbling, score	361	0.16	0.13	**7.3**	4.6
			368	0.24	0.12		
			398	0.51	0.13		
10	37	Melting point, ^oC	361	0.11	0.55	**5.3**	3.4
			368	1.61	0.51		
			398	1.46	0.60		

[a] Position (cM) based on the map of Ihara et al. (2004). [b] logarithm of odds. [c]LD: *M. longissimus dorsi* muscle. [d]ST: *M. semitendinosus* muscle. The highlighted F-values show 1% chromosome-wise significant.

Table 3.2 continued

BTA	Position (cM) [a]	Trait	Family	QTL effect	S.E.	F-Value	LOD[b]
12	98	Eye muscle area, cm^2	361	8.61	3.54	3.8	2.4
			368	0.30	2.13		
			398	5.51	2.41		
12	61	Intramuscular fat content, %	361	0.04	0.29	4.4	2.79
			368	0.10	0.25		
			398	0.98	0.27		
12	25	Meat percent	361	1.65	0.53	3.8	2.4
			368	0.13	0.45		
			398	0.73	0.58		
12	31	Meat to bone ratio	361	0.34	0.01	4.2	2.7
			368	0.01	0.08		
			398	0.03	0.09		
14	22	Carcass length, cm	361	30.12	9.91	**7.0**	4.4
			368	23.61	10.43		
			398	26.27	10.05		
14	16	Cooking loss of LD[c], %	361	0.55	0.33	**8.9**	5.6
			368	1.69	0.35		
			398	0.11	0.35		
14	41	Hot carcass weight, kg	361	24.34	6.79	**7.1**	4.5
			368	16.35	6.97		
			398	12.51	7.03		
15	102	Channel fat, kg	361	0.92	0.62	**5.3**	3.4
			368	0.69	0.71		
			398	2.38	0.66		
15	23	Fat colour on biopsy, score	361	0.08	0.12	**6.1**	3.9
			368	0.21	0.13		
			398	0.51	0.13		
15	75	Liver to carcass weight ratio, %	361	0.05	0.04	**5.2**	3.3
			368	0.14	0.04		
			398	0.06	0.05		
16	2	Marbling, score	361			4.3	2.7
			368				
			398				
17	91	Eye muscle area, cm^2	361	0.13	2.28	**7.2**	4.5
			368	9.66	2.17		
			398	3.95	2.86		

[a] Position (cM) based on the map of Ihara et al. (2004). [b] logarithm of odds. [c]LD: *M. longissimus dorsi* muscle. The highlighted F-values show 1% chromosome-wise significant.

Table 3.2 continued

BTA	Position (cM) [a]	Trait	Family	QTL effect	S.E.	F-Value	LOD[b]
17	95	Hot carcass weight, kg	361	3.21	0.44	4.3	2.7
			368	19.18	2.74		
			398	19.86	2.26		
17	97	Heart to carcass weight ratio, %	361	0.01	0.01	**4.6**	2.9
			368	0.04	0.01		
			398	0.03	0.02		
17	56	Kidney to carcass weight ratio, %	361	0.01	0.01	**5.2**	3.3
			368	0.03	0.01		
			398	0.02	0.01		
17	88	Liver to carcass weight ratio, %	361	0.02	0.04	4.0	2.6
			368	0.14	0.04		
			398	0.02	0.06		
17	40	Meat percent	361	1.40	0.42	**6.2**	3.9
			368	1.13	0.43		
			398	0.14	0.45		
17	90	Meat to bone ratio	361	0.07	0.07	**5.1**	3.2
			368	0.26	0.07		
			398	0.06	0.09		
17	97	Fat depth on the rump (P8), mm	361	0.72	0.90	4.1	2.6
			368	2.82	0.87		
			398	1.20	1.08		
17	87	Fat depth at 400 days, mm	361	0.46	0.21	4.4	2.8
			368	0.49	0.20		
			398	0.22	0.27		
18	41	Peak shear force on LD[c], kg	361	0.17	0.04	**7.4**	4.7
			368	0.04	0.04		
			398	0.02	0.04		
18	44	Meat colour, score	361	0.41	0.14	3.9	2.5
			368	0.02	0.13		
			398	0.23	0.13		
24	8	Butt shape, score	361	0.03	0.09	3.8	2.4
			368	0.15	0.08		
			398	0.26	0.09		
24	55	Intramuscular fat content, %	361	0.14	0.36	4.0	2.5
			368	0.85	0.26		
			398	0.39	0.38		

Detected QTL at least at 5% chromosome-wise significant are reported. [a] Position (cM) based on the map of Ihara et al. (2004). [b] logarithm of odds. [c]LD: *M. longissimus dorsi* muscle. The highlighted F-values show 1% chromosome-wise significant.

Table 3.2 continued

BTA	Position (cM) [a]	Trait	Family	QTL effect	S.E.	F-Value	LOD[b]
24	8	pH of *semitendinosus* muscle	361	0.03	0.02	**4.8**	3
			368	0.05	0.02		
			398	0.00	0.02		
25	46	Carcass length, cm	361	5.72	13.85	4.2	2.7
			368	5.87	8.60		
			398	36.00	10.39		
25	29	Fat depth on the rump (P8), mm	361	2.96	1.75	3.7	2.3
			368	3.44	1.32		
			398	2.00	1.86		
26	13	Fatty acids elongation index, %	361	1.41	0.73	**5.1**	3.3
			368	1.45	0.71		
			398	2.21	0.80		
26	40	Meat colour, score	361	0.53	0.17	4.0	2.5
			368	0.01	0.15		
			398	0.28	0.20		
28	57	Fat depth at ribs 12^{th} and 13^{th}, mm	361	1.83	0.68	3.9	2.5
			368	0.31	0.72		
			398	1.52	0.72		
29	60	Kidney to carcass weight ratio, %	361	0.01	0.01	**5.1**	3.3
			368	0.03	0.01		
			398	0.01	0.01		
29	55	Peak shear force on LD[c], kg	361	0.16	0.05	**7.7**	4.9
			368	0.11	0.03		
			398	0.06	0.04		
29	59	Total monounsaturated fatty acids, %	361	0.05	0.98	3.9	2.5
			368	1.64	0.64		
			398	1.55	0.69		
29	59	Total saturated fatty acids, %	361	0.04	1.00	4.1	2.6
			368	1.73	0.65		
			398	1.61	0.70		

[a] Position (cM) based on the map of Ihara et al. (2004). [b] logarithm of odds. [c]LD: *M. longissimus dorsi* muscle. The highlighted F-values show 1% chromosome-wise significant.

BTA3 exhibited evidence for the presence of a significant QTL (chromosome-wise significance of P<0.01) affecting beef tenderness (aging rate) measured as Warner-Bratzler shear force on *M. semitendinosus* muscle (Table 3.2).

A significant (chromosome-wise significance of P<0.05) QTL with effects on Warner-Bratzler shear force measures of both *M. longissimus dorsi* and *M. semitendinosus*

muscles was identified in the middle of BTA4, close to microsatellite markers MAF50 and RM067.

BTA5 harbours loci affecting hot carcass weight, meat pH and tenderness (Table 3.2). The tenderness QTL was located at 34 cM on this chromosome, close to the hot carcass weight QTL situated at 39 cM.

Across family analysis showed only one QTL on BTA6 for the traits studied. This QTL located at the telomeric end of the chromosome, affecting heart percent (weight defined as percentage of hot carcass weight) and segregating in family 398. Additional putative QTL influencing internal organ weights were detected on BTA15 (QTL for liver percent, situated at 75 cM), BTA17 (QTL for kidney and heart percent, and liver percent, mapped at 56, 88 and 97 cM, respectively) and BTA29 (QTL for kidney percent located at 60 cM) (Table 3.2).

Individual family analysis showed significant QTL (chromosome-wise significance of $P<0.05$) for fat colour (scored on the biopsy samples at weaning) on BTA6 segregating in families 361 and 368 with the allelic effect of 0.25 and 0.26 unit, respectively. However, across family analysis showed no significant QTL for fat colour on this chromosome but significant QTL (chromosome-wise significance of $P<0.05$) for this trait on BTA15.

Based on the individual family analysis, BTA7 seems to contain two QTL linked to rib fat depth (Rbft), one at the telometric end of the chromosome segregating in family 368 with the additive effect of 1.94 mm and the other on the centromeric end of the chromosome, close to microsatellite marker BM9065, with a substitution effect of 2.29 mm.

A significant QTL (chromosome-wise significance of $P<0.01$) for eye muscle area was apparent on BTA8, close to microsatellite markers TGA13 and BMS1341. The detected QTL was segregating in families 361 and 398 with the additive effects of 8.66 and 5.64 cm^2, respectively (Table 3.2). Additionally, individual-family analysis uncovered a significant QTL (chromosome-wise significance of $P<0.01$) for fat colour on BTA8 situated at 49 cM from the beginning of the linkage group (Figure 3.8), close to the BTA8 QTL for eye muscle area.

Linkage analysis indicated significant QTL for marbling (chromosome-wise significance of $P<0.01$) and intramuscular fat content (chromosome-wise significance of $P<0.05$) on BTA9 (Figure 3.9).

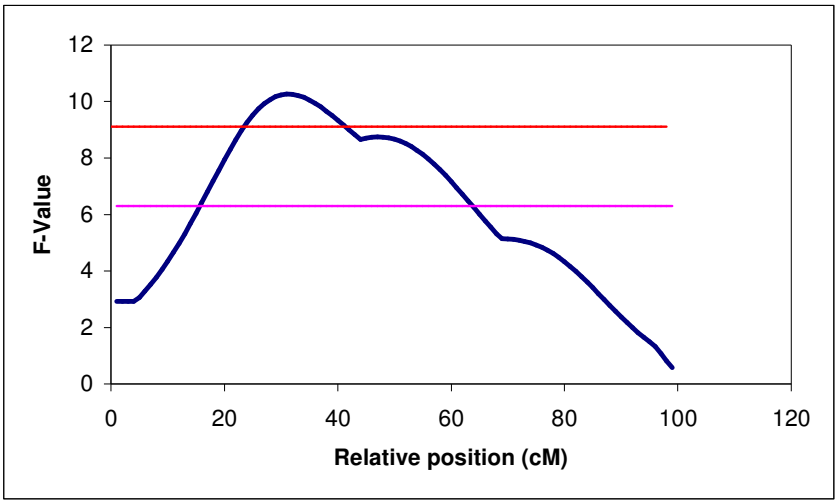

Figure 3.8 F-Ratio profile from individual family analysis (sire family 361) for fat colour on BTA8. The lower and upper horizontal lines represent 5% and 1% chromosome-wise significant evidence of linkage, respectively.

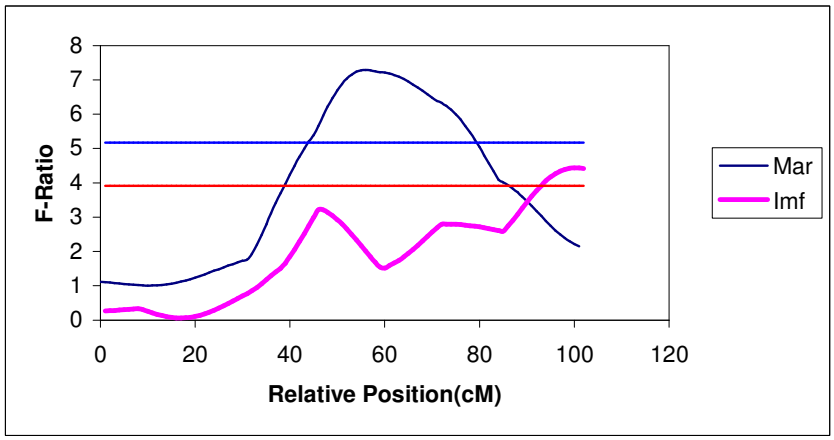

Figure 3.9 F-Ratio profile from across family analysis for marbling (Mar) and intramuscular fat content (Imf) on BTA9. The lower and upper horizontal lines represent 5% and 1% chromosome-wise significant evidence of linkage, respectively.

There is evidence suggesting significant QTL (chromosome-wise significance of $P<0.01$) for fat melting point on BTA10, between microsatellite markers, BMS528 and BMS861, situated from approximately 12 to 30 cM (Figure 3.10). The detected QTL is segregating in family 368 and 398 with the allelic effects of 1.61 and 1.47 unit, respectively.

Individual family analysis of the data for BTA10 indicated a QTL influencing meat colour (chromosome-wise significance of P<0.05) segregating in family 361 with an allelic effect of 0.36 units.

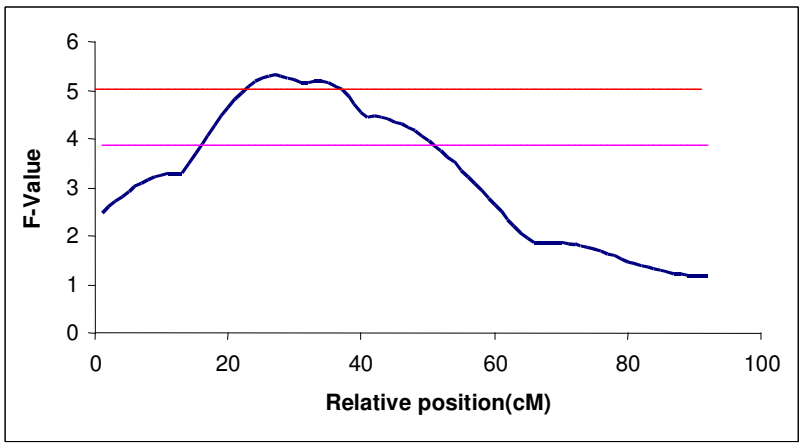

Figure 3.10 F-Ratio profile from across family analysis for melting point of fat tissue on BTA10. The lower and upper horizontal lines represent 5% and 1% chromosome-wise significant evidence of linkage, respectively.

A DNA region with effects on intramuscular fat and eye muscle area was identified on BTA12. The maximum F-values for intramuscular fat and eye muscle area were positioned at 61 and 98 cM, respectively. Additional putative QTL linked to carcass traits were detected on BTA12, affecting meat as a percentage of carcass weight and the ratio of meat to bone (Table 3.2).

Individual family analysis revealed significant QTL (chromosome-wise significance of P<0.01) affecting fat colour on both BTA11 (Figure 3.11) and BTA12 (Figure 3.12).

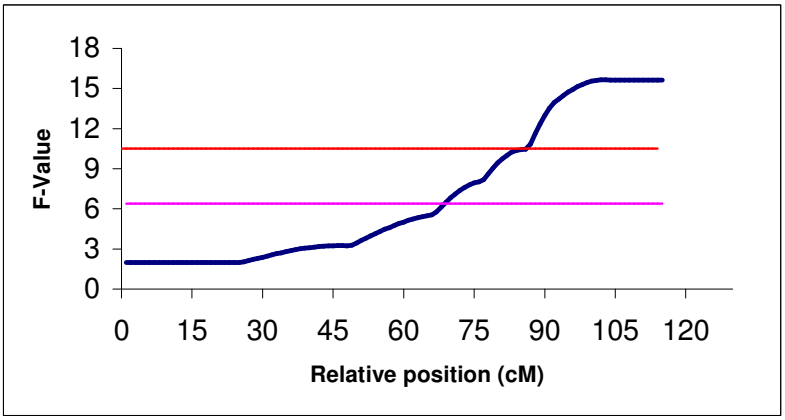

Figure 3.11 F-Ratio profile from individual family analysis (sire family 368) for fat colour (scored on biopsy samples) on BTA11. The lower and upper horizontal lines represent 5% and 1% chromosome-wise significant evidence of linkage, respectively.

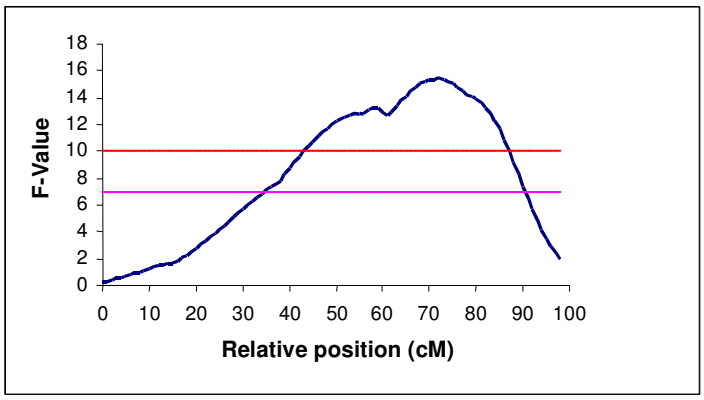

Figure 3.12 F-Ratio profile from individual family analysis (sire family 368) for fat colour on BTA12. The lower and upper horizontal lines represent 5% and 1% chromosome-wise significant evidence of linkage, respectively.

Evidence suggesting the existence of QTL associated with fatty acid elongation (chromosome-wise significance of P<0.01) and meat tenderness (aging rate, chromosome-wise significance of P<0.05) was identified on BTA13 (Figure 3.13). This chromosome also harbours putative QTL for ultimate meat pH (Figure 3.14).

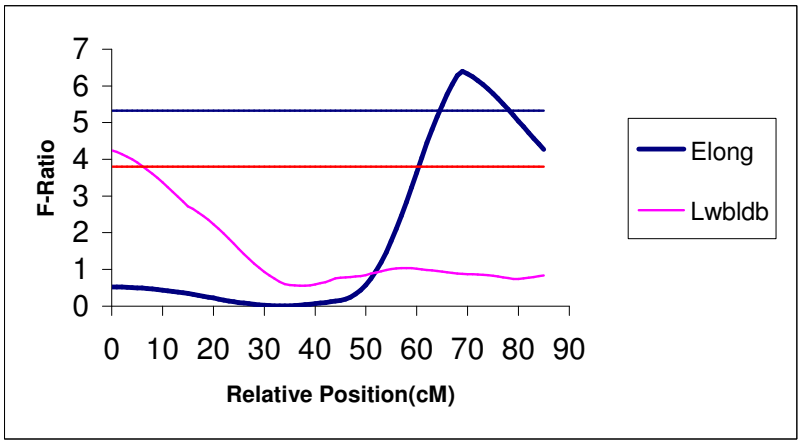

Figure 3.13 F-Ratio profile from across family analysis for aging rate measured on the *M. longissimus dorsi* muscle (Lwbldb) and fatty acids elongation index (Elong) on BTA13. The lower and upper horizontal lines represent 5% and 1% chromosome-wise significant evidence of linkage, respectively.

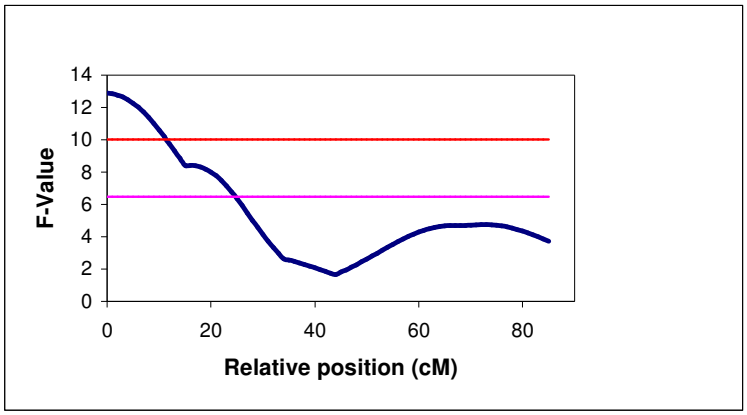

Figure 3.14 F-Ratio profile from individual family analysis (sire family 398) for meat pH on BTA13. The lower and upper horizontal lines represent 5% and 1% chromosome-wise significant evidence of linkage, respectively.

A DNA region near the centromeric end of BTA14 had significant effects (chromosome-wise significance of $P<0.01$) on hot carcass weight, carcass length and cooking loss. BTA14 QTL for carcass length, hot carcass weight and cooking loss were segregating in all families, two families (361 and 368) and one family (368), respectively. Additionally, individual family analysis showed that this chromosome harbours QTL for fat colour, fatty acid elongation and desaturation indices, segregating in families 361, 398 and 368, respectively. The position of all the detected QTL using individual family and across family analyses encompassed 14 to 41 cM (Table 3.2).

A test statistic peak at the telomeric end of BTA15 close to BMS429 indicated a significant QTL (chromosome-wise significance of $P<0.01$) affecting channel fat. The detected QTL is segregating in families 398 with the additive effect of 2.4 unit.

There is evidence suggesting significant QTL (chromosome-wise significance of $P<0.05$) on BTA16 associated with marbling, meat pH and eye muscle area. The maximum F-statistic for the marbling QTL was near the centromeric end of the chromosome, while the pH and eye muscle area QTL were located at 85 cM from the beginning of the linkage group.

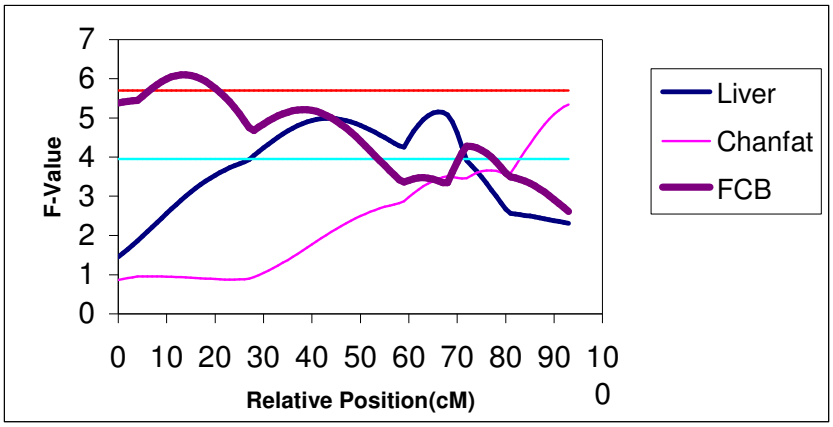

Figure 3.15 F-Ratio profile from across family analysis for liver weight (Liver), fat colour scored on biopsy sample (FCB) and channel fat (Chanfat) on BTA15. The lower and upper horizontal lines represent 5% and 1% chromosome-wise significant evidence of linkage, respectively.

The linkage analysis showed that BTA17 harbours QTL for internal organ weights (heart, liver and kidney weights as a percentage of carcass weight), hot carcass weight, eye muscle area, estimated meat yield, estimated bone yield, meat to bone yield ratio, and backfat thickness (Table 3.2). The positions of the most of the detected QTL on this chromosome were between 87 to 97 cM from the beginning of the linkage group (Figure 3.16), suggesting a gene (or a complex of genes) resides in this region that are responsible for variation in carcass traits.

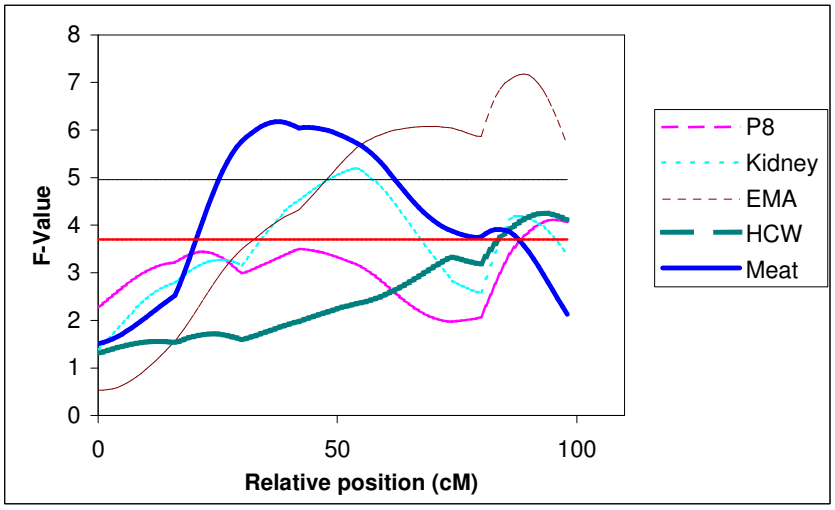

Figure 3.16 F-Ratio profile from across family analysis for backfat thickness (P8), kidney weight (Kidney), eye muscle area (EMA), hot carcass weight (HCW) and estimated meat yield (Meat) on BTA17. The lower and upper horizontal lines represent 5% and 1% chromosome-wise significant evidence of linkage, respectively.

BTA18 harbours significant QTL for meat tenderness (peak force measured on the *M. longissimus dorsi* muscle (Lwblda)) and meat colour (chromosome-wise significance of P<0.01 and P<0.05, respectively). The maximum test statistic peaks for these two traits were at 38 and 44 cM from the beginning of the linkage group, close to microsatellite markers BM8151 and INRA63 (Figure 3.17). The detected QTL is segregating in family 361 with the allelic effects of 0.17 and 0.41 units for Lwblda and meat colour, respectively.

Although across family analysis showed no evidence of association between molecular markers on BTA18 and fatness traits, individual family analysis revealed significant QTL (chromosome-wise significance of P<0.05) linked to channel fat in the middle of the chromosome, segregating in family 361.

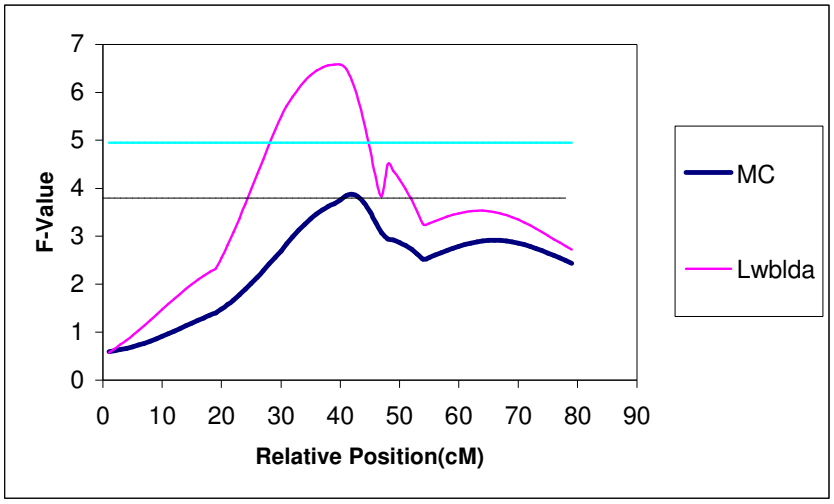

Figure 3.17 F-Ratio profile from across family analysis for meat colour (MC) and tenderness (peak force measured on the *M. longissimus dorsi* muscle, Lwblda) on BTA18. The lower and upper horizontal lines represent 5% and 1% chromosome-wise significant evidence of linkage, respectively.

Across family analysis showed no QTL for carcass and meat traits on BTA19, 20, 21, 22 and 23. However, individual-family analysis identified QTL for meat colour (chromosome-wise significance of P<0.05), fat colour and fatty acid elongation index (chromosome-wise significance of P<0.01) on BTA19, 22 and 23, respectively. The meat colour QTL on BTA19 segregating in family 361, mapped at 23 cM from the beginning of the linkage group, between microsatellite markers HEL10 and BMS2142.

A significant (chromosome-wise significance of P<0.05) F-statistic peak at the centromeric end of BTA24 indicated a putative QTL influencing butt shape, pH and meat aging rate (Lwbstb) (Table 3.2). In addition, individual-family analysis revealed a QTL (chromosome-wise significance of P<0.05) affecting total mono-unsaturated fatty acids in this part of the chromosome. Furthermore, this chromosome was found to harbour QTL (chromosome-wise significance of P<0.05) for cooking loss and intramuscular fat.

A significant QTL (chromosome-wise significance of P<0.05) was detected at the middle of chromosome 25 with an effect on fat thickness (P8). The detected QTL is segregating in family 368 with the allelic effect of 3.4 mm (Table 3.2). Additionally, individual-family analysis showed that this chromosome harbours a significant QTL (chromosome-wise significance of P<0.01) segregating in family 398 for estimated fat yield. The detected QTL was located at the centromeric end of the chromosome, close to microsatellite marker BM4005, with the additive effect of 1.4 %.

A significant QTL (chromosome-wise significance of P<0.01) affecting fatty acid elongation index mapped to BTA26. The allelic substitution effect for this QTL varied between 2.2 and 1.4 units across 3 families. The maximum F-statistic for this QTL was at 13 cM between microsattelite markers BMS651 and BM1314 (Table 3.2). Individual-family analysis revealed a marbling QTL in this part of the chromosome. The marbling QTL is segregating in family 368 with an allelic substitution effect of 0.4 unit. Additionally, this chromosome harbours a QTL (chromosome-wise significance of P<0.05) for meat colour.

A DNA region located at the telomeric end of BTA28 was found to have an effect (chromosome-wise significance of P<0.05) on rib fat thickness (Rbft). The detected QTL is segregating in families 361 and 398 with substitution effects of 1.8 and 1.5mm, respectively (Table 3.2).

Linkage analysis showed that BTA29 harbours QTL linked to four traits, Kidney weight (chromosome-wise significance of P<0.01), total mono-unsaturated fatty acids, summation of saturated fatty acids (chromosome-wise significance of P<0.05) and meat tenderness (peak force, Lwblda) (chromosome-wise significance of P<0.01). The detected QTL for these traits were located at 56 to 60 cM from the beginning of the linkage group, close to marker BMC1206 (Table 3.2, Figure 3.18).

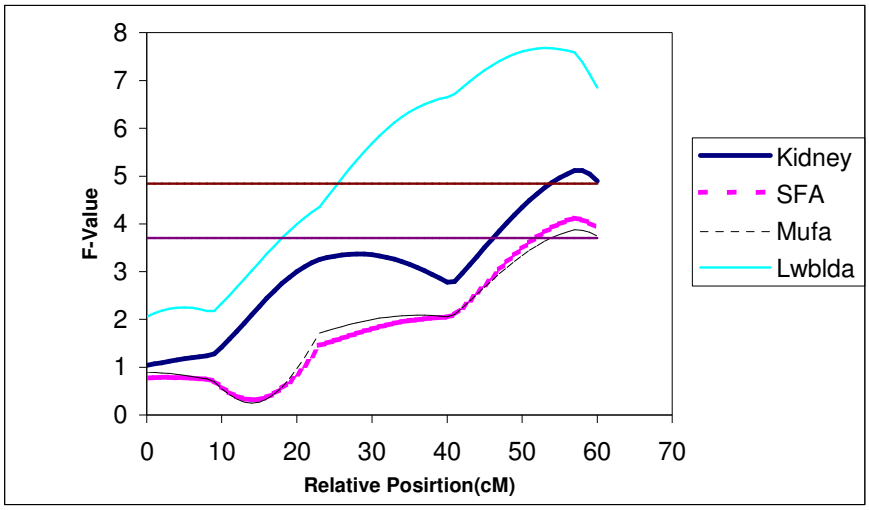

Figure 3.18 F-Ratio profile from across family analysis for kidney weight (Kidney), summation of saturated fatty acids (SFA), total mono-unsaturated fatty acids (Mufa) and meat tenderness (peak force measured on the *M. longissimus dorsi* muscle, Lwblda) on BTA29. The lower and upper horizontal lines represent 5% and 1% chromosome-wise significant evidence of linkage, respectively.

3.3 Discussion

Data from the Australian Limousin × Jersey double backcross population was analysed resulting in convincing evidence for QTL affecting economically importance traits in beef cattle production.

3.3.1 Reproductive traits

The interval mapping results provided significant evidence for QTL affecting birth weight on BTA1 and BTA14. Previous reports indicated the presence of growth QTL on bovine chromosome 1 (Kim *et al*., 2003; Stone *et al*., 1999). Based on data from a half-sib family of a Brahman × Hereford sire, Stone *et al*. (1999) reported a putative QTL for birth weight at 120 cM on BTA1. In addition, the completed scan in all available progeny of this sire confirmed the presence of a QTL for birth weight on BTA1 (Casas *et al*., 2003). However, the support interval for the birth weight QTL in the present study does not include the positions reported by Stone *et al*. (1999) and Casas *et al*. (2003), suggesting more than one QTL for birth weight are segregating on this chromosome.

A putative QTL on BTA5, between microsatellites markers AGLA293 and OARFCB05, was found to affect birth weight. The effects of BTA5 on growth and carcass traits have been reported. A QTL for birth weight was detected in the 70 to 110 cM interval on this linkage group by Davis *et al*. (1998) in a crossbred population. Birth weight QTL

on this chromosome has been reported in families segregating alternative forms of the myostatin gene (Casas *et al.*, 2003). Li *et al.* (2002) also detected QTL on BTA5 for birth weight at 20 to 30 cM and 65 to 75 cM intervals in.

There was evidence supporting the presence of significant QTL on BTA3, 14 and 21 affecting birth weight. Davis *et al.* (1998) reported the presence of a QTL at 0 cM to 42 cM on BTA14 for birth weight, in a similar region found herein affecting birth weight. The evidence of birth weight QTL on BTA3 and BTA21 herein supports a number of published papers (Casas *et al.*, 2004b; Casas *et al.*, 2003; Davis *et al.*, 1998; Kim *et al.*, 2003). The most obvious candidate genes for BTA21QTL is insulin like growth factor 1 receptor.

Davis *et al.* (1998) and Casas *et al.* (2003) detected QTL for birth weight at the centromeric region of BTA21, where the QTL for birth weight was detected in the present study. However, Kim *et al.* (2003) and Casas *et al.* (2004b) reported QTL for birth weight in the telomeric region of BTA21. This inconsistency across studies can partly be explained by the different breeds and designs used in the studies. Additionally, the density and informativity of the markers used in different studies and the large error within each study could lead to QTL not being assigned to the correct genomic positions.

Calf birth weight has been used as an indicator trait to avoid calving difficulties (Eriksson *et al.*, 2004). Thus, identifying loci such as those detected herein (QTL for birth weight on BTA1, 3 and 21), which affect birth weight but not subsequent growth, coupled with marker-assisted selection has potential to overcome these problems. However, the possible effects of this QTL on carcass and meat quality need to be investigated.

Gestation length has been proposed as a breeding objective to reduce birth weight without affecting pre-weaning growth traits (Bourdon and Brinks, 1982; Brinks *et al.*, 1991). The current study revealed suggestive QTL for gestation length on BTA10 and 23. However, no evidence was found for the presence of QTL for birth weight on these chromosomes.

For pelvic area, a significant influence of BTA22 at the centromeric region of the chromosome and suggestive effects of BTA3, 6, 17, and 20 were discovered. There is some evidence supporting QTL affecting calving difficulties traits on BTA3 (Kuhn *et al.*, 2003; Schrooten *et al.*, 2000). Schrooten *et al.* (2000) mapped a QTL for calving ease on BTA3, which was linked to birth weight and pelvic area in the study herein. However, birth weight QTL and pelvic area QTL were mapped in different locations on the chromosome.

Indication of QTL for pelvic width and pelvic area at 35 cM on BTA6 is in agreement with the results from Schrooten *et al*. (2000) and Kuhn *et al*. (2003), who found a QTL in the proximal region of this chromosome for calving ease and stillbirth (direct effects), respectively. Additionally, Casas *et al*. (2000) detected a QTL for birth weight in this region of the chromosome. The putative QTL for pelvic area on BTA6 is further supported by results from Schrooten *et al*. (2000), who mapped a QTL for size and dairy character in the proximal region of BTA6; traits which may influence delivery of a calf Kuhn *et al*. (2003).

On BTA18, where a suggestive QTL for pelvic height was found herein, Kuhn *et al*. (2003) reported a QTL affecting dystocia and stillbirth. Additionally, Ashwell *et al*. (1998) found a QTL on BTA18 for strength and thurl width, which may affect calving ease of a cow (Bellows *et al*., 1971).

BTA12 and BTA16 were linked to the age at puberty. BTA16 harbours live weight and growth QTL in the population studied herein (data have not been presented). The onset of puberty is determined by body weight as heifers start to cycle at approximately 43% of mature body weight (Coffey *et al*., 2006). Therefore, genes that influence growth and live weight, as well as reproductive pathways might be anticipated to play a role in controlling the onset of puberty. Heritability estimates of 0.27 and 0.44 for age and live weight at puberty in beef cattle have been reported previously (Morris *et al*., 2000). McNaughton *et al*. (2005) also reported QTL on BTA16 for puberty defined as proportion of estimated mature live weight at puberty in crossbred Friesian x Jersey animals.

3.3.2 Carcass and beef quality

Several linkage groups were linked to the carcass and meat quality traits. The most important linkage group was BTA2 where a QTL was detected for muscle measurements, meat tenderness and carcass fatness. More compelling evidence for the effects being real comes from the detection of QTL in a similar location in more than one sire family. The position of the maximum F-statistic obtained for all the carcass and meat traits, with the exception of butt shape, which is a subjective trait, ranged from 10 to 18 cM from the beginning of the linkage group, close to microsatellite marker ILSTS26. The *mh* locus (known as myostatin) is a gene that causes muscle hypertrophy in mice (McPherron *et al*., 1997) and has been mapped by Smith *et al*. (1997) to the region where the QTL for carcass and fatness traits herein is located. Thus, the association between this candidate gene and the carcass traits need to be investigated.

Effects of QTL detected (on BTA2) are expected to be in opposite directions for muscling and fat percent. This is in consistent with the observations that double-muscle animals have carcasses with a low percentage of fat. Therefore, mounting evidence suggests that this region of the bovine genome contains a gene or complex of genes with significant effects on carcass composition. However, the linkage analysis showed there are other regions of the bovine genome with effects on carcass and meat traits. Putative QTL for muscling, measured as eye muscle area were found on BTA8, BTA12, BTA16 and BTA17, implying that the QTL on BTA2 may interact with a multitude of loci that influence carcass traits. Thus, further analysis of the data is required to ascertain the relationship between the BTA2 QTL and other regions of the genome. This would be useful for a better understanding the genetic control of the carcass traits.

The amount and distribution of fat has an important impact on carcass and meat quality in beef cattle (Wheeler *et al*., 1994). Employing the individual and across family analyses, the present study revealed a number of putative QTL for fat related traits. BTA1 seems to harbour two QTL for fatness traits, one at the centromeric end of the chromosome, close to microsatellite marker BMS2321, affecting backfat thickness (P8) and the other at 91 cM affecting channel fat. Using a line-cross model, Kim *et al* (2003) mapped a QTL on BTA1, close to microsattelite marker AGLA17, which is responsible for external fat deposition measured as subcutaneous fat thickness between the 12^{th} and 13^{th} ribs. BMS2321 which is close to the detected QTL for external fat in the present study and AGLA17 which is close to the reported QTL for external fat by Kim *et al* (2003) map to 15 and 0 cM on the bovine map (Ihara *et al*., 2004), respectively. Additional putative QTL for external fat were located on BTA3, BTA7 and BTA28 (QTL for rib fat thickness, Rbft) and BTA17 and BTA25 (QTL for backfat thickness, P8).

On BTA17 where QTL for backfat thickness (P8) was detected, MacNeil and Grosz (2002) reported a QTL for internal fat (estimated kidney, pelvic, and heart fat). Additionally, Casas *et al*. (2004b) found a QTL on BTA17 for fat yield. This chromosome harbours QTL for internal organ weights (Heart, Liver and Kidney weights as a percentage of carcass weight), hot carcass weight, eye muscle era, and other carcass traits, indicating the pleiotropic effects of the underlying genes or a hint on multiple genes having different effects in the linkage group. Thus, the present analysis could be extended to handle multiple traits using multitrait QTL analysis. This could provide additional power for chromosomes such as BTA17 where QTL for several traits have been found and also could provide a test of whether there is pleiotropy or close linkage.

BTA17 harbours uncoupling protein 1 (UCP1) which plays a role in heat production by uncoupling oxidative phosphorylation from the respiratory chain. However, there are many genes on this chromosome and further studies are required to test specific hypotheses regarding candidate genes.

Casas *et al.* (2003) reported a QTL for internal fat (estimated kidney, pelvic and heart fat), on BTA15 where a QTL for channel fat was found in the present study. However, the detected QTL in the present study was located at the telomeric end of BTA15 close to BMS429, which does not include the location reported by Casas *et al.* (2003).

The present study, found QTL both intramuscular fat (IMF) and also marbling score. Two QTL were detected for marbling score. One of these is on BTA9 and the other is on BTA16. The former was previously reported in a resource population obtained from a Brahman × Hereford crossbred sire (Casas *et al.*, 2003). Additional putative QTL for intramuscular fat were found on QTL BTA12 and 24. There is no previous report of QTL for this trait on these chromosomes.

A marbling QTL was found on the centromeric region of BTA16. There is previous evidence showing QTL for marbling on this chromosome. Casas *et al.* (2004b) reported a QTL affecting marbling at 44 cM on BTA16, between HUJ1614 and IDVGA68.

No QTL congruency was found for intramuscular fat and other types of fat depots, suggesting that different genes are responsible for intramuscular fat and other types of fat depots. Thus, it should be possible to manipulate marbling accumulation independently of other fat depots.

Concerning fatty acid characteristics, QTL for melting point were found on BTA10 and 13, for desaturation index on BTA14, for elongation index on BTA14, 23 and 26, for total saturated fatty acids and total *cis*-monounsaturated fatty acids on BTA29. Pitchford *et al*. (2002a) reported that melting point of fatty acids is genetically correlated with their calculated desaturation index. However, the present research did not find any DNA regions linked to both melting point and desaturation index.

Apart from its effect on desaturation index and total saturated fatty acids, a QTL on BTA14 was found to affect fat colour scored on a biopsy sample, cooking loss, carcass length and carcass weight. There is ample evidence for a QTL affecting fat deposition located on BTA14 in dairy cattle (Coppieters *et al*., 1998; Heyen *et al*., 1999; Riquet *et al*., 1999) and beef cattle (Casas *et al*., 2000; Moore *et al*., 2003).

In total, five QTL were detected for Warner-Bratzler shear force, a mechanical and objective measure of meat tenderness. Two of these QTL were previously reported. The study herein revealed a QTL for Warner-Bratzler peak force measured on the *M. longissimus dorsi* muscle (Lwblda), mapped to the telomeric end of BTA29. This finding confirms the results of Casas *et al*. (2003; 2000) who found a QTL for tenderness in this region of the bovine genome. Interestingly, the position of the detected QTL in the map used herein coincides with the position of the detected QTL for kidney weight as a percentage of carcass weight), total mono-unsaturated fatty acids and saturated fatty acids, suggesting the pleiotropic effects of the underlying genes or hint of multiple genes having different effects in the identified linkage group.

A DNA region in the middle of BTA4, between microsatellite markers MAF50 and RM067 affected meat tenderness. The detected QTL linked to both peak force and aging rate, influenced the tenderness of both the *M. longissimus dorsi* and *M. semitendinosus* muscles. The evidence of a tenderness QTL on BTA4 in the population herein confirms the results of Casas *et al*. (2001).

A significant QTL was detected on BTA2 affecting meat tenderness, measured as Warner-Bratzler shear force on the *M. semitendinosus* muscle (Lwbsta). The position of the detected QTL coincides with the detected QTL for the muscling traits in the centromeric region of the chromosome. Thus, it would be important to evaluate the effects of *mh* locus on beef tenderness to ascertain the genetic control of meet tenderness in populations segregating myostatin alleles.

A QTL on BTA18 affecting meat tenderness measured as Warner-Bratzler shear force on the *M. longissimus dorsi* (Lwblda) was detected. To date, six different genomic regions on chromosomes 4, 5, 9, 15, 20 and 29 have been reported to be associated with the expression of meat tenderness in cattle. This is the first report of QTL on BTA2 and BTA18 linked to meat tenderness. These results suggest that different genomic regions are involved in the expression of quantitative traits, depending on the genetic background. Alternatively, it may simply be that the QTL are not segregating the relevant alleles in the families under study.

The present study revealed putative QTL for pH on BTA5, 13, 16 and 24 and for meat colour on BTA10, 18, 19 and 26. There is no previously evidence of a QTL affecting meat pH and colour in any chromosome in other QTL experiments in cattle.

In general, individual family analysis revealed a number of QTL which were not significant in the across family analysis. Across family analyses are most powerful when a

QTL is segregating in all the sires (Walling *et al*., 2004). Given the outbred nature of the population and assuming a biallelic QTL, even in the most favourable situation with QTL allele frequencies of 0.5 on average, the effect will not be segregating in 50% of the sires. Given the limited number of sires likely to be heterozygous for the QTL, the residual variation from other nonsegregating families is such that only very large QTL will be found to be statistically significant. Despite this, for any given significance level, the across family analyses are likely to be the most powerful. This study found a greater number of QTL from the individual family analyses, but the results were not adjusted for the number of families tested.

The mapping of QTL involves substantial multiple testing. This study included all 29 bovine autosomes and attempted to correct for multiple positional testing using the chromosome wide thresholds calculated by permutation tests (Churchill and Doerge, 1994). The study did not adjust for the 3 families tested in the individual family analyses nor did it correct for the number of traits analysed. Given the number of tests, it is likely that some results are Type 1 errors (identifying a QTL when there is not one present). However, increasing the stringency of the thresholds would increase the frequency of Type 2 errors (failing to identify a QTL present in the region studied). Adjusting thresholds for the number of traits analysed seems to be too stringent, as results previously declared significant may no longer reach the new required threshold simply because of the addition of a new trait in the analyses. Such a result would be discarded despite no change in the evidence for a putative QTL. Hence, in accordance with the recommendation of Lander and Bostein (1995), suggestive results are reported with the recognition of the potential for false positive results.

3.4 Summary

The preliminary analysis of the Davies cattle gene mapping showed significant association between DNA markers and economically important traits in beef production. A strong QTL was found close to the myostatin gene affecting a number of carcass traits. However, there were also other genomic regions influencing these traits. Thus, investigation of the association between myostatin gene polymorphisms and carcass compositions and probable interactions of this gene with other chromosomal regions could be useful to understand the genetic control of the carcass and meat quality traits. Furthermore, in order to describe the genetic architecture of the traits, it is necessary to

undertake further analysis of the whole data set (the Davies cattle gene mapping and the New Zealand gene-mapping herds) using advanced methods fitting multiple QTL and multitrait QTL models.

Chapter 4: Effects of a myostatin functional SNP on beef traits

4.1 Introduction

Based on the QTL mapping results (Chapter 3), a major QTL was identified with pleiotropic effects on a number of carcass and meat traits. The position of the detected QTL coincided with the mapped position of the myostatin gene (*MSTN*) on bovine chromosome 2 (BTA2).

The transforming growth factor β superfamily (*TGF-β*) encompasses a large group of secreted growth and differentiation factors that play important roles in regulating development and tissue homeostasis (McPherron and Lee, 1997). Myostatin or growth and differentiation factor-8 (*GDF8*) is a member of the *TGF-β* gene superfamily. Mouse studies have shown the gene is expressed predominantly in skeletal muscle of adult animals as well as during early development. Myostatin was first discovered in mice (McPherron *et al*., 1997) and acts as a negative regulator of skeletal muscle mass. In cattle, mutations in *MSTN* are responsible for double muscling, a dramatic increase in skeletal muscle development (Grobet *et al*., 1998; Kambadur *et al*., 1997; McPherron and Lee, 1997).

Traditionally, it was thought that double muscling was inherited as an autosomal recessive and that +/+ and *mh*/+ conferred similar moderate phenotypes and *mh*/*mh* expressed an extreme phenotype. The locus has been termed "partially recessive" because there is some effect of a single copy of the allele, but generally the truly double-muscled phenotype requires that the animal be homozygous (Kambadur *et al*., 1997). Casas *et al*. (1998) documented the "partial recessive character" of *MSTN* in the Belgian Blue cattle, in a study where heterozygous (*mh*/+) animals had a muscle mass 1.6 standard deviations higher than homozygous normal (+/+) animals.

Many distinct mutations have been identified in the bovine myostatin gene that result in an interruption of the production or activity of myostatin (Dunner *et al*., 2003; Grobet *et al*., 1998). Hence, myostatin alleles can be grouped into two functional classes: inactive myostatin (which correspond to *mh*) and active myostatin (which correspond to +). To date, there have been reports of nine mutations in coding regions of myostatin that cause non-synonymous changes, of which three cause mis-sense mutations, including two in

exon 1 and one in exon 2. The remaining six mutations, located in exons 2 and 3, result in premature stop codons, which are the mutations responsible for the double-muscling phenotype (Bellinge *et al*., 2005). One of the mutations in the bovine myostatin gene is a cytosine to adenine transversion in exon 1 at base 413, causing an amino acid substitution of leucine for phenylalanine[94] (Grobet *et al*., 1998). The substitution occurs in a region of the protein known to be the inhibitory domain of the myostatin propeptide (Grobet *et al*., 1998).

Previous studies have examined different mutations in myostatin gene (for example, the 11-base pair deletion in the Belgian Blue and South Devon breeds studied by Casas *et al*. (2004a; 1998) and Wiener *et al*. (2002), respectively). Double-muscled Piedmontese animals have a guanine to adenine transition mutation in exon 3, causing a substitution of tyrosine for cysteine (C313Y) in the signalling portion of the protein (Grobet *et al*., 1998; Kambadur *et al*., 1997). This transition has been predicted to abrogate the function of the protein (Grobet *et al*., 1998; Kambadur *et al*., 1997).

Recent advances in molecular genetics have resulted in assays that accurately identify these *MSTN* mutations, thereby allowing the effects of these genotypes to be examined thoroughly (Fahrenkrug *et al*., 1999). The objective of this study was to document the size and type of the effects of the SNP 413 in the *MSTN* gene on a wide range of traits of economic importance.

4.2 Material and methods

4.2.1 Experimental design

Two-generation resource populations, The University of Adelaide's Davies Gene Mapping and the New Zealand AgResearch Gene Mapping Projects in Australia and New Zealand, respectively, were developed using two phenotypically divergent *Bos taurus* breeds, Jersey (J) and Limousin (L). Three pairs of first–cross half-brothers were generated as first-crosses (X), with one of each pair used for mating in Australia and the other used for mating in New Zealand to both pure Jersey and pure Limousin dams, creating 469 Jersey backcross (XJ) and 315 Limousin backcross (XL) progeny in the two countries (Chapter 2).

4.2.2 DNA extraction and SNP genotyping

DNA was extracted from the New Zealand and Australian cohorts from blood samples collected in their first year of life, with the exception of the XL calves born in New Zealand in 1996 where DNA was extracted from ear cartilage. Genotyping of the SNP 413 in the *MSTN* gene was performed by PCR-RFLP on both Australian and New Zealand progeny (Table 4.1). Amplification of a fragment of the bovine myostatin gene containing the *MSTN* SNP 413 was obtained using the following primer pair (Sellick, 2002):

MSTNaF 5'-ATTCACTGGTGTGGCAAGTTGTCTCTCAGA-3'

MSTNbR 5'-CCCTCCTCCTTACATACAAGCCAGCAG-3'

Table 4.1 Number of animals genotyped for *MSTN* SNP 413

Project	Backcross	SNP 413 genotype			Total
Australia		AA[#]	AC	CC	
	Jersey	0	102	101	203
	Limousin	54	91	15	160
	Total	54	193	116	363
New Zealand					
	Jersey	0	131	125	256
	Limousin	70	69	8	147
	Total	70	200	133	403
Total		124	393	249	766

[#] A=variant allele, C=Normal allele

4.2.3 Statistical analysis

Multivariate analysis of both the Australian and the New Zealand data was conducted using ASReml (Gilmour *et al.*, 2006). The univariate analysis of the individual traits was conducted to provide starting values for the multivariate analysis. For the Australian data, the trait groups were divided as a) live animal measurements, b) carcass traits and c) meat quality, fat quality and behavioural traits. The models included fixed effects of breed of dam (Jersey or Limousin), slaughter group (6 combinations of year of birth and sex= 96H, 96S, 97H, 97S, 98H and 98S), and sire family (three), plus date of birth as a covariate and additive and dominance covariates within breed of dam. Additive

covariates were 0, 1 and 2 to account for zero, one and two variant myostatin allele carrying by CC, AC and AA genotypes, respectively. In order to test for dominance, an additional regression covariate was added with value of 0 for homozygotes and 1 for heterozygotes. A significant result for this covariate was interpreted as evidence of a dominance effect.

An attempt was made to fit a multivariate animal model and also a multivariate sire model in the data set to estimate the polygenic variance and separate it from the residual variance. However, because of the weak pedigree information (only common grandsires across countries and too few dams with multiple progeny), the data did not allow estimation of the additive polygenic correlation between traits in the multivariate framework so the residual effect was the only random term in the model.

For the AgResearch data, trait groups were classed as a) live animal measurements and reproductive traits, b) carcass traits, and c) meat quality and fat quality and behavioural traits. The statistical model used included fixed effects of breed of dam (Jersey or Limousin), farm of birth (three levels) and birth type (single or twin) within breed of dam, slaughter group (28 levels that includes adjustments for sex and year), and sire family (three levels), plus additive and dominance covariates within breed of dam. The residual effect was the only random term in the model.

4.3 Results

The results from multi-trait analysis of growth, carcass and meat and fat quality traits showed that the effects of slaughter group, sire and breed of dam on majority of traits were significant.

Breed of dam. Breed differences were significant for most of the traits. Breed effect was evident in both the Australian and New Zealand progeny. The Limousin backcross (XL) animals were heavier at birth, at weaning, at 400 and 600 days of age and had a heavier hot carcass weight ($P<0.001$) than the Jersey backcross (XJ) progeny. These animals had a larger eye muscle area, heavier *M. longissimus dorsi* muscle, *M. semitendinosus* muscle and Silverside, higher meat yield ($P<0.001$), lower marbling score ($P<0.05$), less intramuscular fat content ($P<0.001$)) and greater fat thickness, and higher fat and bone weights ($P<0.01$) (Table 4.2).

Pelvic area was also affected by breed ($P<0.001$) as the XL animals had larger pelvic area than XJ animals ($P<0.001$).

Breed had significant effects on meat and fat quality traits. Breed affected tenderness measured as Warner-Bratzler shear force. XL animals had more tender *M. semitendinosus* muscle ($P<0.001$) but less tender *M. longissimus dorsi* muscle ($P<0.05$) than the meat cuts from XJ animals. Cooking loss of both *M. semitendinosus* muscle and *M. longissimus dorsi* muscles in the XL animals were higher than XJ animals ($P<0.01$). Breed had no significant effect on meat pH, but the effect of breed on meat colour was significant ($P<0.01$). XJ animals had darker meat than XL progeny ($P<0.01$). Fat colour score, and total monounsaturated fatty acids were higher in the XJ progeny but fat melting point was lower in XJ animals than in XL animals ($P<0.01$). Breed inheritance had no significant effect on behavioral traits.

Myostatin effects. The results indicated that the additive effect of the *MSTN* variant on birth weight was not significant. There was a significant dominance effect of the gene for birth weight recorded in the New Zealand animals. For the Limousin backcross animals in New Zealand, the heterozygous progeny with one copy of the variant *MSTN* allele were 7.6% lighter than the mean of the two homozygous genotypes. However, in general, the *MSTN* variant had a significant positive effect on muscle and negative effect on fatness. The heterozygote was usually similar to the normal homozygote, resulting in many significant dominance estimates (Table 4.2). In the backcross Limousin animals, *MSTN* had both additive and dominance effects on stifle width measured at weaning, eye muscle area, silverside weight, tenderness of *M. semitendinosus* muscle measured on days 1 and 26 *postmortem*. From the Australian data, there was also a dominance effect of *MSTN* for hip width measured on live animals at 600 days of age ($P<0.01$) and docility score ($P<0.05$). On the other hand, only additive effects of *MSTN* were significant for fat depth and stifle width measured on live animals at 600 days of age, meat yield, fat yield, P8 fat depth ($P<0.001$), marbling score ($P<0.01$), intramuscular fat and rib fat ($P<0.05$), tenderness of *M. semitendinosus* muscle measured on days 5 and 12 after slaughter ($P<0.01$) and cooking loss of *M. semitendinosus* muscle ($P<0.001$).

Table 4.2 Additive (*a*) and dominance (*d*) effects (%) of myostatin and breed effects as a percentage of trait means for traits measured on live animals, carcass and beef quality traits.

	Limousin				Jersey			
	a		*d*		*a+d* [a]		Breed difference [b]	
Trait	**AU** [c]	**NZ** [d]	**AU**	**NZ**	**AU**	**NZ**	**AU**	**NZ**
Live traits								
Bwt	2.6	0.7	-3.2	-7.6**	0.9	1.4	32.3***	29.7***
Wwt	1.5	-	-1.5	-	2.0	-	11.2***	-
400W	1.8	0.5	1.3	-3.0	1.0	-1.0	10.1	25.3
600W	0.4	1.7	-1.6	1.6	1.2	0	15. 1***	14.5***
Whip	-0.5	-	-0.6	-	0.8	-	1.7	-
Wsti	2.5*	-	-3.1*	-	1.4	-	10.6***	-
600Hip	-1	-	-2.3**	-	-0.2	-	4.4***	-
600Sti	2.8**	-	-1.7	-	1.4	-	12.3***	-
600Fat	-13.9***	-	-1.7	-	-9.8*	-	12	-
Docility	0.8	2.8	-5.0*	6.8	-1.6	0.3	4.2	-6.7
Carcass traits								
Hcw	2.7	3.4*	-2.5	-3.6	1.2	0.8	22.7***	23.5***
EMA	10.5***	4.8*	-6.3**	-13.0***	4.4*	4.4**	19.1***	36.2***
SS	5.8***	7.2***	-2.7*	-10.4***	1.2	2.5	8.4***	39.1***
P8	-18.7***	-	1.4	-	-2.1	-	26.6**	-
Mar	-17.0**	-	0.7	-	0.7	-	-26.1*	-
Channel fat	-2.2	-5.0	1.3	10.4	-6.4	-11.7**	-13.7*	-39.7***
Omental fat	-1.9	-6.3	-3.1	5.6	0.1	-0.8	-9.1	-27.8**
Rbft	-9.4	-8.8	1.3	11.1	-7.5	-11.2*	4.2	-8.8
Imf	-8.2*	-7.1	2.9	9.0	-4.0	-5.5	-25.9***	-47.4
Meat yield	7.3***	5.9***	-4.2*	-6.7**	2.2	2.1	24.2***	30.4***
Fat yield	-16.5***	-8.1*	1.5	15.0**	-1.7	-7.1*	22.6***	-3.4
Bone yield	-0.8	-0.3	1.1	-1.2	-0.3	-0.5	17.3***	16.7***
PA	-2.2	0	0.1	-0.5	0.9	0.7	14.6***	3.2
Beef quality								
Wbld	-1.3	0.3	1.8	3.9	-0.5	5.7	12.1*	11.3*
Wbst	-4.7**	-	6.6**	-	-2.4	-	-12.0***	-
Clld	0.1	-0.9	1.2	-4.6**	0.6	1.7	4.2**	11.1***
Clst	-2.5***	-	0.8	-	-0.2	-	3.2*	-
Mufa	1.4	-2.2*	1.2	0.2	-0.7	0.9	-6.5***	-1.9

[a] additive plus dominance. [b] Limousin minus Jersey. [c]AU: Australian progeny. [d] NZ: New Zealand progeny. [e] last measurement of tenderness. Bwt: Birth weight, Wwt: Weaning weight, 400W: Body weight at 400 days, Whip: Hip width at weaning, Wsti: Stifle width at weaning, 600Hip: Hip width at 600 days, 600Sti: Stifle width at 600 days, 600Fat: Fat depth at 600 days, Hcw: Hot standard carcass weight, EMA: Eye muscle area, SS: Silverside weight, P8: Carcass fat depth on the rump, Mar: Marbling, Rbft: Fat depth at ribs 12th and 13th, Imf: Intramuscular fat content, PA: Pelvic area, Wbld: Warner-Bratzler shear force measured on the *M. longissimus dorsi* muscle, Wbst: Warner-Bratzler shear force measured on the *M. semitendinosus* muscle, Clld: Cooking loss of the *M. longissimus dorsi* muscle, Clst: Cooking loss of the *M. semitendinosus* muscle, Mufa: Total monounsaturated fatty acids. *: P <0.05, **: P< 0.01, *** : P<0.001

A regression analysis for the average effect of a substitution of a single copy of the variant *MSTN* allele indicated an increase in stifle width at 600 days of age (2.8% of the trait mean, Australian progeny), (eye muscle area 10.5 and 4.8% of the trait mean, Australian and New Zealand progeny, respectively), silverside (5.8 and 7.2%, Australian and New Zealand progeny, respectively), meat yield (7.3 and 5.9%, Australian and New Zealand progeny) and a reduction in fat depth measured at 600 days of age, P8 fat depth and marbling score (–13.9, -18.7, -17.0% of the trait means, Australian progeny), intramuscular fat (-8.2 and –7.1, Australian and New Zealand progeny, respectively), total fat yield (-16.5 and –8.1%, Australian and New Zealand progeny, respectively), Warner-Bratzler shear force and cooking loss of *M. semitendinosus* muscle (–4.7 and –2.5%, respectively, Australian progeny) (Table 4.2).

The analyses indicate that the Limousin backcross calves carrying two copies of the variant *MSTN* allele produced carcasses with about 12-15% more meat, and 16-33% less fat when compared with animals having no copies of the variant *MSTN* allele. Significant additive and dominance effects of the allele variant on meat yield indicate that heterozygous animals produce carcasses with about 3% more meat when compared with animals carrying no copies of the variant *MSTN* allele.

There were only two genotypic groups, AC and CC, in the Jersey backcross progeny. Therefore, it was not possible to separate additive and dominance effects of the gene in this group of animals. Thus, estimated *MSTN* effects for this group are additive effect plus the dominance deviation. The results from the Australian progeny indicated that in this group of animals, *MSTN* had significant effects on fat depth measured on live animals at 600 days of age and eye muscle area ($P<0.05$). The estimated additive plus dominance effect of the *MSTN* gene shows that fat depth measured on live animals was 9.8% lower in heterozygous animals than homozygous normal animals. Also eye muscle area in the heterozygous animals was 4.2% larger than the homozygous normal animals. However, the effect of a single copy of the variant allele was not detectable for most of the traits, including meat and fat yield, in this group of animals.

Similar results from the New Zealand Jersey backcross animals indicated that *MSTN* had significant effects on eye muscle area ($P<0.01$), channel fat ($P<0.01$), rib fat depth ($P<0.05$) and total fat yield ($P<0.05$). The estimated additive plus dominance effect of the *MSTN* gene showed that eye muscle area in heterozygous animals was larger than homozygous normal animals (4.4% of the trait mean, $P<0.01$). The estimated additive plus dominance effects of the *MSTN* gene for rib fat, total fat weight and kidney fat were

-11.2%, -7.1% and –11.6%, respectively, demonstrating that animals carrying one copy of the variant *MSTN* allele produced carcasses with less fat than those of animals carrying no copies of the inactive *MSTN* allele variant.

4.4 Discussion

In Australia the animals were grown on pasture and then finished on grain in the feedlot and were slaughtered at 34-40 months of age, while in New Zealand the animals were grown on pasture from birth to slaughter and were killed at 22-28 months of age.

Pleiotropic effects of a SNP in the myostatin gene in animals with two genetic backgrounds, Limousin and Jersey as breed of dam, were investigated in two different environments, South Australia (where animals were finished on a feedlot and New Zealand (where animals were grown on pasture from birth to slaughter). This is the first report of the effects of the myostatin SNP 413 on many traits in two different environments. In general, the New Zealand progeny were smaller than the Australian progeny because the animals in Australia were both older (slaughter age of 34-40 months and 22-28 months in Australia and New Zealand, respectively) and fatter when slaughtered. The data from two experiments were separately analysed because the traits in two trials differed and the trait measurements were not identical in Australia and New Zealand.

Live animal measurements. The results indicate that the additive effect of the *MSTN* allele variant on birth weight was not significant. This finding is consistent with some of previous reports on myostatin effects (Hanset R., 1991; Menissier, 1982) but heavier weights at birth have been documented in many studies in which double-muscling was involved (Arthur, 1995; Arthur *et al*., 1989; Casas *et al*., 2004a; Casas *et al*., 1999; Casas *et al*., 1998; Cundiff *et al*., 1998; Nott and Rollins, 1979; Short *et al*., 2002). Casas *et al*. (1998) compared animals inheriting one or zero copies of an inactive myostatin allele in Belgian Blue and Piedmontese, and found that the difference between both groups was 4.6 kg, with animals inheriting one copy of the inactive myostatin allele were heavier at birth than animals inheriting zero copies. In another study, Casas *et al*. (1999) reported that homozygous *mh*/*mh* animals were heavier at birth than the heterozygous +/*mh* and homozygous +/+ animals. They reported that the difference between the two homozygotes was 5.2 kg for birth weight. Casas *et al*. (2004a) studied the effect of the Belgian Blue inactive myostatin allele and found a difference of 3.5 and 2.0 kg at birth between the groups inheriting two and one copies, and the groups inheriting one and zero copies of the

inactive myostatin allele, respectively. This magnitude of effect is similar to that observed in studies of the Piedmontese inactive allele *MSTN* Short *et al*. (2002), where differences of 3.1 and 1.3 kg were reported for the same contrasts.

Casas *et al*. (1999) reported that differences in weight were observed at 200 days and 1 year of age in Piedmontese crossbreds segregating an inactivated myostatin allele. Casas *et al*. (2004a) also studied the effect of an inactivated myostatin allele on animal live weights in a crossbred population involving the Belgian Blue breed. They reported that while animals with one copy of the inactive myostatin allele had a postweaning and average daily gain similar to animals with zero copies of the allele, animals with two copies of the inactive myostatin allele had a slower growth rate. Nott and Rollins (1979) and Arthur (1989) found similar performance among these groups. The research presented here did not find a significant additive or dominance effect of the *MSTN* allele variant on animal live weight.

The most likely explanation for conflicting results of the present study and those mentioned above is the type of the mutation in myostatin studied herein. Previous studies have concentrated on inactive *MSTN* alleles, which cause the full double muscling phenotype in the homozygotes. The allele variant described herein should be active as only a single amino acid substitution is present. Both the Belgian Blue and Piedmontese doubled-muscle animals involved in the most studies on myostatin possess an extreme muscularity (Arthur, 1995), which is certainly more pronounced than doubled-muscle animals of Limousin involved in the present research. There may be also some breed effects, which given the Jersey and Limousin genetic backgrounds could affect traits particularly early in life. As the *MSTN* allele variant has been observed in Australian Angus, the effects of the 413 SNP should be investigated in different breeds.

Muscle mass. Although birth weight and other animal live weights were not associated with the myostatin allele variant, there was a significant effect of the allele on the muscularity of live animals and muscle mass measured after slaughter. The effect of the gene on muscularity was evident even at the youngest age examined (stifle width at weaning, 200 d of age) and was maintained in older animals (stifle width at 600 d of age) and even after slaughter (eye muscle area) (Table 4.2). Stifle width is an indicator of muscularity. Both additive and dominance effects of the allele on stifle width measured at weaning were significant. Based on the data from the Australian progeny, the additive effect of the allele for this trait was 2.5%. In other words, AA animals had about 5% wider stifle widths relative to CC animals without differences in weight. Traits that are

considered a more direct measure of muscularity were (e.g., eye muscle area and silverside weight) also increased with one copy of *MSTN* allele variant. More dramatic effects were observed with of two copies of the *MSTN* allele in the Limousin backcross animals. The estimated additive plus dominance effect for eye muscle area in the Jersey backcross progeny was similar to that of the Limousin backcross animals, indicating no interaction between breed of dam and myostatin gene for this trait. Also, the results showed that AA animals from Limousin backcross group had a large increase in meat yield, of 33.6 Kg and 17.6 Kg (Australia and New Zealand, respectively) increase in meat yield compared to CC animals. However, there were no significant additive or dominance effects of *MSTN* on hot carcass weight and the hot carcass weight of CC animals was comparable to that of AA animals in Australian progeny. Although the analysis of the New Zealand data showed a significant additive effect of the SNP in the Limousin backcross animals for hot carcass weight, the effect of the allele in the Jersey backcross animals was not significant. In addition, the size of the additive effect of the gene for hot carcass weight in the Limousin backcross animals was only 3% indicating that AA animals had only 6% higher carcass weights compared to CC animals.

Fatness. Because the animals had normal weights even though differences in skeletal muscle mass observed, the potential redistribution of the body tissues was investigated. The results revealed a significant effect of the allele on fat depots (fat depth measured on live animals, intra muscular fat and external fat measured as fat depth at P8 rump and ribs 12^{th} and 13^{th} at rump). The additive effects of the allele for fat traits were negative showing the AA animals were leaner than CC animals. In order to rule out the possibility that fat stores were simply redistributed in AA animals, the total body fat was analysed. The results showed that total body fat mass was decreased in AA animals compare with CC animals, 33% and 16% based on the Australian and New Zealand data, respectively. Hence, the normal body weight of AA animals, which had higher muscle mass, appeared to result from differences in fat accumulation. McPherron and Lee (2002) reported that mice lacking myostatin have a reduction in total body fat, which is particularly pronounced in older animals. These researchers investigated the effect of the *MSTN* mutation in two genetic models of obesity, agouti lethal yellow (A^y) and obese ($Lep^{ob/ob}$). Their findings indicated that loss of *MSTN* activity led to a partial suppression of fat accumulation and abnormal glucose metabolism. The exact mechanism by which myostatin regulates fat metabolism is unknown. However, it has been suggested that one possibility is that the effects of the myostatin mutations in adipose tissue are an indirect effect of the lack of

myostatin signalling in skeletal muscle so that the anabolic effects of the *MSTN* mutations on skeletal muscle tissue *per se* may shift energy metabolites in such a manner as to prevent fat accumulation elsewhere in the body (McPherron and Lee, 2002). It is possible that the effects on muscle mass and/or fat stores seen in the present study and also in mice studies (McPherron *et al.*, 1997; McPherron and Lee, 2002) may reflect the activity of some other mediator of cachexia whose production or activity is induced by myostatin (indirect effect of myostatin).

Consistent with the findings in the study herein, it is widely accepted that the amount of fat in the carcass of doubled-muscled cattle is significantly less than normal cattle (Arthur, 1995; Casas *et al*., 1998). In particular, intramuscular fat has been reported to be significantly affected by the doubled-muscle phenotype (Casas *et al*., 1998; Hanset R., 1991; Raes *et al*., 2001). The minimal marbling contributes to a reduced flavour rating, and the cause for this reduced marbling is reported to be a reduced subcutaneous and internal fatty tissue adipocyte size. However, the adipocyte size within the intramuscular fat appears to be the same between doubled-muscle and normal cattle (Hocquette *et al*., 1999). Studies by Webb *et al.* (1998) demonstrated that variation also exists in the fatty acids composition of intramuscular fat in the Belgian Blue breed when compared with normal breeds. Based on the New Zealand data, myostatin was associated with composition of fat depots so that the percentage of mono-unsaturated fatty acids (MUFA) in fat obtained from animals inheriting two copies of *MSTN* allele variant was lower than animals inheriting no copies of the allele. Consistent with this result, a higher polyunsaturated fatty acid proportion (PUFA) and a lower MUFA concentration have been found in the intramuscular fat of *mh/mh* genotypes of Belgian Blue breed compared with normal genotype (Raes *et al*., 2001).

Meat quality. The myostain allele was associated with tenderness of the *M. semitendinosus* muscle so that animals carrying two copies of the *MSTN* allele variant had more tender meat than animals with zero or one copy of the allele. In agreement with this finding, the general conclusion from most studies of double-muscled cattle is that meat from double-muscle cattle is more tender than that from normal-muscled cattle (Arthur, 1995; Bailey *et al*., 1982; Hanset R., 1991; Wheeler *et al*., 2001). It has been reported that the muscle of double-muscled cattle contains less connective tissue (Bailey *et al*., 1982), implying a lower background toughness and therefore more tender meat (Bailey *et al*., 1982; Hanset R., 1991).

Wheeler *et al*. (2001) reported that in Piedmontase cattle, both *mh*/+ and *mh*/*mh* genotypes improved the tenderness of *longissimus*, *gluteus medius*, *semimembranosus* and *biceps femoris* muscles relative to the +/+ genotype. On the other hand, Uytterhaegen *et al*. (1994) reported that the *M. longissimus dorsi* in double-muscled cattle is tougher and has more cooking loss than that of normal animals. They reported that lower background toughness, due to decreased levels of collagen (lower connective tissue) in double-muscled animals seems to be largely compensated by decreased myofibrillar tenderization, resulting in tougher meat. Casas *et al*. (1998), however, reported that a single copy of an inactivated *MSTN* allele had no effect on tenderness, measured as by *M. longissimus dorsi* shear force. It seems the inconsistency in reports is due to the measurements of tenderness and the muscle type studied. For example, based on the New Zealand data, there is evidence of significant additive effect of the gene on tenderness of the *M. longissimus dorsi* muscle (tenderness measurements on days 1.3 and 2.3 after *rigor mortis*). However, based on the Australian data where tenderness measurements of two different muscles (*M. longissimus dorsi* and *M. semitendinosus*) were available, the results indicated that the effect of the *MSTN* allele on tenderness is muscle dependent. The allele variant affected the tenderness of the *M. semitendinosus* muscle but not the *M. longissimus dorsi* muscle. *M. semitendinosus* and *M. longissimus dorsi* differ metabolically and histochemically; and *M. longissimus dorsi* is oxidative while *M. semitendinosus* is glycolytic. In addition, as shown by Totland *et al*. (1988), *M. longissimus dorsi* is a relatively homogenous muscle, whereas *M. semitendinosus* is relatively heterogenous in fibre type and has more connective tissue.

Negative aspects of the meat from double-muscled cattle were reported to include pale colour, less taste and reduced water binding (Bailey *et al*., 1982). Meat from double-muscle cattle has been reported to be slightly paler than that from normal-muscled cattle because there is a higher proportion of white muscle (type II B fibres) coupled with lower myoglobin content of the muscles (West, 1974). The study herein investigated the effects of the myostatin allele variant on some of these meat quality attributes. Although there was no evidence for the effect of the allele on meat colour or pH, results indicated that the myostatin allele variant was associated with low cooking loss in *M. longissimus dorsi* and *M. semitendinosus* muscles. Uytterhaegen *et al*. (1994) reported that double muscling in Belgian bulls increased cooking loss in the *M. longissimus dorsi* muscle. However, Wheeler *et al*. (2001) found no differences in cooking loss between +/+, *mh*/+ and *mh*/*mh* genotypes. These contradictions in relative tenderness and cooking loss could be due to inconsistency in cooking, differences in slaughter and chilling conditions, the

measurements of tenderness and the muscle type studied. In addition, there is some question as to whether heterozygotes for the double-muscling mutation have been correctly identified in some of the existing literature (Arthur, 1995). Thus, the magnitude of the effect on tenderness of one and two copies of inactivated myostatin allele is not clear in some experiments because of the uncertainty of genotypes. Contradiction in tenderness effects also could be due to epistatic interactions with other loci (Casas *et al*., 2000; 2001).

It has been reported that myostatin accounts for all of the effects on tenderness of Piedmontese breed (Wheeler *et al*., 2001). However, the results of the present study, showed that after removing the effect of myostatin on the tenderness, the Limousin backcross animals had lower shear force measured on this muscle than that of the Jersey backcross animals, suggesting that myostatin does not account for all of the breed effects on tenderness and that there may be other genomic regions influencing the trait.

Bone. The bones of double-muscled cattle, while significantly hypotrophied, are not affected as drastically as other tissues in the body (Arnold *et al*., 2001). Hans*et alet al*. (1991) reported percent losses in bone mass of double-muscled bulls compared to conventional bulls ranged from -4.8% (tibia) to -9.1% (femur). However, there was no evidence of the *MSTN* effects on bone weight herein. Hamrick *et al*. (2000) demonstrated that despite the impressive musculature of the myostatin-null "Mighty" mouse, its femora is not altered in either shape or size. Their findings indicated that the bone underwent no adaptation whatsoever in response to the increased muscle mass.

Pelvic area. Problems with dystocia and neonatal survival have been consistent problems with double-muscled cattle (Arthur, 1995), and these problems have been the main deterrent to more widespread use of this genetic trait. Bellows *et al*. (1971) showed that the main cause of dystocia is an incompatible relationship between birth weight and pelvic area. Short *et al*. (2002) reported that addition of one and two *mh* alleles linearly increased birth weight and linearly decreased pelvic area. Because pelvic area was not measured in cow, but only in the progeny, the present work was unable to examine the effect of the *MSTN* allele variant on pelvic area as a characteristic of the dam. However, when birth weight and pelvic area were analyzed as traits of the calf, there was no association between the myostatin allele variant and pelvic area or birth weight.

Limousin cattle are described as having a moderate increase in muscling compared with other *Bos taurus* breeds such as Herefords and Angus, but are not considered a double-muscled breed and do not suffer from an increase in dystocia compared with

Hereford cattle in Australia (Short *et al*., 2002). In addition, all purebred Limousin cattle used in the present study were not obviously double-muscled upon live visual assessment.

Gene action. The gene products from the myostatin locus act via autocrine or paracrine mechanisms to control myogenesis (Rios *et al*., 2002). Even though the activity of this gene product was not measured directly herein, some of the traits that were measured in this experiment, such as carcass weight, marbling, eye muscle area, primal cuts, and muscle score are highly related to the effects of this gene and can be used to estimate the genetic control mechanisms. In these traits, there was ample evidence that a major portion of the action of the myostatin gene product is additive. However, there was also evidence of some non-additivity. The non-additivity was always caused by the presence of a second myostatin allele variant, which had a larger effect than one myostatin allele variant. Partial dominance is the term often used to describe this effect. Whether this non-additivity is real or an artifact due to shape of the dose-response curve of myostatin for the traits measured cannot be determined. For example, based on the Australian data animals carrying one and two copies of the allele produced 7.0 kg and 33.6 kg more meat, respectively compared with animals not carrying a copy of the allele. Also animals with two copies of the allele produced 26.6 kg more meat than animals with one copy of the allele. In Australian data, there was evidence of additivity for fatness, but both additive and non-additive (dominance) effects on muscle mass. In New Zealand, evidence of both additive and non-additive (dominance) effects for muscle mass and fatness were observed.

4.5 Summary

The effect of the myostatin SNP 413 on birth, growth, carcass and beef quality traits was investigated in Australia and New Zealand. The study indicated that the effect of myostatin SNP 413 is different from other myostatin mutations. It was shown that despite differences in climate, feeding regimens and age of slaughter in two countries, the allele is expressed similarly in two different environments. Furthermore, the findings showed that along with increased muscling, animals carrying the variant have reduced fat depots and increased meat tenderness with similar birth weight to the normal animals. Thus, the variant is an ideal candidate for genotype-assisted selection to improve carcass composition.

Chapter 5: Multivariate multiple QTL mapping

5.1 Introduction

Initially, for the project herein, a single-QTL model based on regression interval mapping (Knott *et al*., 1996) was used to map QTL for a wide range of economically important traits in the beef industry (chapter 3). The results revealed a major QTL affecting carcass traits on BTA2 close to the map position of the myostatin gene. The pleiotropic effects of the myostatin functional SNP, which underlay this QTL, were then studied (chapter 4). However, given that data from multiple traits and sire families were available, a new approach for incorporating multiple markers together with multiple traits and families was developed to increase the power and accuracy of the genetic mapping of QTL (this chapter).

5.2 Multiple QTL mapping

The most popular approach for QTL mapping is interval mapping, proposed by Lander and Botstein (1989). Interval mapping involves likelihood-ratio tests for each possible QTL by densely covered chromosomes using linkage information in the available marker data. It assumes that a single gene regulates the trait of interest. Under the single-QTL model, interval mapping may fail to separate closely linked QTL and instead report "ghost" QTL that have no true effect on the trait (Haley and Knott, 1992; Martinez and Curnow, 1992). Therefore, procedures for detecting multiple quantitative trait loci (QTL) are of growing interest to geneticists. However, the best procedure for testing for multiple QTL is unclear.

Variable selection based on multiple-regression models of phenotype data on multiple genetic markers has been increasingly accepted as a general framework for mapping multiple QTL, with a large number of proposed methodologies being developed (Broman and Speed, 2002; Hoeschele *et al*., 1997; Jansen, 1993; Piepho and Gauch, 2001; Zeng, 1994). To overcome the required extreme computation times involved in multiple-QTL models, Jansen (1993) and Zeng (1993) independently introduced approximate multiple-QTL models, termed multiple QTL mapping (MQM) and composite interval mapping (CIM) by Jansen (1993) and Zeng (1994), respectively. Both Jansen (1993) and Zeng (1993) highlighted the benefits of including cofactors in the model to account for

QTL segregating elsewhere in the genome. These cofactors have been proposed as linear covariates, simply by including the genotype probabilities at the locations selected. In both multiple QTL mapping and composite interval mapping, markers take over the role of the nearby QTL and are fitted as cofactors while testing for a single QTL elsewhere in the genome. In this manner, the cofactors function as a genetic background control and absorb most of the genetic effects of their nearby QTL from the residual variance. As a result, the power of the QTL analysis is enhanced, while reasonable computation times are retained. However, in the approximate multiple QTL methods there is the additional task of selecting cofactors for controlling the genetic background. However, the choice of method of selecting the location or markers to be included differs between researchers. Kao *et al.* (1999) extended the composite interval mapping approach to multiple-interval mapping (MIM). Multiple-interval mapping directly regresses the trait on a set of markers, which densely cover the chromosomes. Since there has been no convenient way to handle too many intervals simultaneously, intervals must be selectively included in the model, again generating a model selection problem (Xu, 2003). The true multiple-interval mapping should include all intervals defined by markers.

Whittaker *et al*. (1996) showed that the least-squares method for interval mapping is equivalent to standard multiple linear regression of phenotype on marker type. Therefore, the problem of QTL detection essentially reduces to the problem of finding the appropriate pairs of markers. This decreases considerably the computational burden compared to a complete search, but there is still a problem of over-fitting because of the number of models that are fitted. Also, with increasing the number of markers, the majority of genetic markers across a genome will not be linked to QTL for the trait of interest and most of the regression coefficients will have a theoretical value of zero. From a statistical theory perspective, the parameter space in a QTL identification problem is quite sparse (Zhang *et al*., 2005). In addition, the dummy variables will be highly correlated across loci, leading to a high degree of multicolinearity. Furthermore, the number of markers tested can be very large relative to the number of observed individuals (Meuwissen *et al*., 2001; Xu, 2003), a problem that has been notoriously difficult in statistics. When the number of markers exceeds the number of individuals, the ordinary least-squares approach will have no unique solution (Xu, 2003). A number of authors have addressed these problems both in Bayesian (Xu, 2003; Zhang *et al*., 2005) and classical statistical inferences (Gilmour, 2007; Verbyla *et al*., 2007).

Gilmour (2007) proposed a mixed model approach in which all markers are fitted as independent random effects with common variance within the linkage groups. Once the significance of variance associated with a linkage group is established, all the information about the location of the QTL that markers contain is captured in the best linear unbiased predictions (BLUPs) for the markers. The size of a postulated QTL at marker points is predicted from the marker BLUPs and then a QTL profile is predicted from which to postulate the most likely position of the QTL. The location of a putative QTL is then tested by fitting it as a fixed effect in the presence of the random marker effects. If the QTL model is adequate, the random component for the linkage group will become negligible. Otherwise, the above process is repeated.

Verbyla *et al*. (2007) presented an extension of interval mapping that incorporates all intervals simultaneously in the analysis. This approach uses a working model in which the QTL sizes are assumed to be random effects for all intervals across the genome. If the random regression term is found to be significant, there is evidence for at least one QTL. The next step is to use an outlier detection method to locate QTL. Detected QTL are fitted as fixed effects and the above process is repeated until the variance component for the QTL sizes is not significant. Since the genetic link between markers as genetic information and a putative QTL is formulated, this approach seems more realistic than the proposed method by Gilmour (2007).

In this study, a variation on the multiple QTL mapping approach of Gilmour (2007) is presented in which, as Verbyla *et al*. (2007) it is allowed all markers of the entire genome to have common variance. Marker effects (that is, the QTL effects associated with markers) of the entire genome are simultaneously evaluated. If the marker density is relatively high, the markers will detect most of the QTL effects. Using mixed linear models, BLUPs of marker effects can be calculated even if there are more effects to be predicted than data points.

5.3 Multitrait QTL mapping

A QTL may affect more than one trait and this is termed pleiotropic effect. QTL with pleiotropic effects can be mapped using multiple trait analysis. Commonly, in almost all genomic studies including QTL mapping experiments, a great number of measurements are taken. Single and bivariate quantitative analyses are routinely performed on the collected data. However, multiple trait analysis is seldom performed. Multivariate QTL mapping

allows the detection of any possible pleiotropic effects and linked QTL, while exploiting the information from genetic and phenotypic correlations between traits (Gilbert and Le Roy, 2003; Knott and Haley, 2000; Korol *et al*., 1995). This gives potentially more insight into the nature of genetic correlations between different traits. In addition, multivariate approaches can increase the power of the test and the precision of parameter estimates (Gilbert and Le Roy, 2004; Jiang and Zeng, 1995; Korol *et al*., 1995; Meuwissen and Goddard, 2004). Jiang and Zeng (1995) showed that if the true model is a pleiotropic QTL, then analysing the multiple affected traits simultaneously by fitting a pleiotropic QTL increases power and improves the resolution to map QTL.

The simplest way to deal with multivariate data is by mapping individual traits and assessing whether the confidence intervals for QTL overlap for some combinations of traits. However, several approaches have been taken to handle multivariate data collected in gene mapping experiments. In almost all the approaches, multivariate traits are often condensed to allow univariate analysis. One approach is selecting one of the traits as the primary trait and considering the remaining traits as covariates, modifying the mean behaviour of the primary trait. Alternatively, the multivariate trait is replaced by one or more linear combinations of the underlying univariate traits through traditional principal component analysis or factor analysis (Gilbert and Le Roy, 2003, 2004; Stearns *et al.*, 2005; Weller *et al.*, 1996). Neither of these approaches is satisfactory. In the first approach, the traits are treated asymmetrically, with one trait arbitrarily designated as primary. For instance in mapping genes for carcass fatness, treating carcass weight as a covariate runs the risk of masking linkage evidence for genes that impact both traits. In essence, information on the variance and covariances displayed by traits is lost when they are viewed as covariates.

Transforming the original traits into new linear combinations has been approached in several ways, For example, Weller *et al*. (1996) consider principal component analysis, while Gilbert and Le Roy (2003) consider discriminate analysis. Korol *et al*. (2001) use a transformation of the trait space followed by single-trait analysis and subsequent back transformation. Stearns *et al*. (2005) evaluated multivariate and univariate approaches to map QTL and reported that there is a clear gain of power with principal component techniques over univariate models on the original traits, when the traits are influenced by QTL in a manner consistent with the principal component function. These conditions, however, can seldom be completely anticipated.

A possible disadvantage of using principal components in QTL analyses is that the magnitudes of the estimated effects are difficult to interpret directly in terms of traits. A transformation that produces traits that are either phenotypically or genetically uncorrelated does not ensure that the QTL only influences a single canonical trait. This is because different QTL affecting a trait may have different patterns of pleiotropy, for example some QTL affect only one trait whereas others affect two or more traits (Knott and Haley, 2000). In this case, it is not possible to find a canonical transform that ensures all QTL only influence one canonical trait. Consequently, it cannot be assumed that QTL found to be affecting two different canonical variables in the same location are actually different QTL, as stated by Weller *et al.* (1996). One could only conclude that QTL affecting different canonical traits are indeed different if the genetic correlations between traits are the same as the phenotypic correlations and all individual QTL follow the same pattern, a situation that is likely to be rare.

A number of methods to analyse the traits simultaneously have been developed. (Jiang and Zeng, 1995; Knott and Haley, 2000; Korol *et al*., 2001). However, currently, multiple trait approaches suffer in their implementation. These methods have not been widely adopted and this is probably a reflection of their relative statistical complexity. In addition, it is not clear how to proceed with the analysis of data containing many traits (e.g., does one start with single trait analysis or with one multitrait analysis that assumes that there are QTL affecting all traits) (Haley, 1999). Further development is required to determine the most efficient way to select the traits. Additionally, in practice, results are observed that seem intuitively incorrect. For example, Knott (2005) stated that single-trait analyses give evidence for all traits in one region of a linkage group, but when the traits are analyzed together, the best location can move some distance away to where there was no evidence for QTL from the individual trait analyses.

5.4 Joint analysis

The explosion of interest in QTL mapping has led to many different studies being conducted, each based on its own experimental populations. Data from these studies are often analysed separately. The cost of genotyping and collecting phenotypic data is often prohibitive for many studies. Thus, many studies lack a sufficient number of individuals to provide adequate power to detect any but the largest QTL. Thus, one direction for QTL analysis is to combine information from several or many studies (for example, by meta-

analysis of results of studies) (Allison and Heo, 1998; Goffinet and Gerber, 2000; Wood *et al*., 2006) or joint analysis of the original data (Kim *et al.*, 2005; Walling *et al.*, 2000). Where it is not possible to access the original data, meta-analysis can provide valuable insight (Haley, 1999). For example, combining results from several equivocal candidate gene studies can provide a more conclusive analysis (Allison and Heo, 1998). Meta-analysis, however, is limited in its scope by the information that is available, for example, as published in journals. For QTL studies, this information is likely to be a summary of results considered significant at a pre-defined threshold. Thus, for a particular region of the genome, published results are likely to provide a highly censored sample of all results.

The pooling of raw data potentially allows more information to be extracted than meta-analysis. For example, joint analysis of two or more similar populations could lead to more power to detect QTL not found in any individual study or could be used to confirm the presence of QTL detected in only one population (Lander and Kruglyak, 1995). In addition, joint analysis could lead to more precise estimates of the effects and location of a common QTL and could be used to examine differences in QTL effects in different populations (Walling *et al*., 2000).

Joint analysis is applicable to the situations where mapping information is available from different families. In this case, joint analysis can be used to combine information across families. For example, half-sib designs are common for QTL studies in livestock. For a fixed experimental resource, it is often preferable to test more small half-sib families than fewer large half-sib families. Because there is greater chance of detecting a rare allele. In the case of a validation study, significance tests should not be as stringent as for the initial genome scan. Hence, smaller sample sizes suffice, allowing many more families to be screened. However, because within sire analysis of small half-sib families provides limited power to detect QTL, experimenters may desire to incorporate information from all sires.

In order to bridge the gaps outlined, a multitrait multiple QTL technique was developed. The criticism of a multivariate decomposition does not imply that factor analysis is worthless. In the present study, a factor model is integrated more closely with DNA markers to model trait by marker effects for hunting pleiotropic QTL. Moreover, this work will demonstrate how a factor model can be used to combine information across families and traits, and hence, can substantially improve the power and resolution of QTL mapping.

5.5 Multiple marker analysis

5.5.1 Genetic model

A genetic model for a quantitative trait provides the means to interpret the genetic basis of the trait. Consider first the simple case of backcrossing between inbred lines. Two parental strains differing in both marker and QTL genotypes are mated to produce an F1 population. All F1 individuals will have the same heterozygous genotype. The F1 progeny are then mated to one of the parental strains ($Bb \times BB$). The genetic background for this cross is then three-quarters of the recurrent parent, and one-quarter of the other parent. Assume genotypic data for m markers and phenotypic data for one complex trait of interest are collected from n individuals. Further assume the m markers are densely located on the chromosomes of interest such that putative QTL will be co-transmitted with some of these m markers. The alleles are labelled at the ith QTL in the first strain Q_i, and the alleles at the jth marker locus M_j. The alleles in the second strain are labelled q_i and m_j in a corresponding fashion.

The genetic value of each individual and the QTL genotype are unknown and will be denoted as $\mathbf{z}$ and $\mathbf{g} = (g_1, g_2, \ldots, g_n)$, respectively, where g_i labels the number of Q_i alleles at the ith QTL locus as 1 and –1 for the Q_i Q_i and the Q_i q_i, respectively. Herein, it is assumed that QTL combine additively between and within loci, so $\mathbf{z} = \sum_{i=1}^{n} a_i g_i$ where a_i is the effect of the ith QTL.

Most statistical analyses of QTL effects have used a fixed linear model. That is, the phenotype of each backcross individual is modeled as a linear function of a genetic effect and a residual, unexplained, variance (Weller, 2001). Therefore, the basic genetic model is

$$y_i = \mu + g_i + e_i \tag{5-1}$$

where y_i denotes the trait value for ith individual, μ is the mean performance of all individuals, g_i represents the genetic effect of individual i, and e_i the non-genetic (residual) effects which is assume to be distributed as $N(0, \sigma_e^2)$.

For a single QTL model, g_i can be specified as

$$g_i = Q_i a + v_i \tag{5-2}$$

where a is the QTL size of effect, Q_i is unknown, and conditional on the values of the flanking markers, the probability distribution for Q_i can be obtained. The v_i represents the effects of the other QTL, polygenic effects not explained by Q.

To extend the model for multiple QTL situation, following Zeng (1993) consider first each individual in the backcross population has the marker-type $\boldsymbol{x} = (x_1, x_2, \ldots, x_m)$ where x_i is 1 and –1 if the genotype of the individual at the ith marker locus is M_iM_i and M_im_i, respectively. The linear model for y_i is

$$y = \mu + \sum_{j=1}^{m} x_{ij}\beta_j + e_i \qquad (5\text{-}3)$$

where m is the total number of markers in the entire genome, x_{ij} is a dummy variable (defined by 1 and -1) indicating the genotype of the jth marker for individual i, β_j is the QTL effect associated with marker j, and e is residual. β_j are chosen so as to give the linear function of $\boldsymbol{x}$ with maximal covariance with phenotype, and therefore, with genetic value z. Identifying QTL from the markers under investigation using the above multiple-linear-regression model is equivalent to selecting variables x_j, which have non-zero coefficients β_j.

5.5.2 Mixed model for multiple markers

Herein a mixed model approach equivalent to model (5-3) is used which treats the size of the QTL, a, as random rather than fixed. It is assumed that $a \sim N(0, \sigma_a^2)$. σ_a^2 is estimated in the random effects model. Therefore, the related mixed model for the genetic model (model 5-4) can be written in matrix notation as below:

$$\mathbf{y} = \mathbf{X}\boldsymbol{\tau} + \mathbf{Zu} + \mathbf{e} \qquad (5\text{-}4)$$

where $\mathbf{y}$ is a column vector containing the phenotype values for a trait measured in n individuals. It is assumed that this linear mixed model with $p \times 1$ vector of fixed effects ($\boldsymbol{\tau}$) and $m \times 1$ vector of random effects ($\mathbf{u}$) describes the observations adequately. The elements of the vector $\mathbf{u}$ are the marker effects. $\boldsymbol{X}$ and $\boldsymbol{Z}$ are, respectively, $n \times p$ and $n \times m$ incidence matrices, and $\mathbf{e}$ is the $n \times 1$ column vector of residual deviations assumed to be distributed independently of the random effects. Usually, all of the elements of the incidence matrices are equal to 0 or 1, depending upon whether the relevant effect contributes to the individual's phenotype. Herein, $\boldsymbol{Z}$ is a matrix containing marker scores with values of 1 and –1.

Since $E(\mathbf{u}) = E(\mathbf{e}) = \mathbf{0}$, by definition, $E(\mathbf{y}) = \mathbf{X}\boldsymbol{\tau}$. Denote the $n \times n$ covariance matrix associated with the vector $\mathbf{e}$ of residuals by $\mathbf{R}$ and the $m \times m$ covariance matrix associated with the vector $\mathbf{u}$ of random effects by $\mathbf{G}$. Under the assumption that $\mathbf{u}$ and $\mathbf{e}$ are uncorrelated, the distribution and the covariance matrix of the data vector $\mathbf{y}$ is

$$\mathbf{y} \sim N(\mathbf{X\tau}, \mathbf{H}) \tag{5-5}$$

where **H= ZGZ' + R**.

It is assumed that the residuals have constant variance and are uncorrelated, so that **R** is a diagonal matrix, with $\mathbf{R}=\sigma_e^2\,\mathbf{I}$ where **I** is identical matrix.

$\mathbf{G} = \sigma_m^2\mathbf{M}$ where **M** could be a known matrix reflecting the marker correlation coefficients.

$\mathbf{M} = \lfloor \boldsymbol{\rho}_{f\,f'} \rfloor$, (f and $f' = 1,2,...,m$)

In the above formula, m is the total number of markers and $\boldsymbol{\rho}_{f\,f'} = 1 - 2r_{f\,f'}$ is the correlation coefficient between marker m_f and marker $m_{f'}$. $r_{f\,f'}$ is the recombination frequency between marker loci f and f'.

Note that the marker effects are not correlated; it is the marker covariables that are correlated. All of the markers of the entire genome are fitted simultaneously as random effects, allowing one variance for the entire genome. The correlation structure exists among the marker covariates within a linkage group, but a correlation structure on the estimated sizes is not imposed because the aim is simply to shrink the marker effects according to the size of any QTL that they reflect. Hence, $\mathbf{G} = \sigma_m^2\mathbf{I}$. The linkage information is utilized in so far as associating the markers with linkage groups and imputing the missing marker data using an approach based on Martinez and Curnow (1994). The missing markers were replaced by their expected marker-type, conditional on the marker-type of the two markers flanking the marker and the map distances to those markers.

5.5.3 Estimation of the parameters

The estimates of the fixed and random effects in equation (5-4) are obtained as solutions to the mixed-model equations (Lynch and Walsh, 1998), which are given by

$$\begin{pmatrix} \mathbf{X}'\mathbf{R}^{-1}\mathbf{X} & \mathbf{X}'\mathbf{R}^{-1}\mathbf{Z} \\ \mathbf{Z}'\mathbf{R}^{-1}\mathbf{X} & \mathbf{Z}'\mathbf{R}^{-1}\mathbf{Z} + \mathbf{G}^{-1} \end{pmatrix} \begin{pmatrix} \hat{\boldsymbol{\tau}} \\ \tilde{\mathbf{u}} \end{pmatrix} = \begin{pmatrix} \mathbf{X}'\mathbf{R}^{-1}\mathbf{y} \\ \mathbf{Z}'\mathbf{R}^{-1}\mathbf{y} \end{pmatrix}. \tag{5-6}$$

This leads to best linear unbiased estimates (BLUES) of the fixed effects,

$$\hat{\boldsymbol{\tau}} = (\mathbf{X}'\mathbf{H}^{-1}\mathbf{X})^{-1}\mathbf{X}'\mathbf{H}^{-1}\mathbf{y} \tag{5-7}$$

and best linear unbiased predictors (BLUPs) of the random effects ,

$$\tilde{\mathbf{u}} = \mathbf{G}\mathbf{Z}'\mathbf{H}^{-1}(\mathbf{y} - \mathbf{X}\hat{\boldsymbol{\tau}}). \tag{5-8}$$

Since $\mathbf{R}= \sigma_e^2 \mathbf{I}$ and $\mathbf{G} = \sigma_m^2 \mathbf{I}$, implying $\mathbf{R}^{-1} = \sigma_e^{-2} \mathbf{I}$ and $\mathbf{G} = \sigma_m^{-2} \mathbf{I}$, respectively, the mixed model equations (model 6) reduce to

$$\begin{pmatrix} \mathbf{X}'\mathbf{X} & \mathbf{X}'\mathbf{Z} \\ \mathbf{Z}'\mathbf{X} & \mathbf{Z}'\mathbf{Z} + \lambda \mathbf{I} \end{pmatrix} \begin{pmatrix} \hat{\boldsymbol{\tau}} \\ \tilde{\mathbf{u}} \end{pmatrix} = \begin{pmatrix} \mathbf{X}'\mathbf{y} \\ \mathbf{Z}'\mathbf{y} \end{pmatrix} \tag{5-9}$$

where $\lambda = \sigma_e^2 / \sigma_m^2$.

The estimation process to obtain the variance components (σ_e^2 and σ_m^2), BLUPs and BLUEs involves an iterative approach. The most popular method for estimation of the variance parameters in linear mixed models is residual maximum likelihood (REML) (Patterson and Thompson, 1971). REML estimation of the variance parameters involves maximization of the residual log-likelihood that can be written as

$$\ell_R = -\frac{1}{2}\left\{\log|\mathbf{H}| + \log|\mathbf{X}'\mathbf{H}^{-1}\mathbf{X}| + \mathbf{y}'\mathbf{P}\mathbf{y}\right\} \tag{5-10}$$

Let $\boldsymbol{\gamma}$ denote the vector of variance parameters in ***G*** and ***R***; then the score for $\boldsymbol{\gamma}$ is given by

$$U_R(\boldsymbol{\gamma}_i) = \frac{\partial \ell_R}{\partial \boldsymbol{\gamma}_i} = -\frac{1}{2}\left\{tr(\mathbf{P}\mathbf{H}_i) - \mathbf{y}'\mathbf{P}\mathbf{q}_i\right\}, \tag{5-11}$$

where $\mathbf{H}_i = \partial \mathbf{H} / \partial \boldsymbol{\gamma}_i$ and $\mathbf{q}_i = \mathbf{H}_i \mathbf{P}\mathbf{y}$ is the working variate for $\boldsymbol{\gamma}_i$.

The REML estimate of $\boldsymbol{\gamma}$ is obtained by solving the system of equations $U_R(\boldsymbol{\gamma}) = \mathbf{0}$. In general, this requires a numerical solution. Given an estimate $U_R(\boldsymbol{\gamma}) = \mathbf{0}$, an update can be obtained as

$$\boldsymbol{\gamma}^{(m+1)} = \boldsymbol{\gamma}^{(m)} + [\mathbf{I}^{(m)}]^{-1} U_R(\boldsymbol{\gamma}^{(m)}) \tag{5-12}$$

where $\mathbf{I}^{(m)}$ is an information matrix for $\boldsymbol{\gamma}$ evaluated at $\boldsymbol{\gamma}^{(m)}$. Patterson and Thompson (1971) used a Fisher Scoring algorithm that requires calculation of the expected information matrix. The Fisher Scoring algorithm is very computer-intensive, and may be inefficient for large data sets or complex variance models. Several other algorithms have been developed that are less computer-intensive and employ sparse matrix methods (Cullis *et al*., 2004).

In this study, the average information algorithm (Gilmour *et al*., 1995) as implemented in the commercial software ASReml (Gilmour *et al*., 2006) was used to estimate the variance components. This software does not use (5-5) for REML estimation

of variance parameters, but rather uses a distribution free of $\boldsymbol{\tau}$, essentially based on error contrasts or residuals following Verbyla (1990).

The average information algorithm is a modified Fisher Scoring algorithm in which the expected information matrix is replaced by an approximate average of the observed and expected information matrices as given in (5-13).

$$\mathbf{I} = \frac{1}{2}\mathbf{Q}'\mathbf{P}\mathbf{Q} \tag{5-13}$$

where $\mathbf{Q} = [\mathbf{q}_1 ... \mathbf{q}_i]$ and $\mathbf{q}_i$ are working variates corresponding to $\mathbf{q}_i$ as shown above. The calculation of the score and working variables for variance component parameters is detailed in Gilmour *et al*. (1995).

5.4.4 Significance thresholds

Evidence for a QTL

The first step is to fit model (5-4) where markers of the entire genome are fitted simultaneously as random regression genetic effects, allowing one variance for the entire genome. Under the null hypothesis of no QTL, since the markers are neutral, there should be no variance associated with markers. The alternative is that one or more QTL occur on the genome. Then, all of the marker covariables in general, and in particular, those closest to the QTL, will take up some of the variation caused by the QTL, thereby inflating the variance component of the random regression term, σ_m^2. So, if the random regression term is significant, there is evidence for a QTL in the genome, and the process continues to locate QTL (as detailed in the following section). If there is no significant variance component, there is no evidence of QTL, and the process is terminated. The appropriate test for the variance of the marker random regression is the REML likelihood ratio test which is the one-sided test for which –2 times the change in the logarithm of the likelihood ratio is distributed as a mixture of χ_0^2 and χ_1^2 with a 5% critical value of 2.79 (Stram and Lee, 1994).

Locating the QTL

Once the significance of the marker random regression is established, the next stage is to detect the most likely marker linked to the QTL. The BLUPs of the marker effects contain all the information on the QTL. In a fixed effect model, the flanking markers would explain all the QTL variation. However, in a mixed effect model, the QTL effect is

dispersed (unevenly) over all the markers because they are all correlated to some degree with the QTL and each marker effect is shrunk (Gilmour, 2007). In the present study, in order to locate the QTL, individual marker effects were assessed using a probability statement regarding the marker size of effect, *u*, given the data. Following Verbyla (2003):

$$u - \tilde{u} \sim N(0, \sigma^2_{PE}) \tag{5-14}$$

$$u \mid \mathbf{y}_2 \sim N(\tilde{u}, \sigma^2_{PE}) \tag{5-15}$$

where $\boldsymbol{y_2}$ is the portion of the data that defines the residual likelihood, $\tilde{u}$ is the best linear unbiased predictor of *u*, and σ^2_{PE} is the prediction error variance. The relevant probability statement is:

$$\min\{P(u < 0 \mid \mathbf{y}_2), P(u > 0 \mid \mathbf{y}_2)\} \tag{5-16}$$

which can be calculated based on the normal distribution given in (5-15) as:

$$P(u > 0) = P(\frac{u - \tilde{u}}{\sigma_{PE}} > \frac{0 - \tilde{u}}{\sigma_{PE}}) \quad \text{or}$$

$$P(u < 0) = P(Z > \frac{-\tilde{u}}{\sigma_{PE}}) \qquad Z \sim N(0,1) \tag{5-17}$$

A small probability indicates that the distribution of *u* given the data has a distribution that is centred away from zero, and hence, provides evidence that a QTL is close to that marker. These probabilities were converted to log scale using (5-18) (Fisher, 1954)

$$-2ln[\, P(Z \succ \frac{-\tilde{u}}{\sigma_{PE}}) \,] \tag{5-18}$$

This quantity is a distributed χ^2 value with two degrees of freedom (Fisher, 1954). Thus, dividing the values obtained from (5-18) by 4.6 will give an equivalent LOD score for each marker point.

One way to locate QTL is choosing markers, which have reached a pre-defined LOD score threshold, but this will create problem of choosing an appropriate threshold. In the approach herein, however, the marker with highest LOD score is chosen and based on the map information, a QTL covariate is calculated and added to the fixed effect part of the model (5-4), $\boldsymbol{\tau}$, and the above process is repeated. If there is only one QTL and its location has been identified, the marker variance will become non-significant (based on likelihood ratio test) in the presence of the QTL covariate, confirming the location. The correct variance model must be used for any formal testing of the QTL. If the QTL does not

remove all the marker variance, the standard Wald F statistic will assume the residual marker variance is the appropriate divisor and the QTL effect may not appear significant. The appropriate test is performed when any residual marker variance is not fitted in the model since the markers in themselves are assumed neutral. However, the QTL covariate may not explain much or all of the marker variation, leading to the need for further investigation. If there is another QTL not near the first QTL, then the QTL covariate may have explained a substantial amount of the marker variance, but what remains will indicate the location of the second QTL. A covariate is added for the second QTL and the process repeated until the random marker variance becomes effectively zero (that is, non-significant).

5.6 Mixed model for multivariate multiple markers

Suppose that p traits are measured in n individuals. The $(np) \times 1$ dimensional column vector of observations is constructed by concatenating the univariate vectors, where the ith element of the column vector $\mathbf{y}_j$ corresponds to the observations of trait j in the ith individual. The model for combined vector of data across traits is given by:

$$\boldsymbol{y}_j = \boldsymbol{X}_j\boldsymbol{\tau}_j + \boldsymbol{Z}_j\boldsymbol{u}_j + \boldsymbol{e}_j \tag{5-19}$$

where there are q_j fixed effects associated with trait j so that $\mathbf{X}_j$ and $\boldsymbol{\tau}_j$ have, respectively, dimensionality $n \times q_j$ and $q_j \times 1$ for each character. $\mathbf{u}_j^{(bj \times 1)}$ is vector of random effects associated with trait j. Assuming there is a single measurement for each trait in each individual, $\mathbf{Z}_j = \mathbf{I}$, the mixed model can then be written as

$$\begin{pmatrix} \mathbf{y}_1 \\ \mathbf{y}_2 \\ \vdots \\ \mathbf{y}_p \end{pmatrix} = \begin{pmatrix} \mathbf{X}_1 & \mathbf{0} & \dots & \mathbf{0} \\ \mathbf{0} & \mathbf{X}_2 & \dots & \mathbf{0} \\ \vdots & \vdots & \ddots & \vdots \\ \mathbf{0} & \mathbf{0} & \dots & \mathbf{X}_p \end{pmatrix} \begin{pmatrix} \tau_1 \\ \tau_2 \\ \vdots \\ \tau_p \end{pmatrix} + \begin{pmatrix} \mathbf{u}_1 \\ \mathbf{u}_2 \\ \vdots \\ \mathbf{u}_p \end{pmatrix} + \begin{pmatrix} \mathbf{e}_1 \\ \mathbf{e}_2 \\ \vdots \\ \mathbf{e}_p \end{pmatrix} \tag{5-20}$$

It is assumed that the joint distribution of $(\mathbf{u}_j,\ \mathbf{e}_j)$ is Gaussian with zero mean and variance matrix

$$\theta_j \begin{pmatrix} \mathbf{G}_j(\gamma) & \mathbf{0} \\ \mathbf{0} & \mathbf{R}_j(\phi) \end{pmatrix} \tag{5-21}$$

where θ_j is a scale parameter which in the case of multiple trait or multi-trials is fixed to one. However, in mixed effects models with a single residual variance, θ_j is equal to the

residual variance (σ^2). γ and ϕ are vectors of variance parameters. The distribution of the data vector $\mathbf{y}_j$ is thus,

$$\mathbf{y} \sim N(\mathbf{X\tau}, \mathbf{H}) \tag{5-22}$$

where **H= ZGZ' + R**.

R is $(np) \times (np)$ covariance matrix associated with the total vector $\mathbf{e}^T = (\mathbf{e}_1^T, ..., \mathbf{e}_p^T)$ of residuals errors. **G** is covariance matrix associated with the total vector $\mathbf{u}^T = (\mathbf{u}_1^T, ..., \mathbf{u}_p^T)$ of random effects. First, consider the vector of residual effects, $\mathbf{e}^T$. While residual deviations for the same trait measured in different individuals can often be assumed to be uncorrelated, this is not necessarily the case for different characters measured in the same individual, which can exhibit an environmental correlation. The covariance matrix between $\mathbf{e}_i$ and $\mathbf{e}_j$ can be written as $\boldsymbol{\sigma}(\mathbf{e}_i, \mathbf{e}_j) = \sigma_{ij}\,\mathbf{I}$, where $\sigma_{ij} = \sigma_E^2(i, j)$ is the environmental covariance between traits *i* and *j* as expressed in the same individual. Let $\boldsymbol{\Sigma}$ be the $p \times p$ covariance matrix of within-individual environmental effects, with the *ij*th elements being $\sigma_{ij} = \sigma_E(i, j)$. In the Kronecker product notation, the covariance matrix **R** for the total vector of errors, $\mathbf{e}^T$ is

$$\mathbf{R} = \boldsymbol{\Sigma} \otimes \mathbf{I} \tag{5-23}$$

Thus, although **R** is $(np) \times (np)$, its inverse, $\mathbf{R}^{-1}$, can be computed from the inverse of a much smaller $p \times p$ matrix.

The covariance matrix **G**, for the *i*th random term, has many possible forms. In the most general case, **G** could be completely unstructured, comprising $b_i(b_i + 1)/2$ parameters. As stated by Smith *et al.* (2001), interaction terms may be regarded as a vector representation of a t_i dimensional array of effects, where t_i is the number of factors in the interaction. The variance structure for the *j*th dimension is $\mathbf{G}_{ij}$. As with a single dimension random effect, $\mathbf{G}_{ij}$ may take a range of forms. In the model herein, the interaction term is *trait by marker*. Let $\mathbf{u}_m$ be the $mp \times 1$ vector of the effects of *m* markers for *p* traits. A general form for the variance structure of the interaction term is

$$\text{var}(\mathbf{u_g}) = \mathbf{G_p} \otimes \mathbf{G_m} \tag{5-24}$$

where $\mathbf{G_p}$ and $\mathbf{G_m}$ are positive definite symmetric matrices of dimension $p \times p$ and $m \times m$, respectively. The matrix $\mathbf{G_p} = (\sigma_{Mjj})$ is the marker variance matrix. The diagonal elements are the marker variances for traits and the off-diagonal elements are the marker

covariances between pairs of traits. $\mathbf{G_m}$ would be a known matrix reflecting the marker correlation coefficients.

As mentioned above, a correlation structure is not imposed on the estimated effects because the aim is simply to shrink the marker effects according to the size of any QTL that they reflect. Hence, $\mathbf{G_m} = \mathbf{I_m}$ and in the Kronecker product notation, variance of *trait by marker* interaction term, var($\mathbf{u}_g$), is

$$\text{var}(\mathbf{u}_g) = \mathbf{G_p} \otimes \mathbf{I_m} \tag{5-25}$$

The model (5-25) implies that *trait* × *marker* effects are correlated between traits. Separating the marked genetic effects from other random terms, including non-marked polygenic effects, the mixed model (5-19) can then be written as

$$\mathbf{y}_j = \mathbf{X}_j\boldsymbol{\tau}_j + \mathbf{Z}_{0j}\mathbf{u}_{0j} + \mathbf{Z}_{gj}\mathbf{u}_{gj} + \mathbf{e}_j \tag{5-26}$$

where $\mathbf{u}_{gj}$ are the marker effects for trait j with associated design matrix $\mathbf{Z}_{gj}^{(n \times mp)}$ and variance matrix as in (5-25). $\mathbf{u}_{0j}$ comprise any additional random effects (including non-marked polygenic effects) with associated design matrix $\mathbf{Z}_{0j}$ and variance matrix $\mathbf{G}_0$.

A simple structure for $\mathbf{G}_p$ is a diagonal model (DIAG), assuming the markers effects for different traits are regarded as independent so that $\mathbf{G}_p$=diag(σ_{Mjj}), $j=1\ldots p$. The most general form for $\mathbf{G}_p$ is the unstructured variance model which contains $p(p+1)/2$ parameters (i.e. the number of parameters to be estimated increases quadradically with the number of traits). This model will provide the best fit (in a likelihood sense) to the data. However, in cases with a large number of traits and markers, it is difficult to ensure that REML estimates of the variance parameters for such a complex variance model remain within the parameter space. Also estimation of such a structure may be inefficient for a large number of traits and markers so a more parsimonious structure is desirable. This can be achieved using a factor analytic model for the marker effects across traits. The factor analytic model with sufficient multiplicative terms can provide a good and parsimonious approximation to the unstructured form and is generally more computationally robust (Thompson *et al*., 2003). Even for small number of traits, a factor analytic structure is preferred for $\mathbf{G}_p$ as given the purpose that is finding pleiotropic QTL and also trait-specific QTL.

5.6.1 The Factor analysis model

Factor analysis is a branch of multivariate analysis that is concerned with the internal relationships of a set of variates. Initially it was developed mainly by psychologists, and was primarily concerned with hypotheses about the organization of mental ability suggested by the examination of correlation or covariance matrices for sets of cognitive test variates (Lawley and Maxwell, 1971).

The Factor Analysis latent variable model (Johnson and Wichern, 1998) is a method intended to describe the covariance relationships among many variables in a multivariate data set in terms of a few underlying, but unobservable, random quantities called factors. There are two types of factor analytical models, orthogonal (factors are uncorrelated) and oblique (factors are correlated).

Factor analysis is based on a statistical model. The general factor model is linear in the common factors, $\boldsymbol{\xi}$, and is presented in equation (5-27).

$$\mathbf{y}_{(p\times 1)} - \boldsymbol{\mu}_{(p\times 1)} = \boldsymbol{\Lambda}_{(p\times m)}\boldsymbol{\xi}_{(m\times 1)} + \boldsymbol{\delta}_{(p\times 1)} \tag{5-27}$$

In this model, the vector $\mathbf{y}$ represents a multivariate observation, μ a vector of means, $\boldsymbol{\Lambda}$ a matrix of factor loadings (λ_{ij} is loading of the *i*th variable on the *j*th factor), $\boldsymbol{\xi}$ a vector of common factors, and $\boldsymbol{\delta}$ a vector of specific, or residual, factors. The vectors $\boldsymbol{\xi}$ and $\boldsymbol{\delta}$ are generally not observed and assumed independent. With so many unobservable quantities, a direct verification of the factor model from observations on $y_1, y_2,\ldots,y_p$ is hopeless. However, with some additional assumptions about the random vectors $\boldsymbol{\xi}$ and $\boldsymbol{\delta}$, the model in (5-27) implies certain covariance relationships, which can be checked.

Usually, factors are scaled to have unit variances.

$E(\boldsymbol{\xi}) = \mathbf{0}_{(\mathrm{m}\times 1)}$, $\mathrm{Cov}(\boldsymbol{\xi}) = E[\boldsymbol{\xi}\boldsymbol{\xi}'] = \mathbf{I}_{(\mathrm{m}\times \mathrm{m})}$

$E(\boldsymbol{\delta}) = \mathbf{0}_{(\mathrm{p}\times 1)}$, $\mathrm{Cov}(\boldsymbol{\delta}) = E[\boldsymbol{\delta}\boldsymbol{\delta}'] = \boldsymbol{\Psi}_{(p\times p)}$ where $\boldsymbol{\Psi}$ is a diagonal matrix of specific, or residual, variances (Johnson and Wichern, 1998). Under these assumptions, the covariance structure for the model (5-27) is

$$Cov(\mathbf{y}) = \boldsymbol{\Lambda}\boldsymbol{\Lambda}' + \boldsymbol{\Psi} \text{ and } Cov(\mathbf{y},\boldsymbol{\xi}') = \boldsymbol{\Lambda}. \tag{5-28}$$

A property of the factor loading matrix is that it can be rotated by an orthogonal matrix without loss of information (*i.e.* the covariances or correlations between variables are maintained following rotation). The portion of the variance explained by the retained factors for a specific variable is termed the *communality* and the portion not explained by the retained factors is called the *uniqueness* or *specific variance*. The *i*th communality is

the sum of squares of the loadings of the *i*th variable on the *m* common factors (Johnson and Wichern, 1998).

In a mixed model setting, multiplicative models for random interaction terms induce correlations between the interactions. Factor analytic variance structures have been proposed for genotype by environment effects in mixed model analyses of data from multi-environment trials (Cullis *et al.*, 1998; Smith *et al.*, 2001; Smith *et al.*, 2005; Thompson *et al.*, 2003). Smith *et al.* (2001; 2005) use a factor analytic structure to model *variety* by environment interactions, whilst simultaneously estimating a separate spatial correlation structure for the errors for each trial. Herein, the same formulation as Smith *et al.* (2001; 2005) was used to explain the application of factor analysis in modelling *trait by marker* effects.

When applied to the marker effects for each trait, the factor analytic model for marker effects, $\mathbf{u}_g$ will be

$$\mathbf{u}_g = (\boldsymbol{\lambda}_1 \otimes \mathbf{I}_m)\boldsymbol{\xi}_1 + ... + (\boldsymbol{\lambda}_k \otimes \mathbf{I}_m)\boldsymbol{\xi}_k + \boldsymbol{\delta} \tag{5-29}$$

where $\boldsymbol{\xi}_r^{(m\times 1)}$ are a few, random quantities called factors ($r=1...k<p$), the coefficients $\boldsymbol{\lambda}_r^{(p\times 1)}$ are known as loadings, and $\boldsymbol{\delta}^{(mp\times 1)}$ is the vector of residuals or lack of fit for the model.

Equation (5-29) has the form of a random regression on *k* trait covariates $\boldsymbol{\lambda}_1,...,\boldsymbol{\lambda}_k$. However, the underlying, but unobservable, difference between this equation and standard random regression problems is that in this formulation both the covariates and the regression coefficients are unknown and therefore, must be estimated from the data (Smith *et al.*, 2001) .

Matrix notation allows the entire system of equations to be written quite compactly as

$$\mathbf{u}_g = (\boldsymbol{\Lambda} \otimes \mathbf{I}_m)\boldsymbol{\xi} + \boldsymbol{\delta} \tag{5-30}$$

Considering the full model (5-26) the distribution of ($\boldsymbol{\xi}, \boldsymbol{\delta}$, $\mathbf{e}$) is assumed to be multivariate normal, with mean the zero vector and variance matrix

$$\begin{pmatrix} \mathbf{I}_K \otimes \mathbf{I}_m & \mathbf{0} & \mathbf{0} \\ \mathbf{0} & \boldsymbol{\Psi} \otimes \mathbf{I}_m & \mathbf{0} \\ \mathbf{0} & \mathbf{0} & \mathbf{R} \end{pmatrix}, \tag{5-31}$$

where $\boldsymbol{\Psi}$ is a diagonal matrix with elements $(\psi_1, \psi_2, ..., \psi_p)$ and ψ_i is known as the specific variance for the *i*th trait. The variance matrix for the marker effects for each trait, $\text{var}(\mathbf{u}_g)$, is then given by

$$\text{var}(\mathbf{u}_g) = (\boldsymbol{\Lambda} \otimes \mathbf{I}_m)\,\text{var}(\boldsymbol{\xi})\,(\boldsymbol{\Lambda}' \otimes \mathbf{I}_m) + \text{var}(\boldsymbol{\delta}) = (\boldsymbol{\Lambda}\boldsymbol{\Lambda}' + \boldsymbol{\Psi}) \otimes \mathbf{I}_m \qquad (5\text{-}32)$$

Researchers may be interested only in $\boldsymbol{\xi}$ (e.g., in modelling *variety by environment* interaction in plants). However, herein both $\boldsymbol{\xi}$ and $\boldsymbol{\delta}$ are of interest.

5.6.2 Estimation of the parameters

Estimates of the fixed and random effects in equation (5-26) are obtained as solutions to the mixed-model equations (Smith *et al*., 2001), which are given by

$$\begin{pmatrix} \mathbf{X}'\mathbf{R}^{-1}\mathbf{X} & \mathbf{X}'\mathbf{R}^{-1}\mathbf{Z}_g \\ \mathbf{Z}_g'\mathbf{R}^{-1}\mathbf{X} & \mathbf{Z}_g'\mathbf{R}^{-1}\mathbf{Z}_g + \mathbf{G}_p^{-1} \otimes \mathbf{I}_m \end{pmatrix} \begin{pmatrix} \hat{\boldsymbol{\tau}} \\ \tilde{\mathbf{u}}_\mathbf{g} \end{pmatrix} = \begin{pmatrix} \mathbf{X}'\mathbf{R}^{-1}\mathbf{y} \\ \mathbf{Z}_g'\mathbf{R}^{-1}\mathbf{y} \end{pmatrix}. \qquad (5\text{-}33)$$

This leads to best linear unbiased estimates (BLUEs) of the fixed effects,

$$\hat{\boldsymbol{\tau}} = (\mathbf{X}'\mathbf{H}^{-1}\mathbf{X})^{-1}\mathbf{X}'\mathbf{H}^{-1}\mathbf{y} \qquad (5\text{-}34)$$

and best linear unbiased predictors (BLUPs) of the random effects ,

$$\tilde{\mathbf{u}}_\mathbf{g} = (\mathbf{G}_p \otimes \mathbf{I}_\mathbf{m})\mathbf{Z}_g'\mathbf{P}\mathbf{y} \qquad (5\text{-}35)$$

where $\mathbf{H} = \text{var}(y) = \mathbf{Z}_p(\mathbf{G}_p \otimes \mathbf{I}_m)\mathbf{Z}_p' + \mathbf{R}$ (5-36)

and

$$\mathbf{P} = \mathbf{H}^{-1} - \mathbf{H}^{-1}\mathbf{X}(\mathbf{X}'\mathbf{H}^{-1}\mathbf{X})^{-1}\mathbf{X}'\mathbf{H}^{-1} \qquad (5\text{-}37)$$

In practice, BLUEs and BLUPs and the variance components are obtained through an iterative scheme (as in section 5.5.3). However, extra calculations in a factor analytic model are parameters in $\boldsymbol{\Lambda}$ and $\boldsymbol{\Psi}$. In practice, the parameters in $\boldsymbol{\Lambda}$ and $\boldsymbol{\Psi}$ are usually unknown and require estimation from the experimental data. The number of parameters in the factor analytic model with *k* terms is given by *pk+p-k(k-1)/*2. Estimation in factor analysis is a two-stage procedure. First, the parameters in the model are estimated, and then these are used to provide estimates of individual factor scores.

The use of model (5-32) for marker effects can lead to models with variance structures of less than full rank, which may occur when estimates of one or more specific variances tend to zero. In the literature on factor analysis, this is known as the Heywood case (Johnson and Wichern, 1998; Lawley and Maxwell, 1971). In this situation, REML estimation using the average information algorithm (Gilmour *et al*., 1995) or other standard algorithms is no longer possible.

Thompson *et al*. (2003) presented a sparse implementation of the average information algorithm for REML estimation of the factor analytic variance parameters. The algorithm is computationally efficient as exploits the regression underpinning the factor analytic model thereby facilitating substantial time savings. Additionally, the (commonly occurring) case of factor analytic variance structures with less than full rank (reduced rank variance models) has been accommodated in the algorithm, which is useful in the multivariate analysis. The algorithm has been implemented in ASReml (Gilmour *et al*., 2006) and can be accessed via the "XFA" variance model.

5.6.3 Significance thresholds

Testing for pleiotropic QTL

The first step is to fit model (5-26) where markers of the entire genome are fitted simultaneously as random regression genetic effects, considering two covariance models for $\mathbf{G_p}$ (The diagonal covariance model (DIAG) and the factor analytic model with one factor (FA1)). The DIAG model implies no marker covariance between traits (that is, the traits are independent with heterogeneous variances).

Since DIAG and FA1 are nested models, the REMLRT statistic, $2\Delta\ell$ (twice the log likelihood difference) can be approximated by the χ^2 distribution with the degree of freedom equal to the difference in the number of free parameters in the two nested models (Stuart *et al*., 1999).

Rejection of the null hypothesis would provide supporting evidence for the existence of either QTL that cause pleiotropic effects or multiple linked QTL. Under the null hypothesis of no pleiotropic QTL, since the markers are neutral, there should be no covariance associated with markers. The alternative is that one or more QTL affecting two or more than two traits occur on the genome. Then, all of the marker covariables in general, and in particular, those closest to the QTL, will take up some of the covariation caused by the QTL, thereby inflating the covariance component of the random regression term. So, if a likelihood ratio test is significant for a FA1 model for the trait by random regression marker effects, there is evidence for at least one pleiotropic QTL in the genome, this will be fitted as a fixed covariate and the process continues. If there is no significant FA1 model for this term, then there is no evidence of pleiotropic QTL, and the process is terminated.

Locating the pleiotropic QTL

Once the significance of covariance for the marker random regression is established by the factor analytic model, the next stage is to detect the most likely marker linked to the pleiotropic QTL. In order to locate pleiotropic QTL, individual marker effects for individual traits and the factor are converted to LOD scores using (5-18). The marker with highest LOD value for the factor is considered and based on the map information, a QTL covariate is calculated and added to the fixed effect part of the model (5-26) nested within traits. If there is only one pleiotropic QTL and its location is identified, the FA1 model for the marker covariance will become non-significant in the presence of the QTL covariate, confirming the location. However, the QTL covariate may not remove all the marker covariance leading to the need for further investigation. If there is another pleiotropic QTL, then the QTL covariate may have explained a substantial amount of the marker covariance, but the remaining marker covariance will indicate the location of the second pleiotropic QTL. A covariate is added for the second pleiotropic QTL and the process is repeated until the random marker covariance becomes effectively zero (that is, non significant FA1 model compared to the DIAG model). In this stage, only the QTL affecting individual traits (that is, trait specific QTL) remain. In order to locate the trait specific QTL, the analysis is continued using the univariate analysis framework (explained in section 5.5), fitting the detected pleiotropic QTL as fixed covariates and testing the marker variance for that specific trait.

5.7 Simulation study

To investigate the behavior of the approach, extensive simulation studies were conducted. The genetic and statistical models used for creating the simulated data were similar to (5-1) and (5.3), respectively. The variance of model (5-1) can be written as:

$$Var(y)=V_A + V_E \tag{5-38}$$

where V_E, residual variance, was considered as unity. V_A is additive genetic variance and based on the general model of Cockerham (Cockerham, 1954) can be obtained by

$$V_A = \frac{1}{4}\sum_i \alpha_i^2 + \frac{1}{2}\sum_{i \prec j} \alpha_i \alpha_j (1 - 2r_{ij}) \tag{5-39}$$

where r_{ij} is the recombination frequency between QTL *i* and QTL *j*, and α is QTL allele substitution effect.

To assess the empirical type I error rate of the overall test of evidence for QTL, a normally distributed quantitative trait was considered with a residual standard deviation of

unity, with individuals being assigned a random value from this distribution. 1000 populations were generated under the null hypothesis ($\sigma_m^2 = 0$) with 100, 150, 200 and 250 individuals. The simulation scheme for this part of the research involved 11 markers at 10 cM intervals on 9 chromosomes, each with a length of 100 cM with no QTL.

To examine the power of the REMLRT, replicate data sets were simulated under H_1 and analysed to see whether H_0 was rejected by REMLRT. 1000 populations with 100, 150, 200 and 250 individuals were generated. 11 markers at 10 cM intervals on 9 chromosomes each with a length of 100 cM and four QTL having equal size of effects were simulated (Two QTL on chromosome 1 at 30 and 70 cM, one QTL on chromosome 3 at 50 cM and one QTL on chromosome 5 at 0 cM). Three heritabilities were considered for a normally distributed trait ($h^2 = 0.25$, $h^2 = 0.30$ and $h^2 = 0.35$, giving the size of effect for each QTL as 0.5214, 0.5916 and 0.6631 units, respectively).

To investigate the power of the FA1 model, four normally distributed quantitative traits were considered, each with a residual standard deviation of unity, with individuals being assigned a random value from this distribution. The simulation design was based on: sample sizes of 125, 250, 500 and 750, a total chromosome number of 8, 6 markers for each chromosome, and an average marker distance of 20 cM and 1000 replications. Inheritance of all loci was determined assuming random assortment and that recombination events occurred independently, allowing use of Haldane's mapping function (Haldane, 1919). A total of 10 QTL were set (Tables 5.1 and 5.2) for the whole genome. Among these QTL, there are two QTL with pleiotropic effects. Three sets of simulations for each population were generated, QTL with small effects, QTL with medium effects and QTL with large effects, in which each QTL (on average) explains 7, 10 and 13 %, respectively, of the phenotypic variance in the backcross. The variation explained by all the QTL for the four traits was the same. However, the traits had different heritabilities because the four QTL were simulated for traits 1 and 2 and three QTL for traits 3 and 4 (Table 5.2). Two QTL were set in repulsion phase on chromosome 6 affected trait 2. Two QTL on chromosome 5 in coupling phase affected trait 1. Two QTL on chromosome 8 in coupling phase affected trait 4. One QTL in the centromeric position (first marker) of chromosome 2 and another QTL in the telomeric end of chromosome 7 (last marker) both affected trait 2.

Table 5.1 Simulated pleiotropic and trait specific QTL.

	QTL1	**QTL2**	**QTL3**	**QTL4**	**QTL5**	**QTL6**	**QTL7**	**QTL8**	**QTL9**	**QTL10**
	Chr1	Chr2	Chr4	Chr5	Chr5	Chr6	Chr6	Chr7	Chr8	Chr8
Position (cM)	20	0	60	20	80	20	60	100	40	80
Trait 1	PLTC		PLTC	COUP#	COUP#					
Trait 2		#	PLTC			REPL#	REPL#			
Trait 3	PLTC		PLTC					#		
Trait 4	PLTC								COUP#	COUP#

PLTC: Pleiotropic QTL. #: Trait specific QTL. COUP: Two linked QTL in coupling phase, REPL: Two linked QTL in repulsion phase

Table 5.2 Simulated QTL effects on four traits.

		QTL										
		QTL1	QTL2	QTL3	QTL4	QTL5	QTL6	QTL7	QTL8	QTL9	QTL10	
QTL Size	Chromosome	1	2	4	5	5	6	6	7	8	8	
	Position (cM)	20	0	60	20	80	20	60	100	40	80	h^2
Small[a]												
	Trait 1	0.5896[d]		0.5896	0.5896	0.5896						0.28
	Trait 2		0.7182	0.7182			-0.7182	0.7182				0.28
	Trait 3	0.6030		0.6030					0.6030			0.21
	Trait 4	0.5290								0.5290	0.5290	0.21
Medium[b]												
	Trait 1	0.7612		0.7612	0.7612	0.7612						0.40
	Trait 2		0.9273	0.9273			-0.9273	0.9273				0.40
	Trait 3	0.7559		0.7559					0.7559			0.30
	Trait 4	0.6631								0.6631	0.6631	0.30
Large[c]												
	Trait 1	0.9703		0.9703	0.9703	0.9703						0.52
	Trait 2		1.1821	1.1821			-0.1821	1.1821				0.52
	Trait 3	0.9233		0.9233					0.9233			0.39
	Trait 4	0.8099								0.8099	0.8099	0.39

[a] Each QTL on average accounted for 7% of phenotypic variation, [b] Each QTL on average accounted for 10% of phenotypic variation, [c] Each QTL on average accounted for 13% of phenotypic variation. [d] QTL allele effect in units,

5.7.1 Simulation Procedure

The phenotype of an individual is composed of its random residual component plus any genetic effect. The marker genotype data and trait value for each individual can be produced according to the mapping information and the QTL information. The basic simulation strategy is to step along the chromosomes and treat the marker positions and QTL positions alike. The difference between marker and QTL is that if a marker is reached, the marker genotype (0 or 1) is recorded. For a QTL, the additive and epistatic effects of the QTL are added to the trait value for the current individual.

For each individual, the simulation starts from the first marker of each chromosome. By 50% chance, the first marker genotype will be 0 or 1 and is recorded. At the next marker or QTL position, the chance of obtaining a certain genotype is according to the recombination frequency between previous position and the current position. For example, if the distance between these two positions is 10cM and the Haldane map function has

been used, the recombination frequency is 0.091. Therefore, the current genotype will be different to the previous one with probability of 9.1%. After deciding the genotype for current position, the genotype value is recorded or the QTL additive effect is added to the trait value. The procedure continues until all markers and QTL have been reached. After adding the trait mean and the random residual effect, the trait value for current individual is obtained.

5.7.2 Simulation results

Global type I and II errors

A type I error (α) occurs if the null hypothesis H_0 is rejected when it is actually true. The type I error may be higher than the specified "nominal α level" or, conceivably, may be lower than this level. A test is accurate if the type I error rate is not greater than the nominal α level. In the absence of a QTL, it is expected to observe 5% of the analyses resulting in significant marker random regression variance (this being the type I error accepted in setting the significant thresholds in the first part of the method). The empirical size of the REML likelihood ratio test (REMLRT) obtained (Table 5.3).

The type II error of a test (β) occurs if the test fails to reject H_0 when it is false. The power of a test is defined as 1-β and is equal to the probability of rejecting H_0 given that H_0 is wrong and that the alternative hypothesis H_1 is correct. The results showed that the REMLRT is powerful in testing σ_m^2, though the power can be low with low sample size and small QTL size of effect (Table 5.3).

Table 5.3 Type I and type II error rates of the REMLRT.

Sample size	Type I error [a]	Type II error [b]		
		h^2=0.25	h^2=0.30	h^2=0.35
100	0.046	0.130	0.058	0.018
150	0.040	0.010	0.004	0.000
200	0.032	0.001	0.000	0.000
250	0.039	0.000	0.000	0.000

[a]The proportions of replicates which likelihood ratio test statistic exceeding 2.79 (i.e, α=0.05), [b]The proportions of replicates which the test fails to reject H_0 when it is false.

Univariate *vs.* multivariate

Power of pleiotropy test

In terms of the *trait by markers* effects, a DIAG model and a factor analytic model with k=1 factor (denoted FA1) was fitted sequentially. In the simulation study, the DIAG model had 4 parameters and the FA1 model had 8 variance parameters (4 loadings and 4 specific variances for four traits). As the QTL size of effect increased the marker variance estimated from DIAG model for all four traits increased (Table 5.4). In all cases, the marker variances for traits 1 and 2 were higher than those of traits 3 and 4, a result which one would expect as the heritability for these later traits was lower than that of the former (Table 5.2). Since two pleiotropic QTL were simulated (QTL1 and QTL3) for traits 1 and 3 and only one of them was considered to affect one of the traits 2 or 4 (QTL1 for trait 4 and QTL3 for trait 2), the factor loadings on trait 1 and 3 were higher than those for traits 2 and 4.

Three specific QTL (QTL2, 6 and 7) were simulated for trait 2 and only one specific QTL was simulated for trait 3 (QTL8). This fact was reflected in their specific variances so that, in general, traits 2 and 3 had highest and lowest, respectively, specific variances among four traits (Table 5.4). It should be noted that in a few of the replicates, the estimated marker variance using DIAG model for small populations and small QTL size was on the boundary for one trait; that is, it was estimated as zero. Also in some replicates, using the FA1 model led to zero estimation of specific variances for one or two traits when the sample size and QTL effect were small.

Table 5.4 Mean loadings (×10), and marker variances (×100) estimated for four traits and averaged over 1000 replicates.

QTL Effect	Sample Size	Trait	FA1 model		DIAG model
			Loadings	Specific variance	Marker variance
Small[d]					
	125	Trait1	0.61	0.35	0.78
		Trait2	0.37	0.67	0.92
		Trait3	0.59	0.18	0.59
		Trait4	0.23	0.45	0.55
	250	Trait1	0.59	0.35	0.73
		Trait2	0.35	0.80	0.97
		Trait3	0.61	0.18	0.58
		Trait4	0.25	0.43	0.51
	500	Trait1	0.59	0.34	0.71
		Trait2	0.34	0.89	1.03
		Trait3	0.61	0.18	0.57
		Trait4	0.24	0.40	0.47
	750	Trait1	0.60	0.34	0.71
		Trait2	0.34	0.91	1.04
		Trait3	0.61	0.18	0.56
		Trait4	0.24	0.39	0.45
Medium[b]	125	Trait1	0.79	0.57	1.24
		Trait2	0.46	1.27	1.60
		Trait3	0.75	0.29	0.92
		Trait4	0.29	0.70	0.82
	250	Trait1	0.76	0.59	1.18
		Trait2	0.45	1.43	1.68
		Trait3	0.78	0.27	0.91
		Trait4	0.30	0.65	0.76
	500	Trait1	0.76	0.57	1.17
		Trait2	0.44	1.53	1.74
		Trait3	0.77	0.28	0.89
		Trait4	0.31	0.61	0.71
	750	Trait1	0.77	0.56	1.18
		Trait2	0.44	1.56	1.76
		Trait3	0.78	0.28	0.89
		Trait4	0.31	0.59	0.69
Large[c]	125	Trait1	0.99	0.91	1.95
		Trait2	0.58	2.24	2.70
		Trait3	0.92	0.44	1.36
		Trait4	0.35	1.02	1.17
	250	Trait1	0.98	0.92	1.89
		Trait2	0.57	2.43	2.80
		Trait3	0.95	0.42	1.35
		Trait4	0.37	0.94	1.09
	500	Trait1	0.97	0.93	1.91
		Trait2	0.56	2.53	2.86
		Trait3	0.95	0.41	1.33
		Trait4	0.38	0.89	1.03
	750	Trait1	0.98	0.95	1.93
		Trait2	0.55	2.56	2.88
		Trait3	0.96	0.41	1.32
		Trait4	0.38	0.86	1.01

[a] Each QTL accounted for 7% of phenotypic variation, [b] Each QTL accounted for 10% of phenotypic variation,[c] Each QTL accounted for 13% of phenotypic variation

Since the DIAG model is nested within the FA1 model, a direct comparison can be made using a REML likelihood ratio test. Herein, the power of the pleiotropic test is the chance of detecting a common factor (pleiotropic QTL) if that factor really exists. Thus, the power of the pleiotropic test was defined as the number of analyses (out of 1000 replicates) resulting in a significant FA1 model compared with the DIAG model. The power of the test depended on sample size and QTL size of effect (Figure 5.1). For a given QTL effect, as the population size increased, the power of the test increased and there was low power when both QTL effect and sample size were small. A population size of 500 seems to be a critical limit, in which for the small QTL considered in the simulation study, the test can reach up to 80% power. When small QTL were considered, in order to reach a power more than 95%, the sample size needs to be increased to 750 individuals. However, only a small number of real QTL experiments have this number of individuals.

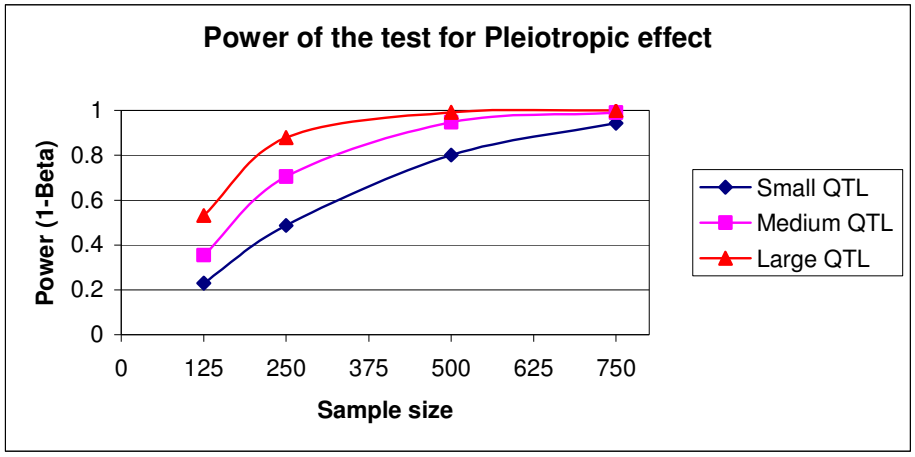

Figure 5.1 Observed statistical power (proportion of replicates with significant (P<0.05) FA1 model compared with DIAG model) for the pleiotropy model test.

Power of QTL detection and false positives

A true positive was declared whenever the LOD score for a marker reached the pre-defined criterion (LOD=2) and a QTL was present at that marker. Conversely, a false positive was declared whenever the LOD score for a marker reached to the pre-defined criterion but a QTL was absent at that marker. In addition, to avoid of identifying adjacent markers as QTL, it was required that the LOD score dropped by at least 1.0 between 'peaks' before declaring that two linked markers were identified as a QTL.

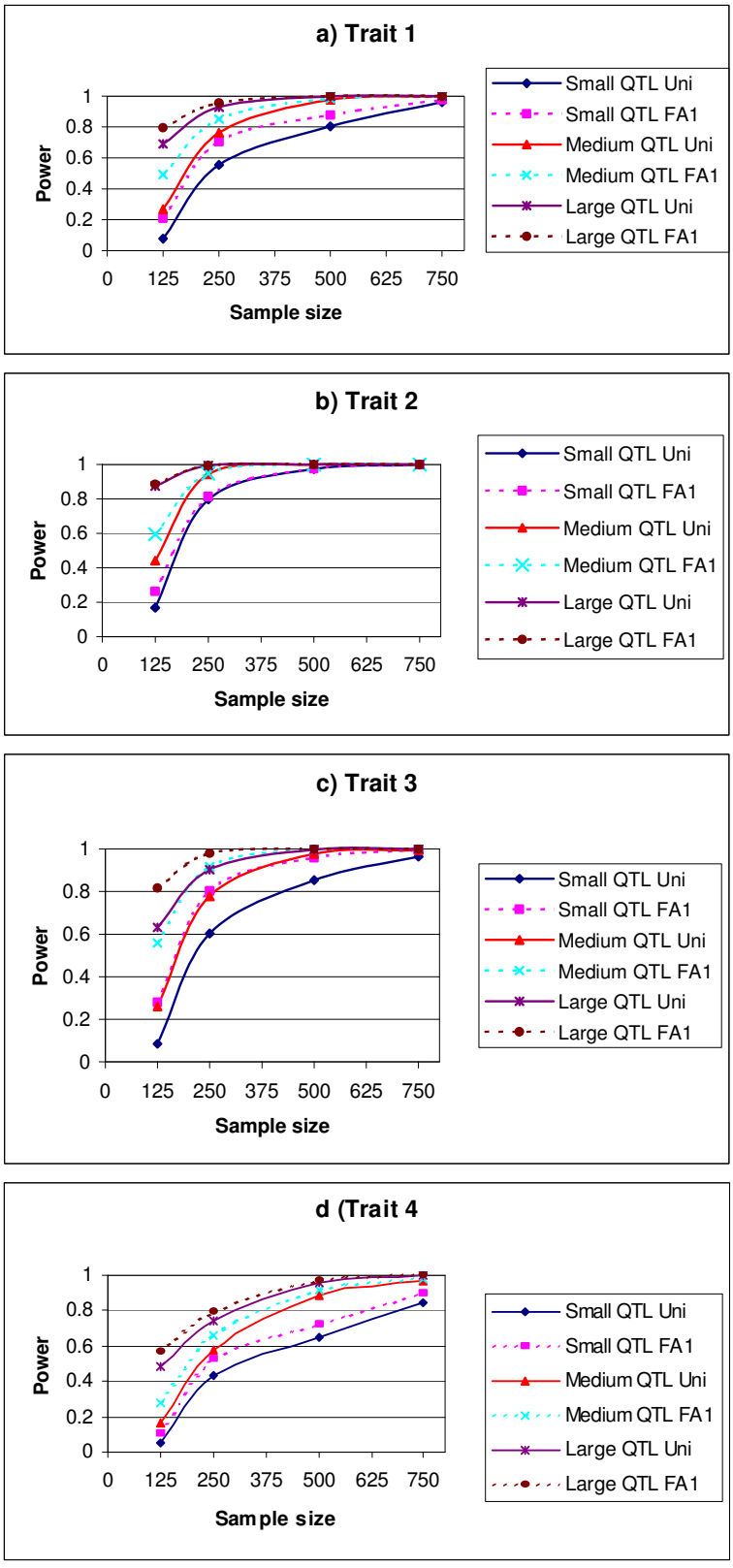

Figure 5.2 Comparison of the power of univariate (Uni) and multivariate (FA1) for QTL detection. The power was defined as average number of QTL detected for each trait divided by the number of QTL present.

The power of the experiment was calculated as the average number of QTL detected divided by the number of QTL present. The results showed that the ability to detect QTL using both univariate and multivariate analyses was strongly influenced by the QTL size of effect, and sample size so that the power to detect QTL improved significantly with increasing sample size and QTL effect (Figure 5.2 and Tables 5.5-5.8).

The overall power of detecting a QTL using the FA1 model was generally higher than that obtained in the univariate analysis. The power of QTL detection using the FA1 was 100% or was almost 100% when the relative QTL effect was large or a relatively large sample size was considered. The increasing power using the FA1 model was more evident when two pleiotropic QTL were affecting the trait (Traits 1 and 3, Figure 5.2). For the large sample size and large QTL effect, the two methods had relatively similar power to identify QTL.

The main feature to be noticed (Tables 5.5-5.8) is the higher ability of the FA1 model compared to univariate analysis to detect QTL1 and QTL3, which were simulated to have common effect on the traits. Multivariate and univariate analyses were equally efficient in detecting trait specific QTL with large effects. However, trait specific QTL with small effect could only be detected with very low efficiency using both multivariate and univariate analyses (Tables 5.5-5.8). In the case of the probability for false QTL detection, in general, both methods gave small likelihoods of finding false QTL. The highest likelihood of detecting false QTL is for small sample size (Tables 5.5-5.8).

In the situation where the two linked QTL were in coupling phase, for a few of the replicates, both methods tended to choose the marker between two correct markers, particularly when the sample size and QTL effect was small. However, in the case of the two linked QTL in repulsion phase, declaring the middle marker as the correct marker rarely happened.

Both univariate and multivariate techniques chose a rather low portion of unlinked loci to a QTL. This effect not evident with large sample sizes. Both approaches seem quite conservative, delivering only about 0-4.7% (univariate) and 0-7.1 % (FA model) of false positive unlinked loci.

Table 5.5 The power of QTL detection (proportion of significant replicates over all 1000 replicates) and the probability of false QTL detected under univariate and multivariate analysis (Trait 1).

QTL Effect	Sample Size	Model	QTL					Pleiotropic [a]			Linked [b]		False QTL [c]		
			1	3	4	5	One	All	One	Two	One	Two	Lk	Uk	Total
Small															
	125	UNI [d]	5.6	6.4	9.7	9.2	24.6	0.1	11.1	0.9	16.9	2.0	2.1	0.8	2.9
		FA1 [e]	28.4	32.6	11.6	11.4	58.2	0.7	48.0	13.0	20.7	2.3	4.2	3.0	7.2
	250	UNI	53.4	50.5	60.2	57.7	95.2	11.0	76.4	27.5	83.6	34.3	8.8	2.2	11.0
		FA1	83.2	85.3	58.2	55.8	99.3	25.3	96.6	71.9	81.0	33.0	10.7	6.0	16.7
	500	UNI	78.7	79.5	82.0	81.2	99.8	42.2	95.2	63.0	97.2	66.0	2.2	0.1	2.3
		FA1	95.4	96.3	78.9	80.3	57.8	100	99.8	91.9	95.5	63.7	1.7	1.0	2.7
	750	UNI	95.5	95.8	96.5	96.0	100	84.8	100	91.3	99.8	92.7	0.6	0.1	0.7
		FA1	99.5	99.7	94.8	96.2	100	90.3	100	99.2	99.9	91.1	0.8	0.2	1.0
Medium	125	UNI	24.6	24.0	29.0	29.3	60.3	2.7	38.2	10.4	47.3	11.0	4.8	1.5	6.3
		FA1	59.3	65.4	35.1	37.0	92.4	7.4	82.8	41.9	57.2	14.9	6.5	5.2	11.7
	250	UNI	75.3	73.3	79.1	77.1	99.5	35.7	92.8	55.8	95.7	60.5	4.1	0.8	4.9
		FA1	94.3	93.9	77.0	75.4	100	52.2	99.7	88.5	57.9	94.5	4.4	2.0	6.4
	500	UNI	96.8	97.4	98.2	97.6	100	90.2	100	94.2	100	95.8	1.2	0.1	1.3
		FA1	99.6	99.7	96.6	97.3	100	93.3	100	99.3	99.9	94.0	1.0	0.4	1.4
	750	UNI	99.7	99.8	99.8	99.6	100	98.9	100	99.5	100	99.4	0.0	0.0	0.0
		FA1	100	100	99.9	99.4	100	99.3	100	99.3	100	99.3	0.1	0.0	0.1
Large	125	UNI	66.1	65.4	72.3	72.7	98.3	26.4	85.0	45.9	92.8	52.2	7.3	2.0	9.5
		FA1	88.6	89.8	69.4	70.2	100	41.1	97.7	80.7	90.3	49.3	8.4	5.7	14.1
	250	UNI	92.6	91.7	93.6	93.1	100	74.2	99.5	84.8	99.5	87.2	1.7	0.4	2.1
		FA1	99.1	98.8	92.1	91.9	100	82.4	99.9	98.0	99.6	84.4	1.6	1.0	2.6
	500	Diag	99.8	99.7	99.9	99.9	100	99.3	100	99.5	100	99.8	0.2	0.0	0.2
		FA1	100	100	99.9	99.5	100	99.6	100	100	100	99.7	0.4	0.2	0.6
	750	UNI	100	100	100	100	100	100	100	100	100	100	0.1	0.0	0.1
		FA1	100	100	100	100	100	100	100	100	100	100	0.3	0.1	0.4

[a] One: percentage of runs in which at least one of the four simulated QTL was identified, All: percentage of runs in which all four simulated QTL were identified, One pleiotropic: percentage of replicates in which at least one of the two simulated pleiotropic QTL was identified, Two pleiotropic: percentage of replicates in which both of the two simulated pleiotropic QTL were identified. [b] One linked: percentage of replicates in which at least one of the two simulated linked QTL was identified, Two linked: percentage of replicates in which both of the two simulated linked QTL were identified. [c] Lk: proportion of falsely chosen markers linked to QTL, Uk: proportion of falsely chosen markers unlinked to QTL, Total false QTL: Lk plus Uk. [d]: univariate. [e]: Factor analytic model

Table 5.6 The power of QTL detection (proportion of significant replicates over all 1000 replicates) and the probability of false QTL detected under univariate and multivariate analysis (Trait 2).

QTL Effect	Sample Size	Model	QTL											
							Repulsion [a]						False QTL [b]	
			2	3	6	7	One	All	One	Two	Mdl	Lk	Uk	Total
Small														
	125	UNI [c]	27.8	16.9	11.3	10.8	41.3	0.8	18.3	3.8	0.0	1.8	0.8	2.6
		FA1 [d]	34.6	41.2	14.3	14.8	64.7	1.4	24.3	4.8	0.0	3.0	3.7	6.7
	250	UNI	93.8	80.6	70.8	73.2	99.5	43.6	89.0	55.0	0.5	8.1	4.7	12.8
		FA1	93.2	90.3	69.9	71.7	99.9	46.6	87.5	54.1	0.6	8.2	7.1	15.3
	500	UNI	99.7	96.5	96.2	96.6	100	89.4	93.1	99.7	0.0	0.8	0.7	1.5
		FA1	99.5	98.6	95.8	95.9	100	90.3	99.7	92.1	0.0	0.9	1.1	2.0
	750	UNI	100	99.6	99.8	99.8	100	99.2	100	99.6	0.1	0.3	0.1	0.4
		FA1	100	99.9	99.8	99.7	100	99.4	100	99.5	0.0	0.4	0.3	0.7
Medium	125	UNI	60.6	45.0	35.9	34.9	71.9	12.9	50.3	20.5	0.0	3.4	1.0	4.4
		FA1	73.8	74.6	45.4	44.1	95.3	17.7	62.4	27.1	0.0	3.5	2.6	6.1
	250	UNI	99.1	94.5	91.6	91.7	100	78.7	98.8	84.5	0.3	3.1	2.3	5.4
		FA1	99.0	97.3	91.2	91.6	100	80.8	98.7	84.1	0.2	2.8	3.8	6.6
	500	UNI	100	99.9	99.9	100	100	99.8	100	99.9	0.0	0.4	0.3	0.7
		FA1	100	100	99.9	99.9	100	99.8	100	99.8	0.0	0.3	0.5	0.8
	750	UNI	100	100	100	100	100	100	100	100	0.0	0.1	0.0	0.1
		FA1	100	100	100	100	100	100	100	100	0.0	0.1	0.0	0.1
Large	125	UNI	96.5	88.5	83.4	81.6	99.9	62.0	94.3	70.7	0.3	6.3	3.0	9.3
		FA1	95.6	94.0	82.7	81.3	99.9	63.0	94.6	69.4	0.2	6.8	3.9	10.7
	250	UNI	100	99.5	98.8	98.9	100	97.2	100	97.7	0.2	1.2	1.4	2.6
		FA1	100	99.7	98.7	98.8	100	97.3	100	97.5	0.3	1.1	2.0	3.1
	500	UNI	100	100	100	100	100	100	100	100	0.0	0.1	0.0	0.1
		FA1	100	100	100	100	100	100	100	100	0.0	0.1	0.1	0.2
	750	UNI	100	100	100	100	100	100	100	100	0.0	0.1	0.0	0.1
		FA1	100	100	100	100	100	100	100	100	0.0	0.1	0.0	0.1

[a] One: percentage or runs in which at least one of the four simulated QTL was identified, All: percentage of runs in which all four simulated QTL were identified, One repulsion: percentage of replicates in which at least one of the two simulated linked QTL in repulsion phase was identified, Two repulsion: percentage of replicates in which both of the two simulated linked QTL in repulsion phase were identified, Mdl: percentage of replicates in which the marker between two linked QTL was chosen. [b] Lk: proportion of falsely chosen markers linked to QTL, Uk: proportion of falsely chosen markers unlinked to QTL, Total false QTL: Lk plus Uk. [c] : Univariate. [d] Factor analytic model

Table 5.7 The power of QTL detection (proportion of significant replicates over all 1000 replicates) and the probability of false QTL detected under univariate and multivariate analysis (Trait 3).

QTL Effect	Sample Size	Model	QTL									
								Pleiotropic [a]		False QTL [b]		
			1	3	8	One	All	One	Two	Lk	Uk	Total
Small												
	125	UNI [c]	5.9	6.4	13.3	21.8	0.1	11.3	1.0	1.2	0.6	1.8
		FA1 [d]	32.6	36.6	14.5	61.1	2.7	55.2	14.0	2.9	3.1	6.0
	250	UNI	53.2	54.2	73.3	91.2	23.7	76.8	30.6	4.7	2.0	6.7
		FA1	84.9	88.0	67.8	99.0	51.8	97.0	75.9	6.1	4.0	10.1
	500	UNI	80.7	79.9	95.3	99.6	63.1	94.8	65.8	0.9	0.1	1.0
		FA1	98.1	97.6	92.1	100	88.7	95.7	92.1	1.1	1.0	2.1
	750	UNI	96.0	94.2	99.3	100	90.0	99.7	90.5	0.4	0.0	0.4
		FA1	99.9	99.4	99.1	100	98.5	100	99.3	0.2	0.8	1.0
Medium	125	UNI	22.8	18.9	36.4	51.6	4.4	7.3	34.4	2.7	1.1	3.8
		FA1	61.7	65.6	40.3	90.6	16.9	85.4	41.9	3.0	2.2	5.2
	250	UNI	71.1	72.3	90.1	98.1	49.0	90.4	53.0	2.7	1.2	3.9
		FA1	94.1	96.3	84.5	100	76.5	99.7	90.7	2.3	2.6	4.9
	500	UNI	97.1	96.4	99.7	100	93.5	99.7	93.8	0.5	0.1	0.6
		FA1	100	99.9	99.3	100	99.2	100	99.9	0.3	0.5	0.8
	750	UNI	99.8	98.7	100	100	98.5	100	98.5	0.0	0.0	0.0
		FA1	100	100	100	100	100	100	100	0.0	0.4	0.4
Large	125	UNI	56.5	55.1	77.7	91.2	29.4	77.0	34.6	5.3	2.4	7.7
		FA1	86.7	88.6	69.6	99.0	54.6	97.7	77.6	6.0	2.0	8.0
	250	UNI	84.9	87.7	98.4	99.9	73.9	98.0	74.6	1.4	0.3	1.7
		FA1	98.9	98.9	96.2	100	94.2	100	97.8	1.1	0.9	2.0
	500	Diag	99.5	99.6	99.9	100	99.0	100	99.1	0.2	0.0	0.2
		FA1	100	100	99.9	100	99.9	100	100	0.1	0.1	0.2
	750	UNI	100	100	100	100	100	100	100	0.0	0.0	0.0
		FA1	100	100	100	100	100	100	100	0.0	0.1	0.1

[a] One: percentage or runs in which at least one of the three simulated QTL was identified, All: percentage of runs in which all three simulated QTL were identified, One pleiotropic: percentage of replicates in which at least one of the two simulated pleiotropic QTL was identified, Two pleiotropic: percentage of replicates in which both of the two simulated pleiotropic QTL were identified. [b] Lk: proportion of false chosen markers linked to QTL, Uk: proportion of false chosen markers unlinked to QTL, Total false QTL: Lk plus Uk. [c]: Univariate. [d]: Factor analytic model

Table 5.8 The power of QTL detection (proportion of significant replicates over all 1000 replicates) and the probability of false QTL detected using univariate and multivariate analysis (Trait 4).

QTL Effect	Sample Size	Model	QTL					Coupling [a]				False QTL [b]	
			1	9	10	One[c]	All[c]	One	Two	Mdl	Lk	Uk	Total
Small													
	125	UNI e	3.0	5.4	6.1	12.8	0.0	10.8	0.7	0.6	1.4	0.4	1.8
		FA1 [f]	14.8	8.0	8.3	26.7	0.2	15.1	1.2	1.3	2.2	2.9	5.1
	250	UNI	32.0	50.0	47.5	79.5	8.7	73.1	24.4	4.7	7.6	1.1	8.7
		FA1	61.1	48.9	47.9	88.8	13.7	73.7	23.1	5.4	8.7	2.1	10.8
	500	UNI	57.5	69.4	68.9	95.8	29.6	91.4	46.9	0.5	0.7	0.3	1.0
		FA1	78.2	69.3	68.2	97.9	38.1	90.4	47.1	0.7	0.8	1.0	1.8
	750	UNI	81.2	86.7	86.5	99.8	60.4	99.2	74.0	0.4	0.7	0.0	0.7
		FA1	93.2	87.3	87.4	100	70.7	99.4	75.3	0.5	0.8	0.5	1.3
Medium	125	UNI	11.0	19.8	18.8	39.0	0.9	34.1	4.5	1.2	2.2	1.1	3.3
		FA1	34.8	25.6	24.2	62.9	2.7	43.7	6.1	2.2	3.6	3.2	6.8
	250	UNI	48.4	62.3	61.3	90.9	20.2	85.7	37.9	2.6	4.0	0.6	4.6
		FA1	74.2	61.5	62.4	95.8	28.9	85.7	38.2	2.9	4.1	2.3	6.4
	500	UNI	84.3	90.3	90.2	100	69.0	99.4	81.1	0.5	0.5	0.1	0.6
		FA1	93.9	89.8	90.3	100	76.2	99.2	80.9	0.6	0.6	0.5	1.1
	750	UNI	95.6	97.0	97.8	100	90.8	100	94.8	0.3	0.4	0.0	0.4
		FA1	98.8	96.8	97.8	100	93.5	100	94.6	0.3	0.4	0.1	0.5
Large	125	UNI	37.2	54.3	54.0	86.1	11.7	80.0	28.3	4.0	7.6	2.4	10.0
		FA1	61.5	53.8	53.8	91.1	19.2	77.9	29.7	4.5	8.7	2.3	12.0
	250	UNI	67.6	77.4	76.5	99.0	39.8	96.1	57.8	1.2	1.3	0.1	1.4
		FA1	86.1	75.2	75.6	99.4	48.3	95.1	55.7	1.7	1.7	1.5	3.2
	500	Diag	94.6	97.6	96.3	100	88.9	100	93.9	0.2	0.2	0.0	0.2
		FA1	98.6	97.4	95.9	100	92.0	100	93.3	0.2	0.2	0.1	0.3
	750	UNI	100	99.8	99.9	100	99.7	100	99.7	0.0	0.1	0.0	0.1
		FA1	100	99.8	99.9	100	99.7	100	99.7	0.1	0.2	0.0	0.3

[a] One: percentage or runs in which at least one of the three simulated QTL was identified. [b] All: percentage of runs in which all three simulated QTL were identified. [c] One coupling: percentage of replicates in which at least one of the two simulated linked QTL in coupling phase was identified, Two coupling: percentage of replicates in which both of the two simulated linked QTL in coupling phase were identified, Mdl: percentage of replicates in which the marker between two linked QTL was chosen. [d] Lk: proportion of false chosen markers linked to QTL, Uk: proportion of false chosen markers unlinked to QTL, Total false QTL: Lk plus Uk. e: Univariate. f: Factor analytic model.

Separate *vs.* joint family analysis

The power of the QTL analysis for the small sample size and small QTL effect was very low and a large sample size (at least 750 individuals based on the simulated data) was needed to identify QTL with small effect with relatively high efficiency (section 5.7.2). However, it is not realistic to expect population sizes of this magnitude in actual planned experiments. In some QTL experiments, mapping information is available from different

families (e.g., half-sib designs), each family having a relatively small size. In this situation, joint analysis can be used to combine information across families.

To explore the efficiency of the factor analytic model for joint analysis of multiple families, a QTL experiment comprising six families (labelled as S1, …, S6) was considered which mimics the real QTL mapping experiment herein (described in chapter 2) in terms of the number of families (six families) and the number of progeny per family (125 individuals per sire family on average). The same parameters as for trait 1 (Tables 5.1 and 5.2) in the case of small QTL effects and small sample sizes were considered. Two situations were simulated: a) all four QTL were segregating in all six families and b) QTL1 and QTL3 were segregating in families S1, S2 and S3 and QTL26 and QTL29 were segregating in families S4, S5 and S6.

A model equivalent to (5-26) was used. However, instead of using the factor analytic model and the DIAG model for the *trait by marker* effects, the FA1 model and the DIAG models we used for *family by marker* interaction effects.

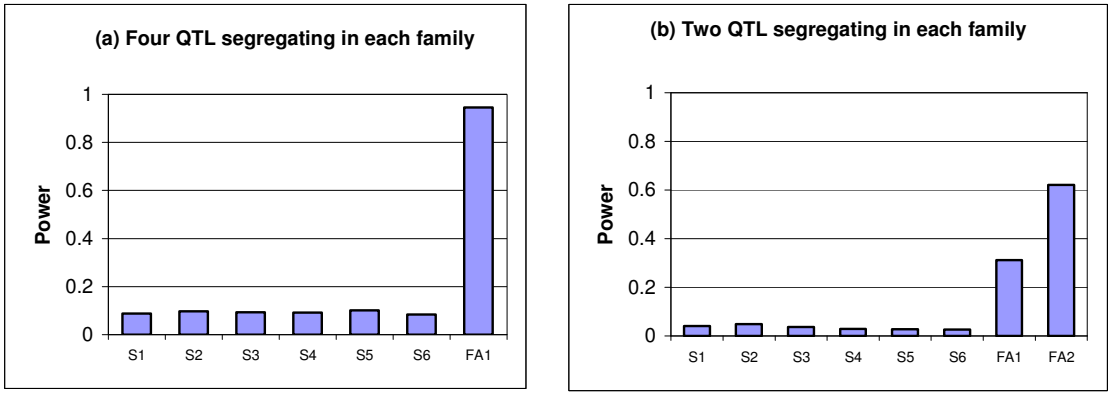

Figure 5.3 The power of QTL detection (average number of detected QTL divided by four) using separate (S1-S6) and joint analysis (FA1 and FA2) of six families (same parameter settings as trait 1). FA1 and FA2: joint analysis employing factor analytic models with one and two factors, respectively.

The benefits to the joint analysis were evident (Figure 5.3, Tables 5.9-5.10). One main advantage was that the evidence for a particular QTL could be substantially increased with the extra information. This was apparent especially when all of the QTL were segregating in all families. However, when the number of common QTL across families or mapping trials decreased, the power of joint analysis tended to be closer to the individual analysis. Furthermore, the LOD score in the joint analysis was substantially higher than

that from the single-family analyses (Figure 5.4). In the real dataset, this could have a subsequent effect on the confidence interval of the QTL location.

Table 5.9 The power of QTL detection[a] and the probability of false QTL detected using separate and joint analysis of six families (same parameter settings as trait 1, four QTL were segregating in all families).

	True QTL				False QTL	
Family[b]	1	3	4	5	Linked	Unlinked
S1	7.9	7.6	10.5	9.5	1.2	0.2
S2	9.2	7.9	10.7	11.3	1.5	0.2
S3	8.9	8.2	9.1	11.4	1.9	0.6
S4	9.7	6.6	10.9	9.6	1.2	0.2
S5	8.8	9.7	10.4	11.8	1.5	0.5
S6	7.7	7.4	10.1	8.9	1.3	0.3
FA1[c]	94.6	94.1	94.9	94.2	0.5	0.1
S750[d]	95.5	95.8	96.5	96.0	0.6	0.1

[a] The percentage of runs (over all 1000 replicates) in which the QTL was identified. [b] Separate analyses of families S1-S6. [c] Joint analysis of the six families using factor analytic model with one factor. [d] Results from Table 5.5 for univariate analysis of trait 1 (small QTL and sample size of 750).

Table 5.10 The power of QTL detection [a] and the probability of false QTL detected using separate and joint analysis of six families (same parameter settings as trait 1, four QTL were not segregating in all families).

	True QTL				False QTL	
Family[b]	1	3	4	5	Linked	Unlinked
S1	4.1	4.0	-	-	0.6	0.1
S2	5.2	4.4	-	-	0.1	0.0
S3	4.6	2.9	-	-	0.4	0.1
S4	-	-	3.3	2.7	0.3	0.0
S5	-	-	3.2	2.6	0.2	0.1
S6	-	-	2.2	3.2	0.0	0.1
FA1[c]	53.8	50.5	10.3	10.6	2.2	0.9
FA2[d]	65.9	66.9	58.1	57.8	2.6	1.4
S750e	95.5	95.8	96.5	96.0	0.6	0.1

[a] The percentage or runs (over all 1000 replicates) in which the QTL was identified. [b] Separate analyses of families S1-S6. [c] Joint analysis of the six families using factor analytic model with one factor. [d] Joint analysis of the six families using factor analytic model with two factors. [e] Results from Table 5.5 for univariate analysis of trait 1(small QTL and sample size of 750).

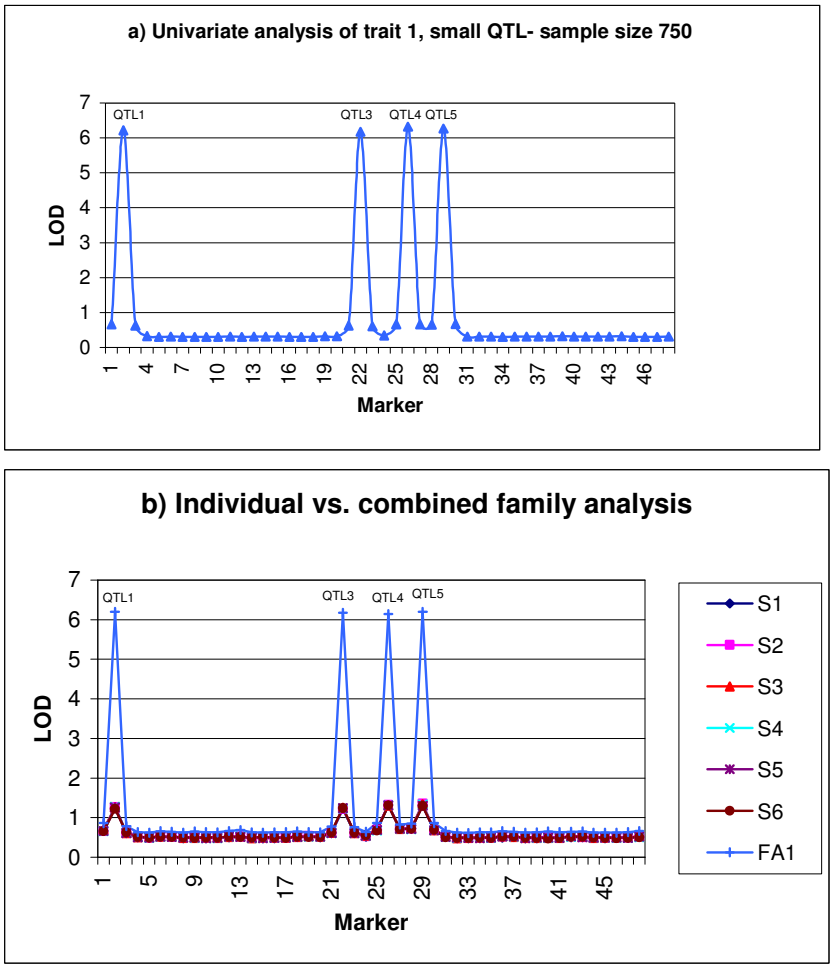

Figure 5.4 The profiles of the LOD score obtained from univariate analysis of trait 1, separate (S1-S6) and joint analysis (FA1) of six families (averaged over 1000 replicates) (same parameter setting as trait 1, four QTL are segregating in all families).

In the case of a small QTL effects for trait 1, increasing the sample size from 125 to 750 could substantially increase the power (Table 5.5). Alternatively, when the QTL were segregating in all families, information from the different families could be combined to extract more information from the available data and reach to similar power as increasing sample size to 750 individuals (Table 5.9).

It should be noticed that when a small number of QTL with small effects were segregating (low trait heritability), the chance of identifying individual QTL, using separate analyses for each family, considerably decreased (scenario a *vs.* scenario b, Tables 5.9 and 5.10). For example, the percentage of the replicates in which QTL26 was detected ranged 10.1-10.9 % and 2.2-3.3% among families S1, S2 and S3 in scenarios a and b,

respectively (Tables 5.9 and 5.10). Thus, given a QTL size, the power of QTL detection largely depended on the trait heritability.

When four QTL were not segregating in all families, more than one factor was required to fully explain the data. Therefore, three models were considered for the *trait by marker* interaction term (DIAG model, FA model with one factor (FA1) and FA model with two factors (FA2)).

Smith *et al.* (2001) discussed the need for constraints for the elements of $\mathbf{\Lambda}$ for identifiability of the variance parameters and demonstrated how these constraints could be easily implemented into the average information algorithm. As discussed by Smith *et al.* (2001), when k>1, k (k-1)/2 independent constraints must be imposed on the elements of $\mathbf{\Lambda}$ in order to ensure identifiability. The implementation of the constrains is that the number of variance parameters in the factor analytic model with k terms is given by pk+p-k(k-1)/2 . One set of constraints that fulfils this requirement is that all k(k-1)/2 elements in the upper triangle of $\mathbf{\Lambda}$ be fixed to zero (Jennrich and Schluchter, 1986). Therefore, in the case of FA2 (k=2), the first loading for the second factor (loading of FA2 on family S1) was set to be zero, leading to 17 parameters (6 loading for first factor, 5 loading for second factor, 6 specific variances) to be estimated.

The analysis of the simulated data set showed that in 81.4% of the replicates the DIAG model was inferior to the FA1 model (REMLRT=17.4 on 6 df, P<0.05, averaged over 1000 replicates), in 92.2% of the runs the FA2 model better fitted than the DIAG model (REMLRT=30.1 on 11 df, P<0.05, averaged over 1000 replicates) and in 62.0% of the replicates FA2 model was superior to the FA1 model (REMLRT=12.7 on 5 df, P<0.05, averaged over 1000 replicates).

The constrained form of $\mathbf{\Lambda}$ described above was only for computational ease. Thus, in order to obtain a meaningful interpretation of the loadings, the factors needed to be rotated. The plot of the first set of loadings against the second shows two clusters of families, namely (1) S1, S2, S3; (2) S4, S3, S6 (Figure 5.5). These results were expected as the families in the first group were simulated for QTL2 and QTL3 and the families in the second group were simulated for QTL4 and QTL5.

While the FA2 model worked well in simulation, in practice it is likely that the best approach is to fit a FA1 model in each run. If the FA1 model is significant, then the marker with highest LOD score is moved to the fixed effects part of the model. This process is repeated until the FA1 term becomes non-significant. In the simulated data herein, this process was undertaken and after two runs, the FA1 model became non-significant. By

using this process, there is no need to interpret the factor loadings. Also, adopting this strategy is much easier computationally.

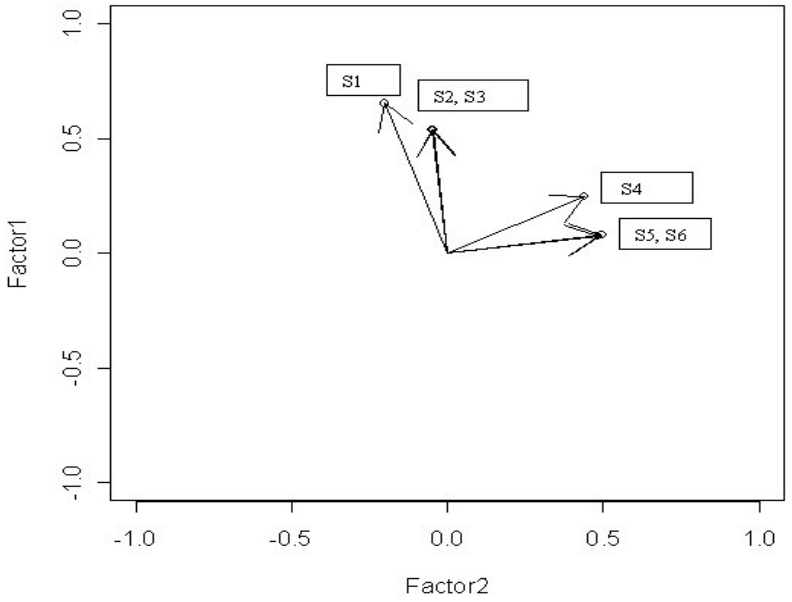

Figure 5.5 Plot of family loadings from the factor analytic model with two factors (FA2) when the QTL were not segregating in all families. S1-S6: Families 1-6.

5.8 Application to experimental data

The computer simulations demonstrated that combining information across traits or families and exploiting this multivariate multiple QTL mapping technique could substantially increase the power and the robustness of mapping studies. As reported in chapter 3, a major QTL on BTA2 is segregating in a number of families affected that a number of carcass traits. The pleiotropic effects of a myostatin functional SNP underlying this QTL were established (chapter 4). Herein, this gene was used as a model to investigate the behaviour of the multivariate multiple QTL approach in the real dataset. It was hypothesized that this gene is responsible for a relatively large part of the genetic correlations among traits and sire families.

Three carcass traits (silverside weight (kg), eye muscle area (cm^2) and channel fat (kg)) from six half-sib families (three families in Australia and three families in New Zealand, on average 128 progeny per sire) (described in chapter 2) were analysed. The model fitted to the data was similar to (5-26) where $\boldsymbol{\tau}$ included fixed effects of traits means, country, country by breed interaction, sire within country, cohort (6 combinations of year of birth and sex) in Australia, farm and birth type within breed of dam, and

slaughter group (28 levels that includes adjustments for sex and year) in the New Zealand data. **u** included the random term *trait by sire by marker* effect. The Limousin backcross animals were allowed to have different residual variation than the Jersey backcross animals.

Allowing two separate residual variance-covariances for the traits measured in the two countries, a DIAG model and a FA1 model were sequentially fitted for the *trait by sire by marker* interaction term ($\mathbf{G_p}$ in (5-25)). Using the DIAG and the FA1 model for $\mathbf{G_p}$ required 18 parameters (18 marker variance for 3 traits and 6 families) and 36 parameters (18 specific variances and 18 factor loadings) to be estimated, respectively. Since these two models are nested, a direct comparison of the models can be made. The values of the log-likelihoods when sequentially fitting two models (DIAG and FA1) to the data were considered. Fitting the DIAG model, assuming there was no marker correlation between traits and families, gave a log-likelihood value of -3125.10, while fitting FA1 model gave a log-likelihood value of -3096.66, leading to a REMLRT of 56.88 on 18 degrees of freedom, which indicated a highly significant ($P<0.0001$) FA1 model compared with the DIAG model.

Results obtained from the DIAG model indicated different markers for different traits and different sires. Using this model, markers with highest LOD score on BTA2 in sires 398, 368, 417, 394 and 402 for channel fat were BMS2626 (75.3 cM), INRA40 (10.9 cM), TGLA377 (30.7 cM), MSTN (6.0 cM), ILSTS26 (10.8 cM), respectively. Markers with the highest LOD score on BTA2 in sires 368, 361, 417 and 402 for eye muscle area were TGLA431 (11.9 cM), MSTN (6.0 cM), INRA40 (10.4 cM), MSTN (6.0 cM), respectively. There was also a peak on BTA2 for sire 402 for silverside and the marker with highest LOD score was TGLA431 (11.9 cM). These results suggested a significant marker on BTA2 but it was not clear which marker is the most significant one.

The FA1 model showed higher LOD scores for markers close to the myostatin gene than other markers in the absence of this gene in the fixed part of the model (Figure 5.6). The highest LOD score was for the myostatin marker (labelled as *MSTN*). A QTL covariate in the position of this marker was calculated and fitted as fixed effect nested within sire families to estimate the allelic effects of the gene (Table 5.11). The above process was repeated and the results from the second run showed a significant FA1 model compared with the DIAG model (REMLRT=33.84, on 18 df, $P<0.05$) indicating that the *MSTN* marker removed substantial covariation among traits and families but still there were other common QTL across traits or families.

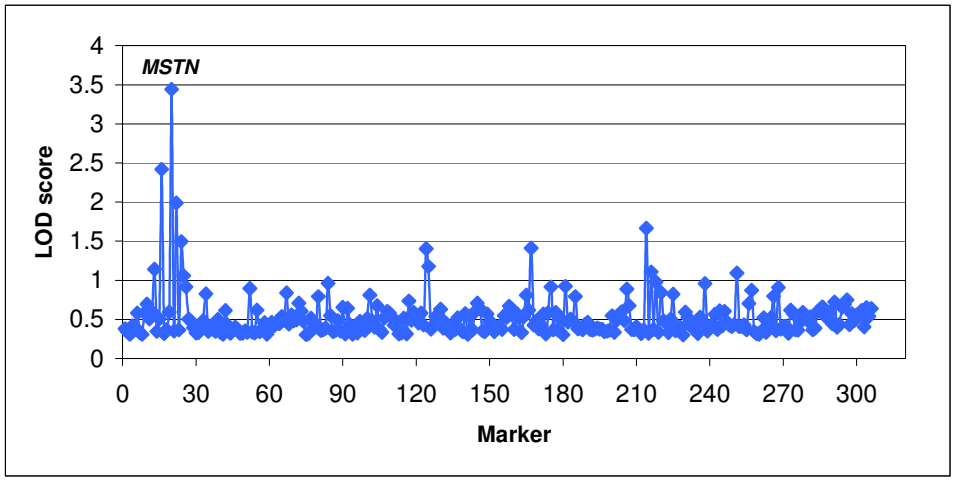

Figure 5.6 The profile of the LOD scores obtained for all of the markers from the factor analytic model (FA1) across three traits (silverside weight, eye muscle area and channel fat) and six sire families. *MSTN*: myostatin marker on BTA2.

Table 5.11 The allelic effect of the pleiotropic QTL linked to the *MSTN* marker when fitted as fixed effect.

Trait	Family	QTL effect [a]	S.E.	Trait mean	QTL effect (%) [b]	LOD
SS(KG)	398	0.29	0.24	9.0	3.2	1.0
SS(KG)	368	0.74	0.21	9.0	8.2	3.6
SS(KG)	361	0.75	0.22	9.0	8.3	3.5
SS(KG)	417	0.28	0.17	9.0	3.1	1.4
SS(KG)	402	0.61	0.16	9.0	6.8	4.3
SS(KG)	394	0.59	0.16	9.0	6.5	4.0
EMA(cm^2)	398	4.74	2.23	83.8	5.7	1.8
EMA(cm^2)	368	7.29	2.00	83.8	8.7	3.9
EMA(cm^2)	361	8.60	2.06	83.8	10.3	4.8
EMA(cm^2)	417	3.14	1.46	63.3	5.0	1.8
EMA(cm^2)	402	6.11	1.31	63.3	9.7	5.8
EMA(cm^2)	394	4.83	1.36	63.3	7.6	3.7
Chanfat (kg)	398	-0.94	0.62	12.9	-7.3	1.2
Chanfat (kg)	368	-0.37	0.55	12.9	-2.9	0.6
Chanfat (kg)	361	-0.59	0.57	12.9	-4.6	0.8
Chanfat (kg)	417	-0.48	0.40	6.5	-7.4	1.0
Chanfat (kg)	402	-1.48	0.36	6.5	-22.8	4.7
Chanfat (kg)	394	-0.57	0.37	6.5	-8.7	1.2

[a]: QTL allele substitution effect, QTL effect as percentage of the trait mean. Signs represent effects of Limousin-derived minus Jersey-derived alleles. SS: Silverside, EMA: eye muscle area, Chanfat: Channel fat

5.9 Discussion

A multitrait multiple QTL approach in the framework of the mixed-effects model was developed, allowing all markers of the entire genome to be included in the analysis simultaneously. It was shown that the method could be used for joint analysis of multiple families in gene mapping studies. Further, the approach was applied to the data from a beef cattle QTL mapping study to combine information across carcass traits and sire families.

The most difficult problem in multiple-QTL analysis comes from the model selection, which has recently become the focus of many QTL-mapping studies (Ball, 2001; Broman and Speed, 2002; Gilmour, 2007; Kao *et al*., 1999; Piepho and Gauch, 2001; Sen and Churchill, 2001; Verbyla *et al*., 2007; Xu, 2003). The multiple marker analysis approach developed and used herein is clearly a model selection strategy fitting relatively few models when compared with other methods. The method focuses first on the null hypothesis of no QTL in the genome (the variance component for the distribution of size of QTL is zero) and only after rejecting the null hypothesis, it is concluded that there is an evidence for QTL and then the QTL are located. The REML likelihood ratio test statistic (REMLRT) allows a genome-wide assessment of significance of the QTL.

In experiments one always wants to know the probability of arriving at the wrong conclusion. In QTL analysis these include (a) the conclusion that there is a segregating QTL whereas in reality there is no QTL, or (b) not detecting a QTL when a QTL is actually present. The first type of error results in a false positive (type I error) and the second in a false negative (type II error). If the type I error rate of a test is greater than nominal α level, the test is relaxed and unreliable. If the type I error rate is less than α, the test is conservative and may lack power.

To assess the accuracy and the robustness of the REMLRT, a series of simulation studies were performed. When no QTL was simulated it was expected to observe 5% of the replicates resulting in significant marker random regression variance based on the REMLRT. It would be best to use the correct distribution of the likelihood ratio test statistic under the null hypothesis, or its close approximation, as then the type I error rate would match the nominal α level. Self and Liang (1987) have discussed a number of special cases of the likelihood ratio test statistics under non-standard conditions. If only one parameter is on the boundary of the parameter space, the LRT statistic is approximately distributed as a mixture $1/2\,\chi_0^2 + 1/2\,\chi_1^2$, provided no other parameter is tested (Self and Liang, 1987; Stram and Lee, 1994). Since only one parameter (σ_m^2), is

being tested, this approximation for testing marker variance could be used. The results showed that the REML likelihood ratio test statistic fitted the above mixture distribution. Consistent with the results herein, a number of simulations (Anisimova *et al*., 2001; Goldman and Whelan, 2000; Ota *et al*., 2000) showed that the likelihood ratio test statistic fits the above mixture distribution well even when the sample size is not very large.

The simulation scheme to evaluate the power of the test consisted of only four QTL located on three chromosomes with no QTL on 6 chromosomes (66 unlinked markers and 29 linked markers). Thus the majority of genetic markers across the genome were not linked to QTL. It might be possible that allowing a common variance for all the markers leads to a low power for the REMLRT. However, the results from the simulation study showed that the REMLRT was remarkably robust.

Genome-wide searches for loci influencing quantitative traits are often plagued by low power and interpretive difficulties. Attempts to remedy these difficulties have typically relied on, and have promoted the use of, larger sample sizes, a greater density of molecular markers, and more-sophisticated statistical modeling. Many of these remedies can be costly to implement. In addition, as pointed out by Broman and Speed (2002), more sophisticated methods may not necessary lead to improved estimates.

There have been numerous publications that address the power issue in QTL mapping. For example, It has been reported that multivariate approaches can increase the power of the test and the precision of parameter estimates (Gilbert and Le Roy, 2004; Jiang and Zeng, 1995; Korol *et al*., 1995; Meuwissen and Goddard, 2004). Meta-analysis of results from different studies (Allison and Heo, 1998; Wood *et al*., 2006) or joint analysis of the original data (Kim *et al.*, 2005; Walling *et al.*, 2000) are other options that have been proposed to improve QTL mapping resolution. By exploiting a multiplicative mixed model approach, the present study has addressed these two views to improve the power of QTL identification.

When modelling multiple trait or multiple trials, it is important to avoid over-parameterization, especially in small experimental populations used for QTL detection (Sillanpaa and Corander, 2002). As Piepho (2000) suggested, to avoid over-parameterization, a certain variance-covariance structure was imposed. The specific multiplicative model considered herein was the factor analytic model. This provided a parsimonious model specification to limit the number of parameters to be estimated.

The proposed approach considered an unstructured covariance model for the residuals for traits and fitted a FA1 and a DIAG model sequentially for the interaction

terms (*trait by marker* term, *family by marker* interaction or *trait by family by marker* interaction). Considering the correlations among QTL effects at a single gene are either +1 or −1 as suggested by Goddard (2001), a factor analytic model is more appropriate structure for a pleiotropic QTL. The aim of fitting FA structure for *trait by marker* effects was to account for the genetic marker covariances among *p* traits in terms of an unknown factor. Because the model was fitted within a mixed-model framework, the importance of the covariance due to the markers could be formally tested using a comparison of a model assuming no marker correlation (the DIAG model) and a model assuming marker correlation (the FA1 model). The DIAG model was nested within the FA1 model. Therefore, residual maximum likelihood ratio tests could be used to compare these models. This provided a formal test for common QTL (across families) or pleiotropic QTL effects. An extensive simulation study was undertaken to investigate the power of the pleiotropy test. The results indicated that the test was robust when the QTL size or sample size were high. However, there was a relatively low power to detect a QTL with small peiotropic effects or in small populations.

Multiplicative models have recently been popularised (Cullis *et al*., 1998; Smith *et al*., 2001; Smith *et al*., 2005; Thompson *et al*., 2003) in the analysis of plant variety trials to model genotype by environment interactions in the analysis of the data from multi-environment trials. The key aims of a multi-environment trial analysis are to provide accurate and precise estimates of overall variety performance and to aid with the interpretation and understanding of *variety by environment* interaction (Smith *et al*., 2001). However, in multiplicative modelling of the *trait by marker* interaction herein, both common and specific factors are of interest. From a breeding point of view, common factors (loci with pleiotropic effects) are important in order to implement an indirect selection program that is getting a response to selection for a trait by selecting on a correlated trait instead. This is particularly the case when the heritability for the secondary trait is smaller (Falconer and Mackay, 1996) or when the secondary trait is difficult or expensive to measure. In addition, knowledge about such loci can be very important for animal breeders who, for example, would like to dissociate the positive correlation between birth weight and carcass weight. Also knowledge about trait-specific genes is important in the case where a breeding objective is to change one trait without affecting other traits.

Specific variances for individual traits sometimes need to be constrained to zero. If more than one trait must be constrained in this way, the factor analytic variance structure

then has less than full rank so that the use of standard information-based estimation techniques (e.g. average information algorithm) is precluded. In the current research, a sparse implementation of the average information algorithm developed by Thompson (2003) was used. The advantage of the algorithm is that convergence for FA models is fast and estimation of parameters in the reduced ranked models is possible. It should be noted that, in practice, for both simulation and the real data set, the occurrence of zero specific variances was observed for one or two traits, which means that the marker effect for the trait was completely determined by the multiplicative part of the model or it was too small to be detected. Also Smith *et al*. (2001) reported this for multi-environment trial data in Australia. In the simulation study when all QTL were segregating in all families the occurrence of zero specific variances for the families was quite common in most of the replicates. This was due to the fact that there were no specific QTL in this case.

The simulation study to examine the multitrait multiple QTL approach contained several situations including some cases, which could present difficulties for a QTL analysis. These cases included QTL near the ends of the chromosome (two QTL), QTL in coupling phase (QTL with loose linkage and QTL with relatively tight linkage), QTL in repulsion phase (two QTL), and pleiotropic QTL (two QTL). The simulation results showed the general behavior and performance of the method with respect to these situations.

In both univariate and multivariate analyses, the focus was on the problem of identifying QTL. Also this study considered an unrealistic situation in which QTL are located exactly at marker loci. However, if the genetic markers are sufficiently dense then one may dispense with interval mapping, considering only the marker loci as putative locations for QTL. Keeping this in mind, the multiple-marker analysis developed in this study will converge the true multiple-interval mapping (including all intervals). If the marker density is low, interval mapping and multiple-interval mapping may offer some advantage over marker analysis if a QTL is located in the middle of a large interval because they can point to one side rather than to two sides of the marker. If a QTL is located right at a marker, interval mapping offers no advantage over single-marker analysis (Xu, 2003). QTL mapping is not simply a gene-finding tool. QTL mapping provides critical information regarding quantitative evolutionary genetic processes. Nobody will try to clone a QTL identified by the interval mapping that is located between two distant markers. One should always try fine mapping using saturated maps in a larger population to further localize the QTL before considering cloning. From that point of view, multiple-

marker analysis used herein and multiple-interval mapping will provide the same amount of information.

Multiple trait QTL analysis should increase the power of detection, and hence, increase the significance of a QTL if the QTL is not a false positive result (Henshall and Goddard, 1999; Jiang and Zeng, 1995; Knott and Haley, 2000; Korol *et al*., 1995; Mangin *et al*., 1998). In terms of the number of QTL correctly identified, the FA1 model performed better than the univariate analysis, though it was only slightly better than univariate analysis for large QTL sizes and large populations. Apart from the issue of power, it is important to understand the nature of a genetic correlation between traits, which can provide relevant information for selection decisions. In this regard, the key advantage of FA1 over univariate analysis is that it provides a formal test for pleiotropic effects. The superior performance of multivariate analysis was due largely to its ability to detect the QTL with common effect on different traits. If the pleiotropic model is the correct one, it would be expected that fitting this model would give highest power and smallest standard deviations especially for location, as in this case, a number of traits are being used to estimate the same parameter (Jiang and Zeng, 1995; Knott and Haley, 2000).

The situation was considered where pleiotropic QTL had the same effect on different traits. In general, multitrait analysis will have a greater benefit when a QTL has small effects on one trait and the same QTL has greater effect on another trait (Sorensen *et al*., 2003) or when the pleiotropic effects of the QTL differ substantially from the most frequently observed effects of the environment and background genes, which is reflected by the environmental and background genetic correlations (Meuwissen and Goddard, 2004).

The power of the FA1 model compared to the univariate analysis was more evident when the pleiotropic effect was small. In general, a pleiotropic locus, too small to be detected by single-trait analyses, can be detected with the help of a multitrait analysis (Mangin *et al*., 1998).

With respect to false QTL detection, in general, both the univariate and multivariate methods chose a rather low portion of linked or unlinked loci to a QTL. In the simulation study herein, a relatively sparse marker density was considered. However, a high marker density may increase the likelihood of choosing linked false markers.

Multivariate and univariate analyses had similar efficiency in detecting trait-specific QTL with large effects. Trait-specific QTL with small effects could only be detected with very low efficiency using both multivariate and univariate analyses. Detecting these QTL

required a relatively large sample size. The results for small QTL when n=750 verified the applicability of the techniques. In practice, however, large mapping families may not be feasible because of the limited resources. Additionally, in most of the livestock QTL experiments, investigators tend to screen a number of families (e.g., half-sib designs), each family having relatively small size. The power of such designs is largely influenced by the number of families included in the experiment and by the size of the individual families (Weller *et al*., 1990). Also, in plants, the breeders tend to conduct marker-based recurrent selection in several populations simultaneously (Bernardo, 2004). The use of larger mapping populations would lead to fewer populations being improved, and many breeders prefer to select in a large number of populations with relatively few progeny, instead of in a few populations with many progeny (Bernardo, 2004). As Lander and Kruglyak (1995) pointed out, joint analysis of two or more similar experiments 1) could lead to more power to detect QTL not found in any individual population or 2) could be used to confirm the presence of QTL detected in only one population. With this in mind, the factor analytic model was applied in order to model the *family by marker* interaction term in simulation replicate data sets comprising six families each having 125 progeny.

The results from the separate analysis of the families showed that there was a very low power to detect QTL with small effect. It should be noted that when a small number of QTL with small effect were segregating (low trait heritability), the chance of identifying individual QTL using separate analysis of each family considerably decreased. The results from joint analysis of six families revealed that gathering information across families could remarkably increase the power. The ability to detect QTL, when QTL were segregating in all families in the simulation data set herein, reached to the same power as increasing sample size to 750 individuals.

To examine the behavior of the approach in actual datasets, the method was tested in data from a beef cattle QTL mapping experiment where the *MSTN* gene was previously found to have pleiotropic effects on meat yield and fatness (chapter 4). The results clearly showed that fitting a FA model across traits and families gave a much better indication of the QTL position than a single trait analysis or separate family analysis.

Only four traits and six families were considered in the simulation data set. However, the model, as formulated in equation (5-26) and shown in the real dataset, is obviously expandable to an unlimited number of phenotypes and families.

Walling *et al*. (2000) and Kim *et al*. (2005) demonstrated the potential benefit of a joint analysis of data from independent mapping experiments. They adopted a two-step

process so that first phenotypic data are standardised to residual standard deviation units for each population, and in the second step, they use the univariate regression interval mapping (Haley *et al*., 1994; Knott *et al*., 1996) to map QTL in independent pig populations. The proposed method herein is a one-stage process, which models residuals and genetic effects simultaneously. In addition, it includes all the markers in one analysis. Moreover, the approach utilises widely available statistical procedures, namely the linear mixed model and restricted maximum likelihood. It can easily accommodate covariates, extra sources of variation, fixed or random including polygenic effects and it can easily be generalized to experimental and crossing designs commonly used.

5.10 Summary

A multiple marker analysis approach in the framework of the mixed-effects model was developed, allowing all markers of the entire genome to be included in the analysis simultaneously. The approach was extended to multitrait and multiple family situations. It was shown through the simulation study that modeling multiple phenotypes and multiple families in a single linkage analysis simultaneously could markedly increase power, compared with modeling of each phenotype or family separately. The practical implementation of the approach in the actual mapping data from beef cattle clearly showed the ability of the method to gather information across traits and families.

Chapter 6: Multiple-trait mapping of QTL for beef traits

6.1 Introduction

The beef industry has been forced to give greater attention to consumer issues in response to the negative publicity regarding health effects of red meat and increased competition from pork and poultry. General trends in consumer preferences are toward leaner meat products with predictable eating qualities and simple preparation requirements (Marshall, 1999). Therefore, carcass and meat quality attributes are becoming increasingly economically important to the beef cattle breeders.

In addition to slaughtering conditions and technological considerations, meat characteristics depend directly on the muscle biology of live animals, which is regulated by genetic, nutritional and biological factors (te Pas and Soumillion, 2001). Among the later, the genetic factors are of prime importance because genetic improvement is both permanent and cumulative when inherited by the next generation. Genetic variation in both quantity and quality of beef is evident through differences between breeds and crossbreeds and between sires within a breed (Pitchford *et al*., 2006). Within-breed variation includes additive genetic effects and also the correlated impacts of additive genetic effects on other economically important productive and adaptive traits that affect beef production (Burrow *et al*., 2001). However, data collection for beef traits in breeding animals is generally difficult and expensive. Use of real-time ultrasound scanning for eye muscle area and fat thickness as a predictor of saleable beef yield is an effective tool for genetic evaluation of carcass quantity in young animals (Bergen *et al*., 1997; Moser *et al*., 1998; Perkins *et al*., 1992; Robinson *et al*., 1993). Ultrasonography is also potentially useful for genetic evaluation of marbling in young animals, particularly in heifers or steer half-sibs (Wilson, 1992). Other than ultrasonography, progeny testing is the only tool currently available to livestock breeders to genetically evaluate carcass and beef quality attributes, but this is a long- term and expensive option.

Over the past decade, there has been an increasing emphasis on the development of molecular genetic tools, such as DNA markers, to improve beef production and quality through marker-assisted selection (MAS). Improving meat quality genetically is difficult by conventional selection methods based on phenotype because most meat quality traits

can only be measured after slaughter. In addition, only phenotypes of relatives can be used to estimate breeding values, which limits their accuracy. These limitations make meat quality traits ideal candidates for the use of MAS. DNA tests can make evaluations available shortly after birth, or even at the embryo stage. This significantly reduces the time needed for evaluation.

In the present study, beef quality is defined by the properties of beef that are appreciated during consumption. Thus, in this definition, beef quality is directly related to the consumer preference, which are primarily focused on meat tenderness and marbling. Focusing on this definition of beef quality, only very limited information on genetic markers is available compared to the relatively large number of publications addressing genetic markers for growth and traits associated with the composition of the whole carcass.

Beef cattle breeding programs aim to select animals with the highest combined economic value for the next generation. Therefore, most breeding programs select for a combination of traits. However, selection for a specific trait can lead to genetic changes in other traits because of the genetic correlations between the traits. The main cause for the existence of a genetic correlation between traits in outbred populations is pleiotropy (Falconer and Mackay, 1996), that is, genes that affect more than one trait. Closely linked QTL can also contribute to genetic correlations because of linkage disequilibrium. Pleiotropic effects of QTL, or closely linked QTL affecting different traits, can influence the value of individual QTL for marker-assisted selection. To avoid negative side effects of selection for certain chromosomal regions or to exploit the positive effects of marker-assisted selection on other traits of interest, the presence of pleiotropic effects should be studied. In addition, characterisation of QTL based upon their pleiotropic effects will provide additional clues for the identification of candidate genes. Based on the proteins or other metabolites involved in the expression of each of those traits, selection of the most likely candidates from a list of candidate genes in that region is possible. Thus, knowledge of the pleiotropic effects should accelerate the identification of the causal DNA variants for the observed QTL effects. The contribution of identified QTL to the overall genetic correlation can be determined by multiple trait QTL analysis.

The results from the interval mapping analysis revealed a number of QTL affecting carcass traits (Chapter 3). It was shown that the *myostatin* mutation accounted for a large amount of variation in carcass traits (Chapter 4). However, it was obvious that there were other genomic regions involved in the expression of carcass and meat quality traits. Consistent with other reports (Jiang and Zeng, 1995; Korol *et al*., 2001; Mangin *et al*.,

1998), it was shown that multitrait analysis could improve the power to detect QTL (Chapter 5). Also the increasing power of QTL detection by combining information using joint analysis of mapping families was evident (Chapter 5). Taking advantage of the multiple-trait multiple marker analysis and the benefits from the joint analysis of multiple families, the goal of the work herein was to identify chromosomal regions affecting multiple traits and QTL affecting individual beef traits.

6.2 Materials and methods

6.2.1 Mapping population

The investigation was carried out on the data from the University of Adelaide's Davies Gene Mapping Project in Australia and the AgResearch cattle-mapping herd in New Zealand (Chapter 2). Briefly, the mapping populations were developed using two phenotypically divergent *Bos taurus* breeds, Jersey (J) and Limousin (L). Six half-sib families were generated by mating six F_1 crossbred sires (LJ) to purebred Jersey or Limousin cows producing 784 backcross animals. Sire derived alleles were determined for 285 microsatellite loci spread across the 29 bovine autosomes. Informative markers in the sires were chosen from the U.S. Meat Animal Research Centre map to cover over 90% of the genome in the F_1 sire families. All of the backcross progeny were genotyped for the heterozygote markers in their sires. Approximately 100 traits were recorded in the two mapping projects. The focus of this chapter is on carcass and meat quality traits.

6.2.2 QTL analysis

In order to choose a number of trait categories, a cluster analysis was conducted on the residual (phenotypic) correlations (Chapter 3) from a wide range of live animal measurements and carcass traits. Based on the results from the cluster analysis and also biological relationships between traits, four categories of beef traits were considered. The trait categories were yield traits (carcass weight, eye muscle area, weight of silverside and weight of striploin), carcass fatness (channel fat, omental fat, back fat thickness, rib fat, intramuscular fat), meat quality traits (tenderness, cooking loss, meat colour and pH) and fat quality traits (fat colour, beta-carotene concentration, melting point of adipose tissue and total mono-unsaturated fatty acids).

Fifteen animals, which had either no marker information or phenotypic data, were excluded from the analysis leaving data from 769 animals (356 and 413 animals in

Australia and New Zealand, respectively) for analysis. Progeny inheriting the first or second allele for each marker from the sire were coded as 1 or 2, respectively and they were converted to −1 or +1, respectively for this analysis. This does not affect the results (except for the value of the intercept and the scale of the effect). Uninformative markers were coded as missing but their marker state, as for other missing genotypes, was imputed from the informative flanking markers following Martinez and Curnow (1994).

Genetic and statistical model

The half-sib design is related to the backcross, that is, the segregation of alleles from one of the parents in their offspring is of interest. In the outbred context, however, genes causing variation within the population (from segregation within family) are targeted. Consider first the backcross design (Chapters 1 and 5), the backcross progeny are divided into two groups, based on their marker genotypes. With a single marker, there are only two marker genotype groups for the backcross design, and only a single contrast can be tested, the difference between the means of the two progeny marker genotype classes (M_1-M_2). However, in a half-sib design, the offspring inheriting two alternative haplotypes from the heterozygous sire are compared. The regression coefficient (Marker contrast, *M1-M2*) in a half-sib design is (Weller, 2001)

$$M1\text{-}M2=a(1-2r)+d(1-2r)(1-2p) \qquad (6\text{-}1)$$

where a, r, d and p are the additive QTL effect, recombination frequency between the genetic marker and the QTL, dominance deviation and QTL allele frequency, respectively.

If the frequencies of two QTL alleles are equal ($p=q=0.5$) in dam population, then similar to the backcross design

$$M1\text{-}M2=a(1-2r). \qquad (6\text{-}2)$$

If $r=0$ (complete linkage) and $q \neq p$ then

$$M1\text{-}M2=a+d(1-2p). \qquad (6\text{-}3)$$

Noting that $q=1-p$ and rearranging (6-3) gives

$$M1\text{-}M2=a+d(q-p) \qquad (6\text{-}4)$$

which is the effect of allele substitution (Falconer and Mackay, 1996).

Therefore, it was straightforward to extend the basic methodology developed from the backcross design (Chapter 5) to the half-sib design.

The basic genetic model (6-5) considered for the data was

$$g_{ij} = Q_{ij}a_j + u_{ij} \qquad (6\text{-}5)$$

where g_{ij} represented the genetic effect of individual i in family j, Q_{ij} was the indicator of the paternal type, either –1 or 1 (Q_{ij} was unknown, and conditional on the values of the flanking markers, the probability distribution for Q_{ij} could be obtained), a_j was the size of the QTL effect at family j (j=1,…,6), and the u_{ij} represented the other genetic effects for individual i at family j not explained by Q.

It was necessary to fit the markers/QTL within families because of the random assignment of the first haplotype, different QTL genotypes between parents, and different phases between markers and QTL between parents. The mixed model multiple QTL mapping method (Chapter 5) was extended here to 'within families'. Consider first the usual ANOVA single marker fixed linear model for the ith sire family (6-6)

$$y_{ijk} = \mu + S_i + M_{ij} + e_{ijk} \quad (6\text{-}6)$$

where y_{ijk} is phenotype of individual k from sire family i, μ represents the mean of the model, S_i is fixed effect of sire family i, M_{ij} is the jth marker fixed effect nested within sire and e_{ijk} represents the associated residuals. The progeny group inheriting sire allele M_1 and the group inheriting sire allele M_2 are compared and the significance of a segregating QTL linked to the genetic marker can be tested using an ANOVA analysis with marker effect nested within family. Under the null hypothesis of no segregating QTL, the ratio of the marker effect mean squares to the residual mean squares should have a central F-distribution. This method requires a separate analysis with each marker in turn. However, the mixed model multiple QTL mapping method (6-7) fits all the markers simultaneously as random effects with common variance within families.

$$y_{ij} = \mu + S_i + \sum_{j=1}^{m} M_{ij} + e_{ij}\,. \quad (6\text{-}7)$$

Under the null hypothesis of no QTL, the variance component for marker random regression variance for each family will be not significant when tested using a one-sided likelihood-ratio test for which twice the change in Log Likelihood is distributed as a mixture of χ_0^2 and χ_1^s with a 5% critical value of 2.79 (Stram and Lee, 1994).

If the random regression term for a family was significant, it was concluded that there was evidence for a QTL in the genome segregating in that family. The marker effects were converted to the LOD scores (Chapter 5) and the marker with highest LOD score was then chosen and added to the model as a fixed covariate nested within families. If this explained all the marker variance for all the families, the process was terminated.

Otherwise, the process was repeated. In those cases where this did not explain all the marker variance for a specific family, the analysis was repeated for that specific family.

It was assumed that the location of the QTL was the same for all families. The size of the QTL across families could, of course, be different. In order to combine information across families, it was deemed that a random effects model for the size of QTL was appropriate, leading to "borrowing strength"(accumulation of evidence) across families.

The mixed model used in this chapter to model multiple traits and multiple families was similar to (5-26) (Chapter 5). However, because of weak pedigree information (only common grandsires across countries and too few dams with multiple progeny), it was not possible to distinguish between the polygenic (unlinked to the markers being considered) and environmental residual variance. Hence, the *trait by family by marker* interaction and the residual effects were the only random effects in the model, simplifying the (5-26) as

$$\mathbf{y}_j = \mathbf{X}_j\boldsymbol{\tau}_j + \mathbf{Z}_{gj}\mathbf{u}_{gj} + \mathbf{e}_j \qquad (6\text{-}8)$$

where the *i*th element of the column vector $\mathbf{y}_j$ corresponds to the observations of trait *j* in the *i*th individual, $\boldsymbol{\tau}_j$ was a vector of fixed effects of traits means, country (Australia or New Zealand), breed of dam (Jersey or Limousin), country by breed interaction, myostatin genotype (three levels) within country, sire within country, cohort (6 combinations of year of birth and sex) in Australia, farm and birth type within breed of dam, and slaughter group (28 levels that included adjustments for sex and year) in the new Zealand data, $\mathbf{X}_j$ was a design matrix relating the fixed effects to the observations for trait *j*, $\boldsymbol{u}_{gj}$ included the random interaction term *trait by sire by marker* effect, $\mathbf{Z}_{gj}$ was a design matrix relating the random marker effects to the observations for trait *j*, and $\boldsymbol{e}_j$ was the column vector of residual deviations for trait *j*. The variance of the $\boldsymbol{u}_{gj}$ was defined by

$$\text{var}(\mathbf{u}_{gj}) = \mathbf{G}_{\mathbf{p}} \otimes \mathbf{I}_{\mathbf{m}} \qquad (6\text{-}9)$$

where **p** indicated the number of the trait by family combinations. The model in (6-9) implies that *trait* × *family* × *marker* effects are correlated between traits and sire families.

The residual variance-covariance matrices for the traits ($\mathbf{R}_j$) were allowed to vary between the two countries. A DIAG model and a FA1 model (Chapter 5) were sequentially fitted for the covariance matrix $\mathbf{G}_{\mathbf{p}}$.

The estimates of the fixed and random effects in equation (6-7) and (6-8) were obtained as solutions to the mixed-model equations (Chapter 5). In this study, the average information algorithm (Gilmour *et al*., 1995) was used to estimate the variance

components. The loadings and specific variances in the FA1 model were estimated using an algorithm described by Thompson *et al*. (2003).

The significance of the FA1 model was tested using the REML likelihood ratio (REMLRT) test. If the FA1 model was significant, the marker with highest LOD score for the factor was chosen and fitted as fixed covariate within families and the traits and the above process was repeated. If the FA1 model was not significant, a model similar to (6-7) was fitted to locate trait or family specific QTL.

Ultimately, the 285 random marker effects were replaced by a small set of fixed QTL covariates for each trait. The QTL effects estimated from the fixed model were converted to the associated Z-scores and P-values. Since LOD scores are asymptotically distributed as chi-squared, they can be estimated from P-values using the expression $LOD = 1/2(\log_{10}e)\ \chi^2 = 0.2172\chi^2$ (Lander and Botstein, 1989). The fixed markers effects, which reached a LOD score of 2.0 (χ^2 of 9.2), were considered as significant and reported in this chapter.

All analyses in this chapter were conducted using the software program ASReml (Gilmour *et al*., 2006), which is a FORTRAN program for mixed-model estimation. A wide range of models can be fitted. ASReml uses sparse matrix methods and the average information algorithm (Gilmour *et al*., 1995) for residual maximum likelihood (REML) estimation of variance parameters (Patterson and Thompson, 1971). As a result, large and complex data sets can be efficiently analysed.

6.3 Results

Four trait groups were analysed (Table 6.1). The trait groups included yield traits (Hot carcass weight, silverside weight, eye muscle area and strip loin weight), carcass fatness (channel fat, omental fat, back fat thickness, rib fat thickness and intramuscular fat), meat quality traits (tenderness, cooking loss, meat colour and pH) and fat quality traits (fat colour, beta-carotene concentration, melting point of adipose tissue and total mono-unsaturated fatty acids). As described in the methods section, the analysis consisted of two steps. In the first step, the significance of the factor model (in the case of multivariate multiple family analysis) or marker random regression variance (in the case of univariate analysis) was established to find an evidence for the genome wide significance of QTL. In the second step, the analysis proceeded to locate QTL and estimate their size of effects (QTL characterisation).

Table 6.1 Summary of the models fitted for the different trait groups

Trait group	Trait	Model
Yield		
	Hot carcass weight (kg), eye muscle area (cm^2), silverside weight (kg)	FA1
	Weight of the M. *longissimus dorsi* muscle (kg)	DIAG
Fatness		
	Channel fat (kg), Omental fat (kg)	FA1
	Intramuscular fat content (%), Fat depth at P8 (mm), Fat depth at rib 10^{th} and 11^{th} (mm)	DIAG
Meat quality		
	Wbld1, Wbld2, Wbld3, Wbld4	FA1
	Wbst1, Wbst2, Wbst3, Wbst4, pH, Clld, Clst, MC	DIAG
Fat quality		
	Melting point (^oC), Total mono-unsaturated fatty acids (%)	FA1
	B-carotene concentration (µg/g fat), Fat colour scored on biopsy sample (score)	FA1

Wbld1, 2, 3 and 4: Shear force measurements (kg) on *M. longissimus dorsi* muscle on days 1, 5, 12 and 26 after slaughter in Australia and days 0, 1/3, 1 and 4 days post mortem in New Zealand. Wbst1, 2, 3 and 4: Shear force measurements (kg) on *M. semitendinosus* muscle on days 1, 5, 12 and 26 after slaughter in Australia. Clld: Cooking loss of *M. longissimus dorsi* muscle (%). Clst: Cooking loss of *M. semitendinosus* muscle (%). MC: Meat colour (score).

6.3.1 Yield traits

Evidence for QTL. Hot carcass weight, silverside weight and eye muscle area were considered as measures of yield. Using the DIAG and the FA1 model, the *trait by sire by marker* interaction term needed 18 parameters (18 marker variance for 3 traits and 6 families) and 36 parameters (18 specific variances and 18 factor loadings) to be estimated, respectively. The values of the log-likelihoods when sequentially fitting two models (DIAG, FA1) to the data were considered. Fitting the DIAG model, assuming there was no marker correlation between traits and families, gave a log-likelihood value of –4293.2, while fitting FA1 model gave a log-likelihood value of –4276.7, leading to REMLRT of 33.0 on 18 degrees of freedom which indicated a significant ($P<0.05$) improvement in using the FA1 model compared with the DIAG model (Table 6.2). The marker with the highest LOD score value for the factor was located at 52 cM on BTA14 (Table 6.4).

This marker was moved to the fixed part of the model nested within trait and sire families and the process was repeated. A further significant FA1 term was found in the presence of the BTA14 QTL in the model. The marker with the highest LOD score value for the factor was located at 92 cM on BTA17. There was no further significant FA1 term when this marker was fitted as fixed effect in the model, leading to the conclusion that

there was no additional significant pleiotropic or common QTL across traits and sire families.

Table 6.2 Summary of residual log-likelihoods (RLL) and sequential likelihood ratio tests (REMLRT) for the models fitted to the *trait × family× marker* interaction effects for the yield traits[a]

Model	Total variance parameters	RLL	REMLRT	df	
DIAG	18	-4293.2			
FA1	36	-4276.7	33.0	18	P<0.05
DIAG + QTL1	18	-4277.4			
FA1+ QTL1	36	-4261.6	31.6	18	P<0.05
DIAG + QTL1+ QTL2	18	-4278.3			
FA1+ QTL1+ QTL2	36	-4265.0	26.6	18	N.S.

[a] Traits included hot carcass weight, silverside and eye muscle area. QTL1=BTA14, 52cM; QTL2=BTA17, 92Cm

A DIAG model was fitted in the presence of these two QTL to locate individual trait or family specific QTL. There was evidence for specific QTL affecting hot carcass weight and silverside (Table 6.3). It is possible that after fitting pleiotropic QTL and common QTL across families still there are pleiotropic QTL segregating in individual families (family-specific pleiotropic QTL). Therefore, a factor model was fitted across eye muscle area, striploin and hot carcass weight for sire families 368 and 394 which had non-zero specific variances for these traits. The results revealed further significant factors for the traits within these sire families. The weight of *M. longissimus dorsi* muscle (striploin) was not analysed in the multivariate analysis, but a univariate analysis was performed for this trait fitting the two identified pleiotropic QTL (BTA14 and BTA17 QTL) as fixed effect nested within sire family for this trait. The results indicated there were two sire families with significant marker variance for striploin weight (Table 6.3).

Table 6.3 Results of residual log-likelihoods (RLL) and likelihood ratio test (REMLRT) for the marker effects for individual yield traits and families.

	HCW		SS		EMA		LD	
Model	RLL	REMLRT	RLL	REMLRT	RLL	REMLRT	RLL	REMLRT
Full	-2758.1		-380.3		-1724.1		-279.0	
Family 398	-2758.1	0.0	-380.3	0.0	-1724.2	0.2	-279.0	0.0
Family 368	-2763.3	**10.4**	-381.5	2.4	-1724.9	1.6	-279.0	0.0
Family 361	-2758.1	0.0	-380.5	0.4	-1724.1	0.0	-281.1	**4.2**
Family 417	-2758.1	0.0	-380.4	0.2	-1724.4	0.6	-279.3	0.6
Family 402	-2758.1	0.0	-380.3	0.0	-1724.3	0.4	-279.0	0.0
Family 394	-2762.0	**7.8**	-381.7	**2.8**	-1724.2	0.2	-282.5	**7.0**

HCW: Hot carcass weight, SS: Silverside, EMA: Eye muscle area, LD: Weight of the M. *longissimus dorsi* muscle. Full model: a DIAG model for the family *by marker* interaction. Family *i* model: A DIAG model for the *family by marker* interaction in which the marker variance for the Family *i*th was fixed to zero. REMLRT was compared with a 5% critical value of 2.79; the highlighted values were significant.

Characterisation of identified QTL. The factor analytic model led to the identification of two pleiotropic QTL on BTA14 and BTA17 affecting yield traits. Individual family analysis in the presence of these two QTL revealed five other family specific-QTL linked to yield traits (three family-specific pleiotropic QTL on BTA3, 5 and 10 and two trait-specific QTL on BTA7 and 15 affecting *M. longissimus dorsi* muscle weight, Table 6.4). A multivariate multiple regression model was fitted to the data considering all the pleiotropic and family-specific QTL as fixed effects and allowing separate residual variances for the two countries. The results showed that a number of the identified QTL had significant effect on *M. longissimus dorsi* muscle weight.

In all cases but the BTA5 QTL, the sign of the QTL effects were in the expected direction, that is, the Limousin allele increased yield. The estimated effect size of the identified QTL for yield traits ranged from –8.7 to 8.7% of the trait means (Table 6.4).

Table 6.4 Identified QTL for yield traits showing their chromosomal (BTA) position and size of effects as a percentage of the trait means.

BTA	Trait	Position [a]	Marker	Family	Mean	Effect (L-J)	LOD
3	EMA	68	HUJ246	394	63.3	5.4	2.0
3	HCW	68	HUJ246	394	237.8	6.3	3.9
3	SS	68	HUJ246	394	9.0	5.3	2.7
5	EMA	52	BL37	368	83.8	-6.2	2.3
5	HCW	52	BL37	368	344.0	-8.0	5.2
5	SS	52	BL37	368	9.0	-8.7	4.5
7	LD	82	INRA192	394	6.4	7.3	3.6
10	EMA	97	TGLA272	394	63.3	5.4	2.0
10	HCW	97	TGLA272	394	237.8	5.8	3.2
10	LD	97	TGLA272	394	6.4	6.5	3.0
10	SS	97	TGLA272	394	9.0	4.9	2.3
14	HCW	52	BM302	361	344.0	6.6	3.8
14	LD	52	BM302	361	6.6	8.7	3.1
14	SS	52	BM302	361	9.0	7.4	3.6
14	HCW	52	BM302	368	344.0	6.6	3.4
14	LD	52	BM302	368	6.6	7.7	2.4
14	SS	52	BM302	368	9.0	7.1	3.0
14	HCW	52	BM302	394	237.8	4.1	2.2
14	SS	52	BM302	394	9.0	4.1	2.0
14	HCW	52	BM302	398	344.0	4.5	2.0
14	SS	52	BM302	398	9.0	5.6	2.1
14	HCW	52	BM302	402	237.8	3.3	2.0
14	SS	52	BM302	402	9.0	4.5	2.6
14	HCW	52	BM302	417	237.8	4.4	2.2
14	SS	52	BM302	417	9.0	4.7	2.3
15	LD	77	BM4325	361	9.0	7.2	3.4
17	EMA	92	BM1233	368	83.8	7.7	3.2
17	HCW	92	BM1233	368	344.0	5.7	3.0
17	LD	92	BM1233	368	6.6	7.6	2.5
17	SS	92	BM1233	368	9.0	6.3	2.7
17	HCW	92	BM1233	398	344.0	5.2	2.0

EMA: Eye muscle area (cm^2), HCW: Hot carcass weight (kg), SS: Silverside weight (kg), LD: Striploin weight (kg), [a] QTL position (cM) based on Ihara *et al*. (2004), signs represent effect of Limousin-derived minus Jersey-derived alleles, LOD: logarithm of odds.

6.3.2 Carcass fatness

Evidence for QTL. The hypothesis in the present research was that the overall fatness was under the control of major genes and a number of individual QTL were underlying specific fat traits. A factor analytic model was formed across the six families and three fatness traits (Channel fat (internal fat), rib fat (external fat) and intramuscular fat). However, the factor model was not significant indicating that there were no pleiotropic QTL affecting these three traits. There were two measurements of internal fatness, channel fat and omental fat. Considering these two traits only, a factor analytic model was fitted for the *trait by sire by marker* interaction term. The results indicated a significant factor model (Table 6.5). The

marker with highest LOD score was located on BTA10. This marker was fitted as a fixed effect within traits and sire families, and the process was repeated until the factor model became not significant. The analysis revealed five pleiotropic QTL affecting channel fat and omental fat.

Table 6.5 Summary of residual log-likelihoods (RLL) and sequential likelihood ratio tests (REMLRT) for the models fitted to the *trait × family × marker* interaction effects for channel and omental fat

Model	Total variance parameters	RLL	REMLRT	df	
DIAG	12	-2343.6			
FA1	24	-2330.5	26.2	12	P<0.01
DIAG + QTL1	12	-1682.6			
FA1+ QTL1	24	-1670.5		12	P<0.05
DIAG + QTL1+ QTL2	12	-1693.9			
FA1+ QTL1+ QTL2	24	-1682.5	24.2	12	P<0.05
DIAG + QTL1+ QTL2+ QTL3	12	-1705.3		12	P<0.05
FA1+ QTL1+ QTL2+ QTL3	24	-1694.6	21.4		
DIAG + QTL1+ QTL2+ QTL3+ QTL4	12	-1710.8			
FA1 + QTL1+ QTL2+ QTL3+ QTL4	24	-1699.6	22.4	12	P<0.05
DIAG + QTL1+ QTL2+ QTL3+ QTL4+ QTL5	12	-1715.5			
FA1 + QTL1+ QTL2+ QTL3+ QTL4+ QTL5	24	-1705.8	19.4		N.S.

QTL1=BTA10, 11 cM; QTL2=BTA9, 65 cM; QTL3=BTA21, 0.0 cM; QTL4=BTA13, 91 cM; QTL5=BTA6, 8 cM

Univariate analyses were conducted in the presence of the five pleiotropic QTL to locate trait-specific QTL or family-specific QTL for the fatness traits. There was evidence for specific QTL for the fatness traits studied herein (Table 6.6).

Table 6.6 Results of residual log-likelihoods (RLL) and likelihood ratio test (REMLRT) for the marker effects for individual fat traits and families

	Kidney fat		Rib fat depth		Omental fat		Intra muscular fat	
Model	RLL	REMLRT	RLL	REMLRT	RLL	REMLRT	RLL	REMLRT
Full	-1066.6		-1059.6		-662.6		-660.3	0.0
Family 398	-1068.5	**3.8**	-1061.1	**3.0**	-663.3	1.4	-662.9	**5.2**
Family 368	-1070.3	**7.4**	-1059.6	0.0	-666.0	**6.8**	-660.3	0.0
Family 361	-1066.7	0.2	-1059.6	0.0	-662.8	0.4	-660.3	0.0
Family 417	-1070.2	**7.2**	-1059.6	0.0	-664.5	**3.8**	-662.3	**4.0**
Family 402	-1079.2	**25.2**	-1059.9	0.6	-664.1	**3.0**	-660.3	0.0
Family 394	-1068.2	**3.2**	-1059.6	0.0	-666.2	**7.2**	-660.3	0.0

Full model: a DIAG model for the *family by marker* interaction. Family *i* model: A DIAG model for the *family by marker* interaction in which the marker variance for the family *i*th was fixed to zero. REMLRT was compared with a 5% critical value of 2.79; the highlighted values were significant.

Characterisation of identified QTL. In total, 34 QTL distributed over 24 autosomes affecting five fatness measurements were found (Table 6.7). The fatness traits were indicators of external fat (P8 and rib fat), internal fat (channel fat and omental fat), and intramuscular fat (IMF). There was no indication of a pleiotropic QTL affecting external, internal and intramuscular fat depositions and as mentioned in the previous section, there was no significant factor model for these traits across six families. However, the factor model formed for omental fat and channel fat across six families was significant, resulting in the identification of chromosomal regions influencing both channel fat and omental fat (BTA6 and BTA13, Table 6.7).

The univariate analysis provided evidence supporting the presence of two QTL on BTA1 affecting P8 and channel fat. The BTA1 QTL for P8 fat was located in the proximal end of the chromosome and the channel fat QTL was on the distal end of the chromosome. The two QTL were segregating in two different families and the Limousin allele was responsible for increasing fatness in both QTL.

A highly significant marker on BTA4 was found to be linked to both intramuscular fat (LOD=2.1-4.3 in different families) and channel fat (LOD=5.3). The QTL was segregating in different families and the signs of effects were different for the two traits. Animals inheriting the Limousin allele from the sire had less channel fat and more intramuscular fat compared to animals inheriting the Jersey allele.

On chromosome 6, a QTL was evident at 8 cM which primarily affected channel fat and omental fat, and was segregating in sire family 417. This QTL was identified using the factor analytic model. The Limousin allele was associated with decreased fatness for this QTL (19 and 35% of the trait mean for omental fat and channel fat, respectively, Table 6.7). Also on chromosome 6, two trait specific QTL were mapped affecting P8 and channel fat. The BTA6 QTL for P8 and channel fat were located in the middle and at the distal end of the chromosome.

The factor analytic model revealed a marker at 65 cM on BTA9 associated with channel fat. The QTL linked to this marker was segregating in three families. The allele substitution effect for this QTL ranged from 12 to 25 % of the trait mean and the Jersey allele was responsible for increased fat. The analysis in the presence of this QTL as fixed effect within sire families revealed another QTL at 42 cM on this linkage group affecting channel fat segregating in two families. The allele substitution effect for this QTL was between 12 to 16% of the trait mean in the two families. Conversely to the other QTL, the Limousin allele was responsible for increased fat in this QTL. There was strong evidence

Table 6.7 Identified QTL for fatness traits with their chromosomal (BTA) position and size of effects as a percentage of the trait means.

BTA	Trait	Position[a]	Marker	Family	Mean	Effect (L-J)	LOD
1	P8am	15	BMS2321	361	12.4	15.7	3.3
1	Chanfat	122	BM1824	398	12.9	18.8	4.1
2	Rbft	97	BM6444	398	9.8	-21.7	2.7
2	Rbft	97	BM6444	417	7.1	30.4	2.0
3	Chanfat	52	BM4129	402	6.5	11.9	2.5
3	Omenfat	94	BMS2145	368	12.1	13.7	2.7
3	Rbft	126	BMC4214	398	9.8	18.8	2.6
4	IMF	102	TGLA159	361	5.2	12.0	2.1
4	IMF	102	TGLA159	398	5.2	17.7	4.3
4	Chanfat	102	TGLA159	402	6.5	-22.6	5.3
5	Chanfat	12	BMS610	402	6.5	-13.3	2.3
6	Chanfat	8	INRA133	417	6.5	-34.6	6.6
6	Omenfat	8	INRA133	417	3.7	-18.7	3.0
6	P8am	35	BM1329	398	12.4	-12.1	3.9
6	Chanfat	78	BM4621	394	6.5	-16.4	2.4
7	Chanfat	0	BM7160	417	6.5	16.0	2.3
9	Chanfat	42	BMS817	398	12.9	14.4	2.0
9	Chanfat	42	BMS817	402	6.5	13.3	2.1
9	Chanfat	65	BMS1290	361	12.9	-11.8	2.1
9	Chanfat	65	BMS1290	368	12.9	-13.6	2.0
9	Chanfat	65	BMS1290	402	6.5	-25.2	5.2
9	IMF	78	TGLA73	361	5.2	-11.6	2.4
9	IMF	78	TGLA73	417	3.9	-46.4	4.2
10	IMF	11	CSSM38	398	5.2	-18.1	4.8
10	Chanfat	11	CSSM38	402	6.5	-16.2	3.4
11	Chanfat	40	RM096	394	6.5	-13.0	2.2
13	Omenfat	91	AGLA232	368	12.1	-13.5	2.7
13	Omenfat	91	AGLA232	394	3.7	15.2	2.9
13	Chanfat	91	AGLA232	402	6.5	15.5	2.6
14	IMF	52	BM302	417	3.9	-30.9	2.7
14	Chanfat	44	RM011	368	12.9	-11.1	2.0
14	Chanfat	44	RM011	402	6.5	-12.1	2.3
15	Chanfat	110	BMS429	398	12.9	19.7	3.0
16	Chanfat	78	BM719	402	6.5	-14.6	2.7
17	Chanfat	66	BM8125	394	6.5	-16.6	2.8
17	P8am	81	BM1862	368	12.4	9.9	3.4
18	Omenfat	48	INRA63	402	3.7	15.4	3.2
19	Chanfat	78	BMS1069	394	6.5	14.6	2.7
21	Chanfat	0	BM8115	402	6.5	24.5	5.5
22	Chanfat	31	AGLA13	394	6.5	14.8	2.7
25	Chanfat	32	BM737	402	6.5	-23.1	5.1
26	Rbft	3	BMS651	361	9.8	-14.7	2.0
26	Rbft	3	BMS651	398	9.8	-15.4	2.0
26	Chanfat	27	BM1314	402	6.5	13.6	2.6
26	Omenfat	52	BM6041	417	3.7	-17.9	3.5
27	Omenfat	64	BM203	394	3.7	-19.7	4.0
28	Rbft	1	BMC2208	398	9.8	27.6	4.4
29	Chanfat	66	BMS1948	368	12.9	-12.1	2.7

P8am: Fat depth at the position 8 on the rump (mm), Chanfat: channel fat, Omenfat: Omental fat Rbft: Fat depth between the 10th and 11th ribs (mm), IMF: Intramuscular fat content, [a] QTL position (cM) based on Ihara *et al*. (2004), signs represent effect of Limousin-derived minus Jersey-derived alleles, LOD: logarithm of odds.

for a QTL associated with intramuscular fat (IMF) on BTA9 (LOD=2.4-4.2 in different families). The QTL was linked to a marker at position 78 cM, adjacent to a marker linked to channel fat at position 65 cM. The allele substitution effect for this QTL for IMF was between 12 to 46% of the trait mean and the individuals inheriting the Jersey allele had higher intramuscular fat than those inherited the Limousin allele.

A marker in the proximal part of BTA10 was associated with both channel fat and IMF. The QTL linked to this marker was segregating in two different families. The Limousin allele decreased both IMF and channel fat.

Using the factor analytic model, a pleiotropic QTL was identified at 91 cM on BTA13 affecting both omental fat and channel fat. In two families, the Limousin allele was associated with increased fatness. Interestingly, in another family, the Jersey allele was responsible for increased fatness.

A number of trait-specific QTL were identified for channel fat, of which the most significant QTL were located on the BTA21 (LOD=5.5) and BTA25 (LOD=5.1). The substitution allele effects for BTA21 QTL and BTA25 QTL were 24 and 23% of the trait mean, respectively. The Limousin allele for the BTA21 QTL and the BTA25 QTL was associated with increased and decreased fatness, respectively.

QTL specific to omental fat were located on BTA18, 26 and 27. The allele substitution effect for these QTL ranged from 15 to 20% of the trait mean (Table 6.7). Lastly, significant association between rib fat and a marker located at the centromeric region of BTA28 was found (LOD=4.4). The QTL linked to this marker was segregating in one family with allele substitution effect of 28% of the trait mean. The animals inherited the Jersey allele at this QTL were leaner than those that inherited the Limousin allele.

6.3.3 Meat quality

Evidence for QTL. A number of traits related to the beef quality were analysed in this chapter. They included tenderness measured as shear-force, cooking loss, meat color and pH. Tenderness measurements of *M. longissimus dorsi* muscle were taken both in Australia and New Zealand. Tenderness of *M. semitendinosus* muscle was also measured in Australia. A factor analytic model with one multiplicative term was fitted across six families and four tenderness measurements (shear force measurements on *M. longissimus dorsi* muscle on days 1, 5, 12 and 26 after slaughter in Australia and tenderness measurements on *M. longissimus dorsi* muscle on days 0, 1/3, 1 and 4 days post mortem in

New Zealand). The FA1 model was superior to the DIAG model (Table 6.8). The results showed that the marker with highest LOD score for the factor was on BTA18. This marker was fitted as fixed covariate within the families and four trait measurements, and the DIAG and FA1 models were sequentially fitted for the *trait by family by marker* interaction term. The better fit of the model to the data was achieved when using FA1 model compared with the DIAG model. The marker with highest LOD score for the factor was on BTA29. Fitting this marker as fixed covariate in the model caused a substantial reduction in the marker covariation across the families and trait measurements, leading to a non-significant FA1 model compared with DIAG model (Table 6.8).

Table 6.8 Summary of residual log-likelihoods (RLL) and sequential likelihood ratio tests (REMLRT) for the models fitted to the *trait × family× marker* interaction effects for meat tenderness[a].

Model	Total variance parameters	RLL	REMLRT	df	
Diag	12	-1694.4			
FA1	24	-1670.5	47.8	24	P<0.01
Diag + QTL1	12	-1728.3			
FA1+ QTL1	24	-1705.7	45.2	24	P<0.01
Diag + QTL1+ QTL2	12	-1766.8			
FA1+ QTL1+ QTL2	24	-1753.0	27.6	24	N.S.

[a] Four tenderness measurements included shear force measurements on *M. longissimus dorci* muscle on days 1, 5, 12 and 26 after slaughter in Australia and tenderness measurements on *M. longissimus dorci* muscle on days 0, 1/3, 1 and 4 days post mortem in New Zealand. QTL1=BTA18, 84 cM QTL2=BTA29, 62 cM.

The BTA18 and BTA29 QTL were fitted as fixed covariates within sire families and the DIAG model was fitted for the individual trait measurements (shear force measurements on *M. longissimus dorsi* muscle (strip loin) in both Australia and New Zealand and shear force measurements on *M. semitendinosus* in Australia). The results indicated significant marker variances for shear force measurements on *M. semitendinosus* on days 1, 12 and 26 after slaughter in family 368 and shear force measurements on *M. semitendinosus* on day 5 after slaughter in family 361 (Table 6.9). Also the marker variances for shear force measured on striploin at *rigor mortis* and shear force measured on striploin on day 26 after slaughter were significant (families 417 and 368, respectively).

With respect to the cooking loss of *M. longissimus dorsi* muscle, the residual log-likelihood for a DIAG model for six sires was –776.5. Dropping marker effects for the families 368, 361 and 417 led to significant decreases in the log-likelihood (log-likelihood

of –778.3, –779.0 and –778.8 and REMLRT of 3.6, 5.0 and 4.6, respectively, P<0.05). The marker variances for other families were not significant.

Table 6.9 Results of residual log-likelihoods (RLL) and likelihood ratio test (REMLRT) for the marker effects for individual tenderness traits and families.

	Wbst1		Wbst2		Wbst3		Wbst4	
Model	RLL	REMLRT	RLL	REMLRT	RLL	REMLRT	RLL	REMLRT
Full	-43.3		-23.7		-0.18		20.9	
Family 398	-43.3	0.0	-23.9	0.4	-0.42		20.2	
Family 368	-45.4	**4.2**	-23.7	0.0	-2.3	**4.2**	14.9	**12.0**
Family 361	-43.4	0.2	-25.3	**3.2**	0.18		20.9	

Wbst1, 2, 3 and 4 are shear force measurements on *M. semitendinosus* muscle on days 1, 5, 12 and 26 after slaughter in Australia. Full model: a DIAG model for the *family by marker* interaction. Family *i* model: A DIAG model for the family *by marker* interaction in which the marker variance for the family *i*th was fixed to zero. REMLRT was compared with a 5% critical value of 2.79; the highlighted values were significant.

Marker random regression variance was not significant for meat color suggesting no evidence for QTL affecting meat color. Also there was no significant factor analytic model for meat pH measured on the *M. semitendinosus* (pHst) or *M. longissimus dorsi* muscles (pHld). However, there were significant marker variances for meat pH in the sire families 368 (pHst and pHld), 368 and 417 (pHld).

Characterisation of identified QTL. Eight linkage groups were established as being associated with meat tenderness (Table 6.10). Two QTL were identified using the factor analytic model having significant effects on tenderness of the *M. longissimus dorsi* muscle. The identified QTL were located at 84 cM and 63 cM on BTA18 and BTA29, respectively. There was no measurement of tenderness on the *M. semitendinosus* muscle in New Zealand project. However, these identified QTL for *M. longissimus dorsi* muscle were tested on *M. semitendinosus* tenderness measured in the Australian project. The results revealed significant effects of these QTL on tenderness of the *M. semitendinosus* muscle measured at day 1 (BTA18 QTL), day 12 and day 26 after slaughter (BTA29 QTL, Table 6.10). The Limousin allele in the BTA29 QTL was associated with increased toughness of both muscles in all the segregating families with associated allelic effects of 6% to 20% of the trait mean. However, for the BTA18 QTL, the Limousin allele was responsible for tenderness of the *M. longissimus dorsi* muscle in sire family 417 and toughness the of *M. longissimus dorsi* and *M. semitendinosus* muscles in sire families 361 and 398, respectively.

The univariate analysis of the Australian shear force data indicated two markers on BTA5 linked to the tenderness of both *M. semitendinosus* and *M. longissimus dorsi* muscles. One of the markers was located at 35 cM and the other marker resided at 65 cM

on this linkage group. The two identified QTL were in favour of the Jersey allele with substitution effects ranging from 7% to 26% of the trait means. The two identified QTL were tested on the tenderness measurements of the *M. longissimus dorsi* muscle in the New Zealand data. The effect of the second QTL (QTL at position 65 cM) was significant on tenderness of the *M. longissimus dorsi* muscle in sire family 394.

Table 6.10 Identified QTL for meat tenderness showing their chromosomal (BTA) position and size of effects as a percentage of the trait means.

BTA	Trait	Position[a]	Marker	Family	Mean	Effect (L-J)	LOD
5	wbst1	35	OARFCB5	361	5.1	7.2	2.7
5	wbst3	35	OARFCB5	361	4.7	7.0	2.7
5	wbst2	35	OARFCB5	361	4.8	8.7	3.5
5	wbld1	65	MAF23	368	4.7	26.4	2.8
5	wbld4	65	MAF23	368	3.8	16.2	2.0
5	wbst1	65	MAF23	368	5.1	12.9	2.7
5	wbst4	65	MAF23	368	4.4	11.9	2.4
5	wbld1	65	MAF23	394	14.6	-11.0	2.3
10	wbld1	24	BMS528	361	4.7	-14.9	2.8
10	wbld2	24	BMS528	361	4.3	-12.2	2.8
10	wbst3	24	BMS528	368	4.7	6.4	2.3
10	wbst2	24	BMS528	398	4.8	-6.9	2.1
13	wbld1	9	TGLA23	398	4.7	12.0	2.0
15	wbld3	39	JAB4	394	7.4	-12.2	2.9
15	wbld2	39	JAB4	394	10.8	-11.9	2.4
18	wbld4	84	TGLA227	361	3.8	10.3	2.2
18	wbst1	84	TGLA227	398	5.1	6.9	2.3
18	wbld1	84	TGLA227	417	14.6	-10.7	3.0
18	wbld3	84	TGLA227	417	7.4	-12.7	2.6
18	wbld2	84	TGLA227	417	10.8	-12.1	2.1
22	wbst1	31	AGLA13	368	5.1	-9.8	4.1
22	wbst3	31	AGLA13	368	4.7	-6.6	2.3
22	wbst4	31	AGLA13	368	4.4	-6.3	2.1
27	wbld4	0	BMS2168	368	3.8	-14.4	3.5
27	wbst3	0	BMS2168	368	4.7	-6.6	2.4
28	wbst1	43	BM6466	368	5.1	5.7	2.2
29	wbst3	63	BMC1206	368	4.7	6.0	2.0
29	wbst4	63	BMC1206	368	4.4	6.2	2.0
29	wbld3	63	BMC1206	417	7.4	20.4	6.0
29	wbld2	63	BMC1206	417	10.8	19.2	4.5

Wbst1, 2, 3 and 4: Shear force measurements on *M. semitendinosus* muscle on days 1, 5, 12 and 26 after slaughter in Australia; Wbld1, 2, 3 and 4: Shear force measurements on *M. longissimus dorsi* muscle on days 1, 5, 12 and 26 after slaughter in Australia and tenderness measurements on *M. longissimus dorsi* muscle on days 0, 1/3, 1 and 4 days post mortem in New Zealand. [a] QTL position (cM) based on Ihara *et al*. (2004), signs represent effect of Limousin-derived minus Jersey-derived alleles. LOD: logarithm of odds.

A QTL influencing meat tenderness was located at the proximal end of BTA10 segregating in families 361, 368 and 398. The effects of the QTL were in favor of the Limousin allele in two families (sires 361 and 398). However, in sire family 368, the Limousin allele was associated with increased toughness.

A marker located on BTA22 was significantly (LOD=2.1-4.1 for different trait measurements) linked to a QTL affecting tenderness of the *M. semitendinosus* muscle measured as Warner-Bratzler shear force at days 1, 12 and 26 after slaughter. The QTL was segregating in sire family 368 and the allelic effects ranged from 6 to 9% for different measurements. The offspring inheriting the Limousin allele had more tender meat than those inheriting the Jersey allele. The other tenderness QTL identified in the present research were located on BTA13, 15 and 27 and 28 (Table 6.10).

There was evidence supporting the association of several linkage groups with meat pH (Table 6.11). However, rather small QTL effects were detected for this trait, which ranged from 0.4 to 2% of the trait mean.

The pH of both *M. longissimus dorsi* and *M. semitendinosus* muscles was measured in the Australian project. With respect to the pH measured on the *M. semitendinosus* muscle (pHst), significant marker random regression variance was established only in one sire family (family 368). The DNA markers linked to the pH in this family were located on BTA5, 10 and 24. The Limousin allele was associated with increased pH in these three QTL. The BTA24 QTL was interesting as it was linked to the pH measured on the *M. longissimus dorsi* muscle (pHld) in this family. In addition, this QTL was segregating in family 402 from New Zealand project as well.

Other markers with effects on pH for *M. longissimus dorsi* muscle were identified on BTA1, 6, 7, 8, 9, 12, 13, 15, 16, 18 and 27 (Table 6.11). BTA6 seems to harbor three QTL for pHld. A QTL in the centromeric region of the chromosome and another QTL in the middle section of the chromosome were both segregating in the sire family 368 with different sign of effects. The sire family 361 was segregating for another QTL located at the distal part of this chromosome. Two markers at 10 and 35 cM on BTA27 were chosen as flanking markers for a QTL affecting on pH segregating on family 368. The markers had similar sign and size of effect.

Regions on BTA6, 14, 15 and 24 contained loci associated with cooking loss of the *M. longissimus dorsi* muscle (Table 6.11). The BTA6 QTL was located at the centromeric region of the chromosome linked to the same marker as for *M. longissimus dorsi* muscle pH. The effect of the Limousin allele in this QTL was an increase in cooking loss with allele substitution effect of 5.5% of the trait mean. The Limousin alleles in the QTL on BTA14 and 24 were also associated with increase cooking loss. However, the identified QTL on BTA15 was in favor of the Limousin allele so that animals inheriting the

Limousin allele had less cooking loss (allele substitution effect of 5% of the trait mean) compared with those inheriting the Jersey allele.

Table 6.11 Identified QTL for pH and cooking with their chromosomal (BTA) position and size of effects as a percentage of the trait means.

BTA	Trait	Position[a]	Marker	Family	Mean	Effect (L-J)	LOD
1	pHld	77	BM6506	417	5.4	-0.7	2.9
1	pHld	99	CSSM032	361	5.6	0.7	2.0
1	pHld	99	CSSM032	368	5.6	1.0	3.0
5	pHst	91	BMS1248	368	5.7	0.8	2.9
6	pHld	8	INRA133	368	5.6	0.8	2.6
6	CLld	8	INRA133	417	23.4	5.5	3.2
6	pHld	54	BM143	368	5.6	-0.9	2.9
6	pHld	127	BM2320	361	5.6	1.0	2.9
7	pHld	91	BMS1331	361	5.6	1.0	2.7
8	pHld	123	BMS836	361	5.6	-0.8	2.3
9	pHld	65	BMS1290	368	5.6	-1.6	5.5
10	pHst	24	BMS525	368	5.7	0.8	2.7
12	pHld	57	BM6404	361	5.6	1.5	5.5
13	pHld	74	RM327	368	5.6	1.1	3.6
14	CLld	14	BMS1678	368	21.7	7.4	5.9
15	CLld	77	BM4325	361	21.7	-5.1	3.3
15	pHld	97	BM848	361	5.6	-1.0	3.0
16	pHld	78	BM719	361	5.6	-1.6	5.5
18	pHld	3	BMS1355	361	5.6	1.4	4.3
24	pHld	8	BM7151	368	5.6	0.8	2.3
24	pHst	8	BM7151	368	5.7	0.6	2.2
24	pHld	8	BM7151	402	5.4	0.4	2.0
24	CLld	44	BMS1743	368	21.7	4.6	3.3
27	pHld	10	BM6526	368	5.6	-2.0	5.9
27	pHld	35	CSSM43	368	5.6	-2.0	5.9

pHld: pH measured on the *M. longissimus dorsi* muscle, pHst: pH measured on the *M. semitendinosus* muscle, CLld: Cooking loss of the *M. longissimus dorsi* muscle. [a] QTL position (cM) based on Ihara *et al.* (2004). Signs represent effect of Limousin-derived minus Jersey-derived alleles. LOD: logarithm of odds.

6.3.4 Fat quality

Evidence for QTL. The traits related to the fat quality were fat color scores of adipose biopsy samples (FCB), β-carotene content (BC) of the fat samples, total mono-unsaturated fatty acids, and melting point of the fat tissue. Preliminary inspection of the fat colour and β-carotene data indicated that the distribution of the β-carotene values was positively skewed. A logarithmic transformation was examined as a means to reduce the skewness (as performed for milk carotenoid concentration by Winkelman *et al.*, 1999). Because the transformation to natural logarithms resulted in a substantial reduction in skewness, the transformed values were used in the analyses.

A factor analytic model was fitted across the six families, fat colour and β-carotene concentration. A substantial increase in log likelihood was observed when the FA1 model was used. The marker with highest LOD score was located on BTA4. This marker was fitted as fixed covariate within the families and traits and the process was repeated. The analysis led to seven pleiotropic QTL (or common QTL across families) (Table 6.12).

In order to map trait or family specific QTL, the DIAG model was fitted in the presence of the seven identified pleiotropic/common markers as fixed covariates. The results revealed significant marker random regression variances for fat colour and β-carotene concentration in the families of sires 398 and 394 (Table 6.13), suggesting that there was evidence for family or trait-specific QTL.

Table 6.12 Summary of residual log-likelihoods (RLL) and sequential likelihood ratio tests (REMLRT) for the models fitted to the *trait × family × marker* interaction effects [a]

Model	Total variance parameters	RLL	REMLRT	df	
Diag	12	364.3			
FA1	24	381.2	33.8	12	P<0.001
Diag + QTL1	12	341.2			
FA1+ QTL1	24	355.2	28.0	12	P<0.01
Diag + QTL1+ QTL2	12	318.7			
FA1+ QTL1+ QTL2	24	332.4	27.4	12	P<0.01
Diag + QTL1+ QTL2+ QTL3	12	294.0			
FA1+ QTL1+ QTL2+ QTL3	24	306.2	24.4	12	P<0.05
Diag + QTL1+ QTL2+ QTL3+ QTL4	12	266.5			
FA1+ QTL1+ QTL2+ QTL3+ QTL4	24	279.4	25.8	12	P<0.05
Diag + QTL1+ QTL2+ QTL3+ QTL4+ QTL5	12	231.9			
FA1 + QTL1+ QTL2+ QTL3+ QTL4+ QTL5	24	249.1	34.4	12	P<0.001
Diag + QTL1+ QTL2+ QTL3+ QTL4+ QTL5+QTL6	12	210.0			
FA1 + QTL1+ QTL2+ QTL3+ QTL4+ QTL5+QTL6	24	221.2	22.4	12	P<0.05
Diag + QTL1+ QTL2+ QTL3+ QTL4+ QTL5+QTL6+QTL7	12	184.8			
FA1 + QTL1+ QTL2+ QTL3+ QTL4+ QTL5+QTL6+QTL7	24	193.1	16.6	12	N.S.

[a] Traits included the fat color scores of adipose biopsy samples and β-carotene concentration. QTL1=BTA4, 12cM; QTL2=BTA1, 15 cM; QTL3=BTA12, 81 cM; QTL4=BTA9, 37 cM; QTL5=BTA19, 59 cM; QTL6=BTA11, 54 cM; QTL7=BTA15, 14 cM.

Table 6.13 Results of residual log-likelihoods (RLL) and likelihood ratio test (REMLRT) for the marker effects for individual fat colour traits and families.

	Fat colour score [a]		β-carotene concentration	
Model	RLL	REMLRT	RLL	REMLRT
Full	-89.6		220.3	
Family 398	-94.3	**9.4**	218.2	**4.2**
Family 368	-89.6	0.0	219.4	1.8
Family 361	-90.0	0.8	220.3	0.0
Family 417	-89.6	0.0	220.3	0.0
Family 402	-90.1	1.0	219.6	1.4
Family 394	-91.1	**3.0**	215.6	**9.4**

Full model: a DIAG model for the family *by marker* interaction. Family *i* model: A DIAG model for the family *by marker* interaction in which the marker variance for the family *i*th was fixed to zero. REMLRT was compared with a 5% critical value of 2.79; the highlighted values were significant. [a] fat color scores of adipose biopsy samples.

Bivariate analysis of the melting point of the adipose tissue and total mono-unsaturated fatty acids was conducted. A factor analytic model was formed across these two traits and the six families. There was a significant FA1 model and the marker with highest LOD score was on BTA26. Fitting this marker as fixed covariate did not remove the marker covariation across traits and families, suggesting there were other common QTL affecting the two traits or segregating in different families. Therefore, another factor model was fitted in the presence of the BTA26 QTL as fixed effect. The likelihood ratio test showed a significant FA1 model. The marker with highest LOD score was located on BTA16. Fitting this marker as fixed covariate removed the marker correlation across the traits and families, leading to non-significant FA1 model compared with DIAG model (Table 6.14).

Table 6.14 Summary of residual log-likelihoods (RLL) and sequential likelihood ratio tests (REMLRT) for the models fitted to the *trait × family × marker* interaction effects [a].

Model	Total variance parameters	RLL	REMLRT	DF	
Diag	12	-2244.1			
FA1	24	-2232.3	23.6	12	P<0.05
Diag + QTL1	12	-2125.4			
FA1+ QTL1	24	-2113.9	23.0	12	P<0.05
Diag + QTL1+ QTL2	12	2014.7			
FA1+ QTL1+ QTL2	24	2009.1	11.2	12	N.S.

[a] Fatty acid traits included mono-unsaturated fatty acids percent and melting point. QTL1=BTA26, 27cM; QTL2=BTA16, 89Cm

Considering the two identified QTL (BTA26 and BTA16 QTL) as fixed covariates, the DIAG model was fitted to test for evidence of trait or family specific QTL. The results showed significant marker random regression variances in sire families 417 and 394 for the

two traits and a significant marker variance for melting point in sire family 368 (Table 6.15), indicating that there was evidence for family or trait-specific QTL.

Table 6.15 Results of residual log-likelihoods (RLL) and likelihood ratio test (REMLRT) for the marker effects for individual fat composition traits and families.

	MUFA		Meltpt	
Model	RLL	REMLRT	RLL	REMLRT
Full	-1193.4		-1059.0	
Family 398	-1193.4	0.0	-1059.0	0.0
Family 368	-1194.2	1.6	-1061.9	**5.8**
Family 361	-1193.4	0.0	-1059.0	0.0
Family 417	-1195.5	**4.2**	-1060.7	**3.4**
Family 402	-1193.4	0.0	-1059.0	0.0
Family 394	-1199.8	**12.8**	-1060.8	**3.6**

Full model: a DIAG model for the family *by marker* interaction. Family *i* model: A DIAG model for the family *by marker* interaction in which the marker variance for family *i*th was fixed to zero. REMLRT was compared with a 5% critical value of 2.79; the highlighted values were significant. MUFA= total mono-unsaturated fatty acids. Meltpt= melting point of the fat tissue.

Characterisation of identified QTL. The analysis of the fat colour score on biopsy samples (FCB) and beta-carotene content (BC) data revealed putative QTL distributed over 17 autosomes; most of them affected both traits (Table 6.16). A marker located at the centromeric region of BTA1 was linked to a QTL with highly significant effects on fat colour score (LOD=7.0). β-carotene content was also implicated with this marker. The estimated allele substitution effects of QTL for fat colour score and β-carotene were 32% and 22% of the traits means, respectively. The animals that inherited the Jersey allele in this QTL deposited more beta-carotene and produced fat with more yellow colour than those who inherited the Limousin allele.

A QTL was located at the proximal end of BTA4 also affected the fat colour score and β-carotene. Similar to the BTA1 QTL, the Jersey allele was associated with more yellow fat colour and higher β-carotene deposition.

The analysis provided significant evidence for a pleiotropic QTL linked to a marker at 37 cM on BTA9 affecting both fat colour score and β-carotene. The QTL was segregating in three families with allele substitution effects ranging from 16 to 36% of the trait means in different sire families. In sire families 394 and 417, the Jersey allele was responsible for more yellow fat but in family 398, the Limousin allele was associated with higher β-carotene concentration and more yellow fat.

Table 6.16 Identified QTL for fat colour and β-carotene content with their chromosomal (BTA) position and size of effects as a percentage of the trait means.

BTA	Trait	Position[a]	Marker	Family	Mean	Effect (L-J)	LOD
1	BC	15	BMS2321	394	1.7	-21.7	2.0
1	FCB	15	BMS2321	398	1.9	-32.1	7.0
2	FCB	109	BMS356	398	1.9	20.3	2.9
4	BC	13	BMS1788	394	1.7	-25.5	3.2
4	FCB	13	BMS1788	398	1.9	-21.5	4.3
8	FCB	21	RM372	361	1.9	15.1	2.5
9	FCB	37	RM216	394	1.6	-15.9	2.3
9	BC	37	RM216	398	1.1	35.9	5.1
9	FCB	37	RM216	398	1.9	15.5	2.6
9	FCB	37	RM216	417	1.6	-20.0	2.8
11	BC	55	BMS1716	398	1.1	24.9	2.9
11	FCB	55	BMS1716	398	1.9	20.0	4.0
11	FCB	81	BMS1048	394	1.6	24.3	3.4
12	FCB	57	BM6404	398	1.9	22.0	2.9
12	BC	81	RM113	361	1.1	-20.0	2.1
12	FCB	81	RM113	398	1.9	-22.9	2.7
13	BC	23	BMS1742	394	1.7	-22.4	3.0
13	FCB	23	BMS1742	394	1.6	-17.4	2.6
14	FCB	14	BMS1678	361	1.9	15.6	3.1
14	BC	14	BMS1678	398	1.1	-45.0	5.4
14	FCB	14	BMS1678	398	1.9	-22.9	3.6
14	FCB	80	BM4513	394	1.6	-17.3	2.5
15	BC	14	BMS2533	398	1.1	-29.3	3.5
15	FCB	14	BMS2533	398	1.9	-28.0	6.5
15	FCB	39	JAB4	402	1.6	-29.7	4.3
16	BC	26	BMS538	394	1.7	-22.0	2.8
19	BC	59	BM17132	398	1.1	-23.4	2.5
19	FCB	59	BM17132	398	1.9	-15.1	2.4
22	BC	83	BM4102	398	1.1	28.4	2.6
22	FCB	83	BM4102	398	1.9	-22.7	3.5
23	BC	42	CYP21	394	1.7	34.2	5.5
23	FCB	42	CYP21	394	1.6	16.1	2.1
24	BC	8	BM7151	394	1.7	-17.5	2.0
24	FCB	8	BM7151	394	1.6	-22.1	3.5
27	FCB	10	BM6526	398	1.9	24.2	3.6
28	BC	25	BL25	394	1.7	19.1	2.1
28	FCB	25	BL25	394	1.6	18.7	2.5
28	BC	25	BL25	398	1.1	-25.8	2.4

BC: β-carotene content. FCB: Fat colour score on biopsy samples. [a] QTL position (cM) based on Ihara *et al*. (2004). Signs represent effect of Limousin-derived minus Jersey-derived alleles. LOD: logarithm of odds.

The factor analytic model revealed a pleiotropic QTL at 55 cM on BTA11 affecting both fat colour score and β-carotene. The QTL was segregating in sire family 398. Fitting this QTL as a fixed covariate in sire family 394 did not remove the marker variance for fat colour score in this family. Individual family analysis in the presence of this QTL as fixed indicated another marker at position 81 cM associated with fat colour score.

BTA14 was significantly linked to the both β-carotene content and fat colour score (LOD=2.5-5.4 for different families and traits). A pleiotropic QTL segregating in sire families 361 and 398 was mapped in the proximal end of the chromosome. In sire family 398, the Jersey allele was associated with higher β-carotene deposition and more yellow fat. The allele substitution effects of the QTL for fat colour score and β-carotene content in this family were 23 to 45% of the trait means, respectively. The individual family analyses in the presence of this QTL as fixed covariate revealed another QTL located at 80 cM on BTA14 segregating in sire family 394 affecting fat colour score.

A marker located in the centromeric region of BTA15 was significantly associated with both β-carotene content (LOD=3.5) and fat colour score (LOD=6.5). The offspring who inherited the Jersey allele in this QTL accumulated more β-carotene and hence had fat with more yellow colour. The other pleiotropic QTL for β-carotene content and fat colour score were located on BTA19, 22, 23, 24,and 28. Trait-specific QTL were also mapped on BTA2, 8 and 27 for fat colour score and on BTA16 for β-carotene content (Table 6.16).

Markers with significant effects on both total mono-unsaturated fatty acids (MUFA), and melting point of the fat tissue (Meltpt) were identified on BTA2, 16 and 26. There were also chromosomal regions linked only to either total mono-unsaturated fatty acids (BTA4, 7, 9, 17, 19 and 28) or Meltpt (BTA3, 13, 22 and 24) (Table 6.17).

A marker located on the distal end of BTA2 was significantly associated with both mono-unsaturated fatty acids and melting point. The QTL linked to this marker was segregating in sire families 398 and 417. However, the QTL affected both traits only in sire family 417. In this family, the offspring inheriting the Limousin allele had higher mono-unsaturated fatty acids and lower melting point values when compared with those inheriting the Jersey allele. This QTL influenced melting point in sire family 398 with the allele substitution effect of 4% of the trait mean. However, in this family, the Limousin allele was associated with higher melting point values.

The effect of a Limousin allele on BTA16 was a decrease in the mono-unsaturated fatty acids, and subsequently, an increase in melting point of the adipose tissue (Table 6.17). The estimated allele substitution effects of the QTL were –3.2 to 3.6 for mono-unsaturated fatty acids and melting point, respectively.

There was evidence supporting the presence of a QTL located at 27 cM on BTA26 segregating in multiple sire families affecting both mono-unsaturated fatty acids and melting point. The effect of the Limousin allele in this QTL for mono-unsaturated fatty

acids and melting point ranged from 2.6 to 4.3% and –3.4 to 3.2% of the trait means, respectively (Table 6.17).

Table 6.17 Identified QTL for fatty acid composition with their chromosomal (BTA) position and size of effects as a percentage of the trait means.

BTA	Trait	Position[a]	Marker	Family	Mean	Effect (L-J)	LOD
2	Meltpt	115	BM2113	398	36.9	4.0	2.1
2	Meltpt	115	BM2113	417	37.5	-3.6	2.6
2	Mufa	115	BM2113	417	50.8	2.4	2.0
3	Meltpt	126	BMC4214	368	36.9	3.2	2.0
4	Mufa	34	BMS1237	394	50.8	-2.3	2.1
7	Mufa	80	BMS792	368	50.4	-3.6	2.0
9	Mufa	91	BM4208	394	50.8	2.8	2.3
13	Meltpt	91	AGLA232	394	37.5	-2.8	2.1
16	Meltpt	89	HUJ625	394	37.5	3.6	3.2
16	Mufa	89	HUJ625	394	50.8	-3.2	3.6
17	Mufa	81	BM1862	417	50.8	-3.0	3.0
19	Mufa	43	BMS2142	394	50.8	2.8	2.9
22	Meltpt	49	BMS390	417	37.5	-3.7	2.6
24	Meltpt	26	CSSM31	368	36.9	3.4	2.0
26	Meltpt	27	BM1314	394	37.5	-3.4	2.0
26	Mufa	27	BM1314	394	50.8	3.2	2.5
26	Mufa	27	BM1314	398	50.4	4.3	2.3
26	Meltpt	27	BM1314	402	37.5	-3.3	2.9
26	Mufa	27	BM1314	402	50.8	2.6	2.7
26	Meltpt	27	BM1314	417	37.5	3.2	2.1
28	Mufa	25	BL25	394	50.8	2.8	2.7

Meltpt: Melting point of the fat tissue, Mufa: Total mono-unsaturated fatty acids. [a] QTL position (cM) based on Ihara *et al.* (2004). Signs represent effect of Limousin-derived minus Jersey-derived alleles. LOD: logarithm of odds.

6.4 Discussion

Multi-trait multiple QTL modeling across six half-sib families was performed to search for DNA markers linked to the traits related to meat yield and quality. The analysis provided evidence for several QTL associated with yield and carcass fatness, plus meat and fat quality. The identified QTL were distributed on almost all the bovine autosomes.

6.4.1 Yield traits

Seven linkage groups (BTA3, 5, 7, 10, 14, 15 and 17) were found for hot carcass weight, eye muscle area, weight of the *M. longissimus dorsi* muscle and silverside weight (Table 6.4). Five of the identified QTL had pleiotropic effects on these traits. In all of the identified QTL, except QTL on BTA5, the Limousin allele increased yield, as was expected based on the breed characteristics. Two QTL specific to the *M. longissimus dorsi*

muscle weight were identified (BTA7 and BTA15). However, there was no indication of QTL specific for eye muscle area as an indication of meat yield.

Evidence for the existence of a pleiotropic QTL associated with yield traits was identified on BTA3. Previous studies reported QTL for carcass traits on BTA3 close to the marker found herein (Casas *et al.*, 2004b; Casas *et al.*, 2003; Casas *et al.*, 2001). Retail product yield is an estimate of the amount of saleable product from a given carcass and is considered an important carcass composition trait (Casas *et al*., 2001). Evidence of a QTL affecting this trait and also marbling score on chromosome 3 has been reported (Casas *et al.*, 2004b; Casas *et al.*, 2001). Casas *et al*. (2003) also reported a QTL on BTA3 for fat yield and carcass fat percent predicted from rib dissection.

BTA5 has been also implicated in the expression of several quantitative traits in beef cattle (Casas *et al.*, 2003; Casas *et al.*, 2000; Mizoshita *et al.*, 2004; Stone *et al.*, 1999). Stone *et al*. (1999) reported a QTL close to marker BL37 on BTA5 affecting bone, dressing percentage and wholesale rib fat yield. This marker is located at 52 cM on BTA5 and was linked to a number of yield traits in the present study. Casas *et al*. (2000) identified QTL on BTA5 for fat thickness, USDA yield grade, retail product yield, and meat tenderness in offspring from a Piedmontese × Angus sire. Casas *et al*. (2003) also found a QTL for eye muscle area, marbling, and fat yield on BTA5 in the same region where yield QTL resided in the present study (Table 6.4). Mizoshita *et al*. (2004) reported QTL for carcass yield estimate and marbling score on BTA5 at 50 cM and 30 cM, respectively. Thus, to date, there are several independent lines of evidence to support the existence of QTL for carcass traits on bovine chromosome 5. Although QTL from different studies lie in the same genomic region, it would be expected that different genes may be affecting different traits. Significant QTL for *M. longissimus dorsi* area are important because this trait is of great value in the beef industry. Further studies are needed to identify whether the same gene or genes are responsible for the observed QTL effects for eye muscle area and the other yield traits.

It is perhaps premature to suggest candidate genes for the observed QTL effects; however, it is interesting that the marker linked to the yield traits on BTA5 was located at 52 cM between two strong candidate genes *MYF-5* and 6 (18 cM) and *IGF-1* (73 cM). Moody *et al*. (1997) found an association between *IGF1* and growth in Hereford cattle. Curi *et al*. (2005) also reported the effects of a polymorphism in the *IGF-I* gene on growth and carcass traits in beef cattle, suggesting the possibility that this or a neighboring gene could be associated with the observed QTL effects on BTA5.

Both the prenatal formation of muscle tissue (myogenesis) and postnatal muscle tissue growth (hypertrophic growth of myofibers without the formation of new myofibers) are regulated by the muscle regulatory factors (MRF) gene family (te Pas and Soumillion, 2001). The MRF gene family consisting of four genes: myogenin, *MyoD1*, *myf-5*, and *myf-6*. The importance of this gene family in relation to meat production has been discussed (te Pas and Soumillion, 2001). While the MRF genes are the central regulators of myogenesis, insulin-like growth factor-I (*IGF-I*) and other growth factors form a regulatory network influencing MRF gene expression pattern. Whether *MYF-5* or *IGF-1* or both of them were associated with the observed QTL effects remains to be discovered.

The myogenic regulatory factor, *MYOD1*, maps to 40 cM on BTA15 and is a transcription factor expressed in during skeletal muscle myogenesis and regeneration (Atchley *et al.*, 1994). A QTL specifically affected weight of the *M. longissimus dorsi* muscle was identified at 77 cM on this chromosome. Although this gene is located 37 cM apart from the marker linked to the QTL, low resolution of the marker span used herein makes it difficult to ascertain whether this gene could possibly underlie the observed QTL effect.

The telomeric region of BTA10 was linked to all of the yield traits. Casas *et al.* (Casas *et al.*, 2003) suggested the presence of a QTL for hot carcass weight in a family obtained from a crossbred Brahman by Hereford sire on chromosome 10, spanning from 0 to 30 cM. However, the pleiotropic QTL identified for yield traits on BTA10 in the study herein was linked to a marker located at 97 cM. It is unlikely that the same gene (or group of genes) on this chromosome is underlying the same trait in the two studies. Differences in breed composition may influence the expression of different genes affecting yield traits.

Compelling evidence was observed for a QTL linked to a marker at 52 cM on BTA14 that affects yield traits. This Limousin-derived pleiotropic QTL affected the carcass size but had no effect on eye muscle area. Kim *et al.* (2003) reported a suggestive QTL for carcass weight on BTA14 linked to RM011, which is located at 44 cM on the map used in the present study. Mizoshita *et al.* (2004) reported QTL for carcass weight and growth traits at 32-49 cM on this chromosome. There are many possible candidate genes in this region. One of the most promising is *myc*, a transcription factor known to activate growth promoting genes and repress growth arresting genes.

This is the first report of evidence for pleiotropic QTL affecting yield traits on BTA10 and 17. Also there is no other report of QTL for yield traits on BTA7 and 15 where the present study found QTL that specifically affected the weight of *M. longissimus dorsi*

muscle. Further research aimed at fine mapping the identified QTL will help to discover the gene or genes responsible for the observed effects.

6.4.2 Carcass fatness

The problem of excess fat in carcass is ubiquitous and has serious consequences for the animal industry on four levels: health perceptions of consumers, wasteful production of an undesired biological component, labour costs associated with trimming waste fat, and lower biological efficiencies of fatter animals (Eisen, 1989). Breeding for optimal fatness distribution is therefore one of the major goals for increasing profitability in the beef industry. Mapping of QTL and identification of causative genes that affect fat metabolism will enhance the progress toward this goal.

Several chromosomes exhibited evidence for the presence of putative QTL for carcass fatness (Table 6.7). Two DNA regions on BTA1 were linked to the fatness traits, one at the centromeric end of the chromosome at 15 cM (affecting external fat measured as fat thickness at P8) and the other at the telomeric end of the linkage group at 122 cM (affecting internal fat). Kim *et al.* (2003), using a random model mapped a QTL at the telomeric end of BTA1 (126 cM), affecting external fat deposition measured as subcutaneous fat thickness between the 12^{th} and 13^{th} ribs but using a line-cross model, they found a QTL at the centromeric region of the chromosome (at 1cM) for this trait from the same data set.

A possible candidate gene for the fatness QTL on BTA1 is cystatin B, which maps to 94 cM on this chromosome. Russo *et al.* (2002) reported association between cathepsin *B* and cystatin *B* genes with backfat thickness and average daily gain in pigs, respectively. Cystatin *B* is a member of the Family 1 (stefin) cysteine proteinase inhibitors and was originally discovered as an inhibitor of cathepsin B (Russo *et al.*, 2002).

It was shown that myostatin gene affected fatness traits in the population studied herein (Chapter 4). However, after removing the effect of this gene, a DNA marker located at 97 cM on BTA2 was linked to subcutaneous fat thickness between the 10^{th} and 11^{th} ribs. MacNeil and Grosz (2002) detected a QTL at 120 cM on chromosome 2 for marbling score using a double backcross derived from a Line 1 Herefords and a Composite Gene Combination line. However, Casas *et al.* (2004b) and Stone *et al.* (1999) showed evidence suggesting the presence of a QTL for marbling score between 25 and 45 cM on chromosome 2. It is possible that two QTL for fatness traits are segregating on this

chromosome, one in the middle of the chromosome and another in the telomeric region of the chromosome. The finding herein supports Casas *et al*. (2004b), who suggested that other genes on BTA2 apart from myostatin are involved in fatness.

Evidence exists for QTL affecting fatness in two regions of chromosome 3, one in the middle of the chromosome, affecting internal fat, and the other in the telomeric region, influencing external fat deposition. QTL on BTA3 for intramuscular fat, measured as marbling score, have been reported between 0-42 cM (Casas *et al.*, 2004b; Casas *et al.*, 2003) and 40-90 cM (Casas *et al*., 2001). Brennan *et al*. (2006) have identified *NHLH2* as a central nervous system regulator of adult body weight, and mapped the bovine *NHLH2* gene on the middle of BTA3. In mice, targeted deletion of the neuronal transcription factor *nhlh2* resulted in adult-onset obesity because of reduced exercise (Brennan *et al*., 2006).

A pleiotropic QTL affecting both internal fat and intramuscular fat was found on BTA4. The Limousin allele was associated with increased intramuscular fat (12-18% of the trait mean) and decreased channel fat (23% of the trait mean). The leptin (87 cM) and insulin-like growth factor binding protein-3 (*IGFBP-3*) (72 cM) genes are on BTA4. The increased leptin production derived from the leptin gene has been associated with fat deposition in swine (Houseknecht *et al*., 1998). Geary *et al*. (2003) reported that serum concentrations of leptin were associated with marbling, back fat depth and kidney, pelvic, and heart fat and quality grad in beef cattle. Polymorphisms in the bovine leptin promoter have been associated with serum leptin concentration, growth, feed intake, feeding behavior, and carcass fatness (Nkrumah *et al*., 2005). However, no association was found between polymorphisms in leptin gene and carcass traits (Sellick, 2002) in the mapping population studied herein.

The centromeric end of BTA6 was associated with both internal and external fat depositions. Additionally, a marker located at 78 cM was linked to the internal fat depots. The Limousin allele in both DNA regions and in all the segregating families was associated with decreased fat. However, whether the two QTL regions represent the same QTL or separate QTL for internal fat and backfat thickness remains to be determined. Li *et al*. (2004) found three haplotypes spanned regions on BTA6 (8.2-11.8 cM, 63.6-68.1 cM and 81.5-83.0 cM) that had significant associations with back fatness. Numerous researchers have studied QTL on BTA 6 in dairy cattle, indicating the presence of multiple QTL affecting milk yield, fat and protein percentage on this chromosome (Freyer *et al*., 2002; Mosig *et al*., 2001; Olsen *et al*., 2002; Spelman *et al*., 1996; Wiener *et al*., 2000).

These results suggest that same gene or a cluster of genes in this part of the bovine genome is responsible for fatness regardless of breed.

Various studies have proposed candidate genes for the QTL on BTA6 based on their putative physiological role on the trait of interest (Cohen-Zinder *et al*., 2005; Sanders *et al*., 2006; Weikard *et al*., 2005). Both the bovine peroxysome proliferator-activated receptor-γ coactivator-1α (*PPARGC1A*, also known as *PGC1α* located at 21 cM on BTA6) and cholecystokinin A receptor (*CCKAR*) genes stand out as strong positional and functional candidates underlying the BTA6 QTL effect on fat. Weikard *et al*. (2005) identified *PPARGC1A* as a plausible positional and functional candidate gene for milk fat QTL on BTA6 because of its chromosomal position and its key role in energy, fat, and glucose metabolism. Weikard *et al*. (2005) observed a significant association between an SNP in intron 9 of the *PPARGC1A* gene and milk fat yield in a major dairy cattle population. The *CCKAR* gene has been implicated in the development of obesity based on the obesity gene map (Snyder, *et al*., 2004). In addition, Cohen-Zinder *et al*. (2005) reported that ATP binding cassette, subfamily, G, member 2 (*ABCG2*) gene located on BTA6 decreased milk yield and increased both protein and fat concentration. Moreover, Olsen *et al*. (2005) postulated that the function of polycystin 2 (*PKD2*) best corresponds with the BTA6 QTL effect, while Cohen-Zinder *et al*. (2005) suggested that the secreted phosphoprotein 1 (*SPP1*) has an essential role in mammary gland differentiation and branching of the mammary epithelial ductal system, and therefore, is a prime candidate. The association of the above mentioned candidate genes for the observed QTL effects on BTA6 for beef fatness needs to be discovered.

BTA9 was linked to both internal and intramuscular fat. Casas *et al*. (2003) reported a QTL for marbling score between 46 and 76 cM on chromosome 9. Marbling score was used as an estimation of the level of intramuscular fat deposition at the region between the 10^{th} to 11^{th} ribs of the *M. longissimus dorsi*. The marbling data were not analysed using the method presented in this chapter. However, the regression interval mapping revealed a marbling QTL in this region of the chromosome (Chapter 2). Georges *et al*. (1995) detected a significant QTL for fat yield in this chromosome in dairy cattle. The QTL for internal fat deposition in the study herein, the marbling QTL and the milk fat yield QTL all reside in a similar location. It could be hypothesized that the same gene or genes in this genomic region on BTA9 could be responsible for production of fat that is deposited intramuscularly and the level of fat in the milk of cattle.

There was evidence suggesting that a QTL in the centromeric region of BTA10 associated with both internal and intramuscular fat deposition. QTL for marbling have been reported at 0 to 28 cM on BTA10 (Casas *et al.*, 2003).

A DNA region between 44-52 cM on BTA14 was linked to carcass fatness. Casas *et al*. (2000) reported a suggestive QTL for fat deposition traits at 33 cM on chromosome 14. Zhang *et al*. (1998) reported QTL in similar location for fat percentage and fat yield in dairy cattle. A number of studies found QTL in the centromeric region of this chromosome in both beef cattle (Moore *et al*., 2003) and dairy cattle (Ashwell *et al.*, 2004; Ashwell *et al.*, 2001; Rodriguez-Zas *et al.*, 2002). It is feasible that BTA14 harbors genes responsible for fat production in cattle, regardless of specialisation (beef versus dairy breeds).

Making use of the information contained in the genetic bovine map (http://www.ensembl.org/Bos_taurus), several positional candidate genes can be identified on BTA14 that are directly related to fat deposition. As an example, the fatty acid binding protein (*A-FABP*) has been reported to be associated with intramuscular fat content in pigs (Gerbens *et al*., 1998) and this could be a candidate gene for the observed QTL effect on intramuscular fat content. Recently, Michal *et al*. (2006) reported that the bovine fatty acid binding protein 4 gene is significantly associated with marbling and subcutaneous fat depth in Wagyu and Limousin cross animals.

Barendse (1999) identified the gene encoding thyroglobolin (*TG*) as a positional candidate, based on its close linkage to the microsatellite locus CSSM66 on BTA14. Furthermore, as its product is the precursor of hormones involved in lipid metabolism, *TG* was also considered as a functional candidate gene. McPeake (2003) indicated that the *TG* gene has been associated with intramuscular fat deposition in long-fed cattle.

The gene encoding diacylglycerol O-acyltransferase 1 (*DGAT1*) enzyme, which catalyses the final step of the triglyceride synthesis, has been demonstrated to affect the fat content of milk (Grisart *et al*., 2002; Winter *et al*., 2002). Thaller *et al*. (2003) reported significant effects of both *TG* and *DGAT1* on the fat content of muscle in German Holstein and Charolais breeds. Moore *et al*. (2003) detected a QTL linked to the CSSM66 locus, which located between *DGAT1* and *TG*, for backfat in a commercial line of *Bos taurus*. However, they reported neither of the two polymorphisms of candidate genes tested, *DGAT1* and *TG*, showed a significant association with backfat. Recently, Casas *et al*., (2005) reported association between a SNP marker in the thyroglobulin gene and fat thickness and eye muscle area but not with marbling score. They found no associations between a SNP in the *DGAT1* gene and carcass composition traits in a population of *Bus*

indicus. The low precision of the QTL mapping makes necessary further studies to evaluate the possible implication of these candidate genes.

The identified QTL on BTA15 for internal fat was linked to a marker located at 110 cM on the chromosome. QTL for internal fat on this chromosome have been reported (Casas *et al.*, 2003; Kim *et al.*, 2003). However, it is unlikely that the QTL found in the study herein represents the same QTL on this chromosome as the position of the suggestive QTL reported by Kim, *et al.* (2003) and Casas, *et al.* (2003) were between 1-61 cM and 21-69 cM.

A marker located at 81 cM on BTA17 was associated with fat depth measured at the P8 site in a sire family from the Australian project. There was also another marker located at 66 cM on BTA17 affecting kidney fat in a sire family from the New Zealand project. The Limousin allele was associated with increased fat thickness and decreased kidney fat on this chromosome. It is likely that the P8 fat QTL on BTA17 represents the same pleiotropic QTL found for yield traits linked to a marker located at 92 cM on this chromosome. MacNeil and Grosz (2002) suggested a QTL for internal fat deposition located at 78 cM on this chromosome. These results, collectively, support the existence of a pleiotropic QTL in this region of the chromosome affecting carcass fatness and yield.

A QTL linked to BMS1069 located at 77cm on BTA19 was associated with internal fat deposition. QTL affecting external fat on this region of the chromosome have been reported (Kim *et al.*, 2003; Li *et al.*, 2004; Taylor *et al.*, 1998). However, there was no indication of the effect of this QTL on external fat in this chromosome. It is possible that a gene or genes involving in bovine fatness are located in this genomic region but their function may differ depending on genetic background and environmental conditions.

A highly significant QTL for kidney fat was located at the centromeric region of BTA21. The fatness QTL on this chromosome is unique as it was not associated with intramuscular fat content, which is important in beef industry.

Two neighboring markers in the centromeric region of BTA26 were linked to channel fat and fat thickness measured at the 10^{th} to 11^{th} ribs. Stone *et al*. (1999) and Casas *et al*. (2004b) suggested the presence of a QTL for retail product yield and fat yield in this region of the chromosome. The present research also found another QTL in the middle region of the chromosome affecting omental fat.

There was evidence for a QTL in the telomeric region of BTA27 for omental fat segregating in sire family 394 linked to the microsatellite marker BM203. QTL have been reported close to the telomeric region of BTA27, affecting marbling (Casas *et al*., 2000),

fat percentage and fat yield (Zhang *et al*., 1998) in dairy cattle. The combined results of these studies suggest that a putative QTL associated with fat deposition is located in this genomic region in cattle. However, more studies would be necessary to establish whether the same genes in this genomic region influence fat deposition in bovine.

6.4.3 Meat quality

Eating quality of meat depends on several important characteristics, including appearance, color, taste, fat content, texture, and tenderness. A number of DNA regions were linked to the beef tenderness, pH and cooking loss.

Tenderness. Meat tenderness, which is a very critical trait in determining consumer satisfaction, can be dominated by a number of environmental influences, although the genetics of the animal can also play a significant role (Shackelford *et al*., 1994). Nine linkage groups have been identified as harboring QTL for meat tenderness (Table 6.10).

A DNA region in the middle of BTA5 was linked to shear force measured on both *M. semitendinosus* and *longissimus* muscles. This finding confirmed the detected QTL for meat tenderness by Casas *et al*. (2000) who reported a QTL located at 70 cM on this chromosome. Additionally, a marker located at 35 cM on this chromosome was linked to shear force measured on the *M. semitendinuosus* muscle. These results suggest two QTL for meat tenderness are located on BTA5, one QTL affecting meat from different muscles and another QTL with muscle-specific effects.

Tenderness of meat is determined by the rate and extent of post-mortem proteolysis. The calpain proteolytic system has been identified biochemically as having a critical role in meat tenderisation. The calpain system is a family of calcium-dependent proteases, consisting of μ-calpain (encoded by the calpain-1 gene and the calpain small subunit gene, and requires micromolar Ca^{2+} for activity), m-calpain (encoded by the calpain-2 gene and the calpain small subunit gene and requires millimolar Ca^{2+} for activity), and calpastatin (endogenous inhibitor of calpains). The calpain-1 large subunit (*CAPN1*), calpain small subunit 1 (*CAPNS1*or *CSS1*), calpain-2 gene (*CAPN2*) and calpastatin (*CAST*) are found on BTA29 (Smith *et al*., 2000), BTA18 (Band *et al*., 2000), BTA16 (http://www.ensembl.org/Bos_taurus) and BTA7 (Bishop *et al*., 1993), respectively. There was evidence of QTL on both BTA29 and BTA18 affecting tenderness of both the *M. semitendinosus* and *M. longissimus dorsi* muscles. However, there was no indication of tenderness QTL on BTA7 and BTA16.

Tenderness QTL on BTA29 have been reported previously (Casas *et al*., 2000) and there are a number of studies showing the association of the DNA variants at *CAPN1* gene with meat tenderness (Casas *et al.*, 2005; Casas *et al.*, 2006; Morris *et al.*, 2006; White *et al.*, 2005). There was association between a SNP in this gene and meat tenderness in the population studied herein (Morris *et al.*, 2006) suggesting that the genetic variation at the *CAPN1* locus could contribute to the heritable component of meat tenderness. However, other DNA variants may be responsible as well.

A QTL linked to a marker located at 24 cM on BTA10 affected the tenderness of both the *M. semitendinosus* and *M. longissimus dorsi* muscles. Interestingly, the calpain-3 (Calcium-activated neutral proteinase 3) gene, assigned at 17 cM (http://www.ensembl.org/Bos_taurus), is on this chromosome.

There was a QTL on BTA22 specifically affecting the tenderness of the *M. semitendinosus* muscle. The QTL was associated with shear force measurements at different times after slaughter and the Limousin allele decreased the shear force values (i.e., the Limousin allele was associated with more tender meat). Calcium channel, voltage-dependent, alpha 2/delta 3 subunit maps to 35 cM on BTA22 and could be a possible candidate gene for this QTL.

There was evidence for QTL linked to a marker located at 39 cM on BTA15. The Limousin allele was associated with meat toughness. A tenderness QTL at 23 cM on BTA15 has been reported in animals with both *Bos indicus* and *Bos taurus* inheritance (Keele *et al*., 1999). The QTL coincides with the region identified herein, suggesting that this DNA region may be involved in beef tenderness regardless of genetic background.

Rexroad *et al*. (2001) proposed calcitonin (*CALCA*) gene and myogenin differentiation 1 (*MyoD1*) as two candidate genes for the detected QTL by Keele *et al*. (1999) for tenderness in this position. Calcitonin involves regulation of intracellular calcium, which is known to play a role in meat tenderness by influencing the calpains (Wheeler *et al*., 1997). Calpain-5 (*CAPN5*) also maps on this chromosome; *MYOD1*, *CALCA* and *CAPN5* are assigned to 40 cM, 43 cM and 35 cM on BTA15, respectively. A direct mechanism by which allelic variation in *MyoD1* during early muscle development might affect postmortem tenderisation is not immediately obvious. However, as pointed out by Rexroad *et al*. (2001), it is possible that genetic variation in this gene (changing its ability to influence the expression of structural components of muscle) could affect meat tenderness. Furthermore, a locus on BTA15 affected the weight of the muscle. Thus, it is

possible that this gene has pleiotropic effects on both muscle development and meat tenderness similar to the indirect effects of myostatin on meat tenderness (Chapter 4).

In summary, in addition to confirming the previously reported tenderness QTL on BTA5, 15 and 29, the present study found evidence for QTL affecting beef tenderness on BTA10, 13, 18, 22, 27 and 28. If validated, these QTL can be used to select animals that produce tender meat. It will be important to fine map these regions to be able to identify candidate genes for these QTL and ascertain their association with these traits in other populations.

pH. The ultimate pH in beef is an economically relevant characteristic, where values higher than 5.5 negatively affect meat attributes such as tenderness and colour (Smith *et al*., 1996). Meat colour is the first criterion used to judge meat quality and acceptability (Conforth, 1994). No QTL were identified for meat colour. However, a number of DNA regions were linked to the ultimate pH of meat (Table 6.11).

While pH is an important meat quality trait, there is no previous evidence of a QTL affecting meat pH in other QTL experiments in cattle. Combined knowledge of gene function and also comparative mapping will be useful to identify candidate genes for the observed effects of the identified QTL in the present study. For example, the 5'-AMP-activated protein kinase (*AMPK*) is part of an ancient stress response system whose primary function is regulation of cellular ATP. Activation of *AMPK*, which is instigated by environmental and nutritional stresses, initiates energy-conserving measures that protect the cell by inhibition and phosphorylation of key enzymes in energy-consuming biochemical pathways (McKay *et al*., 2003). The *RN* (Rendement Napole) gene, which is a mutated *PRKAG3* gene (protein kinase AMP-activated, γ_3 subunit) (Milan *et al*., 2000), is well known to affect meat quality traits (pH, glycogen potential, water-holding capacity) in pigs (Ciobanu *et al*., 2001). The *RN* gene has been mapped to SSC15, which is homeologous to BTA27. This chromosome harbours QTL for pH (Table 6.11). *PRKAG1* is one of the genes that comprise the bovine AMPK family and has been mapped to BTA5 (McKay *et al*., 2003). *PRKAG1* could be a possible candidate gene for the identified QTL for pH measured on the *M. semitendinosus* muscle. Malek *et al.* (2001) reported a QTL for water holding capacity of raw pork on SSC2, which is homeologous to the QTL on BTA7 identified herein, although there are no obvious candidate genes in that region.

Glycogen content of muscle is an important determinant of the pH decreases *post mortem* and hence the ultimate pH. Thus, the level of the muscle glycogen affects meat

colour and tenderness (Shorthose and Harris, 1991). Therefore, genes encoding enzymes involve in the glycogen metabolism can be possible candidate genes for the identified QTL affecting meat pH. In this respect, glucose-6 phosphate transporter (*G6PT*) located on BTA15 can be a candidate because of possible effects on glycolytic processes and potential effects on muscle pH. Since BTA15 was also linked to the meat tenderness, a direct role of allelic variation at this locus on meat tenderness appears straightforward.

Fuji *et al.* (1991) reported the effects of *RYR1* (ryanodine receptor 1) on pork pH, water holding capacity and colour. This gene maps to BTA18 where a QTL affecting pH measured on the *M. longissimus dorsi* muscle was found.

It is worth noting, consequently, that only the QTL for ultimate pH on BTA24 was observed to be in a similar location for the two different muscle types examined herein, the oxidative *M. longissimus dorsi* and the glycolytic *M. semitendinosus.*

Cooking loss. Low cooking loss in meat is associated with improved juiciness as judged by sensory panels (Perry *et al.*, 2001b). The study herein found four linkage groups related to the cooking loss. The results from the "Southern Crossbreeding Project" (Pitchford *et al*., 2002b) showed that the Limousin × Hereford crossbred animals have a high cooking loss compared to the Jersey × Hereford crossbred animals and the current study found three Limousin-derived QTL with increased cooking loss (BTA6, 14, 24, Table 6.11). However, there was also one Limousin derived QTL with decreased cooking loss (BTA15).

Pitchford *et al*. (2002b) reported that the Jersey × Hereford crossbred animals had high intramuscular fat and less cooking loss than the Limousin × Hereford crossbred animals. Although Pitchford *et al*. (2002b) concluded that the breed differences in cooking loss were a function of maintaining cell integrity during storage and cooking, the present study found that BTA14 was linked to both intramuscular fat content and cooking loss supporting that hypothesis that cooking loss could be associated with intramuscular fat content since fat cells have lower moisture content than muscle cells.

Due to their physiological properties, fatty acid-binding protein (*FABP*) loci are candidate genes for intramuscular fat content and possibly on cooking loss. Fatty acid-binding proteins are intracellular transporters that deliver fatty acids either to the sites of fat storage or to the sites of energy production. Damon *et al*. (2006) reported that the adipocyte fatty acid binding protein 4 (*FABP-4*) content in *M. longissimus dorsi* muscle was 2-fold greater in pigs with high lipid concentrations in the *M. longissimus dorsi* muscle than in pigs with low lipid concentrations, and positive correlation coefficients were

reported between the *FABP-4* level and adipocyte number and lipid content. Additionally, significant association between marbling and the bovine fatty acid binding protein 4 gene has been reported (Michal *et al*., 2006). The fatty acid-binding protein, adipocyte gene (*AFABP*) located at 25 cM on BTA14 and the cooking loss QTL are linked to a marker located at 14 cM on this chromosome. The importance of this region of the genome for fatness traits was discussed above. Further, the yield pleiotropic QTL found at 52 cM on this linkage group was associated with intramuscular fat content supporting the hypothesis that the *AFABP* gene may be the underlying gene for both intramuscular fat and cooking loss QTL.

The melanocortin 4 receptor (*MC4R*) is expressed in the appetite-regulating areas of the brain, where it is central in the regulation of feed intake and energy balance. There is evidence that this gene is associated with fatness in pigs (Bruun *et al*., 2006). *MC4R* is located at 43 cM on BTA24 and the cooking loss QTL was linked to a marker located at 44 cM on this chromosome.

Clearly, additional QTL mapping experiments and further research must be undertaken for meat quality traits. However, if the genes and pathways controlling meat quality traits in pork are found to control these traits in beef, then identifying molecular markers for selection in cattle should proceed rapidly.

6.4.4 Fat quality

In some markets, white fat is preferred to yellow fat. The degree of yellowness is associated with concentration of β-carotene (the yellow pigment). On the other hand, high intramuscular fat content and high mono-unsaturated fatty acids are both properties that attract premium prices for beef. Thus, in this study, fat colour and fatty acids characteristics of the intramuscular fat were considered as important fat quality traits. A number of QTL were identified for fat quality traits (Tables 6.16 and 6.17).

Fat colour. Thirteen QTL with pleiotropic effects on fat colour score and β-carotene concentration were identified. Yellow coloration of fat in the meat is mainly due to the presence of carotenoids (beta-carotene) deposited in the adipose tissue. High positive correlations between beef fat colour and the carotene concentration in either blood plasma (0.67) or beef fat (0.92) have been reported (Morgan and Everitt, 1968). Thus, the large number of pleiotropic QTL identified herein could explain the relationship between these two traits. In addition, this indicates that genes involved in β-carotene metabolism are good

candidates for the observed QTL effects for fat colour or β-carotene. For example, beta, beta-carotene 9',10'-dioxygenase (*BCDO2*) maps to BTA15 where the present study found a QTL affected both fat colour and β-carotene. A single nucleotide polymorphism in *BCDO2* gene was associated with fat colour (Tian, 2006) in the population studied herein. The QTL segregated in two sire families, one from the Australian project and another one from the New Zealand project. In the Australian sire family, the QTL affected both fat colour and β-carotene content. However, in the New Zealand sire family, the QTL only influenced fat colour. In both sire families, the Jersey allele was responsible for the fat yellowness, which was expected based on the breed differences reported for Jersey and Limousin (Kruk *et al.*, 1997a; Pitchford *et al.*, 2002a).

β-carotene is a precursor of vitamin A (retinol) and genes involved in vitamin A metabolism are also good candidates for the identified QTL for β-carotene and fat colour. In this respect, the bovine epidermal retinal dehydrogenase 2 (*RDHE2*) gene resides at 11 cM on BTA14 (http://www.ensembl.org/ Bos_taurus). This gene is a member of short-chain alcohol dehydrogenase/reductase (superfamily. *RDHE2* carries out the oxidation of retinol to produce retinal, which is the first and rate-limiting step in the retinoic acid synthetic pathway from retinol. The present study found a pleiotropic QTL linked to a marker located at 14 cM on BTA14, which affected both fat colour and β-carotene concentration. Thus, *RDHE2* could be a strong candidate responsible for the observed QTL effects. Additionally, retinal dehydrogenase 1 maps at 23 cM on BTA8. A QTL affecting fat colour was linked to a marker located at 21 cM on this chromosome. Further, peripherin, a Retinal degeneration slow protein, is located at 17 cM on BTA23 and a marker located at 42 cM on this linkage group was associated with both fat colour and β-carotene. Furthermore, RPE-retinal G protein-coupled receptor (*RGR_BOVIN*) maps to 29.6 cM on BTA28 (http://www.ensembl.org/ Bos_taurus) where the analysis herein found a pleiotropic QTL located at 25 cM on this chromosome affecting fat colour and β-carotene. A number of QTL specifically affected fat colour and had no effect on β-carotene concentration, suggesting other carotenoids or pigments might be involve in fat colour.

Carotenoid-dependent colouration is also obvious in milk fat and studies showed that genetic variation exists in milk fat colour (Winkelman *et al.*, 1999), plasma and milk carotenoid concentration in dairy cattle (Morris *et al.*, 2002). Thus, the findings herein should be useful to characterise individual genes responsible for the genetic variation in fat colour in cattle and should have applications in both beef and dairy cattle breeding programs.

Fatty acids. Fatty acids are involved in various technological aspects of meat quality. Because they have very different melting points, variation in fatty acid composition has an important effect on the firmness or softness of the fat in meat, especially the subcutaneous and intermuscular (carcass fats), but also the high intramuscular (marbling) fat (Wood *et al*., 2004). Eight QTL affecting melting point and nine QTL influencing mono-unsaturated fatty acids were identified, three of which were pleiotropic QTL affecting both melting point and mono-unsaturated fatty acids.

This is the first report of QTL affecting on fat quality in beef cattle and there is no comparable published information for QTL affecting fatty acids in cattle, although there have been some studies in pigs (Clop *et al*., 2003; Perez-Enciso *et al*., 2000) and one study in sheep (Karamichou *et al*., 2006). Karamichou *et al*. (2006) found QTL for fatty acids on sheep chromosome 2 (conservation of synteny with BTA2). A QTL linked to a marker located at 115 cM on BTA2 was identified in the present study, which affected both melting point and total mono-unsaturated fatty acids.

Karamichou *et al*. (2006) reported QTL affecting fatty acids on sheep chromosomes 1 and 5. The study herein found QTL for melting point and total mono-unsaturated fatty acids located on BTA3 and BTA7, which are homeologous to sheep chromosomes 1 and 5, respectively. Additionally, Clop *et al*. (2003) found a QTL for fatty acid composition on pig chromosomes 8 and 12, which are homeologous to bovine chromosomes, BTA17 and BTA19, respectively. The present study found QTL affecting total mono-unsaturated fatty acids on both BTA17 and BTA19. Moreover, both Perez-Enciso *et al*. (2000) and Clop *et al*. (2003) reported QTL for fatty acid composition on pig chromosome 4, which has homeologous regions on both BTA3 and BTA14. There was no indication of QTL affecting fatty acid characteristics on BTA14, but BTA3 was linked to melting point of adipose tissue.

A large number of genes involved in complex metabolic pathways can influence fatty acid metabolism. Several genes might be selected as positional and functional candidate genes for explaining the QTL found in this research. For example, the acetyl-CoA carboxylase A (*ACACA*) gene catalyzes the carboxylation of acetyl-CoA to malonyl-CoA, which is the precursor of de novo synthesized fatty acids (Wakil *et al*., 1983). *ACACA* maps to 25 cM on BTA19 and the BTA19 QTL for total mono-unsaturated fatty acids was linked to a marker located at 43 cM on this chromosome. Similarly, the fatty acid synthase (*FASN*) gene, which involved in the synthesis of long-chain fatty acids (Wakil, 1989), maps to 44 cM on BTA19.

There was compelling evidence for a QTL linked to a marker at 27 cM on BTA26, affecting both total mono-unsaturated fatty acids and melting point. The QTL in both sire families 394 and 402 lowered melting point and increased total mono-unsaturated fatty acids. The stearoyl-coenzyme A (CoA) desaturase (*SCD*) gene encoding the Δ^9-desaturase enzyme has been assigned to 26.7 cM on BTA26 (http://www.ensembl.org/ Bos_taurus), making *SCD* a strong candidate for the identified QTL on BTA26. Δ^9-desaturase is the enzyme responsible for conversion of the saturated fatty acids into monounsaturated fatty acids in mammalian adipocytes. This enzyme is important to the fatty acid composition in both beef and milk in cattle because it is responsible for the majority of monounsaturated fatty acids and the totality of conjugated linoleic acids in these products (Soyeurt *et al*., 2006).

On BTA 24, a QTL for melting point was linked to a marker located at 26cM, which is adjacent to a marker for cooking loss at 44 cM. As mentioned above, the melanocortin-4 receptor (*MC4-R*) gene is also located on BTA24 at 44cM. Association analysis between the allelic variants of these genes and fatty acid content and metabolic ratios should be performed in order to understand the molecular basis of fatty acid composition and its influence on meat quality.

Fatty acid composition in both milk fat and meat fat has received considerable interest in view of its implications for human health. While the nutritional quality of beef and milk fat is important, there is no information regarding the genes involving in the expression of this characteristic in cattle. Genetic variation in fatty acid composition within and between breeds for both milk fat (Soyeurt *et al*., 2006) and beef fat (Pitchford *et al*., 2002a) have been reported. Thus, the findings herein should be useful to characterise individual genes responsible for the genetic variation in fatty acid composition in cattle.

In summary, taking advantage of the multiple trait multiple QTL method developed herein and also the large differences in yield, fatness, meat and fat quality traits between the Jersey and Limousin breeds, the present study was able to identify QTL affecting these traits. However, future research is necessary to identify the genes underlying these QTL that are segregating within breeds or commercial lines.

Chapter 7: General discussion

Carcass and meat quality traits constitute extremely important considerations of modern beef production systems, where consumer health concerns and marketing perspectives play increasingly prominent roles. Consequently, genetic improvement of beef quality has become the subject of several studies during the last decade (Marshall, 1999). Genetic improvement of meat quality by traditional breeding is difficult, and hampered by the need for extensive and expensive measurements of traits on slaughtered relatives. It is expected that for these types of traits, knowledge of the underlying genes will greatly contribute to the efficiency of selection.

Identification of genes and DNA sequence differences that contribute to relatively minor (but still of substantial economic importance) variation in production and carcass traits is a major goal of beef genomic research. Single genes that affect carcass and meat quality attributes provide opportunities for beef breeders to increase meat production and improve meat quality. Using appropriate breeding programs, animals that carry major genes, which affect carcass and meat quality attributes, also provide opportunities to decrease product variability and to exploit differentiation required for specific markets.

A number of studies reported the identification of QTL in beef cattle for a variety of traits (Casas *et al.*, 2004b; Casas *et al.*, 2000; MacNeil and Grosz, 2002; Stone *et al.*, 2005; Stone *et al.*, 1999). However, QTL information for meat quality traits is relatively limited. Thus, the main goal of this study was to identify genomic regions associated with carcass quality in beef cattle. Initially, interval mapping based on regression analysis was used to map QTL for a wide range of economically important traits in beef cattle. Secondly, the effects of a myostatin (*MSTN*) functional single nucleotide polymorphism on growth, carcass and meat quality were studied. Thirdly, a multiple trait QTL mapping technique was developed. Finally, the multiple trait method was applied to map quantitative trait loci influencing carcass and meat quality traits.

7.1 Interval mapping-one QTL model

Regression has always been an important tool for quantitative geneticists. In the first attempt to map QTL for economically important traits in the beef industry, the present

study used the linear regression method described by Knott *et al*. (1996), which uses a model that fits a separate QTL effect for each sire and a common error variance.

The preliminary analysis of the data from the Australian project using the regression interval mapping showed significant association between DNA markers and economically important traits in beef production. Several linkage groups were found to be linked to the different traits studied. However, BTA2 was found to be the most significant linkage group related to the carcass traits that were the main focus of the present research. The identified QTL were located at the centromeric end of BTA2 close to the map location of the myostatin gene. The QTL affected a number of carcass traits including muscling, meat tenderness and carcass fatness. Therefore, in the second step of the research, the association between a previously reported functional SNP (SNP 413) in the myostatin gene and a wide range of growth, carcass and meat quality traits was evaluated.

7.2 Pleiotropic effects of myostatin SNP 413

This research provided strong evidence for the pleiotropic effects of the myostatin SNP 413 on carcass and beef quality. This DNA variant significantly decreased overall adiposity, increased muscle mass and improved meat quality attributes including tenderness and cooking loss. This is the first report of the effects of the myostatin SNP 413 on many traits in two different environments. It was shown that despite differences in climate, feeding regimens and age of slaughter in two countries (Australia and New Zealand), the allele is expressed similarly in two different environments. The present research found no evidence for the effect of the myostatin variant allele on birth weight and growth traits. It can be argued that the allele variant described herein should be active as only a single amino acid substitution is present (Chapter 4). By complete sequencing of the myostatin codon regions and splice junctions, Sellick (2002) has shown the absence of any loss-of-function or deleterious myostatin mutations in the six sire families studies herein.

Overall, the findings suggest an opportunity exists for increasing the efficiency of beef production without the associated increases in calf mortality analogous with complete loss-of-function myostatin mutations (Casas *et al*., 2004a). The use of DNA technology permits the identification of individuals carrying the SNP at an early stage in life, thus allowing flexibility in mating schemes in using this DNA variant to improve carcass composition.

Lastly, while calving problems associated with full double muscling observed in the Belgian Blue and Piedmontese doubled-muscle breeds have historically dominated the controversy over the desirability of breeding for the double-muscled phenotype (Arnold *et al*., 2001), the study herein emphasises that even the drawbacks associated with mutations causing the total loss of function of the myostatin gene do not imply that these mutations are worthless. Appropriate breeding strategies that can take advantage of the useful nature of a myostatin knockout mutation, while selecting against undesirable companion traits, can help to avoid these problems. In addition, increased knowledge about mechanisms of function and expression patterns of myostatin has already hinted at some possible techniques to avoid the deleterious effects of a full myostatin knockout. For example, the work of Lee and McPherron (2001) on agents that block myostatin receptor binding and action may offer a way to "turn off" myostatin function in adult animals, bypassing the problems at parturition and in newborn calves.

7.3 Modelling multiple traits

The current research was directed toward the development of an efficient technique for modelling multiple phenotypes. The developed approach considered an unstructured covariance model for the residuals for the traits and fitted a multiplicative model for the *trait by marker* effects. The particular multiplicative model considered herein was the factor analytic model. This provided a parsimonious model specification to limit the number of parameters to be estimated. Factor analysis is an exploratory statistical method for multivariate data based on the assumption that the observed data are produced by a few unobserved factors. It was the assumption of the present work that these unobserved factors are the QTL with common location across traits or sire families of which the scored markers are constituents.

Combining information across traits exploiting multivariate analysis, such as that proposed in this study can substantially increase the power, as was shown in the simulation study, and the robustness of quantitative trait locus mapping studies. The model proposed has a number of advantages. It is quite flexible and can easily accommodate covariates and multiple linked loci. It requires the sequential running of a series of models. The number of models is directly related to the number of pleiotropic QTL in the data. Therefore, the model is considerably less computational intensive than the number of models required by other methods that might sequentially fit each pair of contiguous markers in a regression to

locate the QTL. In addition, QTL mapping data collected in different trials routinely exhibit heterogeneity of error and genetic variance among trials. The proposed approach can accommodate all of these sources of heterogeneity.

This new method brings QTL detection into the general mixed model framework. One of the nice properties of the mixed model framework is its ability to handle a large number of fixed and random effects. The multiple trait all-marker analysis developed herein has fully taken advantage of this property and the results of the simulation study and data analysis have clearly verified this notion.

The present work has taken the same reasoning as Smith *et al*. (2005), who fitted variety effects as random when studying *genotype x environment* interaction in multi-environment trials. If the main aim of the analysis is to find the most likely position of the QTL or marker selection (to identify the closest marker of those under consideration in the QTL), then rankings of the estimated marker effects are required to be as close as possible to the rankings of the true marker effect. By definition, this implies the use of BLUP so that marker effects should be regarded as random. However, because of the different marker/QTL phases, the sign of the effects is not of interest so the absolute values of the BLUP are taken. It is clear that the aim of the analysis is selection so that the use of random marker effects is appropriate. An additional key advantage with the use of random marker effects is that it allows a valid analysis of data combined across sire families and mapping experiments. The analysis of such data is crucial since it provides a more reliable QTL location by accumulating information across families or mapping projects.

REML and BLUP have been used for many years as the basis for selection and estimation of breeding values and genetic parameters in animal breeding programs. This research has applied a well-known area in animal breeding to analyse the marker and trait associations in the general mixed model framework. To do this, the present work used a "one stage" approach in which the model for residual effects was estimated simultaneously with models for *trait by marker* effects. Hence, in statistical terms, all the available information was used.

Molecular techniques have developed dramatically with the effect that experiments are now possible with many more individuals sampled, many more traits recorded (including expression data on thousands of transcripts) and very dense marker maps. Increasing sample size has only beneficial effects for QTL mapping, irrespective of the approach, and can easily be accommodated with the mixed model framework. With more traits, there is much more, potentially useful, information to help determine the networks or

pathways of genes involved. Multiple trait QTL mapping developed for the current research can easily be applied to many traits. However, the behaviour of the approach using very dense marker maps (e.g., 100,000 SNPs) needs to be investigated. The fact that the same variance for the total markers of the entire genome is used is problematic, since the majority of the markers will not be linked to the QTL and they may dominate the estimate of the marker variance/covariance. Consequently, the estimate of the genome variance/covariance will be close to zero. It should be noted that the extra markers on a chromosome would not dilute the marker variance associated with the chromosome because they also have covariances between them (Gilmour, personal communication). However, adding extra chromosomes may dilute the genome variance, as most of the linkage groups are not linked to the QTL. Therefore, one solution is to allow the markers of the same linkage group have common variance (Gilmour, 2007). The extension of this approach to the multivariate analysis would be removing the linkage groups with non-significant variance for the traits of interest, then those traits that had non-zero variances for a chromosome were combined and the factor model fitted across the chromosome instead of the genome.

Correlations among phenotypes can arise from several different causal processes, which may have different implications for the power and performance of the multivariate linkage analysis. Allison *et al.* (1998) depicted five different models involving a QTL and two phenotypes of interest, labelled "X1" and "X2." In model 1, X1 and X2 are both functions of the QTL. They referred to this situation as "mosaic pleiotropy." In this case, substantial power can be gained by conducting a multivariate linkage analysis (Allison *et al*., 1998; Boomsma, 1996) and the factor model used herein for the *trait by marker* effects performed well in this situation in the simulation study. Model 2, termed as "relational" pleiotropy is where the QTL directly impacts X1, and X1 in turn, directly impacts X2. Model 3 depicts the situation in which X2 might be termed an "exogenous" variable. Here, both the QTL and X2 exert a causal influence on X1. However, the QTL does not influence X2. Model 4 depicts the situation in which X1 and X2 are correlated because each is influenced by an additional variable, Z. However, Z is not observed. Finally, in model 5, X2 is an intermediary phenotype between the QTL and X1 (Allison *et al*., 1998). Obviously, further studies using the approach suggested herein for multitrait QTL mapping that specifically consider these five situations should be undertaken.

7.4 Joint analysis

By exploiting the factor analytic model, the present study has addressed the issue of joint analysis of multiple families or experiments in QTL identification. The proposed approach considered separate residuals for the trials and fitted a factor analytic model for *family by marker* interaction term (or in the case of multiple traits and multiple families, *trait by family by marker* interaction). The results from joint analysis of six simulated families revealed that gathering information across families could remarkably increase the power. The ability to detect QTL, when QTL were segregating in all families, in the simulation data set resulted in the same power as increasing the sample size. Thus, it was shown that a joint analysis is helpful for loci too small to be detected by individual family analyses, if the QTL are segregating in multiple families (chapter 5).

Experience in fitting the factor analytic model has suggested some computational issues when complex *marker by trait by family* models are fitted to the data. Difficulties with convergence of factor analytic models may be due to the choice of starting values. To overcome this problem, the univariate analyses of the traits were performed to estimate the genome variances for the traits in each family and then these estimates were used as initial values to estimate specific variances and loadings across sire families for a particular trait. Finally, these estimates were used as initial values for factor model across traits and sire families.

In practice, however, there are a number of issues that need to be addressed before joint analyses can be performed. For example, phenotype definition differs between studies, that is, the animals are reared in different environments with different testing regimes and different markers may be used in different populations. As far as phenotypes are concerned, it may be possible to adopt a standard trait measurement protocol to some extent, although this may never be achieved completely. Hence, environmental differences and other factors will always need to be considered in such studies. In the proposed approach, it is easily possible to account for the within trial variation while allowing different trials to have different residual variations/covariations. As far as the markers are concerned, with the development of consensus maps and distributed primer sets, it is now much easier to select markers that are used by others. However, markers will vary in the information they provide on different populations and so it would never be possible to completely standardise the marker choice. This being the case, the strategy adopted in the present study was to treat uninformative markers in different sire families as missing and

impute the marker scores using flanking markers as was done for the other missing markers.

Lastly, the present work has demonstrated joint analysis in the case of availability of raw data from different studies. However, access to the raw data may not be feasible. In this case, meta-analysis of published results may be a powerful and informative approach (Allison and Heo, 1998). Meta-analysis would be facilitated if results were reported in the form of LOD scores along the chromosome. Applying the Fisher formula (Fisher, 1954) to results from many studies, the summed LOD score along the chromosome combines information on a QTL at a given point. The summed LOD score is equivalent to one that would be obtained from a model that fits a single QTL at a given location allowing its effects and even the residual variance to vary between trials. However, for general applicability, this strategy would require that LOD profiles were recorded in an accessible format to make future meta-analyses possible.

7.5 QTL congruency for beef traits

The complexity of beef yield and quality traits is one of the problems associated with its genetic analysis. Complexity can arise as a consequence of one carcass quality trait having multiple components such as fatness, tenderness, colour, pH, cooking loss. The genetic covariance that exists between components of beef yield and quality indicates that pleiotropic loci should affect combinations of these components.

Most of the results of QTL findings in beef cattle have been reported on the basis of univariate regression mapping analyses of individual families until now. Such results provide only partial information on the genetic architecture of beef production, and univariate analyses do not optimally use the available information. In this study, the above-mentioned factor analytic model was used in order to combine information across traits and sire families to increase power and to locate possible pleiotropic QTL affecting beef traits. Thus, the main feature of this work was to give a broad picture of QTL influencing carcass and beef quality traits. In order to summarise the identified QTL for yield, carcass fatness, meat and fat quality, each chromosome was divided into approximate 40 cM segments and the related LOD scores for the identified QTL for different traits were placed in these regions (Table 7.1).

The myostatin gene, which had major effect in the expression of many carcass traits (Chapter 4), resides at the centromeric end of BTA2. This research adopted a strategy to

remove the effects of the myostatin variant to identify additional quantitative trait loci scattered throughout the genome associated with carcass traits. To do this in the models used for all of the analyses (Chapter 6), the genotype of the animals for this gene was considered as a fixed factor. Therefore, no QTL can be observed in the region of this gene on BTA2 (Table 7.1). However, in the distal region from the location of the myostatin gene, QTL for fatness (external fat deposition) and fat quality (both fat colour and fatty acids) were mapped. Unlike the myostatin variant, which affected both fatness and yield, this DNA region influenced only fat related traits. This finding supports the observation of Casas *et al*. (2004b) who found other genes apart from the myostatin gene on BTA2 are involved in fatness.

Table 7.1 Summary of the results for yield, fatness, meat and fat quality traits associated with each bovine autosome (BTA) divided into three segments (I, II, III)[a]

	BTA1			BTA2	BTA3		BTA4		BTA5		BTA6			BTA7		BTA8		BTA9			BTA10		BTA11			BTA12		BTA13	
Trait	I	II	III	III	II	III	I	III	I	II	I	II	III	I	II	I	III	I	II	III	I	III	I	II	III	II	III	I	III
EMA					2.0					2.3												2.0							
HCW					3.9					5.2												3.2							
LD															3.6							3.0							
SS					2.7					4.5												2.3							
IMF								4.3											4.2		4.8								
Kidfat			4.1		2.5			5.3	2.3		6.6	2.4		2.3					5.2		3.4		2.2						2.6
Omenfat						2.7					3.0																		2.9
P8am	3.3										3.9																		
Rbft				2.7		2.6																							
CLLD											3.2																		
PHld		3.0									2.6	2.9	2.9		2.7		2.3		5.5							5.5			3.6
PHst																					2.7								
wbld1										2.8											2.8							2.0	
Wbld2																					2.8								
Wbld3																													
Wbld4										2.0																			
wbst1									2.7	2.7																			
Wbst2									3.5												2.1								
Wbst3									2.7												2.3								
Wbst4										2.4																			
BC	2.0						3.2											5.1						2.9			2.1	3.0	
FCS	7.0			2.9			4.3									2.5		2.8						4.0	3.4	2.9	2.7	2.6	
Meltpt				2.6		2.0																							2.1
Mufa				2.0			2.1								2.0					2.3									

Table 7.1 continued

Trait	BTA14			BTA15			BTA16		BTA17	BTA18			BTA19	BTA21	BTA22		BTA23	BTA24		BTA25	BTA26		BTA27		BTA28		BTA29
	I	II	III	I	II	III	I	II	II	I	II	III	II	I	I	II	II	I	II	II	I	II	I	II	I	II	II
EMA									3.2																		
HCW		3.8							3.0																		
LD		3.1			3.4				2.5																		
SS		3.6							2.7																		
IMF		2.7																									
Kidfat		2.3				3.0		2.7	2.8				2.7	5.5	2.7					5.1	2.6						2.7
Omenfat											3.2											3.5		4.0			
P8am									3.4																		
Rbft																					2.0					4.4	
CLLD	5.9				3.3														3.3								
pHld						3.0		5.5		4.3								2.3					5.9				
pHst																		2.2									
wbld1												3.0															
Wbld2				2.4								2.1															4.5
Wbld3				2.9								2.6															6.0
Wbld4												2.2											3.5				
wbst1												2.3			4.1											2.2	2.2
Wbst2																											
Wbst3															2.3								2.4				2.0
Wbst4															2.1												2.0
BC	5.4			3.5			2.8						2.5			2.6		2.0							2.4		
FCS	3.6		2.5	6.5									2.4			3.5		3.5					3.6		2.5		
Meltpt								3.2							2.6		5.5	2.0			2.9						
Mufa								3.6	3.0				2.9				2.1				2.7				2.7		

EMA: Eye muscle area (cm^2), HCW: Hot carcass weight (kg), SS: Silverside weight (kg), LD: Striploin weight (kg), P8am: Fat depth at position 8 on the rump (mm), Kidfat: channel fat, Omenfat: Omental fat Rbft: Fat depth between the 10th and 11th ribs (mm), IMF: Intramuscular fat content, Wbst1, 2, 3 and 4: Shear force measurements on *M. semitendinosus* muscle on days 1, 5, 12 and 26 after slaughter in Australia; Wbld1, 2, 3 and 4: Shear force measurements on M. *longissimus dorsi* muscle on days 1, 5, 12 and 26 after slaughter in Australia and tenderness measurements on *M. longissimus dorsi* muscle on days 0, 1/3, 1 and 4 post mortem in New Zealand, pHld: pH measured on the M. *longissimus dorsi* muscle, pHst: pH measured on the *M. semitendinosus* muscle, CLld: Cooking loss of the M. *longissimus dorsi* muscle, BC: β-carotene content, FCS: Fat colour score on biopsy samples, Meltpt: Melting point of the fat tissue, Mufa: Total mono-unsaturated fatty acids. [a] Values are LOD scores. I, II and III: First, second and third 40 cM segments on the chromosome

A number of studies reported QTL for carcass composition on BTA3, 5, 14 (Casas *et al.*, 2003; Casas *et al.*, 2000; Mizoshita *et al.*, 2004; Stone *et al.*, 1999). The present study confirmed these reported QTL and also found additional loci linked to carcass traits on BTA10 and 17. BTA14 seems to be only linked to a gene or genes controlling the size of the animal. The other pleiotropic markers found on BTA3, 5, 10 and 17 were linked to loci affecting both size and muscling, suggesting that selecting animals based on these markers may lead to increased whole carcass weight (that is, fat, bone and meat yield). However, there was no indication of QTL specific for eye muscle area (which is an indication of meat yield), suggesting that the myostatin variant accounted for most of the variation observed in the expression of muscle mass. In addition, the size of the QTL effects as a percentage of the trait means were similar for all of the yield traits. Further, four of the five pleiotropic QTL (BTA3, 5, 10, 14) were close to the identified birth weight QTL (Chapter 3; Morris *et al.*, 2003) in the population studied herein. The direction of the birth weight QTL effects were similar to those of the yield traits. That is, the Limousin allele on BTA3, 10 and 14 increased birth weight and on BTA5 decreased the trait value. The pleiotropic QTL effects could explain the high positive genetic correlations reported for birth weight, eye muscle area and hot carcass weight in the Australian 'Southern Crossbreeding Project' (Pitchford *et al.*, 2006) in which the Limousin and Jersey breeds were a part of the study. These results collectively suggest that selecting animals based on the identified QTL for yield traits will increase the size of animals and birth weight, and consequently would increase calving difficulties.

It should be noted that above-mentioned pleiotropic QTL were linked to a number of other carcass and beef quality traits (Table 7.1). For example, the DNA region in the middle of BTA5 was linked to both yield and meat tenderness. Moreover, BTA3 was linked to both yield and internal fat. The Limousin allele for this BTA3 QTL increased both yield traits and internal fat. Furthermore, the yield pleiotropic QTL on BTA17 was very close to the P8 fat QTL and the Limousin allele increased all the yield traits and also external fat depositions. These provide additional evidence that the yield pleiotrpic QTL identified herein and reported in several studies involving different breed composition (Casas *et al.*, 2004b; Casas *et al.*, 2003; Casas *et al.*, 2001) will increase the overall body size rather than meat yield. However, the size of the BTA3 QTL for channel fat (12% of the trait mean, Table 6.7) was twice the yield traits (5-6% of the trait means, Table 6.4), indicating that selecting animals against this QTL will decrease internal fat depositions. Similarly, the BTA14 QTL was linked to both yield traits and carcass fatness (Table 7.1).

The Limousin allele for the BTA14 QTL had a negative effect on channel fat and intramuscular fat (allele effects of 11-12% and 31% of the trait means, respectively, Table 6.7) and a positive effect on yield traits (allele effects of 3-9 % of the trait means, Table 6.2). Again this provides an opportunity to decrease internal fat depositions. However, intramuscular fat will be more affected for this QTL.

These results emphasise that the identified QTL for carcass and beef composition in these regions of the genome, which confirmed the results from other studies (Casas *et al.*, 2003; Casas *et al.*, 2000; MacNeil and Grosz, 2002; Mizoshita *et al.*, 2004; Stone *et al.*, 1999), require further investigation. Overall, these findings lead to the question of whether the common genomic regions identified for different traits are representing one gene or closely linked loci.

The proximal region of BTA6 was linked to both internal and external fat depots. In addition, meat quality traits including cooking loss and meat pH were affected by this part of the chromosome. Fatness QTL in this DNA region have been reported in beef cattle (Li *et al*., 2004) and many studies in dairy cattle (Freyer *et al*., 2002; Mosig *et al*., 2001; Olsen *et al*., 2002; Spelman *et al*., 1996; Wiener *et al*., 2000). It is noteworthy that this region of the genome had no effect on intramuscular fat content, offering an opportunity for decreasing fatness without decreasing intramuscular fat (marbling). This is also true for BTA21 where a highly significant QTL located at the centromeric region of the chromosome was found for channel fat. The Limousin allele increased fatness (allele effect of 24% of the trait mean, Table 6.7). Birth weight QTL in this region were also identified. Limousin allele decreased birth weight (by of 9% of the trait mean) (chapter 3). Two independent studies using different beef cattle breeds reported birth weight QTL on this position of the chromosome (Casas *et al.*, 2003; Davis *et al.*, 1998). Since this region was not linked to yield traits and intramuscular fat, it would be a unique opportunity to decrease channel fat without increasing birth weight. Also this selection will not affect intramuscular fat and yield. In this regard, the BTA4 QTL was also interesting because the Limousin allele increased intramuscular fat (12-18% of the trait mean) and decreased channel fat (23% of the trait mean). Therefore, identifying gene(s) responsible for these effects would help to select animals to increase intramuscular fat and decrease internal fat in beef cattle.

A number of fatness and meat quality traits were linked to the centromeric end of BTA10. The identified QTL fell in a region of the chromosome proximal from the identified pleiotropic QTL for yield traits, such that it could be ruled out that they reflect

different QTL. The traits affected by this DNA region were internal fat, intramuscular fat, meat pH and tenderness. A marbling QTL in this genomic region has been reported (Casas *et al.*, 2003). However, this study provided an additional insight into the effects of this QTL on internal fat. Although the QTL were segregating in different sire families, the Limousin allele decreased both intramuscular and internal fat (16 and 18% of the trait means, respectively; Table 6.7). Therefore, selection against these QTL in order to increase intramuscular fat will also lead to increased internal fat. Further research is required to fine map and narrow the identified region affecting multiple traits on this linkage group.

BTA15 was associated with four characteristics measured on the strip loin (pH, cooking loss, tenderness and weight). However, whether there is one gene with pleiotropic effects on the overall muscle characteristics including weight, pH, tenderness and cooking loss or a cluster of closely linked genes each affecting different traits is not clear.

A number of the identified QTL mapped at DNA regions similar for both the fatness traits and fatty acids (BTA2, BTA3, BTA13, BTA16, BTA17, BTA19, BTA22 and BTA26). This is in accordance with the review of De Smet *et al*. (2004), where they reported that differences in fatty acid composition between breeds and genotypes could be largely explained by differences in fatness. However, after correction for fat level, genotype differences in the fatty acid composition have been reported, reflecting the possible genetic differences in fatty acid metabolism independent of fatness (De Smet *et al*., 2004). Siebert *et al*. (1996) also supports this but it depends on how the fat extraction were undertaken. Moreover, there were DNA markers that were only linked to mon-unsaturated fatty acids and melting point herein (BTA4, BTA7, BTA9, BTA23, BTA24, BTA28).

The marker span used in the present study was too coarse for accurate gene targeting and marker-assisted selection. Differentiating the effects of closely linked QTL from the effects of one pleiotropic QTL remains problematic in segregating populations of modest size. Separating multiple linked QTL requires a very large sample size that provides enough recombinants or mapping populations over several generations. A linkage relationship will be broken in the following generations, while pleiotropic effects will remain unaltered.

Finally, theoretical and computer simulation studies have been used to explore the potential increase in selection response that could come from the marker-assisted selection (Meuwissen and Goddard, 1996; Spelman and Bovenhuis, 1998). For instance, Meuwissen and Goddard (1996) found that the use of DNA marker information increased the genetic

gain for a production trait, where records were collected before selection (e.g. growth rate), by almost 9%. However, for a carcass trait, which was recorded by slaughtering half the progeny and selecting amongst the remaining half, these researchers found that marker-assisted selection increased the rate of genetic progress by almost 64%. Presently, meat quality is receiving increasing attention of consumers because of food safety reasons. Thus, if validated, the QTL identified herein could provide valuable information to improve carcass and beef quality using marker assisted-selection.

7.6 Future directions

7.6.1 Confirmation and Validation

The new identified QTL with low statistical support need to be confirmed by other studies. In addition, the results presented in this thesis pertained to alleles that segregate in crosses between the Limousin and the Jersey breeds. To be useful in marker-assisted selection, alleles with important effects that segregate within breeds must be identified. This requires an intensive validation program.

7.6.2 Fine mapping

Fine mapping of the identified QTL in this experiment will enable breeding schemes to employ marker-assisted selection and will allow positional candidate gene analyses to proceed with high levels of accuracy and precision. This would involve comparative mapping between the bovine and human genomes, which is the most efficient way to identify positional candidate genes, as it makes use of the wealth of data available from the human map and biomedical community.

New technology has provided highly saturated single nucleotide polymorphism genetic maps. Using this technology, the cost of genotyping is decreasing to the point that in the future it may become cheaper to genotype populations than to collect the phenotype data necessary for identifying QTL. Eventually, thousands of markers can be used to refine the position of the identified QTL. The approach in this thesis can be used to analyse such data. However, the behavior of the method in the situation of high marker density needs to be investigated.

7.6.3 Epigenetics

Understanding the role of epigenetics is increasingly important in livestock research. For example, epigenetic effects, such as genomic imprinting causing monoallelic gene expression, have been identified as being involved in QTL affecting complex traits such as growth and fatness traits in sheep and pigs (Cockett *et al*., 1996; de Koning *et al*., 2000). However, the design of this experiment did not allow the identification of possible imprinted genes and studying the importance of their role in carcass and beef traits. Also X and Y chromosomes effects have not been tested herein. Thus, the contribution of the non-Mendelian inheritance including paternally imprinted or maternally imprinted gene effects on beef traits may be worth investigating.

7.6.4 Epistasis

The issue of detecting epistatic interactions between pairs of QTL is an important challenge, driven by the biological interest in finding genetic interactions, but hampered by the intense diversity of tests in performing an exhaustive search. Having identified additive QTL, it is straight forward in the proposed approach to test for interactions either among QTL or with other factors. However, this depends on the effects being large enough to appear as significant additive terms in the first instance. Thus, further work requires extending the proposed approach for the epistatic model.

7.6.5 Pleiotropic vs. linked QTL.

The approach presented herein does not distinguish between pleiotropy and linkage. However, if the goal is to look at the possible consequences of marker assisted selection for other traits, it is less relevant if the QTL is a pleiotropic QTL or if there are two closely linked QTL. Distinguishing between these two situations can be important from several perspectives. For gene discovery and positional cloning of gene(s), it is obviously important to know if one or two genes are responsible for the genetic correlation. From an animal breeding perspective, it is also important to know whether the genetic correlation due to a QTL can be dissociated. This could be possible in the case of two linked QTL, but not if the correlation is due to a single pleiotropic QTL. Thus, developing an approach to test for pleiotropy vs. linked QTL is another area that needs more investigation.

7.7 Conclusions

This thesis has quantified the pleiotropic effects of the myostatin SNP 413 on growth, carcass and beef traits in two different environments. It was established that this myostatin variant was associated with increased meat yield, reduced fat deposition and increased meat tenderness, but had no significant effect on birth weight and other live weights. Thus, this DNA variant is an ideal candidate for genotype-assisted selection to improve carcass composition.

The thesis has developed a mixed model multiple marker approach, allowing all markers of the entire genome to be included in the analysis simultaneously. Further, the approach was extended to the multitrait or multiple family situations. It was shown through the simulation study that modeling multiple traits and multiple families in a single linkage analysis simultaneously could substantially increase power, compared to modeling of each phenotype or family separately. To examine the behavior of the approach in experimental data, myostatin was used as a model for pleiotropy gene action and the developed method was tested on this gene. The results clearly showed that fitting a factor analytic model across traits and families gave a much better indication of the QTL position than a single trait or separate family analysis.

Application of the developed approach to the phenotypic data revealed that different genomic regions control beef traits. A number of previously reported QTL were confirmed and new QTL were identified. In addition, trait-specific QTL and QTL with pleiotropic effects were mapped. Overall, the thesis provides a strong basis from which fine mapping can be launched for subsequent gene discovery and marker-assisted selection.

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Appendices

Appendix A Most likely position[a], flanking markers, F-statistic values and allelic effects of putative QTL detected by individual family analysis.

BTA	Trait	Position	Left Marker	Right Marker	Effect	S.E.	F-Value	Family
1	P8 fat depth, mm	16	BMS2321	BMS711	2.8800	0.8266	12.2	361
1	Body length at birth, cm	28	BMS711	BMS4017	1.8381	0.6613	7.7	398
1	Birth weight, kg	46	BMS4017	INRA49	2.7400	0.9600	8.1	398
1	Fat colour on biopsy samples	56	BMS4017	INRA49	0.3176	0.1153	7.6	361
1	Aging rate on LD[b], kg	67	BMS4017	INRA49	0.0036	0.0012	8.9	361
1	Marbling, score	69	INRA49	CSSM032	0.3613	0.1220	8.8	361
1	*M. semitendinosus, kg*	70	INRA49	CSSM032	0.2324	0.0756	9.5	398
1	Silver side, kg	74	INRA49	CSSM032	0.5249	0.1509	12.1	398
1	Channel fat, kg	91	CSSM032	BMS1789	2.3200	0.6882	12.0	398
1	Birth weight, kg	92	CSSM032	BMS1789	2.2800	0.7800	8.6	368
1	Carcass length, cm	107	BMS1789	BM1824	35.1100	13.1100	7.4	398
1	Fat depth at summer, mm	136	BMS2263	DIK5034	0.8573	0.3005	8.1	368
2	Peak force on ST[c], kg	10	ILSTS26	TEXAN2	0.1025	0.0231	19.6	361
2	*M. semitendinosus, kg*	10	ILSTS26	TEXAN2	0.2696	0.0712	14.3	361
2	Silver side, kg	10	ILSTS26	TEXAN2	0.5034	0.1595	10.0	361
2	Meat yield, %	10	ILSTS26	TEXAN2	1.6620	0.4015	17.1	361
2	Eye muscle area, cm^2	10	ILSTS26	TEXAN2	7.3182	2.0734	12.5	361
2	Peak force on ST, kg	14	ILSTS26	TEXAN2	0.0809	0.0262	9.6	398
2	Mono-unsaturated fatty acids, %	15	ILSTS26	TEXAN2	1.9716	0.7475	7.0	361
2	Peak force on ST, kg	16	ILSTS26	TEXAN2	0.1278	0.0237	29.1	368
2	Eye muscle area, cm^2	16	ILSTS26	TEXAN2	10.5970	2.7511	14.8	368
2	Meat to bone ratio	17	ILSTS26	TEXAN2	0.2238	0.0770	8.4	361
2	Meat to bone ratio	19	ILSTS26	TEXAN2	0.2838	0.0866	10.7	368
2	Meat to bone ratio	19	ILSTS26	TEXAN2	0.2903	0.0871	11.1	368
2	Meat yield, %	20	ILSTS26	TEXAN2	2.2804	0.5111	19.9	368
2	Carcass fat,%	20	ILSTS26	TEXAN2	1.6100	0.5424	8.8	368
2	Silver side, kg	21	ILSTS26	TEXAN2	0.7218	0.1889	14.6	368
2	Meat yield, %	26	TEXAN2	OARHH30	1.3992	0.4117	11.6	398
2	Carcass fat,%	27	TEXAN2	OARHH30	1.2324	0.4104	9.0	398
2	*M. semitendinosus, kg*	36	TGLA377	URB042	0.2266	0.0598	14.4	398
2	Silver side, kg	36	TGLA377	URB042	0.3627	0.1272	8.1	398
2	βcarotene concentration, µg/g fat	72	RM356	BMS2626	0.2249	0.0846	7.1	361
2	Body girth at birth, cm	72	RM356	BMS2626	2.0926	0.7596	7.6	398
2	Heart, %	86	BMS2626	BM6444	0.0384	0.0384	8.7	361
2	Liver, %	97	BM6444	BMS356	0.1128	0.0365	9.5	361
2	Melting point, °C	99	BM6444	BMS356	1.5238	0.5679	7.2	398
2	Ultimate pH of ST	109	BMS356	BM2113	0.0353	0.0098	9.3	398
3	Pelvic width, mm	0	INRA006	BMS963	4.4283	1.5845	10.6	361
3	Pelvic area, mm^2	0	INRA006	BMS963	11.9572	3.0682	15.2	361
3	Stifle width at weaning, cm	12	INRA006	BMS963	1.4620	0.4774	9.4	398
3	Fat depth at ribs, mm	14	INRA006	BMS963	2.5413	0.8408	9.1	398
3	Heart, %	16	INRA006	BMS963	0.0389	0.0134	8.3	368
3	Marbling, score	38	BM4129	HUJ246	0.3757	0.1394	7.3	398
3	Ultimate pH of LD	46	BL41	MCM58	0.0244	0.0092	7.0	398
3	Meat yield, %	50	HUJ246	BMS2145	1.2537	0.4284	8.6	398
3	Carcass fat,%	59	HUJ246	BMS2145	1.3376	0.4628	8.3	398

Appendix A continued

BTA	Trait	Position	Left Marker	Right Marker	Effect	S.E.	F-Value	Family
3	Ultimate pH of LD	73	HUJ246	BMS2145	0.0521	0.0192	7.3	361
3	Aging rate on ST, kg	74	HUJ246	BMS2145	0.0030	0.0008	12.0	368
4	Carcass fat,%	45	MAF70	MAF50	1.4502	0.5177	7.8	368
4	*M. semitendinosus, kg*	46	MAF70	MAF50	0.3678	0.1396	7.0	368
4	*M. semitendinosus, kg*	50	MAF70	MAF50	0.1691	0.0575	8.7	398
4	Aging rate on LD, kg	52	MAF50	BMS495	0.0035	0.0010	11.7	361
4	Pelvic width, mm	96	BMS648	TGLA159	7.9348	2.5892	8.6	398
4	Body hight at birth, cm	100	BMS648	TGLA159	2.0247	0.7267	7.8	361
4	Desaturation index, %	100	BMS648	TGLA159	2.1849	0.8148	7.2	361
5	Body hight at birth, cm	27	BMS610	AGLA293	3.3594	0.8921	14.2	398
5	Peak force on ST, kg	33	AGLA293	OARFCB05	0.0818	0.0218	14.1	361
5	Body length at birth, cm	34	AGLA293	OARFCB05	1.7574	0.6468	7.4	398
5	Body girth at birth, cm	34	AGLA293	OARFCB05	2.5308	0.7812	10.5	398
5	Birth weight, kg	34	AGLA293	OARFCB05	2.8957	0.8058	12.9	398
5	*M. semitendinosus, kg*	35	AGLA293	OARFCB05	0.1933	0.0610	10.0	398
5	*M. Longissimus dorsi, kg*	45	OARFCB5	BL37	0.5855	0.1609	13.2	368
5	Hot carcass weight, kg	50	OARFCB5	BL37	26.0647	7.2230	13.0	368
5	Peak force on ST, kg	65	MAF23	CSSM22	0.1350	0.0428	10.0	368
5	Ultimate pH of ST	78	CSSM22	BMS1248	0.0572	0.0216	7.0	368
5	Fat depth at weaning, mm	105	BMS1248	BMS772	0.5338	0.1879	8.1	368
5	FDistance, meter	114	BMS772	BM2830	2.7797	0.8366	11.0	368
6	Aging rate on LD, kg	8	INRA133	BM1329	0.0033	0.0010	8.6	361
6	Eye muscle area, cm^2	17	INRA133	BM1329	6.4681	2.3936	7.3	398
6	Pelvic area, mm3	35	INRA133	BM1329	10.5400	3.2400	8.8	361
6	Meat colour, score	37	BM1329	BM143	0.2994	0.1059	8.0	398
6	Age at Puberty, day	39	BM1329	BM143	22.8040	7.7878	8.0	361
6	Pelvic width, mm	42	BM1329	BM143	5.6720	1.5021	14.3	361
6	Fat colour on biopsy samples	49	BM143	BMS483	0.2524	0.0856	8.7	361
6	Fat colour after slaughter, score	57	BM143	BMS483	0.4611	0.1554	7.4	361
6	Fat colour on biopsy samples	70	BMS483	BM4621	0.2600	0.0901	8.3	368
6	Aging rate on ST, kg	73	BM4621	BM415	0.0026	0.0009	7.7	368
6	Ultimate pH of ST	87	BM415	BM8124	0.0492	0.0183	7.2	368
6	Heart, %	120	BM8124	BM2320	0.0924	0.0253	13.3	398
7	Fat depth at ribs, mm	8	BM7160	RM006	1.9393	0.6157	9.9	368
7	Fat depth at weaning, mm	51	BM741	BM6117	0.5501	0.1960	7.9	368
7	Intramuscular fat content, %	58	BM741	BM6117	0.7880	0.2672	8.7	361
7	Fat depth at ribs, mm	102	BMS1331	BM9065	2.2865	0.7412	9.5	361
7	Peak force on ST, kg	116	BM9065	BMS522	0.0666	0.0222	9.0	398
8	Silver side, kg	21	RM321	RM372	0.3748	0.1378	7.4	398
8	Pelvic width, mm	40	BMS1591	BM4006	4.7783	1.6008	8.9	368
8	Fat depth at winter, mm	45	BMS1591	BM4006	0.4356	0.1390	9.8	368
8	Eye muscle area, cm^2	46	BMS1591	BM4006	6.6710	2.5153	7.0	398
8	Fat colour after slaughter, score	48	BMS1591	BM4006	0.6955	0.2171	10.0	361
8	Eye muscle area, cm^2	62	BMS1341	BMS2072	6.6510	2.0976	10.1	361
8	*M. Longissimus dorsi, kg*	69	BMS2072	BM711	0.6914	0.2573	7.2	361

Appendix A continued

BTA	Trait	Position	Left Marker	Right Marker	Effect	S.E.	F-Value	Family
8	Hot carcass weight, kg	76	BMS2072	BM711	25.1371	8.6334	8.5	361
8	Cooking loss of LD, %	115	BM711	CSSM47	0.7475	0.2730	7.5	398
9	*M. Longissimus dorsi, kg*	42	BMS817	BMS1148	0.4939	0.1787	7.6	361
9	Docility, score	50	BMS817	BMS1290	0.9374	0.3360	7.8	361
9	Marbling, score	58	BMS1148	BMS1290	0.5473	0.1211	20.4	398
9	Eye muscle area, cm^2	66	BMS1290	TGLA73	5.8013	2.0351	8.1	361
9	Fat colour after slaughter, score	75	BMS1290	TGLA73	0.4576	0.1700	7.2	368
9	Marbling, score	76	TGLA73	BM4208	0.3664	0.1328	7.6	368
9	Cooking loss of ST, %	85	TGLA73	BM4208	0.7427	0.2617	8.1	361
9	Intramuscular fat content, %	105	BM4208	BMS1967	0.7760	0.2441	10.1	361
10	Birth weight, kg	11	CSSM38	BMS528	2.0964	0.7476	7.5	398
10	Meat colour, score	23	CSSM38	BMS528	0.2799	0.1038	7.3	398
10	Melting point, ºC	35	BMS528	BMS861	1.5209	0.5300	8.2	368
10	Docility, score	39	BMS528	BMS861	1.0035	0.3624	7.7	361
10	Carcass bone, %	45	BM875	BM888	0.7794	0.2666	8.5	398
10	Body girth at birth, cm	76	BMS1620	TGLA272	2.0969	0.7549	7.7	361
10	Blood cortisol, µg/dL	85	BMS1620	TGLA272	9.1621	3.1621	7.9	361
10	Peak force on LD, kg	87	BMS1620	TGLA272	0.1180	0.0383	9.5	368
10	Gestation length, day	99	TGLA272	BMS2614	3.5524	1.0586	11.3	398
11	Ultimate pH of LD	18	BM827	BMS2131	0.0291	0.0097	7.3	398
11	Aging rate on ST, kg	40	BM304	RM096	0.0026	0.0009	8.1	368
11	Desaturation index, %	50	RM096	INRA111	2.8993	0.8421	11.9	361
11	Birth weight, kg	74	BMS1822	RM150	2.1507	0.7576	8.1	361
11	Carcass bone, %	86	BMS1048	BMS989	0.6565	0.2459	7.1	398
11	Aging rate on ST, kg	98	BMS989	RM363	0.0025	0.0009	7.0	398
11	Fat colour on biopsy samples	122	BMS2315	HEL13	0.3049	0.0770	15.7	368
12	Body hight at weaning, cm	0	BMS410	BM6108	2.4418	0.8167	9.0	361
12	Silver side, kg	3	BMS410	BM6108	0.4593	0.1658	7.7	361
12	Meat yield, %	9	BMS410	BM6108	1.5177	0.4352	12.2	361
12	Age at Puberty, day	11	BMS410	BM6108	25.3900	8.1798	9.6	361
12	Meat to bone ratio	30	BM6108	AGLA226	0.3532	0.0869	16.5	361
12	Meat to bone ratio	31	BM6108	AGLA226	0.3539	0.0876	16.3	361
12	Desaturation index, %	37	BM6108	AGLA226	2.1705	0.8198	7.0	368
12	Carcass bone, %	38	AGLA226	BM6404	1.1806	0.3389	12.1	361
12	Eye muscle area, cm^2	38	AGLA226	BM6404	8.3900	2.6864	9.8	361
12	Intramuscular fat content, %	61	BMS975	RM113	0.9853	0.2993	10.8	398
12	Ultimate pH of LD	64	BMS975	RM113	0.0500	0.0179	7.8	361
12	Cooking loss of ST, %	64	BMS975	RM113	0.7404	0.2648	7.8	368
12	Fat colour after slaughter, score	72	BMS975	RM113	0.6846	0.1743	15.4	368
12	βcarotene concentration, µg/g fat	92	RM113	BMS1316	0.3185	0.1120	8.1	361
12	Ossification, score	92	RM113	BMS1316	28.9910	10.4415	7.7	361
13	Aging rate on ST, kg	9	TGLA23	BMS1742	0.0024	0.0009	7.1	398
13	Aging rate on LD, kg	9	TGLA23	BMS1742	0.0037	0.0011	12.0	361
13	*M. Longissimus dorsi, kg*	9	TGLA23	BMS1742	0.4064	0.1133	12.9	398
13	Ultimate pH of LD	9	TGLA23	BMS1742	0.0303	0.0090	11.3	398
13	Birth weight, kg	43	BMS1742	ILSTS59	1.9811	0.7457	7.1	398
13	Peak force on ST, kg	57	HUJ616	BMS1669	0.0646	0.0219	8.7	368
13	Desaturation index, %	77	RM327	BL1071	1.9274	0.6821	8.0	368

Appendix A continued

BTA	Trait	Position	Left Marker	Right Marker	Effect	S.E.	F-Value	Family
13	Elongation index, %	78	BL1071	AGLA232	3.0103	0.7361	16.7	398
14	Fat colour on biopsy samples	14	BMS1678	ILSTS011	0.3278	0.0841	15.2	361
14	Cooking loss of LD, %	16	BMS1678	ILSTS011	1.6859	0.4508	14.0	368
14	Elongation index, %	16	BMS1678	ILSTS011	2.1189	0.7218	8.6	398
14	Body hight at weaning, cm	16	BMS1678	ILSTS011	2.3510	0.7393	10.1	398
14	Desaturation index, %	20	BMS1678	ILSTS011	2.3925	0.8229	8.4	368
14	Desaturation index, %	20	ILSTS011	RM011	2.3925	0.8230	8.4	368
14	Docility, score	22	BMS1678	ILSTS011	1.0454	0.3586	8.5	368
14	Carcass length, cm	25	ILSTS011	RM011	28.4500	9.4327	9.1	361
14	Body hight at weaning, cm	37	RM011	ILSTS008	3.0471	0.8293	13.5	361
14	Hot carcass weight, kg	42	RM011	ILSTS008	24.4697	7.3781	11.0	361
14	Heart, %	43	RM011	ILSTS008	0.0312	0.0104	9.0	361
14	Liver, %	43	RM011	ILSTS008	0.1030	0.3260	10.0	361
14	Stifle width at weaning, cm	43	RM011	ILSTS008	1.2027	0.4509	7.1	361
14	Body length at birth, cm	43	RM011	ILSTS008	2.1443	0.5457	15.4	361
14	Body hight at birth, cm	43	RM011	ILSTS008	2.4653	0.6662	13.7	361
14	Body hight at birth, cm	43	RM011	ILSTS008	2.4515	0.6839	12.9	361
14	Birth weight, kg	43	RM011	ILSTS008	2.8260	0.6862	17.0	361
14	Weaning weight, kg	43	RM011	ILSTS008	20.3987	5.6869	12.9	361
15	Liver, %	48	JAB4	POTCHA	0.1688	0.0570	8.8	368
15	Fat colour on biopsy samples	49	JAB4	POTCHA	0.5345	0.1958	7.4	398
15	Desaturation index, %	68	POTCHA	BM4325	1.7612	0.6366	7.7	368
15	Channel fat, kg	102	BM848	BMS429	2.3512	0.6530	13.0	398
16	Marbling, score	2	BMS357	HUJ614	0.3818	0.1366	7.8	368
16	βcarotene concentration, µg/g fat	51	BMS1907	CSSM28	0.2205	0.0817	7.3	361
16	Age at Puberty, day	72	CSSM028	BM719	24.7004	6.8891	12.9	398
16	Body length at weaning, cm	81	BM3509	INRA13	3.9044	1.2148	10.3	398
16	Stifle width at weaning, cm	82	BM3509	INRA13	1.2813	0.4600	7.8	398
16	Weaning weight, kg	83	INRA13	HUJ625	20.1550	5.3118	14.4	398
16	Peak force on ST, kg	84	INRA13	HUJ625	0.0628	0.0217	8.4	361
16	Eye muscle area, cm^9	84	INRA13	HUJ625	6.9334	2.5376	7.5	368
16	Pelvic area, mm^2	84	INRA13	HUJ625	10.5077	3.6571	8.3	368
16	*M. Longissimus dorsi, kg*	87	INRA13	HUJ625	0.4485	0.1377	10.6	368
17	Blood cortisol, µg/dL	2	RM156	BMS2220	7.4398	2.4398	8.3	398
17	*M. Longissimus dorsi, kg*	14	RM156	BMS2220	0.3876	0.1383	7.8	398
17	Cooking loss of LD, %	20	BMS2220	BMS941	0.8220	0.3128	7.0	398
17	Elongation index, %	26	BMS2220	BMS941	2.2486	0.7961	8.0	398
17	Silver side, kg	42	OAFCB48	ILSTS23	0.4749	0.1409	11.4	361
17	Meat yield, %	43	BMS941	OARFCB48	1.1140	0.3835	8.4	361
17	Kidney, %	55	OARFCB48	ILSTS23	0.0364	0.0117	9.7	368
17	Meat yield, %	68	ILSTS23	BM8125	1.6441	0.5597	9.0	368
17	Pelvic area, mm^2	74	ILSTS23	BM8125	12.0229	4.4004	7.5	398
17	*M. Longissimus dorsi, kg*	77	ILSTS23	BM8125	0.4062	0.1403	8.4	368
17	βcarotene concentration, µg/g fat	81	BL50	BM1862	0.1981	0.0675	8.6	368
17	Hot carcass weight, kg	86	BM1862	BM1233	20.0303	7.4778	7.2	368
17	Liver, %	88	BM1862	BM1233	0.1393	0.0498	7.8	368

Appendix A continued

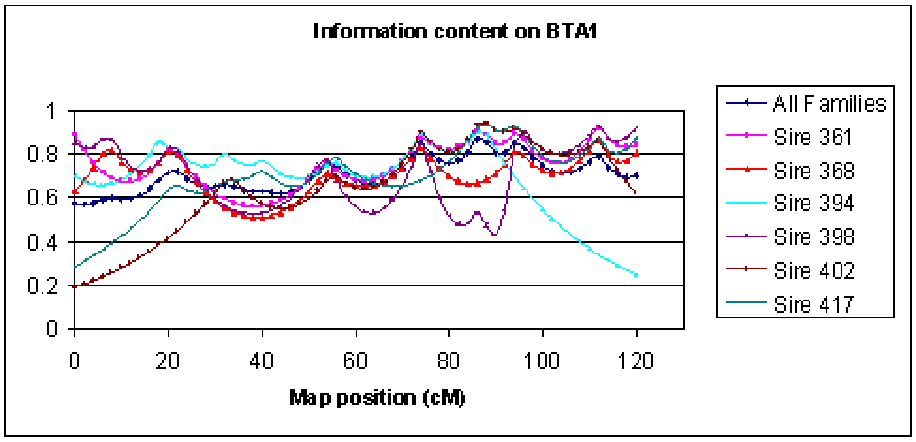
BTA	Trait	Position	Left Marker	Right Marker	Effect	S.E.	F-Value	Family
17	Meat to bone ratio	90	BM1862	BM1233	0.2630	0.0722	13.0	368
17	Meat to bone ratio	90	BM1862	BM1233	0.2617	0.0726	13.3	368
17	Eye muscle area, cm^2	90	BM1862	BM1233	9.4391	2.4401	15.0	368
17	Carcass bone, %	93	BM1862	BM1233	0.8563	0.2609	10.8	368
17	P8 fat depth, mm	93	BM1862	BM1233	3.3244	0.9195	13.1	368
17	Ossification, score	94	BM1862	BM1233	17.2189	6.3332	7.4	368
17	Heart, %	99	BM1862	BM1233	0.0367	0.0139	7.0	368
18	Silver side, kg	16	BMS1355	TEXAN10	0.4703	0.1683	7.8	361
18	Pelvic hight, mm	21	TEXAN10	INRA121	8.0722	2.8584	8.0	398
18	Pelvic width, mm	40	BM8151	INRA63	4.4943	1.6104	7.8	368
18	Peak force on LD, kg	42	BM8151	INRA63	0.1611	0.0382	17.8	361
18	Meat colour, score	43	BM8151	INRA63	0.3411	0.1264	7.3	361
18	Channel fat, kg	44	BM8151	INRA63	1.7126	0.5597	9.4	361
18	Melting point, ºC	81	ILSTS2	TGLA227	1.3255	0.4819	7.6	361
19	FDistance, meter	0	BM9202	HEL10	1.9728	0.7098	7.7	361
19	Aging rate on LD, kg	21	HEL10	BMS2142	0.0043	0.0013	10.4	398
19	Meat colour, score	23	HEL10	BMS2142	0.4093	0.1388	8.7	361
19	Body hight at weaning, cm	45	BMS2142	BP20	2.0183	0.6911	8.5	398
19	Carcass fat,%	76	BM17132	ETH3	1.5298	0.4988	9.4	361
19	Pelvic width, mm	99	RM388	BMS601	4.8343	1.7796	7.4	368
20	Channel fat, kg	9	BM1225	TGLA304	1.6989	0.6306	7.3	368
20	Pelvic hight, mm	11	BM1225	TGLA304	9.0400	3.2881	7.6	398
20	Aging rate on ST, kg	53	BM4107	BMS1120	0.0025	0.0008	8.0	398
21	Birth weight, kg	6	BM8115	RM151	2.2116	0.7117	9.7	368
21	Blood cortisol, µg/dL	15	BM3413	AGLA233	6.9502	2.5058	7.7	398
21	Cooking loss of ST, %	36	BM103	BMC4228	0.7795	0.2567	9.2	361
21	Gestation length, day	36	BM103	BMC4228	2.9361	1.0293	8.1	398
21	Silver side, kg	39	BM103	BMC4228	0.3724	0.1156	10.4	398
22	Fat depth at weaning, mm	3	INRA26	BM1558	0.6958	0.2523	7.6	361
22	Pelvic area, mm2	5	INRA26	BM1558	10.5044	3.0760	11.7	361
22	Body hight at birth, cm	18	BM1558	BM1303	2.3589	0.7070	11.1	368
22	*M. semitendinosus, kg*	36	AGLA13	BMS390	0.1962	0.0741	7.0	361
22	Kidney, %	70	BMS875	HMH1R	0.0294	0.0098	9.0	361
22	Birth weight, kg	79	HMH1R	BM4102	2.5136	0.9198	7.5	368
23	Gestation length, day	20	INRA132	CYP21	4.0898	1.2222	11.2	361
23	Elongation index, %	70	BM1905	BM1443	2.1290	0.6390	11.1	361
23	Hot carcass weight, kg	73	BM1905	BM1443	19.1037	7.0128	7.4	368
24	Mono-unsaturated fatty acids, %	15	BM7151	CSSM31	2.2860	0.7865	8.5	398
24	Saturate fatty acids, %	17	BM7151	CSSM31	2.2587	0.8208	7.6	398
24	Fat depth at weaning, mm	35	CSSM31	BMS1743	0.6318	0.2078	9.2	398
24	Cooking loss of LD, %	52	BMS1743	INRA90	1.1223	0.4072	7.6	368
24	Intramuscular fat content, %	55	BMS1743	INRA90	0.9227	0.2426	14.5	368
25	Peak force on LD, kg	14	BM4005	BP28	0.0995	0.0341	8.5	398

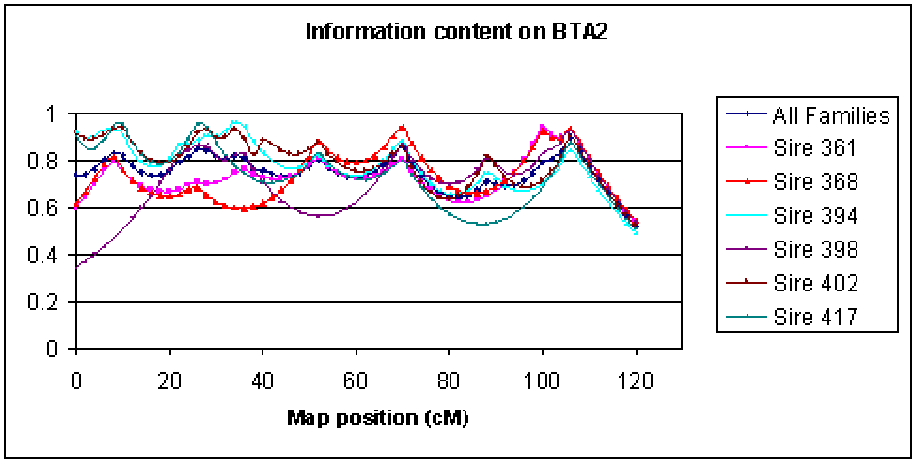
Appendix A continued

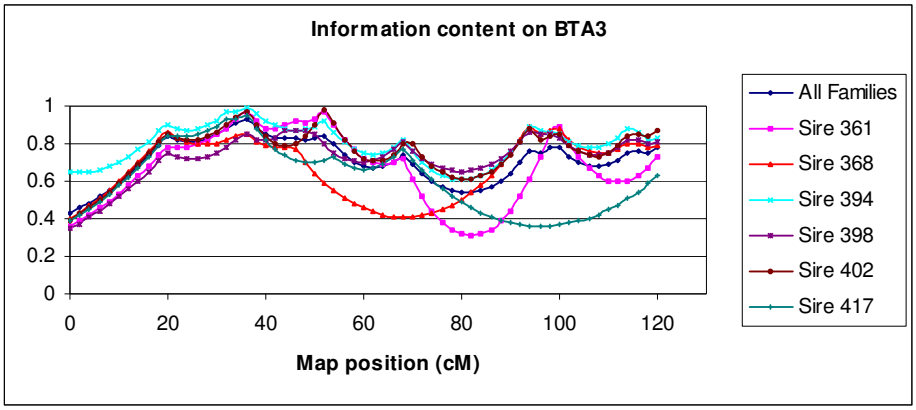
BTA	Trait	Position	Left Marker	Right Marker	Effect	S.E.	F-Value	Family
25	Carcass fat,%	14	BM4005	BP28	1.3589	0.3990	11.6	398
25	Hot carcass weight, kg	41	BM737	BMS1353	23.3609	7.6110	9.4	398
25	Weaning weight, kg	42	BM737	BMS1353	15.8900	5.7238	7.7	398
25	F-Distance, meter	44	BM737	BMS1353	1.9091	0.7186	7.1	398
25	Carcass length, cm	45	BM737	BMS1353	33.4671	10.6295	9.9	398
25	P8 fat depth, mm	47	BM737	BMS1353	2.6197	0.1919	8.1	398
25	Docility, score	47	BM737	BMS1353	0.7986	0.2850	7.8	368
26	Elongation index, %	3	BMS651	BM1314	2.2175	0.7140	9.7	398
26	Fat depth at weaning, mm	17	BMS651	BM1314	0.5861	0.2064	8.1	368
26	Saturate fatty acids, %	23	BMS651	BM1314	2.4430	0.8466	8.3	398
26	Meat colour, score	32	BM1314	BM6041	0.4081	0.1514	7.3	361
27	Silver side, kg	4	BMS2168	BM6526	0.3921	0.1233	10.1	398
27	Meat yield, %	9	BMS2168	BM6526	1.0803	0.4072	7.0	398
27	*M. semitendinosus, kg*	10	BMS2168	BM6526	0.1638	0.0596	7.6	398
27	Stifle width at weaning, cm	20	BM6526	CSSM43	1.5910	0.6000	7.0	361
27	Blood cortisol, µg/dL	43	CSSM43	INRA134	9.5450	2.5037	14.5	398
28	Marbling, score	8	BMC6020	BL25	0.3557	0.1106	10.3	361
28	Aging rate on ST, kg	34	BL25	BM6466	0.0024	0.0009	7.1	368
28	Docility, score	39	BM6466	BMS1714	1.0354	0.2908	12.7	368
28	Body hight at weaning, cm	50	BMS1714	BMC2208	2.3679	0.7897	9.0	368
28	Pelvic width, mm	50	BMS1714	BMC2208	4.3362	1.3845	9.8	361
28	Ossification, score	52	BMS1714	BMC2208	19.6979	6.4625	9.3	368
28	Fat depth at ribs, mm	57	BMS1714	BMC2208	1.7577	0.6133	8.2	361
29	Peak force on LD, kg	49	OARHH22	BMC1206	0.1356	0.0450	9.1	361
29	Peak force on LD, kg	56	OARHH22	BMC1206	0.1126	0.0346	10.6	368
29	Kidney, %	59	BMC1206	BMS1948	0.0301	0.0089	11.3	368
29	Mono-unsaturated fatty acids, %	62	BMC1206	BMS1948	1.9402	0.7018	7.6	398
29	Saturate fatty acids, %	62	BMC1206	BMS1948	2.0309	0.7195	8.0	398

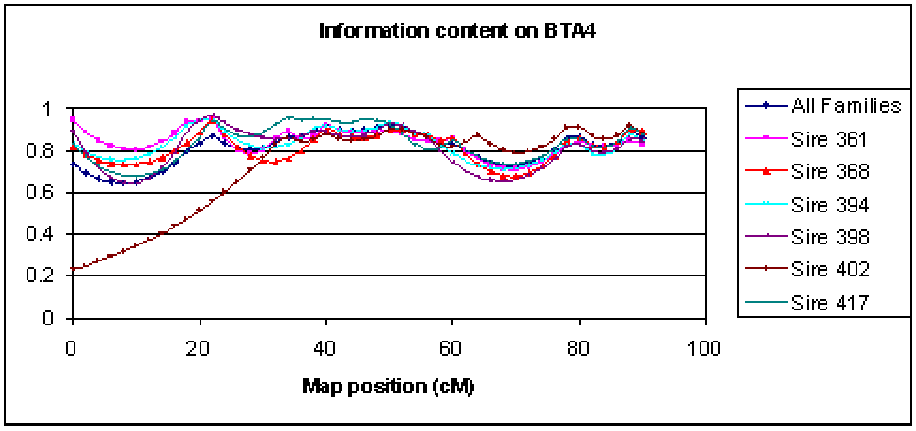
[a] Map position based on Ihara et al. (2004). [b] LD: *M. Longissimus dorsi* muscle. [c]ST: *M. semitendinosus* muscle.

Appendix B Information content derived from 29 Bovine autosomes (BTA)









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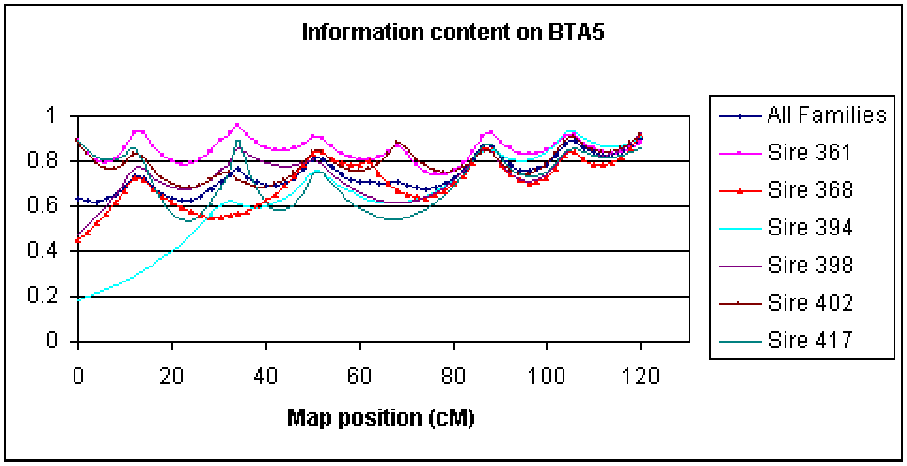

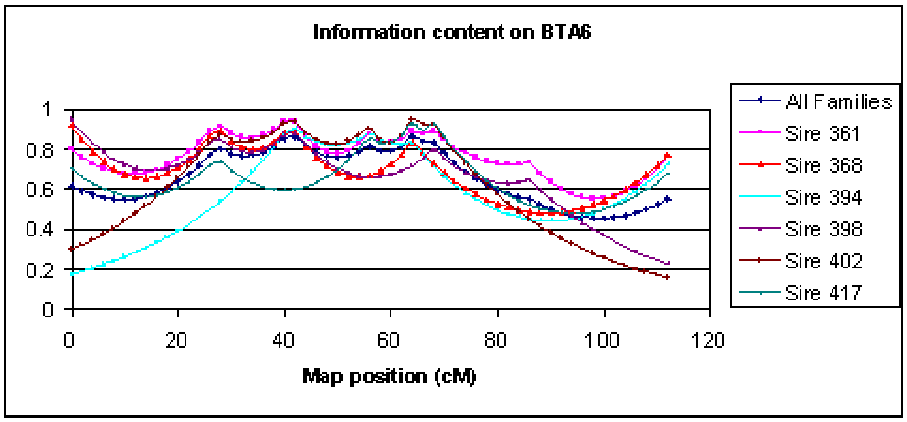

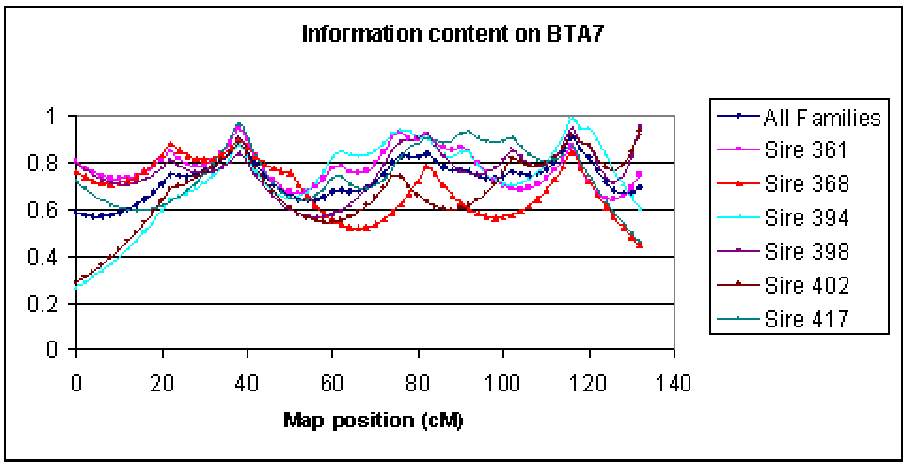

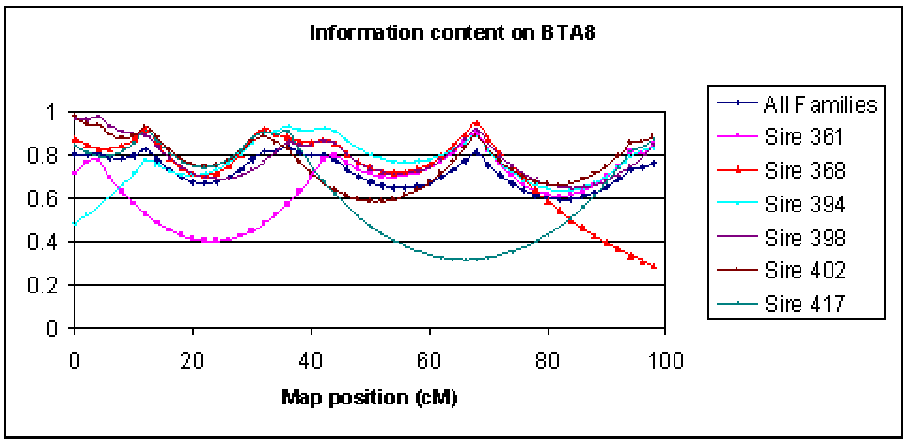

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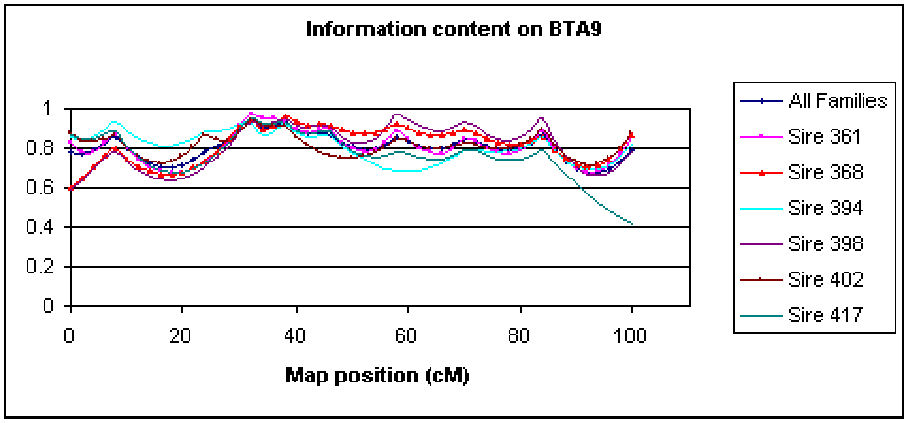

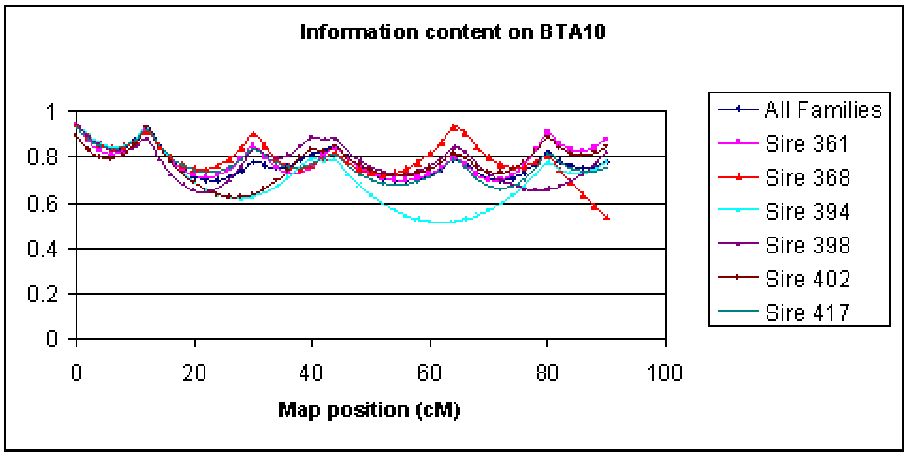

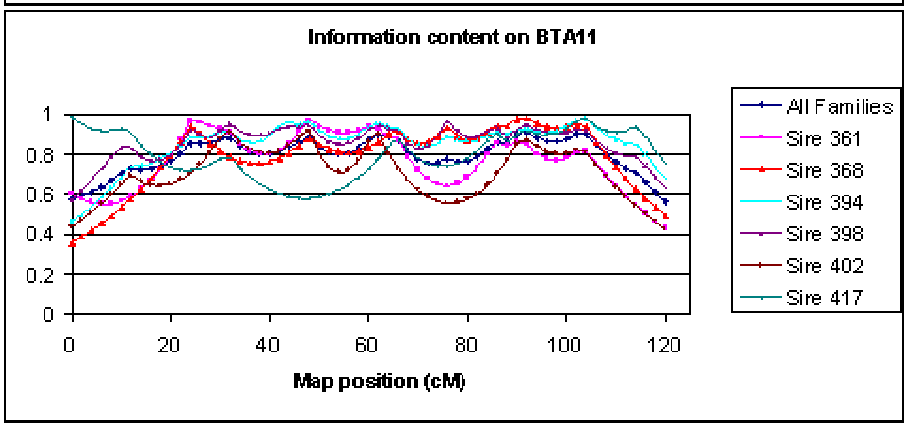

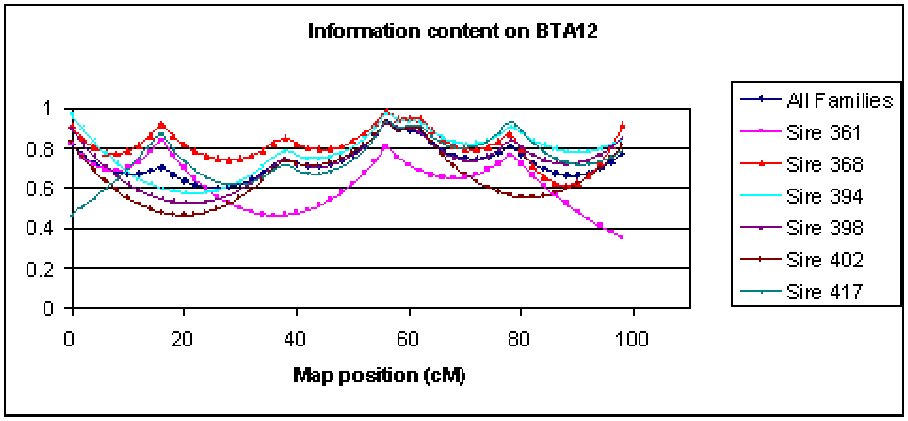

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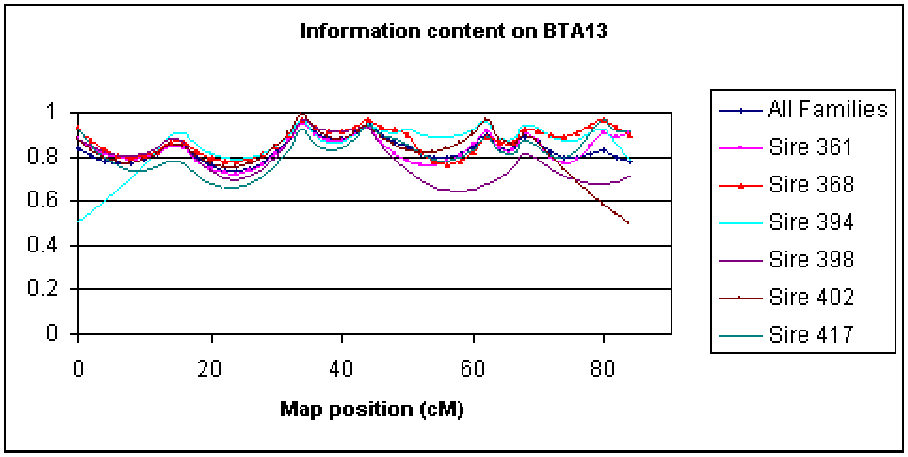

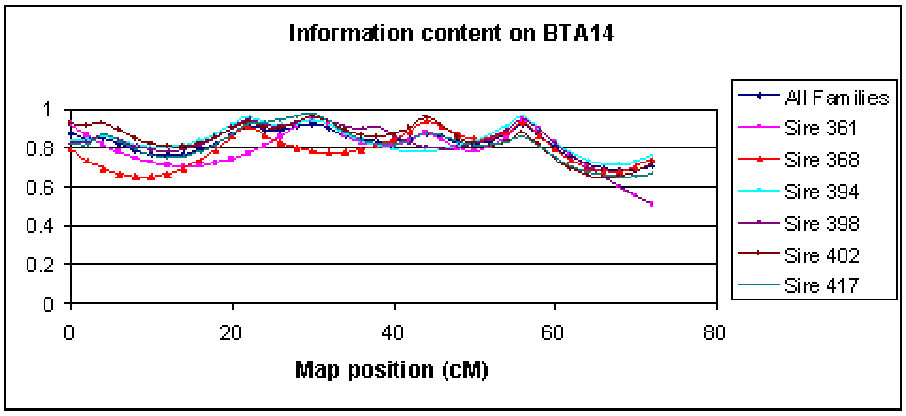

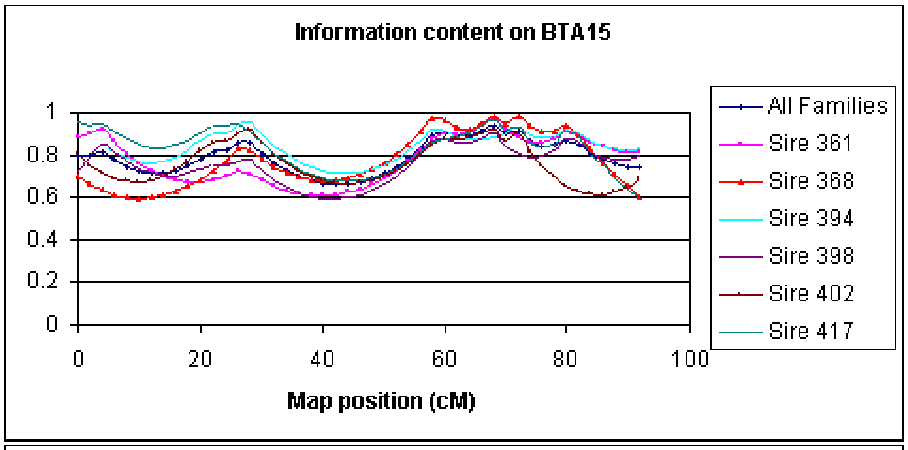

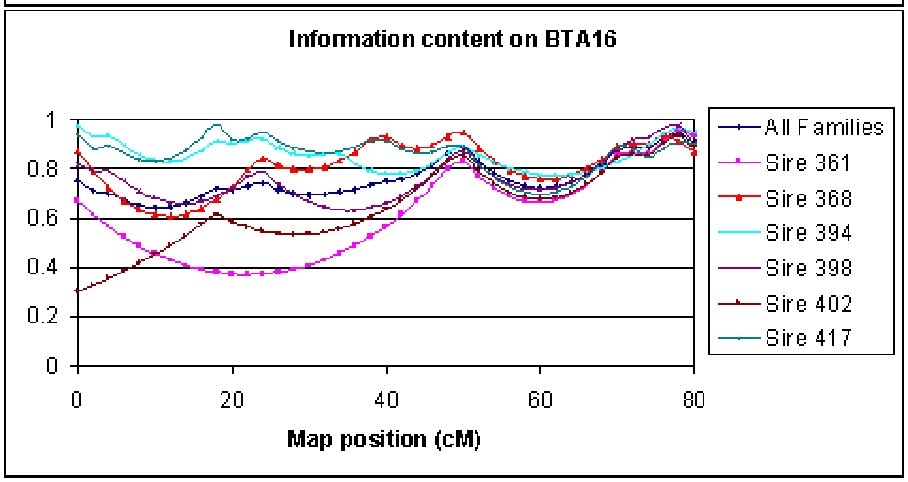

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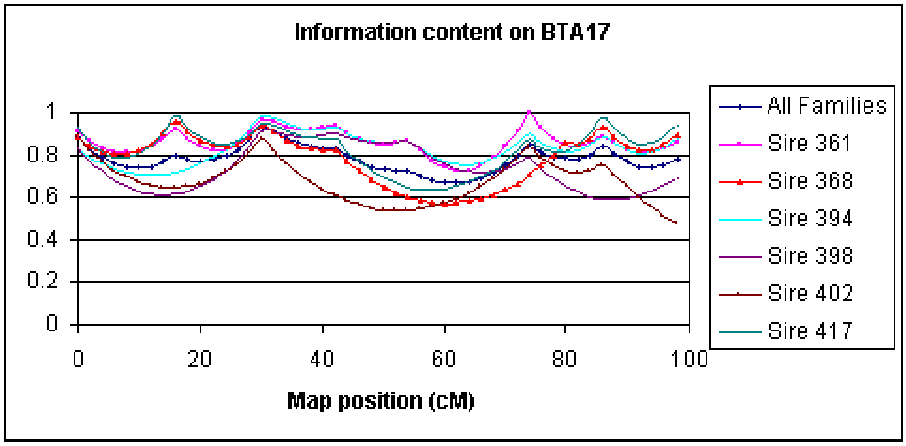

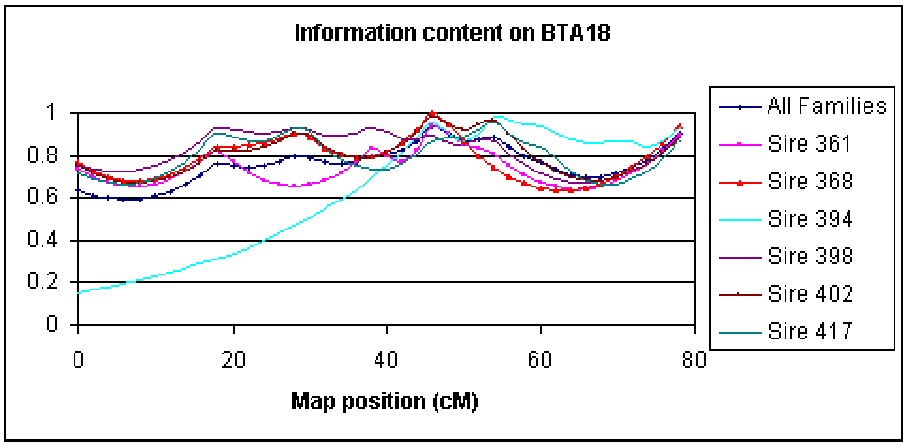

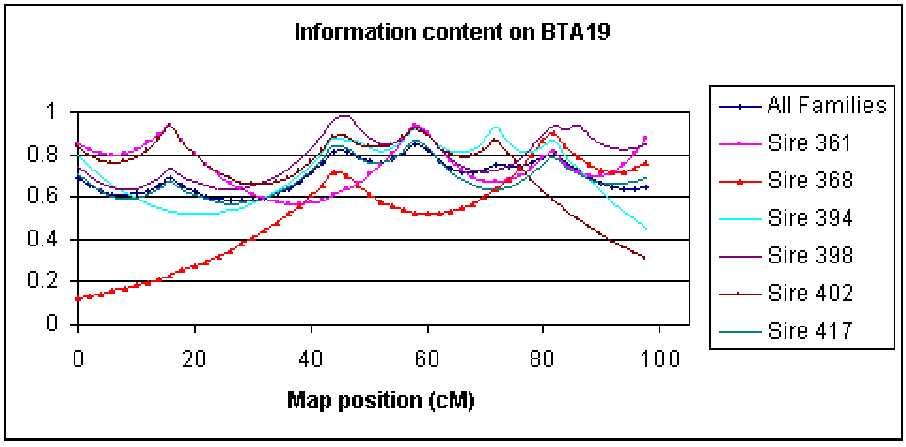

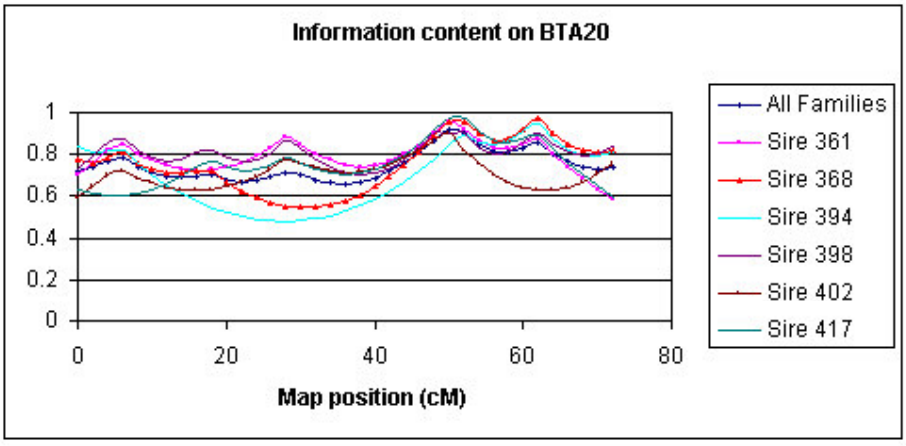

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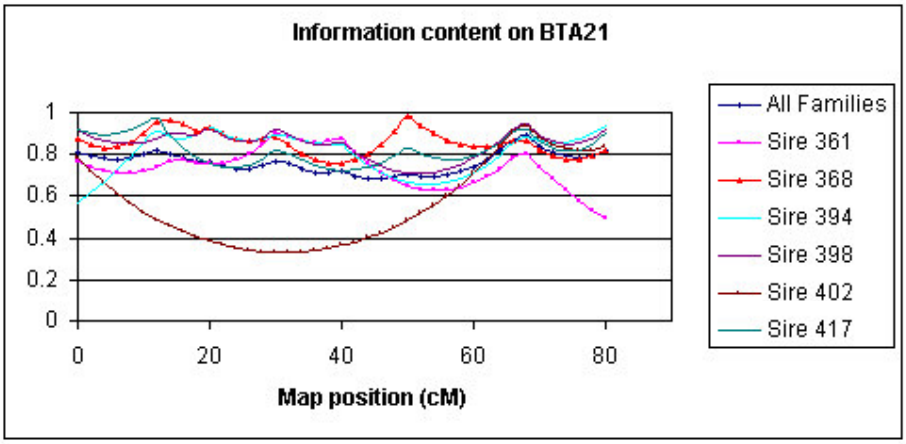

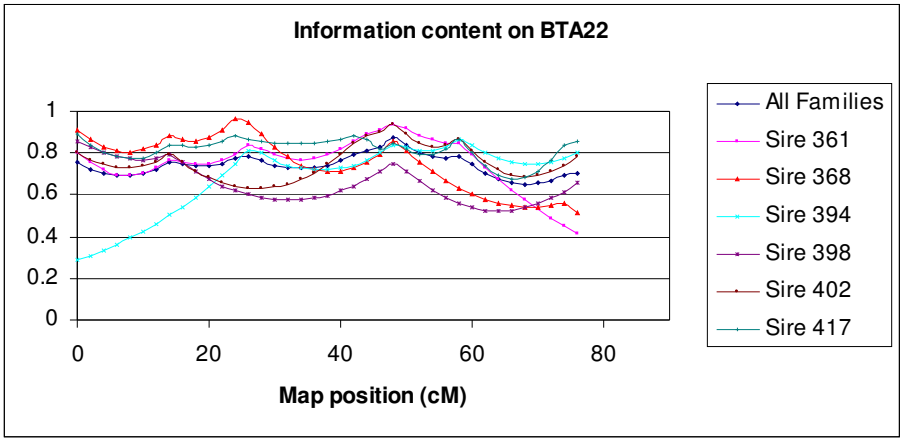

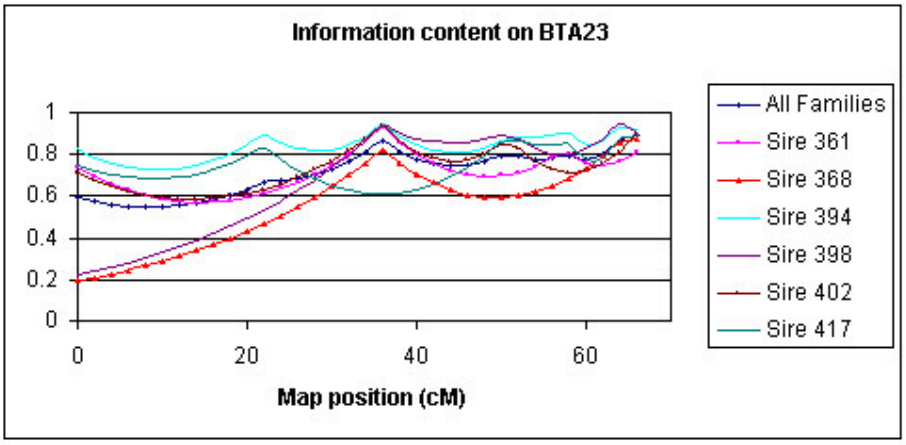

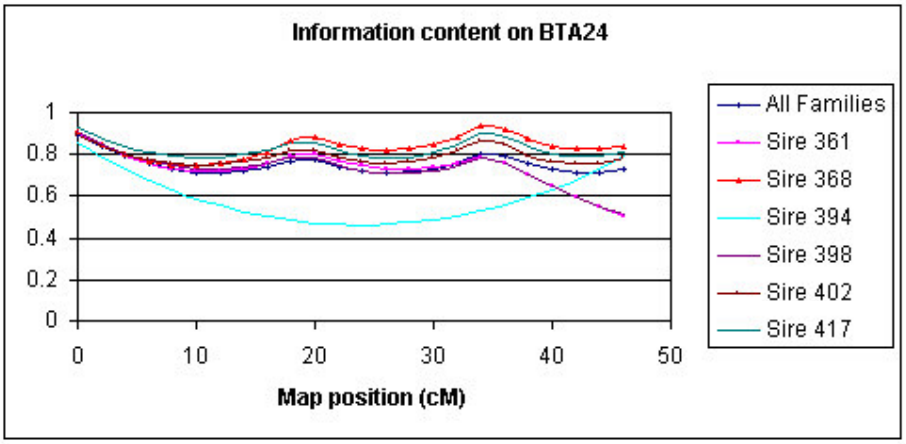

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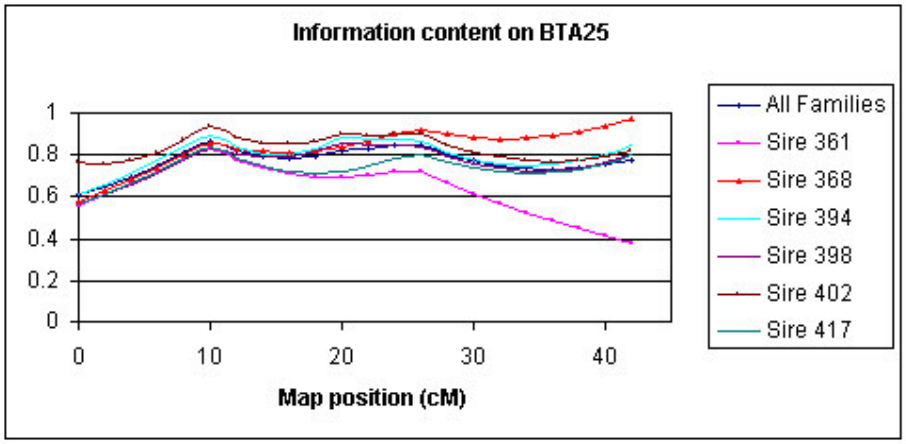

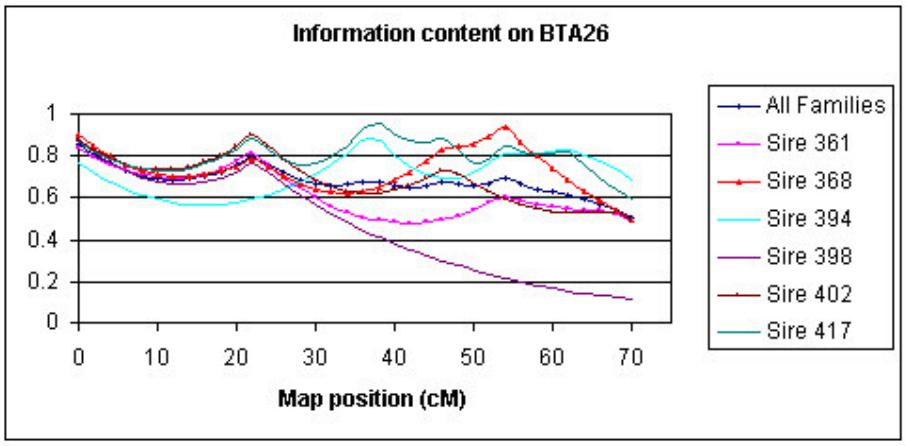

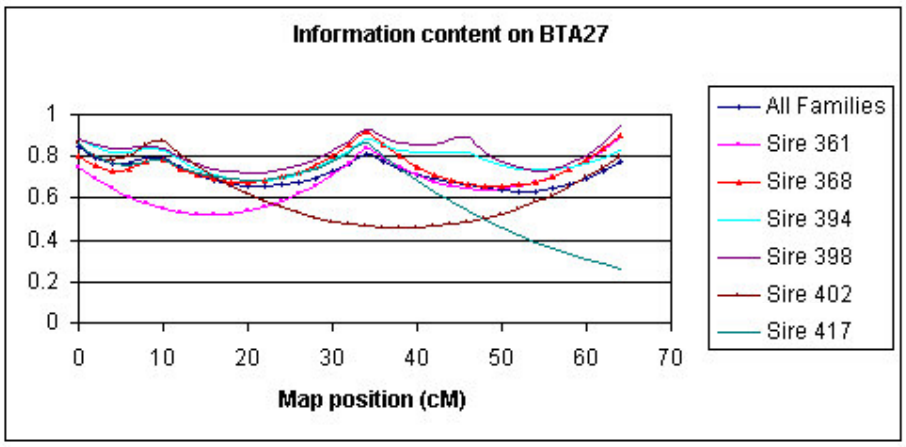

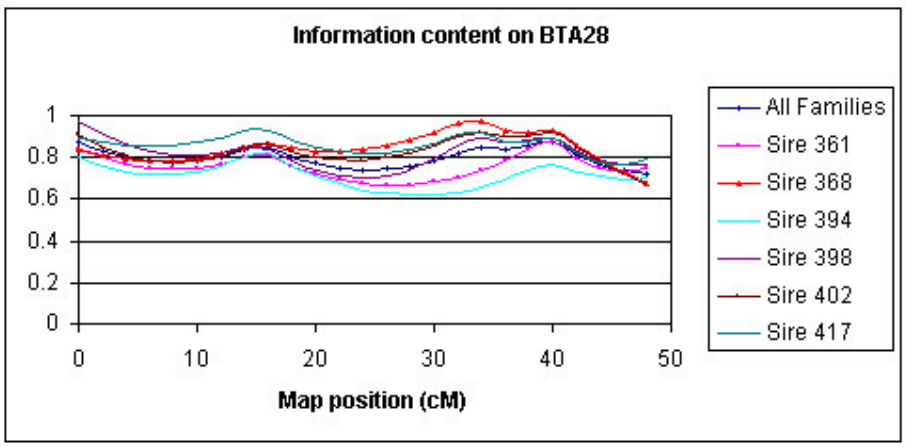

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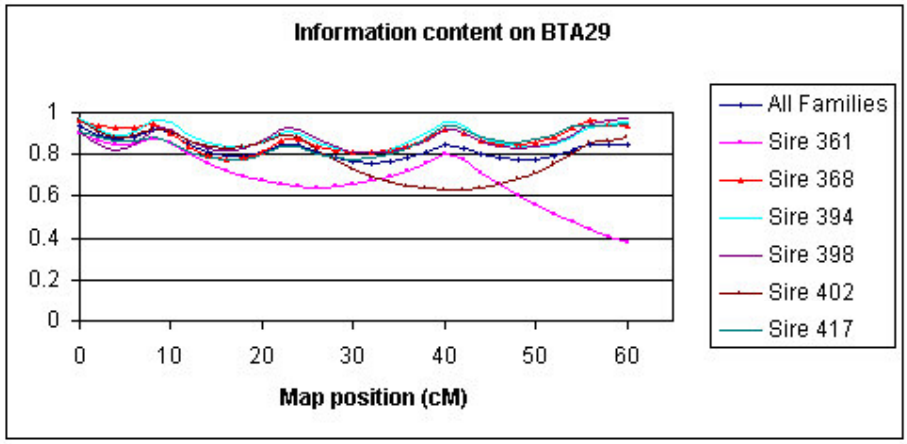

Appendix C Australian results for additive (*a*) and dominance (*d*) effects of myostatin and breed effects for traits measured on live animals, carcass and beef quality traits

Trait	Abbreviation	N	Mean	S.D.	Min	Max	*Limousin* *a* (s.e.)	*Limousin* *d* (s.e.)	*Jersey* $a + d$ (s.e) [#]	Breed difference (L-J)
Birth weight, kg	Bwt	366	26.45	6.09	12.80	44.70	0.68 (0.52)	-0.84 (0.65)	0.24 (0.47)	8.55 (0.98)***
Body height at birth, cm	Birht	366	68.73	5.03	54.00	84.00	-0.26 (0.52)	-0.17 (0.66)	0.18 (0.52)	5.29 (0.10)
Body length at birth, cm	Birlg	366	114.48	6.97	97.00	132.00	0.54 (0.76)	0.12 (0.96)	0.13 (0.76)	7.28 (1.45)***
Body width at birth, cm	Birwd	366	53.36	3.73	38	65.00	0.14 (0.41)	-0.56 (0.52)	0.04 (0.41)	4.01 (0.79)***
Gestation length, day	Gl	363	283.69	5.04	273.00	293.00	0.70 (0.68)	0.13 (0.86)	-0.02 (0.68)	1.05 (1.30)
Weaning weight, kg	Wwt	365	228.55	32.01	112.00	308.00	3.50 (3.53)	-3.36 (4.47)	4.61 (3.54)	25.65 (6.72)***
Body hight at weaning, cm	Wht	359	109.94	4.94	90.00	120.00	0.91 (0.53)	0.71 (0.68)	0.12 (0.53)	3.48 (1.01)
Hip width at weaning, cm	Whip	365	34.72	2.43	26.00	45.00	-0.17 (0.27)	-0.21 (0.34)	0.26 (0.27)	0.59 (0.51)
Stifle width at weaning, cm	Wsti	365	28.11	2.96	18.00	36.00	0.71 (0.29)*	-0.86 (0.37)*	0.38 (0.29)	2.98 (0.55)***
Fat depth at weaning, mm	Wfat	364	0.49	1.14	0.00	5.00	-0.24 (0.16)	-0.13 (0.20)	-0.13 (0.16)	0.39 (0.29)
Body weight at 400 days, kg	400Wt	365	252.30	36.46	137.00	361.00	4.64 (3.40)	-3.16 (4.32)	2.51 (3.42)	25.46 (6.49)***
Body hight at 400 days, cm	400Ht	365	119.41	5.10	100.00	143.00	-0.13 (0.59)	0.66 (0.75)	0.55 (0.59)	4.80 (1.12)***
Hip width at 400 days, cm	400Hip	288	39.05	1.99	32.00	46.00	-0.42 (0.27)	-0.62 (0.34)	0.11 (0.26)	1.50 (0.52)**
Stifle width at 400 days, cm	400Sti	288	28.86	2.82	22.00	40.00	0.47 (0.34)	-0.43 (0.42)	0.60 (0.32)	3.86 (0.64)***
Fat depth at 400 days, mm	400Fat	364	1.06	1.37	0.00	5.00	-0.14 (0.09)	0.00 (0.12)	0.06 (0.09)	0.03 (0.18) [ns]
Body weight at 600 days, kg	600Wt	362	361.38	43.37	235.00	492.00	1.42 (4.21)	-5.59 (5.34)	4.37 (4.24)	54.42 (8.04)***
Body hight at 600 days, cm	600Ht	363	126.02	5.62	108.00	140.00	0.47 (0.50)	0.39 (0.64)	-0.10 (0.51)	5.10 (0.96)***
Hip width at 600 days, cm	600Hip	363	42.99	2.06	37.00	49.00	-0.41 (0.27)	-0.97 (0.34)**	-0.07 (0.27)	1.89 (0.52)***
Stifle width at 600 days, cm	600Sti	363	31.93	3.51	22.00	45.00	0.90 (0.30)**	-0.53 (0.38)	0.45 (0.30)	3.93 (0.57)***
Fat depth at 600 days, mm	600Fat	362	1.63	2.37	0.00	12.00	-0.89 (0.24)***	-0.11 (0.31)	-0.63 (0.25)*	0.77 (0.47) [ns]
Age at puberty, days	AP	181	420.00	47.20	289.00	506.00	-3.38 (4.78)	1.57 (6.43)	-6.32 (5.51)	-0.85 (9.13) [ns]
Marbling, score	Mar	355	1.53	0.79	0.00	4.00	-0.26 (0.09)**	0.01 (0.11)	0.01 (0.09)	-0.40 (0.17)*
Carcass fat depth on the rump, mm	P8	356	12.27	5.23	3.00	30.00	-2.30 (0.61)***	0.17 (0.77)	-0.26 (0.63)	3.26 (1.17)**
Meat colour, score	MC	355	1.95	0.92	1.00	6.00	0.02 (0.10)	-0.01 (0.12)	0.06 (0.10)	-0.24 (0.07)**
Fat colour, score	FC	355	1.78	1.40	0.00	7.00	0.04 (0.13)	0.04 (0.17)	0.15 (0.14)	-0.98 (0.25)***
β-carotene concentration, µg/g fat	Bcbio	363	1.15	0.55	0.10	4.20	0.03 (0.06)	0.10 (0.08)	0.06 (0.07)	-0.24 (0.12)
Fat colour on biopsy samples, score	Fcb	363	2.02	0.61	1.00	5.00	-0.06 (0.08)	0.02 (0.10)	0.03 (0.08)	-0.36 (0.15)*
Eye muscle area, cm^2	EMA	355	80.69	17.00	26.00	166.00	8.50 (1.49)***	-5.08 (1.89)**	3.42 (1.53)*	15.42 (2.85)***
Carcass length, cm	Carclg	356	139.04	5.76	120.0	155.50	-4.42 (6.28)	1.75 (7.96)	-3.02 (6.45)	29.71 (12.02)*
Pelvic area, cm^2	PA	356	278.72	45.06	170.50	451.00	-6.20 (4.77)	0.40 (6.05)	2.51 (4.90)	40.71 (9.13)***
Channel fat, kg	Chanfat	356	12.52	3.86	4.70	22.80	-0.27 (0.43)	0.16 (0.54)	-0.80 (0.44)	-1.71 (0.82)*
Omental fat, kg	Omenfat	266	12.02	4.14	3.30	24.50	-0.23 (0.46)	-0.37 (0.58)	0.01 (0.44)	-1.09 (0.89)
Heart weight, kg	Heart	351	1.81	0.33	0.96	5.96	-0.04 (0.04)	-0.05 (0.05)	-0.02 (0.04)	0.07 (0.08)

Appendix C continued

Trait	Abbreviation	N	Mean	S.D.	Min	Max	*Limousin* a (s.e.)	*Limousin* d (s.e.)	*Jersey* $a + d$ (s.e) [#]	Breed difference (L-J)
Liver weight, kg	Liver	334	5.89	0.96	1.50	9.30	-0.07 (0.11)	-0.09 (0.13)	0.07 (0.11)	0.22 (0.20)
Kidney weight, kg	Kidney	353	1.05	0.17	0.59	1.60	-0.03 (0.02)	0.00 (0.02)	-0.03 (0.02)	0.05 (0.03)
Fat depth at ribs 12th and 13th, mm	Rbft	356	9.68	3.64	3.00	24.00	-0.91 (0.46)*	0.13 (0.58)	-0.73 (0.47)	0.41 (0.87)
Ossification, score	Ossms	356	225.45	47.12	140.00	400.00	-2.95 (4.90)	-2.29 (6.22)	-0.68 (5.04)	14.43 (9.38)
Butt shape, score	Butt	356	2.51	0.68	1.00	4.00	0.16 (0.07)*	-0.20 (0.08)*	0.01 (0.06)	0.81 (0.12)***
Intramuscular fat content, %	Imf	355	5.25	1.71	1.43	11.1	-0.43 (0.19)*	0.15 (0.24)	-0.21 (0.19)	-1.36 (0.36)***
Melting point, ^{o}C	Melpt	355	37.40	3.09	31.00	46.00	-0.34 (0.37)	0.42 (0.46)	0.17 (0.38)	2.12 (0.70)**
Mono-unsaturated fatty acids, % of triacylglyceride	MUFA	355	49.17	5.18	36.02	61.10	0.68 (0.50)	0.59 (0.63)	-0.32 (0.51)	-3.18 (0.95)**
Hot standard carcass weight, kg	Hscw	356	334.71	61.68	168	479.6	8.90 (4.96)	-8.42 (6.29)	3.87 (5.10)	76.07 (9.49)***
Meat weight, kg[#]	Meat	330	230.40	48.47	114.50	355.16	16.82 (3.65)***	-9.74 (4.63)*	4.97 (3.75)	55.84 (6.98)***
Fat weight, kg[#]	Fat	330	45.30	11.32	11.30	82.40	-7.48 (1.32)***	0.67 (1.68)	-0.78 (1.35)	10.22 (2.51)***
Bone weight, kg[#]	Bone	330	58.89	9.98	33.53	88.70	-0.44 (0.78)	0.63 (1.00)	-0.20 (0.80)	10.17 (1.50)***
Flight distance, meter	F-dist	357	9.66	3.82	1.50	23.00	-0.48 (0.51)	0.68 (0.64)	0.62 (0.51)	-0.05 (0.96)
Docility, score	Docsco	362	12.27	1.73	7.50	15.80	0.10 (0.22)	-0.61 (0.28)*	-0.19 (0.22)	0.51 (0.42)
Weight of longissimus dorsi, kg	LD	351	6.27	1.49	3.11	11.48	-0.15 (0.32)	-0.33 (0.39)	0.07 (0.31)	1.83 (0.60)**
Weight of semitendinosus, kg	ST	349	2.49	0.84	1.14	10.00	0.27 (0.14)	-0.17 (0.18)	0.11 (0.15)	1.24 (0.27)***
Weight of silverside, kg	SilverS	346	8.48	2.26	3.82	16.40	0.49 (0.07)***	-0.23 (0.09)*	0.10 (0.07)	0.71 (0.14)***
pH of Longissimus dorsi muscle	pHld	355	5.63	0.12	5.43	6.70	-0.01 (0.01)	0.04 (0.01)	0.02 (0.01)	0.00 (0.02)
pH of semitendinosus muscle	pHst	351	5.69	0.10	5.45	6.38	-0.01 (0.01)	-0.40 (0.01)	-0.01 (0.01)	0.00 (0.02)
Cooking losses of LD muscle, %	Clld	355	21.82	1.89	14.46	39.05	0.01 (0.24)	0.25 (0.30)	0.14 (0.24)	0.91 (0.45)**
Cooking losses of ST muscle, %	Clst	351	26.25	1.81	19.04	31.05	-0.66 (0.18)***	0.20 (0.23)	-0.04 (0.19)	0.83 (0.35)*
SF[a] of LD[b] on day 1 post-mortem, kg	Wbld1	355	4.87	1.34	2.60	13.20	-0.21 (0.16)	-0.03 (0.21)	0.20 (0.17)	0.57 (0.31)
SF of LD on day 5 post-mortem, kg	Wbld2	355	4.42	1.03	2.50	10.30	-0.13 (0.12)	-0.02 (0.16)	-0.09 (0.13)	0.58 (0.24)*
SF of LD on day 12 post-mortem, kg	Wbld3	355	4.19	1.04	1.80	10.60	-0.05 (0.12)	0.05 (0.16)	-0.17 (0.13)	0.39 (0.24)
SF of LD on day 26 post-mortem, kg	Wbld4	355	3.96	0.99	2.10	9.90	-0.05 (0.11)	0.07 (0.14)	-0.02 (0.12)	0.48 (0.22)*
SF of ST[c] on day 1 post-mortem, kg	Wbst1	352	5.30	0.88	3.50	10.30	-0.30 (0.09)**	0.30 (0.12)*	-0.29 (0.10)**	-0.62 (0.18)***
SF of ST on day 5 post-mortem, kg	Wbst2	352	5.07	0.84	2.60	7.40	-0.39 (0.09)**	0.19 (0.11)	-0.28 (0.09)**	-0.39 (0.17)*
SF of ST on day 12 post-mortem, kg	Wbst3	352	4.93	0.84	2.70	8.70	-0.32 (0.08)**	0.17 (0.10)	-0.25 (0.08)**	-0.46 (0.15)**
SF of ST on day 26 post-mortem, kg	Wbst4	351	4.67	0.79	2.70	8.40	-0.22 (0.08)**	0.31 (0.10)**	-0.11 (0.08)	-0.56 (0.15)***

[#] Estimated from prediction equations. [a] SF= Warner-Bratzler Shear force. [b]LD: *M. Longissimus dorsi* muscle. [c]ST: *M. semitendinosus* muscle. *: P <0.05, **: P< 0.01, *** : P<0.001.

Appendix D New Zealand results for additive (*a*) and dominance (*d*) effects of myostatin and breed effects for traits measured on live animals, carcass and beef quality traits

Trait	Abbreviation	N	Mean	S.D.	Min	Max	*Limousin* *a* (s.e.)	*Limousin* *d* (s.e.)	*Jersey* *a* + *d* (s.e) [#]	Breed difference (L-J)
Birth weight, kg	Bwt	306	29.53	5.72	14.00	50.00	0.22 (0.64)	-2.23 (0.80)**	0.40 (0.58) ns	8.78 (1.39)***
Body weight at 400 days, kg	400Wt	241	267.66	38.21	194.00	371.00	1.25 (3.98)	-8.04 (5.14)	-2.62 (3.09)	67.76 (8.08)***
Body weight at 600 days, kg	600Wt	413	435.65	67.05	286.00	642.00	7.27 (6.23)	-7.03 (7.80)	0.13 (4.58)	63.15 (12.89)***
Gestation length, day	Gl	185	284.52	5.86	258.00	301.00	0.04 (0.94)	-0.45 (1.21)	0.69 (1.28)	2.07 (1.45)
Age at puberty, day	Pubert	139	371.19	48.58	199.00	466.00	-2.63 (9.60)	-2.46 (13.00)	1.91 (9.43)	-29.70 (20.12)
Fat weight at side, kg	Fatwt	401	9.48	2.58	3.34	22.81	-0.77 (0.37)*	1.42 (0.46)**	-0.67 (0.27)*	-0.32 (0.76)
Bone weight, kg	Bonewt	401	23.88	3.90	15.89	35.52	-0.06 (0.33)	-0.28 (0.42)	-0.11 (0.25)	3.99 (0.69)***
Meat weight, kg	Meatwt	401	74.13	16.70	44.48	126.09	4.39 (1.26)***	-4.98 (1.58)**	1.58 (0.93)	22.54 (2.61)***
Rump meat weight, kg	Rumeat	406	5.09	1.16	3.02	8.42	0.27 (0.09)**	-0.34 (0.12)**	0.08 (0.07	1.80 (0.19)***
Hot carcass weight, kg	Hscw	413	227.65	43.86	136	369	7.70 (3.72)*	-8.29 (4.65)	1.74 (2.73)	53.39 (7.68)***
Weight of longissimus dorsi, kg	LD	405	6.03	1.35	2.93	10.77	0.36 (0.12)**	-0.14 (0.15)	0.01 (0.08)	1.40 (0.25)***
Weight of silverside, kg	SilverS	406	8.37	2.24	4.36	16.09	0.60 (0.15)***	-0.87 (0.19)***	0.21 (0.11)	3.27 (0.32)***
Eye muscle area, cm^2	EMA	326	58.96	13.62	37.25	111.75	2.84 (1.25)*	-7.64 (1.57)***	2.60 (0.91)**	21.35 (2.60)***
pH of *M. longissimus dorsi* muscle	pHld	413	5.44	0.06	5.34	6.19	-0.00 (0.01)	0.01 (0.01)	0.01 (0.01)	-0.00 (0.02)
SF[a] of LD[b] on *rigor mortis*, kg	Wbld1	413	14.59	2.81	5.36	2.74	-0.16 (0.58)	-1.49 (0.72)*	0.87 (0.42)*	2.78 (1.19)*
SF of LD on day 1.3 after *rigor mortis*, kg	Wbld2	413	10.87	2.88	2.74	20.01	-0.99 (0.44)*	-1.26 (0.54)*	0.75 (0.32)*	4.13 (0.90)***
SF of LD on day 2.0 after *rigor mortis*, kg	Wbld3	413	7.44	1.73	3.31	17.43	0.29 (0.45)	-0.44 (0.57)	0.52 (0.33)	2.27 (0.94)*
SF of LD on day 2.3 after *rigor mortis*, kg	Wbld4	400	6.80	1.39	4.04	16.66	-1.15 (0.53)*	-1.61 (0.65)*	0.15 (0.38)	3.97 (1.10)***
SF of LD on day 4.0 after *rigor mortis*, kg	Wbld5	413	5.83	1.21	3.59	15.55	0.74 (0.47)	-0.02 (0.59)	0.10 (0.34)	0.42 (0.97)
Cooking loss of LD on *rigor mortis*, %	Clld1	413	23.39	4.96	8.65	53.88	0.16 (0.41)	0.86 (0.52)	0.42 (0.30)	-0.57 (0.85)
Cooking loss of LD at day 1.3, %	Clld2	413	23.55	3.99	12.39	39.27	-0.67 (0.45)	0.69 (0.57)	-0.27 (0.33)	0.52 (0.94)
Cooking loss of LD at day 2.0, %	Clld3	413	23.05	3.96	8.79	47.80	-0.67 (0.28)*	-0.25 (0.35)	0.09 (0.20)	1.41 (0.58)*
Cooking loss of LD at day 2.3, %	Clld4	400	21.66	4.05	7.68	45.16	-0.46 (0.23)*	0.03 (0.28)	0.13 (0.17)	1.18 (0.47)*
Cooking loss of LD at day 4.0, %	Clld5	413	23.83	4.19	7.74	48.80	0.02 (0.17)	0.23 (0.21)	0.33 (0.12)	0.66 (0.34)
Fat depth at ribs 12^{th} and 13^{th}, mm	Rbft	325	7.40	2.91	1.00	20.00	-0.65 (0.49)	0.82 (0.62)	-0.83 (0.36)*	-0.65 (1.02)
Meat colour L, score	ColL	411	39.75	1.95	34.07	45.77	0.13 (0.27)	-0.45 (0.34)	-0.03 (0.20)	1.34 (0.56)*
Meat colour a, score	Cola	411	17.22	1.81	11.62	22.25	0.03 (0.26)	-0.14 (0.32)	-0.03 (0.19)	0.38 (0.54)
Meat colour b, score	Colb	411	8.10	1.06	4.12	10.92	-0.08 (0.15)	-0.15 (0.19)	0.08 (0.11)	0.56 (0.31)

Appendix D continued

Trait	Abbreviation	N	Mean	S.D.	Min	Max	*Limousin* *a* (s.e.)	*Limousin* *d* (s.e.)	*Jersey* *a* + *d* (s.e) [#]	Breed difference (L-J)
Side Length, cm	Carclg	413	126.97	4.62	99.60	140.50	0.27 (0.60)	-0.01 (0.74)	-0.43 (0.44)	2.68 (1.23)*
Pelvic area, cm^2	PA	413	212.59	26.33	141.50	288.40	0.01 (3.08)	-0.97(3.85)	1.50 (2.27)	6.88 (6.37)
Kidney fat, kg	Kidfat	413	7.10	3.23	1.50	19.51	-0.35 (0.36)	0.74 (0.45)	-0.83 (0.27)**	-2.82 (0.75)***
Omental fat, kg	Omenfat	413	3.96	2.09	0.58	12.46	-0.25 (0.19)	0.22 (0.24)	-0.03 (0.14)	-1.10 (0.40)**
Pericardial fat, kg	Prcarfat	409	0.51	0.21	0.14	1.76	-0.01 (0.02)	0.02 (0.03)	-0.03 (0.02)	-0.03 (0.06)
Rump fat, kg	Rumfat	406	0.46	0.15	0.00	1.00	-0.03 (0.02)	0.05 (0.02)*	-0.04 (0.01)**	-0.03 (0.04)
Silverside fat, kg	Silversfat	406	0.54	0.14	0.17	1.02	-0.04 (0.02)	0.06 (0.03)*	-0.02 (0.01)	-0.01 (0.04)
Intramuscular fat content, %	Imf	341	4.22	2.27	0.40	12.80	-0.30 (0.34)	0.38 (0.44)	-0.23 (0.26)	-2.00 (0.72)**
Melting point, ^oC	Mp	405	37.23	2.61	28.50	44.00	0.90 (0.40)*	0.24 (0.50)	0.52 (0.29)	0.79 (0.83)
Mono-unsaturated fatty acids, % of triacylglyceride	Mufa	402	51.18	3.03	42.40	59.30	-1.10 (0.44)*	0.10 (0.56)	-0.44 (0.33)	-0.98 (0.92)
Fat colour on biopsy samples, score	Fcb	410	1.69	0.65	1.00	4.00	0.03 (0.10)	0.06 (0.12)	-0.06 (0.07)	-0.60 (0.20)**
Heart weight, kg	Heart	413	1.65	0.24	1.09	2.49	0.02 (0.03)	-0.01 (0.04)	-0.03 (0.02)	0.02 (0.06)
Liver weight, kg	Liver	412	6.22	0.88	3.78	8.98	-0.03 (0.09)	-0.03 (0.12)	-0.03 (0.07)	0.04 (0.20)
Kidney weight, kg	Kidney	413	0.95	0.13	0.62	1.53	-0.01 (0.02)	0.01 (0.02)	-0.02 (0.01)	0.01 (0.03)
Docility, score	Docsco	155	12.67	1.43	9.88	16.8	0.35 (0.42)	0.86 (0.51)	0.04 (0.27)	-0.85 (0.86)

[a] SF: Shear force. [b]LD: *M. Longissimus dorsi* muscle. *: P <0.05, **: P< 0.01, ***: P<0.001.

Appendix E Residual variances, covariances and correlations of live animal measurements (Australian data)[a]

	Bwt	**Birht**	**Gl**	**Wwt**	**Wht**	**Whip**	**Wsti**	**Wfat**	**400wt**	**400ht**	**400hip**	**400sti**	**400fat**	**600wt**	**600ht**	**600hip**	**600sti**	**600fat**	**Ap**
Bwt	**13.47**	0.64	0.04	0.41	0.45	0.27	0.21	-0.07	0.42	0.42	0.30	0.18	-0.10	0.45	0.38	0.35	0.18	0.13	0.00
Birht	8.77	**13.84**	0.10	0.38	0.44	0.30	0.18	-0.07	0.36	0.41	0.30	0.16	-0.10	0.41	0.36	0.33	0.17	0.02	-0.04
Gl	0.69	1.76	**23.32**	0.07	0.01	0.04	0.06	0.07	0.07	0.03	0.02	0.07	-0.02	0.12	0.02	0.06	-0.03	0.04	0.02
Wwt	37.86	35.33	8.01	**627.75**	0.60	0.65	0.60	0.17	0.86	0.55	0.67	0.45	0.14	0.78	0.57	0.54	0.37	0.20	-0.25
Wht	6.17	6.18	0.10	56.39	**14.21**	0.41	0.36	0.08	0.58	0.59	0.43	0.36	0.09	0.58	0.58	0.39	0.28	0.13	-0.09
Whip	1.88	2.11	0.41	31.39	2.93	**3.66**	0.54	0.08	0.60	0.39	0.54	0.30	0.10	0.56	0.40	0.48	0.28	0.14	-0.17
Wsti	1.56	1.38	0.59	31.10	2.77	2.11	**4.21**	0.14	0.59	0.32	0.47	0.39	0.11	0.47	0.30	0.30	0.29	0.14	-0.20
Wfat	-0.29	-0.27	0.40	4.75	0.33	0.16	0.31	**1.23**	0.16	0.02	0.14	0.18	0.40	0.05	0.01	0.12	0.10	0.27	-0.12
400wt	37.35	32.44	7.74	522.96	53.17	27.93	29.51	4.26	**585.17**	0.58	0.70	0.46	0.15	0.81	0.60	0.59	0.43	0.19	-0.24
400ht	6.40	6.37	0.60	57.82	9.32	3.13	2.72	0.10	58.43	**17.52**	0.50	0.31	0.04	0.54	0.56	0.37	0.27	0.12	-0.03
400hip	1.98	1.96	0.18	29.64	2.91	1.84	1.71	0.27	30.03	3.69	**3.16**	0.43	0.12	0.62	0.50	0.60	0.32	0.19	-0.14
400sti	1.40	1.23	0.71	23.69	2.88	1.22	1.70	0.42	23.29	2.76	1.62	**4.40**	0.14	0.40	0.28	0.31	0.32	0.06	-0.21
400fat	-0.25	-0.25	-0.07	2.34	0.22	0.13	0.15	0.30	2.43	0.11	0.14	0.20	**0.46**	0.06	0.02	0.05	0.08	0.19	-0.12
600wt	49.29	45.55	17.25	582.51	65.87	32.06	28.92	1.64	587.09	68.18	33.04	24.89	1.26	**896.41**	0.64	0.62	0.45	0.26	-0.17
600hit	4.97	4.83	0.41	51.21	7.83	2.76	2.22	0.04	51.62	8.36	3.16	2.09	0.05	68.23	**12.71**	0.43	0.29	0.14	-0.02
600hip	2.46	2.37	0.55	26.09	2.84	1.77	1.18	0.26	27.63	3.00	2.06	1.25	0.07	35.88	2.98	**3.72**	0.41	0.18	0.00
600sti	1.40	1.36	-0.32	19.56	2.28	1.14	1.28	0.24	22.09	2.42	1.20	1.42	0.11	28.73	2.20	1.70	**4.54**	0.09	-0.26
600fat	0.85	0.14	0.34	8.52	0.84	0.48	0.50	0.52	8.05	0.85	0.59	0.22	0.22	13.38	0.89	0.59	0.35	**3.02**	-0.14
Ap	0.47	-4.28	2.80	-179.81	-9.62	-9.20	-11.45	-3.90	-165.06	-3.75	-7.29	-12.24	-2.39	-141.89	-2.36	-0.23	-15.45	-7.04	**803.82**

[a]Abbreviations have been defined in Appendix C

Appendix F Residual variances, covariances and correlations of carcass traits (Australian data)[a]

	Ema	Ld	St	Ss	Bs	P8	Mar	Chanfat	Omenfat	Rbft	Imf	Hscw	Heart	Liver	Kidney	Pa	Carclg	Dent	Ossms
Ema	**111.60**	0.24	0.44	0.51	0.32	0.10	0.00	0.08	-0.04	0.01	-0.04	0.57	0.15	0.28	0.19	0.29	0.23	-0.03	0.02
Ld	4.49	**3.22**	0.27	0.30	0.18	0.04	-0.02	0.08	0.01	0.08	0.00	0.42	0.15	0.23	0.15	0.30	0.46	-0.03	0.24
St	4.74	0.49	**1.03**	0.37	0.30	0.28	0.02	0.23	0.22	0.10	0.01	0.69	0.18	0.33	0.23	0.29	0.42	0.04	0.03
Ss	2.82	0.28	0.20	**0.27**	0.31	0.13	-0.08	0.11	-0.04	0.02	-0.09	0.56	0.14	0.23	0.16	0.26	0.33	0.03	0.03
Bs	1.52	0.14	0.13	0.07	**0.20**	0.17	-0.04	0.05	-0.05	0.04	-0.06	0.38	0.10	0.10	0.09	0.24	0.11	0.07	0.08
P8	4.50	0.34	1.22	0.30	0.34	**18.91**	0.06	0.12	0.13	0.25	0.09	0.33	0.07	0.13	0.15	0.03	0.20	0.00	0.09
Mar	0.02	-0.02	0.01	-0.03	-0.01	0.18	**0.40**	0.10	0.11	0.11	0.53	0.03	0.05	0.09	0.04	0.00	0.03	-0.01	-0.03
Chanfat	2.56	0.46	0.71	0.17	0.06	1.63	0.20	**9.28**	0.11	0.08	0.17	0.33	0.10	0.23	0.23	0.08	0.24	0.02	0.00
Omenfat	-1.05	0.04	0.60	-0.06	-0.06	1.55	0.20	0.95	**7.47**	-0.05	0.08	0.11	0.18	-0.14	0.07	0.02	0.11	-0.04	-0.03
Rbft	0.48	0.44	0.33	0.04	0.06	3.56	0.23	0.83	-0.46	**10.47**	0.15	0.18	0.03	0.14	0.05	0.10	0.18	-0.02	0.08
Imf	-0.57	0.01	0.02	-0.06	-0.03	0.55	0.45	0.71	0.31	0.66	**1.80**	0.08	0.02	0.16	0.05	-0.06	0.03	0.02	-0.06
Hcw	211.08	26.42	24.84	10.18	5.95	50.81	0.65	35.05	10.78	20.39	3.73	**1240.96**	0.27	0.53	0.43	0.41	0.63	0.00	0.11
Heart	0.46	0.08	0.05	0.02	0.01	0.09	0.01	0.09	0.14	0.03	0.01	2.81	**0.09**	0.01	0.32	0.18	0.21	-0.04	0.03
Liver	2.22	0.31	0.25	0.09	0.03	0.43	0.04	0.53	-0.27	0.33	0.16	13.87	0.00	**0.55**	0.43	0.18	0.44	0.10	0.11
Kidney	0.27	0.04	0.03	0.01	0.01	0.09	0.00	0.10	0.03	0.02	0.01	2.06	0.01	0.04	**0.02**	0.13	0.37	0.04	0.09
Pa	103.47	18.07	10.03	4.58	3.58	4.88	0.01	8.12	1.64	11.46	-2.59	488.42	1.79	4.58	0.59	**1148.39**	0.37	0.00	0.10
Carclg	106.16	36.57	18.84	7.59	2.10	38.93	0.84	32.14	13.85	25.48	1.73	991.30	2.75	14.56	2.25	560.44	**1988.65**	0.02	0.10
Dent	-0.25	-0.05	0.03	0.02	0.03	0.01	-0.01	0.05	-0.11	-0.05	0.03	-0.04	-0.01	0.07	0.00	-0.02	0.94	**0.83**	-0.09
Ossms	49.52	15.24	1.06	0.58	1.21	13.14	-0.60	-0.39	-2.46	8.83	-2.97	129.42	0.26	2.86	0.42	115.91	161.35	-2.98	**1212.37**

[a]Abbreviations have been defined in Appendix C

Appendix G Residual variances, covariances and correlations of meat and fat quality traits (Australian data)[a]

	Wbld1	Wbld2	Wbld3	Wbld4	Wbst1	Wbst2	Wbst3	Wbst4	Clst	Clld	pHld	pHst	Mc	Fc	Fcb	Bc	Mt	Mufa	Fdist	Docsco
Wbld1	**1.32**	0.73	0.68	0.68	0.28	0.21	0.26	0.16	0.04	0.11	0.19	0.10	0.27	0.01	0.09	0.04	-0.08	0.08	-0.05	0.03
Wbld2	0.73	**0.77**	0.74	0.69	0.29	0.22	0.30	0.22	-0.02	0.10	0.21	0.19	0.31	0.00	0.06	0.07	-0.09	0.06	-0.01	0.01
Wbld3	0.69	0.57	**0.77**	0.72	0.19	0.19	0.21	0.18	0.02	0.11	0.14	0.13	0.29	0.02	0.11	0.06	0.01	0.03	-0.03	-0.01
Wbld4	0.63	0.49	0.51	**0.64**	0.22	0.19	0.24	0.22	0.00	0.04	0.19	0.23	0.29	-0.01	0.09	0.05	-0.08	-0.06	-0.01	0.00
Wbst1	0.22	0.17	0.11	0.12	**0.45**	0.40	0.43	0.40	0.07	0.02	0.12	0.09	0.18	0.12	0.03	0.01	-0.12	0.12	0.12	0.08
Wbst2	0.15	0.12	0.10	0.09	0.17	**0.38**	0.47	0.37	0.07	0.06	-0.03	0.00	0.03	0.04	0.04	0.03	-0.05	0.03	-0.16	-0.37
Wbst3	0.17	0.15	0.11	0.11	0.17	0.17	**0.33**	0.51	0.14	0.09	0.09	0.07	0.10	0.07	0.01	0.01	-0.08	0.09	-0.11	0.08
Wbst4	0.10	0.10	0.09	0.10	0.15	0.13	0.16	**0.30**	0.07	-0.02	0.17	0.23	0.16	0.06	0.02	0.01	-0.05	0.09	-0.10	0.05
Clst	0.05	-0.02	0.02	0.00	0.06	0.06	0.11	0.05	**1.70**	0.33	-0.33	-0.34	-0.26	-0.08	0.01	0.01	0.12	-0.11	-0.03	0.01
Clld	0.21	0.14	0.16	0.05	0.03	0.06	0.09	-0.02	0.71	**2.82**	-0.30	-0.26	-0.15	-0.04	-0.05	0.04	-0.01	-0.01	-0.03	-0.02
pHld	0.02	0.02	0.01	0.01	0.01	0.00	0.01	0.01	-0.04	-0.05	**0.01**	0.70	0.54	0.02	-0.05	0.05	-0.02	0.05	-0.04	0.09
pHst	0.01	0.01	0.01	0.02	0.01	0.00	0.00	0.01	-0.04	-0.04	0.01	**0.01**	0.47	0.02	-0.01	-0.01	-0.02	0.09	0.05	-0.03
Mc	0.21	0.18	0.17	0.16	0.08	0.01	0.04	0.06	-0.23	-0.17	0.04	0.03	**0.47**	0.08	-0.01	0.00	-0.06	0.08	-0.03	0.03
Fc	0.01	0.00	0.01	-0.01	0.07	0.02	0.04	0.03	-0.10	-0.06	0.00	0.00	0.05	**0.90**	0.36	0.29	-0.23	0.19	0.08	-0.06
Fcb	0.06	0.03	0.05	0.04	0.01	0.02	0.00	0.01	0.00	-0.05	0.00	0.00	0.00	0.19	**0.31**	0.51	0.02	0.04	-0.06	0.10
Bc	0.02	0.03	0.03	0.02	0.00	0.01	0.00	0.00	0.01	0.03	0.00	0.00	0.00	0.13	0.13	**0.22**	0.05	0.01	0.02	-0.04
Mp	-0.25	-0.20	-0.17	-0.16	-0.21	-0.08	-0.12	-0.07	0.41	-0.03	-0.01	-0.01	-0.10	-0.56	0.03	0.07	**6.79**	-0.62	0.01	-0.02
Mufa	0.34	0.18	0.09	0.18	0.29	0.06	0.18	0.17	-0.52	-0.03	0.02	0.03	0.19	0.64	0.08	0.01	-5.70	**12.41**	-0.08	0.05
Fdist	-0.20	-0.03	-0.09	-0.02	-0.18	-0.36	-0.24	-0.20	-0.15	-0.17	-0.01	0.02	-0.08	0.28	-0.13	0.04	0.11	-1.06	**12.77**	-0.37
Docsco	0.05	0.02	-0.01	-0.01	0.08	-0.04	0.07	0.04	0.03	-0.04	0.01	0.00	0.03	-0.08	0.08	-0.03	-0.10	0.26	-2.06	**2.46**

[a]Abbreviations have been defined in Appendix C

Appendix H Residual variances, covariances and correlations of meat and fat quality traits (New Zealand data)[d]

	Bonewt	Liver	Kidney	Heart	Carclg	Meatwt	Hcw	Rumeat	Ld	Silverswt	EMA	Rbft	Marb	Fatwt	Chanlfat	Omentfat	Prcarfat	Rumpfat	Silversfat
Bonewt	**3.74**	0.59	0.63	0.65	0.76	0.84	0.88	0.77	0.66	0.78	0.46	0.24	0.11	0.44	0.27	0.21	0.25	0.27	0.28
Liver	0.63	**0.30**	0.54	0.51	0.52	0.60	0.63	0.56	0.49	0.53	0.33	0.22	0.10	0.43	0.33	0.26	0.22	0.26	0.26
Kidney	0.12	0.03	**0.01**	0.53	0.52	0.56	0.59	0.50	0.40	0.50	0.26	0.21	0.09	0.36	0.30	0.21	0.22	0.24	0.20
Heart	0.22	0.05	0.01	**0.03**	0.57	0.61	0.65	0.55	0.55	0.51	0.35	0.17	0.10	0.40	0.39	0.31	0.28	0.28	0.25
Carclg	5.09	0.98	0.18	0.35	**11.87**	0.66	0.70	0.62	0.56	0.60	0.34	0.18	0.09	0.37	0.25	0.21	0.19	0.25	0.26
Meatwt	11.84	2.40	0.40	0.78	16.61	**53.28**	0.98	0.89	0.84	0.93	0.68	0.25	0.12	0.47	0.33	0.28	0.26	0.32	0.26
Hcw	36.52	7.44	1.25	2.44	51.98	152.82	**461.56**	0.88	0.84	0.89	0.62	0.31	0.17	0.62	0.41	0.35	0.32	0.42	0.36
Rummeat	0.81	0.17	0.03	0.05	1.15	3.50	10.23	**0.29**	0.75	0.82	0.58	0.29	0.14	0.48	0.31	0.29	0.19	0.35	0.28
Ld	0.89	0.19	0.03	0.07	1.34	4.28	12.66	0.28	**0.49**	0.72	0.63	0.33	0.13	0.56	0.42	0.33	0.33	0.44	0.31
Silverswt	1.34	0.26	0.04	0.08	1.84	6.01	16.94	0.39	0.45	**0.79**	0.67	0.17	0.05	0.36	0.21	0.17	0.17	0.20	0.17
Ema	6.01	1.23	0.17	0.42	7.87	33.69	91.38	2.14	3.00	4.07	**46.42**	0.01	0.07	0.24	0.21	0.16	0.15	0.15	0.06
Rbft	1.21	0.31	0.05	0.08	1.61	4.73	17.49	0.40	0.60	0.40	0.12	**6.77**	0.07	0.49	0.33	0.31	0.21	0.39	0.28
Mar	0.40	0.10	0.02	0.03	0.58	1.64	6.67	0.14	0.17	0.09	0.85	0.35	**3.55**	0.29	0.20	0.18	0.12	0.26	0.20
Fatwt	1.79	0.49	0.08	0.15	2.73	7.28	28.19	0.54	0.83	0.68	3.50	2.67	1.17	**4.47**	0.60	0.52	0.46	0.67	0.62
Chanlfat	1.11	0.38	0.06	0.14	1.80	5.13	18.65	0.35	0.63	0.39	2.95	1.78	0.79	2.68	**4.43**	0.60	0.40	0.45	0.35
Omenfat	0.46	0.16	0.02	0.06	0.80	2.30	8.34	0.18	0.26	0.16	1.21	0.89	0.38	1.23	1.41	**1.24**	0.31	0.37	0.31
Prcarfat	0.08	0.02	0.00	0.01	0.11	0.30	1.11	0.02	0.04	0.02	0.16	0.09	0.04	0.16	0.14	0.06	**0.03**	0.38	0.25
Rumpfat	0.06	0.02	0.00	0.01	0.10	0.27	1.06	0.02	0.04	0.02	0.12	0.12	0.06	0.17	0.11	0.05	0.01	**0.01**	0.45
Silversfat	0.07	0.02	0.00	0.01	0.11	0.23	0.95	0.02	0.03	0.02	0.05	0.09	0.05	0.16	0.09	0.04	0.01	0.01	**0.02**

[a]Abbreviations have been defined in Appendix D

Appendix I Residual variances, covariances and correlations of meat and fat quality traits (New Zealand data)[a]

	Clld1	Clld2	Clld3	Clld4	Clld5	Wbld1	Wbld2	Wbld3	Wbld4	pHld	ColLAv	ColaAv	ColbAv	FCb	mufa	MP
Clld1	**11.08**	0.22	0.17	0.17	0.13	0.11	-0.03	0.02	0.07	-0.06	0.10	-0.01	0.00	0.01	-0.10	0.09
Clld2	1.87	**6.35**	0.33	0.29	0.25	0.05	0.02	-0.02	0.00	-0.24	0.08	0.08	0.07	0.02	-0.06	0.09
Clld3	1.44	2.14	**6.85**	0.34	0.16	0.11	0.09	0.11	0.07	-0.18	0.04	0.08	0.09	-0.04	-0.14	0.17
Clld4	1.64	2.12	2.62	**8.58**	0.20	-0.01	0.03	0.03	0.02	-0.18	0.06	0.05	0.13	0.07	-0.13	0.13
Clld5	1.22	1.69	1.16	1.61	**7.39**	0.04	0.11	0.08	0.09	-0.18	0.00	0.03	0.04	0.04	-0.05	0.05
Wbld1	0.91	0.30	0.66	-0.05	0.23	**5.72**	0.56	0.50	0.49	0.07	0.00	0.00	-0.03	-0.03	0.00	0.02
Wbld2	-0.22	0.15	0.63	0.27	0.79	3.52	**6.88**	0.78	0.73	0.09	-0.07	-0.04	-0.04	-0.02	-0.02	0.04
Wbld3	0.12	-0.07	0.47	0.12	0.33	1.93	3.31	**2.59**	0.85	0.21	-0.02	-0.09	-0.09	-0.08	-0.04	0.08
Wbld4	0.01	-0.02	-0.01	-0.02	-0.04	-0.02	-0.10	-0.03	**-0.02**	0.34	-0.07	-0.11	-0.09	-0.03	0.02	-0.02
pHld	-0.01	-0.03	-0.02	-0.03	-0.02	0.01	0.01	0.02	0.01	**0.00**	-0.13	-0.28	-0.25	-0.01	0.00	0.00
ColLAv	0.54	0.30	0.18	0.26	-0.02	-0.01	-0.30	-0.05	-0.01	-0.01	**2.47**	-0.41	-0.27	0.08	-0.11	0.09
ColaAv	-0.03	0.30	0.33	0.22	0.13	-0.01	-0.15	-0.22	-0.25	-0.02	-0.97	**2.25**	0.86	-0.07	-0.02	0.04
ColbAv	0.01	0.15	0.21	0.33	0.10	-0.05	-0.08	-0.12	-0.15	-0.01	-0.37	1.13	**0.77**	0.05	-0.04	0.05
FCb	0.01	0.02	-0.05	0.12	0.06	-0.04	-0.03	-0.07	-0.04	0.00	0.07	-0.06	0.03	**0.32**	0.14	-0.10
mufa	-0.82	-0.41	-0.93	-0.98	-0.36	-0.01	-0.15	-0.19	-0.13	0.00	-0.44	-0.09	-0.10	0.20	**6.59**	-0.77
MP	0.73	0.51	1.04	0.88	0.30	0.13	0.25	0.30	0.23	0.00	0.33	0.15	0.11	-0.13	-4.57	**5.33**

[a]Abbreviations have been defined in Appendix D

Appendix J Prediction equations used to estimate carcass composition

Year 1996 and 1997:

Meat percent = 67.28 + 0.69×ts + 1.59×st + 1.07×of + 0.38×ru + 0.45×ld + 1.67×tln + 0.15× rib -1.16×femwt + 1.29×tibwt - 0.33×fqwt - 0.0735×hscw - 0.092×p8 +0.040×ema

Fat percent = 12.48 -0.92×ts - 1.45×st - 0.93×of - 0.35×ru - 0.47×ld - 1.36×tln + 0.20×rib -0.86×femwt - 1.96×tibwt -0.52×fqwt + 0.1196×hscw + 0.103×p8 - 0.037×ema

Bone percent = 20.24 + 0.23×ts - 0.14×st - 0.14×of - 0.04×ru + 0.02×ld - 0.31×tln - 0.34×rib + 2.02×femwt + 0.67×tibwt + 0.85×fqwt - 0.0461×hscw - 0.010×p8 - 0.003×ema

Year 1998:

Meat percent = 66.88 + 2.40×st + 1.18×of + 0.98×kn + 0.51×ld + 3.10×ct -0.46×bones - 0.0747×hscw - 0.075×p8 +0.049×ema

Fat percent = 13.70 -1.93×st - 0.90×of - 0.95×kn - 0.33×ld - 3.12×ct - 2.75×bones + 0.1032×hscw + 0.083×p8 - 0.042×ema

Bone percent = 19.42 - 0.46×st - 0.28×of - 0.02×kn - 0.18×ld + 0.03×ct + 3.12×bones - 0.0285×hscw - 0.009×p8 - 0.007×ema

Meat to bone ratio = Meat percent / Bone percent

Abbreviations:

ts, kg	Topside	femwt, kg	Femur	ct, kg	Chuk tender
st, kg	*M. semitendinosus* muscle	tibwt, kg	Tibia	Bones, kg	Radius/ulna + humerus
of, kg	Outside flat	fqwt, kg	Forequarter bone weight		
ru, kg	Rump	hscw, kg	Hot standard carcass weight		
ld, kg	*M.longissimus dorsi* muscle	p8, mm	Rump fat depth		
tln, kg	Tender loin	ema, cm^2	Eye muscle area		
rib, kg	Ribset	kn, kg	Knuckle		

Appendix K Beef primal cuts (AUS-MEAT Limited, 1998).