MOLECULAR GENETICS OF CATTLE MUSCULARITY

By

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DECLARATION

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ABSTRACT

Genetic improvement is a goal of most livestock industries and molecular information can contribute to the accuracy of selection and hence rate of genetic improvement. The aim of this study was to obtain molecular information that can be used to assist selection for muscularity in cattle. Quantitative trait loci (QTL) mapping for traits related to muscularity (but not growth), candidate gene identification and association studies between the candidate genes and the muscularity related traits were conducted. Interactions between the candidate genes and *myostatin*, a gene known to have a major role on muscularity in cattle, were also examined. Genotype and phenotype data from the JS Davies cattle gene mapping project were used for this study.

QTL for muscularity related traits across 3 sire families were mapped in 366 double back cross progeny from pure Limousin (carrying the *myostatin* F94L variant) and Jersey cows. Cohort and breed were fitted in the model. A model that included the *myostatin* F94L genotype was also fitted to identify chromosomal regions in which gene(s) that may be epistatic with *myostatin* reside. Covariates were used to obtain QTL for carcass traits related to muscularity and not related to growth. In total, all the QTL mapped to 15 regions on 11 chromosomes (BTA 1, 2, 3, 4, 5, 8, 9, 11, 13, 14 and 17). In terms of the traits that best define muscularity, the QTL on cattle chromosomes 2, 3 and 17 were of greatest interest. Fitting the *myostatin* F94L genotype in the model indicated that the QTL on chromosome 17 are likely to be epistatic with *myostatin*.

Six candidate genes were selected based on the QTL results of the study herein and previous studies on the same population. The genes were *activin receptor type 1* (*ACVR1*), *smad nuclear interacting protein 1* (*SNIP1*), similar to *follistatin-like 5* (*FSTL5*), *transforming growth factor β receptor 3* (*TGFBR3*), *insulin like growth factor 1* (*IGF1*) and *follistatin* (*FST*). DNA variants of *FSTL5* were associated with the traits of interest but there was no interaction with *myostatin.* DNA variants in *TGFBR3* and *SNIP1* had no direct effect on muscularity traits but there were significant interactions with *myostatin.* For *IGF1* and *FST*, their DNA variants had direct effects on muscularity related traits and there were significant interactions with *myostatin. FST* SNP7 and the interaction between *IGF1* SNP2 and *myostatin* had the most significant effects on muscularity related traits ($P<0.01$).

The results of this study showed that there are genes affecting muscularity not related to growth and some of these interact epistatically with *myostatin*. Furthermore, potential markers for muscularity have been discovered (eg. *FST*). Further studies in larger cattle populations need to be undertaken to confirm the results herein before these markers can be utilised commercially.

CHAPTER 1

Literature Review

1.1 Introduction

Selection based on observable phenotypes is still the most common method used to improve cattle genetically. Since phenotype is the expression of both the genes and the environment, molecular information can contribute to the accuracy of selection and genetic improvement, and thus, positively affect phenotypic traits. In other words, to maximize its accuracy, selection should be based on all sources of available information including both phenotypic and molecular information. Marker assisted selection is the most common method usually applied to improve the traits using molecular information (Dekkers *et al.* 2002).

Muscularity is a phenotypic trait that is widely utilised by farmers to select cattle for high productivity. Cattle with good muscularity are expected to have more carcass weight and meat. It has been reported that body dimension traits have a moderate to high correlation with growth and carcass traits (Gilbert *et al.* 1993; Reverter *et al.* 2000; Magnabosco *et al.* 2002; Maiwashe *et al.* 2002; Afolayan 2003; Nephawe *et al.* 2004; Afolayan *et al.* 2007; Riley *et al.* 2007). This implies that it should be possible to conduct phenotypic and marker-assisted selection using body dimension traits for carcass yield traits. Studies have also reported heritability estimates for body dimension traits range from moderate to high (37% to 60% for height, 18% to 38% for length and 25-37% for girth) (Gilbert *et al.* 1993; Northcutt and Wilson 1993; Vargas *et al.* 2000; Magnabosco *et al.* 2002; Afolayan 2003; Johnston *et al.* 2003; Nephawe *et al.* 2004; Afolayan *et al.* 2007; Riley *et al.* 2007). Some carcass traits, such as carcass weight, eye muscle area and beef yield, are also relatively highly heritable (Mukai *et al.* 1995; Smith *et al.* 1997; Crews *et al.* 2008) and

estimated breeding values for these traits have been applied in selection programs to breed cattle for specific market requirements.

In order to determine which molecular markers should be utilized for selection of traits, chromosomal regions that contain gene(s) controlling the quantitative traits, namely quantitative trait loci (QTL), are first located. The QTL are then used to identify genes and markers that can be used for the selection. QTL for growth traits and carcass traits have been detected on most cattle chromosomes (Casas *et al.* 2000; Casas *et al.* 2001; Casas *et al.* 2003; Casas *et al.* 2004; Mizoshita *et al.* 2004; Gasparin *et al.* 2005; Mizoguchi *et al.* 2006; Takasuga *et al.* 2007). However, QTL for body dimension and carcass yield traits that describe muscularity need to be located in order to identify the genes controlling these traits. Having QTL for growth, carcass and body dimension traits related to muscularity will enhance the selection of production traits by providing more markers and more accuracy.

There are only a small number of genes that have been identified which control muscle development and carcass traits. These include *callipyge*, *IGF-I* and *myostatin*. *Myostatin* is a gene that is known to have a significant role in muscle development and carcass traits. Knock-out mutations in the *myostatin* gene result in double-muscled cattle (Kambadur *et al.* 1997; McPherron and Lee 1997). There are many other proteins also known to be involved in the *myostatin* regulation of muscle development (McPherron *et al.* 1997; Hill *et al.* 2002; Hill *et al.* 2003; Lee 2004; Dominique and Gerard 2006). This implies that there may be genes that are epistatic and interact with *myostatin* to affect muscle development.

The aim of this study was to locate QTL for body dimension and carcass traits related to muscularity in order to identify genes and markers that reside in the QTL and may be used for selection. Some of the genes chosen may be expected to be epistatic with *myostatin*, and therefore, an additional aim of the study was to examine any interactions between the genes and *myostatin.*. Thus, identifying the genes that control muscularity should be also beneficial for understanding growth and muscle development in cattle.

1.2 Literature review

Muscularity can be defined as "thickness of muscle relative to dimensions of skeleton" (Boer *et al.* 1974). Thus, muscularity in cattle depends on the muscle development or growth. In general, growth is the progressive change of body size (volume, length, height, and girth) and weight of an animal during its life (Marple 2003). Growth can be defined as an enhancement of both the size of individual cells (hypertrophy) and the number of cells (hyperplasia) in a given tissue, including muscle (Marple 2003).

1.2.1 Muscle development in cattle

Muscle development of cattle, as in other mammals, is divided into two periods: the prenatal period (273-292 days) and the postnatal period. At the foetal stage, muscles grow by hyperplasia, but they grow hypertrophically during the postnatal period (Owens *et al.* 1993). During the prenatal period, muscle cells begin with the mitotic division from the third germinal layer and form 40 groups of muscle cells, known as myotomes. Myotomes develop two types of cells, namely primitive connective tissue

cells and primitive muscle cells (or myoblasts). The myoblasts fuse together to form myotubes. By synthesizing myosin and actin, myotubes form myofibrils, which are then structured into skeletal muscles themselves. The number of myofibrils increases during embryonic development but not during the postnatal period (Lawrence and Fowler 1997).

In the postnatal period, muscle growth is typified by increases in the fibre cross-sectional area and increases in fibre length (Lawrence and Fowler 1997). The number of nuclei in muscle fibres during this stage also increases as the cells fuse. 90% of the DNA synthesized during the postnatal period in most muscles of mature animals occurs in the satellite cells (Allen and Goll 2003). Satellite cells are cells that have the function of replicating DNA, dividing, fusing with adjacent fibres and regenerating muscle (Allen and Goll 2003). There are many growth factors that affect satellite cells, including the hepatocyte growth factor, fibroblast growth factors (FGF), insulin-like growth factors (IGF), transforming growth factor-β (TGF-β) and *myostatin*, which is a member of the TGF-β super-gene family.

1.2.2 Genetic parameters for growth traits

The most commonly observed growth traits for estimating genetic parameters are birth weight, weaning weight, yearling weight and body weight before slaughter. The genetic parameters that are usually determined include heritability estimates and genetic correlations between the traits. Variation in the heritability estimates between studies may be caused by differences in the breeds of cattle, environments and/or analytical models utilised.

In general though, most studies have found that the direct heritability is higher than maternal heritability for birth weight, weaning weight and yearling weight (Meyer *et al.* 1993; Splan *et al.* 2002; Norris *et al.* 2004; Phocas and Laloe 2004; Aziz *et al.* 2005). This difference means that there is more genetic influence from the progeny genotype than from the maternal genotype (mainly milk production) for birth weight, weaning weight and yearling weight. However, some direct heritability estimates of weaning weight have been reported to be lower than that for maternal heritability (e.g. 0.18 ± 0.06 and 0.38 ±0.04, respectively (Afolayan *et al.* 2007).

Several direct and maternal heritabilities for birth weight of different cattle breeds have been reported. Direct heritability and maternal heritability for birth weight in Japanese Black cattle were 38% ± 16 and 4% ± 1, respectively (Aziz *et al.* 2005). The direct heritability for Japanese Black cattle was similar to the heritability of Hereford (37% versus 43%), but the maternal heritabilities differed (10% versus 23% for Hereford) (Meyer *et al.* 1993). This difference for maternal heritability may be a result of breed differences. However, Meyer et al (1993) used a different model for the analysis, which could also account for difference in maternal heritability between these two reports. Another study reported that the direct heritability of Charolais, Limousin, Maine-Anjou, Blonde d'Aquitaine cattle for birth weight is 28-36% (Phocas and Laloe 2004). These estimates are similar to the direct heritability reported for Nguni cattle (Norris *et al.* 2004). The variation between heritability estimates may be affected by differences in sex, means and phenotypic standard deviation of the population (Koots *et al.* 1994).

The direct heritability estimates for weaning weight tend to be lower than that of birth weight (Meyer *et al.* 1993; Norris *et al.* 2004; Phocas and Laloe 2004). This trend is likely to be caused by environmental factors that affect weaning weight more. Direct heritability for weaning weight in *Bos taurus* cattle breeds, including Charolais, Limousin, Maine-Anjou and Blonde d'Aquitane, has been estimated to be between 13-32% (Phocas and Laloe 2004). These estimates are similar to the heritabilities reported for Hereford and Wokalup (Hereford, Friesian, Charolais, Angus and Brahman Cross) cattle, which were 17 % and 29% (Meyer *et al.* 1993). Other direct heritabilities for weaning weight for Nguni cattle (from Africa) and Japanese Black cattle were also similar, 29% and 38%, respectively.

1.2.3 Genetic parameters for body dimension traits related to muscularity

The main aim of the beef cattle farmers or breeders is to produce beef cattle with high meat yield. Since muscularity can be defined as muscle depth relative to skeletal dimensions, selection of beef cattle with high meat yield can be achieved by selecting cattle with good muscularity. Muscularity of cattle can be assessed by measuring body dimension traits, such as height, length, girth, stifle and hip width. However, it should to be noted that these body dimension traits are also a good indicator of animal size and not necessarily muscularity if considered individually. For instance, the ratio of stifle to hip width ratio is a better indicator of muscularity than stifle alone. There have been only some studies that have been conducted to determine the genetic parameters of these body dimension traits that are related to muscularity (Afolayan 2003; Afolayan *et al.* 2007).

1.2.3.1 Heritability estimates for body dimension traits related to muscularity

The estimates of heritability for height are higher than the estimates of heritability for body length and girth (Gilbert *et al.* 1993; Magnabosco *et al.* 2002; Afolayan 2003; Afolayan *et al.* 2007). Height has been reported to have moderate to high heritability, ranging from 37% to 60% (Gilbert *et al.* 1993; Northcutt and Wilson 1993; Vargas *et al.* 2000; Magnabosco *et al.* 2002; Afolayan 2003; Johnston *et al.* 2003; Nephawe *et al.* 2004; Afolayan *et al.* 2007; Riley *et al.* 2007). Because of its moderate to high heritability, height can be used to select beef cattle for good muscularity if it considered with carcass weight.

Body length is another body dimension trait related to muscularity. The heritability estimates for body length at weaning, post-weaning and maturity range from 18% to 38% (Gilbert *et al.* 1993; Magnabosco *et al.* 2002; Maiwashe *et al.* 2002; Afolayan 2003; Afolayan *et al.* 2007). Heritability estimated for body length at 600 days for Bonsmara cattle was 18% (Maiwashe *et al.* 2002), whereas Afolayan (2003) obtained moderate heritability estimates for body length at 600 days (27%). These low to moderate heritabilities for body length at 600 days are evidence of a greater influence of environmental factors on length versus height. Johnston *et al.* (2003) estimated that the heritability for scanned eye muscle area ranges from 23% to 32% These results were obtained from different breeds (temperate and tropically adapted breeds) and different ages when the eye muscle areas were measured. The low to moderate heritabilities for body length and eye muscle area does not mean that body length and eye muscle area cannot be used to select cattle. The genetic correlation between body length, eye muscle area, growth and carcass yield traits should be investigated first.

Body girth has more varied estimated heritabilities than body length at all ages. The heritability for girth has been reported to be low to high (9%-40%) at weaning, post weaning and maturity (Gilbert *et al.* 1993; Mukai *et al.* 1995; Afolayan 2003; Afolayan *et al.* 2007). At maturity (about 18 months), heritability for girth has been estimated to be moderate (25%) (Mukai *et al.* 1995) to high (32%-37%) (Afolayan 2003; Afolayan *et al.* 2007). The moderate to high heritability for girth at maturity means that girth can be used as an indicator for selecting beef cattle before slaughter in order to obtain high carcass yields.

Other body dimension measures, such as stifle width and hip width, can be used to predict muscularity of cattle as well. The stifle to hip width ratio, in particular, can be used to predict muscularity of cattle. However, only one study on the genetic parameters for muscularity based on this ratio was conducted. Muscularity was reported to have moderate to high heritability from 19% to 44% (Afolayan 2003; Afolayan *et al.* 2007). These estimates suggest though that muscularity, as measured by the ratio of stifle to hip width, might be a good potential indicator for highly productive beef cattle and can be used for selection.

1.2.3.2 Genetic correlations between body dimension traits related to muscularity with growth and carcass yield traits

Having information on the genetic correlations between traits is important if the aim is to select a trait that is commonly associated with or influenced by other traits. There have been some studies estimating the genetic correlations between body height, length, girth and muscularity with growth traits (especially body weight) and carcass yield traits (hot standard carcass weight, warm carcass weight and cold carcass weight (Table1.1).

Table 1.1 Summary of the genetic correlations between body dimension traits with growth and carcass yield traits

Trait	Weight	Carcass weight	HCW	RBY
Height	0.40-1.00	0.31-0.38	0.69	
Length	0.59-0.89	0.27-0.35		
Girth	0.62-0.92		0.98	
Muscularity	0.35-0.38			
SEMA				0.43-0.97

HCW=hot carcass weight, RBY=percentage of retail beef yield, SEMA=scanned eye muscle area

Sources: (Gilbert *et al.* 1993; Reverter *et al.* 2000; Magnabosco *et al.* 2002; Maiwashe *et al.* 2002; Afolayan 2003; Nephawe *et al.* 2004; Afolayan *et al.* 2007; Riley *et al.* 2007)

Most studies have reported high genetic correlations between weight and body dimension traits (e.g. body length, height and girth) (Gilbert *et al.* 1993; Northcutt and Wilson 1993; Vargas *et al.* 2000; Magnabosco *et al.* 2002; Maiwashe *et al.* 2002; Afolayan 2003; Afolayan *et al.* 2007; Riley *et al.* 2007). The genetic correlations between height and weight at weaning, 400 day weight and weight before slaughter (about 18 months) in these studies ranged from 0.40 to 1.00. Most

studies of genetic correlations between weight and body length and girth also showed high correlations (0.59-0.89 for body weight and length and 0.62-0.92 for weight and girth) (Gilbert *et al.* 1993; Magnabosco *et al.* 2002; Maiwashe *et al.* 2002; Afolayan 2003; Afolayan *et al.* 2007). Afolayan *et al.* (2007) and Afolayan (2003) obtained a moderate genetic correlation between muscularity and weight at 400 days of age. These moderate to high genetic correlations indicate that selection for body dimensions should increase the weight of the cattle.

According to Gilbert et al (1993), the genetic correlations between body dimension traits and carcass traits (specifically, carcass weight), body height and length are moderate (0.31-0.38 for height and 0.27-0.35 for length). Another study reported a high genetic correlation between body height and hot carcass weight for Brahman cattle, approximately 0.69 (Nephawe *et al.* 2004). Genetic analyses of live-animal ultrasound and abattoir measured carcass traits have shown high genetic correlations between the percentage of retail beef yield and scanned eye muscle area (0.43 and 0.97 for Angus and Hereford cattle, respectively) (Reverter *et al.* 2000). In the few studies on the genetic correlations between girth and carcass weight (both warm and cold carcass weights), high genetic correlations were also found (Gilbert *et al.* 1993; Mukai *et al.* 1995). A high and negative correlation (-0.98) was obtained from the study carried out by Gilbert *et al.* (1993). This negative genetic correlation means that selection for greater girth on cattle may lead to a decrease in warm carcass weight. However, positive and high genetic correlations between cold carcass weight and girth at same age have been estimated for Japanese Black cattle, ranging from 0.64 to 0.79 (Mukai *et al.* 1995).

In summary, the genetic correlations between growth traits (e.g. body weight), carcass traits (e.g. carcass weight) and body dimension traits that are related to muscularity (e.g. body length, height, girth) are moderate to high. The high genetic correlations between body weight, carcass weight and some body dimension traits suggest that they may be controlled or affected by the same genes (Vargas *et al.* 2000). These results, therefore, imply that selection based on markers for body dimensions traits can be applied to achieve high carcass weights. Moreover, since body conformation can be defined as muscle compared with skeletal dimensions, these results indicate that selection based on body dimension traits or body shape can be used to select cattle for good muscularity and hence, increased retail beef yield. However, considering that there are only a few studies estimating the genetic correlations of cattle muscularity, more research on the genetics of muscularity in beef cattle should be undertaken.

1.2.4 Genetic parameters of carcass yield traits

Some carcass traits, such as eye muscle area, hot standard carcass weight, meat weight, can explain increased yield in cattle. Therefore, the genetic parameters for carcass traits will be needed in order to decide whether or not using these traits for selection will be beneficial.

In general, most studies have shown that the heritability for eye muscle area (EMA), hot standard carcass weight (HSCW) and yield grade are moderate to high between different breeds of cattle. Heritability of eye muscle area and carcass weight for Simmental, Japanese Black cattle, Angus, Hereford and Brahman cattle have been reported moderate (26%-50%) to high (31%-57%) (Mukai *et al.* 1995; Smith *et al.*

1997; Crews *et al.* 2008). Japanese Black cattle and Brahman cattle have a high heritability for yield grade, 53% and 47%, respectively (Mukai *et al.* 1995; Smith *et al.* 1997; Crews *et al.* 2008). Therefore, estimated breeding values (EBV) for these traits can be used effectively for selection.

1.2.5 Molecular Genetics of muscularity traits

1.2.5.1 Quantitative trait loci (QTL) for growth and carcass traits

Finding quantitative trait loci (QTL) is necessary in order to identify the regions of the genome that might contain genes affecting the traits of interest. QTL for growth and carcass yield traits have been reported (Table 1.2).

Table 1.2. QTL detected in beef cattle for growth and carcass traits

BTA	***Position (cM)***	***Trait[a]***	***Family[b]***	***References***
1	120	BW	BH	(Casas *et al.* 2003)
1	53	RPYD	BA	(Casas *et al.* 2004)
1	50	FATYD	BA	(Casas *et al.* 2004)
1	63	YG	BA	(Casas *et al.* 2004)
1	91	CHESTWT	Wagyu	(Malau-Aduli *et al.* 2005)
2	52	YG	BH	(Casas *et al.* 2003)
2	54	FAT	BH	(Casas *et al.* 2003)
2	59	BW	BH	(Casas *et al.* 2003)
2	54	MAR	BA	(Casas *et al.* 2004)
3	68	RPYD	BM	(Casas *et al.* 2001)
3	65	MAR	BM	(Casas *et al.* 2001)
3	28	MAR	BH	(Casas *et al.* 2003)
3	36	FAT	BH	(Casas *et al.* 2003)
3	59	BW	BH	(Casas *et al.* 2003)
3	56	MAR	BA	(Casas *et al.* 2004)
3	70	RPYD	BA	(Casas *et al.* 2004)
3	79	KHPFat	BA	(Casas *et al.* 2004)
4	33	HCW	BM	(Casas *et al.* 2001)
4		ADG	BM	(Casas *et al.* 2001)
4	19	WBS3	BM	(Casas *et al.* 2001)
4	52-67	LMA	Wagyu	(Mizoshita *et al.* 2004)

Table 1.2. (continued)

BTA	*Position (cM)*	*Trait*[a]	*Family*[b]	*References*
4	59-67	MAR	Wagyu	(Mizoguchi *et al.* 2006)
5	64	BW	GH	(Gasparin *et al.* 2005)
5	45-54	CY	Wagyu	(Mizoshita *et al.* 2004)
5	53-75	Wt	BH	(Casas *et al.* 2003)
5	38-66	LMA	BH	(Casas *et al.* 2003)
5		FAT	BM	(Casas *et al.* 2000)
5		RPYD	PA	(Casas *et al.* 2000)
5		YG	PA	(Casas *et al.* 2000)
5		WBS14	PA	(Casas *et al.* 2000)
6		YW	BM	(Casas *et al.* 2000)
6	9	LMA	BH	(Casas *et al.* 2003)
6		HCW	BM	(Casas *et al.* 2000)
6	9	LMA	BH	(Casas *et al.* 2003)
6	27-58	RT	Wagyu	(Mizoguchi *et al.* 2006)
6	84	MAR	Wagyu	(Takasuga *et al.* 2007)
7	55	FAT	BH	(Casas *et al.* 2003)
8	23	FAT	BM	(Casas *et al.* 2001)
8		MAR	BM	(Casas *et al.* 2001)
8	30	FAT	PA	(Casas *et al.* 2001)
9		WBS14	BM	(Casas *et al.* 2001)
9		MAR	BM	(Casas *et al.* 2001)
9	67	RPYD	MH	(Casas *et al.* 2003)
9	36	MAR	Wagyu	(Takasuga *et al.* 2007)
10	4	HCW	BH	(Casas *et al.* 2003)
10	24	MAR	BH	(Casas *et al.* 2003)
10	50	SW	Wagu	(Takasuga *et al.* 2007)
10	48	CW	Wagyu	(Takasuga *et al.* 2007)
10	72	MAR	Wagyu	(Takasuga *et al.* 2007)
11	66	YG	BH	(Casas *et al.* 2003)
14	16	FAT	BH	(Casas *et al.* 2003)
14	19	YG	Bh	(Casas *et al.* 2003)
14	47	MAR	BH	(Casas *et al.* 2003)
14	33	ADG	Wagyu	(Mizoshita *et al.* 2004)
14	50	CW	Wagyu	(Mizoshita *et al.* 2004)
14	34	SW	Wagyu	(Mizoshita *et al.* 2004)
14	53	MAR	Wagyu	(Mizoshita *et al.* 2004)
14	34,56,68	LMA	Wagyu	(Takasuga *et al.* 2007)
15	45	KHPfat	BH	(Casas *et al.* 2003)
16	56	MAR	BA	(Casas *et al.* 2004)
16	49	HCW	BA	(Casas *et al.* 2004)
16	62	KHPfat	BA	(Casas *et al.* 2004)
17	35	FAT	BA	(Casas *et al.* 2004)
17		MAR	BM	(Casas *et al.* 2000)
18	23	HCW	BH	(Casas *et al.* 2003)
18	85	RPYD	BH	(Casas *et al.* 2003)

Table 1.2. (continued)

BTA	*Position (cM)*	*Trait*[a]	*Family*[b]	*References*
19	18	YG	BH	(Casas *et al.* 2003)
19	5	RPYD	BH	(Casas *et al.* 2003)
20	52	MAR	Wagyu	(Takasuga *et al.* 2007)
20	66	WBS3	BH	(Casas *et al.* 2003)
20	72	WBS4	BH	(Casas *et al.* 2003)
20	12	BW	BA	(Casas *et al.* 2004)
21		BW	BH	(Casas *et al.* 2003)
21	56	BW	BA	(Casas *et al.* 2004)
21	78	MAR	Wagyu	(Takasuga *et al.* 2007)
21	75-84	MAR	Wagyu	(Mizoguchi *et al.* 2006)
23	30	MAR	BH	(Casas *et al.* 2003)
26	26	RPYD	BA	(Casas *et al.* 2004)
26	26	FAT	BA	(Casas *et al.* 2004)
26	26	YG	BA	(Casas *et al.* 2004)
28	56	SFT	Wagyu	(Takasuga *et al.* 2007)
29	54	WBS3	PA	(Casas *et al.* 2000)
29	54	WBS14	PA	(Casas *et al.* 2000)
29	54	HCW	BH	(Casas *et al.* 2003)
29	49	RPYD	BH	(Casas *et al.* 2003)
29		WBS14	BH	(Casas *et al.* 2003)

[a]BW = Birth weight, RPYD=retail product yield, FATYD=fat yield, YG= USDA yield grade, CHESTWT=chest width, FAT=fat depth, KHPFat=kidney, heart and pelvic fat, ADG=postweaning average daily gain, WBS3= meat tenderness measured as Warner-Bratzer shear force at 3d post-mortem, CY=carcass yield, LMA=Longisimus area, YW=yearling weight, RT=rib thickness, CW=cold carcass weight, SW=weight before slaughtering, SFT=Subcutaneous fat thickness [b]BH= sired by Brahman x Hereford bull, BA=sired by Brahman x Angus, Wagyu=Japanese Black Cattle, BM=sired by Belgian blue x MARC III, GH=Gyr x Holstein, PA=sired by Piedmontese x Angus bull, BTA=cattle chromosome

A meta assembly of the literature (Table 1.2) demonstrates that most of the bovine autosomes (BTA) contain regions controlling growth traits (such as weight) and carcass traits (such as carcass yield, fat yield, meat tenderness, marbling, etc.). However, most studies do not indicate the relative position of the QTL. There is some evidence of QTL for growth traits and carcass traits in the same chromosomal regions. For instance, the QTL for the carcass traits of yield grade, fat depth and marbling score and the growth trait of birth weight are located in the same region (52-59 cM) on chromosome 5 (Casas *et al.* 2003; Casas *et al.* 2004). This suggests a gene or a group of genes affecting growth traits and carcass traits are located in the

same chromosomal region or that the same gene(s) control both sets of traits (Casas *et al.* 2000).

QTL for both growth traits and carcass traits are often found on different chromosomes, such as the QTL for marbling score. In Wagyu cattle (Japanese Black cattle), QTL have been reported for marbling on several chromosomes (chromosomes 4, 6, 10, 14 and 21) (Mizoguchi *et al.* 2006; Takasuga *et al.* 2007) and this indicates that the trait is affected by several different genes. QTL for *M. longissimus dorsi* muscle area in Wagyu cattle have been found also in two different genomic regions (Takasuga *et al.* 2007). The explanation again is that for most quantitative traits, different genomic regions will be involved in the expression of the quantitative trait (Casas *et al.* 2001).

The results of the studies illustrate that QTL affecting growth traits and carcass traits are located on most of the bovine chromosomes. However, studies on other carcass traits that are related to muscularity, such as eye muscle area (EMA), meat to bone ratio, etc, have yet to be undertaken.

1.2.5.2 Genes involved in muscle development and carcass traits

Only a few genes have been identified for muscle development and carcass traits. However, *IGF-1* and *myostatin* are two of the major genes with significant roles in muscle development that appear to affect carcass traits in livestock.

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1.2.5.2a *Insulin-like growth factor 1* (*IGF1*)

IGF1 is a member of the insulin-like growth factor family (IGFs). IGFs are growth factors that are known to have prominent roles in growth. Insulin-like growth factor 1 is a single chain protein (7.5 kDa), produced in the liver and elsewhere in the body (Oksbjerg *et al.* 2004). Studies indicate that IGFs have important roles in muscle development and both IGF1 and IGF2 can stimulate muscle cell proliferation and differentiation through their interaction with the type 1 IGF receptor (Oksbjerg *et al.* 2004). Mathews *et al.* (1988) found that when the level of IGF1 is 50% above normal, the growth of bone and muscle in transgenic animals was increased 30% (cited in Florini *et al.* 1996). Indeed, the deletion of *IGF1* disrupts skeletal muscle development (Bass *et al.* 1999)

In cattle, the *IGF1* gene is located on chromosome 5 at 73 cM (Kappes *et al.* 1997). There are a limited number of studies on the activity of IGF1 in cattle. Studies on the association of *IGF1* gene-specific SNP markers and growth traits have shown a significant dominance effect of *IGF1* on birth weight (Li et al 2004). Studies on the relationship of *IGF1* genotypes with growth and carcass traits in swine found that that *IGF1* is associated with average daily gain and rib backfat (Casas *et al.* 1997).

1.2.5.2b *Myostatin*

In 1997, a new member of TGF-β superfamily was identified, called growth differentiation factor 8 (GDF8) or myostatin (McPherron *et al.* 1997). Like other TGF-β superfamily members, myostatin is produced as a precursor protein, which is cleaved by proteolysis for activation. Myostatin consists of a signal sequence, an N

terminal propeptide domain, and a C terminal domain that contains the active peptide (Dominique and Gerard 2006).

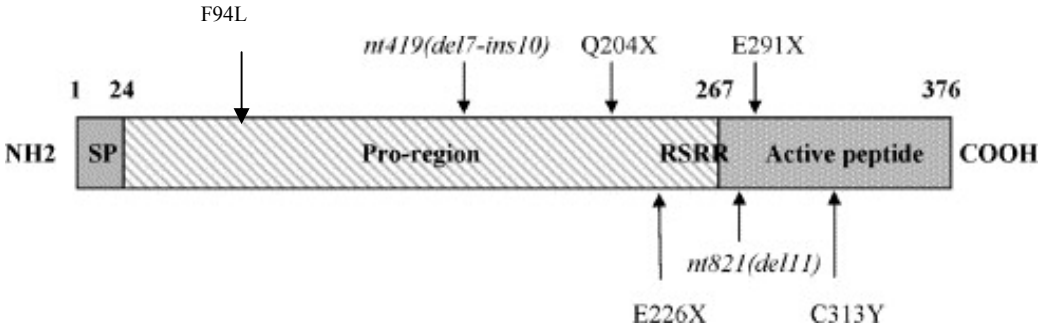


Figure.1 Structure of myostatin protein, SP= signal peptide. The arrows indicate the position of inactivating mutations that are responsible for the increased muscle growth observed in some cattle breeds (Dominique and Gerard 2006) .

McPherron *et al.* (1997) determined the biological function of myostatin by knocking out the gene in mice and showed that the mutant mice were larger than the wild type mice as a result of increased muscle mass. This suggested that myostatin has an important role in skeletal muscle development by inhibiting muscle overgrowth. The increased skeletal muscle mass results from both hyperplasia and hypertrophy, and this means that myostatin inhibits muscle fibre development both pre-natally and post-natally. Myostatin appears to affect the proliferation and/or differentiation of both myoblasts (during fetal development) and satellite cells (during the post natal period) (Lee 2004).

The same phenomenon of excessive muscle development has been found in some cattle breeds, so called the double-muscled cattle. Since the identification of myostatin and its effect on mice muscle mass, studies have examined the effect of myostatin on cattle skeletal muscle mass, specifically in double-muscled cattle. The *MH* (muscle hypertrophy) locus causing the double-muscling in cattle was located in

bovine chromosome 2 at the centromeric end (Charlier *et al.* 1995). The position of the *MH* locus was located at the same chromosomal interval as bovine *myostatin* gene, thus verifying the causal relationship (Smith *et al.* 1997). The alignment of the myostatin amino acid sequence of murine, cat, human, baboon, bovine, porcine, ovine, chicken, turkey and zebrafish shows that myostatin is highly conserved between species and suggests that the function of myostatin has been also conserved (McPherron and Lee 1997).

After studies located the *MH* locus as the *myostatin* gene, *myostatin* mutations in double- muscled cattle were identified. In Belgian Blue cattle, double muscling is caused by a deletion of 11 base pairs in the *myostatin* coding sequence for the bioactive carboxyl-terminal domain, which results in a stop codon (Grobet *et al.* 1997; Kambadur *et al.* 1997; McPherron and Lee 1997). The deletion of the coding sequence for the bioactive carboxyl-terminal (C-terminal) in the *myostatin* gene would lead to the inactivation of the myostatin since the C-terminal is the active part of myostatin protein that binds the receptors.

The *myostatin* mutation that causes double-muscling in Piedmontese cattle is a G to A transition in exon 3, which causes the substitution of cysteine with tyrosine at amino acid 313 (Kambadur *et al.* 1997; McPherron and Lee 1997). In Limousin cattle, there is a C to A transition at nucleotide 282 in exon 1, known as F94L, resulting in the substitution of leucine for phenylalanine at amino acid 94 (McPherron and Lee 1997). This variant is not as severe as double muscle mutations and only results in a moderate increase in muscle mass (Sellick et al 2007).

The role of myostatin in muscle mass regulation involves initiating a signal transduction pathway in its target cells (in this case, the muscle fibre cells). Before the signal transduction pathway is initiated, myostatin is activated from its latent state. This activation can be only completed after the pro-peptide is cleaved proteolytically by members of the BMP-1 (bone morphogenetic protein) or tolloid family (Lee 2004). After cleavage of the pro-peptide, the pro-region and active peptide form a dimer. The dimer must dissociate for the active peptide to bind its receptors. There are several proteins that have been identified which have the ability to bind the mature dimer and inhibit the activation of myostatin (Table 1.3) (Dominique and Gerard 2006).

Table.1.3 Myostatin binding proteins

Localisation	Binding molecule	Myostatin form bound	Consequence of binding
Serum	Myostatin propeptide	Mature myostatin	Inhibits myostatin receptor binding
	GASP-1	Mature myostatin and myostatin propeptide	Inhibits myostatin activation
	FLRG	Mature myostatin	Inhibits myostatin receptor binding
Skeletal muscle	hSGT	Myostatin N-term signal peptide region	Inhibits myostatin secretion and activation
	Titin cap	Mature cap	Inhibits myostatin latent complex formation and secretion
	Follistatin	Myostatin	Inhibits myostatin receptor binding

Source: (Dominique and Gerard 2006)

The myostatin binding proteins have a function to either inhibit myostatin activation or myostatin receptor binding. For example, over-expression of follistatin or the myostatin pro-peptide results in an increase of muscle mass in mice (Lee and McPherron 2001). Follistatin can also block the activation of BMP-11, another TGF-β superfamily member, which is highly related to myostatin (Hill *et al.* 2002). The highly similar follistatin related (FLRG) protein has been also reported to have a direct interaction with the mature myostatin protein and has been suggested to act as a major negative regulator of myostatin (Hill *et al.* 2002). Another binding protein that has been identified to have the ability to bind and inhibit myostatin is GASP-1. GASP-1 has been reported to bind and inhibit myostatin (and other growth factors including BMP-11), by preventing the activation of the myostatin latent complex (Hill *et al.* 2003).

Once myostatin has been activated, it will bind its receptor (ActRIIB) and initiate a signal transduction in the target cells. As with the other TGF-β superfamily members, the myostatin receptors are divided into two sub-families : type I receptors and type II receptors (Massague 1998). Usually, the ligand (active myostatin peptide) will bind first to a type II receptor kinase and this activates a type I receptor kinase, which will then phosphorylate the Smad proteins (Table 1.4). The Smad proteins have an important role in regulating the expression of many downstream proteins (Lee 2004) .

Table 1.4. Elements of the TGF-β ligand family pathway

Ligand	Type II receptor	Type I receptor	R-Smad
Activins	ActRII	ActRIB/ALK-4	Smad 2
Myostatin	ActRIIB	unknown	Smad 3
TGF-β	TβRII	TβRI/ALK5, ALK1, ActRI/AlK3	Smad 1
BMPs	BMPRII	BMPRI/ALK3	Smad 5
GDFs	ActRII ACtRIIB	BMPRIB/ALK6 ActRI/ALK2	Smad 8

Source :(Dominique and Gerard 2006)

There are other proteins, besides the binding proteins and receptors, which are affected during the regulation of muscle mass by myostatin. Bouley *et al.* (2005) studied these affected proteins in doubled muscle cattle and 13 proteins were identified that are influenced by myostatin. The proteins included eight proteins related to the contractile apparatus, several myosin light chains (MLC2, MLC3, MLC2s, MLC1sa, MLC1sb), troponin T (slow TnT and fast TnT) and myostatin binding protein H. Two other proteins are engaged in metabolic pathways, phosphoglucomutase (PGM) and heart fatty acid-binding protein (H-FABP). The other three proteins are sarcosin, sarcoplasmic reticulum 53 kDA glycoprotein, and p20.

All of these results suggest that there are many proteins which interact with myostatin and there are likely to be other proteins that have not yet been identified. Since many proteins interact with myostatin, there may be many genes that could be

epistatic with *myostatin*. Therefore, studying the interactions between *myostatin* and other genes will be useful for understanding the biology underlying muscularity and body conformation traits.

1.2.6 Summary

In summary, body dimension traits, such as height, length and girth have moderate to high heritabilities. Close genetic relationships between these body dimension traits with growth and carcass yield traits (especially for carcass weight) have been reported, so that it should be efficient to conduct selection for production traits based on body dimension traits and their gene markers. However, only a few studies have located QTL for eye muscle area, meat to bone ratio, and other traits that can indicate muscularity not related to growth in beef cattle. Therefore, QTL mapping for these traits needs to be conducted in order to locate genomic regions controlling these traits. Once these genomic regions have been found, identification of the causative genes can be achieved by studying the most likely candidate genes that are located in the region.

Only a few genes have been identified for muscle development and carcass traits. *Myostatin* is one of these genes and has an important role in muscle development and carcass traits in cattle. It is likely that the *myostatin* gene will interact with other genes to regulate muscle development. The objectives of this study were to locate the QTL for muscularity, to identify the genes that involved in these traits, study the interaction between these genes and *myostatin*, and to study the association between these genes and the traits in order to develop markers for the selection of carcass production traits.

1.3 Research objectives

It was hypothesized that there will be QTL for muscularity related traits that are not related to growth and that there will be interactions between *myostatin* and gene(s) in some of these QTL controlling muscularity. Based on these hypotheses, a molecular approach was utilized to identify the genes and study their association with muscularity. This approach included quantitative trait loci (QTL) mapping of muscularity related traits and candidate gene(s) selection based on the the location of the QTL. Analyses of association between candidate gene(s) and muscularity related traits including examining potential epistatic interactions with *myostatin*. Thus, the specific aims of this study are:

1. to locate quantitative trait loci (QTL) for cattle muscularity,
2. to identify the genes controlling muscularity from these QTL,
3. to study the association between the genes with muscularity, and
4. to study the relationship between these genes with the *myostatin* gene.

From the results of the study herein, it was expected that markers may be discovered for selection of muscularity in cattle. Moreover, a better understanding of the biology that underlies muscle development as well as greater knowledge of *myostatin* effects on muscularity and interactions with other gene(s) would be provided.

CHAPTER 2

Materials and Methods

2.1 JS Davies cattle gene mapping project

This study was based on the JS Davies Cattle Mapping Project. The aims of this project were to investigate the inheritance of various beef cattle traits such as growth, meat production, meat quality and muscle structural composition and to map and identify genes controlling those traits.

Two very different cattle breeds (Jersey and Limousin) were used in the project in order to obtain maximum variation in the traits of interest of the progeny from their crosses. Jersey is a small dairy breed and Limousin is a beef cattle breed. These two breeds have different phenotypes for most traits, including body size, weight, growth rate, retail beef yield, degree of muscling, marbling, meat tenderness, fat content and fat distribution.

The design was double-backcross. Three Limousin x Jersey F1 bulls were mated to both purebred Jersey and Limousin dams in Australia (Figure 2.1). 161 Limousin cross progeny and 205 Jersey cross progeny were born in Australia from 1996 to 1998.

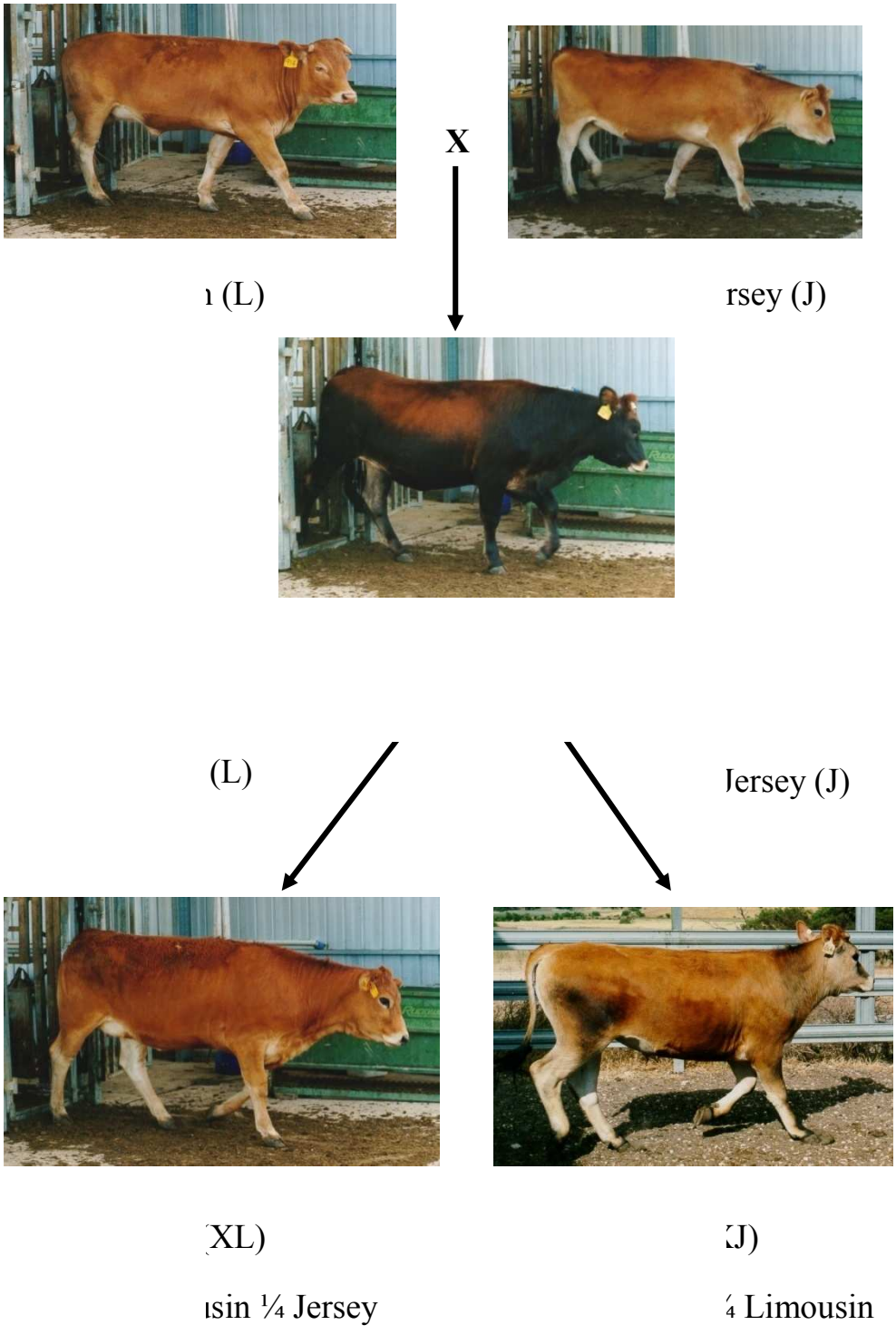


Figure 2.1 JS Davies cattle gene mapping herd

All calves were measured for weight, height (distance between hip and ground), length (the distance between spinal cord bone on shoulder and pin-bone), and girth at birth and every 50 days. When they were weaned (about 250 days), 400 days old and 600 days old, they were also measured for weight, height, length, girth, fat depth (scanned at P8 site on the rump using Ezi-scan®), hip width (distance between the two hip bones), and stifle width (distance between the two muscles of the back legs) and muscularity (stifle width/hip width) (Figure 2.2). The calves were finished on grain concentrates for at least 180 days.

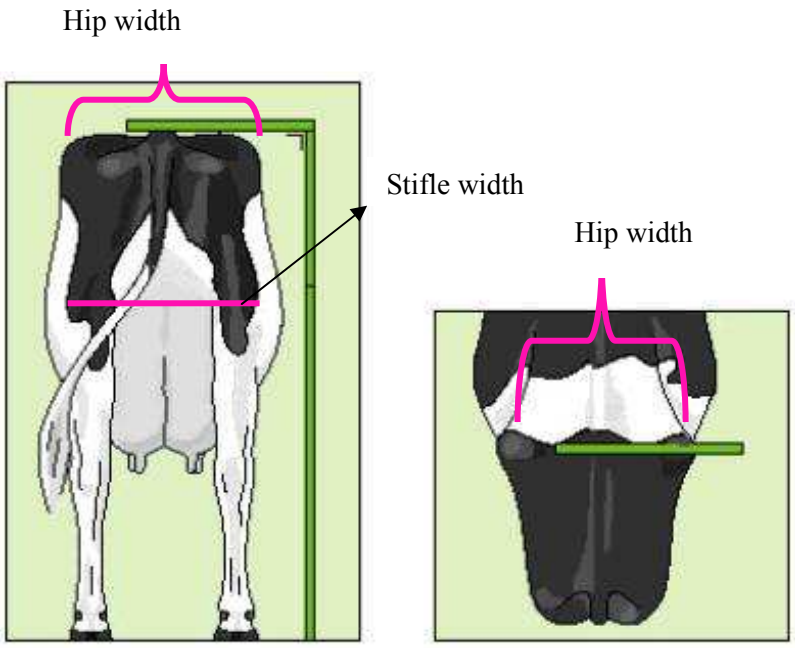


Figure 2.2. Stifle width and hip width measurements (height, stifle width and hip width) where muscularity = stifle width/hip width.

At 36-40 months of age, the progeny were slaughtered, after finishing on grain, to obtain carcass trait data, including hot carcass weight and length, P8 fat depth, dentition, butt shape, pelvic area, marbling, eye muscle area, fat cover and depth, fat and meat colour, meat texture, meat fat content, tenderness, fatty acid composition, and β-carotene concentration.

Meat percentage and bone percentage were estimated using prediction equations based on the differences in saleable beef yield of 241 steers from 10 diverse *Bos Taurus* breed (including Limousin and Jersey). There were two equations applied based on the year of slaughter (1996+1997 and 1998) because of the differences in carcass measurements collected at the different abattoirs.

Equation for year 1996 and 1997:

$$\text{Meat\%} = 67.28 + 0.69\times ts + 1.59\times st + 1.07\times of + 0.38\times ru + 0.45\times ld + 1.67\times tln + 0.15\times rib - 1.16\times femwt + 1.29\times tibwt - 0.33\times fqwt - 0.0735\times hscw - 0.092\times p8 + 0.04\times ema$$

$$\text{Bone\%} = 20.24 + 0.23\times ts - 0.14\times st - 0.14\times of - 0.04\times ru + 0.02\times ld - 0.31\times tln - 0.34\times rib + 2.02\times femwt + 0.67\times tibwt + 0.85\times fqwt - 0.0461\times hscw - 0.01\times p8 - 0.003\times ema$$

Equation for year 1998:

$$\text{Meat\%} = 66.88 + 2.40\times st + 1.18\times of + 0.98\times kn + 0.51\times ld + 3.10\times ct - 0.46\times bones - 0.747\times hscw - 0.075\times p8 + 0.049\times ema$$

$$\text{Bone\%} = 19.42 - 0.46\times st - 0.28\times of - 0.02\times kn - 0.18\times ld + 0.03\times ct + 3.12\times bones - 0.0285\times hscw - 0.009\times p8 - 0.007\times ema$$

Abbreviations:

ts	Topside (kg)	tibwt	Tibia (kg)
st	*M. semitendinosus* muscle (kg)	fqwt	Forequarter bone weight (kg)
of	Outside flat (kg)	hscw	Hot standard carcass weight (kg)
ru	Rump (kg)	p8	Rump fat depth (mm)
ld	*M.longissimus dorsi* muscle (kg)	ema	Eye muscle area (cm^2)
tln	Tender loin (kg)	kn	Knuckle (kg)
rib	Ribset (kg)	ct	Chuck tender (kg)
femwt	Femur (kg)	Bones	Radius/ulna + humerus (kg)

This study focused on hot standard carcass weight, meat weight, meat percentage, eye muscle area, silverside weight, meat to bone ratio, muscularity as measured by the ratio of stifle to hip width, stifle width, bone weight, bone percentage and hip width (Table 2.1). The correlations between traits of interest was also measured (Table 2.2).

Table 2.1 Summary statistics of the traits of interest

Trait	Mean	S.D	Min	Max	%CV[a]
Hot standard carcass weight	334.7	61.8	168	479.6	18.5
Meat weight	230.3	48.6	114.5	355.2	21.1
					4.3[b]
					13.73[c]
Meat percentage	68.62	2.99	62.90	80.26	4.36
					3.91[d]
Eye muscle area	80.7	17	26	166	21.1
					12.66[b]
Silverside weight	8.47	2.23	3.82	15.51	26.38
					10.53[b]
Measured meat to bone ratio	3.91	0.534	2.79	6.62	13.64
Muscularity (ratio of stifle to hip width)	74.30	7.42	59.46	102.27	9.9
Stifle width	31.94	3.51	22	45	10.99
					9.97[e]
Bone weight	58.87	10	33.53	88.7	16.99
Bone percentage	17.76	1.79	11.40	23.85	10.1
Hip width	42.99	2.07	37	49	4.8

[a]Coefficient of variation (%), [b]%CV after adjusted HSCW, [c]%CV after adjusted bone weight, [d]%CV after adjusted bone percentage, [e]%CV after adjusted hip width

Table 2.2 Raw correlations between traits

Trait	BoneWt	Bone%	EMA	HSCW	MeatWt	Meat%	Mttobn	SilvWt	HipWdt	Mus
BoneWt										
Bone%	0.10									
EMA	0.62	-0.42								
HSCW	0.82	-0.48	0.80							
MeatWt	0.76	-0.52	0.85	0.98						
Meat%	0.21	-0.44	0.66	0.46	0.63					
Mttobn	-0.01	-0.94	0.55	0.54	0.63	0.69				
SilvWt	0.72	-0.47	0.84	0.92	0.97	0.73	0.62			
HipWdt	0.29	-0.21	0.26	0.37	0.33	0.002	0.16	0.28		
Mus	0.28	-0.50	0.45	0.54	0.60	0.54	0.58	0.60	-0.05	
StiWdt	0.38	-0.56	0.53	0.66	0.69	0.50	0.61	0.68	0.39	0.90

BoneWt=bone weight, Bone%=bone percentage, EMA=eye muscle area, HSCW=hot standard carcass weight, MeatWt= meat weight, Meat%=meat percentage, Mttobn=meat to bone ratio, SilvWt=silverside weight, HipWdt=hip width, Mus=muscularity, StiWdt=stifle width

2.2 Mapping quantitative trait loci (QTL)

QTL mapping was performed to locate the chromosomal regions that may contain genes controlling the phenotypic traits of interest. QTL Express software (http://qtl.cap.ed.ac.uk/) was used to map the QTL by regression analysis of the phenotypes (600 day stifle width, 600 day hip width, muscularity, hot standard carcass weight, meat weight, meat percentage, bone weight, bone percentage, meat to bone ratio, eye muscle area and silverside weight) and genotypes (genotype data were from 150 microsatellite markers approximately 20 cM apart and covering all the autosomes) obtained for all the backcross progeny.

The QTL Express software is suitable for half-sib outbred and F2 populations (both inbred and outbred crosses) (Seaton *et al.* 2002). The multiple marker approach for interval mapping in half sib families was used as described by Knott *et al.* (1996) and completed at 1 cM interval along each the chromosome. Based on Knott *et al*.

(1996), three steps were applied. Firstly, informative marker alleles from the sires (361, 368 and 398) were identified to determine which allele the progeny inherited. Each marker was informative for at least for one sire. Secondly, the probability of the individual progeny inheriting either allele 1 or 2 from the sires was calculated. Then, these probabilities were combined, providing coefficients upon which the phenotypic data were regressed. Cohort (combination of year and sex), breed (Limousin cross and Jersey cross), and the *myostatin* F94L genotype were included as fixed effects and were nested within the sire (Model 1). The *myostatin* F94L genotypes were included in the model to identify chromosomal regions in which gene(s) that may be epistatic with *myostatin* reside.

In order to obtain QTL for carcass traits related to muscularity and not related to growth, four covariates were used for this study. The covariates were:

1. hot standard carcass weight as a covariate for meat weight, eye muscle area (EMA) and silverside weight,
2. bone weight as a covariate for meat weight,
3. bone percentage as a covariate for meat percentage, and
4. hip width as a covariate for stifle width.

$$Y_{ijklm} = \mu + \eta(x_{ijklm} - \bar{x}) + \alpha_i + \beta_j + (\theta_l) + b_k x_k + \varepsilon_{ijklm} \qquad \text{(Model 1)}$$

Where:

Y_{ijkm} is the response variable (phenotypic trait)

μ is the overall mean

α_i is the effect of i^{th} cohort

β_j is the effect of j^{th} breed

θ_l is the effect the l^{th} *myostatin* F94L genotype (when include *myostatin* F94L genotype in the model)

η is the effect of the covariate (when covariate is used)

$b_k x_k$ b_k is the allele substitution effect of the QTL within family k, x_k is the probability that animal m inherited the (randomly assigned) first haplotype of sire k

ε_{ijkl} is the residual effect

Significant QTL were defined by selecting the QTL maxima with F-values greater than 4 as the threshold for the across all 3 sire family analysis (Churchill and Doerge 1994), which is equivalent to $P<0.05$ threshold. The positions of the QTL peaks were used to select the candidate genes based on the Ensembl bovine database (www.ensembl.org) (see section 2.3).

2.3 Identification of candidate genes

Using the chromosome regions that have been located using QTL Express, the positions of the markers were noted. The positions of the markers in centiMorgans (cM) were converted to base pairs (1.000.000 base pairs per centiMorgan) to identify candidate genes using the Ensembl database (www.ensembl.org). Candidate genes were chosen based on their known function or potential involvement with muscle development.

2.4 Optimization of PCR condition

After the candidate genes were selected, DNA variants in these genes were identified. To find the DNA variants, the first step was to amplify the candidate gene coding regions by polymerase chain reaction (PCR) using DNA from the 3 Australian mapping sires. PCR conditions were optimised so that a single product with the expected size was amplified. The reactions were carried out in 96 well full skirt PCR microplate (*Axygen)* and the samples were amplified using a Corbett Palm-Cycler. 25 µl reactions were used for each sample and contained 2.5 µl dNTPs (1.25 mM), 2.5 µl $MgCl_2$ (25mM), 2.5 µl 10x buffer, 2.5 µM forward primer and 2.5 µM reverse primer (*Geneworks and Sigma)*, 0.5 U Amplitaq Gold Polymerase (*Roche*), 50 ng DNA template and 13.5 µl H_2O. A touch-down program was used and the conditions were as follows:

Touchdown Program

Cycle 1 (1 repeat):	95 ºC for 10 minutes
Cycle 2 (40 repeats):	95 ºC for 1 minute
	TA for 1 minute
	72 ºC for 1 minute
Cycle 3 (1 repeat):	72 ºC for 10 minutes
	2. 4 ºC for 4 minutes

The first annealing temperature trialled was 60 ºC. If no band was produced, the magnesium concentration was increased or the initial annealing temperature was adjusted to 70 ºC or 55 ºC. When extra products were present, the magnesium concentration was decreased. In those cases where no single product could be

produced by changing magnesium concentration and annealing temperature, another polymerase (such as Kapa taq) was used or new primers designed.

The amplified products from PCR were confirmed by gel electrophoresis using 2.0% agarose (A*pplichem*) gel electrophoresis in 1X TAE buffer (containing 10 mM Tris buffer, 10 mM EDTA, 1 M glacial acetic acid and Milli Q water) for 40-45 minutes at 110 V. Gels were stained using 0.5µg/ml ethidium bromide for 10-15 minutes and the product visualized under UV illumination using a Gel Documentation 1000 system (*Biorad*). The amount of PCR product analysed using gel electrophoresis were 5 µl for the PCR optimization and 2 µl for the sequencing from the sires or grandparent DNA templates.

2.5 DNA purification from PCR reactions

After the amplification, the PCR products of correct size and quantity were purified to remove any contaminants using Ultra Clean PCR Clean-up Kits (*MoBio*). The manufacture's protocol (Appendix 2.1) was used for this purification.

2.6 Sequencing reaction of PCR product

Once the DNA has been purified, the concentration of the DNA was measured using both gel electrophoresis and a spectrophotometer ND-100 (*NanoDrop*). The DNA concentration measurements were used to determine the quantity of DNA needed for the sequencing reactions (50-100ng in 3-5 µl).

10 µl reactions were used for sequencing each PCR sample and contained 1-3 µl H_2O, 2 µl Big Dye Terminator (*ABI*), 1 µl glycogen (*Roche*), 1 µl 5 µM primer and

3-5 µl DNA. The reactions were performed in a Corbett's Palm-Cycler in 0.2 µl tubes (A*xygen*). The following conditions were used for sequencing:

Cycle 1 (1 repeat):	95ºC, 5 seconds
Cycle 2 (24 repeats):	96 ºC, 30 seconds
	50ºC, 15 seconds
	60ºC, 4 minutes
Cycle 3:	hold at 4ºC

The sequencing reaction products were then purified using 75% isopropanol (Appendix 2.2)

The dried samples were sent to Institute of Medical and Veterinary Science (IMVS), Adelaide, South Australia for automated sequencing on an AB 373 or ABI 3700 DNA sequencer (Table 2.3).

Table 2.3 Sequenced regions of the candidate genes

Gene	Σ Exons	Sequenced regions
ACVR1	10	10 exons, 5'UTR, 3'UTR
SNIP1	4	4 exons, 5'UTR, 3'UTR
TGFBR3	16	16 exons, 5'UTR, 3'UTR
FSTL5	16	14 exons, part of 3'UTR

2.7 Genotyping using high resolution melts

After DNA variants of sires had been identified and verified in the grandparents by sequencing, genotyping of single nucleotide polymorphisms (SNPs) for the progeny was performed using high-resolution melting (HRM) analysis. A Rotor-Gene 6000 (*Corbett*) was used to the amplify progeny DNA and genotype the product using

HRM. 20 µl reactions were used for each sample and contained 2.2 µl H_2O, 10 µl pre-mixed SensiMix (*Quantace*), 1 µl of 2.5 µM forward and reverse primers (*Geneworks or Sigma*), 0.8 µl EvaGreen (*Biotium Inc*) and 5 µl of 10 ng/ µl DNA template. Alternatively, the master mix consisted of 6.2 µl H_2O, 2.5 µl dNTPs (1.25 mM), 2.5 µl buffer A (*Kapa Biosystems*), 1 µl of 2.5 µM forward and reverse primer (*Geneworks or Sigma*), 1 µl of 0.5 U Kapa Taq (*Kapa Biosystems)*, 0.8 µl EvaGreen dye (*Biotium Inc*) and 5 µl of 10 ng/ µl DNA template This alternative mix was only used if the amplification and HRM products were insufficient using SensiMix.

The PCR and HRM conditions were optimised before commencing the genotyping of the progeny samples. The touchdown program above was used to amplify the DNA with 60ºC as the initial annealing temperature. If there was insufficient product for genotyping, the annealing temperate was adjusted (to 70ºC or 55ºC) and magnesium added. For HRM, a melting temperature range of 60ºC- 95ºC was used as the initial melting temperature range for the first optimization. The HRM signal was detected at 470-510 nm (green channel). The melting temperature range for each SNP was determined based on the melting curve results of the optimization. The following conditions were used for genotyping:

Hold 1:	95ºC, 10 minutes
Cycling (40-50 repeats):	95ºC, 20 seconds clarify
	TA, 30 seconds (first 11 cycles were touchdown, TA)
	72ºC, 20 seconds
Hold 2:	72ºC, 10 minutes
HRM:	60ºC- 95ºC

2.8 Statistical analysis of SNPs

The effects of the SNPs on phenotypic traits were analysed using an unbalanced design analysis of variance (*Genstat*) with fixed effects. The fixed effects were:

- Cohort (sex and year interaction: 96H, 96S, 97H, 97S, 98H, 98S)
- Breed (XJ= ¾Jersey and ¼ Limousin; XL= ¾ Limousin and ¼ Jersey)
- Sire (361, 368, or 398)
- F94L *myostatin* genotype (CC, CA or AA)
- Covariates (HSCW, bone weight and bone percentage) for some traits
- Genotype of the progeny for each SNP in a given gene

Before analysing the effect of the SNP on the traits of interest, the variance ratio of cohort, breed and sire were calculated to determine how much variation was caused by the fixed effects (Table 2.4).

Table 2.4 Variance ratio of cohort, breed and sire

Trait	Variance ratio		
	cohort	**breed**	**sire**
HSCW	52.69***	435.83***	11.17***
Meat weight	41.61***	505.58***	9.25***
Meat weight (with HSCW as covariate)	19.89***	52.67***	0.24
Meat weight (with bone weight as covariate)	56.06***	131.17***	0.466
Meat%	14.43***	242.62***	0.47
Meat% (with bone% as covariate)	13.09***	155.80***	0.89
Eye muscle area (EMA)	23.43***	290.77***	8.17***
EMA (with HSCW as covariate)	7.41***	16.02***	4.45*
Silverside weight	24.06***	695.81***	11.88***
Silverside weight (with HSCW as covariate)	17.97***	105.11***	3.01*
Meat to bone ratio	48.03***	155.18***	0.73
Muscularity	38.96***	303.93***	0.54
Stifle width	42.25***	339.64***	8.03***
Stifle width (with hip width as covariate)	43.14***	343.05***	2.37

***F probability <0.001, *F probability<0.05

There were three models used to analyse the effects of the SNPs on the phenotypic traits. The first model fitted cohort, breed, sire, *myostatin* F94L genotype and the SNP genotype of the candidate gene:

$$Y_{ijkm} = \mu + \alpha_i + \beta_j + \gamma_k + \theta_l + \eta(x_{ijklm} - \bar{x}) + \lambda_m + \varepsilon_{ijklm} \quad \text{(Model 2)}$$

Where:

Y_{ijkm} is the response variable (phenotypic trait)

μ is the overall mean

α_i is the effect of i^{th} cohort

β_j is the effect of j^{th} breed

γ_k is the effect of k^{th} sire

θ_l is the effect the l^{th} *myostatin* F94L genotype

η is the effect of the covariate (when covariate is used)

λ_m is the effect of m^{th} SNP genotype

ε_{ijkl} is the residual effect

The second model was applied to study the interaction between the SNP genotype and the *myostatin* F94L genotype:

$$Y_{ijklm} = \mu + \alpha_i + \beta_j + \gamma_k + \theta_l + \eta\,(x_{ijklm} - \bar{x}) + \lambda_m + (\theta\lambda)_{lm} + \quad \text{(Model 3)}$$

Where:

$(\theta\lambda)_{lm}$ is the interaction between l^{th} *myostatin* F94L genotype and m^{th} SNP genotype

Interactions between each SNP genotype within a gene were examined by fitting the other SNP in the model and its interaction with other SNP:

$$Y_{ijklmn} = \mu + \alpha_i + \beta_j + \gamma_k + \theta_l + \eta(x_{ijklm} - \bar{x}) + \lambda_m + \delta_n + (\lambda\delta)_{mn} + \quad \text{(Model 4)}$$

Where:

δ_n is the effects of n^{th} SNP B

$(\lambda\delta)_{mn}$ is the interaction between m^{th} SNP A genotype and n^{th} SNP B

When there was a significant interaction between SNPs of a gene associated with traits of interest, a haplotype analysis was conducted. Haplotypes were formed using the PHASE program (Pirinen et al. 2008). Combinations of halpotype were used to analyse (using unbalanced ANOVA) their effect on the traits. The model used was similar to the first model described above but the SNP effect was replaced with the haplotype (Model 5).

$$Y_{ijkm} = \mu + \alpha_i + \beta_j + \gamma_k + \theta_l + \eta(x_{ijklm} - \bar{x}) + \varphi_m + \quad \text{(Model 5)}$$

Where:

φ_m is the effect of m^{th} haplotype

In addition, if there was significant association found between the DNA SNP variants of the candidate genes and traits of interest, the additive and dominance effect was estimated using a "general linear model" (*Genstat*) by including these effects as variates. For the additive effect, each genotype was given a different code, such as 2 for AA; 1 for AG and 0 for GG, whereas for the dominance effect, 0 and 1 were used to code for homozygous and heterozygous animals, respectively. Cohort, breed, sire, *myostatin* F94L genotype, additive and dominance effects were fitted in the model and covariates were included for some traits (Model 6).

$$Y_{i=} \beta_0 + \beta_{Cov} X_{iCov} + \alpha_i + \beta_i + \gamma_i + \theta_l + \beta_{Ad} X_{iAd} + \beta_{Dom} X_{i\,Dom} + \varepsilon_i$$

(Model 6)

Where:

Y_i is the phenotype

B_0 is the constant

β_{Cov} is regression coefficient of covariate

β_{Ad} is regression coefficient of additive effect

β_{Dom} is regression coefficient of dominance effect

X_i is the regressor or i^{th} value of each parameter (covariate, cohort, breed, sire, myostatin, additive and dominance)

ε_i is residual variation

Analysis using a "linear mixed model" (*Genstat*) was conducted to measure the variance of each observed SNP. Cohort, breed, sire and covariates were fitted as fixed effects, while the *myostatin* F94L genotype and SNP genotypes were included as random effects (Model 7).

$$Y_{i=} \mu + \beta_{Cov} X_{iCov} + \beta_{Ct} X_{iCt} + \beta_{Bd} X_{iBd} + \beta_{Sr} X_{iSr} + \sigma^2_{Ms} Z_{iMs} + \sigma^2_{SNP} Z_{iSNP} + \sigma^2_{Ms.SNP} Z_{i\,Ms.SNP} + \varepsilon_i$$

(Model 7)

Where:

Y_i is the phenotype

μ is overall mean

β_{Cov} is regression coefficient of covariate

β_{Ct} is regression coefficient of cohort

β_{Bd} is regression coefficient of breed

β_{Sr} is regression coefficient of sire

σ^2_{Ms} is random effect coefficient of *myostatin* (*myostatin* variance)

σ^2_{SNP} is random effect coefficient of SNP (SNP variance)

$\sigma^2_{Ms.SNP}$ is random effect coefficient of the *myostatin* and SNP interaction (*myostatin* and SNP interaction variance)

X_i is the regressor or i^{th} value of each fixed effect (covariate, cohort, breed, sire)

Zi is the regressor or i^{th} value of each random effect (*myostatin*, SNP, interaction of *myostatin* and SNP)

ε_i is residual variation

CHAPTER 3

QTL Mapping and Candidate Gene Selection

3.1 Introduction

Selection for muscularity is an essential step to improve the productivity of beef cattle. To maximize the accuracy of selection, information from both phenotypic and molecular level is required. Phenotypic information can be obtained by measuring or observing traits related to muscularity and hence, retail beef yield (eg body weight and stifle width). Ideally, these traits would be related to increased muscle mass relative to the skeleton rather than related to growth *per se*. For molecular information, quantitative trait loci (QTL) mapping is the first step towards obtaining markers. By mapping traits such as meat percent or by using covariates such as carcass weight for traits like eye muscle area herein (Chapter 2), regions of the genome that contain gene(s) that may affect muscularity and retail beef yield rather than growth or overall animal size could be identified.

3.2 Results

3.2.1 QTL for muscularity related traits

QTL for stifle width, muscularity as predicted by the ratio of stifle to hip width, hot standard carcass weight (HSCW), meat weight, meat percentage (meat %), eye muscle area (EMA), meat-to-bone ratio, silverside weight, bone weight and bone percentage (bone%) were mapped using cohort and breed as fixed effects. Since some traits, such as stifle width, meat weight, eye muscle area and silverside weight, may be related to the overall size of the animal rather than an increase in specific muscles, covariates (hot standard carcass weight, hip width, bone weight and bone %) were included where appropriate. Hot standard carcass weight was used as a covariate for meat weight, eye muscle area and silverside weight; hip width as a

covariate for stifle width; bone weight as a covariate for meat weight; and bone percentage as a covariate for meat percentage.

Stifle width, muscularity (ratio of stifle width with the hip width), hot standard carcass weight and meat weight (with hot standard carcass weight as covariate) had four QTL for each trait identified across the sire families (Table 3.1). Three significant QTL segregated for meat weight (with bone weight as covariate) and meat percentage (without and with bone weight as covariate). There was one QTL identified for eye muscle area, bone weight and bone percentage and two QTL mapped for meat-to-bone ratio and silverside weight.

QTL for most of the traits (stifle width, muscularity, meat weight with hot carcass weight and bone weight as covariate, meat percentage, eye muscle area and silverside weight and meat-to-bone ratio) were found on BTA 2 (Table 3.1). QTL for all these traits were found within 8 cM of each other at the centromeric end of the chromosome, apart from the muscularity QTL, which was located at 27 cM. This suggests that the QTL for these traits (apart from muscularity) may be controlled by the same gene. The QTL identified on BTA 3 for meat weight and meat percentage were located at the same location (100cM) as were the QTL on BTA 9 for stifle width and muscularity (104cM).

Table 3.1 QTL identified for muscularity related traits with cohort and breed as fixed effects

Traits	BTA	QTL location (cM)	F-value	Sire effect (±standard error)		
				361	368	398
Stifle width	2	0	4.93	-1.2 (±0.43)	-0.24 (±0.41)	1.45 (±0.59)
	9	104	4.33	-0.52(±0.39)	1.13(±0.37)	-0.59(±0.43)
	13	65	4.22	0.45(±0.39)	0.47(±0.39)	1.6(±0.5)
	18	4	4.66	-0.35(±0.46)	-1.03(±0.43)	1.32(±0.47)
Muscularity	2	27	5.2	-1.65(±1.03)	-0.42(±1.13)	3.65(±1.02)
	4	9	4.32	-0.17(±0.99)	-2.55(±1.02)	3.02(±1.17)
	9	104	4.85	-1.33(±0.9)	2.59(±0.88)	-1.91(0.99)
	11	84	4.17	0.99(±0.97)	0.75(±0.89)	3.31(±1.01)
Hot standard carcass weight	1	87	4.6	-0.4(±7.1)	-20.15(±7.17)	-18.48(±7.55)
	5	41	6.08	2.41(±6.98)	31.54(±8.05)	12.62(±7.72)
	14	36	6.74	-24.09(±6.76)	-15.82(±7.05)	-11.43(±7.01)
	17	85	4.09	-3.44(±7.27)	17.93(±7.04)	-20.96(8.79)
Meat weight with HSCW as covariate	2	6	17.27	-7.37(±1.81)	-8.64(±1.78)	6.9(±2.08)
	3	100	4.06	-5.5(±2.06)	2.23(±1.71)	3.32(±1.82)
	4	37	4.28	-1.89(±1.7)	-4.22(±1.66)	4.03(±1.77)
	17	37	4.87	-5.2(±1.63)	3.43(±1.64)	-0.29(±1.75)
Meat weight with bone weight as covariate	2	8	31.3	-14.82(±4.8)	-21.35(±4.73)	8.83(±5.27)
	8	17	4.19	-5.44(±4.45)	-11.59(±4.12)	-7.61(±4.40)
	17	82	4.74	3.7(±4.44)	14.96(±4.21)	-5.47(±5.55)

Table 3.1 continued - QTL identified for muscularity related traits with cohort and breed as fixed effects

Traits	BTA	QTL location (cM)	F-value	Sire effect (±standard error)		
				361	368	398
Meat percentage	2	6	20.2	-1.9(±0.46)	-2.5(±0.45)	1.82(±0.53)
	3	100	4.12	-1.31(±0.54)	-0.73(±0.44)	-0.91(±0.47)
	17	38	6.07	-1.41(±0.42)	1.12(±0.42)	-0.16(±0.45)
Meat percentage with bone percentage as	2	5	17.31	-1.75(±0.45)	-2.27(±0.45)	1.78(±0.54)
covariate	3	100	4.45	-1.25(±0.52)	-0.78(±0.43)	-0.95(±0.46)
	17	38	5.11	-1.35(±0.41)	0.88(±0.42)	-0.17(0.44)
Eye muscle area	2	2	11.22	-6.07(±1.98)	-7.92(±1.91)	6.54(±2.53)
Meat-to-bone ratio	2	8	9.24	-.021(±0.08)	-0.34(±0.08)	0.19(±0.09)
	17	82	4.42	0.07(±0.08)	0.25(±0.07)	-0.07(±0.09)
Siverside weight	2	4	12.64	-0.61(±0.15)	-0.59(±0.15)	0.45(±0.18)
	17	40	6.16	-0.55(±0.14)	-0.19(±0.14)	-0.02(±0.14)
Bone weight	14	18	10.84	-5.21(±1.23)	-2.69(±1.25)	-3.84(±1.21)
Bone percentage	8	17	4.34	0.46(±0.26)	0.65(±0.25)	0.43(±0.26)

On BTA 17, there were QTL for hot standard carcass weight, meat weight (with bone weight as covariate) and meat-to-bone ratio at 82-85 cM, while another QTL for meat weight (with hot standard carcass weight as covariate), meat percentage and silverside weight was located at 37-40 cM.

The level of significance as indicated by the F-value for all the QTL was mirrored in the size of effect. That is, QTL with higher F-values had a greater size of effect in those sire families that were segregating the trait (Table 3.1).

3.2.2 Effects of *Myostatin* F94L genotype on QTL

The *myostatin* F94L DNA variant is known to significantly impact muscularity traits in the cattle used in the study herein (Sellick *et al.* 2007). Given that this variant is found in Limousin cattle and is segregating in the mapping population herein, the *myostatin* F94L genotype was fitted as fixed effect and the QTL mapped for comparison. The inclusion of the *myostatin* F94L genotype as a fixed effect in the model affected the QTL results for some of the chromosomes. Some significant QTL were no longer significant after the *myostatin* F94L genotype was included in the model, whereas other QTL were not affected (Table 3.2).

As expected since the *myostatin* gene is located at 0 cM on BTA 2, the QTL effects identified on BTA 2 significantly decreased when the *myostatin* F94L genotype was fitted as fixed effect and none of the QTL were no longer significant. This included the muscularity QTL at 28 cM on BTA 2. The size of effect also decreased for all the QTL on BTA 2.

All QTL on BTA 17 were also eliminated by the inclusion of the *myostatin* F94L genotype in the model. Fitting *myostatin* F94L genotype as a fixed effect also affected the QTL found on BTA 4 (for muscularity), 8 (for bone percentage), 9 (for stifle width) and 18 (for stifle width) but not as dramatically. The maximum of the QTL peak for stifle width on BTA 13 shifted from 15cM to significant at 88cM after the *myostatin* F94L genotype was added to the model.

The QTL on BTA 1, 3, 5 and 8 (for meat weight with bone weight as covariate), 9 (for muscularity), 11 and 14 were not affected by the inclusion of the *myostatin* F94L genotype in the model. However, using this new model with the *myostatin* F94L genotype, there were additional QTL identified for muscularity on chromosome 18 and bone weight on chromosome 25.

Table 3.2. QTL results after fitting *myostatin* F94L genotype in the model*

Traits	BTA	QTL location (cM)	F-value	Sire effect (±standard error)		
				361	368	398
Stifle width	2	86	2.8	-0.54(±0.47)	-0.7(±0.44)	0.92(±0.43)
	9	104	3.94	-0.52(±0.37)	1.06(±0.36)	-0.45(±0.42)
	13	88	4.51	0.72(±0.38)	-0.02(±0.38)	1.47(±0.46)
	18	2	5.28	-0.47(±0.44)	-1.04(±0.41)	1.30(±0.45)
Muscularity	2	86	2.47	-1.40(±1.08)	-0.97(±1.05)	2.24(±1.01)
	4	78	1.13	-0.13(±0.97)	-2.08(±1)	2.83(±1.15
	9	104	4.47	-1.37(±0.88)	2.5(±0.86)	2.83(±1.15)
	11	85	4.51	0.83(±0.95)	0.54(±0.87)	3.47(±0.98)
	18	4	4.13	-0.96(±1.06)	-2.81(±1.01)	2.13(±1.08)
Hot standard carcass weight	1	87	4.44	0.17(±7.04)	-19.73(±7.16)	-18.12(±7.48)
	5	42	5.66	1.67(±6.96)	30.75(±7.9)	10.24(±7.75)
	14	34	6.9	23.51(±6.9)	-17.34(±6.93)	-11.77(±7.02)
	17	86	3.66	-4.91(±7.22)	16.64(±6.99)	-19.32(±8.66)
Meat weight with HSCW as covariate	2	29	2.31	-0.85(±1.54)	-4.4(±1.69)	0.74(±1.53)
	3	95	4.62	-2.96(±1.67)	-2.30(±1.44)	-4.43(±1.55)
	4	39	3.49	-1.58(±1.32)	-3.06(±1.31)	2.71(±1.43)
	17	38	2.05	-2.67(±1.35)	2.01(±1.34)	0.31(±1.42)
Meat weight with bone weight as covariate	2	24	2.39	-4.01(±4.54)	-11.39(±4.8)	-3.36(±4.62)
	8	8	4.28	-8.67(±4.09)	-10.88(±3.8)	1.49(±4.18)
	17	82	3.92	3.6(±3.96)	12.25(±3.77)	-3.28(±4.95)

Table 3.2. QTL result after fitting *myostatin* F94L genotype in the model* (continued)

Traits	BTA	QTL location (cM)	F-value	Sire effect (±standard error)		
				361	368	398
Meat percentage	2	24	2.72	-0.11(±0.41)	-1.25(±0.44)	0.08(±0.42)
	3	98	4.92	-0.63(±0.43)	-0.73(0.36)	-1.13(±0.39)
	17	39	2.92	-0.75(±0.35)	0.70(±0.34)	-0.01(±0.36)
Meat percentage with bone percentage as covariate	2	23	2.44	-0.05(±0.42)	-1.18(±0.44)	-0.09(±0.42)
	3	98	5.13	-0.64(±0.43)	-0.75(±0.36)	-1.15(±0.39)
	17	39	2.65	-0.76(±0.34)	0.62(±0.35)	-0.02(±0.36)
Eye muscle area	2	104	1.39	3.29(±1.70)	-1.12(±1.62)	-0.16(±1.76)
Meat-to-bone ratio	2	26	1.44	-0.06(±0.08)	-0.07(±0.08)	-0.005(±0.07)
	17	82	3.63	0.06(±0.07)	0.21(±0.07)	-0.04(±0.09)
Siverside weight	2	48	1.72	-0.20(±0.13)	-0.21(±0.12)	0.09(±0.17)
	17	40	3.81	-0.38(±0.12)	0.13(±0.12)	0.06(±0.12)
Bone weight	14	17	10.7	-5.23(±1.23)	-2.49(±1.27)	-3.84(±1.2)
	25	21	4.01	2.39(±1.28)	-1.05(±1.06)	3.47(±1.26)
Bone percentage	8	15	3.91	0.55(±0.26)	0.63(±0.25)	0.23(±0.27)

*Shaded QTL substantially altered by the inclusion of the *myostatin* F94L genotype in the model

3.2.3 Candidate gene selection

QTL for muscularity related carcass traits were identified on BTA 2, 3, 4, 5, 8, 9, 11, 14, 17 and 18. However, the QTL found on BTA 2, 3 and 17 represented the most traits of interest. The QTL identified on BTA 2 were associated with *myostatin,* a gene known to have a major role on muscle development (Figures 3.1 and 3.2). It is not clear whether there is another QTL on BTA 2 for muscularity located at 27 cM. This QTL was also no longer significant with the inclusion of the myostatin F94L genotype in the model (Figure 3.3). This indicates that either the *myostatin* F94L variant is the quantitative trait nucleotide (QTN) for the muscularity QTL at 27 cM or there is another gene(s) controlling muscularity on BTA 2 that may be epistatic with *myostatin*.

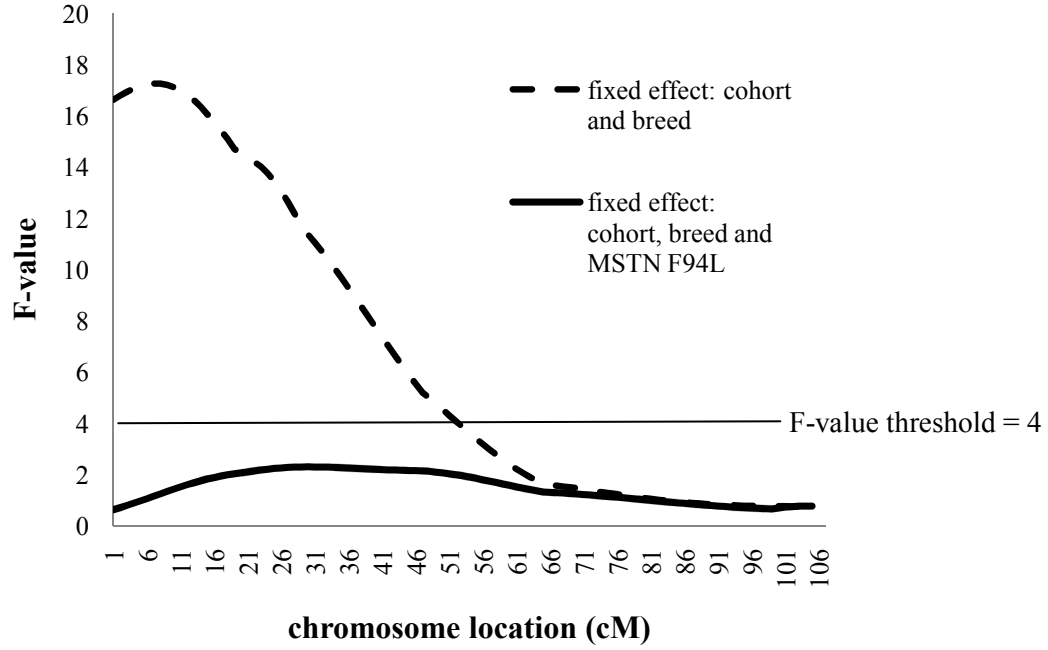


Figure 3.1 QTL for meat weight (with HSCW as covariate) on BTA 2, with and without the inclusion of MSTN F94L genotype

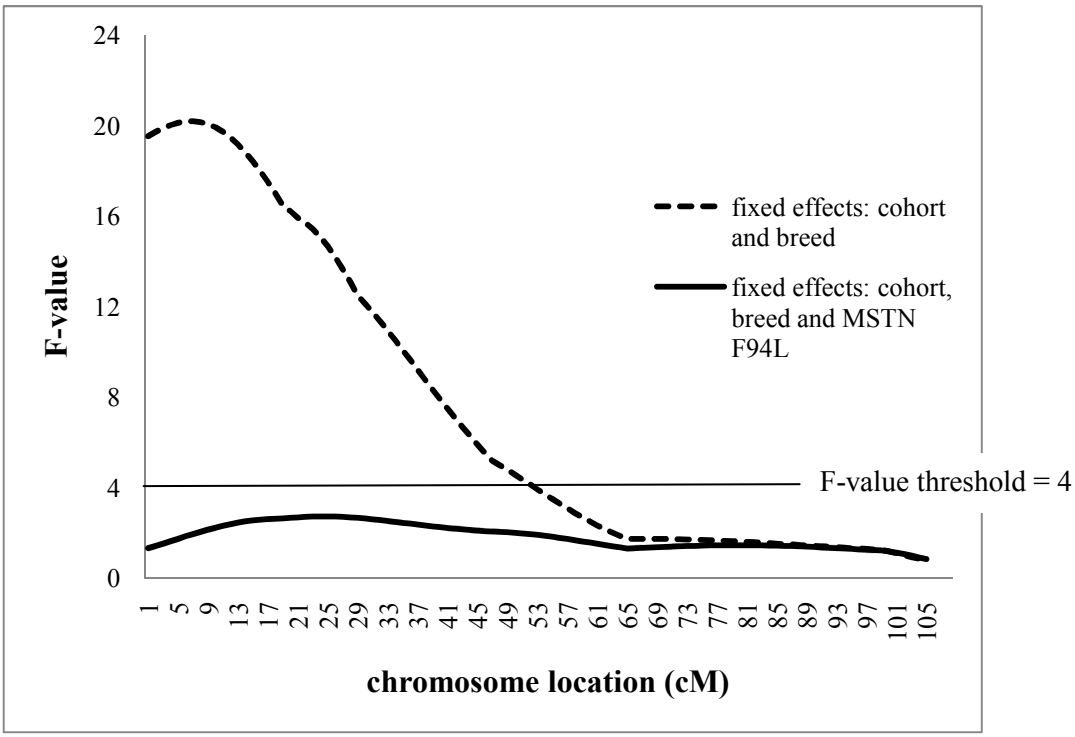


Figure 3.2 QTL for meat percentage on BTA 2, with and without the inclusion of MSTN F94L genotype

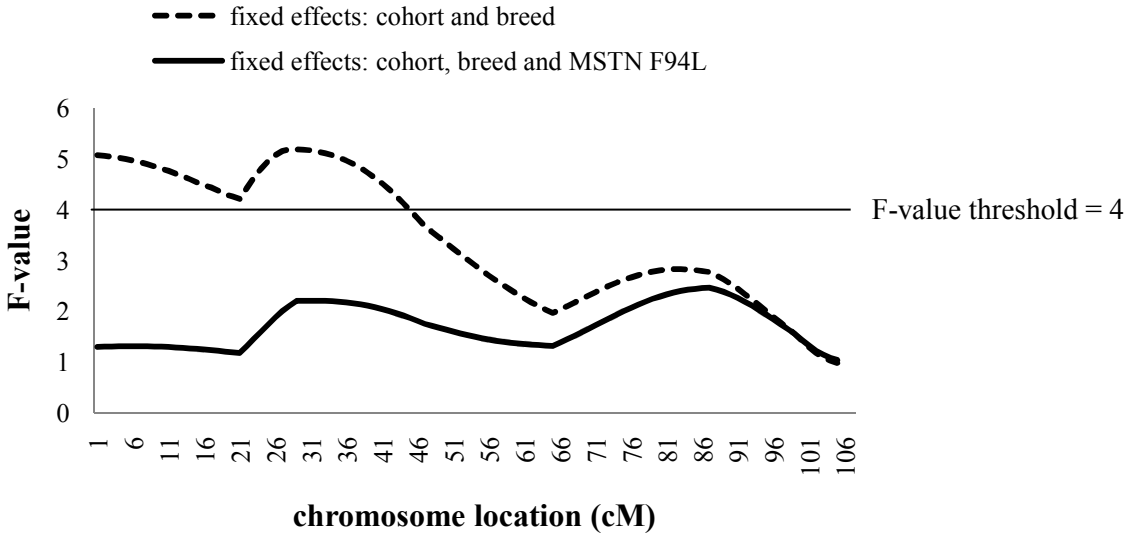


Figure 3.3 QTL for muscularity on BTA 2, with and without the inclusion of MSTN F94L inclusion

For BTA 17, the QTL for muscularity related carcass traits, such as meat weight (with HSCW as covariate) and meat percentage, also no longer significant with the inclusion of the *myostatin* F94L genotype in the model (Figures 3.4 and 3.5). Again there may be genes on BTA 17 that are acting epistatically with *myostatin.*

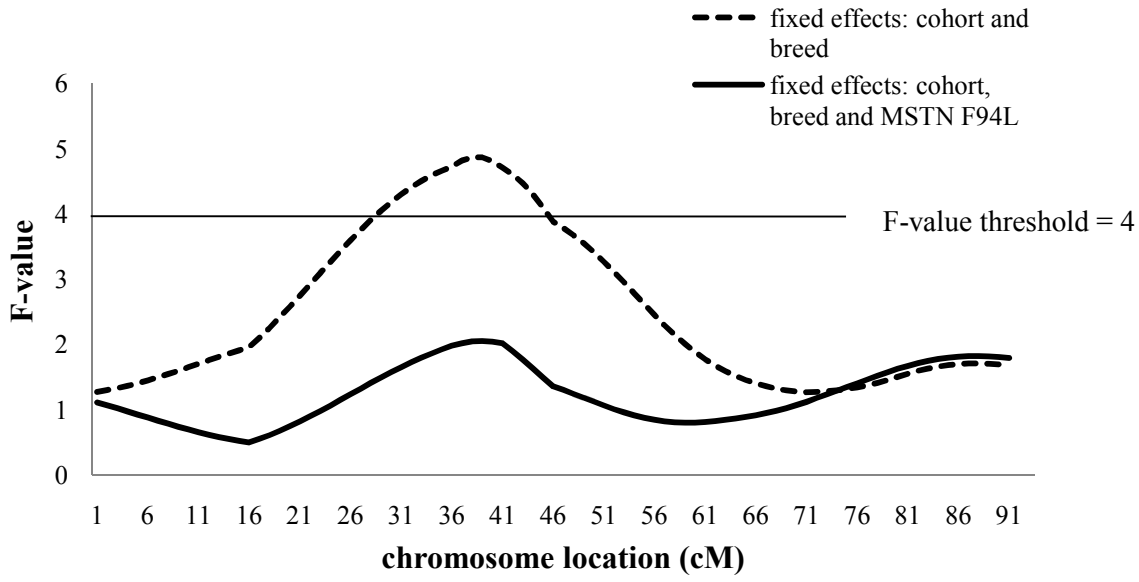


Figure 3.4 QTL for meat weight (with HSCW as covariate) on BTA 17, with and without the inclusion of MSTN F94L genotype

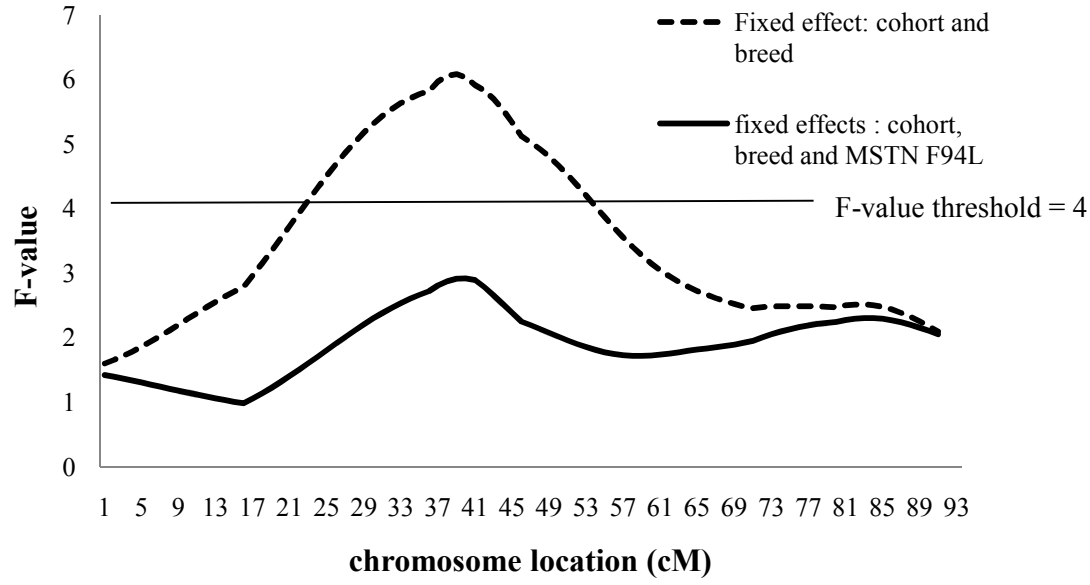


Figure 3.5 QTL for meat percentage on BTA 17, with and without the inclusion of MSTN F94L genotype

QTL on BTA 3 were significant for meat weight (with hot carcass weight as covariate) and meat percentage with or without the *myostatin* F94L genotype fitted as a fixed effect (Figures 3.6 and 3.7). This result indicates that gene(s) affecting muscularity might be located on this chromosome but are unlikely to interact with *myostatin.*

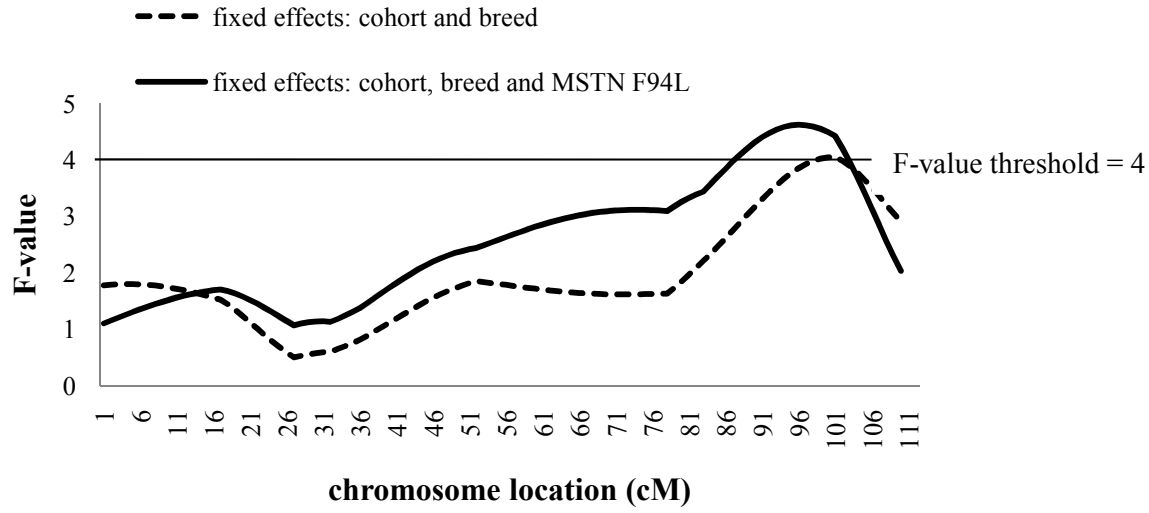


Figure 3.6 QTL for meat weight (with HSCW as covariate) on BTA 3, with and without the inclusion of MSTN F94L genotype

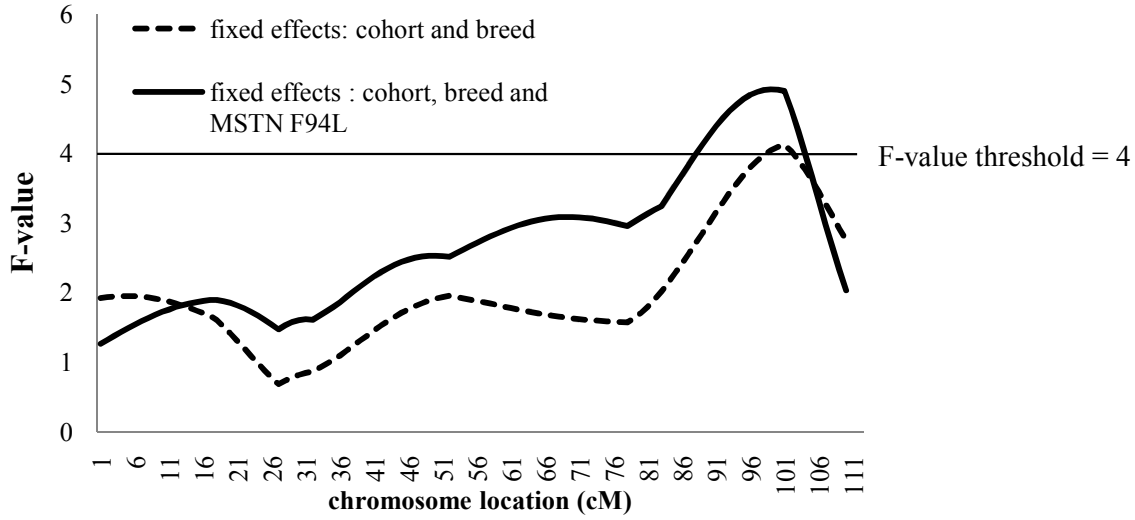


Figure 3.7 QTL for meat percentage on BTA 3, with and without the inclusion of MSTN F94L genotype

The relative positions (in cM) of the QTL on these three chromosomes (BTA 2, 3 and 17) were located using microsatellite markers (Table 3.3). The identified markers were utilised to convert the QTL relative position from centiMorgans (cM) to base pairs (bp) using the bovine human comparative map database (http://www.animalgenome.org/cattle/maps/RHMap3/). That is, the markers were used to locate the equivalent position of these QTL in the human genome sequence in addition to their position in the bovine genome sequence.

Table 3.3 Relative position and markers for identified QTL on BTA 2, 3 and 17

BTA	Traits	Relative position (cM)	Markers
2	Stifle width	0	ILSTS26
2	Muscularity	27	OARHH30-TGLA377
2	MeatWt with HSCW as covariate	6	ILST26 - TEXAN2
2	MeatWt with BoneWt as covariate	8	ILST26 - TEXAN2
2	Meat%	6	ILST26 - TEXAN2
2	Meat% with bone% as covariate	5	ILST26 - TEXAN2
2	EMA	2	ILST26 - TEXAN2
2	Mttobn	8	ILST26 - TEXAN2
2	Silverside weight	4	ILST26 - TEXAN2
3	MeatWt with HSCW as covariate	100	BMS896 – BMC4214
3	Meat%	100	BMS896 – BMC4214
3	Meat% with bone% as covariate	100	BMS896 – BMC4214
17	HSCW	85	BL50 – BM1862
17	MeatWt with HSCW as covariate	37	BM941 – OARFCB48
17	MeatWt with boneWt as covariate	82	BL50 – BM1862
17	Meat%	38	BM941 – OARFCB48
17	Meat% with bone% as covariate	38	BM941 – OARFCB48
17	Mttobn	82	BL50 – BM1862
17	Silverside weight	40	BM941 – OARFCB48

MeatWt= meat weight; HSCW= hot standard carcass weight; BoneWt= bone weight, Mttobn= meat-to-bone ratio; EMA= eye muscle area

Based on the position of the QTL in base pairs, all the genes that reside in the region were determined using the Ensembl data base (www.ensembl.org). There were several potential candidate genes residing within these QTL regions but only three genes were selected for further study. They were the *activin receptor type 1* (*ACVR1*), *smad nuclear interacting protein 1* (*SNIP1*) and similar to *follistatin-like 5* (*FSTL5*) (Table 3.4). These were selected based on the function of these genes in muscle development and their association with *myostatin*. The *follistatin-like 5* gene sequence was compared to the human sequence to confirm it was homologous.

Table 3.4 Candidate gene list

Genes	BTA	Position (bp)
Activin receptor type 1 (*ACVR1*)	2	39,926,385-40,003,195
Smad nuclear interacting protein (*SNIP1*)	3	115,537,805-115,552,855
Follistatin-like 5 (*FSTL5*)	17	33,809,229-34,543,747

Apart from the candidate genes identified from the QTL results, there were several other candidate genes that were selected based on QTL mapping result from the previous studies using. These included *transforming growth factor β receptor 3* (*TGFBR3*) on chromosome 3 at 50 cM (Koshkoih 2006), *insulin like growth factor 1* (*IGF1*) on chromosome 5 (Sellick 2002), *follistatin* (*FST*) on chromosome 20 (Naik 2007). Again these were selected because they may affect muscle growth and may be epistatic or interact with *myostatin.*

3.3 Discussion

3.3.1 QTL for muscularity related traits

With the exception of hot standard carcass weight, all of the traits of interest had significant QTL on BTA 2 with peaks at 2-8 cM, except for muscularity which was located at 27 cM. The significant QTL at 2-8 cM suggested that most of the traits may be controlled by the same gene(s). QTL on BTA 2 at the same location using the same mapping herd had been previously identified with effects on meat percentage, eye muscle area and silverside percentage (Sellick *et al.* 2007). It was shown that the F94L *myostatin* genotype underlies these QTL (Sellick *et al.* 2007), which was confirmed herein (chapter 3.2.2). Moreover, based on information from the cattle QTL database (http://www.animalgenome.org/cgi-bin/QTLdb), QTL on BTA 2 at the same location have been identified for most of the meat traits, including meat percentage, eye muscle area and carcass weight, demonstrating that *myostatin* is a pleiotropic gene.

Some overlapping QTL were detected herein. The QTL identified on BTA 3 for meat weight and meat percentage were located at the same location (100 cM) as were the QTL on BTA 9 for stifle width and muscularity as measured by the ratio of stifle to hip width (104 cM). These results suggest that same gene(s) on BTA 3 may affect meat weight and meat percentage and the same gene(s) on BTA 9 may affect stifle width and muscularity.

QTL for hot standard carcass weight and meat percentage have been identified in other studies and some correspond with the QTL of this study. The QTL for hot standard carcass weight identified on BTA 14 at 34-36 cM is in the same region as

previously reported at 29-42 cM (Mizoshita *et al.* 2004) and 26-48 cM (Takasuga *et al.* 2007) for Japanese Black cattle. Moreover, a study by Kim *et al.* (2003) also detected a QTL for hot carcass weight on chromosome 5 at 75 cM. This QTL is relatively close to QTL found at 87 cM. For meat percentage, the QTL found on chromosome 3 at 100 cM corresponds with a previous study by Casas *et al.* (2001) who found a QTL for retail product yield on the same chromosome although it was 30 cM from the region identified herein. However, given the limited resolution of the mapping, this may represent the same QTL.

The QTL mapping results for eye muscle area and silverside weight (excluding the QTL on BTA 2) did not correspond to any previous studies (http://www.animalgenome.org/cgi-bin/QTLdb). There have been no studies reporting QTL for either stifle width or meat-to-bone ratio. It is not surprising that not all the QTL mapping results have not been replicated. Differences in QTL mapping can arise from differences in the method of analysis, model and/or cattle breed. For example, the animals used herein were included in a larger analysis with progeny from New Zealand. In that study, Morris *et al.(2009)* used another model in a combined sire analysis that included both Australian and New Zealand sires to map QTL and the QTL results often varied from those herein. For example, the same QTL were found on BTA 2 but not on BTA 17. Moreover, the QTL results changed if eye muscle area and silverside weight were analysed using hot standard carcass weight as the covariate (Table 3.1).

3.3.2 Effects of *myostatin* F94L genotype on QTL

It was demonstrated that by fitting the *myostatin* F94L genotype in the model, some QTL were no longer significant, in particular, those on BTA 2 (Figures 3.1 and 3.2). The lack of QTL on BTA 2 after fitting *myostatin* in the model indicates that the F94L variant is likely to be the quantitative trait nucleotide (QTN) and it accounts for most of the variation in carcass muscularity traits from BTA2.

Interestingly, the QTL for muscularity was at 27 cM and was not found at the same region as *myostatin* (at 0 cM). However, this QTL was no longer significant; the F value decreasing from 5.20 to 2.47, after the *myostatin* F94L genotype was included in the model (Figure 3.3). This finding provides indirect support that the *myostatin* F94L variant may be the functional quantitative trait nucleotide (QTN) for this muscularity QTL at 27 cM. The mapping resolution in the cattle population utilised herein is relatively low because of the restricted number of informative meioses. Therefore, the QTL peaks are quite broad and the possibility that the QTL at 0 cM and at 28 cM on BTA 2 represent the same gene cannot be ruled out. However, an alternative explanation for the results would be that there is another gene(s) controlling muscularity on BTA 2 which is epistatic with *myostatin*.

The test statistics (F value) for other QTL on BTA 8, 9 and 17 also decreased slightly when *myostatin* was fitted in the model. The QTL on chromosome 4 for muscularity decreased more dramatically. A similar observation was made for the QTL on chromosome 17. Two QTL regions were found at 37-40 cM for meat weight and meat percentage and at 82-85 cM for hot standard carcass weight, meat weight with bone weight as covariate and meat-to-bone ratio. The F values for QTL

identified at 37-40 cM (particularly for meat percentage) decreased greatly compared to the QTL at 82-85 cM when *myostatin* was included in the model. The hypothesis from this result was there may be gene(s) on chromosome 17 that are acting epistatically with myostatin.

An alternative hypothesis to epistasis could be that there is co-linearity between the genotypes on BTA 2 and BTA 17. Thus, a chi-square test was conducted to test for independence of the genotypes. The frequency of the cattle that carried the same allele combination on OARFCB48 (the nearest marker to the QTL found on BTA 17) and the *myostatin* F94L genotype was determined (Table 3.5) as well as frequency of the cattle that carried the same sire allele and the *myostatin* F94L genotype (Table 3.6). The chi-square test showed that the probability of obtaining the expected distribution were 0.59 (for *OARFCB48* allele combination) and 0.99 (for sire allele). These results suggest that the *myostatin* allele and the marker alleles are independent so that the QTL disappearance on BTA 17 is likely to be a consequence of an epistatic effect with *myostatin,* not co-llinarity.

Table 3.5 Frequency of cattle carrying each *myostatin* F94L genotype and *OARFCB48* allele combinations. [Note: A=Limousin DNA variant allele]

Allele	**MSTN genotype**			
combinations	**CC**	**AC**	**AA**	**Total**
11	11	22	2	**35**
12	42	55	24	**121**
13	17	28	8	**53**
22	32	46	13	**91**
23	9	33	5	**47**
Total	**111**	**184**	**52**	**347**

Table 3.6 Frequency of cattle carrying each *mysotatin* F94L genotype and *OARFCB48* sire allele

Sire allele	MSTN genotype			
	CC	AC	AA	Total
1	28	50	10	**88**
2	41	79	18	**138**
3	42	55	24	**121**
Total	**111**	**184**	**52**	**347**

There were two new QTL detected for muscularity (on chromosome 18) and bone weight (on chromosome 25) after fitting *myostatin* F94L in the model. This is presumably because inclusion of the *myostatin* F94L genotype in the model explained some of the residual variation, thereby increasing the power to detect other QTL.

3.3.3 Candidate gene selection

Using the results from QTL mapping herein and previous work (Sellick 2002 ;Koshkoih 2006; Naik 2007), candidate genes were selected based on their potential function in muscle development. They were *activin type 1 receptor* (*ACVR1*), *smad nuclear interacting protein 1* (*SNIP1*), *transforming growth factor β receptor III* (*TGFBR3*), *similar to follistatin-like 5* (*FSTL5*), *insulin like growth factor 1* (*IGF1*), and *follistatin* (*FST*).

ACVR1 is located on the same chromosome as *myostatin* (BTA 2) but is at 39 cM. This gene was selected because there was a QTL identified for muscularity located at 27 cM on BTA 2 but it is not clear whether this is a separate QTL or an epistatic QTL with *myostatin* (see above section 3.3.2). ACVR1 (also called ALK2) is a type

1 receptor for transforming growth factor β (TGF-β) family members and it is essential for the TGF-β signalling pathway (Massague 1998). TGF-β itself plays an important role in development, particularly for cell proliferation for all tissues including skeletal muscle (Massague 1998). The type 1 receptor for myostatin has not been yet identified but ACVR1 is likely to be the type 1 receptor for myostatin (Dominique 2006).

TGFBR3 is a candidate gene identified on chromosome 3. TGFBR3 is also known as betaglycan. Similar to ACVR1, this protein is part of the TGF-β pathway. The binding of TGF-β members to their type II receptors is facilitated by this protein (Massague 1998).

SNIP1 (located on chromosome 3) was selected because it is also involved in TGF-β pathway. SNIP1 has been demonstrated to control the TGF-β signalling pathway by its interaction with the Smad proteins (Kim *et al.* 2000). Smad proteins have an important role in facilitating the signal transduction of the TGF-β family members from membrane to nucleus and in regulating the consequent changes in gene expression (Schmierer and Hill 2007).

A gene similar to *FSTL5* was found on chromosome 17 using the bovine genome sequence database. Studies have not reported the function of FSTL5. However, two follistatin family members, FSTL3 (follistatin like 3/follistatin related gene) and FST (follistatin) are acknowledged for their contribution in the myostatin pathway (Dominique and Gérard 2006). They inhibit myostatin from binding to its receptor. Follistatin is also known to have role on muscle growth (Amthor *et al.* 1996; Amthor

et al. 2002). Deficiency of follistatin in mice can cause muscle decrease (Matzuk *et al.* 1995). Presumably, these effects of follistatin are through its role of inhibiting myostatin. Therefore, it is suggested that FSTL5 may have similar effect on skeletal muscle.

IGF1 was the only candidate gene that did not involve on the TGF-β pathway. *IGF1* is a member of the insulin-like growth factor family (IGFs). IGFs are growth factors that are known to have prominent roles in growth. However, *IGF1* also stimulates myoblast differentiation and mediates muscle hyperthrophy (Oksbjerg *et al.* 2004; Barton 2006) and therefore, was another obvious candidate gene for muscularity.

3.4 Summary

Four QTL were identified for stifle width, muscularity, hot standard carcass weight and meat weight (with hot standard carcass weight as covariate) , 3 QTL were found for meat weight (with bone weight as covariate) and meat percentage, 2 QTL were mapped for meat-to-bone ratio and silverside weight and 1 QTL for eye muscle area. In terms of the traits that best define muscularity, the QTL on chromosomes 2, 3 and 17 are of greatest interest. When the *myostatin* F94L genotype was fitted in the model, the QTL on BTA 2 and 17 were no longer significant. The *myostatin* F94L genotype is the likely QTN for the QTL on BTA 2. However, the QTL on BTA 17 are likely to be epistatic with *myostatin*, thus explaining the disappearance of the QTL. From these QTL and previous studies, candidate genes were selected based on their potential function in muscle development and/or their possible roles in the *myostatin* pathway.

CHAPTER 4

SNP Association Studies

4.1 Introduction

Only a few QTL have been mapped for muscularity related carcass traits and as a result, only a small number of genomic regions and genes controlling muscularity (but not related to growth) have been identified. *Myostatin* is a gene known to have a major effect on skeletal muscle mass by inhibiting muscle overgrowth (McPheron *et.al*. 1997). *Myostatin* deletions cause double muscling in cattle, but other variants, such as the *myostatin* F94L, of this gene that also affect muscle mass (Dominique and Gérard 2006). The m*yostatin* F94L variant is commonly found in Limousin cattle. This variant has been shown to affect eye muscle area, silverside percentage, meat percentage and hot standard carcass weight in the animals used for QTL mapping herein (Sellick *et al.* 2007). There are many other proteins involved in the *myostatin* regulatory pathway of muscle development (McPherron *et al.* 1997; Hill *et al.* 2002; Hill *et al.* 2003; Lee 2004; Dominique and Gerard 2006) so it is hypothesised that there will be genes which are epistatic with *myostatin* and affect muscularity.

In order to identify additional candidate genes controlling muscle development and that may possibly interact with *myostatin*, the QTL results for muscularity were used (Chapter 3) to choose three candidate genes (Table 3.6) for further study. Three additional candidate genes were also selected based the previous studies (Selllick ; Koshkoih 2006; Naik 2007). The DNA variants of these genes were analysed to study their possible association with the muscularity traits of interest and their potential relationship with *myostatin*.

4.2 Results and discussion

4.2.1 Candidate gene polymorphism identification

In order to study the association between the candidate genes and the traits of interest, polymorphisms in each gene were found. The polymorphisms were identified by sequencing of the genomic DNA of the gene mapping sires. DNA variants discovered included single nucleotide polymorphisms (SNP) and insertion/deletions (in/del). Any DNA variants that changed the amino acid composition or occurred within the untranslated regions or splice junctions may be considered to be potentially functional. In total, there were 43 DNA variants found in the candidate genes including 10 in/dels and 33 SNPs (Appendix 13).

There were 14 SNPs found in *ACVR1* but all of them were located in intronic regions (Table 4.1). Three DNA variants were found in *SNIP1* including 1 insertion/deletion and 2 SNPs. The insertion/deletion was identified in the promoter region, one SNP was located in an intron, while the other SNP was located in the 3' untranslated region. *TGFBR3* had 8 DNA variants; they included 3 SNPs and 5 in/dels. One SNP was a potential functional SNP causing the substitution of glutamine^{640} by arginine. The other SNPs were in introns and the 3' untranslated region. Four of the identified in/dels were located in introns and one was located in 3' untranslated region. For the *FSTL5* gene, 16 DNA variants were detected. Four were in/dels and the others were SNPs. One SNP in exon 2 was a potential functional SNP as it replaces leucine^{2} with phenylalanine.

Table 4.1 DNA variants of the candidate genes

Gene	Total # DNA variants	Types of DNA variants	Potentially functional DNA variants
ACVR1	14	14 SNPs (intronic)	-
SNIP1	3	1 in/del (promoter)	-
		1 SNP (intronic)	
		1 SNP (3'UTR)	
TGFBR3	8	5 in/del (intronic)	1 SNP in exon 12
		1 SNP (exonic)	(glutamine^{640} → arginine)
		1 SNP (intronic)	
		1 SNP (3'UTR)	
FSTL5	16	4 in/del (intronic)	1 SNP in exon 2
		10 SNPs (intronic)	(leucine^{2} → phenylalanine)
		1 SNP (exonic)	
		1 SNP (3'flanking region)	

The identification of the DNA variants for *IGF1* and *FST* and genotyping of the backcross progeny were completed in a previous study (Sellick 2002; Naik 2007). These SNPs were located in introns, except for the SNP1 of *IGF1* located in the 5' untranslated region (Table 4.2).

Table 4.2 Genotyped DNA variants for *IGF1 and FST*

SNP name	DNA variant	Location	Sire genotype		
			361	368	398
IGF1 SNP1	CT	5'UTR	CT	CC	TT
IGF1 SNP2	CT	intron 4	CC	CC	CT
FST SNP5	AG	intron 3	AA	AA	AG
FST SNP6	AG	intron 4	GG	AG	GG

4.2.2 SNP association analysis

After the polymorphisms were identified in the candidate genes, they were verified in the grandparents. This assisted the formation of the sire haplotypes and the selection of the DNA variants for genotyping (Table 4.3).

Table 4.3 Genotyped DNA variants for *ACVR1, SNIP1, TGFBR3 and FSTL5*

SNP name	DNA variant	Location	Sire genotype		
			361	368	398
ACVR1 SNP6	TC	intron 5	TC	TT	TT
ACVR1 SNP7	CA	intron 5	CA	CA	CA
SNIP1 SNP2	AG	intron 3	GG	GA	GA
SNIP1 SNP3	CT	3'UTR	CC	CT	CC
FSTL5 SNP2	CT (leucine^{2}→phenylalanine)	exon 2	CC	CC	CT
FSTL5 SNP5	AG	intron 3	AA	AG	AA
FSTL5 SNP8	GA	intron 6	GA	GA	GA
FSTL5 SNP14	CT	exon15	CT	CC	CC
TGFBR3 SNP5	AG SNP (glutamine^{640}→arginine)	exon 12	AG	AA	AG
TGFBR3 SNP6	AG SNP	3'UTR	AG	AG	AG

After genotyping the selected SNPs in the backcross progeny using high resolution melt, the associations between the candidate gene SNPs and the traits of interest were analysed using unbalanced analysis of variance (Table 4.3).

Most of the SNPs had no significant effects on traits of interest (Table 4.4). However, there were significant effects found for the *FST*L 5 SNP5 on meat weight with hot standard carcass weight as covariate and meat-to-bone ratio. It should be noted, however, that the *FSTL5* SNP5 was found to have a low genotype frequency for cattle carrying the GG genotype (only 7 cattle).

The *FSTL* 5 SNP8 had a significant effect on meat percentage and eye muscle area. Hot standard carcass weight was associated with *IGF1* SNP1. *FST* SNP7 had more associations with the traits of interest than the other candidate gene SNPs. It had significant effects on meat weight (with hot carcass weight as covariate), meat percentage (with and without bone percentage as covariate), eye muscle area and silverside weight.

Any SNP that was associated with the traits of interest was also analysed to determine whether the alleles had additive and/or dominance effects (Table 4.5). SNP5 in *FSTL5* had no significant additive and dominance effects. However, the A allele of the *FSTL5* SNP8 had a negative additive effect on meat weight (with hot standard carcass weight as covariate), meat percentage and eye muscle area (Table 4.5).

Positive additive effects for the A allele of *FST* SNP7 were found for meat weight (with hot carcass weight as covariate), meat percentage, eye muscle area and silverside weight. Moreover, there were negative dominance effects for all of these traits as well. For the *IGF*1 SNP1, there was a significant dominance effect for hot standard carcass weight (Table 4.5).

Table 4.4 Association of candidate gene SNPs with muscularity traits

SNP name	F probability of the traits									
	HSCW	**MtWt[a]**	**MtWt[b]**	**Mt%**	**Mt%[a]**	**EMA**	**SilversideWt**	**Mttobn**	**Mus**	**StifWdth**
ACVR1 SNP6	0.22	0.68	0.92	0.58	0.56	0.76	0.30	0.79	0.59	0.39
ACVR1 SNP7	0.65	0.6	0.93	0.40	0.42	0.99	0.68	0.93	0.86	0.53
SNIP1 SNP2	0.50	0.70	0.75	0.70	0.64	0.44	0.43	0.70	0.19	0.15
SNIP1 SNP3	0.36	0.38	0.69	0.43	0.37	0.87	0.23	0.90	0.19	0.13
FSTL5 SNP2	0.67	0.82	0.62	0.76	0.65	0.82	0.83	0.73	0.10	0.18
FSTL5 SNP5	0.24	0.47	**0.04***	0.14	0.23	0.12	0.32	**0.02***	0.78	0.71
FSTL5 SNP8	0.30	0.16	0.06	**0.04***	0.07	**0.03***	0.53	0.06	0.91	0.95
FSTL5 SNP14	0.69	0.59	0.72	0.54	0.57	0.21	0.11	0.71	0.95	0.92
TGFBR3 SNP5	0.57	0.51	0.69	0.46	0.51	0.37	0.61	0.73	0.52	0.54
TGFBR3 SNP6	0.16	0.66	0.76	0.72	0.7	0.78	0.21	0.68	0.87	0.89
IGF1 SNP1	**0.01***	0.50	0.19	0.31	0.35	0.8	0.24	0.33	0.85	0.85
IGF1 SNP2	0.11	0.75	0.52	0.78	0.67	0.13	0.99	0.80	0.95	0.78
FST SNP5	0.71	0.87	0.67	0.96	0.92	0.89	0.86	0.53	0.96	0.88
FST SNP7	0.82	**<0.001*****	0.42	**<0.001*****	**<0.001*****	**0.001****	**0.01***	0.66	0.18	0.18

HSCW= hot standard carcass weight, MtWt[a]= meat weight with HSCW as covariate, MtWt[b]= meat weight with bone weight as covariate, Mt%= meat percentage, Mt%[c]= meat percentage with bone percentage as covariate, EMA= eye muscle area with HSCW as covariate, SilversideWt= silverside weight with HSCW as covariate, Mttobn= meat-to-bone ratio, Mus= muscularity as measured by the stifle-to-hip width ratio, StifWdth= stifle width with hip width as covariate *** P<0.001; **P<0.01; *P<0.05.Boldface= effects with F values < 0.05

An analysis using a linear mixed model (*Genstat*) (Model 7) was performed to determine how much of the total phenotypic variance could be accounted by the significant SNPs (Appendix 16). Most of the analysed SNPs accounted for less than 10% of the phenotypic variance (Table 4.5). However, *FST* SNP7 accounted for 34%, 34%, 35%, 20% and 19% of the phenotypic variance for meat weight (with HSCW as covariate), meat percentage, meat percentage (with bone percentage as covariate), eye muscle area and silverside weight, respectively.

4.5 Additive and dominance effects of significant SNPs

SNP name	Traits	Estimate parameter±(S.E)		
		Additive	Dominance	V_{SNP} (%)
***FSTL5* SNP5**	Meat Wt (with HSCW as covariate)	0.28±1.43^{ns}	1.40±1.56^{ns}	0.08%
	Meat to bone ratio	-0.03±0.07^{ns}	0.1±0.08^{ns}	2.20%
***FSTL5* SNP8**	Meat Wt (with bone wt as covariate)	-3.15±1.57*	3.12±2.15^{ns}	0.45%
	Meat %	-0.29±0.14*	0.33±0.2^{ns}	0.95%
	Eye muscle area	-1.8±0.69**	0.97±0.95^{ns}	2.16%
***IGF1* SNP1**	HSCW	4.86±3.56^{ns}	11.15±4.06**	4.65%
***FST* SNP7**	Meat Wt (with HSCW as covariate)	8.13±1.94***	-7.22±2.07***	34.17%
	Meat %	2.08±0.5***	-1.96±0.54***	33.70%
	Meat % (with bone% as covariate)	2.09±0.5***	-1.95±0.53***	35.48%
	Eye muscle area	5.18±2.55*	-9.07±2.72***	19.73%
	Silverside weight	0.54±0.18**	-0.44±0.19*	19.27%

*** P<0.001; **P<0.01; *P<0.05; ns: not significant, S.E=standard error

4.2.2.1 Effects of *FSTL5* SNP5

The effects of the significant SNPs were examined further in terms of the specific genotypes. The *FSTL5* SNP5 had a significant effect on meat weight (with bone weight as covariate) and meat-to-bone ratio (Table 4.4). Cattle that carried the AG and GG genotypes were found to have the heaviest meat weight (with bone weight as

covariate) with a mean of 235.9 kg and 234.9 kg, respectively, while the AA genotype cattle had the lowest meat weight (228.6 kg) (Figure 4.1).

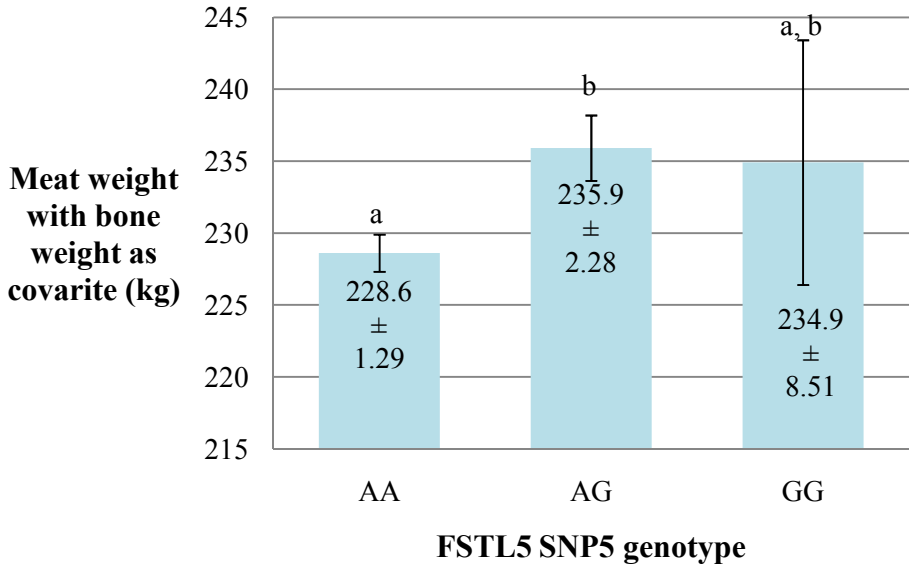


Figure 4.1 Effect of *FSTL5* SNP5 genotype on meat weight (with bone weight as covariate)

The same trend also occurred for the meat-to-bone ratio; the animals with either the AG or GG genotypes also had a higher meat-to-bone ratio (4 and 3.95, respectively) compared to the AA genotype animals (Figure 4.2). The AA genotype cattle were statistically different from the heterozygous AG cattle but not from the homozygous GG cattle for both meat weight and meat-to-bone ratio. However, when the data were analysed formally to estimate additive or dominance effects, there were no significant additive or dominance effects. This presumably occurred because the accuracy of the estimates relies upon a sufficient number of animals with each genotype and there were only 7 cattle with the GG genotype (Appendix 15). As a result, the standard error was high for the data from the GG genotype animals. However, given the significant difference between AA and AG cattle, the effects of this polymorphism should be studied in other larger populations.

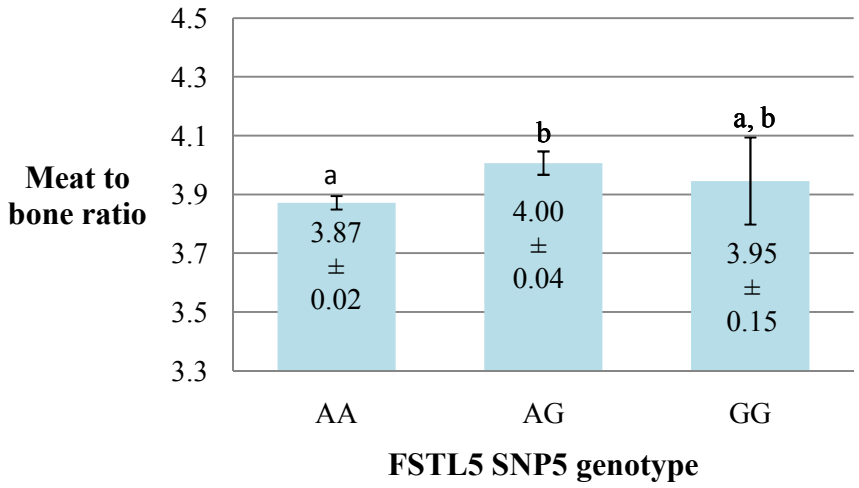


Figure 4.2 Effect of *FSTL5* SNP5 genotype on meat-to-bone ratio

In total, the *FSTL* SNP5 explained 0.08% and 2.2% of the phenotype variation of meat weight (with HSCW as covariate) and meat-to-bone ratio, respectively (Table 4.5). This result was not unexpected since the *myostatin* F94L variant was also included in the model and it explains more than 50% of the genetic variance for these traits (Appendix 16).

4.2.2.2 Effects of *FSTL5* SNP8

The *FSTL5* SNP8 had a significant effect on meat percentage and eye muscle area. The heterozygous animals (AG) and homozygous AA animals had the highest meat percentage (68.77%±0.14 and 68.73%±0.18, respectively). In contrast, the GG homozygous animals had the lowest meat percentage (68.18%±0.22) (Figure 4.3).

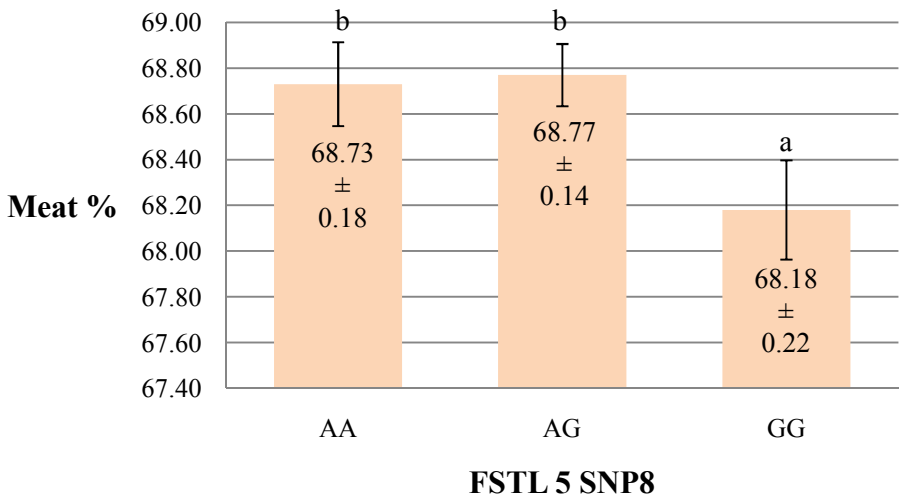


Figure 4.3 Effect of *FSTL5* SNP8 genotype on meat percentage

GG homozygous cattle for this SNP also had the smallest eye muscle area compared to homozygous cattle (AA) and heterozygous cattle (AG) (Figure 4.4). This result indicates that G allele for this SNP has a negative effect on eye muscle area, which was confirmed by the additive effect results from the general linear regression analysis. It was observed that the additive effect for the G allele was negative (1.80 cm^2 ± 0.69). The same results were observed for the effect of this SNP on meat percentage; the G allele had a negative additive effect on meat percentage. The dominance effect was in opposite direction; the dominance effect for this SNP was found to be positive for meat percentage and eye muscle area. This implies that cattle that carried A allele (can be AA or AG genotype) will be expected to have a higher meat percentage and larger eye muscle area compared to cattle that only carry the G allele.

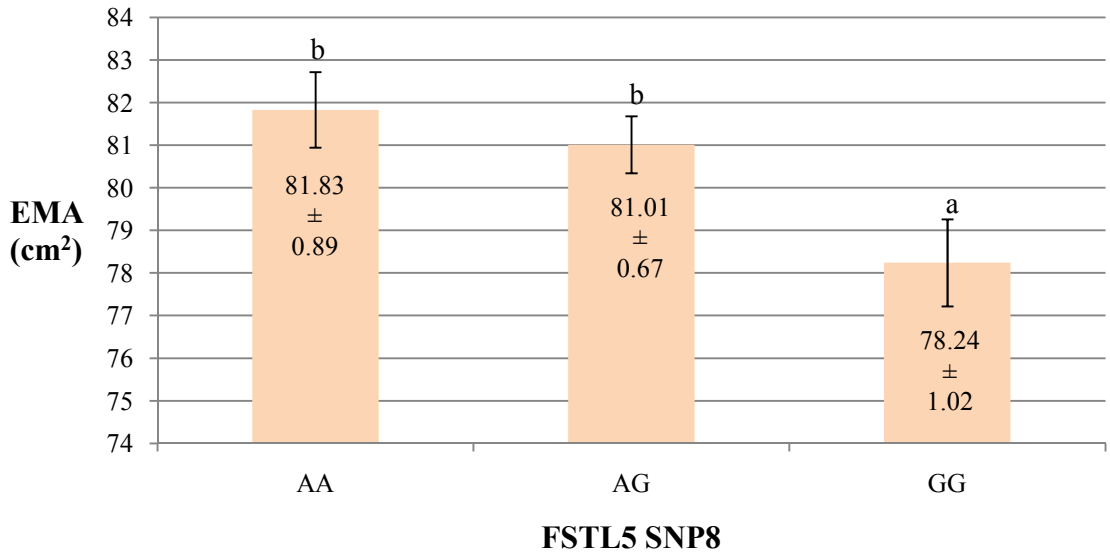


Figure 4.4 Effect of *FSTL5* SNP8 genotype on eye muscle area

The *FSTL5* SNP8 accounted for 0.95% and 2.16% of the phenotype variance for meat percentage and eye muscle area, respectively (Table 4.5). These percentages are the total percentage of the *FSTL5* SNP8 accounting for the phenotype variance (meat percentage and eye muscle area) and include the genetic variance from the additive and dominance effects of this SNP. As with the *FSTL5* SNP5, the *myostatin* F94L variant also accounted for most of the phenotype variation for meat percentage (66.32%) and for eye muscle area (40.11%) (Appendix. 16).

4.3.2.3 Effect of *IGF1* SNP1

The *IGF1* SNP1 was associated with hot standard carcass weight (HSCW) and accounted for 4.65% of the phenotypic variance. The heterozygous CT cattle and the homozygous TT cattle had the highest HSCW and the homozygous CC animals had the lowest HSCW (321.8 kg) (Figure 4.5).

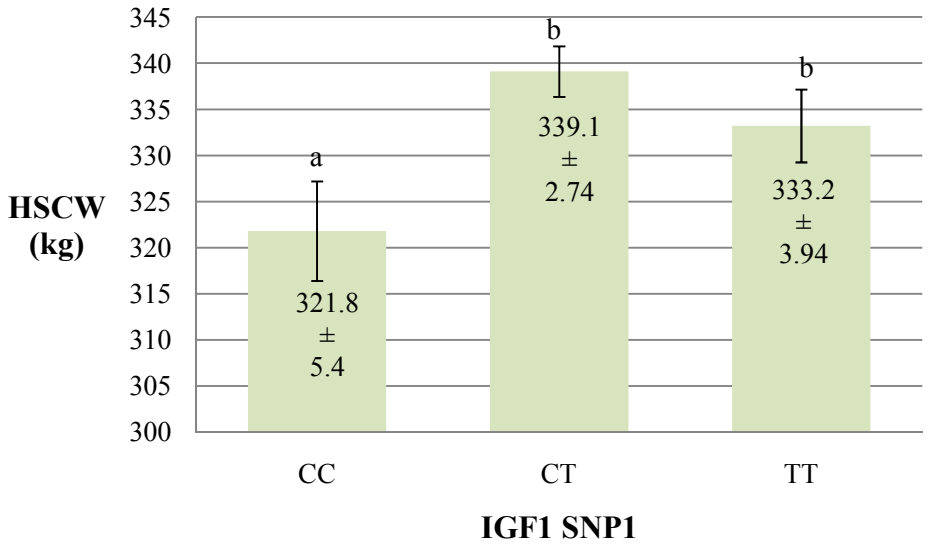


Figure 4.5 Effect of *IGF1* SNP1 genotype on hot standard carcass weight (HSCW)

Thus, the T allele for this SNP is responsible for the increased hot standard carcass weight in the cattle. The *IGF1* SNP1 had a positive dominance effect of 11.15 kg ±4.06 for hot carcass weight but it had no significant additive effect. There were few studies on the association between *IGF1* polymorphisms and beef carcass traits. A polymorphism in the 5' flanking region (*Sna*BI) was found at 512 bp before the first exon in Angus cattle which alters thymine to cytosine (Ge *et al.* 2001). The polymorphism is relatively close to the *IGF1* SNP1 in the study herein which is also located in the 5' flanking region at 313 bp before exon 1 and causes a substitution of thymine by cytosine. The *IGF1/Sna*BI polymorphism has been reported to be associated with hot standard carcass weight, cold carcass weight and lean weights of valuable cuts (Curi *et al.* 2005 and Siadkowska 2006). Both of these studies showed that the heterozygous cattle had the heaviest hot standard carcass weight, cold carcass weight and lean weights of valuable cuts. These results correspond with observations herein although the polymorphisms differ.

4.2.2.3 Effects of *FST* SNP7

The *FST* SNP7 was associated with more traits than the other SNPs. It was associated with meat weight (with hot carcass weight as covariate), meat percentage, meat percentage (with bone weight as covariate), eye muscle area and silverside weight. Cattle carrying the AA homozygous genotype tended to have more meat weight, meat percentage, eye muscle area and silverside weight compared to GG homozygous and AG heterozygous cattle (Figure 4.6, 4.7, 4.8, 4.9 and 4.10). There was no difference observed for meat weight, meat percentage, eye muscle area and silverside weight between the GG genotype and AG genotype cattle. The standard error for AA genotype was relatively high as a result of the limited number of cattle with the AA genotype (only 3 cattle). Three cattle had the AA genotype, 66 cattle had the AG genotype and 288 cattle had the GG genotype (Appendix 15). Clearly, the "A" allele frequency in the dams must have been low to result in so few homozygous AA progeny.

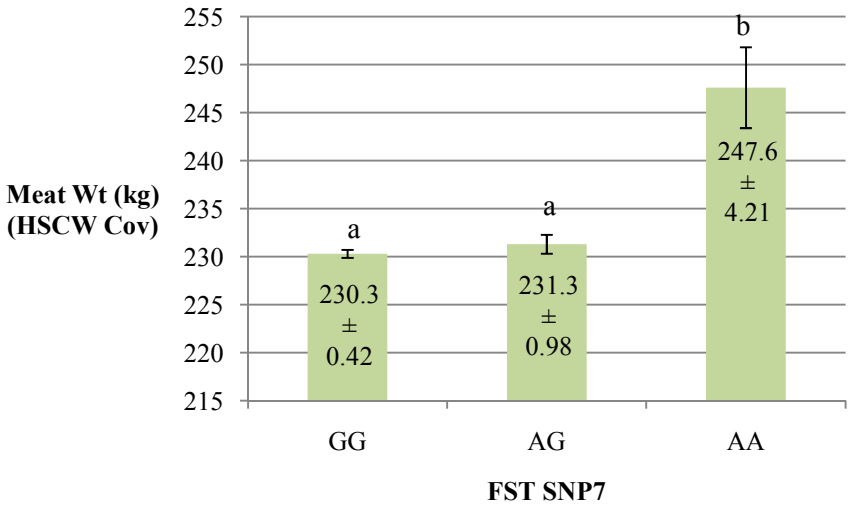


Figure 4.6 Effect of *FST* SNP7 genotype on meat weight (with HSCW as covariate)

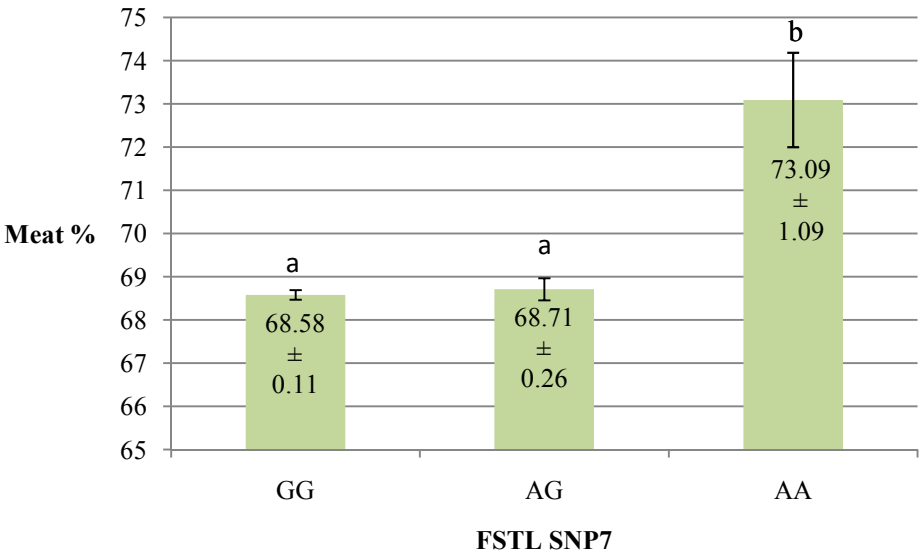


Figure 4.7 Effect of *FST* SNP7 genotype on meat percentage

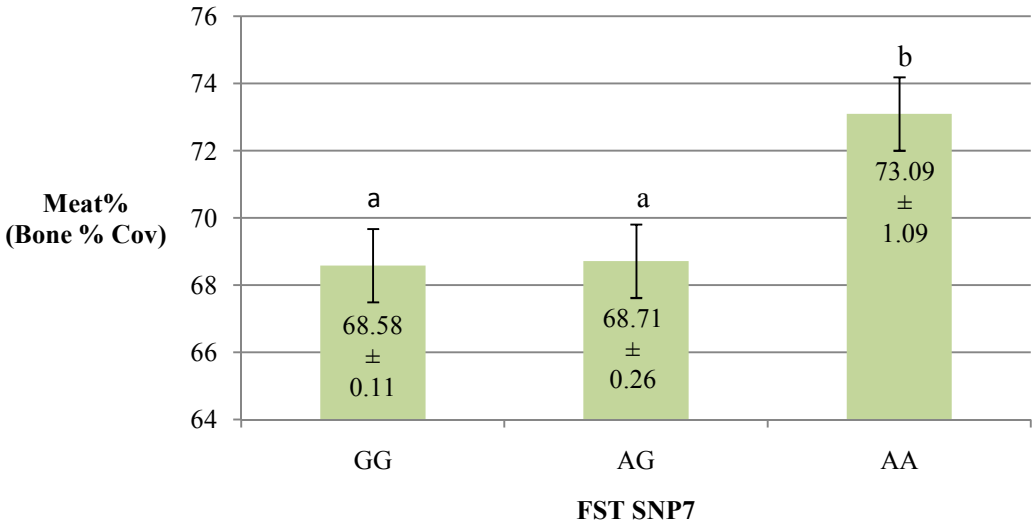


Figure 4.8 Effect of *FST* SNP7 genotype on meat percentage (with bone percentage as a covariate)

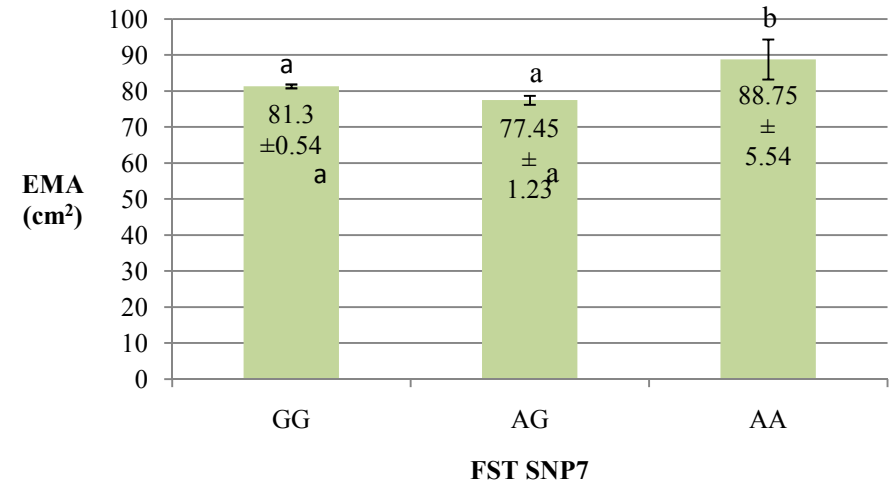


Figure 4.9 Effect of *FST* SNP7 genotype on eye muscle area

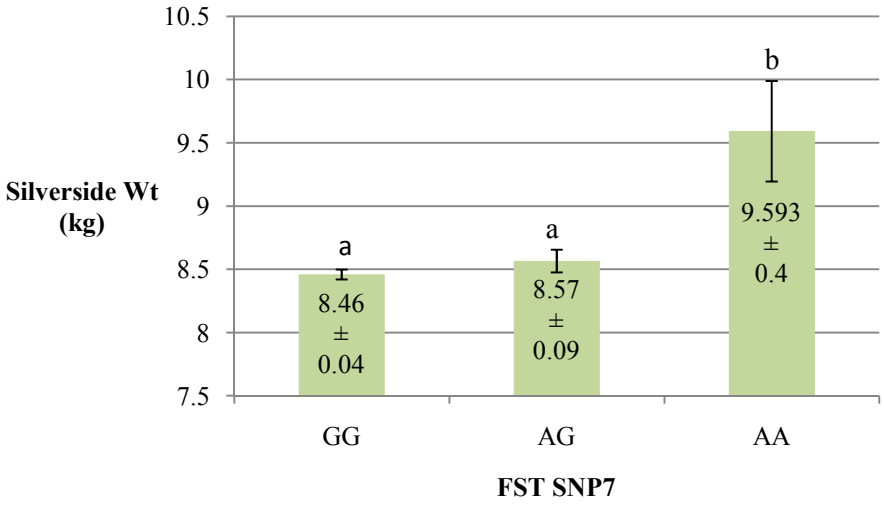


Figure 4.10 Effect of *FST* SNP7 genotype on silverside weight

The general linear regression analysis indicated that there were negative dominance effects for all of these traits. The G allele for this SNP appears to be the dominant allele, whereas A allele is recessive (Figure 4.6 – 4.10). Cattle carrying one or two G alleles had less meat weight, meat percentage, eye muscle area and silverside weight compared to those carrying two copies of the A allele, indicating that A allele is recessive or partially recessive. The additive effects were positive for the A allele.

By calculating the genotype frequency for each breed, it was determined that the Limousin backcross progeny had a higher A allele frequency (about 8% higher) compared to the Jersey backcross progeny. Furthermore, this SNP explains a large proportion of the phenotypic variance (differ only 8-27% with the *myostatin* variance) (Table 4.6). These results suggest that the *FST* is another gene with major effects on muscularity besides *myostatin.*

However, there are no studies reporting associations between *follistatin* polymorphisms and carcass traits. Several studies have examined the function of this gene either *in vivo* or *in vitro* (Matzuk et al. 1995; Amthor et al. 1996; Amthor et al. 2002). These studies have shown that *FST* is expressed in chick embryos in the somites of developing muscle (Amthor *et al.* 1996). Moreover, mice with *FST* deletions have decreased muscle development (Matzuk *et al.* 1995).

Table 4.6 *FST* SNP7 and *myostatin* F94L variance

Traits	**V_P[a]**	**V_{MSTN}[b] (%)**	**$V_{FST\ SNP7}$[c] (%)**
Meat weight (with HSCW as covariate)	202.21	45.25	34.17
Meat%	13.59	45.70	33.70
Meat % (with bone % as covariate)	13.11	43.44	35.48
Eye muscle area	152.24	32.30	19.73
Silverside weight	1.06	45.80	19.27

[a] phenotype variance, [b] *myostatin* variance, [c] FST SNP7 variance

In order to identify the proportion of additive, dominance and interaction variance accounted from the total SNP variance, an analysis using a linear mixed model was conducted by fitting additive, dominance and interactions between the *FST* SNP7 and *myostatin* as random effects. It was observed that for meat weight (with HSCW as covariate), meat percentage, eye muscle area and silverside weight, both additive and dominance effects explained the *FST* SNP7 variance (Table 4.7). However, interactions or epistatic effects only accounted for 0.11% of the total SNP variance for meat weight (with HSCW as covariate). In general, the additive effects accounted for more of the total SNP variance compared to the dominance effects. For eye muscle area, the dominance effect variance was bigger than the additive effect variance and this result confirms the additive and dominance analysis using general linear regression (Table 4.5). Considering that the additive effect of this SNP accounted for about 23% of the meat weight and meat percentage variance, this SNP may be a potential marker for the selection of cattle for higher meat production.

Table 4.7 Additive, dominance and epistatic effects with of *FST* SNP7

Trait	V_{Add}[a](%)	V_{Dom}[b](%)	V_I[c](%)
Meat Wt (HSCW as covariate)	23.51	18.43	0.11
Meat%	22.96	18.97	0.00
Meat% (bone% as covariate)	23.92	20.07	0.00
EMA	6.44	26.79	0.00
Silverside weight	13.59	5.88	0.00

[a] additive effect variance, [b] dominance effect variance, [c] interaction/epistasis variance

4.2.3 SNP interactions with the *myostatin* F94L variant

There were significant effects found for the interaction between *mysotatin* and some of the identified SNPs (Table 4.8). SNP3 of the *SNIP1* gene and the *myostatin* F94L variant were observed to have a significant epistatic effect on eye muscle area. SNP6 for *TGFBR3* found to have a significant interaction with the *myostatin* F94L variant on meat weight (with HSCW as covariate) and eye muscle area. Both SNPs of *IGF1* had a significant interaction with *myostatin* F94L variant on silverside weight (for SNP1), HSCW, meat weight (with bone weight as covariate) and meat-to-bone ratio (for SNP2). *FST* SNP7 interaction with *myostatin* significantly affected muscularity (measured as the ratio of stifle width to hip width).

Table 4.8 Test of significance of interactions between *myostatin* F94L genotype and candidate gene SNP genotypes

SNPs	F probability of the traits									
	HSCW	**MtWt[a]**	**MtWt[b]**	**Mt%**	**Mt%[c]**	**EMA**	**SilversideWt**	**Mttobn**	**Mus**	**StifWdth**
MSTN×*ACVR1* SNP6	0.82	0.94	0.82	0.70	0.66	0.50	0.53	0.90	0.54	0.85
MSTN×*ACVR1* SNP7	0.06	0.81	0.65	0.82	0.79	0.40	0.98	0.17	0.07	0.12
MSTN×*SNIP1* SNP2	0.54	0.99	0.62	0.99	0.99	0.48	0.30	0.61	0.94	0.87
MSTN×*SNIP1* SNP3	0.12	0.07	0.17	0.16	0.20	**0.02***	0.15	0.47	0.53	0.76
MSTN×*FSTL5* SNP2	0.73	0.22	0.44	0.32	0.29	0.22	0.55	0.42	0.19	0.21
MSTN×*FSTL5* SNP5	0.10	0.05	0.67	0.24	0.20	0.65	0.69	0.74	0.50	0.40
MSTN×*FSTL5* SNP8	0.92	0.76	0.63	0.73	0.77	0.30	0.75	0.50	0.95	0.97
MSTN×*FSTL5* SNP14	0.56	0.54	0.53	0.75	0.63	0.11	0.70	0.42	0.52	0.41
MSTN×*TGFBR3* SNP5	0.58	0.76	0.32	0.92	0.85	0.60	0.88	0.11	0.15	0.39
MSTN×*TGFBR3* SNP6	0.40	**0.04***	0.70	0.12	0.10	**0.04***	0.19	0.67	0.57	0.93
MSTN×*IGF1* SNP1	0.73	0.29	0.95	0.26	0.23	0.6	**0.04***	0.94	0.81	0.83
MSTN×*IGF1* SNP2	**0.02***	0.17	**0.004****	0.37	0.56	0.55	0.33	**0.02***	0.23	0.22
MSTN×*FST* SNP5	0.45	0.34	0.80	0.35	0.34	0.22	0.18	0.89	0.14	0.13
MSTN×*FST* SNP7	0.74	0.37	0.49	0.61	0.43	0.49	0.91	0.34	**0.03***	0.10

HSCW= hot standard carcass weight, MtWt[a]= meat weight with HSCW as covariate, MtWt[b]= meat weight with bone weight as covariate, Mt%= meat percentage, Mt%[c]= meat percentage with bone percentage as covariate, EMA= eye muscle area with HSCW as covariate, SilversideWt= silverside weight with HSCW as covariate, Mttobn= meat-to-bone ratio, Mus= muscularity as measured by the ratio of stifle to hip width, StifWdth= stifle width with hip width as covariate, BnWt=bone weight. *** P<0.001; **P<0.01; *P<0.05

4.2.3.1 Interactions between *myostatin* F94L and *SNIP1* SNP3, *TGFBR3* SNP6 and *IGF1* SNP1

SNP3 of the *SNIP1* gene interaction with *myostatin* F94L significantly affected eye muscle area. In general, cattle that carried homozygous variant allele for *myostatin* F94L (AA) had the largest eye muscle area compared to the CC homozygous and AC heterozygous cattle. However, cattle that carried the AA genotype for *myostatin* F94L and the TT homozygous genotype for the *SNIP1* SNP3 had the largest eye muscle area (107.03 cm^2 ±6.24) amongst the backcross progeny (Figure 4.11). It should be noted that there were only two TT homozygous cattle that had the AA homozygous genotype for *myostatin* F94L, however, and as a result, the standard error was large (6.24) (Appendix 15).

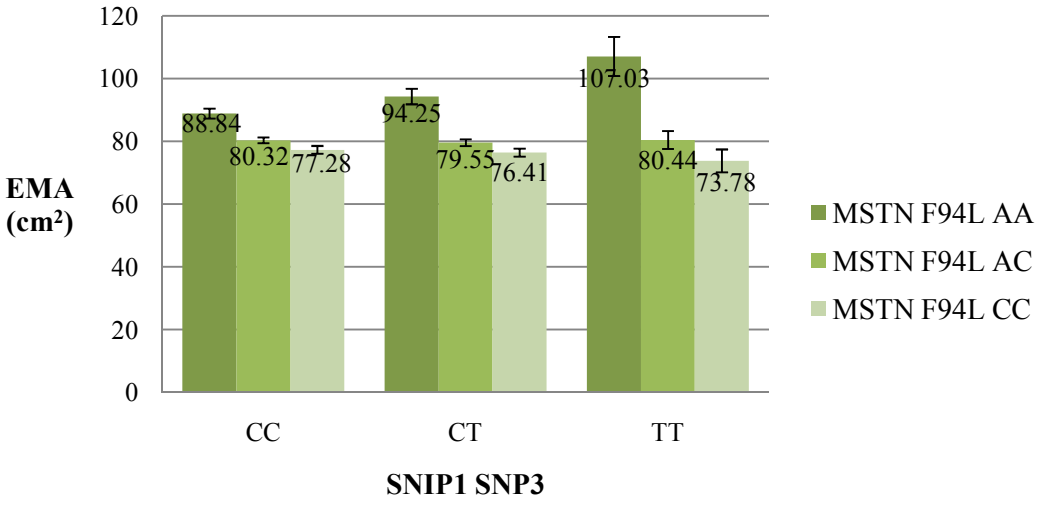


Figure 4.11 Effect of interaction between *myostatin*F94L and *SNIP1* SNP3 on eye muscle area

For *TGFBR3* SNP6, its interaction with *myostatin* F94L significantly affected meat weight (with HSCW as covariate) and eye muscle area (EMA). GG homozygous cattle for the *TGFBR3* SNP6 that also carried the homozygous (AA) variant for the

myostatin F94L had the heaviest meat weight (249.6±2.28 kg) and the largest eye muscle area (96.49 cm^2 ±2.61). Similar to the effect of the interaction between *SNIP1* SNP3 and *myostatin* F94L on eye muscle area, heterozygous and homozygous *TGFBR3* SNP6 cattle that also had the variant homozygous genotype (AA) for the *myostatin F94L* always had the heaviest meat weight and largest eye muscle area.

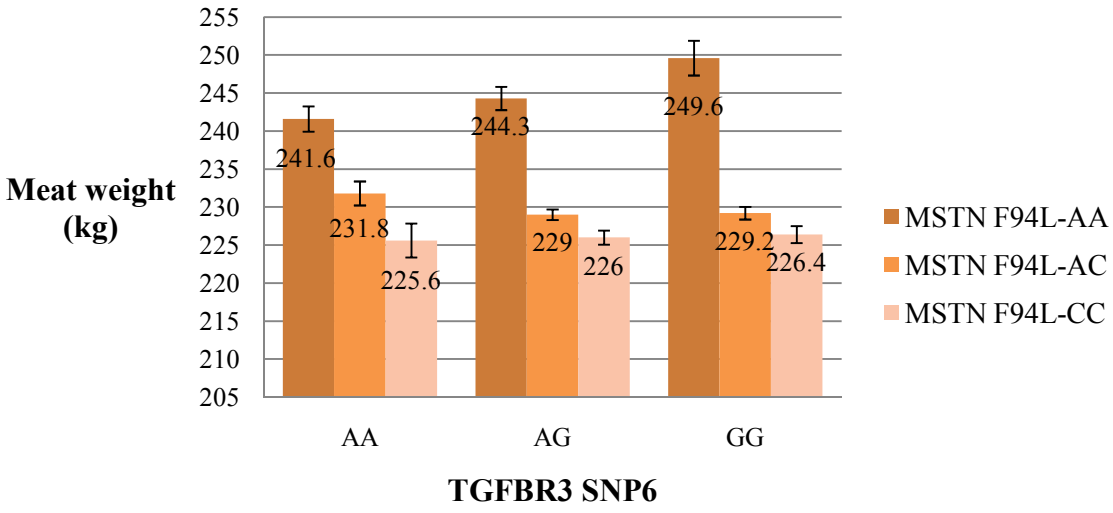


Figure 4.12 Effect of interaction between *myostatin* F94L and *TGFBR3* SNP6 on meat weight (with HSCW as covariate)

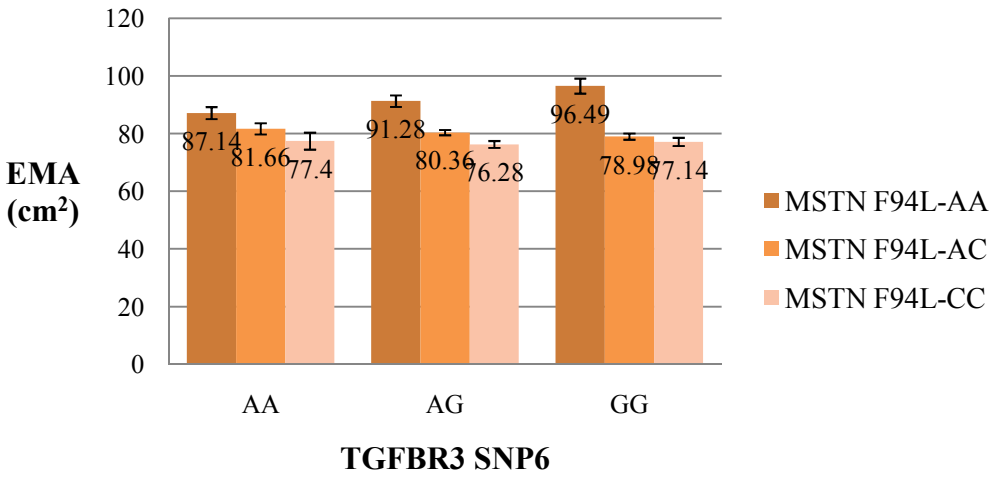


Figure 4.13 Effect of interaction between *myostatin* F94L and *TGFBR3* SNP6 on eye muscle area (with HSCW as covariate)

The *IGF1* SNP1 interaction with the *myostatin* F94L genotype also had a significant effect on silverside weight. The silverside weight of all cattle with the AA genotype for *myostatin* F94L were significantly different from the cattle carrying the AC and CC genotypes of the *myostatin* F94L. There was no significant variation in the silverside weights of the cattle that carried the AA *myostatin* F94L genotypes and the different *IGF1* SNP1 genotypes. The mean silverside weight of the CC homozygous cattle for *IGF1* SNP1 with AA myostatin genotype was 9.43±0.17 kg; CT heterozygous with AA homozygous *myostatin* genotype had a weight of 9.48±0.14 kg and TT genotype with AA *myostatin* genotype cattle had a mean weight of weight 9.40±0.16 kg. However, there was a significant difference between the silverside weights of the AC *myostatin* F94L genotype cattle that carried the CC genotype versus the CT genotype for the *IGF1* SNP1. A significant difference was also found for CC *myostatin* F94L genotype cattle with the CC genotype versus the TT genotype for *IGF1* SNP1. For CC *myostatin* homozygous cattle, the CT *IGF1* SNP1 cattle statistically had lower silverside weights than those with the TT genotype.

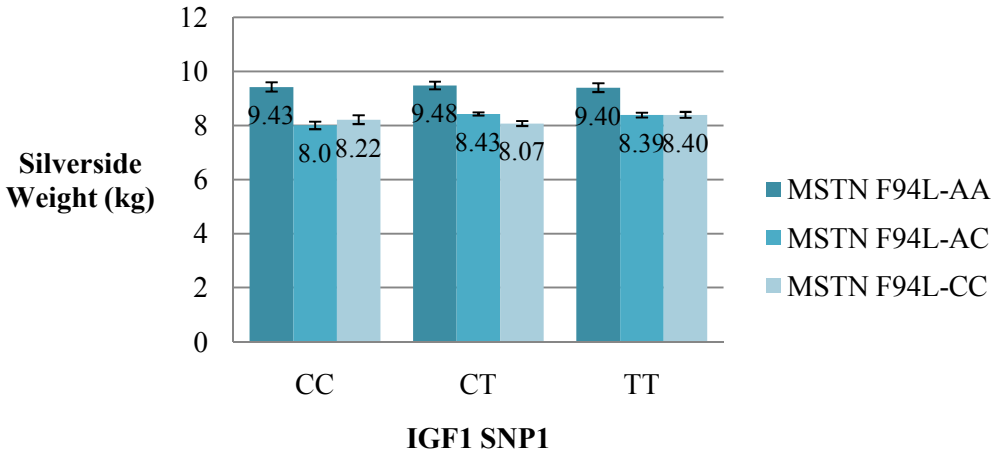


Figure 4.14 Effect of interaction between *myostatin* F94L and *IGF1* SNP1 on silverside weight

In general, for *SNIP1* SNP1, *TGFBR3* SNP6 and *IGF1* SNP1, the combination of the AA *myostatin* homozygous cattle with any genotype for these SNPs had a higher eye muscle area, meat weight and silverside weight compared to the AC and CC *myostatin* genotype cattle. This was expected as the A allele was shown in these cattle to be responsible for an increase of eye muscle area, meat percentage and silverside percentage and is partially recessive (Sellick *et al.* 2007)

4.2.3.2 Interaction between *myostatin* F94L and *IGF1* SNP2 and *FST* SNP7

Unlike the effect of the interactions between the *myostatin* F94L and the other SNPs, cattle that carried the combination of homozygous AA genotype for *myostatin* F94L and the homozygous TT genotype of *IGF1* SNP2 did not have the greatest hot standard carcass weight, meat weight (with bone weight as covariate) and meat-to-bone ratio (Figure 4.15, 4.16 and 4.17). The TT homozygous cattle for *IGF1* SNP2 that also carried m*yostatin* F94L AA homozygous had less hot standard carcass weight, meat weight and meat to bone ratio than those carried the AC genotype for the *myostatin* F94L. However, there were no significant differences between cattle carrying the combination of the AA *myostatin* genotype and the CC *IGF1* SNP2 and cattle that had the CT *IGF1* SNP2 genotype.

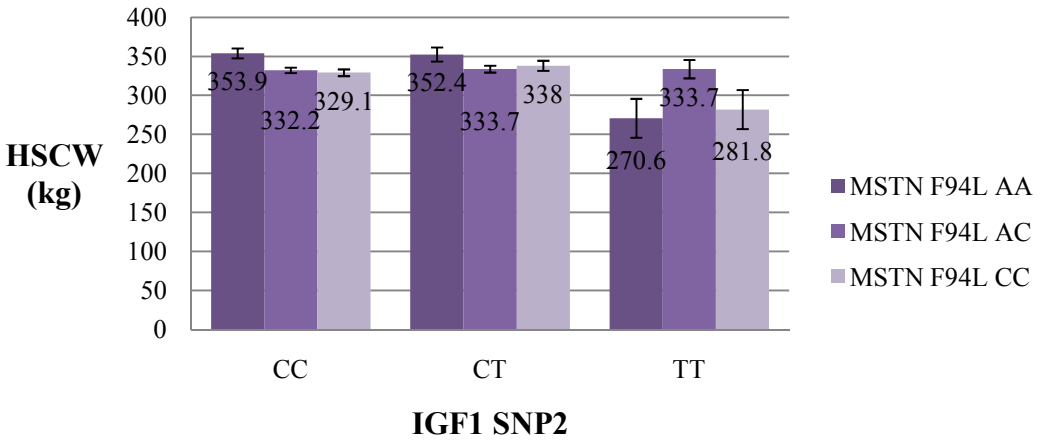


Figure 4.15 Effect of interaction between *myostatin* F94L and *IGF1* SNP2 on hot standard carcass weight (HSCW)

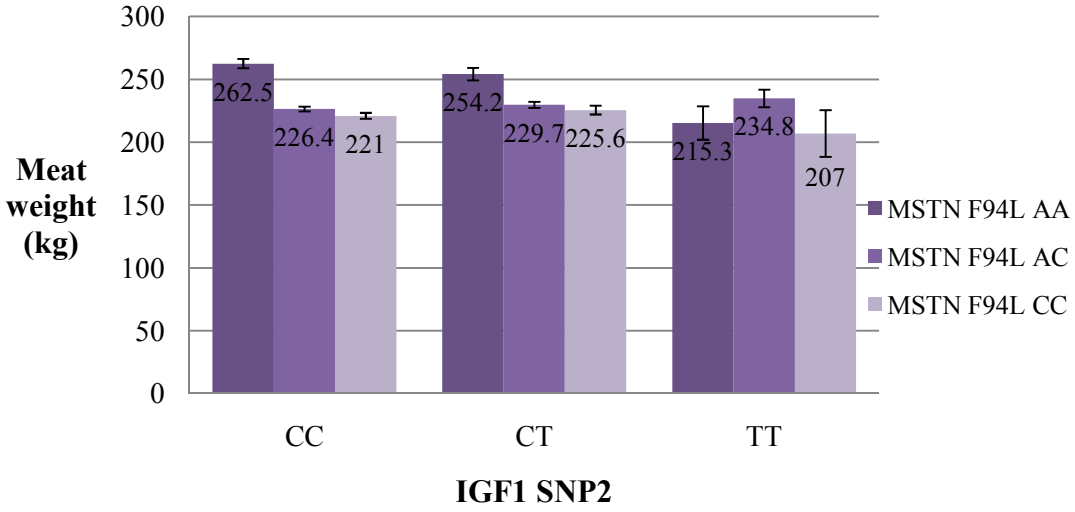


Figure 4.16 Effect of interaction between *myostatin* F94L and *IGF1* SNP2 on meat weight (with bone weight as covariate)

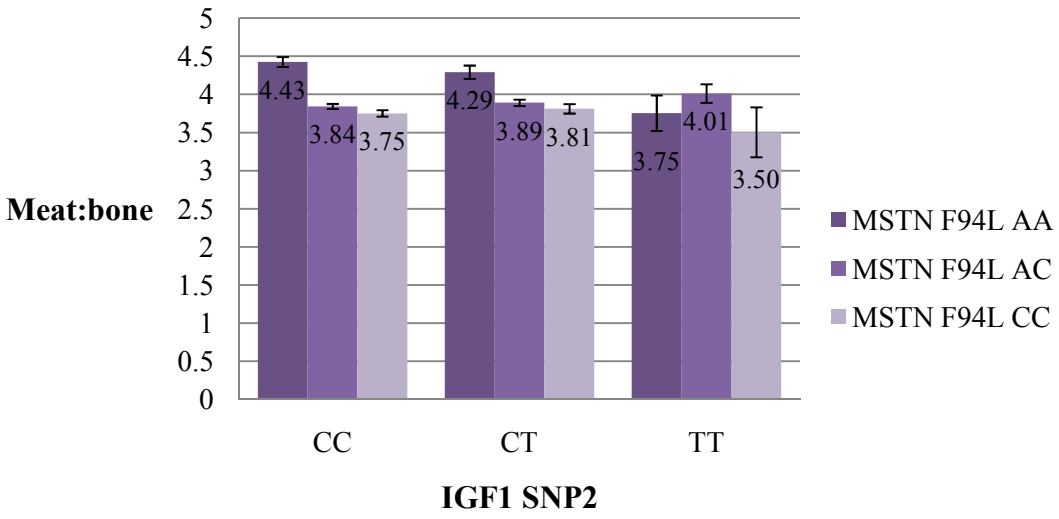


Figure 4.17 Effect of interaction between *myostatin* F94L and *IGF1* SNP2 on meat-to-bone ratio

The only significant interaction between the *myostatin* F94L genotype and the *FST* SNP7 was found for muscularity (as measured by the ratio of stifle width to hip width). There were no significant differences observed for cattle carrying the homozygous AA genotype for *myostatin* F94L and any of the possible *FST* SNP1 genotypes. Interestingly though, the greatest muscularity was detected for cattle carrying the heterozygous AC genotype for the *myostatin* F94L and homozygous AA genotype for the *FST* SNP7.

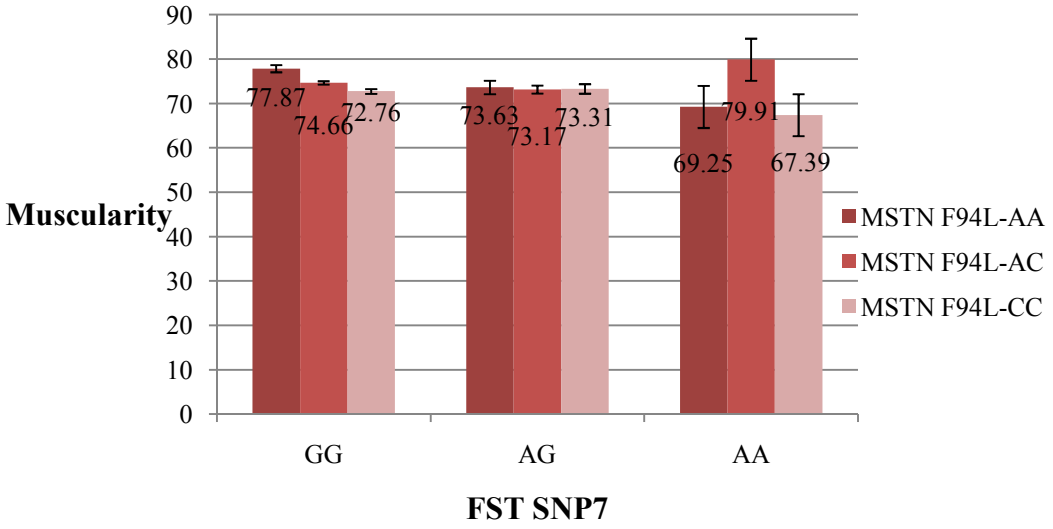


Figure 4.18 Effect of interaction between *myostatin*F94L and *FST* SNP7 on meat-to-bone ratio

The interaction results between the *myostatin* F94L with the *IGF* SNP2 and *FST* SNP 7 genotypes and the other SNPs may indicate that the *SNIP1* SNP3, *TGFBR3* SNP6 and *IGF1* SNP1 interact with *myostatin* differently and this may be anticipated by the biological function. No studies thus far have reported an interaction between the polymorphisms of these genes with beef cattle carcass traits or muscularity. There are several *in vitro* and *in vivo* studies though which have identified interactions between follistatin and myostatin on skeletal muscle development (Gamer *et al.* 1999; Lee and McPherron 2001; Lee 2004). Indirect interactions between myostatin and IGF1 had been also observed in a few *in vitro* studies (Kamanga-Sollo *et al.* 2003; Kamanga-Sollo *et al.* 2005).

4.2.4 Interactions between candidate gene SNP genotypes

In addition to detecting interactions between the *myostatin* F94L variant and candidate gene SNPs, interactions between the SNPs within each gene were also examined. However, not many interactions were observed between the SNPs in any given gene (Table 4.9).

Interactions were only identified for SNP6 and SNP7 in *ACVR1* and SNP2 and SNP8 in *FSTL5.* The interaction between *ACVR1* SNP6 and *ACVR1* SNP7 was associated with meat weight (with bone weight as covariate), eye muscle area, silverside weight and meat-to-bone ratio, whereas the *FSTL5* SNP2 and *FSTL5* SNP8 was only associated with hot standard carcass weight.

For those interactions detected, another ANOVA was completed to examine the haplotype effect for these SNPs. The haplotypes were formed using the program *PHASE* for those SNPs with significant interactions (Pirinen et al. 2008). For *FSTL5*, the haplotypes were formed using SNP2 (C>T 38190903) and SNP8 (A>G38776959), while for *ACVR1,* SNP6 (T>C 39975631) and SNP7 (C>A 39979144) were used to form the haplotypes (Table 4.10). The haplotypes of the progeny was analysed for their association with the traits of interest.

Table 4.9 Test of significance of interactions between SNPs within candidate genes.

SNPs	F probability of the traits									
	HSCW	**MtWt[a]**	**MtWt[b]**	**Mt%**	**Mt%[c]**	**EMA**	**SilversideWt**	**Mttobn**	**Mus**	**StiffWdth**
ACVR1 SNP6**ACVR1* SNP7	0.39	0.14	**0.03***	0.06	0.09	**0.02***	**0.03***	**0.009****	0.62	0.76
SNIP1 SNP2**SNIP1* SNP3	0.62	0.25	0.79	0.29	0.28	0.57	0.35	0.78	0.19	0.19
FSTL5 SNP2**FSTL5* SNP5	0.68	0.42	0.31	0.32	0.30	0.81	0.48	0.35	0.62	0.74
FSTL5 SNP2**FSTL5* SNP8	**0.01***	0.62	0.43	0.78	0.70	0.92	0.18	0.46	0.46	0.38
FSTL5 SNP2**FSTL5* SNP14	0.10	0.79	0.80	0.73	0.71	0.23	0.16	0.93	0.39	0.24
FSTL5 SNP5**FSTL5* SNP8	0.83	0.33	0.78	0.43	0.43	0.44	0.91	0.90	0.16	0.2
FSTL5 SNP5**FSTL5* SNP14	0.79	9.57	0.22	0.64	0.65	0.65	0.59	0.18	0.43	0.54
FSTL5 SNP8**FSTL5* SNP14	0.53	0.69	0.44	0.84	0.85	0.22	0.78	0.44	0.35	0.35
TGFBR3 SNP5**TGFBR3* SNP6	0.87	0.95	0.49	0.96	0.98	0.35	0.48	0.25	0.27	0.37
IGF1 SNP1* *IGF1* SNP2	0.19	0.08	0.45	0.11	0.12	0.33	0.85	0.37	0.58	0.29
FST SNP5 **FST* SNP7	0.15	0.35	0.67	0.56	0.57	0.15	0.46	0.55	0.93	0.85

HSCW= hot standard carcass weight, MtWt[a]= meat weight with HSCW as covariate, MtWt[b]= meat weight with bone weight as covariate, Mt%= meat percentage, Mt%[c]= meat percentage with bone percentage as covariate, EMA= eye muscle area with HSCW as covariate, SilversideWt= silverside weight with HSCW as covariate, Mttobn= meat to one ratio, Mus= muscularity prediction, StiffWdth= stifle width with hip width as covariate, BnWt=bone weight. *** P<0.001; **P<0.01; *P<0.05

Table 4.10 *FSTL5* and *ACVR1* haplotypes

Haplotype number	*FSTL5*		ACVR1	
	C>T	A>G	T>C	C>A
	38190903 allele	38776959 allele	39975631 allele	39979144 allele
1	C	A	T	A
2	C	G	T	C
3	T	G	C	C
4	T	A	C	A

The haplotypes were not significantly associated with most of the traits of interest (Table 4.11). The haplotypes for *FSTL5* were not quite significantly associated with hot carcass weight (P = 0.06), while the *ACVR1* haplotypes were only just significantly associated with meat-to-bone ratio (P = 0.048).

Table 4.11 Test of significance of haplotype of *FSTL5* and *ACVR1* on traits of interest

Traits	F-probability	
	***FSTL5* haplotype**	***ACVR1* haplotype**
HSCW	**0.06**	0.25
Meat weight[a]	0.51	0.30
Meat weight[b]	0.28	0.13
Meat %	0.25	0.17
Meat%[c]	0.27	0.23
Eye muscle area	0.20	0.12
Silverside weight	0.42	0.19
Meat-to-bone ratio	0.29	**0.048***
Muscularity	0.41	0.74
Stifle width	0.45	0.71

[a]Meat weight with HSCW as covariate, [b]Meat weight with bone weight as covariate, [c]Meat percentage with bone percentage as covariate. *** P<0.001; **P<0.01; *P<0.05

4.2.4.1 *ACVR1* haplotype effect on meat-to-bone ratio

From the haplotype analysis, only the *ACVR1* SNP haplotypes showed an association with meat-to-bone ratio. There were 7 haplotype combinations formed for *ACVR1* (with 6 degrees of freedom). The 2/2 and 3/3 haplotypes had the highest meat-to-bone ratio compared to other haplotypes (Table 4.12). However, the standard error of the 3/3 haplotype is high and there was no statistical difference between the animals with the 1/1, 1/3 and 1/4 haplotypes verus the 2/2 and 3/3 haplotypes (Figure 4.19). There was also a high standard error for 1/4 haplotype since only two cattle carried this haplotyp.

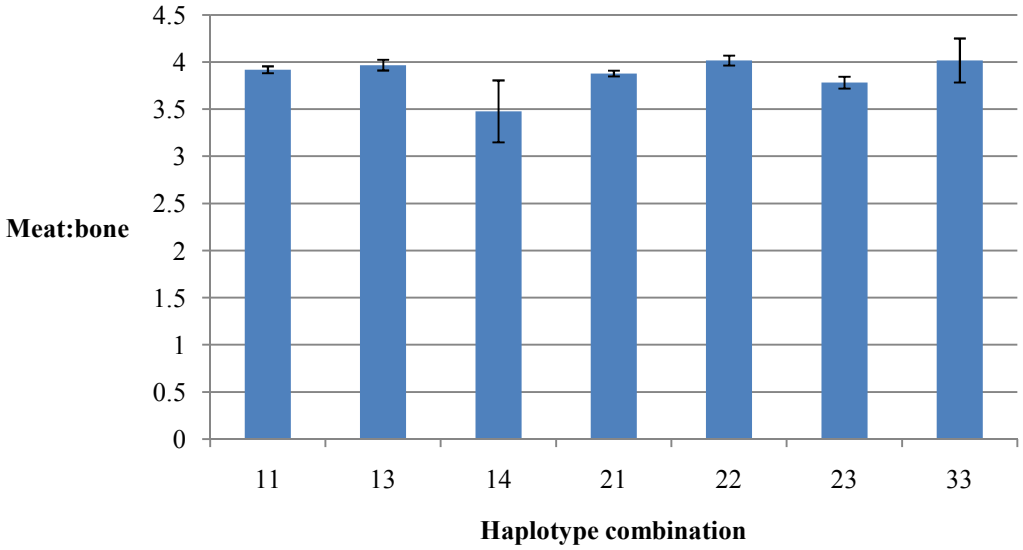


Figure 4.19 *ACVR1* haplotype effects on meat-to-bone ratio

Table 4.12 Meat-to-bone ratio means and standard error for *ACVR1* haplotypes

Haplotype	Mean	Standard error	#Cattle
1/1	3.92	0.04	94
1/3	3.97	0.06	45
1/4	3.48	0.33	2
2/1	3.88	0.03	141
2/2	4.02	0.05	49
2/3	3.78	0.06	37
3/3	4.02	0.23	2

4.2.4.2 *FSTL5* haplotype effect on hot standard carcass weight

The *FSTL5* SNP haplotype was close to significant for hot standard carcass weight with an F-value of 0.06 (with 6 as degree of freedom). The 3/3 haplotype had the heaviest hot standard carcass weight (Table 4.13). However, the other haplotypes were not significantly different, except for the 4/3 haplotype which had the lowest hot standard carcass weight (Figure 4.20).

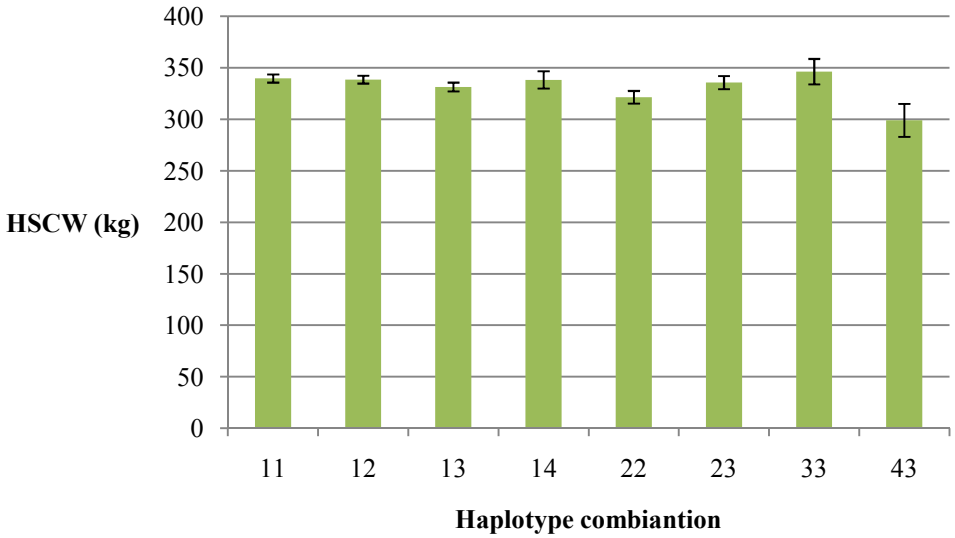


Figure 4.20 *FSTL5* haplotype effects on hot standard carcass weight

Table 4.13 Hot standard carcass weight means and standard error for *FSTL5* haplotypes

Haplotype	Mean	Standard error	#Cattle
1/1	339.5	3.99	86
1/2	338.4	3.77	96
1/3	331.3	4.20	78
1/4	338.2	8.40	20
2/2	321.3	6.18	35
2/3	335.6	6.39	36
3/3	346.2	12.35	9
4/3	298.9	16.04	5

4.2.5 QTL mapping

Additional QTL mapping by fitting the *FSTL5 SNPs* into the model was conducted to confirm whether the *FSTL5* underlies the QTL on chromosome 17. The *FSTL5* SNP5 and *FSTL5* SNP8 had significant effects on several traits of interest; therefore, these two SNPs specifically were included in the model for the QTL mapping.

Two QTL had been found on chromosome 17 (Chapter 3). The QTL for meat weight (with hot standard carcass weight as covariate), meat percentage (with and without bone percentage as covariate) and silverside weight were located on 37-40 cM, while the QTL for hot standard carcass weight, meat weight (with bone weight as covariate) and meat-to-bone ratio were located on 82-85 cM. Overall, the F-values only slightly decreased after fitting the *FSTL5* SNPs as fixed effects for the QTL for meat weight (with HSCW as covariate), meat percentage and silverside weight located at 36-40cM (Table 4.14). There was a marginally greater decline in the F-

values for the QTL for hot standard carcass weight, meat weight (with bone weight as covariate) and meat-to-bone ratio located at 82-85 cM.

Table 4.14 QTL mapping results for BTA 17 with the inclusion of *FSTL5* SNP genotypes in the model

Traits	Presence of *FSTL5* SNPs	Position	F-value
Meat weight	Without *FSTL5* SNPs	37	4.87*
(with HSCW as covariate)	With *FSTL5* SNP5	37	4.33*
	With *FSTL5* SNP8	36	4.08*
Meat percentage	Without *FSTL5* SNPs	38	6.07**
	With *FSTL5* SNP5	38	4.84*
	With *FSTL5* SNP8	36	4.83*
Meat percentage	Without *FSTL5* SNPs	38	5.11*
(with bone% as covariate)	With *FSTL5* SNP5	38	4.43*
	With *FSTL5* SNP8	37	4.19*
Silverside weight	Without *FSTL5* SNPs	40	6.16**
	With *FSTL5* SNP5	40	4.7*
	With *FSTL5* SNP8	39	5.78*
Hot standard carcass weight	Without *FSTL5* SNPs	85	4.09*
	With *FSTL5* SNP5	98	3.49
	With *FSTL5* SNP8	92	3.98
Meat weight	Without *FSTL5* SNPs	82	4.74*
(with bone weight as covariate)	With *FSTL5* SNP5	92	3.13
	With *FSTL5* SNP8	90	4.16*
Meat-to-bone ratio	Without *FSTL5* SNPs	82	4.42*
	With *FSTL5* SNP5	92	2.74
	With *FSTL5* SNP8	90	3.78

*F-value =4-6, **F-value=6-10

These results suggest that *FSTL5* may affect the QTL on BTA 17 but the SNPs examined are probably not the QTN for muscularity. It was of interest that the *FSTL5* also influenced the QTL located at 82-85cM even though this gene is located at about 36-40cM from the QTL peak. However, since the mapping resolution is poor due to the limited number of informative meioses, this may actually be the same QTL.

4.3 Summary

There were 43 DNA variants found in the *ACVR1, SNIP1, TGFBR and FSTL5* genes including 19 insertion/deletions and 33 SNPs with two potentially functional SNPs that change amino acids. Four more DNA variants were identified from previous studies (Sellick 2002 and Naik 2007) in *IGF1* and *FST* that were genotyped. Only four SNPs (*FSTL5* SNP5, *FSTL5* SNP8, *IGF1* SNP1 and *FST* SNP7) were associated with the traits of interest. The *FSTL5* SNP5 was associated with meat weight (with bone weight as covariate) and meat-to-bone ratio; the *FSTL5* SNP8 was associated with meat percentage and eye muscle area; the *IGF1* SNP1 was associated with hot standard carcass weight and the *FST* SNP7 was associated with meat weight (with HSCW as covariate), meat percentage (with and without bone percentage as covariate), eye muscle area and silverside weight. Most of these SNPs had significant additive effects, except for the *FSTL5* SNP5 and *IGF1 SNP1*. Dominance effects were found for *IGF1* SNP1 and *FST* SNP7. Surprisingly, the *FST* SNP7 accounted 19.27% -34.17% of the variances for traits of interest. However, these results require confirmation given the limited number of homozygous progeny.

The interactions of several SNPs with the *myostatin* F94L variant affected the traits of interest. *SNIP1* SNP3 interactions with *myostatin* F94L affected eye muscle area.

The interaction between *TGFBR3* SNP6 and *myostatin* F94L was associated with meat weight (with HSCW as covariate) and meat percentage. The *IGF1* SNP1 interaction with *myostatin* F94L affected silverside weight; the interaction between *IGF1* SNP2 and *myostatin* F94L was associated with HSCW and meat weight (with bone weight as covariate). Lastly, the interaction between *myostatin* F94L and *FST* SNP7 was associated with muscularity.

To confirm whether the *FSTL5* represents the QTL on chromosome 17, additional QTL mapping fitting the *FSTL5* SNP5 and SNP8 was undertaken. For the QTL located at 36-40 cM, the F-value of the QTL only slightly decreased and was not significantly different. For the QTL located at 82-85 cM for HSCW, meat weight (with bone weight as covariate) and meat-to-bone ratio, the F-values decreased just below the level of significance ($F<4$) although *FSTL5* is not located on this region. The results suggest that these SNPs near *FSTL5* are not the QTN for muscularity.

CHAPTER 5

General Discussion

5.1 Introduction

The aims of this study were to locate QTL for cattle muscularity; to identify candidate genes controlling muscularity from the QTL; to study the association between the genes and muscularity, and to study the relationship between these genes and *myostatin*. There were many QTL mapped for muscularity related carcass traits in this study. 4 QTL were identified for stifle width, muscularity, hot standard carcass weight and meat weight (with hot standard carcass weight as covariate), 3 QTL were found for meat weight (with bone weight as covariate) and meat percentage, 2 QTL were mapped for meat-to-bone ratio and silverside weight and 1 QTL for eye muscle area (Table 3.1). Overlapping QTL were found on BTA 2, 3, 8 and 17, suggesting that some traits are controlled by the same gene(s). In total, all the QTL mapped to 15 regions on 11 chromosomes (BTA 1, 2, 3, 4, 5, 8, 9, 11, 13, 14 and 17). Many more QTL have been mapped for growth traits (eg. body weight and daily gain weight) and meat traits that do not define muscularity (eg. eye muscle area without the inclusion of hot carcass weight in the model) (http://www.animalgenome.org/cgi-bin/QTLdb). In terms of the traits that best define muscularity, the QTL of greatest interest were on 3 chromosomes, BTA 2, 3 and 17. The QTL on BTA 2 was explained by the *myostatin* F94L variant as shown previously (Sellick*, et al*. 2007).

Based on the QTL results for BTA 2, 3 and 17, three candidate genes were chosen, *ACVR1, SNIP1* and *FSTL5*. Three additional candidate genes (*TGFBR3, IGF1* and *FST*) that were not located within these QTL regions were also chosen for further study. These candidate genes were selected based on their potential function in muscle development and/or their possible role in the myostatin pathway.

Myostatin (GDF8), a member of transforming growth factor-β superfamily, was first identified in 1997. It was found that "knock-out" mutations of this gene in mice dramatically increased skeletal muscle mass, indicating that myostatin is a negative regulator of skeletal muscle growth (McPherron *et al.* 1997). In cattle, deletions of *myostatin* cause double-muscling, such as is observed in Belgian Blue cattle. However, several other non-deletion mutations in *myostatin* have been also identified that affect muscle mass (Grobet *et al.* 1997; Kambadur *et al.* 1997; McPherron and Lee 1997; Karim *et al.* 2000). The F94L variant of *myostatin* in Limousin cattle is associated with increased carcass yield but produces intermediate muscled cattle rather than double-muscling (Sellick *et al.* 2007).

Like other TGF- β superfamily members, myostatin is produced as a precursor protein, which is cleaved by proteolysis for activation. The activation of myostatin from its latent complex is mediated by pro-peptide cleavage and amino acid residues 98 to 115 represent the pro-peptide cleavage site (Jiang et al. 2004). This site is relatively close to amino acid 94 where the variant in the *myostatin* gene occurs in Limousin cattle, suggesting that the change of phenylalanine^{94} to leucine in the myostatin protein may affect the pro-peptide cleavage and hence, myostatin activation. Interestingly, the amino acid residue at position 94 is completely conserved as tyrosine in all species sequenced to-date, except cattle which have phenylalanine at this position (Figure 5.1).

NOTE:
This figure is included on page 104 of the print copy of the thesis held in the University of Adelaide Library.

Figure 5.1. Conservation of *myostatin* gene sequence. The position of the F94L variant (highlighted in red) is tyrosine in all species except cattle. (Adapted from McPherron & Lee, 1997)

The increased skeletal muscle mass for both double-muscling and the F94L intermediate non-double muscling cattle appears to be due to hyperplasia (Wegner *et al.* 2000), as myostatin normally inhibits myoblast proliferation and hence, inhibits muscle development (Thomas *et al.* 2000; Joulia *et al.* 2003). Myostatin causes accumulation of myoblasts in the G0/G1 phase of the cell cycle by causing cell cycle arrest. As a result, the muscle stops growing (Thomas *et al.* 2000; Joulia *et al.* 2003).

During muscle development, myostatin is thought to act through the same signalling pathway as the other TGF-β family members (Figure 5.2). Myostatin initiates the signalling pathway by binding to its type II receptor; this binding activates the type I receptor by inducing phosphorylation. The next step is the phosphorylation of the SMAD proteins, which are the signal transducers that facilitate the translocation of

the complex into the nucleus for the activation of target gene(s) transcription (Massague 1998; Dominique and Gérard 2006). However, there are many other proteins that are involved in the myostatin pathway. This includes a series of proteins that either inhibit or activate myostatin cleavage and/or myostatin binding to its receptor, including follistatin (FST) and follistatin related gene (FLRG) (Figure 5.2). Thus, it is likely that there will be genes epistatic with *myostatin*, and these should be good candidates in the search for genes affecting muscling in cattle.

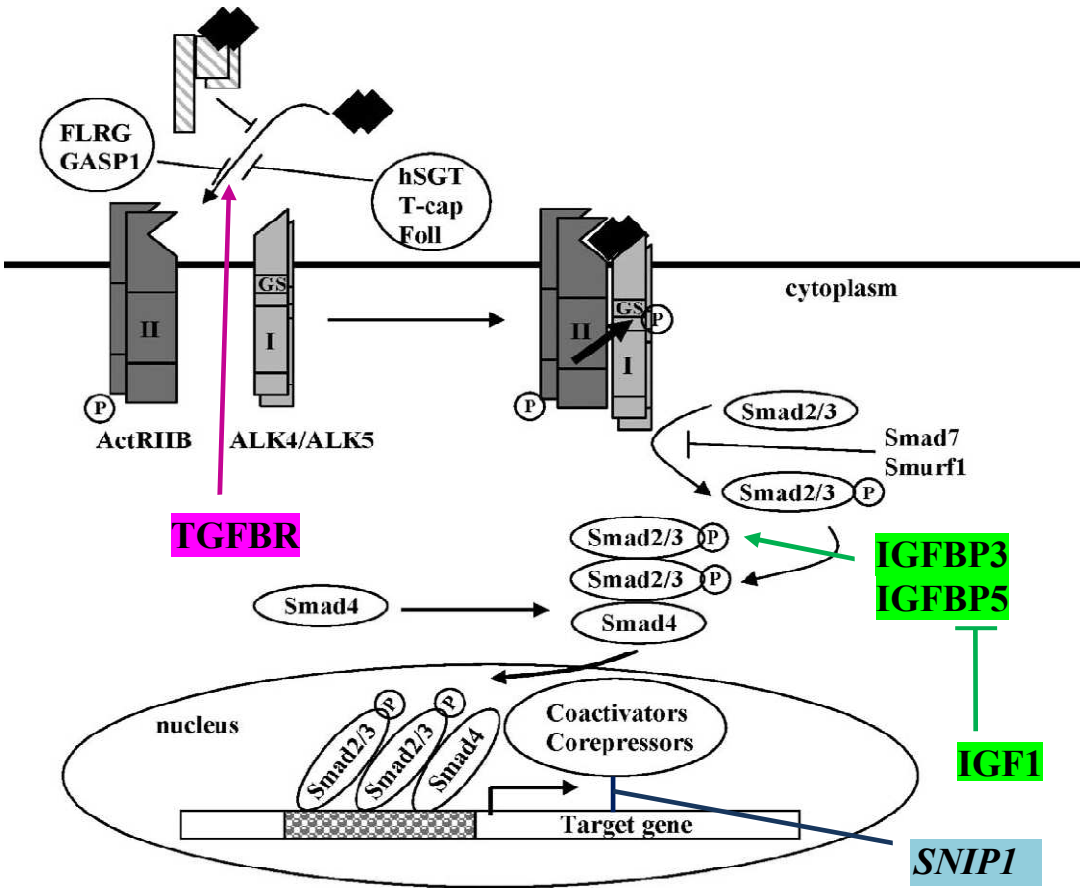


Figure 5.2 Myostatin pathway and potential interactions of the candidate gene proteins. (Adapted from (Dominique and Gérard 2006))

DNA variants in *FSTL5* (SNP5 and SNP8), *IGF1* (SNP1) and *FST* (SNP7) had significant associations with muscularity related traits (hot standard carcass weight, meat weight, meat percentage, meat-to-bone ratio, eye muscle area and silverside weight). However, most of these variants accounted for only a small proportion (less than 10%) of the trait phenotypic variance compared to the *myostatin* F94L variant (Table 4.5). The exception was the *FST* SNP7 that accounted for 19-35% of the phenotypic variance of several traits (Appendix 16).

This study also showed that there were interactions between the DNA variants of the *SNIP1, TGFBR3, IGF1* and *FST* with the *myostatin* F94L genotypes that significantly affected muscularity related carcass traits. The direct pathways for these interactions were not previously documented for most of these candidate genes, except *FST*. However, their contribution to muscle development and their role in TGF-β family member related pathways have been shown.

Interestingly, two potentially functional SNPs in *FSTL5* (SNP2) and *TGFBR3* (SNP5), which caused amino acid substitutions, were not associated with any of the muscularity traits, nor did they interact with *myostatin*. This is despite the fact that the amino acid substitutions are not conservative (leucine to phenylalanine for *FSTL5* SNP2 and glutamine to arginine for *TGFBR3* SNP5). Yet, other SNPs in these genes were associated with muscularity (*FSTL5* SNP5 and *FSTL5* SNP8) or were epistatic with *myostatin* (*TGFBR3* SNP6).

5.2 Interactions between *SNIP1* and *myostatin*

There was an interaction between the *Smad nuclear interacting protein 1* (*SNIP1*) DNA SNP3 variant and the *mysotatin* F94L genotype affecting eye muscle area (EMA) (Chapter 4). SNIP1 is a novel nuclear protein with 396 amino acids, which consists of a nuclear localization signal (NLS) domain and a forkhead-associated (FHA) domain (Kim *et al.* 2000). Not many studies have examined the function of SNIP1. However, Kim *et al.* (2000) demonstrated that SNIP1 can suppress the TGF-β signalling pathway by interacting and competing with SMAD4 to bind to the coactivator p300.

SMAD proteins are the signal transducers of receptors and the target genes in the nucleus, and thereby, regulate gene expression (Massague 1998; Schmierer and Hill 2007). Based on their function, they can be divided into 3 sub families: the receptor regulated SMADs (R-SMADs) that are the direct substrates of the TGF-β receptors, the co-SMADs that mediate the signalling of the receptor regulated SMADs, and the antagonistic/inhibitory SMADs (I SMADs) that inhibit the function of the two other types of SMADs in the signalling process (Massague 1998). R-SMADs (receptor regulated SMAD) specificity depends upon the type 1 receptor (Figure 5.3). For myostatin, SMAD 2 and SMAD 3 are the R-SMADs, SMAD 4 is the co SMAD and SMAD 7 is the I SMAD (Dominique and Gérard 2006). SMAD 2 and SMAD 3 together with SMAD 4 form a complex that accumulates in the nucleus with SMAD 4 controlling the transcription of the target gene(s) (Schmierer and Hill 2007).

NOTE:
This figure is included on page 108
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the University of Adelaide Library.

Figure 5.3 Core signalling in the mammalian TGF-β –SMAD pathway
(Schmierer and Hill 2007)

(Kim *et al.* 2000) reported that SNIP1 interacts with SMAD4 at the MH2 domain, which mediates the interactions with R-SMAD and the transcription factors (co-activators and co-repressors). Since SNIP1 competes with SMAD4 to bind to the coactivator, the interaction between *SNIP1* and *myostatin* is likely to occur at the level of the transcription of the target gene. As a result of the changes in the transcriptional activity, muscle development may be affected (Figure 5.2). That is, the presence of the SNIP1 may suppress the inhibitory activity of myostatin, resulting in increased muscle growth and meat yield.

5.3 Interactions between *TGFBR3 (betaglycan)* and *myostatin*

TGFBR3 (also known as betaglycan) is one of the accessory receptors of the TGF-β family members and is one of the type III receptors. Betaglycan does not have a direct function in the signalling pathway, but it does mediate the access of the TGF-β ligand to the signalling receptors (Massague 1998). Betaglycan binds the TGF-β via its core protein, which then facilitates the binding of the type II receptors, continuing the signal transduction process (Santander and Brandan 2006).

There were significant interactions between the *myostatin* F94L genotypes and the *TGFBR3* SNP6 genotypes affecting meat weight (with HSCW as covariate) and eye muscle area. Since myostatin may share pathways with the other TGF-β superfamily members, an epistatic interaction between *mysostatin* and *betaglycan* can be explained the indirect role of betaglycan in the signalling pathway. Betaglycan may facilitate the binding of myostatin to its type II receptor as described above. This, in turn, will activate the type I receptor and together with the SMAD proteins alter the target gene transcription (Figure 5.2).

However, in LLC-PK1 cells, betaglycan has been also shown to inhibit the TGF-β signalling pathways by inhibiting the formation of type II and type I receptor complexes. Thus, the role of betaglycan may be more complex than original thought and may depend upon the cell type (Eickelberg *et al.* 2002). Interestingly, studies on betaglycan using the skeletal muscle cell line C2C12 showed that the expression of *betaglycan* is up-regulated during skeletal muscle differentiation and appears to support the effect of TGF-β during differentiation (Lopez-Casillas *et al.* 2003). Myostatin was also affected during differentiation by betaglycan.

5.4 IGF1 role in muscularity and its interactions with myostatin

IGF1 is a member of the insulin-like growth factor family and is a single chain polypeptide protein (7.5 kDa), produced in the liver. It is involved in the proliferation and differentiation of all cell types, and hence, can affect muscle cell fibre number (Cohick and Clemmons 1993; Bass *et al.* 1999; Oksbjerg *et al.* 2004). Indeed, the deletion of *IGF1* disrupts skeletal muscle development (Bass *et al.* 1999). IGF1 affects both muscle hyperplasia and hypertrophy through the Ras-Erk MAP kinase (Erk-MAPK) and the PI3K-Akt1 pathways, respectively (Figures 5.4 and 5.5) (Chang 2007). These two pathways are induced by the type 1 receptor of IGF1 (IGF1R). Herein, the association between *IGF1* polymorphisms and hot standard carcass weight (HSCW) (Table 4.3) may be a result of either or both of these two major pathways.

NOTE:
This figure is included on page 110 of the print copy of the thesis held in the University of Adelaide Library.

Figure 5.4 Erk-MAPK signalling pathway of hyperplasia (Chang 2007)

NOTE:
This figure is included on page 111 of the print copy of the thesis held in the University of Adelaide Library.

Figure 5.5 PI3K-Akt1 signalling pathway of muscle differentiation and hypertrophy (Chang 2007)

Of more interest, an interaction between the *myostatin* F94L genotypes and *IGF1* DNA variants were also found to affect HSCW, meat weight and silverside weight. Although myostatin and IGF1 seem to have different pathways affecting muscle development, the possible epistatic interactions raise a question about whether there is a common pathway in which myostatin interacts with IGF1 to regulate the skeletal muscle cell development. (Florini et al. 1996) showed that IGF1 and TGF-β family member(s) are connected at the level of myogenesis, as IGF1 has been shown to control the expression and secretion of TGF-β proteins although the specific TGF-β proteins have not been identified.

Growth hormone induction in skeletal muscles and C2C12 cells both *in vivo* and *in vitro* has been also demonstrated to inhibit *myostatin* mRNA synthesis and this

action of the growth hormone in muscle is mediated by IGFs, include IGF1 *(Florini et al. 1996; Liu et al. 2003).* In addition, an *in vitro* study, using the porcine embryonic myogenic cell line PEMC cultured with myostatin, demonstrated an increase of the insulin-like growth factor binding protein 3 (IGFBP3) (Kamanga-Sollo *et al.* 2003). IGFBPs are the carrier proteins as well as modulators of IGF1 activity (Florini *et al.* 1996). IGFBP3, specifically, can both inhibit and stimulate IGF1 activity (Baxter 2000). Further studies using PEMC cells showed that IGFBP3 and IGFBP5 can affect myostatin inhibition through the receptor-mediated SMAD phosphorylation mechanism (Kamanga-Sollo *et al.* 2005). These results suggest that there is an antagonistic interaction between myostatin and IGF1 although the interaction is indirect (Figure 5.2).

5.5 *Follistatin* role in muscularity and its interaction with *myostatin*

Follistatin (FST) is a 31-42 kDa monomeric polypeptide and was named based on its ability to inhibit follicle stimulating hormone. It was originally thought to only have a function in reproduction (Patel 1998). However, studies have found that FST also plays important role in muscle development (Matzuk *et al.* 1995; Amthor *et al.* 1996; Amthor *et al.* 2002). A study of FST in chick embryo showed that *FST* is expressed in somites in developing muscle (Amthor *et al.* 1996) and *FST* mutant deletion mice have decreased muscle mass (Matzuk *et al.* 1995). The role of FST in muscle development is suggested to be due to its interaction with the bone morphogenetic proteins (BMPs), particularly BMP2 and BMP7 (Amthor *et al.* 2002). BMPs are TGF-β family members and regulate embryonic muscle development by up-regulating *Pax-3* to promote muscle growth but which also can inhibit muscle development by inducing apoptosis (Massague 1998; Amthor *et al.* 2002).

Although FST is a BMP binding protein, it is believed not to be an antagonist for BMPs like other BMP binding proteins, such as noggin and chordin (Amthor *et al.* 2002). FST activiates BMP7 to induce muscle growth and prevents apoptosis or muscle loss from BMP7 activity. This pathway may explain the significant association between the *FST* DNA variants and some of the traits of interest (Table 4.4).

However, the result of the study herein (Chapter 4) also showed an interaction between the *myostatin* F94L genotypes and the *FST* SNP genotypes. FST is known to play an antagonist role with myostatin by preventing myostatin binding to its receptor. As a result, the ability of myostatin to inhibit muscle development is disrupted (Lee and McPherron 2001; Lee 2004). As a consequence, mice with over-expressed *FST* have increased muscle mass. Based on *in vitro* studies, FST is believed to block the ability of the active part of myostatin (C-terminal dimer) to bind to Act RIIB, the type II receptor of myostatin (Lee and McPherron 2001). Moreover, FST interactions with other ligands, such as GDF 11/BMP11 that is closely related to myostatin, could also possibly explain the FST interaction with myostatin (McPherron *et al.* 1997; Gamer *et al.* 1999; Nakashima *et al.* 1999). 90% and 88% of the GDF11 amino acid sequence is identical to myostatin in mouse and rat, respectively (Nakashima *et al.* 1999) and FST is able to bind and inhibit BMP11 activity (Gamer *et al.* 1999).

Thus, follistatin could affect muscle development through a number of mechanisms. It is not clear though whether the epistatic effects between *follistatin* and *myostatin*

observed herein are more likely to be through a direct interaction between FST and myostatin or through an indirect interaction involving the BMPs or a combination of both.

5.6 Future experiments

Studies on *myostatin* gene expression of the *myostatin* mutant doubled-muscled cattle (Belgian Blue and Piedmontese) showed that these mutants have higher levels of *myostatin* mRNA compared to normal cattle (Bass *et al.* 1999; Oldham *et al.* 2001; Forbes *et al.* 2006; Lehnert *et al.* 2007). The high mRNA level in double-muscled cattle is believed to be a consequence of up-regulation of the non-functional *myostatin* gene in order to compensate for the lack of functional myostatin protein (Forbes *et al.* 2006; Lehnert *et al.* 2007). However, studies on the level of *myostatin* mRNA in Limousin cattle carrying the *myostatin* F94L variant have not been conducted. It would be predicted that since the myostatin protein is not fully functional in the F94L homozygous variants, then the homozygous F94L variants may have increased *myostatin* gene expression compared to other cattle though perhaps as not up-regulated as in double-muscled cattle. A gene expression study on the F94L homozygous variants would provide a better understanding of any feedback mechanisms during muscle development which involves the regulation of *myostatin* gene expression. Moreover, the study herein showed interactions between candidate gene DNA variants (SNPs in *SNIP1, TGFBR3* and *FST*) and the F94L *myostatin* genotypes. Based on these associations at the DNA level, conducting gene expression studies for these genes would help determine whether these genes are epistatic with *mysotatin* at the mRNA level or at the protein level.

Identification of DNA variants and association studies of other candidate genes would also be valuable. One such candidate gene for muscularity worthy of further investigation is the *mitogen-activated protein kinase-activated protein kinase 5* (*MAPKAPK5*). *MAPKAPK5* is located in the QTL on chromosome 17 at 84-87 cM (65,511,719-65,533,819 bp). *MAPKAPK5* is believed to be phosphorylated and activated by Erk and p38 kinase (Ni *et al.* 1998). As described earlier, the Erk-MAPK pathway has a role in muscle hyperplasia through IGF1 to regulate muscle development (Change 2007) and p38 MAPK has a major role in myogenesis during skeletal muscle development (Keren *et al.* 2006; Chang 2007).

Other family members of the IGFBPs, such as *IGFBP2*, *IGFBP3* and *IGFBP5*, are also potential candidate genes for muscularity. Since IGFBPs are the carrier proteins and modulators of IGFs that either inhibit or stimulate IGF action, IGFBPs play a prominent role in muscle development (Florini *et al.* 1996). An *in vitro* study of the IGFBP3 and IGFBP5 activity demonstrated their ability to affect *myostatin* activity through receptor-mediated SMAD phosphorylation (Kamanga-Sollo *et al.* 2005). Association studies of the IGFBP DNA variants may provide a better understanding of the biology behind the epistatic interaction between *IGF1* and *myostatin*.

Thus, examining more candidate genes would be useful for both a better understanding of the genetics behind muscularity in cattle and for more markers to assist in selection. It is clear that even the best marker which explained the most phenotypic variance for muscle traits (*FST* SNP7) found herein is unlikely to the QTN. Therefore, the promoter, flanking regions and introns of *FST,* in particular, should be fully sequenced for more variants.

Lastly, considering the limited number of cattle genotyped in this study, all SNPs associated with muscularity traits herein should be verified by genotyping a larger number of additional non-related cattle. A major difficulty with the study herein is that there were too few animals with specific genotypes for some SNPs (eg FST SNP 7), and consequently, the size of effect could not be accurately estimated. Therefore, it is important that such SNPs of interest are investigated further. Preferably, several thousand animals with good phenotypic records would be genotyped to verify the size of effect and determine if the direction of the effect (ie, negative or positive) is always the same. This is of particular importance for estimating any epistatic effects.

5.7 Summary

The identification and study of candidate genes that may affect muscularity in cattle was deemed a suitable approach herein for both increasing our understanding of muscle development in livestock and for providing additional markers for selection. The ratio of stifle width to hip width was used as an indicator for muscularity since these measurements represent muscle relative to the dimensions of the skeleton. Other carcass traits were also used as indicators of muscularity (instead of growth) by including the appropriate co-variates in the models. However, inaccuracy in measuring traits, such as stifle width and hip width, may occur. Repetitive measurements must be taken and growth must be taken into account. Therefore, selection based on molecular information is a reasonable option provided that markers have been identified which significantly account for the phenotypic variance of muscularity related carcass traits. *Myostatin* is one gene that has been shown to account for a large proportion of the phenotypic variance in muscularity related

traits, such as hot standard carcass weight, eye muscle area and silverside weight (Sellick *et al.* 2007). In this study on the same cattle population, *follistatin* was also observed to have a significant effect on muscularity related carcass traits. It should be noted that even if the level of significance was more conservative ($P<0.01$), then the effect of *FST* (SNP7) on muscularity traits and the interaction between *IGF1* SNP2 and *myostatin* would still be significant. Thus, the DNA variants of *follistatin* and *insulin-like growth factor 1* should be examined further for their potential use in selection programs to increase retail beef yield.

This study suggested that the myostatin pathway is quite complex and provided evidence of interactions with other pathways. For example, TGFBR3 and IGF1 seem to have no direct function in the myostatin signalling pathway but they are possibly related as epistatic interactions were detected. TGFBR3 (betaglycan) is known to facilitate TGF-β family member binding to their type II receptors to induce signal transduction activity (Massague 1998; Santander and Brandan 2006). Although there are no studies thus far showing direct interactions between myostatin and betaglycan, the results herein suggest that myostatin and betaglycan interact through the same pathway as the other TGF-β family members, and that myostatin does indeed share the same relationship with betaglycan as other TGF-β family members.

IGF1 is another candidate gene that has no known direct function in the *myostatin* signalling pathway, although growth hormone induction which is mediated by IGFs in skeletal muscle cells has been shown to inhibit *myostatin* mRNA synthesis (Florini *et al.* 1996; Liu *et al.* 2003). However, the most likely interaction between mysotatin and IGF1 is presumably through IGFBP interactions with SMAD proteins. IGFBPs are modulators of IGF1 action (Florini *et al.* 1996) and IGFBP3 and

IGFBP5 are involved in myostatin inhibition through the phosphorylation of receptor-mediated SMADs (Kamanga-Sollo *et al.* 2005).

SNIP1 and *FST* are other candidate genes that showed epistatic effects with *myostatin*. There have been more studies examining the role of these proteins in the myostatin pathway and their interactions are easier to postulate. *SNIP1* is known to suppress the TGF-β signalling pathway by competing with SMAD4 to bind with the coactivator and control the target gene transcription (Kim *et al.* 2000; Schmierer and Hill 2007). SMAD4 is known to be the co-SMAD for myostatin and facilitates SMAD2 and SMAD3 (the R-SMADs for *myostatin*) translocation to the nucleus and commencement of target gene transcription (Dominique and Gérard 2006; Schmierer and Hill 2007). *FST* is known for its ability to prevent myostatin binding with its receptor and as a result, myostatin inhibition of muscle development is disrupted (Lee and McPherron 2001; Lee 2004).

5.8 Conclusions

The results provided three interesting findings related to the biological pathways underlying muscularity. Firstly, it seems that IGF1 must be involved in the myostatin pathway, at least indirectly. Secondly, the results suggest that SNIP1 must play a larger role in the myostatin pathway than anticipated. Thirdly, follistatin appears to be able to act independently of myostatin to affect muscularity. Based on these results, further experiments to elucidate the myostatin pathway are warranted in order to clarify the roles of these genes in muscularity.

As originally hypothesized, the results of this study have shown that there are QTL for muscularity traits. Many but not all of these muscularity QTL overlap with growth QTL. Based on the location of the muscularity QTL, candidate genes that may affect muscularity traits were identified. SNPs in some of these candidate genes were shown to affect muscularity directly while other genes were shown to interact with *myostatin*. This included both genes that were anticipated to be involved in the myostatin pathway (e.g. *FST*) and genes that were not necessarily expected to interact with *myostatin* (e.g.*IGF1*). In addition to the epistatic effects, large dominance effects were seen. As a consequence, the dominance and epistatic effects of the SNPs must be considered if these SNPs are used in breeding programs. However, further analysis of these SNPs in larger cattle populations needs to be undertaken to confirm the results herein before these markers can be utilised commercially.

APPENDICES

Appendix 1. PCR condition of the candidate genes

Gene	Region	Primers sequence		PCR Condition			
		Forward primer	Reverse primer	Program	Taq	[Primer]	[$MgCl_2$]
ACVR1	Exon1 5'UTR	CTACTGGCTTTTGTGTCATTTA	GAAAAGAATGGCAGAAGCA	TD2	AmpliTaq	2.5μM	2.5mM
ACVR1	Exon2	GTCCTTTACGAATACCCATCC	AATCCCTTTGCCTTCCTTT	TD2	AmpliTaq	2.5μM	2.5mM
ACVR1	Exon3	GCCCACCAGACCAAAGATAC	CCCTAATACGCCCATCCTC	TD2	AmpliTaq	2.5μM	1mM
ACVR1	Exon4	CTGTCTGTGAGGGCTTGATG	TTGTCTGTCCCTGTCCCTTT	TD2	AmpliTaq	2.5μM	2.5mM
ACVR1	Exon5	CCTTTTATTTTTGCTCCACTTT	AGCAGAAGCACAGCCACTC	TD2	AmpliTaq	2.5μM	2.5mM
ACVR1	Exon6	CACCTTGCTTTTGGATGTCT	TTCAAAAAGGGTGGGGTAA	TD2	AmpliTaq	2.5μM	2.5mM
ACVR1	Exon7	CTGCTGATGCCTCTGTAAAC	TCTTGACAGGGATTTGGAC	TD2	AmpliTaq	2.5μM	2.5mM
ACVR1	Exon8	CTTTTTCAGGGATAGAGCAG	CGACCAGCCATAAATCAA	TD2	AmpliTaq	2.5μM	2.5mM
ACVR1	Exon9	CCGAAGGGGATGGATTATT	GGGAGAGAAAGCAACAGAACA	TD2	AmpliTaq	2.5μM	2.5mM
ACVR1	Exon10	CCATCAAGGGGTCCAGA	GTGTTCCTCCAGTTCCCTAC	TD2	AmpliTaq	2.5μM	2.5mM
ACVR1	Exon10 3'UTR	GCCCTGCGTATCAAAAAG	TTCAGCATCATTGTAAACATCA	TD2	AmpliTaq	2.5μM	2.5mM
ACVR1	3'UTR	TGCCTGTGCTTCTCTTCTTT	ATTAGTTTTGTGCTTCTGTTTCC	TD1	KapaTaq	5μM	

Gene	Region	Primers Sequence		PCR Condition			
		Forward sequence	Reverse sequence	Program	Taq	[Primer]	[$MgCl_2$]
SNIP1	Exon1 5'UTR	ACTTGAATCTCCCGCCTCTC	CTCGTTCAAGGTGCGTTTCT	TD1	AmpliTaq	2.5μM	1.5mM
SNIP1	Exon2	TGGCAGAAAACAGGAAAAGT	GGAGGGAACTGGGTGTGA	TD2	AmpliTaq	2.5μM	2.5mM
SNIP1	Exon3	TGGGGTCGCAAAGAGTCA	CCCGAAAGGTGTTAGTGTCC	TD1	AmpliTaq	2.5μM	3.5mM
SNIP1	Exon3	GAGCACCGCCAGAAGAAC	CGGCTTTACTTCCATTTACC	TD2	AmpliTaq	2.5μM	2.5mM
SNIP1	Exon4	CTGTGCCTTCCCATTGAGTT	TTCTGGAGGCTATTTTGGTGA	TD2	AmpliTaq	2.5μM	2.5mM
SNIP1	3'UTR	CTGTGGGCATTTATTTTCCT	TGCGGCTCTTTCACACAC	TD1	Kapa	10μM	
SNIP1	3'UTR	TCAGAAGACGGTTGTGTGGT	TGCCCCAACTCTGACTCTT	TD2	AmpliTaq	2.5μM	2.5mM
TGFBR3	Exon1 5'UTR	GTTTGCTTGAGGGCTGTCT	CTGCGTCACCAACATTCG	TD3	AmpliTaq	2.5μM	1.5mM
TGFBR3	Exon2	CCCGTGGAGAAGGAGATG	GGGCAGGAAAGATGAAAAG	TD2	AmpliTaq	2.5μM	2.5mM
TGFBR3	Exon3	GTGCTGTGCTGAGTGGTTTT	GGGAGGAAGAAGGGGAAG	TD2	AmpliTaq	2.5μM	2.5mM
TGFBR3	Exon4	AGAAAAGGAAGGGGTTAGATTG	GGAAAAAGGGAAGGAGAGGA	TD3	AmpliTaq	2.5μM	3.5mM
TGFBR3	Exon5	GAGGAAATGGGTGAGGACAA	CCGAGGGGTATCTGAAAACA	TD3	AmpliTaq	2.5μM	3.5mM
TGFBR3	Exon6	TGAGCCTGAAGAAGCAACAA	ACAACAAGACTGGAAAGCACAC	TD2	AmpliTaq	2.5μM	2.5mM
TGFBR3	Exon7	TCTCAGCCCAAGGAAAGTAG	TGTCCCAACCCCTCTGTAT	TD3	AmpliTaq	2.5μM	3.5mM

Gene	Region	Primers Sequence		PCR Condition			
		Forward sequence	Reverse sequence	Program	Taq	[Primer]	[$MgCl_2$]
TGFBR3	Exon8	GGGAAACCAAAGCACAGAGA	ACAGCCAAGGGGAAGTGAG	TD2	AmpliTaq	2.5μM	2.5mM
TGFBR3	Exon9	TGGTAGGCTGAAAGGAGGTAAG	GGAAGGAAGGGTGGGAAAG	TD2	AmpliTaq	2.5μM	2.5mM
TGFBR3	Exon10 Exon11	TGGGCTTCTTCTTTTTCATTT	AGGGGCTTTCACTCATGCT	TD2	AmpliTaq	2.5μM	2.5mM
TGFBR3	Exon12	CAAATCACGCAATGTCTCTCA	CCCAAACCAAAAACCTCAAC	TD2	AmpliTaq	2.5μM	2.5mM
TGFBR3	Exon13	CTACCATCACAAATCACAAACA	AGCAGGAGAACCAACTAAACA	TD2	AmpliTaq	2.5μM	2.5mM
TGFBR3	Exon14	GGAGGAGGAATAAAGACGCT	AAAAAGGGGTGAGGGAGAA	TD2	AmpliTaq	2.5μM	2.5mM
TGFBR3	Exon15	TAGTTGGTTCTCAGCCTTTC	TCCTCGCTTACTTCCTGTC	TD2	AmpliTaq	2.5μM	2.5mM
TGFBR3	Exon16	TGAAAAGAGCCCCAGATGA	GCAGCAAGGTGAGAAGTGTG	TD2	AmpliTaq	2.5μM	2.5mM
TGFBR3	3'UTR	GATAGCAGAGCCAGTGAGGA	ACCAACAGAAAACCAAAAGAA	TD2	AmpliTaq	2.5μM	2.5mM
FSTL5	Exon2	CTTACCTATTATGGATTCAGTTGG	TGGCAAATTCAGGAACATAAC	TD2	AmpliTaq	2.5μM	2.5mM
FSTL5	Exon3	TCCATTGCTTGCCATTA	TGAGATGGCAGTGACTAAAT	TD2	AmpliTaq	2.5μM	2.5mM
FSTL5	Exon4	CCTAAGAACAACTGAACCCTGAT	TGGAATTGGCTTGTTGACTG	TD2	AmpliTaq	2.5μM	2.5mM
FSTL5	Exon5	CCCATTCTTTTCTCCTTGTGTAG	GAAACCTGTCAAAAGTAGATGCC	TD2	AmpliTaq	2.5μM	2.5mM
FSTL5	Exon6	AATGTTCAAGTTTTCTGGGTCC	TGATAAGCAGTGACAAACAATGAA	TD2	AmpliTaq	2.5μM	2.5mM
FSTL5	Exon7	AGCCAGCAATGATCTCACC	AGGCAATTTCACCAACACAG	TD2	AmpliTaq	2.5μM	2.5mM

Gene	Region	Primers Sequence		PCR Condition			
		Forward sequence	Reverse sequence	Program	Taq	[Primer]	[$MgCl_2$]
FSTL5	Exon8	ATAGCCCATCAAGTCTTCCA	CTCAGTTCAGTTCAGTTCAGTCA	TD2	AmpliTaq	2.5µM	2.5mM
FSTL5	Exon9	TATTTCACCTGGTACAGTCTC	TTCAATTATTCTGTTCAGCAT	TD2	AmpliTaq	2.5µM	2.5mM
FSTL5	Exon10	GCAAGGTAGGGAGTATGAAGTG	TGAGTGTGATGTGTGTGTATGTATG	TD2	AmpliTaq	2.5µM	2.5mM
FSTL5	Exon11	AGAAGAATTGGCTATCACGAC	TGAACCTACAACCCCTGC	TD2	AmpliTaq	2.5µM	2.5mM
FSTL5	Exon12	AATCTGTGTATCCAAGGGTTC	GAAAAATAAGAAAAACTAAAGGG	TD2	AmpliTaq	2.5µM	2.5mM
FSTL5	Exon13	GGGATGGATGAAAGGACTAT	GGTGCCATTGTAACTAGGATA	TD2	Kapa	10µM	
FSTL5	Exon14	TACTCCCACCCAACCATAAA	CATCATAGTCACCCCCTCCT	TD2	AmpliTaq	2.5µM	2.5mM
FSTL5	Exon15	GGAAGGAAGGAAAAGGGA	GCAAAAACATAATCAGAAGAAC	TD2	AmpliTaq	2.5µM	2.5mM
FSTL5	3’UTR	CCAAATGACTTCTGCTTAACTC	TTCTACCCCATCCCATCAC	TD3	AmpliTaq	2.5µM	3.5mM

Appendix 2. Purification protocols

2.1 DNA purifications from PCR reactions steps

1. add 5 volume of Spin Bind to the PCR reaction and transfer to a spin filter unit tube
2. centrifuge about 40 second at minimum 13,000 rpm
3. discard the liquid from the tube and put back the spin filter basket to the tube
4. add 400 µl Spin Clean buffer and centrifuge for 60 seconds
5. discard the liquid and centrifuge for another 2 minutes
6. transfer the spin filter basket to the collection tube and add 30 µl elution buffer, centrifuge for 60 seconds
7. remove the spin filter basket from the collection tube.

2.2 Purification of sequencing reaction product

1. transfer the sequencing reaction product to 1.5 ml tube
2. add 80 µl 75% isopropanol and vortex
3. leave at room temperatures for 15 minutes
4. centrifuge for 20 minutes at 13,000 rpm
5. discard the liquid completely
6. add 250 µl isopropanol to the tube, vortex and centrifuge again for 20 minutes
7. discard the liquid and dry the sample in the incubator 60ºC for 40 minutes.

Appendix 3. QTL results (cohort and breed as fixed effects)

BTA	Trait	Position	F-value	Likelihood ratio
1	Hip width	107cM	2.31	6.86
1	Muscularity	34cM	2.9	8.61
1	Hot standard carcass weight	87cM	4.6	13.55
1	Meat%	0cM	2.47	7.32
1	Bone weight	84cM	3.07	9.08
1	Bone%	0cM	2.16	6.43
1	Meat to bone ratio	0cM	3.42	10.12
2	Hip width	20cM	3.2	9.48
2	Muscularity	27cM	5.2	15.28
2	Hot standard carcass weight	5cM	2.78	8.26
2	Meat%	6cM	20.2	55.45
2	Bone weight	98cM	2.72	8.06
2	Bone%	11cM	3.52	10.4
2	Meat to bone ratio	8cM	9.24	26.6
3	Hip width	43cM	1.16	3.48
3	Muscularity	0cM	1.54	4.61
3	Hot standard carcass weight	51cM	1.25	3.72
3	Meat%	100cM	4.12	12.15
3	Bone weight	7cM	2.35	6.97
3	Bone%	14cM	1.43	4.26
3	Meat to bone ratio	11cM	1.87	5.56
4	Hip width	33cM	2.32	6.89
4	Muscularity	9cM	4.32	12.75
4	Hot standard carcass weight	9cM	1.04	3.12
4	Meat%	36cM	3.47	10.26
4	Bone weight	78cM	1.29	3.84
4	Bone%	89cM	1.78	5.32
4	Meat to bone ratio	13cM	1.61	4.8
5	Hip width	52cM	1.95	5.82
5	Muscularity	86cM	2.74	8.12
5	Hot standard carcass weight	41cM	6.08	17.78
5	Meat%	109cM	1.72	5.13
5	Bone weight	44cM	2.42	7.2
5	Bone%	35cM	1.88	5.61
5	Meat to bone ratio	33cM	1.63	4.86

BTA	Trait	Position	F-value	Likelihood ratio
6	Hip width	0cM	1.68	5.01
6	Muscularity	2cM	2.97	8.8
6	Hot standard carcass weight	119cM	2.55	7.59
6	Meat%	37cM	1.88	5.61
6	Bone weight	41cM	1.46	4.36
6	Bone%	119cM	1.8	5.37
6	Meat to bone ratio	30cM	2.01	5.99
7	Hip width	0cM	5.16	15.17
7	Muscularity	86cM	2.36	7.03
7	Hot standard carcass weight	0cM	1.98	5.9
7	Meat%	89cM	2.42	7.18
7	Bone weight	5cM	1.46	4.37
7	Bone%	118cM	2.28	6.77
7	Meat to bone ratio	0cM	2.33	6.91
8	Hip width	17cM	2.95	8.74
8	Muscularity	8cM	3.21	9.52
8	Hot standard carcass weight	56cM	3.37	9.99
8	Meat%	58cM	2.82	8.35
8	Bone weight	68cM	1.91	5.7
8	Bone%	17cM	4.34	12.77
8	Meat to bone ratio	17cM	3.9	11.49
9	Hip width	80cM	0.73	2.17
9	Muscularity	104cM	4.85	14.27
9	Hot standard carcass weight	60cM	2.13	6.33
9	Meat%	104cM	0.69	2.06
9	Bone weight	60cM	2.57	7.62
9	Bone%	103cM	1.91	5.67
9	Meat to bone ratio	100cM	2.21	6.58
10	Hip width	43cM	2.14	6.36
10	Muscularity	98cM	2.27	6.76
10	Hot standard carcass weight	62cM	2.08	6.19
10	Meat%	98cM	1.35	4.04
10	Bone weight	18cM	1.29	3.85
10	Bone%	35cM	2.87	8.52
10	Meat to bone ratio	49cM	2.66	7.88
11	Hip width	79cM	3.52	10.41
11	Muscularity	84cM	4.17	12.3
11	Hot standard carcass weight	111cM	1.3	3.88
11	Meat%	60cM	1.31	3.92
11	Bone weight	10cM	1.44	4.29
11	Bone%	70cM	3.08	9.13
11	Meat to bone ratio	70cM	3.08	9.12

BTA	**Trait**	**Position**	**F-value**	**Likelihood ratio**
12	Hip width	57cM	2.53	7.51
12	Muscularity	15cM	3.15	9.34
12	Hot standard carcass weight	102cM	0.91	2.71
12	Meat%	25cM	3.93	11.58
12	Bone weight	5cM	3.21	9.51
12	Meat to bone ratio	31cM	4.23	12.46
12	Bone%	38cM	3.47	10.26
13	Hip width	27cM	2.35	7
13	Muscularity	5cM	3.76	11.1
13	Hot standard carcass weight	0cM	1.12	3.35
13	Meat%	27cM	1.63	4.87
13	Bone weight	32cM	1.94	5.77
13	Bone%	72cM	1.28	3.83
13	Meat to bone ratio	43cM	1.17	3.51
14	Hip width	48cM	1.79	5.33
14	Muscularity	75cM	1.6	4.79
14	Hot standard carcass weight	36cM	6.74	19.67
14	Meat%	30cM	0.69	2.06
14	Bone weight	18cM	10.84	30.97
14	Bone%	66cM	2.15	6.4
14	Meat to bone ratio	66cM	1.92	5.71
15	Hip width	41cM	0.84	2.51
15	Muscularity	0cM	0.77	2.31
15	Hot standard carcass weight	11cM	1.99	5.93
15	Meat%	76cM	1.57	4.68
15	Bone weight	89cM	2.17	6.45
15	Bone%	29cM	1.52	4.53
15	Meat to bone ratio	80cM	1.93	5.75
16	Hip width	84cM	1.92	5.73
16	Muscularity	0cM	2.14	6.36
16	Hot standard carcass weight	84cM	2.31	6.88
16	Meat%	28cM	2.62	7.76
16	Bone weight	84cM	1.62	4.84
16	Bone%	81cM	0.7	2.08
16	Meat to bone ratio	11cM	1.48	4.41

BTA	Trait	Position	F-value	Lilkelihood ratio
17	Hip width	88cM	1.07	3.21
17	Muscularity	0cM	2.04	6.08
17	Hot standard carcass weight	85cM	4.09	12.06
17	Meat%	38cM	6.07	17.74
17	Bone weight	90cM	1.71	5.09
17	Bone%	83cM	3.9	11.51
17	Meat to bone ratio	82cM	4.42	13
18	Hip width	0cM	0.62	1.87
18	Muscularity	6cM	3.66	10.83
18	Hot standard carcass weight	0cM	1.95	5.82
18	Meat%	3cM	2.91	8.62
18	Bone weight	81cM	1.82	5.41
18	Bone%	57cM	2.42	7.2
18	Meat to bone ratio	45cM	2.84	8.41
19	Hip width	16cM	1.24	3.7
19	Muscularity	16cM	1.25	3.73
19	Hot standard carcass weight	59cM	1.5	4.48
19	Meat%	90cM	1.45	4.32
19	Bone weight	59cM	0.93	2.79
19	Bone%	71cM	0.68	2.02
19	Meat to bone ratio	46cM	0.53	1.58
20	Hip width	0cM	1.65	4.93
20	Muscularity	54cM	1.42	4.24
20	Hot standard carcass weight	56cM	1.18	3.52
20	Meat%	56cM	1.61	4.8
20	Bone weight	36cM	1.71	5.1
20	Bone%	16cM	2.3	6.84
20	Meat to bone ratio	56cM	1.83	5.46
21	Hip width	74cM	2.81	8.33
21	Muscularity	45cM	1.32	3.94
21	Hot standard carcass weight	0cM	1.31	3.9
21	Meat%	67cM	2.79	8.28
21	Bone weight	1cM	2.76	8.2
21	Bone%	30cM	2.13	6.34
21	Meat to bone ratio	30cM	2.19	6.51
22	Hip width	80cM	0.86	2.58
22	Muscularity	46cM	0.73	2.18
22	Hot standard carcass weight	28cM	1.96	5.84
22	Meat%	63cM	0.92	2.76
22	Bone weight	28cM	2.47	7.32
22	Bone%	26cM	1.24	3.71
22	Meat to bone ratio	26cM	1.05	3.13

BTA	Trait	Position	F-value	Likelihood ratio
23	Hip width	15cM	1.26	3.77
23	Muscularity	69cM	1.63	4.85
23	Hot standard carcass weight	69cM	2.52	7.48
23	Meat%	42cM	0.59	1.76
23	Bone weight	17cM	2.96	8.78
23	Bone%	47cM	1.69	5.05
23	Meat to bone ratio	50cM	1.74	5.2
24	Hip width	0cM	2.31	6.88
24	Muscularity	0cM	1.79	5.32
24	Hot standard carcass weight	0cM	1.26	3.78
24	Meat%	48cM	0.76	2.27
24	Bone weight	0cM	1.86	5.53
24	Bone%	0cM	0.47	1.41
24	Meat to bone ratio	0cM	0.68	2.03
25	Hip width	21cM	3	8.89
25	Muscularity	0cM	2.38	7.09
25	Hot standard carcass weight	28cM	2.52	7.5
25	Meat%	0cM	1.37	4.1
25	Bone weight	22cM	3.97	11.71
25	Bone%	12cM	1.87	5.58
25	Meat to bone ratio	9cM	0.78	2.34
26	Hip width	60cM	1.67	4.98
26	Muscularity	69cM	0.81	2.42
26	Hot standard carcass weight	56cM	1.51	4.51
26	Meat%	0cM	1.57	4.7
26	Bone weight	69cM	0.79	2.35
26	Bone%	0cM	0.75	2.26
26	Meat to bone ratio	0cM	1.54	4.58
27	Hip width	0cM	0.98	2.94
27	Muscularity	45cM	1.6	4.78
27	Hot standard carcass weight	45cM	1.23	3.66
27	Meat%	10cM	2.28	6.76
27	Bone weight	45cM	2.1	6.24
27	Bone%	10cM	1.15	3.44
27	Meat to bone ratio	10cM	2.21	6.58
28	Hip width	41cM	1.01	3.01
28	Muscularity	0cM	0.65	1.95
28	Hot standard carcass weight	50cM	1.78	5.31
28	Meat%	0cM	0.79	2.37
28	Bone weight	35cM	0.96	2.87
28	Bone%	52cM	1.85	5.5
28	Meat to bone ratio	52cM	1.32	3.94

BTA	Trait	Position	F-value	Likelihood ratio
29	Hip width	20cM	1.5	4.48
29	Muscularity	40cM	1.4	4.19
29	Hot standard carcass weight	24cM	1.25	3.72
29	Meat%	61cM	1.2	3.6
29	Bone weight	1cM	2.74	8.12
29	Bone%	0cM	1.32	3.93
29	Meat to bone ratio	1cM	1.84	5.48

Appendix 4. QTL results with hot standard carcass weight as covariate (cohort and breed as fixed effects)

BTA	Trait	Position	F-value	Likelihood ratio
1	Meat weight	0cM	2.47	7.33
1	Eye muscle area	133cM	2.31	6.86
1	Silverside weight	60cM	3.76	11.12
2	Meat weight	6cM	17.27	47.99
2	Eye muscle area	2cM	11.22	32.11
2	Silverside weight	4cM	12.64	35.94
3	Meat weight	100cM	4.06	11.95
3	Eye muscle area	0cM	2.07	6.15
3	Silverside weight	100cM	2.26	6.72
4	Meat weight	37cM	4.28	12.59
4	Eye muscle area	21cM	2.50	7.42
4	Silverside weight	12cM	3.04	9.01
5	Meat weight	109cM	1.68	5.02
5	Eye muscle area	109cM	1.45	4.33
5	Silverside weight	80cM	1.21	3.61
6	Meat weight	39cM	1.93	5.74
6	Eye muscle area	0cM	2.50	7.44
6	Silverside weight	60cM	1.12	3.36
7	Meat weight	90cM	2.49	7.40
7	Eye muscle area	60cM	1.67	4.98
7	Silverside weight	88cM	2.09	6.21
8	Meat weight	67cM	1.75	5.22
8	Eye muscle area	53cM	2.62	7.78
8	Silverside weight	0cM	1.19	3.55
9	Meat weight	101cM	0.66	1.97
9	Eye muscle area	46cM	1.72	5.14
9	Silverside weight	104cM	0.77	2.32
10	Meat weight	98cM	1.49	4.44
10	Eye muscle area	13cM	2.93	8.69
10	Silverside weight	96cM	1.78	5.31
11	Meat weight	50cM	1.00	2.98
11	Eye muscle area	7cM	2.24	6.68
11	Silverside weight	80cM	1.92	5.73
12	Meat weight	25cM	3.65	10.78
12	Eye muscle area	52cM	3.89	11.48
12	Silverside weight	4cM	3.76	11.12
13	Meat weight	3cM	1.73	5.16
13	Eye muscle area	50cM	2.08	6.19
13	Silverside weight	52cM	1.51	4.50

BTA	Trait	Position	F-value	Likelihood ratio
14	Meat weight	0cM	0.75	2.25
14	Eye muscle area	12cM	2.88	8.55
14	Silverside weight	32cM	0.68	2.05
15	Meat weight	76cM	1.39	4.15
15	Eye muscle area	23cM	1.43	4.28
15	Silverside weight	100cM	1.73	5.17
16	Meat weight	30cM	2.71	8.03
16	Eye muscle area	30cM	3.31	9.81
16	Silverside weight	12cM	1.62	4.82
17	Meat weight	37cM	4.87	14.29
17	Eye muscle area	76cM	3.68	10.89
17	Silverside weight	40cM	6.16	18.00
18	Meat weight	5cM	2.73	8.09
18	Eye muscle area	44cM	2.33	6.92
18	Silverside weight	8cM	3.15	9.34
19	Meat weight	87cM	1.86	5.54
19	Eye muscle area	92cM	1.08	3.24
19	Silverside weight	8cM	0.53	1.59
20	Meat weight	56cM	1.46	4.34
20	Eye muscle area	31cM	2.60	7.72
20	Silverside weight	0cM	1.90	5.65
21	Meat weight	66cM	2.40	7.13
21	Eye muscle area	63cM	1.72	5.14
21	Silverside weight	72cM	2.20	6.54
22	Meat weight	74cM	1.27	3.78
22	Eye muscle area	0cM	1.41	4.22
22	Silverside weight	0cM	1.50	4.46
23	Meat weight	37cM	0.56	1.67
23	Eye muscle area	37cM	1.04	3.12
23	Silverside weight	56cM	0.95	2.84
24	Meat weight	48cM	1.03	3.09
24	Eye muscle area	0cM	0.72	2.17
24	Silverside weight	48cM	0.89	2.67
25	Meat weight	0cM	1.89	5.62
25	Eye muscle area	0cM	1.74	5.19
25	Silverside weight	0cM	1.06	3.16
26	Meat weight	0cM	1.52	4.54
26	Eye muscle area	0cM	1.06	3.18
26	Silverside weight	4cM	2.14	6.35
27	Meat weight	8cM	2.13	6.33
27	Eye muscle area	45cM	0.67	2
27	Silverside weight	8cM	2.97	8.81

BTA	Trait	Position	F-value	Likelihood ratio
28	Meat weight	0cM	0.74	2.20
28	Eye muscle area	17cM	1.83	5.45
28	Silverside weight	0cM	0.76	2.27
29	Meat weight	61cM	1.21	3.63
29	Eye muscle area	0cM	0.37	1.12
29	Silverside weight	60cM	1.81	5.38

Appendix 5. QTL results for meat weight with bone weight as covariate (cohort and breed as fixed effects)

BTA	Traits	Position	F-value	Likelihood ratio
1	Meat weight	98cM	3.47	10.26
2	Meat weight	8cM	10.96	31.3
3	Meat weight	95cM	1.35	4.03
4	Meat weight	12cM	2.01	5.99
5	Meat weight	32cM	2.2	6.55
6	Meat weight	119cM	2.41	7.16
7	Meat weight	0cM	2.3	6.82
8	Meat weight	17cM	4.19	12.34
9	Meat weight	101cM	2.21	6.57
10	Meat weight	49cM	2.88	8.54
11	Meat weight	70cM	2.45	7.26
12	Meat weight	30cM	3.29	9.74
13	Meat weight	43cM	1.14	3.41
14	Meat weight	68cM	1.52	4.54
15	Meat weight	80cM	2.15	6.39
16	Meat weight	11cM	1.26	3.77
17	Meat weight	82cM	4.74	13.93
18	Meat weight	45cM	3.28	9.7
19	Meat weight	46cM	0.33	0.99
20	Meat weight	56cM	1.99	5.91
21	Meat weight	30cM	1.95	5.79
22	Meat weight	26cM	1.1	3.3
23	Meat weight	50cM	1.69	5.05
24	Meat weight	0cM	0.81	2.41
25	Meat weight	9cM	0.62	1.85
26	Meat weight	0cM	1.49	4.45
27	Meat weight	10cM	2.05	6.1
28	Meat weight	52cM	1.79	5.32
29	Meat weight	2cM	0.99	2.97

Appendix 6. QTL results for meat percentage with bone percentage as covariate (cohort and breed as fixed effects)

BTA	Traits	Position	F-value	Likelihood ratio
1	Meat%	0cM	1.72	5.11
2	Meat%	5cM	17.31	48.07
3	Meat%	100cM	4.45	13.1
4	Meat%	36cM	3.61	10.65
5	Meat%	109cM	1.84	5.48
6	Meat%	41cM	1.66	4.94
7	Meat%	91cM	2.61	7.74
8	Meat%	60cM	1.88	5.6
9	Meat%	104cM	0.36	1.08
10	Meat%	96cM	1.47	4.39
11	Meat%	59cM	1.04	3.11
12	Meat%	23cM	2.94	8.72
13	Meat%	27cM	1.67	4.98
14	Meat%	0cM	0.91	2.72
15	Meat%	76cM	1.16	3.47
16	Meat%	26cM	2.66	7.89
17	Meat%	38cM	5.11	14.98
18	Meat%	3cM	3.05	9.03
19	Meat%	90cM	1.94	5.78
20	Meat%	17cM	1.3	3.89
21	Meat%	68cM	2.76	8.2
22	Meat%	61cM	1.08	3.22
23	Meat%	37cM	0.33	1
24	Meat%	48cM	0.7	2.09
25	Meat%	0cM	2.26	6.71
26	Meat%	2cM	1.19	3.54
27	Meat%	8cM	1.88	5.59
28	Meat%	0cM	0.93	2.78
29	Meat%	61cM	1.23	3.68

Appendix 7. QTL results for stifle width with hip width as covariate (cohort and breed as fixed effects)

BTA	Traits	Positon	F-value	Likelihood ratio
1	Stifle width	34cM	3.5	10.33
2	Stifle width	0cM	4.93	14.46
3	Stifle width	0cM	1.52	4.54
4	Stifle width	11cM	3.56	10.5
5	Stifle width	84cM	2.76	8.19
6	Stifle width	0cM	1.37	4.09
7	Stifle width	84cM	2.81	8.33
8	Stifle width	8cM	2.6	7.71
9	Stifle width	104cM	4.33	12.75
10	Stifle width	51cM	2.03	6.04
11	Stifle width	85cM	2.47	7.33
12	Stifle width	102cM	1.75	5.21
13	Stifle width	65cM	4.42	13
14	Stifle width	66cM	1.52	4.54
15	Stifle width	0cM	1.34	4.01
16	Stifle width	86cM	2.16	6.43
17	Stifle width	0cM	3.15	9.32
18	Stifle width	4cM	4.66	13.69
19	Stifle width	108cM	0.82	2.47
20	Stifle width	49cM	1.75	5.21
21	Stifle width	0cM	2.11	6.27
22	Stifle width	51cM	1.07	3.21
23	Stifle width	69cM	2.24	6.65
24	Stifle width	0cM	3.5	10.33
25	Stifle width	0cM	1.42	4.24
26	Stifle width	0cM	0.78	2.34
27	Stifle width	45cM	1.46	4.37
28	Stifle width	41cM	1.16	3.48
29	Stifle width	40cM	1.44	4.31

Appendix 8. QTL results (cohort, breed and *myostatin* F94L as fixed effects)

BTA	Trait	Position	F-value	Likelihood ratio
1	Hip Width	107cM	2.36	7.00
1	Muscularity	35cM	2.93	8.70
1	Hot standard carcass weight	87cM	4.44	13.09
1	Meat %	4cM	1.49	4.45
1	Bone weight	84cM	3.24	9.58
1	Bone%	70cM	2.21	6.57
1	Meat to bone ratio	97cM	2.23	6.62
2	Hip Width	20cM	3.60	10.65
2	Muscularity	86cM	2.47	7.34
2	Hot standard carcass weight	27cM	2.15	6.39
2	Meat %	24cM	2.72	8.08
2	Bone weight	98cM	2.96	8.76
2	Bone%	29cM	1.19	3.56
2	Meat to bone ratio	26cM	1.44	4.30
3	Hip Width	44cM	1.22	3.64
3	Muscularity	0cM	1.77	5.28
3	Hot standard carcass weight	51cM	1.21	3.61
3	Meat %	98cM	4.92	14.44
3	Bone weight	8cM	2.25	6.69
3	Bone%	16cM	1.29	3.85
3	Meat to bone ratio	16cM	1.74	5.19
4	Hip Width	33cM	2.62	7.78
4	Muscularity	9cM	3.44	10.19
4	Hot standard carcass weight	78cM	1.13	3.39
4	Meat %	38cM	2.62	7.78
4	Bone weight	78cM	1.22	3.65
4	Bone%	89cM	1.96	5.84
4	Meat to bone ratio	13cM	1.40	4.19
5	Hip Width	52cM	1.79	5.34
5	Muscularity	86cM	2.26	6.72
5	Hot standard carcass weight	42cM	5.66	16.58
5	Meat %	0cM	1.03	3.09
5	Bone weight	42cM	2.54	7.53
5	Bone%	36cM	1.81	5.39
5	Meat to bone ratio	30cM	1.30	3.88

BTA	Trait	Position	F-value	Likelihood ratio
6	Hip Width	0cM	1.77	5.28
6	Muscularity	2cM	3.13	9.27
6	Hot standard carcass weight	119cM	2.24	6.66
6	Meat %	32cM	1.82	5.41
6	Bone weight	41cM	1.50	4.49
6	Bone%	119cM	1.54	4.60
6	Meat to bone ratio	60cM	1.79	5.34
7	Hip Width	0cM	4.85	14.28
7	Muscularity	82cM	2.19	6.52
7	Hot standard carcass weight	0cM	1.68	5.01
7	Meat %	62cM	2.52	7.47
7	Bone weight	10cM	1.66	4.95
7	Bone%	117cM	2.28	6.78
7	Meat to bone ratio	0cM	2.02	6.02
8	Hip Width	17cM	2.78	8.25
8	Muscularity	8cM	2.51	7.46
8	Hot standard carcass weight	8cM	3.04	9.01
8	Meat %	48cM	1.08	3.23
8	Bone weight	66cM	2.28	6.77
8	Bone%	15cM	3.91	11.54
8	Meat to bone ratio	9cM	3.42	10.11
9	Hip Width	60cM	0.69	2.06
9	Muscularity	104cM	4.47	13.17
9	Hot standard carcass weight	60cM	2.15	6.39
9	Meat %	46cM	0.39	1.16
9	Bone weight	60cM	2.67	7.91
9	Bone%	22cM	1.60	4.78
9	Meat to bone ratio	98cM	1.87	5.56
10	Hip Width	43cM	2.06	6.14
10	Muscularity	98cM	2.81	8.35
10	Hot standard carcass weight	58cM	1.74	5.20
10	Meat %	90cM	2.01	5.99
10	Bone weight	16cM	1.38	4.13
10	Bone%	37cM	2.97	8.80
10	Meat to bone ratio	49cM	2.98	8.84
11	Hip Width	78cM	3.45	10.20
11	Muscularity	85cM	4.51	13.28
11	Hot standard carcass weight	111cM	1.10	3.28
11	Meat %	50cM	0.73	2.19
11	Bone weight	10cM	1.61	4.81
11	Bone%	70cM	2.41	7.17
11	Meat to bone ratio	70cM	2.15	6.39

BTA	Trait	Position	F-value	Likelihood ratio
12	Hip Width	57cM	2.74	8.13
12	Muscularity	15cM	3.43	10.14
12	Hot standard carcass weight	0cM	0.90	2.70
12	Meat %	9cM	2.34	6.97
12	Bone weight	4cM	3.12	9.23
12	Bone%	37cM	2.80	8.32
12	Meat to bone ratio	28cM	3.04	9.00
13	Hip Width	26cM	2.35	6.98
13	Muscularity	2cM	3.14	9.29
13	Hot standard carcass weight	0cM	1.40	4.17
13	Meat %	88cM	0.94	2.81
13	Bone weight	33cM	1.77	5.28
13	Bone%	43cM	0.94	2.81
13	Meat to bone ratio	43cM	1.05	3.15
14	Hip Width	37cM	1.61	4.80
14	Muscularity	77cM	1.13	3.39
14	Hot standard carcass weight	34cM	6.90	20.11
14	Meat %	54cM	1.20	3.58
14	Bone weight	17cM	10.70	30.59
14	Bone%	66cM	2.38	7.07
14	Meat to bone ratio	66cM	2.52	7.49
15	Hip Width	44cM	0.77	2.31
15	Muscularity	0cM	0.77	2.30
15	Hot standard carcass weight	8cM	1.76	5.24
15	Meat %	80cM	1.43	4.26
15	Bone weight	89cM	2.17	6.45
15	Bone%	29cM	1.17	3.50
15	Meat to bone ratio	80cM	1.87	5.57
16	Hip Width	84cM	1.80	5.36
16	Muscularity	0cM	1.79	5.33
16	Hot standard carcass weight	84cM	2.05	6.09
16	Meat %	11cM	0.40	1.19
16	Bone weight	84cM	1.84	5.49
16	Bone%	5cM	0.75	2.23
16	Meat to bone ratio	7cM	1.12	3.35

BTA	Trait	Position	F-value	Likelihood ratio
17	Hip Width	90cM	1.23	3.69
17	Muscularity	0cM	2.21	6.58
17	Hot standard carcass weight	86cM	3.66	10.82
17	Meat %	39cM	2.92	8.64
17	Bone weight	90cM	1.76	5.25
17	Bone%	83cM	3.25	9.60
17	Meat to bone ratio	82cM	3.63	10.72
18	Hip Width	0cM	0.55	1.66
18	Muscularity	4cM	4.13	12.19
18	Hot standard carcass weight	0cM	1.56	4.66
18	Meat %	3cM	2.43	7.22
18	Bone weight	81cM	1.80	5.37
18	Bone%	60cM	2.03	6.03
18	Meat to bone ratio	57cM	1.89	5.63
19	Hip Width	16cM	1.31	3.92
19	Muscularity	100cM	1.06	3.17
19	Hot standard carcass weight	59cM	1.67	4.97
19	Meat %	96cM	1.97	5.86
19	Bone weight	57cM	0.94	2.82
19	Bone%	74cM	0.89	2.65
19	Meat to bone ratio	0cM	1.44	4.29
20	Hip Width	54cM	1.47	4.39
20	Muscularity	49cM	0.91	2.72
20	Hot standard carcass weight	54cM	0.86	2.58
20	Meat %	10cM	1.94	5.77
20	Bone weight	35cM	1.83	5.46
20	Bone%	17cM	2.31	6.86
20	Meat to bone ratio	17cM	1.78	5.29
21	Hip Width	75cM	2.75	8.15
21	Muscularity	0cM	1.05	3.14
21	Hot standard carcass weight	0cM	1.53	4.57
21	Meat %	69cM	2.50	7.43
21	Bone weight	0cM	2.86	8.47
21	Bone%	30cM	1.69	5.03
21	Meat to bone ratio	29cM	1.73	5.14
22	Hip Width	80cM	0.75	2.24
22	Muscularity	46cM	1.08	3.24
22	Hot standard carcass weight	28cM	1.82	5.42
22	Meat %	61cM	0.79	2.37
22	Bone weight	28cM	2.52	7.48
22	Bone%	0cM	1.12	3.35
22	Meat to bone ratio	0cM	1.16	3.47

BTA	Trait	Position	F-value	Likelihood ratio
23	Hip Width	14cM	1.36	4.07
23	Muscularity	69cM	1.64	4.89
23	Hot standard carcass weight	53cM	2.35	7.00
23	Meat %	53cM	0.99	2.97
23	Bone weight	15cM	3.05	9.04
23	Bone%	49cM	1.73	5.16
23	Meat to bone ratio	53cM	2.13	6.35
24	Hip Width	0cM	2.27	6.76
24	Muscularity	0cM	2.03	6.06
24	Hot standard carcass weight	0cM	1.22	3.64
24	Meat %	0cM	0.30	0.89
24	Bone weight	0cM	1.96	5.84
24	Bone%	36cM	0.53	1.57
24	Meat to bone ratio	0cM	0.70	2.10
25	Hip Width	22cM	2.95	8.74
25	Muscularity	0cM	2.64	7.85
25	Hot standard carcass weight	29cM	2.60	7.73
25	Meat %	32cM	1.38	4.11
25	Bone weight	21cM	4.01	11.83
25	Bone%	12cM	2.35	6.99
25	Meat to bone ratio	11cM	1.47	4.38
26	Hip Width	60cM	2.04	6.08
26	Muscularity	69cM	0.79	2.35
26	Hot standard carcass weight	57cM	1.44	4.29
26	Meat %	0cM	0.21	0.63
26	Bone weight	69cM	0.73	2.20
26	Bone%	45cM	0.55	1.64
26	Meat to bone ratio	0cM	0.67	1.99
27	Hip Width	0cM	1.13	3.37
27	Muscularity	44cM	1.91	5.68
27	Hot standard carcass weight	50cM	1.40	4.19
27	Meat %	47cM	0.89	2.66
27	Bone weight	45cM	1.97	5.87
27	Bone%	64cM	0.70	2.08
27	Meat to bone ratio	64cM	0.91	2.72

BTA	Trait	Position	F-value	Likelihood ratio
28	Hip Width	41cM	1.13	3.39
28	Muscularity	41cM	0.54	1.61
28	Hot standard carcass weight	51cM	1.96	5.83
28	Meat %	0cM	1.80	5.37
28	Bone weight	52cM	0.88	2.63
28	Bone%	52cM	2.08	6.18
28	Meat to bone ratio	52cM	2.00	5.96
29	Hip Width	21cM	1.58	4.71
29	Muscularity	40cM	1.24	3.71
29	Hot standard carcass weight	22cM	1.31	3.90
29	Meat %	22cM	0.34	1.02
29	Bone weight	1cM	2.56	7.59
29	Bone%	0cM	1.12	3.36
29	Meat to bone ratio	0cM	1.54	4.61

Appendix 9. QTL results with hot standard carcass weight as covariate (cohort, breed and MSTN F94L as fixed effect)

BTA	Trait	Position	F-value	Likelihood ratio
1	Meat weight	1cM	1.40	4.18
1	Eye muscle area	133cM	1.92	5.73
1	Silverside weight	60cM	2.05	6.11
2	Meat weight	29cM	2.31	6.86
2	Eye muscle area	104cM	1.39	4.16
2	Silverside weight	48cM	1.72	5.13
3	Meat weight	95cM	4.62	13.59
3	Eye muscle area	40cM	1.83	5.45
3	Silverside weight	96cM	2.9	8.59
4	Meat weight	39cM	3.49	10.30
4	Eye muscle area	24cM	1.83	5.46
4	Silverside weight	12cM	2.04	6.07
5	Meat weight	0cM	1.56	4.66
5	Eye muscle area	109cM	0.99	2.95
5	Silverside weight	12cM	1.2	3.58
6	Meat weight	36cM	1.97	5.86
6	Eye muscle area	0cM	2.35	7.00
6	Silverside weight	60cM	1	2.99
7	Meat weight	62cM	2.85	8.44
7	Eye muscle area	43cM	2.27	6.76
7	Silverside weight	68cM	1.83	5.46
8	Meat weight	90cM	1.11	3.32
8	Eye muscle area	53cM	1.76	5.25
8	Silverside weight	88cM	1.47	4.37
9	Meat weight	73cM	0.51	1.52
9	Eye muscle area	46cM	1.90	5.65
9	Silverside weight	104cM	0.86	2.57
10	Meat weight	92cM	2.18	6.47
10	Eye muscle area	13cM	3.51	10.39
10	Silverside weight	92cM	1.97	5.87
11	Meat weight	51cM	0.98	2.92
11	Eye muscle area	16cM	1.54	4.60
11	Silverside weight	80cM	2.16	6.44
12	Meat weight	6cM	2.38	7.07
12	Eye muscle area	58cM	3.47	10.27
12	Silverside weight	0cM	2.98	8.83
13	Meat weight	88cM	1.31	3.92
13	Eye muscle area	50cM	1.26	3.76
13	Silverside weight	84cM	1	2.98

BTA	Trait	Position	F-value	Likelihood ratio
14	Meat weight	0cM	1.00	3.00
14	Eye muscle area	12cM	3.36	9.96
14	Silverside weight	52cM	1.73	5.15
15	Meat weight	80cM	1.45	4.34
15	Eye muscle area	17cM	1.44	4.31
15	Silverside weight	100cM	1.05	3.13
16	Meat weight	41cM	0.79	2.35
16	Eye muscle area	85cM	3.33	9.87
16	Silverside weight	0cM	0.39	1.16
17	Meat weight	38cM	2.05	6.11
17	Eye muscle area	77cM	3.66	10.81
17	Silverside weight	40cM	3.81	11.26
18	Meat weight	4cM	2.47	7.33
18	Eye muscle area	52cM	2.06	6.14
18	Silverside weight	12cM	2.92	8.65
19	Meat weight	0cM	2.56	7.61
19	Eye muscle area	7cM	0.95	2.84
19	Silverside weight	16cM	0.94	2.8
20	Meat weight	15cM	1.70	5.07
20	Eye muscle area	5cM	2.80	8.30
20	Silverside weight	0cM	3.07	9.09
21	Meat weight	69cM	2.20	6.55
21	Eye muscle area	63cM	1.51	4.52
21	Silverside weight	72cM	1.43	4.26
22	Meat weight	61cM	1.04	3.10
22	Eye muscle area	10cM	2.05	6.11
22	Silverside weight	64cM	1.55	4.63
23	Meat weight	53cM	0.87	2.61
23	Eye muscle area	37cM	0.88	2.64
23	Silverside weight	56cM	1.16	3.46
24	Meat weight	0cM	0.45	1.34
24	Eye muscle area	0cM	0.74	2.20
24	Silverside weight	44cM	0.5	1.51
25	Meat weight	0cM	1.59	4.74
25	Eye muscle area	0cM	1.46	4.34
25	Silverside weight	0cM	0.75	2.25
26	Meat weight	0cM	0.34	1.01
26	Eye muscle area	60cM	0.78	2.33
26	Silverside weight	4cM	0.99	2.96
27	Meat weight	48cM	1.08	3.24
27	Eye muscle area	39cM	2.00	5.96
27	Silverside weight	8cM	1.92	5.73

BTA	Trait	Position	F-value	Likelihood ratio
28	Meat weight	0cM	1.39	4.16
28	Eye muscle area	41cM	3.08	9.13
28	Silverside weight	0cM	0.66	1.98
29	Meat weight	40cM	0.45	1.34
29	Eye muscle area	40cM	0.40	1.20
29	Silverside weight	64cM	1.23	3.66

Appendix 10. QTL result for meat weight with bone weight as covariate (cohort, breed and MSTN F94L as fixed effects)

BTA	Traits	Position	F-value	Likelihood ratio
1	Meat weight	99cM	2.85	8.44
2	Meat weight	24cM	2.39	7.1
3	Meat weight	16cM	1.17	3.49
4	Meat weight	12cM	1.51	4.52
5	Meat weight	30cM	1.57	4.69
6	Meat weight	119cM	2.03	6.04
7	Meat weight	0cM	1.77	5.29
8	Meat weight	8cM	4.28	12.59
9	Meat weight	60cM	2.12	6.3
10	Meat weight	49cM	3.29	9.72
11	Meat weight	70cM	1.59	4.73
12	Meat weight	7cM	2.32	6.88
13	Meat weight	43cM	1.02	3.06
14	Meat weight	64cM	2.15	6.39
15	Meat weight	80cM	2.04	6.07
16	Meat weight	1cM	1.13	3.38
17	Meat weight	82cM	3.92	11.57
18	Meat weight	57cM	2.15	6.41
19	Meat weight	5cM	1.16	3.46
20	Meat weight	17cM	1.82	5.42
21	Meat weight	27cM	1.48	4.42
22	Meat weight	45cM	1.17	3.5
23	Meat weight	53cM	2.16	6.41
24	Meat weight	0cM	0.6	1.81
25	Meat weight	9cM	1.21	3.61
26	Meat weight	49cM	0.83	2.5
27	Meat weight	64cM	1.49	4.43
28	Meat weight	52cM	2.54	7.54
29	Meat weight	0cM	0.86	2.57

Appendix 11. QTL results for traits using bone percentage as covariate (cohort, breed and MSTN F94L as fixed effect)

BTA	Traits	Position	F-value	Likelihood ratio
1	Meat %	3cM	1.34	4.01
2	Meat %	23cM	2.44	7.24
3	Meat %	98cM	5.13	15.04
4	Meat %	39cM	2.76	8.17
5	Meat %	0cM	1.18	3.52
6	Meat %	34cM	1.72	5.12
7	Meat %	62cM	2.56	7.61
8	Meat %	48cM	0.82	2.44
9	Meat %	46cM	0.3	0.89
10	Meat %	89cM	2.12	6.31
11	Meat %	111cM	0.87	2.6
12	Meat %	8cM	2.01	5.99
13	Meat %	5cM	1.1	3.27
14	Meat %	0cM	1.1	3.27
15	Meat %	80cM	1.2	3.58
16	Meat %	72cM	0.43	1.28
17	Meat %	39cM	2.65	7.85
18	Meat %	3cM	2.54	7.55
19	Meat %	95cM	2.18	6.49
20	Meat %	11cM	2.14	6.38
21	Meat %	70cM	2.61	7.76
22	Meat %	61cM	0.82	2.47
23	Meat %	53cM	0.77	2.32
24	Meat %	0cM	0.31	0.92
25	Meat %	0cM	1.76	5.23
26	Meat %	0cM	0.16	0.49
27	Meat %	35cM	0.92	2.76
28	Meat %	0cM	1.87	5.56
29	Meat %	64cM	0.35	1.05

Appendix 12. QTL results for stifle width with hip width as covariate (cohort, breed and MSTN F94L as fixed effects)

BTA	Traits	Position	F-value	Likelihood ratio
1	Stifle width	35cM	3.6	10.64
2	Stifle width	86cM	2.8	8.29
3	Stifle width	0cM	1.5	4.47
4	Stifle width	11cM	2.7	8.02
5	Stifle width	85cM	2.61	7.74
6	Stifle width	2cM	1.45	4.31
7	Stifle width	82cM	2.3	6.82
8	Stifle width	8cM	2.1	6.24
9	Stifle width	104cM	3.94	11.61
10	Stifle width	98cM	2.23	6.64
11	Stifle width	85cM	3.06	9.05
12	Stifle width	102cM	2.07	6.16
13	Stifle width	88cM	4.51	13.25
14	Stifle width	71cM	1.14	3.41
15	Stifle width	0cM	1.41	4.22
16	Stifle width	86cM	2.04	6.06
17	Stifle width	0cM	3.66	10.81
18	Stifle width	2cM	5.28	15.47
19	Stifle width	108cM	1.16	3.45
20	Stifle width	49cM	1.6	4.76
21	Stifle width	0cM	1.99	5.93
22	Stifle width	51cM	1.41	4.2
23	Stifle width	69cM	2.13	6.34
24	Stifle width	1cM	3.94	11.62
25	Stifle width	0cM	1.76	5.25
26	Stifle width	0cM	1.08	3.23
27	Stifle width	41cM	1.89	5.61
28	Stifle width	41cM	0.84	2.5
29	Stifle width	40cM	1.06	3.16

Appendix 13. Identified DNA variants of the candidate genes

SNP name	DNA variants	Sequence context	SNP location	Sires genotype		
				361	368	398
ACVR1 SNP1 A>C 39926559	AC	GTAGCCAAG(A/C)TTTTACC	intron 1	AA	AC	AA
ACVR1 SNP2 G>C 39926633	GC	TTTTATGA(G/C)ACGGGAAAT	intron1	GG	GC	GG
ACVR1 SNP3 C>G 39968723	CG	GTCTTGGAGAC/GACTGCGTGAT	intron 2	CC	CC	CG
ACVR1 SNP4 A>G 39975281	AG	TAAAACTCTGG(A/G)TATAAGAA	intron 4	AG	AA	AA
ACVR1 SNP5 G>A 39975439	GA	GCAGCCTCC(G/A)TGCAAGTTA	intron 4	GA	GG	GG
ACVR1 SNP6 T>C 39975631	TC	TAATTCTCTT(T/C)TCCCTTTCTTT	intron 5	TC	TT	TT
ACVR1 SNP7 C>A 39979144	CA	CACAGTGTT(C/A)ATTGGTGACCTG	intron 5	CA	CA	CA
ACVR1 SNP8 G>C 39986350	GC	ATGGGTGAT(G/C)TCTACTATATG	intron 6	GC	GG	GG
ACVR1 SNP9 A>T 39986726	AT	TAAATTTTT(A/T)AATTTACTA	intron 7	AT	AA	AA
ACVR1 SNP10 G>C 39986801	GC	TAACACTGTTA(G/C)GAAAAATTT	intron7	GC	GG	GG
ACVR1 SNP11 G>A 39986860	GA	TGTATTTACC(G/A)CCTAGATTC	intron 7	GA	GG	GG
ACVR1 SNP12 G>C 39986987	GC	TGGCTTCA(G/C)CATACACACCAC	intron 7	GC	GG	GG

SNP name	DNA variants	Sequence context	SNP location	Sires genotype		
				361	368	398
ACVR1 SNP13 T>A 40000965	TA	TATTTTTT(T/A)AAGACCTAC	intron 8	TA	TT	TT
ACVR1 SNP14 A>C 40000992	AC	TTTAGGATC(A/C)TCTTGAAGTTT	intron 8	AC	AA	AA
SNIP1 SNP1 8bpdel 115537490	8 bp del	GCC(GATCCCTT)CTCC	promoter	no del	8 bp del	no del
SNIP1 SNP2 A>G 115551553	AG	TGTGTGCACA(G/A)GAAGCCCA	intron3	GG	GA	GA
SNIP1 SNP3 C>T 115552932	CT	GGCACCAC/TGGTGCTCTCT	3'UTR	CC	CT	CC
TGFBR3 SNP1 T del 55101724	T del	TAACCACCTTTTTTTTTT(T)CCCAATG	intron 5	T del	T del	T del
TGFBR3 SNP2 G ins 55108232	G in	AATACCCCCCCCC(C)AAATGTGCTT	intron 7	no in	G in	no in
TGFBR3 SNP3 G>A 55110914	AG	TGTTTTGTTGGTGA(G/A)TGTGTGGGAGTTG	intron 9	AG	GG	AG
TGFBR3 SNP4 Tdel 55113488	T IN DEL	CTTGGAGTTTTTTTTTTTT(T)AATTAAGTC	intron 9	in/del	in/del	in/del
TGFBR3 SNP5 A>G 55120372 (Q2R)	AG	TCCTATCCCAC(A/G)AGCCGAG	exon12	AG	AA	AG
TGFBR3 SNP6 A>G 55145704	AG	TTACTAAATTAATGT(A/G)TAAACAGGGGCA	3' flanking region	AG	AG	AG
TGFBR3 SNP7 Adel 55145115	1 bp del	CATGTGAGAAA(A)GCTAAGGTG	3'utr	del/no del	del/no del	del/no del
TGFBR3 SNP8 8bpdel 55123571	8 bp del	GCTGCTGCT(GCTGCTGCTA)AGTCGCTT	intron 13	no del	8 bp del	no del
FSTL5 SNP1 T>C 38190824	CT	TTGAATATTTT(T/C)GATATATTTC	intron 1	TT	TT	CT
FSTL5 SNP2 C>T 38190903 (L2F)	CT	AATAAGGATG(C/T)TTAAGTGCTGGT	exon 2	CC	CC	CT L-F

SNP name	DNA variants	Sequence context	SNP location	Sires genotype		
				361	368	398
FSTL5 SNP3 T>C38191098	CT	CACCTAAGAG(T/C)ACTTATTGCA	intron 2	TT	TT	CT
FSTL5 SNP4 C>A38299977	AC	ATTTATTTAT(C/A)AAATCATTTGT	intron 2	AC	AA	AC
FSTL5 SNP5 A>G38300299	AG	TAAGAAAAA(A/G)AATTGATGCT	intron 3	AA	AG	AA
FSTL5 SNP6 6bpdel 38300026	6bp indel	ATGTACACT(AAGTAT)GATTACTGC	intron2	no del	6 bp del	no del
FSTL5 SNP7 A>G38605563	GA	ACATTGTTT(A/G)ACTGTAGGCA	intron 5	GG	GA	GA
FSTL5 SNP8 A>G38776959	GA	CCTGACACTTT(A/G)TCTTTTCAG	intron 6	GA	GA	GA
FSTL5 SNP9 A>T38777176	AT	TGTGTATCT(A/T)GTGTGATATA	intron 7	AT	TT	AT
FSTL5 SNP10 A>G38868797	AG	GTGGGATT(A/G)TTTTAATT	intron8	GA	GG	GA
FSTL5 SNP11 Gdel38920507	G DEL	AAAAAAAGTTAT(G)TTAGTACCTA	intron9	no del	G del	G del
FSTL5 SNP12 A>C39017181	AC	TCTTTTTTGAA(A/C)GTGAAAGTAG	intron 13	AC	AA	AC
FSTL5 SNP13 A>C38999331	AC	GGGGGAAA(A/C)AAAAGTTGA	intron14	AA	AA	AC
FSTL5 SNP14 C>T38995159	CT	TGGACGATTT(C/T)TTCATTCCCA	exon15	CT	CC	CC
FSTL5 SNP15 Tdel38643498	T del	TTTTTT(T)GTCTAC	intron 3	T del	T del	T del
FSTL5 SNP16 In/del38925460	in/del	CACACACACACACACACGG	intron 10	in/del	in/del	in/del

Appendix 14. Genotyping condition for genotyped SNPs

SNP Name	SNP sequence	Primers Sequence	HRM Condition			
			Hold	Cycling	Hold 2	Melting
ACVR6 SNP6	CACAGTGTT(C/A)ATTGGTGACC	CTGCTGGAGTGTGTTGGT (F) CACAGAACCCACAAAGAAA (R)	95°, 10 min	Cycle repeat 45x 95º, 20 sec 60º, 30sec (touchdown) 72º, 20sec (acquiring on green)	72º,10 min	60º-90º
ACVR6 SNP7	ATGGGTGAT(G/C)TCTACTATAT	AAGGGAGGGTTGAGAGTGG (F) GTGACACTGGGCAGGAGG (R)	95°, 10 min	Cycle repeat 45x 95º, 20 sec 60º, 30sec (touchdown) 72º, 20sec (acquiring on green)	72º,10 min	65º-90º
SNIP1 SNP2	TGTGTGCACA(G/A)GAAGCCCA	CATCTGCGGAGGAAACCA (F) GCAGGTTCAGCCAAAAAAAT (R)	95°, 10 min	Cycle repeat 45x 95º, 20 sec 60º, 30sec (touchdown) 72º, 20sec (acquiring on green)	72º,10 min	65º-90º
SNIP1 SNP3	GTGTGCACA(G/A)GAAGCCCAT	ACGAGGAAGAGGAAGAGGTG (F) TGGTCAGCAACAGAAGAAAGA (R)	95°, 10 min	Cycle repeat 45x 95º, 20 sec 60º, 30sec (touchdown) 72º, 20sec (acquiring on green)	-	75º-90º

SNP Name	SNP sequence	Primers sequence	HRM Condition			
			Hold	Cycling	Hold 2	Melting
TGFBR3 SNP5	TCCTATCCCAC(A/G)AGCCGAG	CATTGAGAACATTTGTCCTA (F) TCCTTTTCCTTTTTGGTA (R)	95°, 10 min	Cycle repeat 50x 95º, 20 sec 60º, 30sec (touchdown) 72º, 20sec (acquiring on green)	-	70º-95º
TGFBR3 SNP6	ACTAAATTAATGT(A/G)TAAAC	CCAGGCTCACACACCAAC (F) ATCACTGTGCAAATGCCT (R)	95°, 10 min	Cycle repeat 45x 95º, 20 sec 60º, 30sec (touchdown) 72º, 20sec (acquiring on green)	72º,10 min	65º-90º
FSTL5 SNP2	AATAAGGATG(C/T)TTAAGTGCT	CTTACCTATTATGGATTCAGTTG (F) ATCCTTATTGCTTTTCACCT (R)	95°, 10 min	Cycle repeat 45x 95º, 20 sec 60º, 30sec (touchdown) 72º, 20sec (acquiring on green)	72º,10 min	70º-90º
FSTL5 SNP5	TAAGAAAAA(A/G)AATTGATGC	TCAAAGGTAAGGAGTAACAAT (F) ATTTCAATTATACTTTCAAGGT (R)	95°, 10 min	Cycle repeat 50x 95º, 20 sec 55º, 30sec (touchdown) 72º, 20sec (acquiring on green)	72º,10 min	65º-90º

SNP Name	SNP sequence	Primers sequence	HRM Condition			
			Hold	Cycling	Hold 2	Melting
FSTL5 SNP8	CCTGACACTTT(A/G)TCTTTTCA	CTTTTCTCCATTTTATACAA (F) GCTGTGACGCTTAGTTT (R)	95°, 10 min	Cycle repeat 45x 95º, 20 sec 55º, 30sec (touchdown) 72º, 20sec (acquiring on green)	-	75º-90º
FSTL5 SNP14	TGGACGATTT(C/T)TTCATTCCC	TACCACACCACACCATCCAC (F) TGCTCATCCAATCACTCACC (R)	95°, 10 min	Cycle repeat 40x 95º, 20 sec 60º, 30sec (touchdown) 72º, 20sec (acquiring on green)	-	70º-90º

(F)= forward primer, (R)= reverse primer

Appendix 15. Genotype frequency of each SNPs and *myostatin* F94L genotype

SNPs	MSTN									ΣSNPs genotype			
	CC (Σ=116)			AC (Σ=188)			AA(Σ=54)						
	1	2	3	1	2	3	1	2	3	1	2	3	
ACVR1 SNP6	2	20	94	0	44	142	1	15	37	**3**	**79**	**273**	1(CC),2(CT),3(TT)
ACVR1 SNP7	42	54	20	39	107	42	10	20	24	**91**	**181**	**86**	1(AA),2(CA),3(CC)
SNIP1 SNP2	25	61	30	36	97	55	7	24	23	**68**	**182**	**108**	1(AA),2(GA),3(GG
SNIP1 SNP3	54	55	7	102	76	10	38	14	2	**194**	**145**	**19**	1(CC),2(CT),3(TT)
TGFBR3 SNP5	20	72	24	64	102	22	34	17	3	**118**	**191**	**49**	1(AA),2(AG),3(GG)
TGFBR3 SNP6	9	62	45	23	94	71	20	22	12	**52**	**178**	**128**	1(AA),2(AG),3(GG)
FSTL5 SNP2	83	31	2	114	66	8	17	33	4	**214**	**130**	**14**	1(CC),2(CT),3(TT)
FSTL5 SNP5	88	26	2	137	47	4	35	18	1	**260**	**91**	**7**	1(AA),2(AG),3(GG)
FSTL5 SNP8	43	54	19	51	95	42	12	26	16	**106**	**175**	**77**	1(AA),2(AG),3(GG)
FSTL5 SNP14	95	20	0	155	32	1	42	12	0	**292**	**64**	**0**	1(CC),2(CT),3(TT)
IGF1 SNP1	17	55	44	23	107	58	14	24	16	**54**	**186**	**118**	1(CC),2(CT),3(TT)
IGF1 SNP2	84	30	2	112	65	11	34	18	2	**230**	**113**	**15**	1(CC),2(CT),3(TT)
FST SNP5	13	65	38	12	87	87	0	7	46	**25**	**159**	**171**	1(GG),2(AG),3(AA)
FST SNP7	94	21	1	152	34	1	42	11	1	**288**	**66**	**3**	1(GG),2(AG),3(AA)

Highlighted rows= SNP that interact with *myostatin*

Appendix 16. Variance of phenotypes, MSTN and SNP from each gene associated with traits

SNP name	Trait	V_P	V_{MSTN} (%)	V_{SNP} (%)
SNIP1 SNP3	HSCW	15049.17	0.46	0.03
	Meat Wt (HSCW as covariate)	134.07	67.17	0.34
	Meat Wt (bone Wt as covariate)	689.60	49.19	0.00
	Meat	9.05	67.63	0.00
	Meat (bone as covariate)	8.52	65.98	0.00
	EMA	121.53	37.44	0.00
	Silverside weight	0.86	55.62	0.20
	Meat : bone	0.19	44.07	0.00
	Muscularity	26.12	14.62	0.04
	Stifle width	4.61	17.05	0.39
TGFBR3 SNP6	HSCW	1313.00	5.10	0.84
	Meat Wt (HSCW as covariate)	129.22	65.93	0.08
	Meat Wt (bone Wt as covariate)	683.40	48.79	0.00
	Meat	9.07	67.70	0.00
	Meat (bone as covariate)	8.53	66.05	0.00
	EMA	127.14	40.39	0.35
	Silverside weight	0.84	54.44	0.34
	Meat : bone	0.20	46.34	0.00
	Muscularity	26.13	14.16	0.27
	Stifle width	4.54	15.21	0.44
FSTL5 SNP5	HSCW	1318.00	5.01	1.21
	Meat Wt (HSCW as covariate)	133.54	67.28	0.08
	Meat Wt (bone Wt as covariate)	691.10	48.01	2.20
	Meat	9.07	67.17	0.93
	Meat (bone as covariate)	8.59	65.82	0.85
	EMA	127.13	39.56	1.47
	Silverside weight	0.84	54.68	0.41
	Meat : bone	0.20	44.26	2.68
	Muscularity	26.12	14.47	0.00
	Stifle width	4.59	16.87	0.00
FSTL5 SNP8	HSCW	1325.00	5.43	0.53
	Meat Wt (HSCW as covariate)	133.73	67.02	0.45
	Meat Wt (bone Wt as covariate)	698.90	47.98	2.20
	Meat	9.16	66.32	0.95
	Meat (bone as covariate)	8.55	65.71	0.80
	EMA	128.33	40.11	2.16
	Silverside weight	0.84	54.95	0.00
	Meat : bone	0.20	45.11	1.22
	Muscularity	26.18	14.29	0.00
	Stifle width	4.66	17.99	0.00

SNP name	Trait	V_P	V_{MSTN} (%)	V_{SNP} (%)
IGF1 SNP1	HSCW	1355.00	5.24	4.65
	Meat Wt (HSCW as covariate)	133.42	66.98	0.42
	Meat Wt (bone Wt as covariate)	691.30	49.27	0.20
	Meat	9.02	67.38	0.43
	Meat (bone as covariate)	8.48	65.70	0.47
	EMA	126.41	39.86	0.00
	Silverside weight	0.84	54.95	0.00
	Meat : bone	0.20	45.13	0.00
	Muscularity	26.35	15.07	0.00
	Stifle width	4.63	17.41	0.00
IGF1 SNP2	HSCW	1311.00	5.26	0.00
	Meat Wt (HSCW as covariate)	133.88	67.37	0.00
	Meat Wt (bone Wt as covariate)	617.60	37.40	7.29
	Meat	9.07	67.69	0.00
	Meat (bone as covariate)	8.54	66.09	0.00
	EMA	126.63	39.82	0.95
	Silverside weight	0.84	54.95	0.00
	Meat : bone	0.19	44.37	0.62
	Muscularity	26.00	13.81	0.00
	Stifle width	4.60	17.07	0.00
FST SNP7	HSCW	1317.00	5.47	0.00
	Meat Wt (HSCW as covariate)	202.21	45.25	34.17
	Meat Wt (bone Wt as covariate)	689.50	49.24	0.00
	Meat	13.59	45.70	33.70
	Meat (bone as covariate)	13.11	43.44	35.48
	EMA	152.24	32.30	19.73
	Silverside weight	1.06	45.80	19.27
	Meat : bone	1.10	90.14	0.00
	Muscularity	24.20	1.28	8.26
	Stifle width	4.42	7.69	7.05

V_p= phenotype variance, V_{MSTN}=*myostatin* variance, V_{SNP}= SNP variance

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