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Effects of the myostatin F94L substitution on beef traits[1]

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ABSTRACT: This study investigated the effects of a SNP in the *myostatin* gene (*MSTN* or *growth differentiation factor 8*, *GDF8*) on birth, growth, carcass, and beef quality traits in Australia (Aust.) and New Zealand (NZ). The SNP is a cytosine to adenine transversion in exon 1, causing an amino acid substitution of leucine for phenylalanine^{94} (F94L). The experiment used crosses between the Jersey and Limousin breeds, with the design being a backcross using first-cross bulls of Jersey × Limousin or Limousin × Jersey breeding, mated to Jersey and Limousin cows. Progeny were genotyped for the *myostatin* SNP and phenotyped in Aust., with finishing on feedlot (366 calves, over 3 birth years) and in NZ with finishing on pasture (416 calves, over 2 birth years). The effect of the F94L allele (A allele) on birth and growth traits was not significant. The F94L allele in Limousin backcross calves was associated with an increase in meat weight (7.3 and 5.9% of the trait mean in Aust. and NZ, respectively, $P < 0.001$), and a reduction in fat depth (–13.9 and –18.7% of the trait means on live calves (600 d) and carcasses, respectively, Aust. only, $P < 0.001$), intramuscular fat content (–8.2% of the trait mean in Aust., $P < 0.05$; –7.1% in NZ, not significant), total carcass fat weight (–16.5 and –8.1% of the trait mean, Aust. and NZ; $P < 0.001$ and $P < 0.05$, respectively). Meat tenderness, pH, and cooking loss of the M. longissimus dorsi were not affected by the F94L variant. In the Jersey backcross calves, additive and dominance effects were confounded because the F94L allele was not segregating in the Jersey dams. The combined effects, however, were significant on LM area (4.4% in both Aust., $P < 0.05$, and NZ, $P < 0.01$), channel fat (–11.7%, NZ only, $P < 0.01$), rib fat depth (–11.2%, NZ only, $P < 0.05$), and carcass fat weight (–7.1%, NZ only, $P < 0.05$). The results provide strong evidence that this *myostatin* F94L variant provides an intermediate and more useful phenotype than the more severe double-muscling phenotype caused by knockout mutations in the *myostatin* gene.

Key words: beef cattle, carcass trait, GDF8, F94L, meat quality, myostatin

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INTRODUCTION

The analysis of data from a bovine QTL mapping experiment revealed a major QTL in 2 different environments, South Australia (**Aust.**) and New Zealand (**NZ**). The QTL has pleiotropic effects on several carcass and meat traits. The position of the detected QTL coincides with the mapped position of the *myostatin* gene (**GDF8**) on bovine chromosome 2 (BTA2; Sellick et al., 2007).

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

Many different mutations have been identified in the bovine *myostatin* gene that result in an interruption of

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Table 1. Summary statistics of the traits (Australia)

Trait	n	Mean	RSD[1]	Minimum	Maximum
Birth weight, kg	366	27.2	3.7	12.8	44.7
Weaning weight, kg	365	234.8	25.0	112.0	308.0
Hip width at weaning, cm	365	35.4	1.9	26.0	45.0
Stifle width at weaning, cm	365	28.1	2.1	18.0	36.0
Fat depth at weaning, mm	365	0.5	1.1	0.0	5.0
Body weight at 400 d, kg	365	259.8	24.2	137.0	361.0
Body weight at 600 d, kg	362	370.4	29.9	235.0	492.0
Hip width at 600 d, cm	363	44.0	1.9	37.0	49.0
Stifle width at 600 d, cm	363	32.7	2.1	22.0	45.0
Fat depth at 600 d, mm	362	1.7	1.7	0.0	12.0
Docility, score	362	12.2	1.6	7.5	15.8
LMA, cm^2	355	83.8	10.6	26.0	166.0
Hot standard carcass weight, kg	356	344.0	35.2	168.0	479.6
Weight of silverside, kg	346	9.0	0.5	3.8	16.4
Meat weight,[2] kg	330	236.2	27.4	114.5	355.2
Fat weight,[2] kg	330	46.5	6.2	11.3	82.4
Bone weight,[2] kg	330	60.5	5.7	33.5	88.7
Pelvic area, cm^2	356	285.2	33.9	170.5	451.0
Channel fat, kg	356	12.9	3.0	4.7	22.8
Omental fat, kg	266	12.1	2.7	3.3	24.5
Carcass rump fat depth, mm	356	12.4	4.3	3.0	30.0
Fat depth at 10th to 11th rib, mm	356	9.8	3.2	3.0	24.0
Intramuscular fat content, %	355	5.2	1.3	1.4	11.1
Monounsaturated fatty acids, % of TG[3]	355	50.4	3.5	36.0	61.1
LM shear force,[4] kg	355	3.8	0.8	2.1	9.9
LM cooking loss, %	355	21.7	1.7	14.5	39.1
LM pH, unit	355	5.6	0.1	5.4	6.4

[1]RSD = residual (phenotypic) SD.
[2]Estimated from prediction equations.
[3]TG = triacylglyceride.
[4]Tenderness measured as shear force on M. longissimus dorsi (LM) at 26 d after slaughter.

the production or activity of myostatin (Grobet et al., 1998; Dunner et al., 2003). One of the mutations in the bovine *myostatin* gene is a cytosine (C) to adenine (A) transversion in exon 1 (*GDF8*g.433C > A), causing an amino acid substitution of leucine for phenylalanine[94] (**F94L**; Grobet et al., 1998; Dunner et al., 2003). This F94L substitution occurs in a region of the protein known to be the inhibitory domain of the myostatin propeptide (Grobet et al., 1998; Dunner et al., 2003; Lee, 2004). The substitution of F94L has been shown recently to affect muscling in Jersey (**J**) and Limousin (**L**) backcross cattle (Sellick et al., 2007).

The objective of this study was to document the size and type of the effects of the F94L variant in the *myostatin* gene on a wide range of traits of economic importance in beef cattle.

MATERIALS AND METHODS

Experimental protocols were approved by the University of Adelaide Animal Ethics Committee.

Experimental Design

Two-generation cattle resource populations, the University of Adelaide Davies Gene Mapping and the AgResearch Gene Mapping Projects in Aust. and NZ, respectively, were developed using 2 phenotypically divergent *Bos taurus* breeds, J and L. Three pairs of half-brothers were generated as first-crosses (X = J × L or L × J), and 1 of each pair was used for mating in either Aust. or NZ over both J and L cows, generating in total 782 backcross progeny (469 XJ and 313 XL) in the 2 countries.

In the mid-north of Aust., 366 backcross calves (205 XJ and 161 XL) were born in the autumn over 3 yr (1996 to 1998). The calves were weaned at approximately 250 d, grown out on pasture until about 2 yr of age, and finished in a feedlot (approximately 65% grain) for at least 180 d as part of an intensive feed efficiency trial.

In the North Island of NZ, 261 experimental backcross calves (162 XJ and 99 XL) were born in spring 1996, and another 155 were born in spring 1997 (102 XJ and 53 XL). The J backcrosses were born in J herds and were bucket-reared, whereas the Limousin backcrosses were born in 1996, after embryo transplantation, as singles or twins to Hereford × Friesian recipients, and in 1997, they were born as singles in 2 Limousin herds. In both years, the XL calves were reared on their dams and weaned at approximately 180 d of age.

Traits Analyzed

Live Animal Measurements. Birth weights were recorded within 24 h of birth. Calves were weighed every 30 to 50 d with those at weaning, 400 d, and 600 d

reported herein. Other measurements included rump fat depth scanned at position 8, as described by Arthur et al. (2001), using an Ezi-scan sonic device (Amac Pty. Ltd., Armidale, Australia) plus hip width (bone) and stifle width (muscle) measured using calipers. Hip and stifle widths were not measured on calves born in 1996.

Slaughter and Dissection Data. In Aust., all calves born in 1996 and 1997 were slaughtered at the Stockyard Abattoir, Grantham, Queensland, with those born in 1998 slaughtered at the T&R Murray Bridge Abattoir, Aust. (age at slaughter 34 to 40 mo). The calves were killed using a captive bolt, immobilization, and throat-cut in a commercial plant. Carcasses were electrically stimulated with a low voltage (peak 45V, 200mA) rectal-nostril stimulator for 40 s within 5 min of sticking. After standard line processing, the carcasses were split, weighed (hot standard carcass weight, HSCW), and stored in a chiller (0 to 4°C) overnight. Approximately 18 h after slaughter, carcasses were quartered at the 10th to 11th rib and carcass assessment was performed by an accredited AUS-MEAT grader. Carcass traits evaluated include the cross-sectional area of the M. longissimus dorsi (LM area, LMA, cm^2), fat depth at position 8 on the rump (P8; mm), and subcutaneous fat at the 10th to 11th rib over the M. longissimus dorsi muscle (Rbft, mm; Table 1). Pelvic height and half-width were measured on sides (split carcasses), and the area was estimated by multiplying height by width.

Carcass meat, fat, and bone weight were estimated from prediction equations that were developed on cattle of equivalent BW and genotype in a preliminary study. The equations estimated percentage components, and these were simply multiplied by carcass weight to get predicted weights. The 1998-born calves had different information available to those born in 1996 and 1997, and so a second series of prediction equations was used.

For the 1996- and 1997-born calves:

$$\text{Meat (\%)} = 67.28 + 0.69(\text{ts}) + 1.59(\text{st}) + 1.07(\text{of}) + 0.38(\text{ru}) + 0.45(\text{LM}) + 1.67(\text{tln}) + 0.15(\text{rib}) - 1.16(\text{femwt}) + 1.29(\text{tibwt}) - 0.33(\text{fqwt}) - 0.0735(\text{HSCW}) - 0.092(\text{P8}) + 0.040(\text{LMA}),\ R^2 = 0.85;$$

$$\text{Fat (\%)} = 12.48 - 0.92(\text{ts}) - 1.45(\text{st}) - 0.93(\text{of}) - 0.35(\text{ru}) - 0.47(\text{LM}) - 1.36(\text{tln}) + 0.20(\text{rib}) - 0.86(\text{femwt}) - 1.96(\text{tibwt}) - 0.52(\text{fqwt}) + 0.1196(\text{HSCW}) + 0.103(\text{P8}) - 0.037(\text{LMA}),\ R^2 = 0.81;\ \text{and}$$

$$\text{Bone (\%)} = 20.24 + 0.23(\text{ts}) - 0.14(\text{st}) - 0.14(\text{of}) - 0.04(\text{ru}) + 0.02(\text{LM}) - 0.31(\text{tln}) - 0.34(\text{rib}) + 2.02(\text{femwt}) + 0.67(\text{tibwt}) + 0.85(\text{fqwt}) - 0.0461(\text{HSCW}) - 0.010(\text{P8}) - 0.003(\text{LMA}),\ R^2 = 0.92;$$

and for 1998-born calves:

$$\text{Meat (\%)} = 66.88 + 2.40(\text{st}) + 1.18(\text{of}) + 0.98(\text{kn}) + 0.51(\text{LM}) + 3.10(\text{ct}) - 0.46(\text{bones}) - 0.0747(\text{HSCW}) - 0.075(\text{P8}) + 0.049(\text{LMA}),\ R^2 = 0.83;$$

$$\text{Fat (\%)} = 13.70 - 1.93(\text{st}) - 0.90(\text{of}) - 0.95(\text{kn}) - 0.33(\text{LM}) - 3.12(\text{ct}) - 2.75(\text{bones}) + 0.1032(\text{HSCW}) + 0.083(\text{P8}) - 0.042(\text{LMA}),\ R^2 = 0.79;\ \text{and}$$

$$\text{Bone (\%)} = 19.42 - 0.46(\text{st}) - 0.28(\text{of}) - 0.02(\text{kn}) - 0.18(\text{LM}) + 0.03(\text{ct}) + 3.21(\text{bones}) - 0.0285(\text{HSCW}) - 0.009(\text{P8}) - 0.007(\text{LMA}),\ R^2 = 0.81;$$

where ts = weight of topside, st = weight of eye round (M. semitendinosus), of = weight of outside flat (remainder of silverside), ru = weight of rump, LM = weight of striploin (M. longissimus dorsi), tln = weight of tenderloin (M. psoas major), rib = weight of ribset, femwt = weight of femur, tibwt = weight of tibia, fqwt = weight of ribs and spine from forequarter, kn = weight of knuckle, ct = weight of chuck tender, and bones = combined weight of radius, ulna, and humerus (AUS-MEAT Limited, 1998).

The left half-carcass from each animal was deboned, and samples were collected from the striploin (M. longissimus dorsi). Samples of 2.5-cm-thick steaks were vacuum-packed, randomly assigned to different aging treatment groups at 0 to 1°C (26-d values reported herein), and frozen (–20°C) after the completion of the aging treatment. Before the tenderness measurements, the steaks were thawed overnight at 2°C and trimmed to 80- to 100-g samples. The pH was recorded before cooking using a WP-80 pH, mV, Temp-meter (TPS Pty. Ltd., Springwood, Australia). The pH was consistent across the aging treatments, so a simple average was used as the best indicator of ultimate pH. Samples were cooked at 70°C for 40 min in a water bath and cooled in running water. After storage overnight in the chiller, rectangular strips (15.0 × 6.6 mm) were cut parallel to the fibers and Warner-Bratzler shear force measurements were performed on a Lloyd pressure tester (model LRX, Lloyd Instruments, Hampshire, UK) according to the method described Bouton and Harris (1972). Fat samples were taken from the lateral part of the striploin for determination of intramuscular fat content and fatty acid composition using a method described by Pitchford et al. (2002).

In NZ, calves were preallocated to slaughter groups in the spring of 1998 and 1999 over 18 and 10 kill days, respectively (once a week, with approximately 15 same-sex calves per slaughter group, 18 groups in 1998 and 10 in 1999). Preallocation was based on breed of calf, sire, and balanced as far as possible within breed for live BW before the first slaughter day. Offspring (steers and heifers) were slaughtered off pasture in 28 groups based on year of birth and sex, at ages ranging from 22 to 28 mo at the Ruakura Abattoir in Hamilton, NZ.

Table 2. Summary statistics of the traits (New Zealand)

Trait	n	Mean	RSD[4]	Minimum	Maximum
Birth weight, kg	306	30.3	3.7	14.0	50.0
Body weight at 400 d, kg	415	278.8	20.6	194.0	371.0
Body weight at 600 d, kg	413	450.4	36.0	286.0	642.0
Docility, score	155	12.5	1.4	9.9	16.8
LMA, cm^2	326	63.3	6.8	37.3	111.7
Hot standard carcass weight, kg	413	237.8	21.5	136.0	369.0
Weight of silverside, kg	406	9.0	0.9	4.4	16.1
Meat weight in side,[1] kg	401	77.7	7.3	44.5	126.1
Fat weight in side,[1] kg	401	9.9	2.1	3.3	22.8
Bone weight in side,[1] kg	401	25.2	1.9	15.9	35.5
Fat depth at 10 to 11th rib, mm	325	7.1	2.6	1.0	20.0
Pelvic area, cm^2	413	215.6	17.8	141.5	288.4
Channel fat, kg	413	6.5	2.1	1.5	19.5
Omental fat, kg	413	3.7	1.1	0.6	12.5
Intramuscular fat content, %	341	3.9	1.9	0.4	12.8
Monounsaturated fatty acids, % of TG[2]	402	50.2	2.6	42.4	59.3
LM shear force,[3] kg	413	6.0	1.3	3.6	15.6
LM cooking loss, %	400	23.4	2.7	10.1	31.7
LM pH, unit	413	5.4	0.1	5.3	6.2

[1]The actual measurement for a side.
[2]TG = triacylglyceride.
[3]Tenderness measured as shear force on M. longissimus dorsi (LM) at 4.0 d after slaughter.
[4]RSD = residual (phenotypic) standard deviation.

Each animal was stunned by captive bolt and then slaughtered; no electrical stimulation was applied immediately after slaughter. After splitting the carcass and weighing the 2 sides to obtain the HSCW, the right strip-loin was removed and the right side was stored in a chiller for 24 h before quartering at the 10 to 11th rib. A butcher's dissection of the right fore- and hindquarters was then carried out to record, for each joint, the weights of saleable meat plus meat trim (the combined total being referred to as meat), trimmed fat (referred to as fat) leaving a fat cover of approximately 2 mm, and bone (Table 2).

Initial pH of the striploin was recorded within approximately 30 min of slaughter and then monitored at intervals for approximately 24 h until rigor mortis (pH < 5.5), while the striploin was held in a controlled-temperature cabinet at 15°C. Ultimate pH was the lowest pH attained in the first 24 h, defining the full development of rigor mortis. Steak portions (5) were then cut from the strip-loin for cooking and shear-force measurements.

The first steak was processed on reaching rigor mortis, and the 4 other steaks were cooked at intervals (approximately 1.3, 2.0, 2.3, and 4.0 d postmortem), after continued aging at 15°C. For cooking, each steak was placed inside a plastic cooking bag, heated in a boiling water bath to an internal temperature of 75°C, then removed and cooled rapidly in ice to an internal temperature of 2°C. Shear-force measurements were then recorded using a MIRINZ tenderometer (Fraserhurst and MacFarlane, 1983), taking the average from measurements of ten 1 × 1-cm cores, aligned with the fibers running longitudinally along the core. The weight of each steak, before and after cooking, was also recorded. The percentage weight loss of each steak during cooking was calculated from the steak weights before and after cooking. The average (across aging treatments) cooking loss is reported herein (Table 2).

DNA Extraction and SNP Genotyping

The DNA was extracted from the NZ and Australian calves from blood samples collected in their first year of life, with the exception of the XL calves born in NZ in 1996 where DNA was extracted from ear cartilage. Genotyping of the F94L variant was performed by PCR-RFLP on Australian and NZ progeny (Table 3). Amplification of a fragment of the bovine myostatin gene containing the F94L variant was performed using the following primer pair (Sellick et al., 2007):

MSTNaF 5′-ATTCACTGGTGTGGCAAGTTGTCTCTCAGA-3′, and

MSTNbR 5′-CCCTCCTCCTTACATACAAGCCAGCAG-3′,

which were the forward and reverse primers, respectively.

Statistical Analysis

The data from 2 experiments were analyzed separately because the trait measurements were not identical. Univariate and multivariate analyses of both the Australian and NZ data was conducted using ASREML (Gilmour et al., 2006). The trait groups were live animal measurements and carcass traits. The model fitted for the Australian data included fixed effects of breed of dam (J or L), cohort (6 combinations of year of birth and sex), and sire family (3 levels), plus date of birth as a

covariate, and additive and dominance covariates within breed of dam. Additive covariates were 0, 1, and 2 to account for the number of variant F94L *myostatin* alleles for the CC, AC, and AA genotypes, respectively. To test for dominance, an additional regression covariate was added with value of 0 for homozygotes and 1 for heterozygotes; this was possible only in the XL calves. A significant result for this covariate was interpreted as evidence of a dominance effect.

The statistical model used for the NZ data included fixed effects of breed of dam (J or L), farm of birth (3 levels), and birth type (single or twin) within breed of dam, slaughter group (28 levels that includes adjustments for sex and year), and sire family (3 levels), plus additive and dominance covariates within breed of dam.

RESULTS

The results from multi-trait analysis of growth, carcass, and meat and fat quality traits showed that the effects of slaughter group, sire, and breed of dam on the majority of traits were significant. The additive effect of the F94L variant on birth weight was not significant. However, in general, the F94L variant had a significant positive effect on muscle and negative effect on fatness (Table 4). In the backcross Limousin calves, the F94L variant had both additive and dominance effects on stifle width measured at weaning, LMA, and silverside weight. From the Australian data, there was also a dominance effect of F94L for hip width measured on live calves at 600 d of age ($P < 0.01$). On the other hand, only the additive effects of F94L were significant for fat depth and stifle width measured on live calves at 600 d of age, meat weight, fat weight, P8 fat depth ($P < 0.001$), and intramuscular fat and rib fat ($P < 0.05$).

The results for the average effect of substituting a single copy of the variant F94L allele indicated an increase in stifle width at 600 d of age (2.8% of the trait mean, Australian progeny, Table 4), LMA (10.5 and 4.8% of the trait mean, Australian and NZ progeny, respectively), silverside (5.8 and 7.2%, Australian and NZ progeny, respectively), and meat weight (7.3 and 5.9%, Australian and NZ progeny). There was a reduction in P8 fat depth at 600 d and slaughter (–13.9, –18.7% of the trait means, Australian progeny), intramuscular fat (–8.2 and –7.1%, Australian and NZ progeny, respectively), and carcass fat weight (–16.5 and –8.1%, Australian and NZ progeny, respectively) (Table 4).

Limousin backcross calves carrying 2 copies of the variant F94L allele produced carcasses with approximately 12 to 15% more meat and 16 to 33% less fat compared with calves with no copies of the variant F94L allele. Significant additive and dominance effects of the allele variant on meat weight indicate that heterozygous calves produce carcasses with about 3% more meat weight compared with calves carrying no copies of the variant F94L allele.

There were only 2 genotypic groups, AC and CC, in the J backcross progeny. Therefore, it was not possible to separate additive and dominance effects of the gene in this group of calves. Thus, the estimated F94L effects for this group included the additive effect plus the dominance deviation. The results from the Australian progeny indicated that in this group of calves, F94L had significant effects on fat depth measured on live calves at 600 d of age and on the LMA ($P < 0.05$). The estimated additive plus dominance effect of the F94L variant shows that fat depth measured on live calves was 9.8% lower in heterozygous calves than homozygous normal calves. Also the LMA in the heterozygous calves was 4.4% larger than the homozygous normal calves. However, the effect of a single copy of the variant allele was not detectable for most of the traits, including total meat and fat weight, in this group of calves.

Similar results from the NZ J backcross calves indicated that F94L had significant effects on LMA ($P < 0.01$), pelvic fat ($P < 0.01$), rib fat depth ($P < 0.05$), and carcass fat weight ($P < 0.05$). The estimated additive plus dominance effect of the F94L gene showed that the LMA in heterozygous calves was larger than homozygous normal calves (4.4% of the trait mean, $P < 0.01$). The estimated additive plus dominance effects of the F94L gene for rib fat, fat weight, and pelvic fat were –11.2, –7.1, and –11.7%, respectively, demonstrating that calves carrying 1 copy of the variant F94L allele produced carcasses with less fat than those of calves with no copies of the F94L allele variant.

Table 3. Number of calves genotyped for F94L *myostatin* variant[1]

		F94L genotype			
Location	Backcross	AA	AC	CC	Total
Australia	Jersey	0	102	101	203
	Limousin	54	91	15	160
	Total	54	193	116	363
New Zealand	Jersey	0	131	125	256
	Limousin	70	69	8	147
	Total	70	200	133	403
Total		124	393	249	766

[1]A = F94L variant allele and C = normal allele.

DISCUSSION

The pleiotropic effects of the F94L variant of the *myostatin* gene in calves from 2 genetic backgrounds, Limousin and J as breed of dam, were investigated in 2 different environments. In general, the NZ progeny were smaller than the Australian progeny because the calves in Aust. were both fatter and older when slaughtered (NZ 22 to 28 mo compared with Aust. 34 to 40 mo).

Live Animal Measurements

The results indicated that the additive effect of the F94L allele variant on birth weight was not significant. This finding is consistent with some previous reports on

Table 4. Additive (*a*) and dominance (*d*) effects (%) of F94L *myostatin* variant, and breed effects as a percentage of trait means

	Limousin				Jersey			
	a		*d*		*a*+*d*[2]		Breed difference[3]	
Trait[1]	Aust.[4]	NZ[5]	Aust.	NZ	Aust.	NZ	Aust.	NZ
Birth weight	2.6	0.7	–3.2	–7.6	0.9	1.4	32.3***	29.7***
Wwt	1.5	—	–1.5	—	2.0	—	11.2***	—
400W	1.8	0.5	1.3	–3.0	1.0	–1.0	10.1	25.3***
600W	0.4	1.7	–1.6	1.6	1.2	0.1	15.1***	14.5***
Whip	–0.5	—	–0.6	—	0.8	—	1.7	—
Wsti	2.5*	—	–3.1*	—	1.4	—	10.6***	—
WFat	–9.6*	—	–5.2		–5.0		4.0	—
600Hip	–1.0	—	–2.3**	—	–0.2	—	4.4***	—
600Sti	2.8**	—	–1.7	—	1.4	—	12.3***	—
600Fat	–13.9***	—	–1.7	—	–9.8*	—	12.0	—
Docility	0.8	2.8	–5.0*	6.8	–1.6	0.3	4.2	–6.7
Carcass trait								
HSCW	2.7	3.4*	–2.5	–3.6	1.2	0.8	22.7***	23.5***
LMA	10.5***	4.8*	–6.3**	–13.0***	4.4*	4.4**	19.1***	36.2***
Silverside	5.8***	7.2***	–2.7*	–10.4***	1.2	2.5	8.4***	39.1***
Rbft	–9.4*	–8.8*	1.3	11.1	–7.5	–11.2*	4.2	–8.8
P8	–18.7***	—	1.4	—	–2.1	—	26.6**	—
IMF	–8.2*	–7.1	2.9	9.0	–4.0	–5.5	–25.9***	–47.4**
Channel fat	–2.2	–5.0	1.3	10.4	–6.4	–11.7**	–13.7*	–39.7***
Omental fat	–1.9	–6.3	–3.1	5.6	0.1	–0.8	–9.1	–27.8**
Total MUFA	1.4	–2.2*	1.2	0.2	–0.7	0.9	–6.5***	–1.9
Meat weight	7.3***	5.9***	–4.2*	–6.7**	2.2	2.1	24.2***	30.4***
Fat weight	–16.5***	–8.1*	1.5	15.0**	–1.7	–7.1*	22.6***	–3.4
Bone weight	–0.8	–0.3	1.1	–1.2	–0.3	–0.5	17.3***	16.7***
PA	–2.2	0.1	0.1	–0.5	0.9	0.7	14.6***	3.2
LM shear force	–1.3	0.3	1.8	3.9	–0.5	5.7	12.1*	11.3*
LM cooking loss	0.1	–0.9	1.2	–4.6*	0.6	1.7	4.2*	11.1***
LM pH	–0.2	–0.1	0.7	0.3	0.3	0.1	0.2	–0.1

[1]Wwt = weaning weight, 400W = BW at 400 d, Whip = hip width at weaning, Wsti = stifle width at weaning, WFat = fat depth at weaning, 600Hip = hip width at 600 d, 600Sti = stifle width at 600 d, 600Fat = fat depth at 600 d, HSCW = hot standard carcass weight, LMA = LM area, SS = silverside weight, P8 = carcass fat depth on the rump, Rbft = fat depth at 12th and 13th ribs, IMF = intramuscular fat content, and PA = pelvic area.
[2]*a*+*d* = additive plus dominance.
[3]Breed difference = Limousin minus Jersey.
[4]Aust. = Australian progeny.
[5]NZ = New Zealand progeny.
*$P < 0.05$, **$P < 0.01$, and ***$P < 0.001$.

myostatin effects (Menissier, 1982; Hanset, 1991), but heavier weights at birth have been documented in many studies in which double-muscling gene knockout mutations were involved (Nott and Rollins, 1979; Arthur et al., 1989; Arthur, 1995; Casas et al., 1998, 1999, 2004; Cundiff et al., 1998; Short et al., 2002). Casas et al. (1998) compared animals inheriting 1 or 0 copies of an inactive *myostatin* allele in Belgian Blue and Piedmontese, and found that the genotype difference in both groups was 4.6 kg, with animals inheriting 1 copy of the inactive *myostatin* allele being heavier at birth than animals inheriting no copies. In another study, Casas et al. (1999) reported that homozygous *mh*/*mh* animals were heavier at birth than the heterozygous +/*mh* and homozygous +/+ animals. They reported that the difference between the 2 homozygotes was 5.2 kg for birth weight. Casas et al. (2004) studied the effect of the Belgian Blue inactive *myostatin* allele (11 base pair deletion) and found a difference of 3.5 and 2.0 kg at birth between the groups inheriting 2 and 1 copies and the groups inheriting 1 and 0 copies of the inactive *myostatin* allele, respectively. This magnitude of effect is similar to that observed in studies of the Piedmontese inactive allele *myostatin* (Short et al., 2002), where differences of 3.1 and 1.3 kg were reported for the same comparisons.

Casas et al. (1999) reported that differences in weight were observed at 200 d and 1 yr of age in Piedmontese crossbreds segregating the C313Y *myostatin* allele (a guanine to adenine transition mutation in exon 3 of the *myostatin* gene, causing a substitution of tyrosine for cysteine in the signaling portion of the protein). Casas et al. (2004) also studied the effect of an inactivated *myostatin* allele on animal live BW in a crossbred population involving the Belgian Blue breed. They reported that while animals with 1 copy of the inactive *myostatin* allele had a postweaning gain and average daily gain

similar to animals with 0 copies of the allele, animals with 2 copies of the inactive *myostatin* allele had a slower growth rate. Nott and Rollins (1979) and Arthur et al. (1989) found similar results. The research presented here did not find a significant additive or dominance effect of the *myostatin* F94L allele variant on animal live BW.

The most likely explanation for conflicting results of the present study and previous reports is the type of *myostatin* mutation studied herein. Previous studies have concentrated on inactive *myostatin* alleles, which cause the full double muscling phenotype in the homozygotes. The F94L allele variant described herein is likely to be active, as only a single amino acid substitution is present in the inhibitory domain of the propeptide. Both the Belgian Blue and Piedmontese doubled-muscle animals involved in most studies on myostatin possess an extreme muscular phenotype (Arthur, 1995), which is certainly more pronounced than the heavily muscled Limousin animals used in the present research. There may be also some breed effects, which given the J and Limousin genetic backgrounds, could affect traits, particularly early in life. As the F94L allele variant is present in some Australian Angus (Sellick et al., 2006), the effects of this variant should be investigated in different breeds.

Muscle Mass

Although birth weight and other animal live BW were not associated with the *myostatin* F94L allele variant, there was a significant effect of the allele on the muscularity of live animals and carcass muscle mass. The effect of the F94L variant on muscularity was evident even at the youngest age examined (stifle width at weaning, 200 d of age) and was maintained in older animals (stifle width at 600 d of age) and at slaughter (LMA; Table 4). Stifle width is used as an indicator of muscularity, and both the additive and dominance effects of the F94L allele on stifle width measured at weaning were significant. Based on the data from the Australian progeny, the additive effect of the F94L allele for this trait was 2.5%. In other words, AA animals had about 5% greater stifle widths relative to CC animals without differences in weight. Traits that are considered a more direct measure of muscularity (e.g., LMA and silverside weight) also increased with 1 copy of the F94L allele variant. More dramatic effects were observed with 2 copies of the F94L allele in the Limousin backcross animals. The estimated additive plus dominance effect for LMA in the J backcross progeny was similar to that of the Limousin backcross animals (Aust.), indicating no interaction between breed of dam and the *myostatin* gene for this trait. Also, the results showed that AA animals from the Limousin backcross had a large increase in meat weight in the carcass (34.5 and 18.3 kg in Aust. and NZ, respectively) compared with CC animals. However, it should be noted that there were limited numbers of Limousin backcross animals with the CC genotype. There were no significant additive or dominance effects of F94L on hot carcass weight in the Aust. progeny. Although the analysis of the NZ data showed a significant additive effect of the F94L in the Limousin backcross animals for hot carcass weight, the effect of the allele in the J backcross animals was not significant. In addition, the size of the additive effect of the gene for hot carcass weight in the Limousin backcross animals was only approximately 3% indicating that AA animals had only 6% greater carcass weights than CC animals.

Fatness

Because F94L had negligible effect on carcass weight, the potential redistribution of body tissues was investigated. The results revealed a significant effect of the variant F94L allele on most fat depots, although not omental fat weight. The additive effects of the variant allele for fat traits were negative showing the AA animals were leaner than CC animals. To rule out the possibility that fat stores were simply redistributed in AA animals, the total carcass fat was analyzed. Carcass fat mass was decreased in AA animals compared with CC animals, 33 and 16% based on the Australian and NZ data, respectively (Table 4). Hence, the normal body weight of AA animals, which had greater muscle mass, appeared to be associated with differences in fat accumulation. At first glance, it would appear that the effects of F94L allele on fat (–16.5%, Table 4) were larger than on meat (7.3%). However, the actual increase in meat weight (7.3% of 236.2 kg = 17.2 kg per A allele) was more than double the decrease in fat weight (16.5% of 46.5 kg = 7.7 kg).

McPherron and Lee (2002) reported that mice lacking myostatin have a reduction in total body fat, which is particularly pronounced in older animals. These researchers investigated the effect of the *myostatin* mutations in 2 genetic models of obesity, agouti lethal yellow (A^y) and obese ($Lep^{ob/ob}$). Their findings indicated that loss of myostatin activity led to a partial suppression of fat accumulation and abnormal glucose metabolism. The exact mechanism by which myostatin regulates fat metabolism is unknown. However, it has been suggested that one possibility is that the effects of the *myostatin* mutations in adipose tissue are an indirect effect of the lack of myostatin signaling in skeletal muscle so that the anabolic effects of the *myostatin* mutations on skeletal muscle tissue per se may shift energy metabolites in such a manner as to prevent fat accumulation elsewhere in the body (McPherron and Lee, 2002). It is possible that the effects on muscle mass and/or fat stores seen in the present study and also in mice studies (McPherron et al., 1997; McPherron and Lee, 2002) may reflect the activity of some other mediator of cachexia whose production or activity is induced by myostatin.

Consistent with the findings in the study herein, it is widely accepted that the amount of fat in the carcass of doubled-muscled cattle is significantly less than normal cattle (Arthur, 1995; Casas et al., 1998). In particular,

intramuscular fat has been reported to be significantly affected by the doubled-muscle phenotype (Casas et al., 1998; Raes et al., 2001). Based on the NZ data, the *myostatin* F94L allele variant was associated with composition of fat depots so that the percentage of MUFA in fat obtained from animals inheriting 2 copies of F94L allele variant was lower than animals inheriting no copies of the allele. Consistent with this result, a greater PUFA proportion and a lower MUFA concentration have been found in the intramuscular fat of *mh*/*mh* genotypes of Belgian Blue breed compared with normal genotype (Raes et al., 2001).

Meat Quality

Negative aspects of the meat from double-muscled cattle have been reported to include pale color, less taste, and reduced water binding (Bailey et al., 1982). This study has investigated the effects of myostatin on several the same meat quality attributes. There was no evidence for the effect of the F94L variant on meat pH, tenderness, and cooking loss measured in the M. longissimus dorsi muscle. Casas et al. (1998) reported that a single copy of the inactivated *myostatin* allele had no effect on tenderness, measured as M. longissimus dorsi shear force. However, it has been reported that the muscle of double-muscled cattle contains less connective tissue, implying a lower background toughness and more tender meat (Bailey et al., 1982). Therefore, the effect of the gene on other muscles such as M. semitendinosus, which has more connective tissue than M. longissimus dorsi, should be investigated.

Bone

The bones of double-muscled cattle are less affected by myostatin than other tissues in the body (Arnold et al., 2001). Hamrick et al. (2000) demonstrated that despite the impressive musculature of the myostatin-null "Mighty" mouse, its femora were not altered in either shape or size. Their findings indicated that the bone underwent no adaptation whatsoever in response to the increased muscle mass. In the present study, there was also no effect on bone weight.

Pelvic Area

Dystocia and neonatal survival have been consistent problems with double-muscled cattle (Arthur, 1995), and these problems have been the main deterrent to more widespread use of this genetic trait. Bellows et al. (1971) showed that the main cause of dystocia is an incompatible relationship between birth weight and pelvic area. Short et al. (2002) reported that addition of 1 and 2 *mh* alleles linearly increased birth weight and linearly decreased pelvic area. Because pelvic area was not measured in the cows herein, but only in the progeny, the present work was unable to examine the effect of the F94L allele variant on pelvic area as a characteristic of the dam. However, when birth weight and pelvic area were analyzed as traits of the calf, the *myostatin* F94L affected neither pelvic area nor birth weight.

Gene Action

The gene product from the *myostatin* locus acts via autocrine or paracrine mechanisms to control myogenesis (Rios et al., 2002). Even though the activity of myostatin was not measured directly herein, some of the traits that were measured in this experiment, such as carcass weight, marbling, LMA, primal cuts, and muscle score are highly related to the effects of this gene and can be used to estimate the genetic control mechanisms. In these traits, there was ample evidence that a major portion of the action of the *myostatin* gene product is additive. However, there was also evidence of some nonadditivity. For example, based on the Australian data, animals carrying 1 and 2 copies of the F94L allele produced 7.3 kg and 34.5 kg more meat, respectively, compared with animals not carrying a copy of the allele. In the Australian data, there was evidence of additivity for fatness, but both additive and dominant effects on muscle mass (Table 4). In NZ, there was evidence of both additive and nonadditive (dominance) effects for muscle mass and fatness. Although the results appear consistent with previous reports of dominance at this locus (Short et al., 2002; Casas et al., 2004), it should be noted that dominance was estimated with low accuracy because of the low number of Limousin backcross progeny that were homozygous for the F94L allele (Table 3).

F94L Substitution

The F94L substitution occurs in a conserved region of the N-terminal propeptide or prodomain of the myostatin protein. Interestingly, overexpression of the myostatin propeptide in transgenic mice results in increased muscling as a consequence of myofiber hypertrophy (Yang et al., 2001). The propeptide is cleaved from the C-terminal biologically active myostatin protein. The propeptide then binds to the mature myostatin protein to form a latent complex (Lee, 2004). The propeptide can also bind G-protein coupled receptor-associated sorting protein 1 (GPRASP1) and small glutamine-rich tetratricopeptide repeat (TPR)-containing, α (STGA), which prevent myostatin activation and secretion (Dominique and Gerard, 2006). The F94L substitution is predicted to change the tertiary structure of the propeptide by reducing the number of loops in the region. Therefore, the F94L substitution may affect the propeptide binding to the mature myostatin protein, GPRASP1, SGTA, or both, and hence, affect the activation of myostatin.

This is the first report of the effects of the *myostatin* F94L on many cattle traits in 2 different environments. The conclusion of the study is that the effect of this variant is different from other *myostatin* mutations. It was shown that despite differences in climate, feeding regimens, and age of slaughter in 2 countries, the F94L allele had similar effects in both systems. Furthermore,

the findings showed that along with increased muscling, animals carrying the *myostatin* F94L allele variant have reduced fat depots but similar birth weights to the normal animals. Thus, the F94L variant appears to have many positive effects without correlated negative effects, and is therefore, an ideal candidate for genotype-assisted selection.

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