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CORRELATION ANALYSIS BETWEEN MYOSTATIN GENE POLYMORPHISMS AND CARCASS TRAITS IN NEW ZEALAND ROMNEY SHEEP

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The identification of genes that affect economically important traits for sheep meat would improve selective breeding programs for sheep production. Myostatin is one such gene. It is a member of the transforming growth factor β superfamily. The members of this family regulate cell growth and differentiation in both embryonic and adult tissues. In mammals, myostatin is highly expressed in developing and adult muscles and acts as a negative regulatory factor by inhibiting myogenic factor 5 (MYF5) and myogenic differentiation factor (MyoD). These factors are involved in the differentiation of muscle precursor cells into myoblasts (McPherron *et al*., 1997).

Myostatin loss-off function leads to increase skeletal muscle mass (double muscling) in mice (McPherron *et al*., 1997; Szabo *et al*., 1998; Lin *et al*., 2002; Whittemore *et al*., 2003; Mendias *et al*., 2008). In addition, myostatin deficiency reduces adipogensis (Lin *et al*., 2002; McPherron and Lee, 2002), as a result of reduced production and secretion of leptin (McPherron and Lee, 2002). Variation in other species including sheep (Clop *et al*., 2006; Kijas *et al*., 2007; Hickford *et al*., 2009; Han *et al*., 2010; Haynes *et al*., 2013), cattle (McPherron and Lee, 1997; Grobet *et al*., 1997; Kambadur *et al*., 1997; Wiener *et al*., 2002; Marchitelli *et al*., 2003; Grisolia *et al*., 2009) and human

(Schuelke *et al*., 2004) was reported to affect muscling.

The effect of myostatin on myogenic and adipogenic differentiation potentially has important implications for growth and carcass traits of sheep. The variation in the ovine myostatin gene and its effect on important production traits in sheep has been described in a number of reports. For example a single nucleotide polymorphism (SNP; g.6223G>A) has been detected in the 3'- UTR of myostatin gene in Belgian Texel sheep (Clop *et al*., 2006). The same SNP was also detected in other breeds including Australian Texel sheep (Kijas *et al*., 2007), Charollais sheep (Hadjipavlou *et al*., 2008), New Zealand Texel sheep (Johnson *et al*., 2009) and White Suffolk, Poll Dorest and Lincoln sheep in Australia (Kijas *et al*., 2007). This SNP has been found to affect muscle hypertrophy in Belgian Texel sheep (Clop *et al*., 2006), muscle depth in Charollais sheep (Hadjipavlou *et al*., 2008) and birth weight, mean lean yield and total muscle yield in New Zealand Romney sheep (Han *et al*., 2010). Additional SNPs (g- 41C>A, g+ 4036A>C and g+ 6223G>A) have been identified in the promoter and intron 2 regions and showed significant effects on slaughter measurements of muscling and fatness (Kijas *et al*., 2007). Another two SNPs (C.2360G>A and C.960delG) have been detected and are reported to reduce fatness and increase muscle mass in Norwegian White sheep (Boman *et al*., 2010). A single strand conformational polymorphism analysis (SSCP) of the 473-bp of the exon 1- intron 1 region of myostatin gene has revealed three allelic variants in NZ Romney sheep (Zhou *et al*., 2008). In the same breed, five SSCP allelic variants have been detected in intron 1 region of myostatin gene and showed significant effects on leg yield, loin yield, loin yield% and total yield (Hickford *et al*., 2009).

The ovine myostatin gene consists of three exons, divided by two introns (Bellinge *et al*., 2005) and located on chromosome 2.

The objectives of the present study are to further investigate allelic and genotypic polymorphisms of intron 1 of the ovine myostatin gene and to test their association with carcass traits in New Zealand Romney sheep.

MATERIALS AND METHODS

Animals and data collection

Carcass data were collected from 529 of male lambs of NZ Romney sheep. The animals were the progeny of 17 sires and 398 dams and born over six years in ten fully pedigree-recorded research resource flocks. All lambs were ear-tagged with a unique identification number within 12 h of birth, and date of birth, weight of birth, type of birth, gender of lamb and dam number were recorded. All of the ewes and lambs were brought together at tailing (lambs aged between 2-6 weeks old) and remained together until weaning. Lambs were weaned at an average of 12 weeks of age and were fed *ad libitum* on high quality pastures until slaughtering.

Slaughters were carried out at an average 6 months of age. Video imaging analysis (VIASCAN®Sastek), developed by Meat and Livestock Australia and described by Hopkins *et al*. (2004), was used to estimate the following carcass traits: lean meat yield in the shoulder (shoulder yield), loin (loin yield), leg (leg yield), total yield (the sum of the leg, loin and shoulder yields for any given carcass), the shoulder yield%, the loin yield% and the leg yield%.

DNA purification and genotyping

At weaning, blood samples were collected on FTA cards (Whatman). The blood was allowed to air dry and was stored in darkness at room temperature. For each sample, a disc of 1.2 mm in diameter was punched and the genomic DNA was purified from the dried blood spot using a two-step procedure described by Zhou *et al*. (2006).

A 414 bp fragment containing intron 1 of myostatin gene was amplified using a pair of specific primers. The sequences of these two primers are described in the report of Hickford *et al*. (2009) and are as follows: F: 5'-GAAACGGTCATTACCATGC-3' and R: 5'-CATATTTCAGGCA-ACCAAATG-3'. PCR amplification was carried out in a total reaction volume of 20 µl containing the genomic DNA on the FTA card, 0.25 µM of each primer, 150 µM of Mg^{++}, 0.5 U of Taq DNA polymerase and 1x reaction buffer supplied. The reaction conditions were as follow: an initial DNA template denaturation at 94°C for 2 min, followed by 35 cycles of denaturation at 94°C for 30 sec; annealing at 61°C for 30 sec, and extension at 72°C for 40 sec and final extension at 72°C for 5 min.

One µl of each amplicon was mixed with 10 µl of loading dye (98% formamide, 10 mM EDTA, 0.025 % bromophenol blue, 0.025% xylene cyanol). After denaturizing at 105°C for 5 min, samples were rapidly cooled on wet ice and then loaded on 12% acrylamide gels. Amplicons representive of the five known myostatin alleles (Zhou *et al*., 2008) were also included in each polyacrylamide gel to use their banding patterns as a standard for determining the alleles present in individual lambs. Electrophoresis was performed using Protean IIxi cells (Bio-Rad), at 350V and 12°C for 18 h in 0.5x TBE buffer. Gels were silver-stained according to the method described by Byun *et al*. (2009).

Sequencing and analysis of allelic polymorphisms

Three PCR amplicons representative of each homozygous genotype were directly sequenced at the DNA Sequencing Facility, Lincoln University, New Zealand. Also, alleles which were found in heterozygous sheep were sequenced using a rapid approach described by Gong *et al.* (2011). Briefly, one of the unique bands representing the novel allele was cut out of the polyacrylamide gel, washed twice with 200 µl 1x TE buffer in a 1.5 ml tube, mashed up with a pipette tip in 50 µl 1x TE buffer and incubated for 1 h at 55°C.

Then, this was used as the DNA template for a re-amplification and sequencing. Sequence alignment, translations and comparisons with the sheep NCBI dbSNP database (http://www.ncbi.nlm.nih.gov/SNP/index.html) were carried out using DNAMAN (version 5.2.10, Lynnon BioSoft, Canada).

Statistical analysis

Statistical analyses exploring the effects of non-genetic factors and the variation in myostatin gene on carcass traits of NZ Romney sheep were undertaken using the general linear model (GLM) procedure of SAS (2000).

Three different sets of modeling approaches were used to test these effects. The first set of general linear mixed models (GLMMs) was used to assess the effect of myostatin genotypes on carcass traits, the second set of GLMMs was used to explore the effect of the presence/absence of each myostatin allele on carcass traits and the third set of GLMMs was performed to test the effect of the number of each myostatin allele copies present on carcass traits. Variation of myostatin gene, farm, year of lambing, parity of ewe and type of birth were fitted as fixed factors while age at slaughtering was included in the model as a co-variant. Where significant, these were further explored using pairwise comparison (Duncan test; $P<0.05$). Only the common genotypes AA, AB, AC, BB and BC were included, as the frequency of CC was less than 1%.

The general mathematical model used to analyze carcass traits can be written as follows:

$$Y_{ijklmno} = \acute{u} + G_i + F_i + Y_k + P_l + T_m + \mathrm{bAS}_{ijklmn} + e_{ijklmno},$$

Where:

$Y_{ijklmno}$ = the observed records on the traits,

$\acute{u}$ = a constant not equal to the overall mean,

G_i = the fixed effect of i^{th} myostatin genotype (i = 1,…5) in the first set of GLMMs, the fixed effect of the presence/ absence of each detected allele in the second set of GLMMs (i = 1, 2) or the fixed effect of the number of copies of each detected allele in the third set of GLMMs (i = 1, 2, 3),

F_j = the fixed effect of j^{th} of farm, j = 1, ..., 10,

Y_k = the fixed effect of k^{th} year of lambing: k= 1,…, 6,

P_l = the fixed effect of l^{th} parity of ewe, l = 1, …, 5,

T_m = the fixed effect of m^{th} type of birth, m = 1, 2, 3,

bAS_{ijklmn} = the partial regression coefficient of carcass traits on age at slaughtering as a covariate and

$e_{ijklmno}$ = the random residual component; assumed to be $(0, \sigma^2 e)$.

RESULTS AND DISCUSSION

Allelic and genotypic polymorphisms

Amplicons of the expected size (414 bp) were obtained from all sheep

blood samples. Using the SSCP analysis, these amplicons exhibited six genotypes (AA, AB, AC, BB, BC and CC with frequencies of 0.107, 0.368, 0.100, 0.289, 0.129 and 0.005, respectively) representing three alleles A, B and C with frequencies of 0.34, 0.54 and 0.12, respectively). Fifteen SSCP genotypes representing five alleles (A, B, C, D and E) were detected in a variety of breeds and composite breeds in New Zealand (Zhou *et al*., 2008). Variation in the same region of genome in New Zealand Romney sheep has been also described by Hickford *et al.* (2009). They also reported six myostatin genotypes AA (0.466), AB (0.302), AC (0.133), BB (0.058), BC (0.035) and CC (0.06), derived from three alleles A (0.683), B (0.227) and C (0.09).

Sequence variation in the intron 1 of myostatin gene

Sequencing the PCR amplicons for homozygous and heterozygous lambs identified six SNPs at positions 18, 241, 243, 249, 259 and 323 which gave rise to the three detected alleles (Fig. 1). These SNPs were non-synonymous substitutions and resulted in amino acid changes [c.373+18 (cysteine/glycine)], [c.373+241 (phenylalanine/serine)], [c.373+243 (methionine/valine)], [c.373+249 (tyrosine/histidine)], [c.373+259 (valine/glycine)] and [c.373+323 (serine/leucine)]. The number of SNPs that were found here was less than those reported by Clop *et al.* (2006) for a Romanov × Texel F_2 population in which 8 SNPs were identified. The other two SNPs were located at positions c.373+101 and c.373+240. The sequences of these three alleles shared high similarity with the published myostatin sequences from cattle (94%) and goats (98%) species.

Effect of non-genetic factors on carcass traits

The Significance effects of non-genetic factors on carcass traits are presented in Table (1). The lambing year showed significant effects ($P<0.05$) on slaughtering weight and dressing% and high significant ($P<0.01$) effects on leg yield, shoulder yield and leg yield%. These differences might be due to the changes in pasture quality that vary from year to year depending on the rainfall level.

Farm significantly ($P<0.5$) affected loin yield, leg yield, total yield and loin yield%. These results suggest that differences in carcass traits may due to differences in management practices applied on different farms. Management at different farms should be optimized to produce lambs that perform well during the growth period and produce desirable carcasses.

As a covariate, age at slaughtering influenced ($P<0.05$) significantly leg yield, shoulder yield% and leg yield%.

No associations were found for parity of ewe and type of birth with all carcass traits. These findings correspond to lower maternal effects for post-weaning growth and then lamb carcass traits.

Effect of myostatin genotype on carcass traits

The effects of myostatin genotype on carcass traits are shown in Table (2). The GLMM results suggested significant effects ($P<0.05$) for the genotype on the slaughtering weight and total yield. In addition, the results suggested high significant associations ($P<0.001$) between the genotype and each dressing percentage, leg yield, shoulder yield%, loin yield% and leg yield%.

Least square mean results showed that lambs with the genotype AC had the highest slaughtering weight and lowest dressing%. Also, the genotype AA had the highest values and the genotype BC had the lowest values for leg yield, leg yield% and total yield. In contrast, loin yield% was higher in lambs with BB genotype and was lower in lambs with AA genotype. These results are partially consistent with the observations made by Hickford *et al.* (2009) who found that the myostatin genotypes in intron 1 significantly affected leg yield, loin yield, total yield and loin yield% and tended to affect shoulder%. Boman *et al.* (2010) detected two myostatin mutation (c.2360G>A and c.960delG) in the Norwegian White Sheep. Both mutations affected conformation and fat class that caused yielding carcasses with less fat and increased muscle mass. Two studies concerned the identification of quantitative trait loci (QTL) affecting the muscling phenotype in Texel sheep. In the first, Marcq *et al.* (1998), used the myostatin as a starting point and found no sequence difference in the coding region of the myostatin gene of Belgian Texel or Romanov sheep. In the second, two microsatellites markers (BM81124 & BULGE20) were detected in the flanking region of myostatin gene and were found to affect the weights of shoulder and leg cuts of Belgian Texel sheep (Laville *et al.*, 2004).

Effect of the presence/ absence of myostatin allele in animal genotype on carcass traits

The effect of the presence/ absence of the detected myostatin alleles on carcass traits are presented in Table (3). The presence of A allele in animal genotype was significantly ($P<0.01$) associated with the higher leg yield, leg yield% and the lower loin yield%. The increased shoulder yield% and loin yield% and the decreased leg yield, leg yield% and total yield were significantly ($P<0.001$) observed in the presence of B allele. Hickford *et al.* (2009) proved that, the presence of allele A was associated with the decreased leg and total yield, whereas the presence of allele B was associated with increased loin yield and loin yield%. Testing the presence/absence of A allele in NZ Texel sheep proved that the presence of A allele was associated with higher slaughter weight, shoulder yield%, loin yield%, leg yield% and total yield (Johnson *et al.*, 2009).

Effect of the number of myostatin allele copies present in animal genotype on carcass traits

The associations between number of myostatin allele copies present in ani-

mal genotype and carcass traits of NZ Romney sheep are presented in Table (4). The presence of two copies of A allele was associated with higher dressing% ($P<0.05$), leg yield ($P<0.001$), leg yield% ($P<0.001$) and total yield ($P<0.05$). Having two copies of B allele also was associated with higher shoulder yield% ($P<0.05$) and loin yield% ($P<0.001$). There were no associations between the number of C allele copies present and all traits.

The effect of number of allele copies present for myostatin gene was investigated by Hickford *et al.* (2009). They found that the presence of two copies of B allele were associated with a decrease in shoulder yield%, whereas two copies of A allele were associated with a decrease in loin yield%. Han *et al.* (2015) investigated the haplotypic diversity of myostatin gene in NZ Romney sheep and identified five haplotypes (H1, H2, H3, H5 and H7) affecting carcass traits. Their results proved that the presence of H1 was associated with an increase in loin yield, leg yield, total yield and loin yield% of lamb carcasses, the presence of H2 was associated with an increase in loin, shoulder and total yield of lean meat. H3 was associated with an increase in leg yield and total yield of lean meat. The presence of H5 in lambs was associated with a decrease in loin yield, shoulder yield and total yield of lean meat. The association of H7 with a decrease in leg yield was identified for the first time in this study.

The variation in intron 1 of myostatin gene is mainly correlated with leg yield, leg yield%, total yield and loin yield% of the lamb carcasses. Leg and loin primal cuts are the most important carcass cuts of lambs that provide optimal returns to the farmers. The loin meat is very tender and is invariably cooked using a dry-heat method and also may be deboned to produce boneless roasts or chops that consumers prefer. Furthermore, the primal leg is the large section of lamb carcass and partially or fully boned to be roasted for buffet service or braised with vegetables or beans for a hearty dish.

However, the genetic variation was found in intronic DNA, which makes it difficult to explain how the variation affected the activity of myostatin. Possibilities include that the intronic sequence may harbor important functional elements that affect gene expression and RNA splicing (Lomelin *et al*., 2010). It may also be linked to nucleotide variation in critical gene control regions (Hickford *et al*., 2009).

Livestock's myostatin gene knockdown has potential advantages for meat production, although, double muscled animals have many defects, such as decreasing the size of internal organs, reducing the fertility of female, delaying the sexual maturation, and decreasing the viability of offspring (Kambadur *et al*., 1997), but it seems that the advantages are greater than the disadvantages. So many researchers make efforts to produce double-muscled livestock that have more muscle mass by developing methods to suppress the expression of myostatin gene

through knockdown or RNA interference. Zhang *et al.* (2007) and Jain *et al.* (2010) have focused on using knockout methods to inhibit the myostatin gene in sheep using a recombinant myostatin. Knocking out this gene includes a large genomic deletion might affect the function of its contiguous genes. Moreover, myostatin is not only expressed in muscle tissue but also in other tissues, and an endogenous basal production of myostatin could have a significant function in an animal's normal growth and development (Zheng *et al*., 2012). Here, the researchers have to imitate the natural mutations in intron 1 to inactivate the myostatin in Romney sheep. These mutations introduced a target to increase the lean yields in loin and shoulder cuts.

The final conclusion, PCR-SSCP is an appropriate tool to detect the variability of the candidate genes affecting important traits of farm animals. Myostatin gene promises higher meat yield form leg and loin areas which are the most preference cuts of lamb carcass for consumers. Genetic improvement is possible for leg cuts through genomic selection for A alleles and also possible for loin cut through genomic selection for B allele. Further investigations are needed to assess the effect of variation in another region in this gene on growth and carcass traits of New Zealand Romney sheep and other breeds.

SUMMARY

Using genetic markers can aid identifying those animals with the highest values for economically important traits in sheep. The current study was designed to detect the allelic and genotypic polymorphisms of the intron 1 of myostatin gene and to test their associations with carcass traits (slaughtering weight, dressing%, shoulder yield, loin yield, leg yield, total yield, shoulder yield%, loin yield% and leg yield%) in 529 male lambs of New Zealand Romney sheep.

The polymerase chain reaction-single strand conformational polymorphism (PCR-SSCP) analysis was used to identify the allelic and genotypic polymorphisms in intron 1 of myostatin gene for 529 males from New Zealand Romney lambs. Associations of the variation in the intron 1 of myostatin gene with carcass traits were determined using the general linear model (GLM) procedure of SAS (2000).

The SSCP analysis revealed six SSCP genotypes: AA, AB, AC, BB, BC and CC with frequencies of 0.107, 0.368, 0.100, 0.289, 0.129 and 0.005, respectively, that derived from three identified alleles: A, B and C with frequency 0.34, 0.54 and 0.12, respectively.

Myostatin genotype significantly affected ($P<0.05$) slaughtering weight and total yield, and highly significant affected ($P<0.001$) dressing%, leg yield, shoulder yield%, loin yield% and leg yield%. The presence of A allele in animal genotype was associated with higher leg yield and leg yield%, however, the presence of B allele was associated with higher loin yield and loin yield%. The LSM showed that, lambs with two copies of A allele had

the highest dressing%, leg yield, leg yield% and total yield, however, lambs with two copies of B allele had the highest shoulder yield, shoulder yield% and loin yield%. The results presented here give valuable information to select for A and B alleles and against C allele to improve the most important primal cuts of lambs across most production systems.

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Table (1): Significance effect of non-genetic factors on carcass traits of New Zealand Romney sheep

Trait	Non genetic factors				
	Year of lambing	Parity of ewe	Type of birth	Farm	Age at slaughtering
Slaughtering weight	*	ns	ns	ns	ns
Dressing%	*	ns	ns	ns	ns
Shoulder yield	ns	ns	ns	ns	ns
Loin yield	ns	ns	ns	*	ns
Leg yield	**	ns	ns	*	*
Total yield	ns	ns	ns	*	ns
Shoulder yield%	**	ns	ns	ns	*
Loin yield%	ns	ns	ns	*	ns
Leg yield%	**	ns	ns	ns	*

ns: refers to non significance ($P > 0.05$); *: refers to significance at ($P < 0.05$); ** refers to significance at ($P < 0.01$).

Table (2): Least square means and their standard errors for carcass traits in New Zealand Romney sheep according to the myostatin genotypes.

Trait	Myostatin genotype					Significance
	AA	AB	AC	BB	BC	
Slaughtering weight (Kg)	114.30^{b} ± 2.42	116.52^{b} ± 0.68	122.45^{a} ± 1.17	117.31^{b} ± 0.75	115.04^{b} ± 1.22	*
Dressing%	47.60^{a} ± 0.50	46.57^{ab} ± 0.23	44.78^{c} ± 0.36	46.26^{b} ± 0.26	46.80^{ab} ± 0.40	***
Shoulder yield (Kg)	17.38 ± 0.09	17.44 ± 0.06	17.63 ± 0.09	17.40 ± 0.07	17.33 ± 0.09	ns
Loin yield (Kg)	14.80 ± 0.08	14.78 ± 0.06	15.02 ± 0.11	14.95 ± 0.07	14.68 ± 0.09	ns
Leg yield (Kg)	22.78^{a} ± 0.08	21.77^{bc} ± 0.08	21.99^{b} ± 0.14	21.67^{bc} ± 0.09	21.54^{c} ± 0.14	***
Total yield (Kg)	54.97^{a} ± 0.19	54.01^{bc} ± 0.17	54.65^{ab} ± 0.27	54.03^{bc} ± 0.21	53.56^{c} ± 0.29	*
Shoulder yield%	31.61^{ab} ± 0.12	32.31^{ab} ± 0.07	32.27^{a} ± 0.15	32.22^{ab} ± 0.07	32.38^{b} ± 0.12	***
Loin yield %	26.93^{c} ± 0.09	27.36^{b} ± 0.06	27.49^{ab} ± 0.11	27.66^{a} ± 0.08	27.40^{ab} ± 0.08	***
Leg yield%	41.44^{a} ± 0.11	40.31^{b} ± 0.06	40.23^{b} ± 0.12	40.10^{b} ± 0.08	40.21b ± 0.12	***

Means of the same trait with the same letter do not significantly ($P < 0.05$) differ from each other; ns: refers to non significance ($P > 0.05$); *: refers to significance at ($P < 0.05$); ** refers to significance at ($P < 0.01$); *** refers to significance at ($P < 0.001$).

Table (3): Association of the presence / absence of myostatin alleles with carcass traits in New Zealand Romney sheep.

Trait	Allele being assessed	LSM ± SE				Significance
		N	Absent allele	N	Present allele	
Slaughtering weight (Kg)	A	221	116.61 ± 0.644	306	117.12 ± 0.679	ns
	B	110	118.19 ± 1.431	416	116.57 ± 0.469	*
	C	406	116.50 ± 0.555	121	118.28 ± 0.919	ns
Dressing%	A	221	46.43 ± 0.220	306	46.45 ± 0.192	ns
	B	110	46.24 ± 0.342	416	46.49 ± 0.159	ns
	C	406	46.60 ± 0.166	121	45.92 ± 0.292	ns
Shoulder yield (Kg)	A	221	17.38 ± 0.057	306	17.46 ± 0.045	ns
	B	110	17.50 ± 0.068	416	17.41 ± 0.041	ns
	C	406	17.42 ± 0.041	121	17.46 ± 0.069	ns
Loin yield (Kg)	A	221	14.86 ± 0.061	306	14.83 ± 0.046	ns
	B	110	14.91 ± 0.070	416	14.83 ± 0.043	ns
	C	406	14.85 ± 0.043	121	14.83 ± 0.075	ns
Leg yield (Kg)	A	221	21.63 ± 0.081	306	22.00 ± 0.064	**
	B	110	22.40 ± 0.089	416	21.70 ± 0.058	***
	C	406	21.88 ± 0.059	121	21.73 ± 0.102	ns
Total yield (Kg)	A	221	53.88 ± 0.169	306	54.30 ± 0.127	ns
	B	110	54.82 ± 0.166	416	53.94 ± 0.121	***
	C	406	54.15 ± 0.118	121	54.03 ± 0.206	ns
Shoulder yield%	A	221	32.27 ± 0.066	306	32.17 ± 0.061	ns
	B	110	31.93 ± 0.102	416	32.29 ± 0.049	**
	C	406	32.18 ± 0.050	121	32.33 ± 0.098	ns
Loin yield%	A	221	27.58 ± 0.062	306	27.30 ± 0.047	**
	B	110	27.19 ± 0.080	416	27.48 ± 0.043	**
	C	406	27.41 ± 0.045	121	27.44 ± 0.069	ns
Leg yield%	A	221	40.13 ± 0.070	306	40.51 ± 0.059	**
	B	110	40.86 ± 0.102	416	40.22 ± 0.049	***
	C	406	40.39 ± 0.054	121	40.22 ± 0.087	ns

ns: refers to non significance ($P > 0.05$); *: refers to significance at ($P < 0.05$); ** refers to significance at ($P < 0.01$); *** refers to significance at ($P < 0.001$).

Table (4): Association of myostatin allele copy number with carcass traits of New Zealand Romney sheep.

Trait	Allele being assessed	LSM ± SE						Significance
		Allele absent	N	Allele 1 copy	N	Allele 2 copies	N	
Slaughtering weight (Kg)	A	116.61 ± 0.64	221	117.78 ± 0.61	248	114.30 ± 2.42	57	ns
	B	118.19 ± 1.43	110	116.13 ± 0.60	263	117.31 ± 0.75	153	*
	C	116.50 ± 0.55	406	118.28 ± 0.91	121	-	0	ns
Dressing%	A	46.43^{b} ± 0.22	221	46.19^{b} ± 0.20	248	47.60^{a} ± 0.50	57	*
	B	46.24 ± 0.34	110	46.63 ± 0.20	263	46.26 ± 0.26	153	ns
	C	46.60 ± 0.16	406	45.92 ± 0.29	121	-	0	ns
Shoulder yield (Kg)	A	17.38 ± 0.05	221	17.48 ± 0.05	248	17.38 ± 0.09	57	ns
	B	17.50 ± 0.06	110	17.41 ± 0.05	263	17.40 ± 0.07	153	ns
	C	17.42 ± 0.04	406	17.46 ± 0.06	121	-	0	ns
Loin yield (Kg)	A	14.86 ± 0.06	221	14.83 ± 0.05	248	14.80 ± 0.08	57	ns
	B	14.91 ± 0.07	110	14.75 ± 0.05	263	14.95 ± 0.07	153	ns
	C	14.85 ± 0.04	406	14.83 ± 0.07	121	-	0	ns
Leg yield (Kg)	A	21.63^{b} ± 0.08	221	21.82^{b} ± 0.02	248	22.78^{a} ± 0.08	57	***
	B	22.40^{a} ± 0.08	110	21.71^{b} ± 0.07	263	21.67^{b} ± 0.09	153	***
	C	21.88 ± 0.05	406	21.73 ± 0.10	121	-	0	ns
Total yield (Kg)	A	53.88^{b} ± 0.16	221	54.15^{b} ± 0.14	248	54.97^{a} ± 0.19	57	*
	B	54.82^{a} ± 0.16	110	53.89^{b} ± 0.14	263	54.03^{b} ± 0.20	153	***
	C	54.15 ± 0.11	406	54.03 ± 0.20	121	-	0	ns
Shoulder yield %	A	32.27^{a} ± 0.06	221	32.30^{a} ± 0.06	248	31.61^{b} ± 0.12	57	***
	B	31.93^{b} ± 0.10	110	32.33^{a} ± 0.06	263	32.22^{a} ± 0.06	153	*
	C	32.18 ± 0.05	406	32.33 ± 0.09	121	-	0	ns
Loin yield%	A	27.58^{a} ± 0.06	221	27.39^{a} ± 0.05	248	26.93^{b} ± 0.09	57	***
	B	27.19^{b} ± 0.08	110	27.37^{b} ± 0.04	263	27.66^{a} ± 0.08	153	***
	C	27.41 ± 0.04	406	27.44 ± 0.06	121	-	0	ns
Leg yield%	A	40.93^{b} ± 0.07	221	40.29^{b} ± 0.06	248	41.44^{a} ± 0.11	57	***
	B	40.86^{a} ± 0.10	110	40.28^{b} ± 0.06	263	40.10^{b} ± 0.08	153	***
	C	40.39 ± 0.05	406	40.22 ± 0.08	121	-	0	ns

Means of the same trait with the same letter do not significantly ($P < 0.05$) differ from each other; ns: refers to non significance ($P > 0.05$); *: refers to significance at ($P < 0.05$); ** refers to significance at ($P < 0.01$); *** refers to significance at ($P < 0.001$).

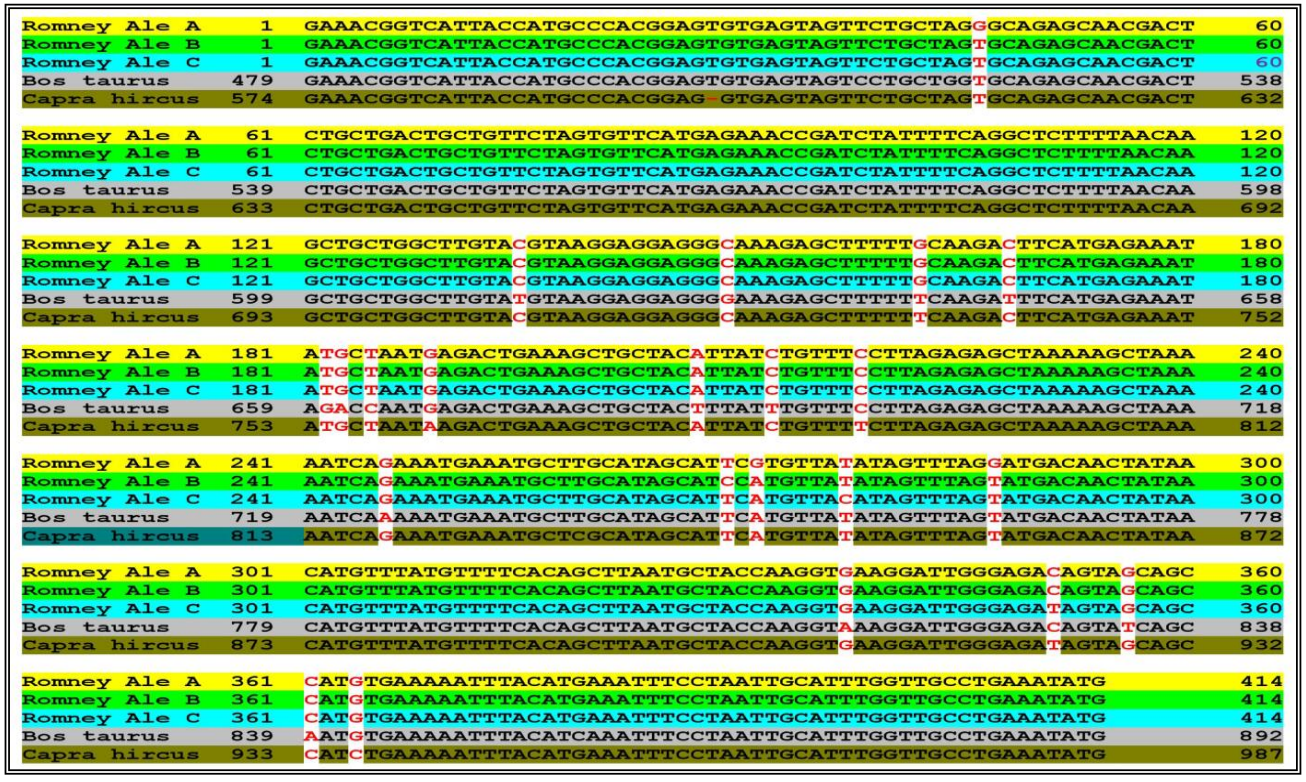

Fig. (1): Sequences of the three detected alleles of the myostatin gene in New Zealand Romney sheep and their corresponding regions in *Bos taurus* and *Capra hircus* species.