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PCR-RFLP of the Ovine Calpastatin Gene and its Association with Growth

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ABSTRACT

This study was designed to investigate effects of the calpastatin (CAST) genotypes determined by PCR-RFLP (polymerase chain reaction-restriction fragment length polymorphism) on weight traits (birth, weaning, post-weaning weights and average daily gain). A total of 359 animals (116 purebred Polypay, 110 purebred Targhee and 133 crossbred sheep) born at the Ohio Agricultural Research and Development Center (OARDC) were used. Selection of PCR primers for the bovine CAST gene was based on exons from 24 to 28 corresponded to a calcium binding domain. RFLP analysis with the Taq I restriction enzyme revealed 3 restriction sites at nucleotide positions 208, 467 and 1,198, resulting in fragments of 731, 467 (259 and 208) and 125 bp. After verification of individual sequences, differences revealed substitutions of nucleotides (A to G) at a nucleotide position 208 located in a non-coding region and newly identified sequences were submitted into GenBank with an accession number (AF285630). Genotypes were significantly associated with Birth weight (BW) and Average Daily Gain (ADG) and significant additive effects for BW and ADG and dominance effects for PW and ADG were estimated. The mixed breed revealed significantly high values for all traits comparing with purebreds, assuming that the maximized genetic effects were caused by heterosis. Analysis of breed compositions with genotypes revealed that 75% of Suffolk with AA genotypes and 50% of Targhee with BB genotypes showed significantly low PW. The calpastatin genotypes discovered by PCR-RFLP may explain variations of growth and be useful as genetic markers for a marker assisted selection program with BW and ADG.

Key words: Calpastatin, ovine, SNP, growth

INTRODUCTION

Major subjects of sheep industry are understanding biochemical and physiological mechanisms underlying growth. Several studies regarding characterization of growth patterns have been focused on growth factors and hormones that influence maximization of economic benefits (Banerjee *et al.*, 2010; Cam *et al.*, 2010; Crisa *et al.*, 2010; Hickford *et al.*, 2010; Zeng *et al.*, 2011). In addition, the most difficult issue in animal breeding is that we actually do not have clear answers for what kinds of and how many genes are involved in the process of growth. It is, therefore, required to understand mechanisms of growth using molecular techniques to identify genetic and environmental effects.

Calpastatin, which is a widely distributed endogenous inhibitor (EC 3.4.22.17, Ca^{2+} dependent cysteine proteinase) of calpains (u-and m-calpain), plays a central role in regulation of the calpain activity in cells (Goll *et al.*, 1998; Maki *et al.*, 1989) and is considered to be one of major modulators of calpains. Calpains are fully inhibited in the cell depending on their relative degree of associations with calpastatin in the intracellular environment and domains II and IV were responsible for protease activity and binding Ca^{2+} , respectively (Maki *et al.*, 1989; Kawasaki *et al.*, 1993).

Calpastatin seems to be the variable component of the calpain system (Hirwa *et al.*, 2011) and the skeletal muscle calpastatin activity is highly related to the rate of muscle protein turn over. The current paradigm is that high calpastatin activity decreases the rate of muscle protein turnover and is associated with an increased rate of skeletal muscle growth (Goll *et al.*, 1998). The rate and extent of skeletal muscle growth depend on three factors such as the rate of muscle protein synthesis, rate of muscle protein degradation and the number of size of skeletal muscle cells. Studies have shown that the calpastatin activity is significantly related to muscle growth (Parr *et al.*, 1992; Pringle *et al.*, 1993). Furthermore, the calpastatin activity is associated with muscle hypertrophy in sheep carrying the callipyge gene (Duckett *et al.*, 2000). In addition, the callipyge phenotypes, which are inherited as an autosomal in sheep (Cockett *et al.*, 2005; Qanbari *et al.*, 2007), have shown that the calpastatin activities in the affected muscles from the callipyge lambs were 68-126% higher than in the same muscles from normal lambs (Koochmaraie *et al.*, 1995). A study also reported that CAST was used to slow muscle wastage in experimental animals (Tidball and Spencer, 2002). Moreover, the increase of calpain/CAST mRNA ratio caused the increase in the calpain catalytic activity (Pringle *et al.*, 1993) under conditions of muscle breakdown induced by starvation (Salem *et al.*, 2005). Overall, these results suggest that increased rates of skeletal muscle growth can be resulted from a decrease the rate of muscle protein degradation that is associated with decreases in activity of the calpain system, due principally to a large increase in the calpastatin activity (Goll *et al.*, 1998). With supporting the idea regarding CAST related to growth traits, Byun *et al.* (2008) reported associations of the ovine calpastatin gene with birth weight and growth rates to weaning. According to genetic mechanisms between calpastatin and calpain, understanding genetic variation of CAST in molecular levels may explain variations of growth.

Several studies discovered genetic variants in the calpastatin gene focusing on exon 1 (Nassiry *et al.*, 2007), exon 6 (Chung *et al.*, 2001), exon 12 (Zhou *et al.*, 2007), intron 12 (Juszczuk-Kubiak *et al.*, 2008) and domains from II to IV (Cockett *et al.*, 1995). The domain IV contains four EF-hand structures consisting of single helix-loop-helix (Sorimachi *et al.*, 1996; Raser *et al.*, 1996) structures, which are calcium binding modules responsible for calcium dependent regulation of calpain. Each EF-hand structure potentially binds one calcium ion, but actually 2 or 3 calcium ions can bind to the domain IV (Minami *et al.*, 1987), suggesting that the EF-hand roughly corresponds to the calcium concentration required for its activity. However, it is not clear whether calpastatin inhibits the binding of calpain to cell membranes by a regulatory inhibition site or the inhibiting sequences (Kawasaki *et al.*, 1993).

Calpastatin has been cloned using swine (Maki *et al.*, 1987), rat (Ishida *et al.*, 1991), human (Maki *et al.*, 1989) and bovine (Killefer and Koochmariae, 1994), comprising 603 amino acid residues with 4 repetitive sequences (TIPPLYL, nucleotide positions at 1,832-1,852 corresponded to the domain IV based on L14450), which are responsible for the calpain inhibitory activity. However, the calcium binding domain (domain IV), which is very important because both calpain and calpastatin were under the major influence of calcium binding activity according to calpain/CAST

ratio (Salem *et al.*, 2005), has not been studied to search genetic variants. In addition, the intracellular free Ca^{2+} concentration is elevated in the muscular dystrophies and stimulates the calpain and calpastatin activities (Turner *et al.*, 1991). Thus, it may be useful tools that genetic variants in genomic regions corresponded to the calcium binding domain, which essentially regulates the calpastatin activity, may account for variations of growth. If calpastatin is highly heritable with high genetic correlations for economic traits of interest, its use in a selection program may increase the rate of genetic changes. This study was, therefore, carried out to find genetic variations in the calcium binding domain of the calpastatin gene, to investigate effects of genotypes on growth traits and to provide useful information for the selection criteria of sheep industry.

MATERIALS AND METHODS

Animals: A total of 359 sheep (116 purebred Polypay, 110 purebred Targhee and 133 crossbred sheep) born at the Ohio Agricultural Research and Development Center (OARDC) were used for identification of genetic variation and association tests. In the pedigree analysis, the selected animals did not have significant genetic relationships between individuals. The mixed population, which was constructed with Hampshire, Polypay, Rambouillet, Dorset, Suffolk and Targhee breeds using rotational and terminal crossing systems, contained average genetic materials of Polypay (11.23%), Targhee (30.43%), Suffolk (51.81%) and others (6.52%). Lambs were weaned at an average age of approximately 60 days and post weaning weights were measured at an average age of approximately 200 days in accordance with guidelines of the National Sheep Improvement Program (NSIP). Animals were fed with a post weaning diet formulated to meet nutrient requirements of lambs according to NRC (1985). Least squares means and standard errors of birth (BW), weaning (WW), post-weaning (PW) weights and Average Daily Gain (ADG) by the calpastatin genotypes and breeding groups were estimated. Individuals contained only 75 and 50% of genetic materials for each Suffolk and Targhee breeds in the mixed population were selected to estimate actual breed effects with genotypes for weight traits. For the testing of Mendelian segregation, approximately 122 DNA samples from eight sheep reference families at the Agricultural Research Center in New Zealand were used.

Genomic DNA preparation: Approximately, 10 mL of whole blood was taken from the jugular vein with heparin and red cells were washed out four times using ammonium chloride. Cell lysis was conducted using a buffer (10 mM tris-HCl, 400 mM NaCl and 2 mM EDTA, pH 8.2) with 20% SDS and 10 mg of proteinase K (Ahmed, 2006). Genomic DNA was precipitated with two volumes of ethanol and 7 M of sodium acetate.

Primer design: Selection of primers focusing on exons from 24 to 28 corresponded to the calcium binding domain of CAST was based on the coding sequences (GenBank accession no. L14450) using the PRIMER SELECT in DNASTAR version 6.0 with options for an optimal TM (57°C) and amplification size (200 bp). For the primer C2425 containing exons 24 and 25, forward and reverse primers were ATGCC CTGGA TCAAC TTTCT G (nucleotide positions 1,698-1,718) and TGGTA TTTAG GTGGG ATGGT GTCA (1,825-1,848), respectively. For the primer C2728 containing exons 27 and 28, forward and reverse primers were GTGCC CAGGA CCCC TTGA (1,941-1,959) and ATCCT TTGCT TTCCC GCCAT TC (2,065-2,086), respectively.

PCR-restriction fragment length polymorphism (RFLP): Two microliters of 10 X reaction buffer (10 mM Tris, pH 8.3, 50 mM KCl, 0.1% Triton X-100, 1.5 mM MgCl_2), 2.5 mM dNTP, 10 pmol of each primer, 50 ng of the genomic DNA and 1 unit of Tag DNA polymerase (Gibco BRL, Grand

Island, NY) in a final volume of 20 μ L were used. After heating at 95°C for 2 min, a total of 35 cycles were adapted for denaturation at 94°C/1 min, annealing at 59°C/1 min and polymerization at 72°C/1.5 min (MJ Research, PT-200, Watertown, MA). PCR-generated fragments of the ovine calpastatin gene were identified on 1.2% agarose gels. RFLP analysis was performed using 8 μ L of PCR products and 2 U of Taq I restriction enzyme and the mixture was incubated at 65°C for 2 h. After digestion, electrophoresis analysis visualized genetic variants on agarose gels containing ethidium bromide.

Confirmation of PCR products: Animals having different homozygous genotypes were selected to verify Single Nucleotide Polymorphisms (SNPs). Individual DNA samples showing different RFLP banding patterns were purified using the NucleoTrap gel purification system (Clontech, Palo Alto, CA). Direct sequencing was conducted to verify genetic variants with an ABI3730 Genetic Analyzer (Applied Biosystems) at the National Institute of Animal Science (KY, Korea).

Statistical analysis: Analysis of variance was conducted to investigate effects of genotypes on Birth Weight (BW), Weaning Weight (WW), Post Weaning Weight (PW) and Average Daily Gain (ADG). Least squares means and standard errors were estimated. A statistical model was fitted:

$$y_{ijklm} = u + g_i + tb_j + tr_k + bg_l + \beta a_m + e_{ijklm}$$

where, y_{ijklm} is trait measured, u is overall mean; g_i is genotype effect ($i = AA, AB$ and BB); tb_j is type of birth effect ($j = 1-4$, easy to difficult); tr_k is type of rearing effect ($k = 1-3$); bg_l is breed effect (Polypay, mixed and Targhee); βa_m is age as a covariate and e_{ijklm} is residual errors. Analysis of variance was performed using the Statistical Analysis System (version 9.1) with the General Linear Model (GLM) procedures and least squares means were compared using Fisher's the least significant difference test (SAS, 2009).

RESULTS

Analysis of PCR-generated fragments: Primer sets targeting genomic regions amplified approximately 1,323 bp (C2425) and 1,505 bp (C2728) of segments that were identified on 1.2% agarose gels. RFLP analysis for C2425 segments with the Taq I restriction enzyme revealed 3 banding patterns presenting fragments of 731, 467 (259 and 208) and 125 bp (Fig. 1), whereas,

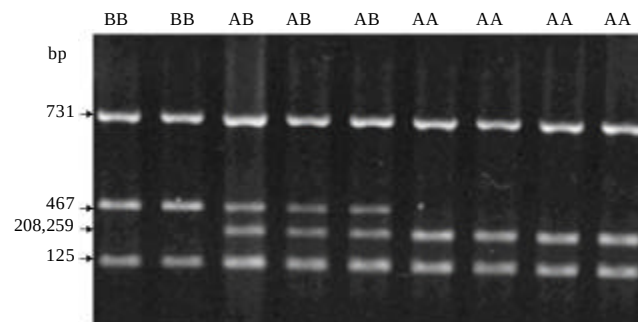


Fig. 1: PCR-RFLP with the Taq I restriction enzyme for segments located in exons 24 and 25 of the ovine calpastatin gene, demonstrating the three genotypes (AA, AB and BB), PCR products (8 μ L) were cut using 2 units of the Taq I restriction enzyme with incubation at 65°C for 2 h and 4 fragments [731, 467 (259 and 208) and 125 bp] were produced

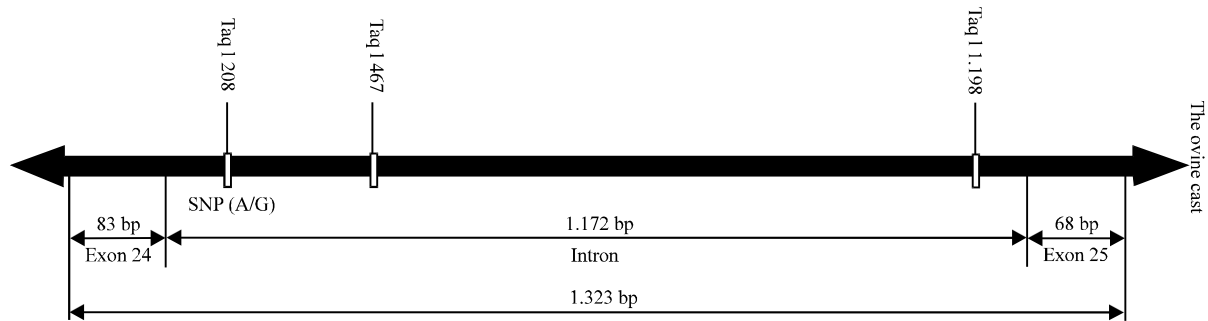


Fig. 2: A map has been constructed with the identified RFLPs using the Taq I restriction enzyme for the ovine calpastatin gene showing 3 restriction sites and nucleotide substitutions at a nucleotide position 208 (A/G) based on sequences with an GenBank accession number (AF285630)

no genetic polymorphisms were detected for C2728 segments. The verification of SNPs was conducted with animals presenting different genotypes in RFLP analysis and sequence differences revealed substitutions of nucleotides (A to G) at a nucleotide position 208 locating in a non-coding region. Newly identified genetic variants, which were observed firstly in this study, were submitted into GenBank with an accession number (AF285630). Sequence analysis showed that the restriction sites by the Taq I enzyme revealed at nucleotide positions 208, 467 and 1,198 (Fig. 2).

Estimation of allele frequency: Co-dominant Mendelian segregation of alleles was confirmed using 8 full-sib reference families of IMF (international managed flock) presenting allele frequencies of A (0.442) and B (0.558). To estimate allele frequencies that may show different distributional patterns according to breed specificity, the estimation of allele frequencies revealed no significant deviations from the Hardy-Weinberg equilibrium in purebreds (Polypay and Targhee) and crossbred (mixed breed) animals (Table 1). Significant differences of frequencies for allele A have been detected between Polypay (0.524) and Targhee (0.182). The most interesting finding was that allele frequencies in mixed population, which has been created by terminal and rotational crosses with 6 breeds, underlie between frequencies of Polypay and Targhee breeds. Overall frequencies of alleles A and B were estimated to 0.373 and 0.627, respectively.

Association test: As shown in Table 2, genotypes of the ovine calpastatin gene were significantly associated with BW and ADG, while significant genotypic effects were not detected on WW and PW. The statistical analysis revealed that least square means were significantly high in BW with animals having AA genotypes than that of animals with other genotypes (AA 5.157>AB 4.905>BB 4.679 kg). Significant mean differences between genotypes were observed in ADG, which showed that AA genotypes (0.222 kg) were greater than AB (0.217 kg) and BB (0.201 kg) genotypes.

In general, sheep breeds are used for several purposes that consider characterizing breeds to maximize economic benefits in commercial mating systems. As expected, breed effects accounted for variations of all traits except PW. Polypay and Targhee breeds showed low values, while the mixed breed showed significantly high values in all traits. Even though Polypay showed low BW comparing with Targhee, differences of PW were not detected at the end of performance testing periods. As expected that there was a heterosis effect from mating systems between purebreds, we found significantly high ADG in the mixed population, comparing with purebreds. In addition,

Table 1: Allele frequencies of the ovine calpastatin gene segments (C2425) for pure and crossbred populations

Allele	Polypay (P)			Mixed (M) ¹			Targhee (T)			Overall	
	Frequency	N	HWE	Frequency	N	HWE	Frequency	N	HWE	Frequency	N
A	0.524	116	5.121	0.419	133	4.092	0.182	110	2.974	0.373	359
B	0.476			0.581			0.818			0.627	

¹The mixed breed was consisted of Hampshire, Targhee, Polypay, Rambouillet, Dorset and Suffolk breeds containing average 11.23, 30.43, 51.81 and 6.52%, respectively, HWE: Hardy-Weinberg equilibrium

Table 2: Least squares means and standard errors of birth weight (BW), weaning weight (WW), post-weaning weights (PW) and average daily gain (ADG) by the calpastatin genotypes and breeding groups

Item	Genotype	N	BW (p = 0.007)	WW (p = 0.368)	PW (p = 0.915)	ADG (p = 0.006)
C2425	AA	136	5.157±0.08 ^a	22.340±0.40	58.159±0.89	0.222±0.01 ^a
	AB	142	4.905±0.08 ^{ab}	22.906±0.41	58.621±0.83	0.217±0.01 ^a
	BB	81	4.679±0.12 ^b	21.878±0.63	58.642±1.45	0.201±0.01 ^b
			p = 0.001	p = 0.001	p = 0.622	p = 0.001
Breed	Polypay	116	4.195±0.07 ^c	21.659±0.46 ^b	57.757±1.03	0.187±0.01 ^c
	Mixed ¹	133	5.442±0.07 ^a	24.105±0.42 ^a	59.035±0.87	0.236±0.01 ^a
	Targhee	110	5.131±0.07 ^b	20.319±0.56 ^b	58.396±1.16	0.206±0.01 ^b

¹The mixed breed consisted of Hampshire, Targhee, Polypay, Rambouillet, Dorset and Suffolk, Different letters mean significant differences, All weight measurements were reported in kg

Table 3: Genetic effects of the ovine calpastatin gene on growth traits

Traits	Breed	Genetic effect			
		Additive	p-value	Dominance	p-value
BW	Polypay	0.706±0.25	0.006	-0.435±0.21	0.413
	Mixed	0.065±0.21	0.756	0.146±0.20	0.626
	Targhee	0.060±0.59	0.919	0.420±0.60	0.537
	All population	0.459±0.17	0.008	0.052±0.17	0.828
WW	Polypay	2.491±1.47	0.095	1.387±1.25	0.497
	Mixed	-0.350±1.25	0.781	1.429±0.20	0.424
	Targhee	3.523±3.06	0.253	3.794±3.12	0.280
	All population	0.112±0.85	0.895	1.839±0.85	0.131
PW	Polypay	-3.080±4.16	0.461	-6.539±3.55	0.259
	Mixed	0.432±1.65	0.794	5.957±1.58	0.012
	Targhee	10.033±5.98	0.097	9.689±6.07	0.158
	All population	-0.489±1.65	0.767	0.461±1.63	0.844
ADG	Polypay	0.019±0.01	0.056	-0.007±0.01	0.571
	Mixed	0.022±0.01	0.009	0.025±0.01	0.033
	Targhee	0.021±0.02	0.338	0.013±0.02	0.586
	All population	0.025±0.01	0.001	0.011±0.01	0.226

¹BW, WW, PW and ADG are standing for birth, weaning and post-weaning weight and average daily gain, respectively

Targhee revealed a significantly high ADG than Polypay. The types of birth and rearing were already known factors for weight traits in sheep analysis and therefore, no further results were described. Additive and dominance effects were observed for each breed including all populations to describe particular allele actions on breeds (Table 3). Significant additive effects were determined in BW for Polypay and all populations and ADG for the mixed and all populations. Only mixed breed showed dominance effects in PW and ADG.

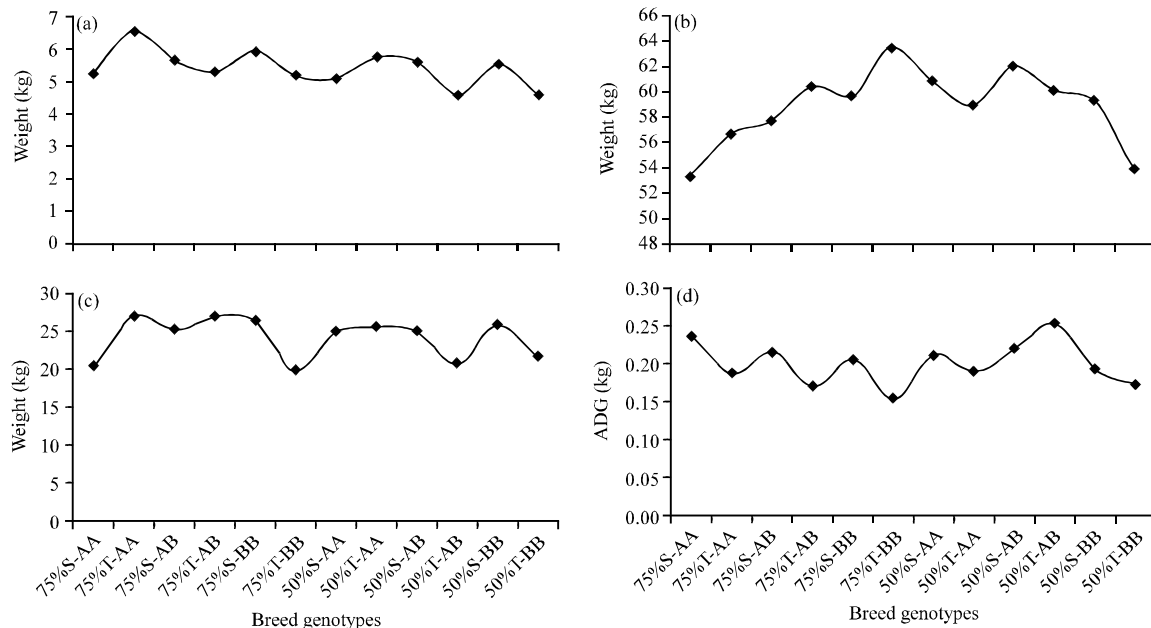


Fig. 3(a-d): Differences of weight traits by breed compositions (X-axis) with the calpastatin genotypes, in the mixed population, individuals contained only 75 and 50% of genetic materials for each Suffolk and Targhee breeds were selected to estimate actual breed and genotype effects for growth traits, (a) Birth, (b) Post-weaning, (c) Weaning and (d) Average daily gain

To verify effects by breed compositions with genotypes in the mixed population, animals containing exactly 75 and 50% of genetic materials for each Suffolk and Targhee breeds were selected because these breeds were openly used for rotational and terminal mating systems. As shown in Fig. 3, significant differences between breed compositions with genotypes for all measured traits were observed. The 75% of Targhee with AA genotypes (75%T-AA) showed extremely high BW (6.545 kg) while low BW (4.575 kg) was observed in 50% of T with AB and BB genotypes (50%T-AB and BB). The 75% S-AA, 50% T-AB and 75% T-BB showed relatively low WW than others. The 75% S-AA and 50% T-BB showed extremely significant low PW (average 53.603 kg) while 75% T-BB showed the highest PW (63.423 kg). The highest ADG was detected in 50% T-AB while 75% T-BB showed the lowest values. ADG of 75% Suffolk with all genotypes tended to be higher than 75% Targhee, whereas 75% Suffolk was smaller than 75% Targhee in PW.

DISCUSSION

Genetic variation of the ovine calpastatin gene: Since the calpastatin gene was, firstly, cloned for swine (Maki *et al.*, 1987), bovine (Killefer and Koohmariae, 1994) has been revealed approximately 2,117 bp length of mRNA comprising 723 amino acids. Genetic variations located in 3' UTR and introns (5, 6 and 12) in CAST have been intensively studied (Chung *et al.*, 2001; Juszczuk-Kubiak *et al.*, 2008; Barendse, 2003). In addition, SNPs of CAST are currently used in commercial areas as the IGENITY TenderGene marker in the Gene-Star (Schenkel *et al.*, 2006).

Calpastatin has repeated sequences (TIPPLYL), which are located in nucleotide positions from 1,832 to 1,852 corresponded to the calcium binding domain of CAST based on a GenBank accession number (L14450) and believed responsible for the calpain inhibitory activity. However, variations have not been reported in ovine and therefore, genetic information in this genomic region may give

us useful clues to understand variations of muscle organization and growth. In general, genetic variants are interested in understanding genetic effects that may be associated with economically important traits regarding selection markers for animals at early growth stages. Furthermore, utilization of genetic markers may maximize economic benefits when mutations influencing gene codes are responsible for quantitative traits.

Significant differences of allele frequencies have been detected between pure and mixed populations in this analysis. In addition, we found significance differences of allele frequencies between purebreds, implying that genetic constitution of Targhee for calpastatin was extremely different with Polypay as well as other purebreds and crossbreeds. The identified genetic variants, therefore, may provide useful information to select sires and dams for pure and cross breeds in mating systems of commercial populations regarding a variety of production traits.

Association: Our results confirmed genotypic effects of CAST on BW and ADG using purebreds and crossbred populations while Byun *et al.* (2008) reported significant genotypic effect using Romney lambs on BW. The most interesting finding in our results was that animals with BB genotypes showed low BW and WW, but there were no significant differences in PW. The results strongly suggested that the genotypes may be helpful for animal breeders to reduce types of birth difficulty that is critically related to income while considering BB genotypes in mating systems. Therefore, the identified genotypic effects in this study will give us many advantages for mating systems considering that the calpastatin genotypes may be directly used to selection programs.

Nonneman and Koochmaraie (1999) suggested that calpain has an important role in muscle development and growth, calpastatin should be considered regardless animal growth. Actually, breeding schemes focus on heterosis to maximize economic benefits when the determination of breeds for mating systems is required. ADG and weight traits have relatively high priorities regarding heterosis in sheep industry and as supporting the fact, Hielscher *et al.* (2006) reported that the high heterosis for ADG and weight traits are significantly related to carcass traits.

As expected significant weight differences in breeding populations, Polypay and Targhee breeds clearly showed significant mean differences for all weight traits in this analysis. Iman and Slyter (1996) also reported differences of breeds for significant effects on weaning and litter weights using Targhee and Dorset breeds. They also proposed that differences may be caused by characterization of milking behavior to take care of animals, assuming that characterization of some breeds described in better performance ability than other breeds. As we observed significant effects by breeds on weight traits, characterization of purebreds and crossbreeds may be used in commercial markets to maximize population benefits for particular purposes such as growth, wool, milk and meat traits. However, without specified information for characterization of breeds, any breeding scheme should be difficult to increase economic benefits or to reach goals that have been established by commercial breeders. The most important issue that we have to consider in animal breeding is heritability of growth traits because mating or breeding plans should be adjusted when the heritability of target traits is lower than expected. As a matter of fact, weight traits are generally tended to show high heritability and several studies estimated a direct heritability of birth (0.12-0.25), weaning (0.19-0.35) and fleece weights (0.54) (Van Vleck *et al.*, 2005; Hanford *et al.*, 2003; Gicheha *et al.*, 2006; Lavvaf and Noshary, 2008). Bromley *et al.* (2000) also reported that heritability of ADG was 0.26 with Columbia, Polypay, Rambouillet and Targhee breeds.

Al-Wahab (2003) found that a crossbred showed higher effects on weight traits than that of pure breeds, our results may provide guidelines for utilization of breeds in mating systems,

assuming that combinations of alleles may be considered to have the best benefits. In addition, the results of additive and dominance effects according to pure and cross breeds may provide lots of benefits to animal breeders when significant allele effects are confirmed. The most important benefit was that we may select animals using the identified genetic variants at early growth stages when the demands of consumers have been aimed at improving quantitative traits through crossings (Cemal *et al.*, 2007).

Significant differences between 75% S-AA (53.303 kg) and 50% S-AA (60.747 kg) in PW revealed that genetic compositions are significantly differed even though the same genotypes existed (Fig. 3). In other words, Suffolk breed with AA genotypes should be depended on terminal crosses because 50% S-AA was from rotational crossing systems. The same result was detected between 75% S-AB (57.660 kg) and 50% S-AB (61.947 kg). Therefore, we may conclude that Suffolk breed may not have high priorities in terms of terminal crosses with AA and AB genotypes. On the other hand, no significance was detected between 75% T and 50% T with AA and AB genotypes, but 75% T-BB (63.421 kg) revealed extremely high PW than 50% T-BB (53.907 kg), assuming that Targhee breed may be preferred to terminal mating systems with BB genotypes. However, in this study we only compared breed compositions of Suffolk and Targhee in the mixed population and sheep breeds are normally used differently according to target products.

CONCLUSION

From the result of significant genotypic effects in BW and ADG, we may conclude that the calpastatin genotypes discovered by PCR-RFLP may explain variations of either muscle development or growth regarding useful genetic markers for the marker assisted selection criteria. However, selection of animals with genotypes is still a controversial issue because genetic effects become clouded after birth due to interactions between genetic and non-genetic effects for muscle growth. We may, thereby, suggest that further studies are still required for selection areas considering mating systems by several breeds with identified genes. Further studies focusing on these possibilities and the role of imprinting of CAST would be highly relevant to elucidate the influence of this genomic region in relation to observed growth traits.

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