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Short Communication

Genotypic frequency of *calpastatin* gene in lori sheep by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method

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Calpastatin is a natural occurring inhibitor of calpastatin (CAST) and consequently the balance of calpain-calpastatin activity in muscles is believed to dictate the rate of tenderization in post-mortem meat. Genomic DNA was extracted from 100 sheep blood sample. Polymerase chain reaction was performed to amplify a 622 bp fragment of this gene. Restriction reaction of polymerase chain reaction (PCR) products was done using MspI enzyme. The MspI digestion of the PCR products produced digestion fragments of 336 and 286 bp. The results show that in the population, genotypes AA, AB and BB, respectively, had frequencies 32.2, 63.2 and 4.6, and that this locus was not at Hardy - Weinberg equilibrium in the lori sheep strain (P<0.05).

Key words: *Calpastatin* gene, polymorphism, lori sheep, polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP).

INTRODUCTION

Increase in sheep production will help increase mutton production and study on *calpastatin* gene combined with other molecular techniques such as marker assisted selection (MAS) can play very important part to better sheep production in Iran. The effect of calpains gene polymorphism on the analyses meat quality traits are discussed in detail in another paper (Goll et al., 1998; Chung et al., 2002; Forsberg et al 1989). The protein encoded by this gene is an endogenous calpain (calcium-dependent cysteine protease) inhibitor. It consists of an N-terminal domain L and four repetitive calpain-inhibition domains (domains 1-4), and it is involved in the proteolysis of amyloid precursor protein. Of the five domains, the N-terminal leader (L) domain does not appear to have any calpains inhibitory activity, but maybe involved in targeting or intracellular localization (Takano et al., 1999), while the other domains (I-IV) are highly homologous and are each independently capable of

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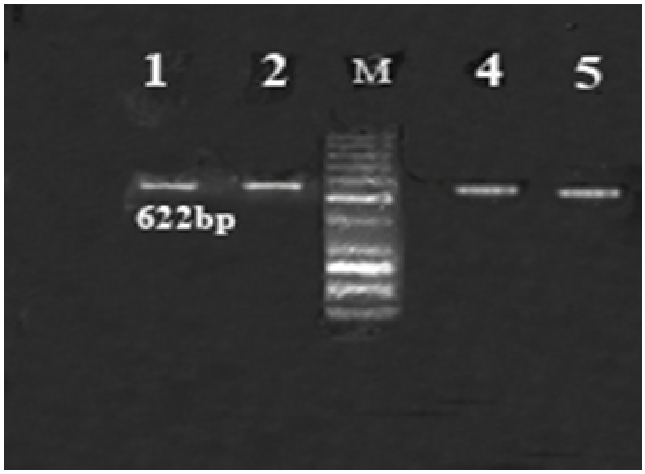

Figure 1. PCR product analyzed by electrophoresis (622 bp).

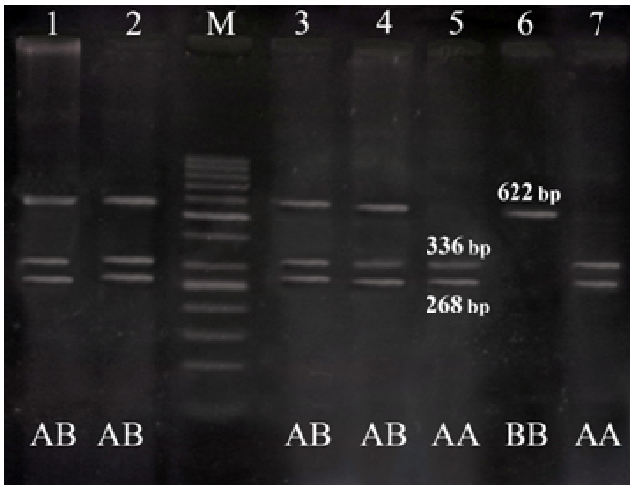

Figure 2. Genotype AA, AB and BB digestion with MspI.

inhibiting calpains (Cong et al., 1998). This Indicates that the inhibitory domains of calpastatin contain three highly conserved regions, A, B and C, of which A, played a regulatory role by altering phosphorylation patterns on the protein (Takano et al., 1999). Calpastatin (*CAST*) gene is located on the fifth chromosome of sheep and plays important roles in the formation of muscles, degradation and meat tenderness after slaughter. Increased rate of skeletal muscle growth can result from a decreesed rate of muscle protein degradation, and this is associated with a decrease in activity of the calpain system, due principally to a large increase in calpastatin activity (Goll et al., 1998).

Associations have been reported between variation in CAST, and carcass and meat quality traits in cattle (Casas et al., 2006; Schenkel et al., 2006), but in sheep, a genetic variation in the CAST gene has been investigated too (Palmer et al., 2000; Zhou et al., 2007). In our research, we have studied the position of the *calpastatin* gene in the lori sheep breeds in Iran.

MATERIALS AND METHODS

In this study, random blood samples were collected from 100 lori sheep from different regions in Lorestan province of Iran. Approximately, 5 ml blood sample was gathered from vena in ethylene diaminetetraacetic acid (EDTA) tube and was transferred to -20°C freezer. Genomic DNA was extracted from whole blood. Exon and intron region from a portion of the first repetitive domain of the ovine calpastatin gene were amplified to a product of 622 bp using primers based on the sequence of the bovine (Killefer and Koohmaraie, 1994; Gen bank accession no L14450) and ovine calpastatin genes. In this research, DNA primers described by Palmer (1998) were used for PCR amplification; primers were obtained from Cinnagen Company in a lyophilized form (non-sensitive to temperature).

F:5'-TGGGGCCCAATGACGCCATCGATG-3'
R:5'-GGTGGAGCAGCACTTCTGATCACC-3'

The polymerase chain reaction (PCR) was performed using a buffer PCR 1X, 200 µM dNTPs, 1.5 µM $MgCL_2$, 10 pmol each primer, 1.25 U taq DNA polymerase, 50 ng ovine gnomic DNA and H_2O up to a total volume of 25 µl. 33 cycle of preliminary denaturation at 95°C (5 min), denaturation at 94°C (1 min), annealing at 60°C (1 min), extension at 72°C (2 min) and final extension at 72°C (8 min). The PCR products were separated by 1.2% (w/v) agarose gel electrophoresis. The amplified fragment of calpastatin was digested with *Msp*I. 15 µl of PCR production with 2 µl buffer, U (0.5) of *Msp*I and 11.5 µl H_2O up to a total volume of 29 µl, following the manufacturers instruction for 12-16 h at 37°C. The digestion products were electrophoresed on 2% agarose gel in 1X TBE and visualized by ethidium bromide staining for 1 h at 85 V. Estimates genotype and alleles frequencies and Hardy-Weinberg equilibrium was analysis with Pop Gene 32 package (Yeh et al., 1999).The relative frequency of particular allele in a population is called the allele frequency (Nei and Kumar, 2000).

Description:
$\chi 2$ = Hardy-Weinberg equilibrium test
O = observed number of genotype A11
E = expected number of genotype A11

RESULTS AND DISCUSSION

The amplified calpastatin resulted in a DNA fragment with 622 bp including the sequences of exon and intron regions from a portion with PCR technique (Figure 1). Due to the digestion of 622 bp PCR product for *CAST* gene with restriction endonucleases MspI, three different genotypes were observed (AA, BB and AB). The first genotype (AA) showed the two band pattern (bands of ~336 and 286 bp). In the second genotype (AB), due to a mutation in one of the alleles, bands 622, 336 and 286 bp were observed. In the third genotype (BB), one band pattern (~ 622 was observed (Figure 2).

This result shows that the polymorphism was detected in CAST I segment, as previously reported in a variety of other sheep in the world such as the dorset sheep (Palmer

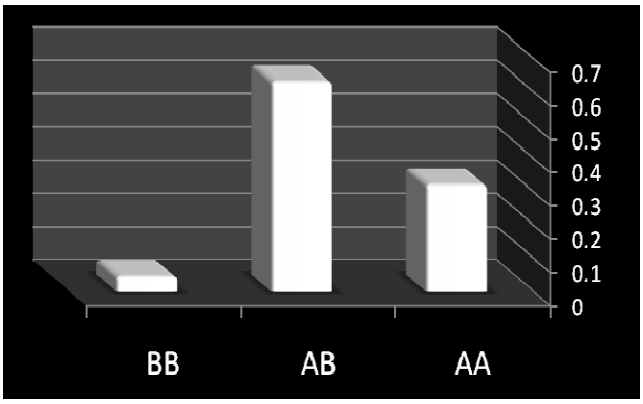

Figure 3. Genotype frequencies of the calpastatin in lori sheep.

Table 1. Chi-square test of the *calpastatin* gene in lori sheep.

Genotype	Observed frequency	Expected frequency	p
AA	0.322	0.407	
AB	0.362	0.462	0.001281 **
BB	0.044	0.131	

et al., 1998), Kurdi sheep in Iran (Nassiry et al., 2006), Merino, Corriedale, Romney, Poll Dorset, and crossbred NZ sheep in New Zealand (Zhou et al., 2007) and Sutikno (2011), and Ghezel sheep (Elyasi et al 2009). After assessment of the samples, the frequencies of A and B alleles were calculated as 0.638 and 0.362, respectively. Also the frequencies of AA, AB and BB genotypes were calculated as 0.332, 0.632 and 0.046, respectively (Figure 3). In their present researches, the fragments size, the number of alleles and genotypes observed were similar to those of Palmer et al. (1998). They reported the three fragments of 286 and 336 bp length, and hence two alleles with three different genotypes. According to the obtained data analysis in their present researches, the results were significant in both tests used and sheep populations were not in Hardy Weinberg equilibrium (Table 1).

Hardy-Weinberg equilibrium can be affected by inbreeding, assortative mating, natural selection and population subdivision (Nei and Kumar, 2000). Lack of Hardy Weinberg equilibrium for *calpastatin* gene in other populations have been reported by researchers (Elyasi et al., 2009; Mohammadi et al., 2008) and (Gabor et al., 2009). The results indicate that it could be useful to consider genetic diversity at calpastatin locus in lori sheep.

Conflict of Interests

The author(s) have not declared any conflict of interests.

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