

Genotypic Frequency of Calpastatin Gene in Atabi Sheep by PBR Method

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Abstract. Calpastatin have role in regulation muscle growth and meat tenderness after slaughter that its coding gene located on ovine chromosome 5. Studies have shown that this gene is polymorphic in many breeds of sheep and is related with weight gain and carcass traits. In this study blood samples were collected from 120 Atabi sheep located in northern and western Golistan of Iran. Genomic DNA was extracted from blood sample. Gel monitoring and spectrophotometer methods were used to determination quality and quantity of DNA. Exon and entron I from L domain of the ovine calpastatin gene was amplified to produce a 622 bp fragment. The PCR products were digested by restriction endonucleases MspI. Digested products were separated by electrophoresis on 2.5% agarose gel and visualized after staining with ethidium bromide on UV transillumination. The MspI digestion of the PCR products produced digestion fragments of 336 bp and 286 bp. Data analysis was done using PopGen32 software (ver.1.32). In this population, AA, AB, BB genotype have been identified with the 67.5, 27.5, 5.0% frequencies, respectively. The population was found to follow Hardy-Weinberg equilibrium.

Keywords: Calpastatin gene, polymorphism, Atabi Sheep, PBR Method.

1. Introduction

Genetic polymorphism in native breeds is a major concern considering the necessity of preserving genetic resources. It is very important to characterize genetically indigenous breeds (Bastos et al., 2001). Marker assisted selection is one of the new DNA based methods that improves accuracy and progress of selection in animal programmers'. Calpastatin (CAST) gene is located on the fifth chromosome of sheep and plays important roles in formation of muscles, degradation and meat tenderness after slaughter. The rate and extent of skeletal muscle growth ultimately depends mainly on three factors: rate of muscle protein synthesis, rate of muscle protein degradation, and the number and size of skeletal muscle cells. Recent studies have shown that calpain activity is required for myoblast fusion [Balcerzak 1995, Barnoy 1997] and cell proliferation in addition to cell growth [Mellegren 1997]. The calpain system may also affect the number of skeletal muscle cells (fibres) in domestic animals by altering rate of myoblast proliferation and modulating myoblast fusion. A number of studies have shown that the calpain system is also important in normal skeletal muscle growth. Increased rate of skeletal muscle growth can result from a decreased 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). Calpastatin, which is an endogenous inhibitor (Ca⁺² dependent cysteine proteinase), plays a central role in regulation of calpain activity in cells (Murachi et al., 1981; Murachi, 1983; Forsberg et al., 1989) and is considered to be one of the major modulators of the calpains. Therefore, calpastatin may affect proteolysis of myofibrils due to regulation of calpain, which can initiate postmortem degradation of myofibril proteins (Goll et al., 1992; Hufflonergar et al., 1996). At the

protein structural level, calpastatin is a five-domain inhibitory protein (Figure 1) (Killefer and Koochmaraie, 1994). 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 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, and C, bind calpain in a strictly Ca^{2+} -dependent manner but have no inhibitory activity, whereas region B inhibits calpain on its own. It is also found that the removal of the XL domain played a regulatory role by altering phosphorylation patterns on the protein (Takano et al., 1999). These observations suggest that genes coding for calpains and calpastatin may be considered as candidate genes in muscle growth efficiency and meat quality in sheep. The aim of the present investigation was to analyse the polymorphism of the CAST gene in Atabi sheep and evaluate its association with daily weight gain traits.

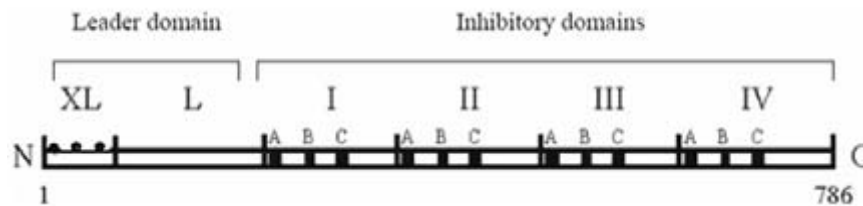


Fig. 1: Structure of calpastatin's polypeptide domains.

2. Material and Method

Random blood samples were collected from 120 Atabi sheep from Golistan province of Iran. Approximately, 5 ml blood sample was gathered from vena in EDTA tube and was transferred to $-20^{\circ}C$ freezer. Genomic DNA was isolated by using DNA Extraction Kit and was based on Boom et al. (1989) method. 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 Koochmaraie, 1994; Gen bank accession no L14450) and ovine calpastatin genes. Spectrophotometer was used for investigating quality and quantity of DNA. The full sequence of primer: CAST 1C 5'-TGGGGCCCAATGACGCCATCGATG - 3' CAST 1D 5'-GGTGGAGCAGCACTTCTGATCACC - 3' The polymerase chain reaction (PCR) was performed using a buffer PCR 1X, 200 μ M dNTPs, 1.5 μ M MgCL₂, 10 pmol each primer, 1.25 U Taq DNA polymerase, 50 ng ovine genomic DNA and H₂O up to a total volume of 25 μ L. Thirty-three cycle of preliminary denaturation at 95 $^{\circ}C$ (5 min), denaturation at 94 $^{\circ}C$ (1 min), annealing at 60 $^{\circ}C$ (1 min), extension at 72 $^{\circ}C$ (2 min) and final extension at 72 $^{\circ}C$ (8 min). The PCR products were separated by 2% (w/v) agarose gel electrophoresis. The amplified fragment of calpastatin was digested with MspI enzyme. Digestion was conducted at 37 $^{\circ}C$ for 12-16 h and in a 29 μ L reaction solution including 11.5 μ L of distilled H₂O, 2 μ L buffer, 0.5 μ L (5 units) of restriction endonucleases (MspI), and 15 μ L of PCR product solution. The digestion products were electrophoresed on 2.5% 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).

3. 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 (Figure2). Within the analyzed, two alleles (A and B) were observed, resulting in three genotypes. The MspI digestion of the PCR products produced digestion fragments of 336 bp and 286 bp (Figure3). The animals with both alleles were assigned as AB genotype, whereas those possessing only A or B alleles as AA or BB genotypes, respectively. Genotype AA showed the two band pattern (bands of approximately 339 and 286 bp). Genotype BB one band pattern (approximately 622), while AB animals displayed a pattern with all three band (622, 336, 286) (Figure 3). This result shows that the polymorphism was detected in CAST I segment, as previously observed by Palmer et al. (1998) and Chung et al. (2001). The genotypes of all animals were used to determine the allele frequencies. A and B allele frequencies were 0.81 and 0.19, respectively. The observed genotype frequencies

were 0.675 for AA, 0.05 for BB and 0.275 for AB. The sheep populations were in Hardy-Weinberg equilibrium. The observed and expected heterozygosity were 0.275 and 0.306, respectively. Effective allele and true allele are estimated 1.44 and 2, respectively. This difference between effective allele number and true allele number and low diversity is due to the higher frequency of allele A compared to allele B, which reduced the frequency in any locus. This number is higher if there are more loci with the same combination of alleles. Atabi sheep shows a low degree of genetic diversity for the calpastatin locus. This may be explained by the conservation and breeding method that has been carried out. Although we observed the low variability for this locus, on the other hand, this data provides evidence that Iranian Atabi sheep breeds have a good polymorphism for the calpastatin gene, which opens interesting prospects for future selection programs, especially marker-assisted selection between different genotypes of different loci and milk, gain and meat traits. Present results showed that PCR-RFLP (PBR) is an appropriate tool for evaluating genetic variability.

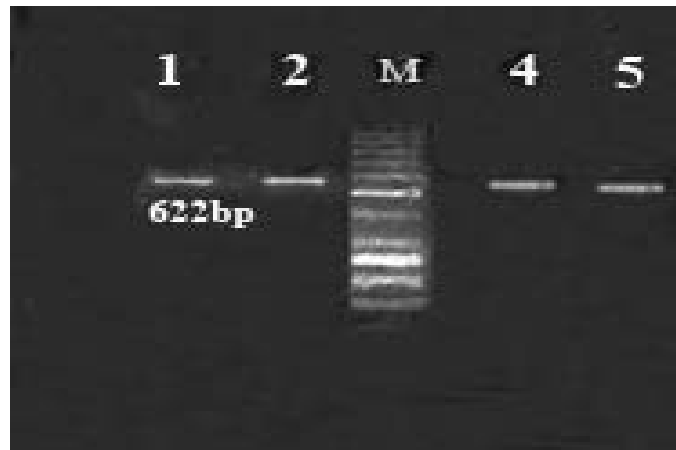


Fig. 2: PCR product analyzed by electrophoresis (622 bp).

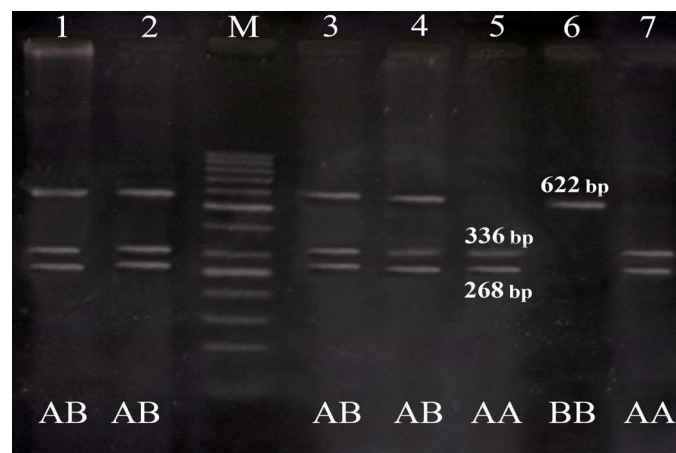


Fig. 3: Genotype AA, AB and BB digestion with MspI on 2.5% agarose gel.

4. Acknowledgements

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