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Short Communication

Polymorphism of Calpastatin Gene in Crossbreed Dalagh Sheep Using PCR-RFLP

Saber Khederzadeh

Faculty of Biological Sciences, Varamin Pishva branch, Islamic Azad University, Varamin Pishva, Iran. E-mail: saber_a14@yahoo.com.

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In modern programs of animal breeding, the genetic polymorphisms of production traits can be used as marker systems. Calpastatin gene is located on ovine chromosome 5 and is polymorphic in many breeds of sheep. Calpastatin has a role in meat tenderness after slaughter. Blood samples were collected from 120 crossbreeds of Dalagh sheep located in Golistan province of Iran. Genomic DNA was extracted from blood sample. Gel monitoring and spectrophotometer methods were used to determine the quality and quantity of DNA. Intron I from L domain of the ovine calpastatin gene was amplified to produce a 565 bp fragment. The PCR products were digested by restriction endonucleases *Msp*I. Digested products were separated by electrophoresis on 1.5% agarose gel and visualized after staining with ethidium bromide on UV transillumination. The *Msp*I digestion of the PCR products produced digestion fragments of 306 and 259 bp. Data analysis was done using PopGen32 software. In this population, MM, MN and NN genotype were identified with 65.5, 29, 5.5% frequencies, respectively. The population was found to follow Hardy-Weinberg equilibrium.

Key words: Calpastatin gene, polymorphism, crossbreed, Dalagh sheep.

INTRODUCTION

Genetic polymorphism in native breeds is a major concern when considering the necessity of preserving genetic resources. It is very important to characterize genetically indigenous breeds (Bastos et al., 2001). 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. 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. 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 as one of the major modulators of the calpain. 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 (Killefer and Koohmaraie, 1994). Of the five domains, the N-terminal leader (L) domain does not appear to have any calpains inhibitory activity (Figure 1), 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 (Figure 2). 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.,

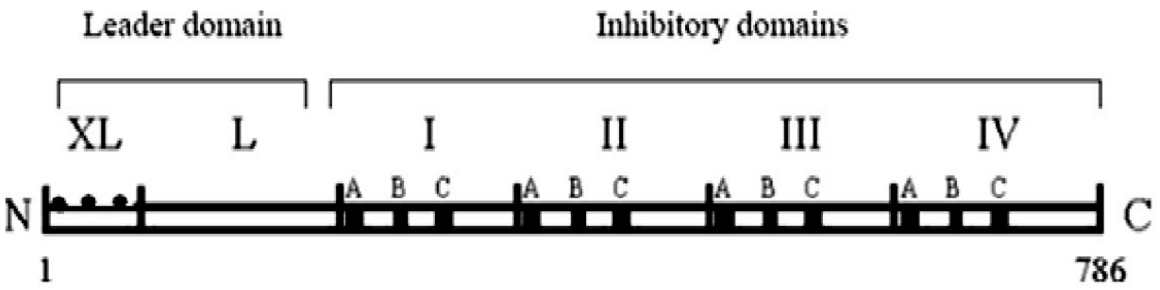

Figure 1. Structure of calpastatin's polypeptide domains.

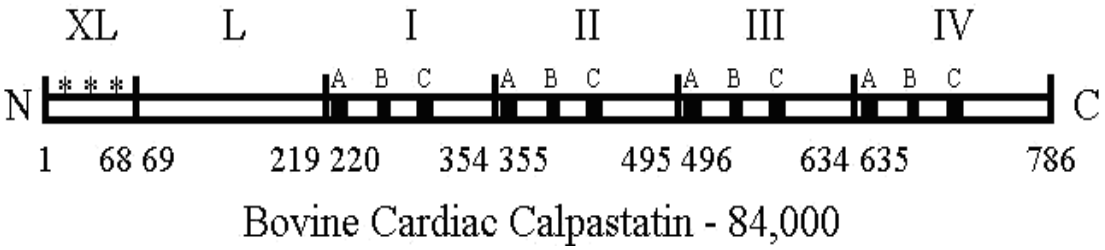

Figure 2. Bovine cardiac calpastatin.

1999). These observations suggest that genes coding for calpain and calpastatin may be considered as candidate genes in muscle growth efficiency and meat quality in sheep. The aim of this study was to analyse the polymerphism of the calpastatin (CAST) gene in crossbreed of Dalagh sheep.

MATERIALS AND METHODS

Random blood samples were collected from 120 crossbreeds of Dalagh sheep from Golistan province of Iran. Approximately, 5 ml blood sample was gathered from venom in EDTA tube and was transferred to -20°C freezer. Genomic DNA was isolated by using DNA extraction kit and was based on Boom et al. (1989) method. Exon and intron regions from a portion of the first repetitive domain of the ovine calpastatin gene were amplified to a product of 565 bp using primers based on the sequence of the bovine (Roberts et al., 1996; Gen bank accession no AF016006.1) and ovine calpastatin genes. Spectrophotometer was used for investigating quality and quantity of DNA using NanoDrop1000. The full sequence of the primer was: CAST F: 5'-CCT TGT CAT CAG ACT TCA CC-3' and CAST R: 5'-ACT GAG CTT TTA AAG CCT CT-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 U Taq DNA polymerase, 50 ng ovine gnomic DNA and H_2O up to a total volume of 25 µl, with thirty-three cycles 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 2% (w/v) agarose gel electrophoresis. The amplified fragment of calpastatin was digested with *Msp*I enzyme. Digestion was conducted at 37°C for 12 to 16 h and in a 29 µl reaction solution including 11.5 µL distilled H2O, 2 µl buffer, 0.5 µL (5 units) restriction endonucleases (*Msp*I), and 15 µl PCR product solution. The digestion products were electrophoresed on 1.5% agarose gel in 1X TBE and visualized by ethidium bromide staining for 1 h at 85 V. Estimates of genotype and alleles frequencies and Hardy-Weinberg equilibrium was analysis with Pop Gene 32 package (Yeh et al., 1999).

RESULTS AND DISCUSSION

The amplified calpastatin resulted in a DNA fragment with 565 bp including the sequences of exon and intron regions from a portion with PCR technique. From the analysis, two alleles (M and N) were observed, resulting in three genotypes. The *MspI* digestion of the PCR products produced digestion fragments of 306 and 259 bp. The animals with both alleles were assigned with MN genotype, whereas those possessing only M or N alleles were assigned with MM or NN genotypes, respectively. Genotype MM showed the two band pattern (bands of approximately 306 and 259 bp). Genotype NN showed one band pattern (approximately 565 bp), while MN animals displayed a pattern with all three bands (565, 306 and 259). The genotypes of all animals were used to determine the allele frequencies. M and N allele frequencies were 0.80 and 0.20, respectively. The observed genotype frequencies were 0.655 for MM, 0.055 for NN and 0.290 for MN. The sheep population was in Hardy-Weinberg equilibrium. The observed and expected heterozygosity were 0.300 and 0.320, respectively. Effective allele and true allele estimates were 1.47 and 2.00, respectively. This difference between all effective and true allele number and low diversity is due to more frequency of allele M as compared to allele N, which reduced frequency in any locus. This number is larger,

when there are more loci with same combination of alleles. Crossbreeds of Dalagh sheep showed a low degree of genetic diversity for the calpastatin locus. Although, a low variability for this locus was observed, this data provide evidence that Iranian crossbreed of Dalagh sheep have a good polymorphism for calpastatin gene, which opens interesting prospects for future selection programs, especially marker assistant selection between different genotypes of different locus, and milk, gain and meat traits. This results showed that PCR-RFLP (PBR) is an appropriate tool for evaluating genetic variability.

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