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Short Communication

Polymorphism of calpastatin gene in Arabic sheep using PCR- RFLP

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Calpastatin has been known as candidate gene in muscle growth efficiency and meat quality. This gene has been located to chromosome 5 of sheep. In order to evaluate the calpastatin gene polymorphism, random blood sample were collected from 111 Arabic ram sheep from different regions. The DNA extraction was based on Boom et al. (1989) method. Exon and entron I from L domain of the ovine calpastatin gene was amplified to produce a 622 bp fragment. The PCR products were electrophoresed on 1.2% agarose gel and stained by etidium bromide. Then, they were digested with restriction enzyme *MspI* and then electrophoresed on 2.5% agarose gel with ethidium bromide and revealed two alleles, allele A and allele B. Data were analysed using PopGene32 package. In this population, AA, AB, BB genotype have been identified with the 70.27, 28.82, 0.9% frequencies. A and B allele's frequencies were 0.85, 0.15, respectively. The population was found to follow Hardy-Weinberg equilibrium.

Key words: Calpastatin gene, polymorphism, PCR-RFLP.

INTRODUCTION

Producing meat tenderness that consumers desire is one of the major problems facing the industry, because meat tenderization during the postmortem period is highly variable between carcasses. Therefore, studies of biochemical mechanisms for muscle breakdown are essential at the molecular level. 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. The calpain activity is required for myoblast fusion (Kuryl et al., 2003; Barnoy et al, 1997) and cell proliferation in addition to cell growth (Mellegren, 1997). The calpain system may also affect the number of skeletal muscle cell (fibres) in domestic animals by altering rate of myoblast proliferation and modulation myoblast fusion. 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).

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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 Koohmaraie, 1994).

Calpastatin is present in all tissues expressing calpains and in skeletal muscle. Calpastatin is expressed at a higher level of activity then the calpains themselves. 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

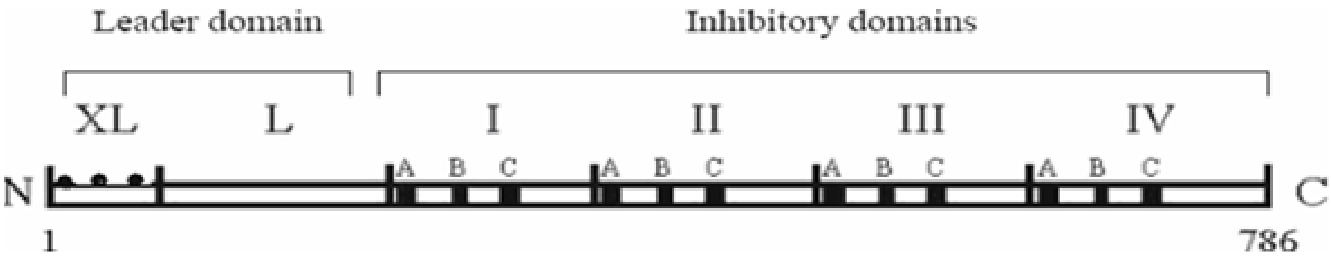

Figure 1. Structure of calpastatin's polypeptide domains.

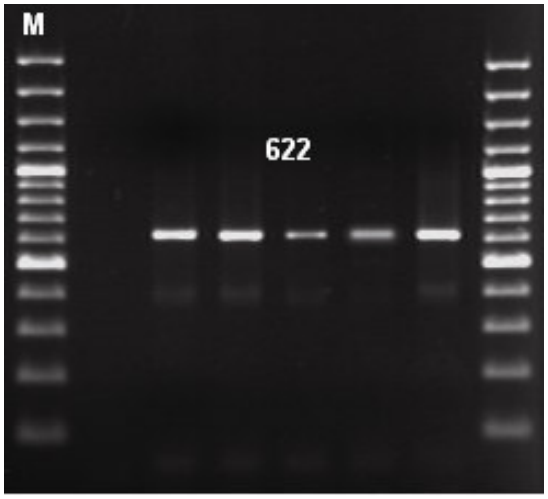

Figure 2. PCR product analyzed by electrophoresis (622 bp).

played a regulatory role by altering phosphorylation patterns on the protein (Takano et al., 1999). The purpose of the present investigation was to analyses the polymorphism of the CAST gene in Arabic sheep and evaluate its association with daily weight gain traits.

MATERIALS AND METHODS

In this study random blood samples were collected from 111 (Arabic) ram, sheep from different regions in southwestern of Iran. Approximately, 5 ml blood sample was gathered from vena 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 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. 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'

Method of detection

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), extention at 72˚C (2 min) and final extension at 72˚C (8 min). The PCR products were separated by 1.2% (w/v) agaros gel electrophoresis. The amplified fragment of calpastatin was digested with *Msp*I. 15 µl of PCR production with 2 µl buffer, 5U (0.5) of *Msp*I and 11.5 µl H2o 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 2.4% agarose gel in 1X TBE and visualized by eithdium bromide staining for 1 h at 85 V.

Statistical analysis

Estimates genotype and alleles frequencies and Hardy-Weinberg equilibrium was analysis with Pop Gene 32 package (Yeh et al., 1999).

RESULT 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 2). Within the analyzed, two alleles (A and B) were observed, resulting in three genotypes. The MspI digests the allele A amplimer, but not allele B. 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 were detected in CAST I segment, as previously observed by Palmer et al. (1998) and chung et al. (2001).

A and B allele frequencies were 0.85 and 0.15, respectively. The genotype frequencies within 111 animals examined were 70.27 for AA, 0.9 for BB and 28.82 for AB (in 78,1,32 ram, sheep, respectively). $\chi 2$ in this population showed Hardy – Weinberg equilibrium. The observed and expected hetrozygosity were 0.26 and 0.28, respectively.

Effective allele and true allele are estimated 1.38 and 2, respectively. This different between effective all and true allele number and low diversity is due to more frequency of allele A compare to allele B, that reduced frequency in any locus. This number is more, if there are

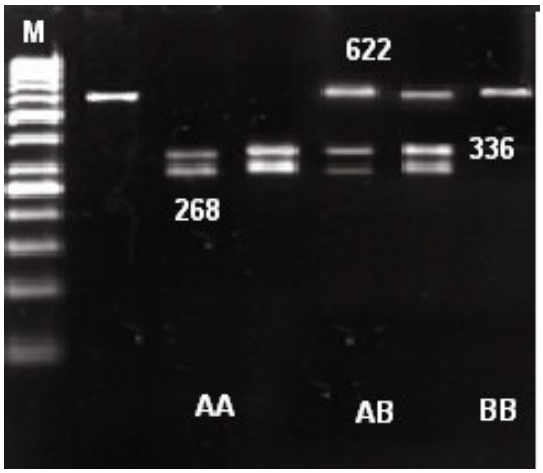

Figure 3. Genotype AA, AB and BB digestion with MspI on 2.5% agarose gel. The band sizes indicated in the figure are in base pairs (bp).

more loci with same combination of alleles.

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