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Full Length Research Paper

Polymorphism investigation of calpastatin gene in Zel sheep population of Iran by PCR-RFLP method

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Meat tenderness is an important quality characteristic for which consumers are interested. Calpastatin is the calpain inhibitor enzyme and plays an important role in muscle growth and meat quality. The calpastin gene is located on sheep chromosome 5 and its polymorphisms are associated with economic traits. This study was performed to identify calpastatin gene polymorphisms using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method. Random blood samples were collected from 100 Zel sheep. DNA was extracted by the salting out method and was amplified using PCR. The quantity and quality of the extracted DNA were determined by spectrophotometry and agarose gel electrophoresis. For amplification, a 622 bp fragment exon 1 of the calpastatin gene was used. Amplified fragments were digested by Msp1 restriction enzyme to determine the calpastatin genotypes, and two alleles. M and N were identified. The genotype frequencies of the MM, MN and NN were 0.62, 0.26 and 0.12, respectively in the population under study. Also, the allelic frequency of M and N were 0.75 and 0.25, respectively. Investigation of population genetic structure of Zel sheep revealed that it was not in Hardy-Weinberg equilibrium ($p<0.05$). The results indicate that it could be useful to consider genetic diversity at calpastatin locus in Zel sheep.

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

INTRODUCTION

Common source of animal protein in Iran is the mutton. Currently, more than 42% of total meat production is obtained from sheep. The amount of prorductive meat is not enough for the needs of the growing population. So, increasing the efficiency of sheep production is especially important (Saadatnuri and Siahmansur, 2002).

Meat tendereness is one of the most important quality characteristics of interest to consumers. Meat industry is focused on meat quality grading system. The carcasses based on expected palatability levels are classified. Inconsistency in the meat tendereness is a combination of two factors (Palmer et al., 1998): 1) the inability to produce consistently tender meat, and 2) the difficulty to identify animals that produce tough meat.

Results of researches conducted on the quality of the meat can be summarized as follows (Koohmaraie, 1992; Koohmaraie et al., 1988; Koohmaraie, 1987; Koohmaraie, 1992):

1. From all the proteolytic systems endogenous to skeletal muscle, only calpain system is involved in meat tenderization.
2. Calpain system consists of two enzymatic systems (μ and m) and a specific inhibitor called the calpastatin.

Calpains require calcium ion for activity. After slaughtering the animal, factors such as time, the electrical excitability of muscle, pH, muscle tremors during the slaughtering and meat-cooking methods affect the quality of meat (Zhou et al., 2007). The first protein of calpain family was identified in 1976, and has a key role in the

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Table 1. Primer sequences used for calpastatin gene (Palmer et al., 1998).

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

breakdown of muscle and flesh of slaughtered animals (Sorimachi et al., 1989). Calpain system is a complex enzyme. It has been found in muscle cells. Calpain enzymes cause the myofibril breakdowns, which controls muscle growth and after slaughter effect on marketing and meat tenderness (Bahrampour et al., 2008; Falconer, 1996). So one of the methods used to estimate the amount of meat tenderness is determination of the calpain and calpastatin amounts (Shackelford et al., 1991; Shackelford et al., 1994; Whipple and Koohmaraie, 1992).

Calpastatin gene is located on sheep chromosome 5 (Huang and Forsberg, 1998) and can cause muscle protein turnover at animal growths after translation to protein (Forsberg et al., 1989).

By selecting genetically best animals, meat quality can be improved. Development of molecular genetics technology in achieving breeding goals is effective. One of the fundamental works is the study of the polymorphism of genes affecting meat quality and finding alleles associated with economic traits (Hosseini Salkadeh et al., 2005).

Molecular genetic studies of Iranian sheep breeds, such as Zel are rare. It seems imperative that research should be done in these fields. This study aimed to evaluate the presence or absence of polymorphism in the calpastatin gene of the Iranian Zel sheep using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method.

MATERIALS AND METHODS

Zel is the only breed of sheep in Iran, which has tail instead of fat-tail. It is native of two Northern provinces of the country. The average live weight of adult rams and ewes of the small size breed are 50 and 32 kg, respectively. The coat is colored. The breeding technique for this breed is similar to other breeds in Iran, and has summer and winter quarters. Herds of this breed are kept on pasture and desert. Zel meat quality is good. The meat produced by the Zel sheep provides part of the market demand of the Northern provinces and Tehran (Saadatnuri and Siahmansur, 2002).

Sampling

Blood samples were taken from 100 random Zel sheep in northern of Iran from the jugular vein. To extract DNA, the samples were kept at temperature of - 20°C.

DNA extraction

DNA was extracted by the salting out method and according to the experimental protocol (Miller et al., 1988). Materials used for DNA extraction were: 1) buffers, 2) sodium dodecyl sulphate (SDS 10%), 3) chloroform, 4) 70% ethanol, 5) sodium acetate solution (3.2 M) and 6) sodium chloride solution (5.7 M). 1.5 ml of blood was pippeted from samples and with the extraction materials were added to the process. Centrifugation for DNA extraction was performed twice: first, for 5 min at the speed of 7000 rpm; and secondly, for 15 min at the speed of 2500 rpm. To separate the DNA, agarose gel (1.2%) was prepared. Agarose gel was stained with ethidium bromide. The quality and quantity of extracted DNA were assessed by two different methods (agarose gel and spectrophotometer).

Table 2. Concentrations of materials used in the PCR-RFLP reaction.

Material	Concentration
Template DNA	200 ng
PCR buffer	1 X
dNTPs	200 µl
Forward primer	10 p mol/µl
Reverse primer	10 p mol/µl
Magnesium chloride	2.5 MM
Polymerase	1.25 U
Distilled water	-

PCR-RFLP, Polymerase chain reaction-restriction fragment length polymorphism.

Primers

Primers were obtained from Cinnagen Company and in form of the lyophilized (non-sensitive to temperature). The sequences of the primers used for calpastatin gene are presented in Table 1.

Polymerase chain reaction (PCR)

PCR machine in the lab, with the temperature gradient was fully automated and contained 12 rows of eight set sink. Materials for PCR of the primer used in this study are presented in Table 2.

All reaction components (except DNA) according to the number of samples used for the PCR in a mixture form were poured into separate tubes. After preparation of the base mix, 23.5 µl of it were added to each of the DNA containing tubes. To uniformly mix the reaction components, the tubes were centrifuged at 3000 rpm for 15 s. Then, the tubes were placed in the thermocycler (for PCR). Replication and amplification of the sequences in the PCR was performed in three stages. These steps include: denaturation of the templates fragment, annealing and elongation of the primer.

After PCR, agarose gel (1.2%) was used for diagnosis of the amplified bands, and to determine their molecular weight for revealing different alleles for the gene under study, Msp1 restricted enzyme was used for studying the calpastatin polymorphism. This enzyme recognizes the sequences 5'-CCGG-3' and breaks them into two pieces: 5'-C and CGG-3'. For detection of the fragments from PCR of the calpastatin gene, polyacrylamide gel (8%) was used.

Population genetic structure

After the amplified fragments were digested by the enzyme, the

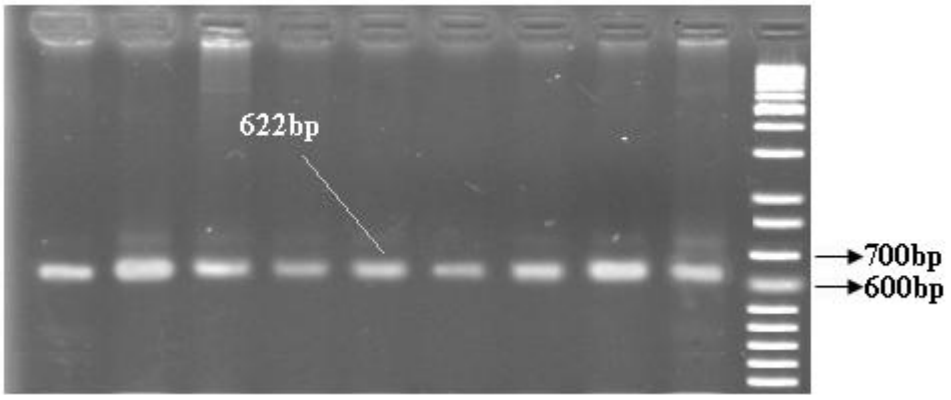

Figure 1. PCR products obtained from different samples.

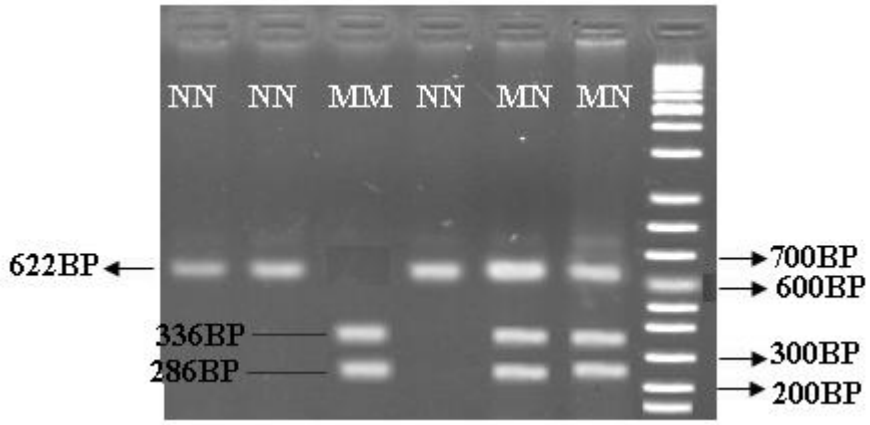

Figure 2. Products of enzymatic digestion of the samples using Msp1.

alleles and genotypes were identified based on the numbers of fragments produced. Using genotype information, gene and genotypic frequencies were calculated. To investigate the presence or absence of Hardy Weinberg equilibrium in the population, Chi square test was used.

RESULTS AND DISCUSSION

RFLP analysis of the calpastatin gene

Mspl enzyme cuts the PCR fragments (Figure 1) after the second base (that is, C), and two sticky end fragments with the length of 336 and 286 bp were created. Due to the digestion of the PCR product by the enzyme (Mspl), three different genotypes were observed. In the first genotype (NN), due to a mutation in the enzyme cutting site, the site of the incision was not recognized and only a band of 622 bp was revealed. In the second genotype (MN), due to a mutation in one of the alleles, bands of 622, 336 and 286 bp were observed. In the third genotype (MM), only 336 and 286 bp bands were observed. Observation of this genotype indicates that both DNA strands are cut by the enzyme (Figure 2). To achieve greater accuracy in the test results, enzymatic digestion of PCR products was repeated three times consecutively. Finally, polymorphism in calpastatin gene was confirmed in the Iranian Zel sheep.

After assessment of the samples, the frequencies of M and N alleles were calculated as 0.75 and 0.25, respectively. Also, the frequencies of MM, MN and NN genotypes were calculated as 0.62, 0.26 and 0.12, respectively.

Palmer et al. (1998) studied the calpastatin gene polymorphism using Mspl enzyme in Dorset Down sheep in New Zealand. They are recognized as the first researchers in this field. They reported the three fragments of 286 and 336 bp length, and hence two alleles with three different genotypes. In their present researches, the fragments size, the number of alleles and genotypes observed were similar to those of Palmer et al. (1998). The results obtained here are in agreement with the previous works of Nikmard (2007), Nassiry et al. (2006), Mohammadi (2009), Bahrampour et al. (2008) and Torabi et al. (2008). In the study of Nassiry et al.

(2006) on Iranian Kurdish sheep, NN genotype with the band size of 622 bp was not observed.

The Chi-square test for deviation from Hardy Weinberg equilibrium in the population for the studied locus indicated that there were no equilibrium ($p>0.05$). The results reflect the fact that: 1) PCR-RFLP method is appropriate for the study of the calpastatin gene and its relationship with meat tendeneness. 2) Genetic selection of superior animals for meat tenderness could be efficient by genotyping animals for the calpastatin locus.

REFERENCES

Bahrampour W, Mohammad Abadi M, Mirzaee H, Baghizadeh A, Dashab G, Mohammadi A, Soflaee M, Hesali A (2008). Molecular analysis of Calpastatin gene in Kermani sheep. Iran. Agric. Resour. Sci. 131: 124-130.

Falconer DS (1996). Introduction to quantitative genetic. Longman Scientific & technical, USA.

Forsberg NE, Ilian MA, Ali-Bar A, Cheeke PR, Wehr NB (1989). Effects of cimaterol on rabbit growth and myofibrillar protein degradation and on calcium dependent proteinase and calpastatin activities in skeletal muscle. J. Anim. Sci. 67: 3313-3321.

Hosseini Salkadeh G, Gharayazi B, Naghavi M (2005). Molecular markers. Tehran university publication, Tehran, Iran.

Huang J, Forsberg NE (1998). Role of calpain in skeletal-muscle protein degradation. Proc. Natl. Acad. Sci. USA. 95: 12100-12105.

Koohmaraie M (1992). Ovine skeletal muscle multicatalytic proteinase complex (proteasome): purification, characterization, and comparison of its effects on myofibrils with mu-calpains. J. Anim. Sci. 70: 3697-3708.

Koohmaraie M (1992). The role of Ca-dependent proteases (Calpain) in postmortem proteolysis and meat tenderness. Biochemistry, 74: 239-245.

Koohmaraie M, Babiker AS, Schroeder AL, Merkel RA, Dutson TR (1988). Acceleration of postmortem tenderization in ovine carcasses through activation of Ca2+dependent proteases. J. Food Sci. 53: 1638-1641.

Koohmaraie M, Seideman SC, Schollmeyer JE, Dutson TR, Crouse JD (1987). Effect of post-mortem stage on Ca+2-dependent proteases, their inhibitor and myofibril fragmentation. Meat Sci. 19: 187-196.

Miller SA, Dykes DD, Paletsky HF (1988). A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acid Res. 16(3): 1215-1220.

Mohammadi M (2009). Study of Calpastatin gene polymorphism and its relation with production traits in Arabic sheep. Iran J. Agric. Sci. 25: 32-35.

Nassiry MR, Tahmoorespour M, Javadmanesh A, Soltani M, Foroutani Far S (2006). Calpastatin polymorphism and its association with daily gain in Kurdi sheep. Iran J. Biotech. 4: 188-192.

Nikmard M (2007). Study of Calpastatin gene polymorphism and its relation with growth traits in Afshari sheep. Iran J. Agric. Sci. 2: 35-41.

Palmer BR, Roberts N, Hickford GG, Bickerstaffe R (1998). PCR-RFLP for MspI and NcoI in the ovine calpastatin gene. J. Anim. Sci. 76: 1499-1500.

Saadatnuri M, Siahmansur S (2002). Founamation of sheep husbandari. Ashrafi Publication, Tehran, Iran.

Shackelford SD, Koohmaraie M, Waipple G, Wheeler TL, Miller MF, Crouse JD, Reagan JO (1991b). Predictors of beef tenderness: Development and verification. J. Food Sci. 56: 1130-1135.

Shackelford SD, Koohmaraie M, Cundiff LV, Gregory KE, Rohrer GA, Savell JW (1994). Heritabilities and phenotypic and genetic correlations for bovine postrigor calpastatin activity, intramuscular fat content, Warner Bratzlewr Shear force, retail product yield and growth rate. J. Anim. Sci. 72: 857-863.

Sorimmachi H, Imajoh-Ohmi S, Emori Y, Kawaskai H, Ohno S, Minami Y, Suzuki K (1989). Molecular cloning of a novel mammalian calcium-dependent protease distinct from both mand μ-types: Specific expression of the mRNA in skeletal muscle. J. Biol. Chem. 264: 20106-20111.

Torabi A, Shoja J, Pirani N, Elyasi G, Valizadeh M (2008). Study of the Calpastatin gene in Moghani sheep by PCR-RFLP. Iran Agric. Resour. Sci. 141: 140-148.

Whipple G, Koohmaraie M (1992). Effects of lamb age, muscle type, and 24-h activity of endogenous proteinases on postmortem proteolysis. J. Anim. Sci. 70: 798-804.

Zhou H, Hickford JG, Gong H (2007). Polymorphism of the ovine Calpastatin gene. Mol. Cell Prob. 21: 242-244.