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PCR-RFLP

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Polymorphism of Calpastatin Gene in Moghani Sheep Breed Using PCR-RFLP A Torabi¹, J Shodja², N Pirani^{2*}, G Elyasi³ and M Valizadeh⁴

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Abstract

Tenderness is one of the important characteristics of meat and desired to consumers. Calpastatin is one of the genes which affects meat quality and growth of the animals. The aim of this study was to determine genotypic variation of calpastatin gene in Iranian Moghani sheep breed using PCR-RFLP. For conducting this study, the blood samples of 176 sheep were collected. After extraction of genomic DNA, the L region of exon 1 of calpastatin gene with 622 bp was amplified with specific primers. The *MspI* and *NcoI* restriction enzymes were used to cut the PCR products. The mentioned enzymes cut the amplicons in complementary manner and alleles of M and N with frequency of 0.54 and 0.46, respectively were produced. Genetic variation (heterozygosity) in the Moghani sheep breed was moderate (0.49) and the population was in Hardy-Weinberg equilibrium. The results confirmed that the PCR-RFLP can be used to identify different genotypic variation in this breed.

Keywords: Calpastatin, Moghani sheep breed, PCR-RFLP, Polymorphism

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¹House Keeping
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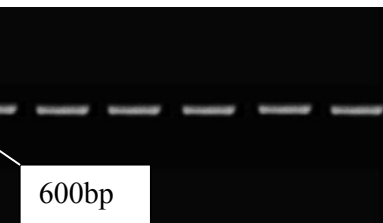
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Ovine 1D: 5' GG TGGAGCAGC
ACTTCTGATCACC 3' (exon 1D)
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dNTP

¹Salting out

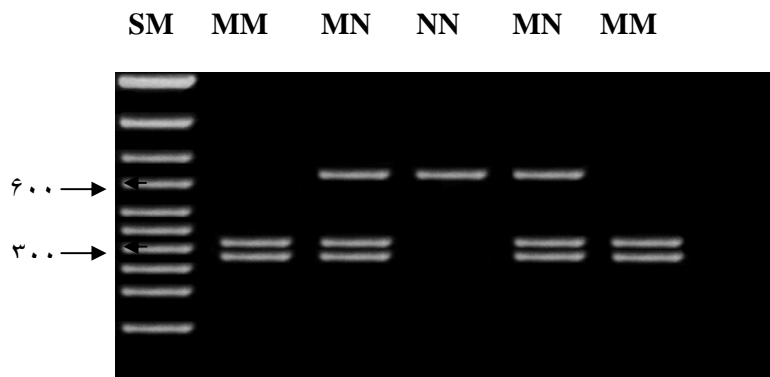
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² Standard size marker

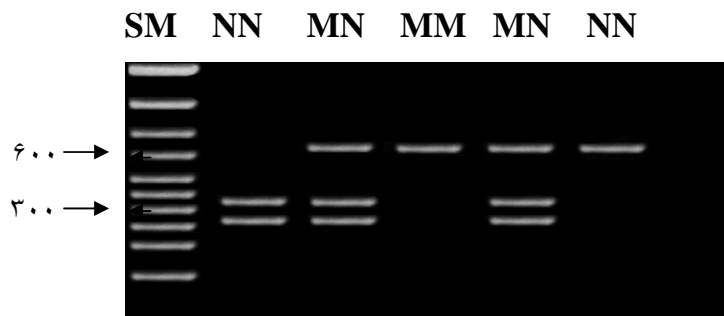


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- Ciobanu DC, Bastiaansen JW, Longergan SM, Thomsen H, Dekkers JC, Plastow GS and Rothschild MF, 2004. New alleles in calpastatin gene are associated with meat quality traits in pigs. *J Anim Sci* 82: 2829-2839.
- Collingwood KM, Gilmour RS, Speck PA, Tucker GA, Bardsley RG and Buttery PJ, 1992. cDNA sequence and ontogenetic expression of ovine calpastatin. Ninth International ICOP Conference on Proteolytic and Protein Turnover. Williamsburg, VA.
- Elyasi G, Shodja J and Nassiry MR, 2004. Polymorphism of β -lactoglobulin gene in Iranian sheep breed using PCR-RFLP. Pp: 66-72. Proceeding of the Joint Agric Natl Reso Symp, May 14-16. Ganja/Azerbaijan.
- Goll DE, Otsuka Y, Nagainis PA, Shannon JD, Sathe SK and Muguruma M, 1998. Role of muscle proteinases in maintenance of muscle integrity and mass. *J Food Biochem* 7: 137.
- Hong MR, Hong QY, Tanko E, Hatanaka M and Maki M, 1994. Amino terminal conserved region in proteinase inhibitor domine of calpastatin is calpain inhibitory activitiy by interaction with calmadolin like domain of the proteinase. *J Biol Chem* 269: 2440-2443.
- Killefer J and Koohmaraie M, 1994. Bovine skeletal muscle Calpastatin: Cloning, sequence analysis, and steady-state mRNA expression. *J Anim Sci* 72: 606-614.
- Koohmaraie M, 1992. The role of Ca^{2+} -dependent proteases (calpain) in postmortem proteolysis and meat tenderness. *Biochimie* 74: 239-245.
- Koohmaraie M, 1994. Muscle proteinases and meat aging. *J Meat Sci*, 36: 93-104.
- Koohmaraie M, Babiker AS, Schroeder AL, Merkel RA and Dutson TR, 1988. Acceleration of postmortem tenderization in ovine carcasses through activation of Ca^{2+} -dependent proteases. *J Food Sci* 53: 1638-1641.
- Koohmaraie M, Seideman SC, Schollmeyer JE, Dutson, TR and Crouse JD, 1987. Effect of post-mortem stage on Ca^{++} -dependent proteases, their inhibitor and myofibril fragmentation. *J Meat Sci* 19: 187-196.
- Miller SA, Dykes DD and Polesky HF, 1988. A simple salting out procedure for extraction of DNA from human nucleated cells. *Nucl Acid Res* 16: 1215.
- Morgan JB, Savell JW, Hale DS, Miller RK, Griffin DB, Cross HR and Shackelford SD, 1991. National Beef Tenderness Survey. *J Anim Sci* 69:3274-3283.

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- Nassiry MR, Eftekhari Shahroudi F, Tahmoorespur M and Javadmanesh A, 2007. Genetic variability and population structure in beta-lactoglobulin, calpastatin and calpain loci in Iranian Kurdi sheep. *Pak J Biol Sci* 10: 1062-1067.
- Nei M, 1987. Estimation of average heterozygosity and genetic distance from a small number of individuals. *Genetics* 89: 583-590.
- Palmer BR, Roberts N, Hickford GG and Bickerstaffe R, 1998. PCR-RFLP for *MspI* and *NcoI* in the ovine calpastatin gene. *J Anim Sci* 76: 1499-1500.
- Palmer BR, 1999. A candidate gene approach to animal quality traits. *Proceeding of the New Zealand Society of Anim Prod* 57: 294-296.
- Savell IW, Harris JJ, Cross HR, Hale DS and Beasley L, 1991. National beef market basket survey. *J Anim Sci* 69: 2883-2893.
- Shackelford SD, Koohmaraie M, Cundiff LV, Gregory KE, Rohrer, GA and 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.
- Shackelford SD, Koohmaraie M, Miller MF, Crouse JD and Reagan JO, 1991a. An evaluation of tenderness of the longissimus muscle of Angus by Hereford versus Brahman crossbred heifers. *J Anim Sci* 69: 171-177.
- Shackelford SD, Koohmaraie M, Whipple G, Wheeler TL, Miller MF, Crouse JD and Reagan JO, 1991b. Predictors of beef tenderness: development and verification. *J Food Sci* 56: 1130-1135.
- Veiseth E, Shakelford SD, Wheeler TL and Koohmaraie M, 2004. Factors regulating lamb longissimus tenderness are affected by age at slaughter. *Meat Sci* 68: 635-640.
- Whipple G, Koohmaraie M, Dikeman ME, Crouse JD, Hunt MC and Klemm RD, 1990. Evaluation of attributes that affect longissimus muscle tenderness in *Bos taurus* and *Bos indicus* cattle. *J Anim Sci* 68: 2716-2728.
- Wulf DM, Tatum JD, Green RD, Morgan JB, Golden BL and Smith GC, 1996. Genetic influences on beef longissimus palatability in Charolais and Limousine steers and heifers. *J Anim Sci* 74: 2394-2405.