International Journal of Agriculture Innovations and Research
Volume 5, Issue 4, ISSN (Online) 2319-1473
Manuscript Processing Details (dd/mm/yyyy) :
Received: 12/12/2016 | Accepted on: 17/12/2016 | Published: 03/02/2017

# Analysis of the Third Exon of Myostatin Gene and the First Intron of Calpastatin Gene in Gray Shirazi Sheep Populations

**Saber Khederzadeh*, Asghar Mobaraki, Habib Allah Haghi and Leila Ezeddinloo**
Natural History Museum and Genetic Resources, Department of Environment,
Pardisan Eco-Park, 738314155, Tehran, Iran.
*Corresponding author email id: Saber_a14@yahoo.com

*Abstract* – **The Myostatin is a member of the transforming growth factor β superfamily that normally acts to limit skeletal muscle mass by regulating both the number and the growth of muscle fibers. Calpastatin gene is a specific inhibitor of the ubiquitous calcium-dependent proteases– μ-calpain and m-calpain, found in mammalian tissues. The level of components included into the calpain-calpastatin system determines the rate of postmortem tenderization of meat. Calpastatin inhibits both the rate and extent of postmortem proteolysis and plays a role in muscle growth and meat quality. Therefore, they are considered as candidate genes for meat and growth traits. In current study, Genomic DNA was extracted from blood sample of Gray Shirazy sheep. Gel monitoring and spectrophotometer methods were used to determinate the quality and quantity of DNA. Third Exon of myostatin gene and Intron I from L domain of the ovine calpastatin gene were amplified to produce a 337 and 622 bp fragment, respectively. The PCR products were digested by restriction endonuclease enzymes *Hha*III and *Msp*I. Digested products were separated by electrophoresis on 1.5% agarose gel and visualized after staining with GelRed on UV transillumination. The *Hha*III digestion of the PCR products produced digestion fragments of 81, 123 and 131bp. The *Msp*I digestion produced fragments of 286 and 336bp. Data analysis was done using PopGen32 software. In this population, mm genotype and AA, AB and BB genotypes were identified with 100% and 78.4, 19.6, 2% frequencies for myostatin and calpastatin, respectively. The population was found to follow Hardy-Weinberg equilibrium.**

*Keywords* – **Myostatin, Calpastatin, Polymorphism, Gray Shirazy Sheep.**

## I. INTRODUCTION

The Livestock industry requires the development of very standard sheep and cattle population to fulfill its commercial needs by selection practices in breeding programs. Genetic diversity and gene polymorphism found in population allows farmers to develop new characteristics in response to changes in environment, diseases or market conditions [21]. The extensive selection and multiplication of superior animals cause a significant decrease in the genetic variation needed for the improvement of economic traits and breeds [19]. Genetic diversity and gene polymorphism data also can be used to monitor the genetic structure of populations and detect changes in the frequency of genes due to breeding programs, which makes it possible to preserve the biological diversity of farm animals [20, 21 & 22]. The physiological regulation of muscle growth in animals is under the control of multiple genes. Polymorphisms in these genes, which show associations with specific economically important traits, are useful markers for marker-assisted selection. Single nucleotide polymorphisms (SNP) are the most frequently occurring forms of variation in the genome and they can be used to study associations between them and the production traits of individuals [29]. 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 [3]. This is an increasingly common approach to genetic association studies. Myostatin (MSTN) or growth differentiation factor-8 (GDF-8) is a member of the mammalian growth transforming family (TGF-beta superfamily), which plays a role in the regulation 2 of embryonic development and tissue homeostasis in adults [27]. They are known to block myogenesis, hematogenesis and enhance chondrogenesis as well as epithelial cell differentiation in vitro. In mice, null mutants are significantly larger than wild-type animals, with 200–300 % more skeletal-muscle mass, because of hyperplasia and hypertrophy [23]. Muscular hypertrophy (mh), also known as "double-muscling" in cattle, has been recognized as a physiological character for years [1] and is seen in Belgian Blue, and Piedmontese cattle [15]. These animals had less bone, less fat, and 20 % more muscle on an average [5 & 12]. Mutations within myostatin gene led to muscular hypertrophy allele (mh allele) in the double muscle breeds [15]. Such a major effect of a single gene on processing yields opened a potential channel for improving processing yields of animals using knockout technology. 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. Studies have shown that calpain activity is required for myoblast fusion [2] and cell proliferation in addition to cell growth. 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 [10]. Calpastatin, which is an endogenous inhibitor ($Ca^{+2}$ dependent cysteine proteinase), plays a central role in regulation of calpain activity in cells [8] and is considered 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 [11 & 13]. At the protein structural level, calpastatin is a five-domain inhibitory protein [17]. Of the five domains, the N-terminal leader (L) domain does not appear to have any calpains inhibitory activity (Fig 1), but maybe involved in targeting or intracellular localization [29], while the other domains (I-IV) are highly homologous and are each independently capable of inhibiting calpains [6]. 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 [29]. 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. Gray Shirazy sheep are fat tail breed adapted to the dry and harsh climatic conditions. They are primarily raised for mutton, with milk and wool being of secondary importance [9]. The aim of the present investigation was to analyze the polymorphism of the Myostatin (*MSTN*) and Calpastatin (*CAST*) gene in Gray Shirazy sheep breed.

## II. Materials and Methods

### *2.1. Animals and Sampling*

In current study, Random blood samples were collected from 102 Gray Shirazy sheep from six populations involve: Abadeh, Marv Dasht, Fasa, Darab, Estahban and Neyriz cities of Iran (Fig 1).


Fig. 1. Geographical location of the studied populations

Approximately, 3 ml blood sample was gathered from venom in EDTA tube and brought on ice to laboratory and was transferred to-20°C freezer or used directly for extraction.

### *2.2. DNA Extraction and PCR Amplification*

About 500-1000 µL of EDTA blood was sampled and immediately Genomic DNA was isolated by using DNA Extraction Kit (Diatom) and was based on Boom et al. (1989) method with minor modifications (4). Quantity was determined by measuring the absorbance at 260 nm and the concentration, purity and quality were determined by measuring the absorbance at 260/280 nm and 230/260 ratios using a NanoDrop TM 1000 spectrophotometer (NanoDrop Technologies Inc., Wilmington, DE, USA). DNA extractions were appropriately labeled and stored at -20°C for analysis. Two loci were selected for investigation. The exon 3 region from ovine myostatin gene was amplified to a product of 337 bp using primers based on the sequence of the ovine myostatin genes (Table 1). The full sequence of primer:

MSTN F: 5'- CCGGAGAGACTTTGGGCTTGA -3' and MSTN R: 5'-GGTGGAGCAGCACTTCTGATCACC-3'. The polymerase chain reaction (PCR) was performed using a buffer PCR 1X, 200 µM dNTPs, 1µM $MgCL_2$, 0.4 pmol of each primer, 0.7 U Taq DNA polymerase, 100 ng ovine gnomic DNA and $H_2O$ up to a total volume of 20 µl. Amplification of DNA samples from individual isolates was carried out in a Thermocycler (eppendorf, Garmany) under the following conditions: Thirty five cycle of preliminary denaturation at 95°C (5 min), denaturation at 94°C (30 sec), annealing at 62°C (40 sec), extension at 72°C (40 sec) and final extension at 72°C (5 min). Confirmation of successful PCR amplifications was carried out by electrophoresis on a 1% (w/v) agarose gel for 1 h at 80V, stained with GelRed staining (Biotium, USA) and photographed. The exon and intron regions from a portion of the first repetitive domain of the ovine calpastatin gene was amplified to a product of 622 bp using primers based on the sequence of the bovine and ovine calpastatin gene (Table 1). The full sequence of primer:

CAST F: 5'-TGGGGCCCAATGACGCCATCGATG-3' and CAST R: 5'-GGTGGAGCAGCACTTCTGATCACC-3'. The polymerase chain reaction (PCR) was performed using a buffer PCR 1X, 200 µM dNTPs, 1.5 µM $MgCL_2$, 0.4 pmol of each primer, 1 U Taq DNA polymerase, 50 ng ovine gnomic DNA and $H_2O$ up to a total volume of 20 µl.

Table 1. Primer used in the amplification (suggested by Timothy et al., 1997)[30]

| Locus | Primer Sequence | PCR Product Size (bp) |
|---|---|---|
| MSTN | CCGGAGAGACTTTGGGCTTGA<br>TCATGAGCACCCACAGCGGTC | 337 |
| CAST | TGGGGCCCAATGACGCCATCGAG<br>GGTGGAGCAGCACTTCTGATCACC | 622 |

Thirty five cycle of preliminary denaturation at 95°C (5 min), denaturation at 94°C (30 sec), annealing at 62°C (40 sec), extension at 72°C (50 sec) and final extension at 72°C (5 min). Confirmation of successful PCR amplifications was carried out by electrophoresis on a 1% (w/v) agarose gel for 1 h at 80V, stained with GelRed staining (Biotium, USA) and photographed.

*2.3. Enzyme Digestion and Analysis*

The amplified fragment of Myostatin was digested with *HhaIII* enzyme. Digestion was conducted at 37°C for 12-16 h and in a 20 µL reaction solution including 12.5µL distilled $H_2O$, 2 µL buffer10X, 0.5µL (5 unit) restriction endonucleases (*Hha*III) and 5 µL PCR product solutions. The digestion products were electrophoresed on 1.5% agarose gel in 1X TBE and visualized by eithdium bromide staining for 45 min at 80V. The amplified fragment of calpastatin was digested with *Msp*I enzyme. Digestion was conducted at 37°C for 12-16 h and in a 20 µL reaction solution including 12.5µL distilled $H_2O$, 2µL buffer10X, 0.5µL (5 unit) restriction endonucleases (*Msp*I) and 5µL PCR product solutions. The digestion products were electrophoresed on 1.5% agarose gel in 1X TBE and visualized by GelRed staining (Biotium, USA) for 1 h at 80V. Estimates genotype and alleles frequencies and Hardy-Weinberg equilibrium were calculated using Pop Gene 32 software [31].

## III. Results

In Current reasearch, DNA was obtained either directly from the bloods with high quality (Fig 2).

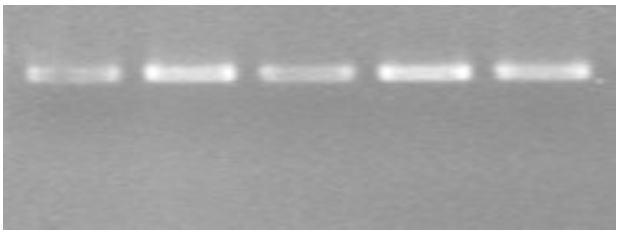

Fig. 2. Extracted DNA from sheep blood

A 337 bp fragment for third exon of MSTN locus was amplied (Fig 3).

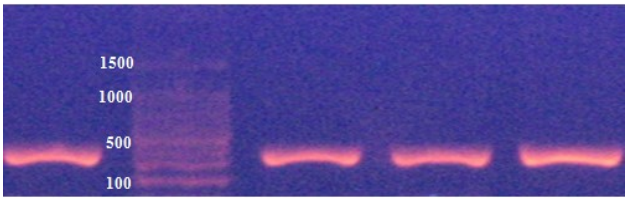


Fig. 3. PCR products of MSTN (Obtained size: 337 bp)

HaeIII restriction enzyme was used to digest the PCR products. The HaeIII digests the m allele, but not M allele. Digestion of the m allele produced three fragments of 83, 123, and 131 bp (Fig 4).

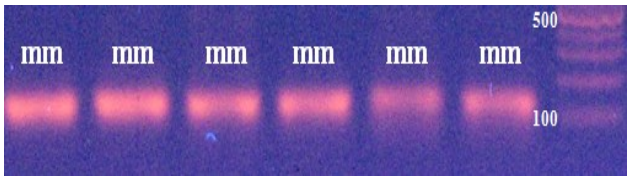


Fig. 4. MSTN genotyping by PBR (2% agaros gel)

As a result, MSTN gene was monomorph. Although myostatin locus was monomorphic in the populations, the results showed acceptable polymorphism for calpastatin and calpain loci, which may open interesting prospects for future selection programs, especially using marker-assisted selection for improving weight gain and meat quality. The amplified calpastatin resulted in a DNA fragment with 622bp including the sequences of Exon and intron regions from a portion with PCR technique (Fig 5).


Fig. 5. PCR products of CAST (Obtained size: 622 bp)

Two alleles (A and B) were observed, resulting in three genotypes. The MspI digestion of the PCR products produced digestion fragments of 306bp and 259bp. 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 306 and 259bp). Genotype AA one band pattern (approximately 565bp), while AB animals displayed a pattern with all three band (565, 306, 259) (Fig 6).

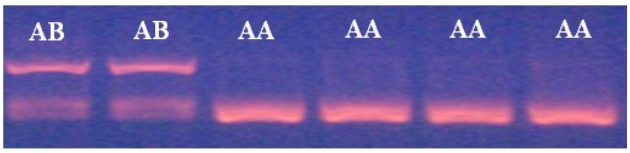


Fig. 6. CAST genotyping by PBR (1.5% agaros gel)

The genotypes of all animals were used to determine the allele frequencies. A and B allele frequencies were 0.8824 and 0.1176, respectively. The observed genotype frequencies were 0.784 for AA, 0.020 for BB and 0.196 for AB (Table 2). The sheep population was in Hardy-Weinberg equilibrium ($P \leq 0.05$). The observed and expected heterozygosity were 0.1961 and 0.2086, respectively. Effective allele and true allele estimates were 1.262 and 2.000, respectively (Table 3). This difference between effective all and observed 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 larger, if there are more loci with same combination of alleles.

Table 2. Genotype and Allele frequencies in all regions

| | Genotype frequencies | | | Allele frequencies | |
|---|---|---|---|---|---|
| All Regions | AA | AB | BB | A | B |
| Mean | 0.784 | 0.196 | 0.020 | 0.8824 | 0.1176 |

Table 3.The Observed number of alleles (Na), Effective number of alleles (Ne), observed and expected heterozygosity in all regions

| All Regions | Na | Ne | observed Heterozygosity | expected Heterozygosity |
|---|---|---|---|---|
| Mean | 2.000 | 1.262 | 0.1961 | 0.2086 |

This difference between effective all and observed 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 larger, if there are more loci with same combination of alleles. We observed the high variability for this locus. On the other hand, this data provide evidence that Iranian Gray Shirazy sheep show 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. Present results showed that PCR-RFLP (PBR) is appropriate tools for evaluating genetic variability.

## IV. Discussion

The Candida genes have known biological functions related to the development or physiology of an important trait. We observed the no variability for Myostatin locus (monomorph). Also, This locus dosen't have any polymorphism in other studied of iranian sheep breeds such as native dalagh sheep (mm genotype) and The nn and mn genotypes were not found. On the contrary Soufy et al. [28] observed polymorphism for MSTN gene in Sanjabi Sheep and native kermanian sheep. This inconsistency may be ascribed to breed differences, population and sampling size, environmental factors, mating strategies, geographical position effect and frequency distribution of genetic variants.The random genetic drift can be one of the causes of this homozygosity. In current study, the data reaveled that the Gray Shirazi sheep have a good polymorphism for calpastatin gene. The highest differentiation in terms of genotype occurrence in the examined breed was found in the Gray Shirazi sheep and all possible genotypes occurred (AA (78.4%), AB (19.6%) and BB (2%) genotypes). The A and B allele frequencies were 0.8824 and 0.1176, respectively. Also, This locus have high polymorphism in other studied of Iranian sheep breeds such as native Lori, Arabi, dalagh, Zel and other native sheep breeds in Iran. The Polymorphisms of Calpastatin Gene In the examination population of Dalagh sheep from North of Iran, occurrence of M and N alleles was identified in CAST/*Msp*I locus with frequencies of 0.80 and 0.20 respectively [16]. Furthermore, frequencies of M/*Msp*I and N/*Msp*I alleles similar to those detected in the authors's own research were found in Iran in Kurdi sheep. At the same time, genotype frequency differed, as no NN homozygotes were identified in this case and the MM and MN genotype frequencies were 76% and 24% respectively [25]. In contrast to the results of the authors' own research, where the M/*Msp*I allele frequency was quite high from 76.2% in the Polish Merino sheep up to 95.0% in the Ile de France sheep, the research conducted by Elyasi (2009) showed frequency of the M allele between 48% and 69% in particular breeds [7]. As has been said earlier, in the examined group of sheep the presence of all genotypes identified with the use of the *Msp*I enzyme was found. The only exception was the sheep of the Berrichon du Cher and Ile de France breeds, in which the NN homozygotes were not present. Whereas in the research conducted by Kaczor (2006), 100% of Polish Mountain Sheep were established to be MM homozygotes [14]. In the known literature, no information has so far appeared on allele and genotype frequencies in the calpastatin gene in sheep identified using the NcoI restrictase. The presence of 3 genotypes was found in the analysis of CAST/*Msp*I locus (MM, MN, and NN), the frequency of which reflects the allele frequency. Analyzing the results of the authors' own research and the contents of specialist literature one may conclude that the frequency of alleles and CAST/*Msp*I genotypes is influenced by the breed factor. Whereas the examination of CAST/*Nco*I locus showed low differentiation of genotype frequencies and high frequency of the M allele (in the Polish Merino, Berrichon du Cher, and Ile de France breeds no NN homozygotes and heterozygotes were found at all).

## V. Conclusion

The Gray Shirazi sheep breed showed no degree of heterozygosity and variation for the Myostatin (monomorph) but Calpastatin (CAST) genes showed high variation. In the other hand, this data provide evidence that Gray Shirazi sheep populations have a good polymorphism for some gene which open interesting prospects for future selection programs, especially marker assistant selection (*MAS*) and gene assistant selection (*GAS*) between different genotypes of different locus and gain and meat traits. The gene variation in Present Results showed that PCR-RFLP (PBR) is appropriate tools for evaluating genetic variability.

## Acknowledgment

The current study has been supported by biotechnology division of natural history museum and

genetic resources of Department of Environment in Pardisan Eco-Park of Tehran, Iran.

## REFERENCES


[1] Arthur, P.F., 1995. Double muscling in cattle: A review. Aust. J. Agric. Res., vol. 46, 1995, p. 1493-1515.

[2] Barnoy, S., T. Glaser and N.S. Kosower, 1997. Calpain and calpastatin in myoblast differentiation and fusion: Effects of inhibitors. Biochim. Biophys. Acta, 1358: 181-188. DOI: 10.1016/S0167-4889(97)00068-2

[3] Bastos, E., A. Cravador, J. Azevedo and H. Guedes-Pinto, 2001. Single Strand Conformation Polymorphism (SSCP) detection in six genes in the Portuguese indigenous sheep breed "Churra da Terra Quente". Biochtechnol. Agron. Soc. Environ., 5: 7-15. https://sapientia.ualg.pt/handle/10400.1/1209

[4] Boom, R., C.J. Sol, M.M. Salimans, C.L. Jansen and P.M.W.V. Dillen *et al*., 1990. Rapid and simple method for purification of nucleic acids. J. Clin. Microbiol., 28: 495-503. http://jcm.asm.org/content/28/3/495.short

[5] Casas, E., J.W. Keele, S.D. Shackelford, M. Koohmaraie and T.S. Sonstegard *et al*., 1998. Association of the muscle hypertrophy locus with carcass traits in beef cattle. J. Anim. Sci., 76: 468-473. http://www.animal science.org/content/76/2/468.short

[6] Cong, M., V.F. Thompson, D.E. Goll and P.B. Antin, 1998. The bovine calpastatin gene promoter and a new n-terminal region of the protein are targets for cAMP-dependent protein kinase activity. J. Biol. Chem., 273: 660-666. DOI: 10.1074/jbc.273.1.660

[7] Elyasi, G., J. Shoja, M.R. Nassiry, O. Pirahary and A. Javanmard, 2009. Allelic and genotypic frequency of Calpastatin gene in Ghezel and Arkhamerino sheeps and their crossbreds, J. New Agr. Sci. 13: 3.

[8] Forsberg, N.E., M.A. Ilian, A. Ali-Bar, P.R. Cheeke and N.B. Wehr, 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. PMID: 2482286

[9] Ghafouri-Kesbi, F. and M.P. Eskandarinasab, 2008. An evaluation of maternal influences on growth traits: the Zandi sheep breed of Iran as an example. J. Anim. Feed Sci., 17: 519-529. http://psjc.icm.edu.pl/psjc/cgi-bin/getdoc.cgi?AAAA022640

[10] Goll, D.E., V.F. Thompson, R.G. Taylor and A. Ouali, 1998. The calpain system and skeletal muscle growth. Canadian J. Anim. Sci., 78: 503-512. http://direct.bl.uk/bld/PlaceOrder.do?UIN=058792652&ETOC=RN&from=searchengine

[11] Goll, D.E., V.F. Thompson, R.G. Taylor and T. Zaleweska, 1992. Is calpain activity regulated by membranes and autolysis or by calcium and calpastatin? BioEssays, 14: 549-556. DOI: 10.1002/bies.950140810

[12] Hanset, R., 1991. The Major Gene of Muscular Hypertrophy in the Belgian Blue Cattle Breed. In: Breeding for Disease Resistance in Farm Animals, Owen, J.B. (Ed.), CAB International, Wallingford, ISBN-10: 0851987109, pp: 499.

[13] Huff-Lonergan, E., T. Mitsuhashi, D.D. Beekman, F.C. Parrish and D.G. Olson *et al*., 1996. Proteolysis of specific muscle structural proteins by mu-calpain at low pH and temperature is similar to degradation in postmortem bovine muscle. J. Anim. Sci.,74:993-1008.http://www.journalofanimalscience.org/content/74/5/993.short

[14] Kaczor, U. 2006. Identyfikacja polimorfizmu (MspI) genu kalpastatyny (locus CAST) wwybranych populacjach owiec, Komunikaty naukowe LXXI Zjazdu Polskiego Towarzystwa Zootechnicznego w Bydgoszczy, 4: 16.

[15] Kambadur, R. M. Sharma, T.P.L. Smith and J.J. Bass, 1997. Mutations in myostatin (GDF8) in double-muscled belgian blue and piedmontese cattle. Genet. Res., 7: 910-915. DOI: 10.1101/gr.7.9.910

[16] Khederzadeh, S. 2011. Polymorphism of Calpastatin Gene in Crossbreed Dalagh Sheep Using PCR-RFLP. African Journal of Biotechnology. 10 (53): 10839-10841.

[17] Killefer, J. and M. Koohmaraie, 1994. Bovine skeletal muscle calpastatin: Cloning, sequence analysis and steady-state mRNA expression. J. Anim. Sci., 72: 606-614. http://www.journalofanimalscience.org/content/72/3/606.short

[18] Kocabas, A.M., H. Kucuktas, R.A. Dunham and Z. Liu, 2002. Molecular characterization and differential expression of the myostatin gene in channel catfish (*Ictalurus punctatus*). Bioch. Biophys. Acta, 1575: 99-107. DOI: 10.1016/S0167-4781(02)00289-0

[19] Machado, A., S. Ivan, M. Mário Luiz and C.R. Ana Lúcia, 2003. Genetic Diversity of Four Cattle Breeds Using Microsatellite Markers Bras. Zootec. 32(1): 93-98.

[20] Martín-Burriel, I., E. García-Muro and P. Zaragoza, 1999. Genetic diversity analysis of six Spanish native cattle breeds using microsatellites.Anim. Genet. 30: 177-182.

[21] Maudet, C., G. Luikart and P. Taberlet, 2002. Genetic diversity and assignment tests among seven French cattle breeds based on microsatellite DNA analysis, J. Anim. Sci. 80: 942-950.

[22] Moazami-Goudarzi, K., D. Laloë, J.P. Furet and F. Grosclaude, 1997. Analysis of genetic relationships between 10 cattle breeds with 17 microsatellites. Anim. Genet. 28: 338-345.

[23] Mcpherron, A.C., A.M. Lawler and S.J. Lee, 1997. Regulation of skeletal muscle mass in mice by a new TGF-beta superfamily member. Nature, 387: 83-90. PMID: 9139826

[24] Nassiry, M.R., M. Tahmoorespour, A. Javadmanesh, M. Soltani and S.F. Far, 2006. Calpastatin polymorphism and its association with daily gain in Kurdi sheep, Iran. J. Biotechnol. 4: 188-192.

[25] Nassiry, M.R., F.E. Shahroudi, M.Tahmoorespur and A. Javadmanesh, 2007. Genetic variability and population structure in beta-lactoglobulin, calpastatin and calpain loci in Iranian Kurdi sheep, Pak. J. Biol. Sci. 10: 1062-1067.

[26] Palmer, B. R., Morton, J. D., Roberts, N., Ilian, M.A., and Bickerstaffe .R.1999. Marker –assisted selection for meat quality and the ovine calpastatin gene Proceeding of the New Zealand Society of Ani. Produ. 59:266-268

[27] Sonstegard, T.S., G.A. Rohrer and T.P.L. Smith, 1998. Myostatin maps to porcine chromosome 15 by Linkage and physical analyses. Anim. Genet., 29: 19-22. DOI: 10.1046/j.1365-2052.1998.00229.x

[28] Soufy, B., M.R. Abadi, K. Shojaeian, A. Baghizadeh and S. Ferasaty *et al*., 2009b. Evaluation of myostatin gene polymorphism in Sanjabi sheep by PCR-RFLP method. Slovak J. Anim. Sci., Res., 19: 81-89. https://docs.google.com/viewer?a=v&q=cache:mODI3bSGC_sJ:www.sid.ir/fa/vewssid/j_pdf/41713880109.pdf+&hl=en&pid=bl&srcid=ADGEESgrIZ7PuUQF1N1LmHcMQ5Urm7f44mLAKDtO9H3s2iaGORt7agGlOO6it4QC78vgseYjYuJyVC7Q2Ny8Ria7_knreghrxH_7dGYxtKEEdTcwmgsionVOKHKrWFag-MCO9t8O4gtx&sig=AHIEtbRJdAqOmx-E57KtJCLd1vb2JSjgMg

[29] Takano, J., T. Kawamura, M. Murase, K. Hitomi and M. Maki, 1999. Structure of mouse calpastatin isoforms: Implications of species-common and species-specific alternative splicing. Biochem. Biophys. Res. Commun., 260: 339-345. PMID: 10403772

[30] Timothy, P.L., N.L. Lopez-Corrales, S.M. Kappes, T.S. Sonstegard, 1997. Myostatin maps to the interval containing the bovine mh locus. Mamm. Genome, 8: 742-744.

[31] Yeh, F., C. Yang and T. Boyle, 1999. POPGENE version 1.31 Microsoft window-based freeware for Population Genetic Analysis. University of Alberta. Edmonton. AB. Canada.