http://www.pjbs.org

PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Pakistan Journal of Biological Sciences 10 (7): 1062-1067, 2007
ISSN 1028-8880

Genetic Variability and Population Structure in Beta-lactoglobulin, Calpastain and Calpain Loci in Iranian Kurdi Sheep

Mohammad Reza Nassiry, Feridoun Eftekhari Shahroudi,
Mojtaba Tahmoorespur and Ali Javadmanesh
Center of Excellence for Animal Science, College of Agriculture,
Ferdowsi University of Mashhad, Mashhad, Iran

Abstract: The genotypes for Beta-Lactoglobulin (BLG) and calpastatin (CAST) were determined by Polymerase Chain Reaction (PCR) and restriction enzyme digestion and genotyped for calpain (CAPN) by PCR-SSCP method in an Iranian breed sheep, Kurdi. Blood samples were collected from 100 pure bred Kurdi sheep from Kurdi breeding station located in Shirvan, Mashhad. The extraction of genomic DNA was based on Guanidin Thiocyanate-Silica gel method. After PCR reaction, amplicons were digested with restriction enzymes *Msp*I and *Rsa*I for beta-lactoglobulin and calpastatin genes, respectively. The beta-lactoglobulin locus had 3 genotypes with frequencies of 0.24, 0.54 and 0.22 for AA, AB and BB, respectively; calpastatin genotypes had 2 genotypes with frequencies of 0.76 and 0.24 for MM and MN genotypes, respectively. Calpain genotypes were analyzed with SSCP method, which had 2 genotypes with frequencies of 0.92 and 0.08 for AA and AB, respectively. Heterozygosity value for beta-lactoglobulin locus was 49% and for calpastatin and calpain loci was very low (24 and 8%, respectively). χ^2 test confirmed the Hardy-Weinberg equilibrium for three loci in this population. These data provide evidence that Iranian's Kurdi sheep breed have a variability, which opens interesting prospects for future selection programs, especially marker-assistant selection between different genotypes of different locus and milk and cheese characteristics, gain and meat traits and also for preservation strategies.

Key words: Beta-lactoglobulin, calpastatin, calpain, polymorphism, RFLP, SSCP, Kurdi sheep

INTRODUCTION

Genetic variability in indigenous breeds is a major concern considering the necessity of preserving what may be a precious and irreplaceable richness, regarding new productive demands. Conservation should be based on a deep knowledge of the genetic resources of the specific breed. It is therefore important to make efforts in order to characterize genetically indigenous breeds (Bastos *et al*., 2001). Several DNA polymorphisms have been considered as potential tools for selection of dairy and meat ruminants. DNA-based molecular methods have made possible genotyping of animals of any age and sex for milk and meat genes, thus providing a potentially more efficient and flexible selection tool. Selection efficiency, however, depends on allelic frequencies in the breeds and on the effect of these polymorphisms on dairy and meat traits and technological properties of milk and meat. In case of sheep, research on the genetics polymorphism of beta-lactoglobulin, calpastatin and calpain have been performed. Among specific genes that may affect economically important traits in sheep, the beta-lactoglobulin locus has been extensively studied (Barillet *et al*., 2005). Beta-lactoglobulin accounts for about 75% of the albumin fraction (Golijow *et al*., 1999). The genetic variants A and B differ in an amino acid at position 20 (Tyr to Hys) and this base substitution gives rise to an *Rsa*I polymorphism (Nassiry *et al*., 2002). Ali *et al*. (1999) compared the DNA sequences of alleles A and B. The genotype BB of beta-lactoglobulin seems to be associated with higher milk yield; on the other hand genotypes AA and AB seem to be superior in protein and casein content and crude yield (Garzon and Martinez, 1992). The level of postmortem calpastatin appears critical in determining the ultimate tenderness of aging muscles.

In using a molecular genetic approach to study meat quality in sheep, Palmer *et al*. (1999) have chosen the ovine calpastatin gene (CAST) as a candidate gene for meat quality. Palmer *et al*. (1999) have described a two alleles system of polymorphic variant (M and N) in a region of the ovine CAST (exon and intron regions from a portion of the first repetitive domain) by PCR-RFLP method. Digestion with restriction endonucleases *Msp*I and *Nco*I differentiates alleles M and N. Polymorphism of

Corresponding Author: Mohammad Reza Nassiry, Center of Excellence for Animal Science, College of Agriculture, Ferdowsi University of Mashhad, P.O. Box 91775-1163, Mashhad, Iran Fax: +98 511 8787430

CAST has been detected in several farm animal breeds and studies of the affect of CAST genotypes on meat production traits. Since 1997, Palmer *et al.* (1999) have carried out slaughter trials on small groups of Dorset down hogget's and Dorset down × Coop worth lambs to ascertain any association between meat quality traits and the molecular markers in CAST. Sheep with the genotype ac for CAST locus (in PCR-SSCP method) were compared for traits indicate an association with increased live weight gain (+12-17%, $p<0.05$), increased age-corrected carcass weight (+15-18%, $p<0.05$), but increased Longissimus dorsi shear force (+4-12%, no significant) compared to sheep with the CAST genotype AA (Palmer *et al.*, 1999). Calpastatin is the endogenous and specific inhibitor of calpains. It regulates the rate and extent of post mortem tenderization (Kocwin and Kuryl, 2003). Another genes intensively investigated in farm animal are that of calpastatin (CAST) and calpain (CAPN). Calpastatin and calpain deserves special attention because of their major role in meat production. The Calpain-Calpastatin System (CCS) comprises a family of calcium dependent neutral proteases. The calpain and calpastatin are specific inhibitors of the calpain which regulates their *in vivo* activity. The CCS is found in most animal tissue and influences many important processes including muscle development and degradation, meat tenderization postmortem, cataract formation and fertility (Chung *et al.*, 1999). The calpains have been shown to play the major role in post mortem tenderization in beef, lamb and pork by degrading specific muscle structural proteins. 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. It is now accepted that calpain-mediated degradation of myofibrillar proteins is responsible for the post mortem meat tenderization, which occurs during storage at refrigeration temperatures. Two CAPN alleles (A and B) from exons 5 and 6 have been identified and are easily detected by PCR amplification and SSCP process (Chung *et al.*, 1999).

Kurdi native coarswooled is a native Iranian meat type sheep breed. The aim of the present study was to identify genotypes of beta-lactoglobulin, calpastatin and calpain genes in Kurdi sheep breed by PCR.

MATERIALS AND METHODS

Animals and DNA extraction: Blood samples were randomly collected from 100 pure bred Kurdi sheep from Kurdi breeding station located in Shirvan, Mashhad, Iran. DNA was extracted from 100 μL of blood as described by Boom *et al.* (1990). Quality and quantity of DNA were measured by spectrophotometer by taking the optical density at wavelength of 260 and 280 nm, respectively.

PCR: One microlitre DNA was amplified in a total volume of 25 μL PCR mix using the Biometra T Personal Ver: 1.11 thermocycler. The PCR mix contained: 2.5 μL PCR buffer 10-X (200 mM $(NH_4)_2SO_4$, 0.1 mM Tween 20%, 750 mM Tris-HCl pH = 8.8), 2.5 mM $MgCl_2$, 200 μM dNTPs and 3 μL mix of oligonucleotids (10 pm from each primer), 1 U Taq DNA polymerase and 11 μL ddH_2O.

Beta-lactoglobulin: Amplification was done for 34 cycles at the following program: denaturation at 94°C for 50 sec, annealing at 59°C for 30 sec and extension at 72°C for 40 sec. Primers were designed by Primer Premier 5 software according to X12817 Gene Bank accession number. The sequences are BLG1 (5'-CTCTTTGGGTTCAGTGTGAGTCTGG-3') and BLG2 (5'-CACCATTTCTGCAGCAGGATCTC-3')thatamplified a 301 bp fragment from the exon II of the ovine β-lactoglobulin gene.

Calpastatin: Thirty-five cycles of 95°C (1 min), 62°C (1 min) and 72°C (2 min) followed by 72°C (8 min). Exon (IC, ID) of the ovine calpastatin gene (CAST I) were amplified to a product of 622 bp fragment using primers based on the sequences bovine calpastatin gene. Primer sequences were ovine 1C: 5'-TGGGGCCCAATGACGCCATCGATG-3' and ovine 1D: 5'-GGTGGAGCAGCACTTCTGATCACC-3' (Gene Bank accession No. AY834765).

Calpain: PCR program included a preliminary denaturizing at 95°C, followed by 35 cycles, denaturing at 94°C for 45 sec annealing at 59°C for 1 min, extension at 72°C for 1.5 and 10 min at 72°C as final extension. The ovine m-calpain regulatory gene, exon 5 and 6 including intron (CAPN456), was amplified with primers (CAPN456F: 5'-AACATTCTCAACAAAGTGGTG-3' and CAPN456R: 5'-ACATCCATTACAGCCACCAT-3') designed according to the published bovine nucleotide cDNA sequence (Gene Bank accession No. J05065).

Products of amplification were recognized by electrophoresis on 1.5% agarose gel stained with etithium bromide.

RFLP: Five microliter of PCR products were incubated for 5 h at 37°C with 5 units of *Rsa*I and *Msp*I enzymes for

beta-lactoglobulin and calpastatin genes, respectively in separate reactions. Digestion products of beta-lactoglobulin were separated by electrophoresis on 8% non-denaturant polyacrylamid gel and visualized after silver staining. Digestion products of calpastatin were separated by electrophoresis on 2% agarose gel stained with etithium bromide.

SSCP: For the genotyping of calpain locus, PCR products were diluted with 12 μL of running buffer. Running buffer included: 800 μL formamid, 100 μL bromophenol blue 1%, 100 μL xylenecyanol 1%, 2 μL 0.5 M EDTA and 1 μL 10 M NaOH. After heating at 95°C for 5 min, they were immediately placed on ice. Polymorphisms were detected using 8% non-denaturing polyacrylamid gel with 10% glycerol. The mixture was electrophoresed for 3-4 h at 250 V and 10°C. DNA fragments were visualized using silver staining method.

Statistical analysis: The frequencies of genotypes, alleles, mean expected, observed and Nei's heterozygosities and Hardy-Weinberg equilibrium test were calculated using PopGene32 (ver 1.31) program (http://cc.oulu.fi/~jaspi/popgen/popdown.htm).

RESULTS

Beta-lactoglobulin: A 301 bp fragment of the ovine β-Lactoglobulin gene from exon II was amplified successfully. Digestion with restriction endonuclease *Rsa*I differentiates alleles A and B. The digested AA PCR product revealed two fragments of 241 and 60 bp, AB genotype exhibited 241, 175, 66 and 60 bp fragments and BB genotype had 175, 66 and 60 bp fragments (Fig. 1).

Calpastatin: A 622 bp fragment from CAST I was amplified. Digestion with restriction endonuclease *Msp*I differentiates alleles M and N. The *Msp*I digests the allele M amplicon, but not allele N. The MM genotype exhibited two fragments of 336 and 286 bp, MN genotype exhibited 622, 336 and 286 bp fragments and NN genotypes exhibited a 622 bp fragment (Fig. 2). The allelic frequencies were 88% and 12% for M and N, respectively. The genotype distribution in Kurdi sheep were 76, 24 and 0.0% for MM, MN and NN, respectively (Table 1). NN genotype was not detected in this study.

Calpain: The ovine calpain II regulatory gene, exon 5 and 6 including intron (CAPN456), was amplified. Under the SSCP analysis condition, different conformations were separated by electrophoresis on non-denaturing condition (Fig. 3). Two alleles (A and B) were observed with frequencies of 0.96 and 0.04, respectively. Genotype frequencies were 0.92 for AA, 0.08 for AB and 0.00 for BB (Table 1).

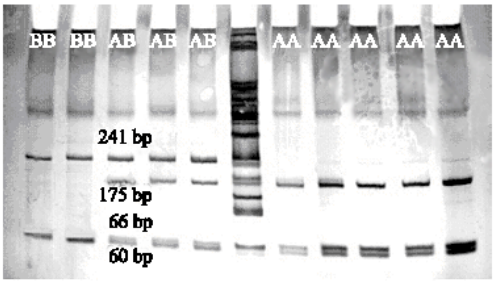

Fig. 1: Restriction patterns of beta-lactoglobulin gene after digesting with *Rsa*I in a 8% non-denatured polyacrylamide gel. Molecular marker is pUC19/*Msp*I (501, 489, 404, 331, 242, 190, 147, 111, 110, 67, 34 and 26 bp)

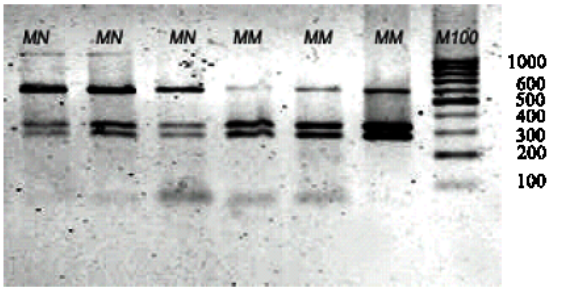

Fig. 2: Restriction patterns of 622 bp fragments of CAST I after digesting with *Msp*I in 2% agarose gel. Molecular markers are M100 (1000, 900, 800, 700, 600, 500, 400, 300, 200 and 100 bp)

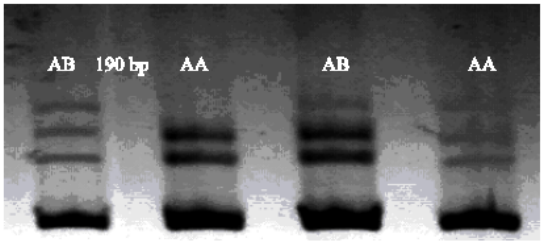

Fig. 3: SSCP pattern of the ovine CAPN regulatory gene in exons 5 and 6 including intron (190 bp), on 8% denatured polyacrylamide gel. Two patterns demonstrating the 2 genotypes

Table 1: Allelic and genotype frequencies, observed heterozigosity, expected heterozigosity, average heterozigosity and Nei values for BLG, CAPN and CAST loci

Locus	A	B	AA	AB	BB	Obs_Het	Exp_Het*	Nei**	Ave_Het	χ^2
BLG	0.51	0.49	24%	54%	22%	0.5400	0.5023	0.4998	0.4998	0.5686
CAPN	0.96	0.04	92%	8%	0%	0.0802	0.0794	0.0790	0.0790	0.1336
CAST***	0.88	0.12	76%	24%	0%	0.2400	0.2123	0.2112	0.2112	1.7743

*Expected heterozygosity were computed using Levene (1949), ** Nei's and Li (1979) expected heterozygosity, ***Alleles for CAST locus have shown in text with M and N but in table M = A and N = B

DISCUSSION

The three loci were polymorphic in Iranian Kurdi sheep. The genotypes AA/AB/BB for the beta-lactoglobulin, MM/MN for the calpastatin and AA/AB for the calpain locus were observed. Table 1 shows the allele frequencies for beta-lactoglobulin, calpastatin and calpain genes in the Iranian Kurdi sheep.

Among specific genes that may affect economically important traits in sheep, the beta-lactoglobulin locus has been extensively studied. Two genetic variants A and B with allele frequencies of 0.51 and 0.49, respectively, were identified for beta-lactogllobulin. Similar results observed in Lithuanian Blackface (Kucinskiene *et al*., 2005), Savetski Merino and Oparinski (Nassiry *et al* ., 2002), Arkhar Merino, Makui, Ghezel (Elyasi *et al*., 2004), Merino, Lacaune, Manchega, Massa (Barillet *et al*., 2005). The most frequent genotype in Kurdi breed which was 54% for the individuals was AB (Table 1). Homozygous genotypes AA and BB were observed at frequencies of 24.00 and 22.00%, respectively. Mean observed heterozygosity was slightly higher than mean expected heterozygosity, Nei's heterozygosity value was similar to the mean expected heterozygosity. Relatively, similar amount of heterozygous individuals (54%) was observed also in Ghezel (Elyasi *et al*., 2004) and Karakul (Nassiry *et al*., 2002) breeds. However, the frequency of AA genotype in Kurdi was smaller in comparison to the group of sheep, represented by Lithuanian Native Coarsewooled (Kucinskiene *et al*., 2005), British Milk sheep, Hungarian Merino (Anton *et al*., 1997) and Romney-Marsh (Bochkarev *et al*., 1997) Ghezel, Makui (Elyasi *et al*., 2004). Nassiry *et al*., (2002) didn't find BB genotype in Russian and Iranian Karakul sheep breed. Significant associations have been made between beta-lactoglobulin genotypes and some milk traits. Carolie *et al*. (1995) and Faraghi *et al*. (1996) reported a positive effect of the B allele on milk yield in the Sarda breed; while Lopez-Galvez *et al*. (1993) found that the beta-lactoglobulin AA genotype milk had better cheese-making properties than beta- lactoglobulin BB or AB genotypes in the Manchega breed. Similarly, Gutierrez-Gil *et al*. (2001) found that AA homozygous animals showed a higher cheese yield than AA or BB ewes in the Churra breed.

One of the candidate genes in order to meat tenderness and gain is calpastatin gene. Our results showed that the calpastatin locus had two alleles in Iranian Kurdi sheep breed. The allele frequency was 88 and 12% for M and N, respectively. The genotype distribution in Kurdi sheep were 76, 24 and 0.0% for MM, MN and NN, respectively. Similar results for Calpastatin genotypes in Iranian Karakul sheep was obtained by Eftekhari, who found a high frequencies of the M allele (0.79) than the N allele (0.21) (Eftekhari Shahroudi *et al*., 2005) In contrast to our study, Elyasi *et al*. (2005) reported that both M and N allele's frequency were 0.50 and for calpastatin locus in Ghezel× Arkharomerino sheep. They observed MN genotype in high value for Arkharmerino (47.62%) and Ghezel×Arkharomerino (46.67%), They have not detected NN genotype. A high degree of calpastatin polymorphism has also been reported in studies with Dorset Down hoggets, Dorset Down×Coopworth sheep, Corriedale rams, Angus bulls, crossbred steer and pigs. According to Palmer *et al*. (1997) the calpastatin genotypes (MM, MN, NN) were detected in unrelated Corridale rams and allele frequency was 77% for allele M and 12% for allele N, which is in agreement with present results. In comparison to bovine, in Angus bulls, (Chung *et al*., 1999) observed genotype were AA, AB and BB for CASTI and CAST5 loci and AA, BB, CC, AB, AC and BC for CAST10 locus. Kurly *et al*. (2002) identified the polymorphism of calpastatin gene with three restriction enzymes (*Hif*I, *Msp*I, *Rsa*I) in stambeek (Dutch large whith × Dutch Landrrace) pig breed. All of the heterozygosity of this locus had a low value.

The calpain gene was investigated as a potential candidate gene for quantitative trait locus (QTL) affecting meat tenderness (Chung *et al*., 2001). Under the SSCP analysis, different conformations are than Separated by gel electrophoresis on non-denaturing condition. Allelic frequencies (A and B) were calculated as 0.96 and 0.04, respectively. A higher allele frequency for A allele was observed at CAPN456 locus. This result is in contrast to those obtained by Tahmoorespour *et al*. (2005), who found a frequencies of the A allele (0.56) than the B allele (0.44) for CAPN in wool and Iranian meat sheep of Baluchi. Observed heterozygosity (8.24%), expected heterozygosity (7.94%), Nei's heterozygosity (7.90%) and average heterozygosity (7.90 %) of CAPN locus for Kurdi sheep were slightly high.

This herd has been kept in closed station with no selection for these loci; therefore we observed Hardy-Weinberg equilibrium for three loci in this breed. Kurdi sheep shows a low degree of genetic variability for the beta-lactoglobulin, calpastatin and calpain loci. This may be explained by the conservation and breeding method, has been carried out. In this station of Kurdi sheep only a few rams have been selected and used (only from this station) for breeding. With respect to low effective number of population the inbreeding rate is high and so heterozigosity and genetic variability is low. For this problem it seems that in this station must be use the rams from other stocks. Although we observed the low variability for those loci, in the other hand, these data provide evidence that Iranian's Kurdi sheep breed have a good polymorphism for beta-lactoglobulin, Calpastatin and Calpain loci, 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 and PCR-SSCP are appropriate tools for evaluating genetic variability. It was the study first using polymorphism of beta-lactoglobulin, calpastatin and calpain loci to understand genetic variability of Kurdi sheep in Iran. Very little information is currently available to compare different Iranian breeds. The present study may be regarded as the beginning of attempts to understand the genetic variability of native sheep breed in Khorasan region and identification of association between genotypic variants and productive parameters for further studies.

REFERENCES

Ali, S., M. McGlenaghan, J.P. Simons and A.J. Clark, 1999. Characterization of the alleles encoding ovine β-Lactoglobulin A and B. Gene, 91: 202-207.

Anton, I., A. Zsolani and S. Kulkovics, 1997. Genetic Polymorphisms of Milk Proteins in Hungarian Dairy Sheep Breeds and Crosses. Proceeding of the workshop held in Budapest. Hungary, pp: 224-226.

Barillet, F., J. Arranaz and A. Carta, 2005. Mapping quantitative trait loci for milk production and genetic polymorphisms of milk proteins in dairy sheep. Genet. Sel. Evol., 37: 109-123.

Bastos, E., A. Cravador, J. Azevedo and H. Guedes, 2001. Single Strand Conformation Polymorphism (SSCP) detection in six genes in Portuguese Indigenous sheep breeds Churra da Terra Quente. Biochtechnol. Agron. Soc. Environ., 5: 7-15.

Bochkarev, V.V., 1998. The molecular analysis of beta-lactoglobulin locus in the different sheep breeds. Dubrovicy. Ph.D Thesis, pp: 1-93.

Boom, R., C.J. Sol and M.M. Salimans, 1990. Rapid and simple method for purification of nucleic acids. J. Clin. Microbiol., 28: 495-503.

Caroli, A., P. Bolla and A. Spanu, 1995. Effect of beta-lactoglobulin genotype on milk yield in the Sardinian sheep. Procceding 11th Cong. Nat. ASPA, Udine, Italy, pp: 181.

Chung, H.Y., M.E. Davis and H.C. Hines and D.M. Wulf, 1999. Effect of the calpain proteolysis and calpain genotype on meat tenderness of Angus Bulls. J. Anim. Sci., 77, Suppl.1. pp: 31.

Chung, H.Y., M.S. Davis and H.C. Hines, 2001. Effect of calpain and calpastatin genotypes on growth of Angus Bulls, Research and reviews: Beef and Sheep. Bulletin of animal sciences research and reviews, Ohio State University. Special Circular No., 156: 18-21.

Eftekhari Shahroudi, F., M.R. Nassiry and R. Valizadeh, 2003. The genetic polymorphism of calpastatin gene in karakul sheep. J. Agric. Sci. Natl. Reso. Khazar, 2: 1-10.

Elyasi, G., J. Shoja and M.R. Nassiry, 2004. Polymorphisms of β-lactoglobulin gene in Iranian Sheep Breed Using PCR-RFLP. Proceeding of The Joint Agric. Natl. Reso. Symposium. May 14-16. Ganja/Azerbaijan, pp: 66-72.

Faraghi, A., A. Carta and F. Pilla, 1996. Beta-lactoglobulin polymorphism in sarda dairy sheep. Procceding of 47th annul meeting of the European association for animal production, pp: 42.

Garzon, A.I. and J. Martinez, 1992. β-Lactoglobulin in Manchega sheep breed: Relationship with milk technological index in handcraft manufacture of manchego cheesse. Anim. Genet. 23, suppl. 1, pp: 106.

Golijow, C.D., G. Giovambattista and M.V. Ripoli, 1999. Genetic variability and population structure in loci related to milk production trait in native Argentine Creole and commercial Argentine Holstein cattle. Genet. Mol. Biol., 22: 395-398.

Gutierrez, Gil, B., J.J. Arranz and M.H. Othmane, 2001. Influencia del genotipo de la beta-lactoglobulina ovina sobre caracteres cualitativosy rendimiento quesero individual en la raza Churra. ITEA., 22: 15-17.

Kocwin, M.P. and J. Kuryl, 2003. The effect of interaction between genotype at loci CAST, RYRI and RN on pig carcass quality and pork traits. Anim. Sci. Paper and Report, 21: 61-65.

Kucinskiene, J., G. Vagonis, J. Maleviciute and I. Tapio, 2005. Genetic polymorphism of β-lactoglobulin in Lithuanian blackface and Lithuanian native coarse-woled sheep. Veterinarija ir zootechnika, 29: 90-92.

Kurly, J., W. Kapelanski and M. Pierzchala, 2002. Preliminary observations on the effect of calpastatin gene polymorphism in carcass trait in pigs. Anim. Sci. Paper and Report, 2: 87-95.

Levene, H., 1949. On a matching problem in genetics. Ann. Math. Stat., 20: 91-94.

Lopez-Galvez, G., M. Ramos and P.J. Martin-Alvarez, 1993. Influence of milk protein polymorphism on cheese producing ability in the milk of Manchega ewes breed, cheese yield and factors affecting its control. Int. Dairy Fed Seminar, Cork, pp: 167-173.

Nassiry, M.R., J. Shoja and G. Elyasi, 2002. The determination of beta-lactoglobulin gene polymorphism in some Iranian and Russian sheep breeds. Veterinarija ir zootechnika. 19: 112-115.

Nei, M. and W.H. Li, 1979. Mathematical model for studying genetic variation in terms of the restriction endonucleases. Proc. Natl. Acad. Sci. USA., 76: 5269-5273.

Palmer, B.R., J.G.H. Hickford and R. Bickerstaffe, 1997. A candidate gene approach to animal quality traits. Proc. New Zealand Soci. Anim. Prod., 57: 294-296.

Palmer, B.R., J.D. Morton and N. Roberts, 1999. Marker-assisted selection for meat quality and the ovine calpastatin gene. Proc. New Zealand Soc. Anim. Prod., 59: 266-268.

Tahmoorespour, M., 2005. Study on the calpain calpastatin gene polymorphisms by polymerase chain reaction-single strand conformation polymorphism and its relation to average daily gain in iranian baluchi sheep. Ph.D Thesis. Ferdowsi University, Mashhad, Iran.