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Polymorphism of Candidate Genes for Meat Production in Lori Sheep

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Abstract

Calpastatin and callipyge have been known as two of the candidate genes in meat quality and quantity. Calpastatin gene and callipyge gene has been located to chromosome 5 and 18 of sheep. The objective of present study was to identification of calpastatin and callipyge genes polymorphism and survey of genotype structure in population of Lori sheep kept in Iran. Blood samples were taken from 120 Lori Sheep breed and Genomic DNA was extracted by salting out method. Polymorphism was identified applying the PCR-RFLP technique. The PCR products were digested with MspI and FaqI endonucleases for calpastatin gene and callipyge gene, respectively. In this population, three patterns were observed and genotype frequencies for AA, AB and BB detected 0.32, 0.63, and 0.05 for calpastatin gene respectively. The results obtained for the callipyge gene revealed that only the wild-type allele A was observed indicating that, only genotype AA was present in the population under consideration. So, the results confirmed that PCR-RFLP technique can be used to identify genetic variation in this breed and is a useful tool for selection plans based on marker-assisted selection.

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Keywords: Polymorphism; Calpastatin; Callipyge; PCR-RFLP; Lori sheep

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1. Introduction

The amount of genetic gain that can be obtained in direct election for Characteristics of a meat is depends on genetic variation. Improvement comes from the use of markers in selection programs depends on a variety of candidate genes that are expressed. (John L. Williams, 2008).

Identification of major genes affecting a trait variation provides Conditions to use genetic data in election schemes. Selecting animals with the most desirable genes at worthy genes, the amount of animal gain could be dramatically raised. Indeed, genomic procedures have the suitable capacity for easier to the development of traits that are problem to select via the conventional procedures. The knowledge on polymorphisms in genes influencing Special traits, and comprehension the biological effects of these Variations, will allow genomic data to be applied most effectively in animal selection Protocols. Genetic markers close to the genes for economically important traits have the potential to be used in selection programs which are called marker-assisted selection (Dekkers, 2004). The first stage is the recognition of mutations in interested major genes that can be surveyed in association studies in particular picked examinations. Based on role play, calpastatin (CAST) and callipyge (CLPG) genes can be considered desirable genes for meat Characteristics.

Calpastatin gene is placed on 5 chromosome of sheep and has important functions in foundation of skeletal muscles, degradation and meat tenderness after carnage. Calpastatin function is significantly related to the rate of muscle protein operation and amount of postmortem tenderization (Goll et al., 2003; Amanda et al., 2004). Calpastatin, which is an interior repressor (ca^{+2} dependent cysteine proteinase), has an remarkable impress in alignment of calpain function in cells and is weighed to be one of the key regulator of the calpains (Forsberg et al., 1989). So, calpastatin may alter proteolysis of myofibrils because of regulation of calpain, which can commence postmortem erosion of myofibril proteins (Goll et al., 1992; Hufflonergar et al., 1996).

Callipyge gene is placed in the telomeric zone on ovine chromosome eighteen, within a class of imprinted alleles. The Callipyge (CLPG) shape in sheep is a muscular hypertrophy that is most apperceived in the muscles of the pelvic limb. Callipyge lambs display several favorable production properties and meat quality properties. Superior dressing percentages, major longissimus (loin eye) region, preferable lean composition, and higher leg scores Specified for Callipyge carcasses. Also Callipyge animals produce leaner, higher construction cadavers, but there is some relevance with reduced tenderness of the loin [Jackson et al., 1997 and Koohmaraie et al., 1995]. The Callipyge phenotype in sheep illustrate A new model of inheritance called “polar overdominance” where only heterozygous individuals having inherited the callipyge mutation from their sire represent the phenotype (i.e. the +M/CLPGP genotype). The other genotypes are natural in appearance. (Cockett et al., 1996).

The name of this breed is derived from Lori tribe, which is one of the main nomadic groups of Zagros Mountain in western parts of the country. This sheep have strong constitution, good traveling ability with suitable conformation as a mountain sheep (Mansouri et al., 1995).

Meat production in Iran is one of the most valuable traits for livestock Breeders. Also, Sheep meat has tremendous values that rather than the meat of other animals and is popular between people. So, the purposes of present study were to distinguish the genetic diversity of the CAST and CLPG genes can be considered as interested genes for meat yield in Lori sheep population.

2. Materials and Methods

2.1. Genomic DNA extraction

In this study, the blood samples of 120 Lori sheep were randomly collected from Lorestan Province of Iran. About 5 ml of blood were gathered from each animal in vacutainer including EDTA and stored at 4°C.

Genomic DNA was exploited by salting out procedure according to (Miller et al. 1988) with minor modifications.

2.2. PCR-RFLP analysis

CAST: A 622 bp fragment of calpastatin gene in the exon 1 zone was amplified by using PCR to study of polymorphism in Lori sheep breed. Spectrophotometer was used for determine quality and quantity of DNA. Sequence of forward and reverse primers was according to following sequences: F: 5'- TGGGGCCCAATGACGCCATCGATG - 3' and R: 5'- GGTGGAGCAGCACTTCTGATCACC - 3'. PCR was implemented using a buffer PCR 1X, 200 µM dNTPs, 1.5 µM $MgCL_2$, 10 pmol each primer, 1 U Taq DNA polymerase, 50 ng ovine genomic DNA and H_2O up to a total volume of 25 µl. 33 cycle of initial denaturation at 95°C (5 min), denaturation at 94°C (60 sec), renaturation at 60°C (60 sec), amplification at 72°C (2 min) and final amplification at 72°C (8 min). The PCR products were isolated by 2% (w/v) agarose gel electrophoresis. The amplified fragment of calpastatin was digested with MspI endonuclease. Digestion was carried out at 37°C for 12-16 h and in a 29 µL reaction solution including 11.5 µL of distilled H_2O, 2 µl buffer, 5 units of MspI Enzyme, and 15 µL of PCR supply solution.

CLPG: DNA primers described by Freking [10] were applied to PCR amplification: forward primer 5´- TGA AAA CGT GAA CCC AGA AGC - 3´ and reverse primer 5´ - GTC CTA AAT AGG TCC TCT CG - 3´. Polymerase chain reaction were performed in 15 µl volume including 50 ng DNA, 1 U Taq polymerase (Fermentas), 1 x PCR buffer $(NH4)_2SO_2$, 2mM $MgCl_2$, 200 µM dNTP, 2 µM of each primer. The PCR reaction was optimized in the thermocycler MJ Mini. The following extension components were used: 95°C for 4 minutes followed by 35 cycles: 94°C for 20 seconds, 58°C for 30 seconds, 72°C for 1 min. The PCR products of 426 bp were digested with 10 units of the FaqI (BsmFI) endonuclease.

The digestion products of CAST and CLPG gene were isolated separately on 2% agarose gel in 1X TBE and conceived by ethidium bromide dyed for 1 h at 85 V.

2.3. Statistical analysis

The genotype and gene frequencies of CAST and CLPG gene polymorphisms and X2 test were used to determine whether the population is in HWE using POPGENE32 software (Yeh et al., 1999).

3. Results and Discussion

To assess the population genetics, parameters such as allelic and genotypic frequencies, observed and expected heterozygosity, effective allele number and real allele number, and the population is in Hardy - Weinberg Equilibrium or not, is used which as a measure of polymorphisms are known. So the like other researchers, to study these candidate genes in Lori sheep population these parameters were measured.

The CAST locus was polymorphic in Lori sheep. The genotypes AA, AB and BB for Calpastatin locus were observed. But this study showed that the CLPG mutation was not present in Lori sheep and CLPG locus was monomorphic in this population.

3.1. Calpastatin

Digestion of the 622 bp fragment in calpastatin gene with MspI restriction enzyme revealed three genotypes. The AA genotype exhibited 2 fragments of 336 and 286 bp, AB genotype exhibited 622, 336 and 286 bp fragments and BB genotypes exhibited a 622 bp fragment (Fig. 1). The gene frequencies were 0.635

and 0.365 for A and B, respectively. The genotypic frequencies for AA, AB and BB in Lori sheep were 0.32, 0.63 and 0.05, respectively. The Lori sheep were not in HWE and it was concluded that breeding based on selection for Calpastatin gene was done ($p < 0.001$). The H_O and H_E were 0.63 and 0.46, respectively. N_E and N_A are computed 1.87 and 2, respectively. These results indicate that Lori sheep breed have a suitable diversity for calpastatin gene, that can be used in future breeding programs. Present results showed that PBR is a useful tool for the study of genetic diversity.

The diversity perceived in the present study was consistent with reports on other breeds (Mohammadi et al., 2008; Tohidi et al., 2013; Nassiry et al., 2006). Mohammadi et al. (2008) studied polymorphism of calpastatin allele in One hundred and eleven Arabic sheep breed and reported the genotypic frequencies of 0.703, 0.283 and 0.014 of the AA, AB and BB respectively. Similar data were found by Elyasi et al., (2005) that a significant frequency of genotype MN in Arkharomerino (0.476) and Ghezel and Arkharomerino crossbred (0.467) sheep was showed (Corresponding to AB genotype). Also they stated that, in the studied population for this gene, selection plan so far was not done. Also Zhou et al., (2007) reported a substantially diversity in the intron district of the ovine calpastatin locus.

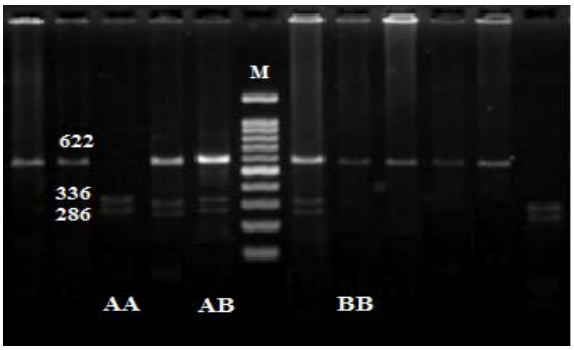

Fig. 1. PCR-RFLP analysis of 622 bp fragment of calpastatin gene by MspI enzyme on 2 % agarose gel.

3.2. Callipyge

The polymorphism of CLPG gene was detected in 426 bp PCR product by restriction endonuclease FaqI (Fig. 2). The FaqI digestion of the PCR products produced segments of 395 bp and 31 bp for mutant allele G and 278 bp, 117 bp and 31 bp for wild allele A (Fig. 2). In the present study, the same digestion patterns were observed and all Lori sheep exhibited AA genotype. Therefore, segments of 278, 117 and 31 bp were obtained from endonuclease digestion. As expected, this suggests that the Lori sheep was monomorphic for callipyge locus.

These results suggest that the restriction site surrounding an A → G transition as functional mutation of callipyge phenotype was not present in the studied population. Also, in the frequency of genotype AA was 1.00 therefore the genetic equilibrium of analyzed population was not evaluated on the base X2 test.

Jackson et al. (1997) have shown the occurrence the mutant allele G for breeds of sheep Dorset, Rambouillet and Hampshire. In a study Gabor et al., (2009) for the callipyge gene in sheep of breed's tsigai, improved valachian, east friesian, lacaune and crossbreds lacaune and tsigai detected homozygous genotype AA – 1.00 only. The present study showed the same result reported by Quanbari et al., (2007), that reported no CLPG mutation in experimental flock of Afshari breed.

To participate in genetic improvement of animals, further studies with adequate samples from animals with accurate information on animal phenotypes are necessary to better understanding the regulatory mechanisms

of polymorphism on gene expression of CLPG. This locus is important for the producers and the need to consider the other breeds as well.

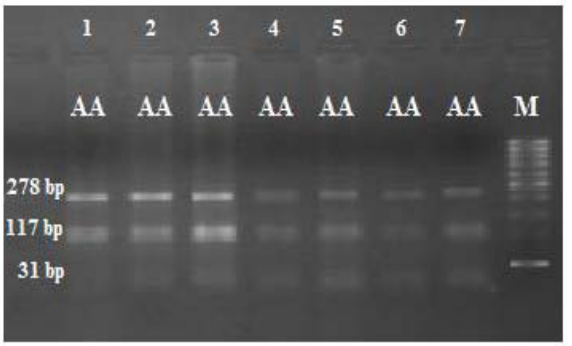

Fig. 2. PCR-RFLP analysis of 426 bp fragment of CLPG gene by FaqI enzyme on 2 % agarose gel

4. Conclusion

The first step in the application of the candidacy locus procedure is genotyping of the base flock for the favorable gene. In this paper, Lori sheep population revealed variation for calpastatin gene. The essence of sufficient diversity in the Calpastatin locus in Lori sheep illustrated that the quality of meat in this breed can be accommodated by selecting schemes. As respects, the results show that frequency distribution of CLPG gene was all of the wild-type (AA) genotype. Polymorphisms result could be applied for marker in the selection of local sheep with meat quality in future. This research is a beginning stage to find candidate genes for selection of quantity and quality meat traits. According to these results, we concluded that these genes can be regarded as a candidate gene of sheep high quantity and quality meat traits. Also, genetic markers are the key to the usage of these genes in the sheep industry and have been a useful tool for study the genetic basis of high meat production in different breeds.

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