Middle-East Journal of Scientific Research 23 (9): 2001-2004, 2015
ISSN 1990-9233

DOI: 10.5829/idosi.mejsr.2015.23.09.93165

Polymorphism of Candidate Genes for Meat Quality in Sheep

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Abstract: A number of candidate genes have been identified as potentially relevant to sheep meat quality traits. Studies provide molecular evidences that the degradation of myofibrillar proteins in muscle related to the calpain system shows strong impact on the variation of meat characteristics. Indeed, the functional candidate gene, Calpastatin (CAST) is a specific inhibitor of calpains, making the CAST gene an excellent candidate for meat tenderness of sheep. The objective of the study was to evaluate the polymorphism on one of these candidate genes in lori sheep strain. Genomic DNA was extracted from 100 Sheep blood sample. Polymerase chain reaction was performed to amplify a 622bp fragment of exon and intron I from L domain of the ovine calpastatin. Restriction reaction of PCR products was done using MspI enzyme. The MspI digestion of the PCR products produced digestion fragments of 336 bp and 286 bp. The results showed genotypes AA, AB and BB had a frequencies 32.2, 63.2 and 4.6 respectively. Also results showed that this locus was not at Hardy – Weinberg equilibrium in the Lori sheep strain.

Key words: Meat Quality · Calpastatin gene · Polymorphism

INTRODUCTION

Increase in sheep production will help increase mutton production and study on Calpastatin gene combined with other molecular techniques such as marker assisted selection (MAS) can play very important part to better sheep production in Iran. The Lori is an economically influential breed of sheep prized for its meat, particularly in the Nomadic herders (Figure 1). The effect of Calpain gene polymorphism on the analyses of meat quality traits are discussed in previously [1]. The protein encoded by this gene is an endogenous Calpain (calcium-dependent cysteine protease) inhibitor. It consists of an N-terminal domain L and four repetitive Calpain-inhibition domains (domains 1-4) and it is involved in the proteolysis of amyloid precursor protein. Of the five domains, the N-terminal leader (L) domain does not appear to have any Calpains inhibitory activity, but may be involved in targeting or intracellular localization [2]. While the other domains (I-IV) are highly homologous and are each independently capable of inhibiting Calpains [3]. This Indicates that the inhibitory domains

Fig. 1: Lori sheep farming methods in Iran

of Calpastatin contain three highly conserved regions, A, B and C, of which A, played a regulatory role by altering phosphorylation patterns on the protein [2]. Calpastatin (CAST) gene is located on the fifth chromosome of sheep and plays important roles in the formation of muscles, degradation and meat tenderness after slaughter. Increased rate of skeletal muscle growth can result from a decreased rate of muscle protein degradation and this is

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associated with a decrease in activity of the Calpain system, due principally to a large increase in Calpastatin activity [1]. Associations have been reported between variation in CAST and carcass and meat quality traits in cattle [4, 5]. Also in sheep a genetic variation in the CAST gene has been investigated [6,7]. In our research we have studied the position of the Calpastatin gene in the Lori sheep breeds in Iran.

MATERIALS AND METHODS

Sampling and DNA Extraction: Random blood samples were collected from 100 Lori sheep from different regions in Lorestan province of Iran. Approximately, 5 ml blood sample was gathered from vena in EDTA tube and was transferred to -20°C freezer. Genomic DNA was extracted from whole blood.

Amplification of the Exon 1 of CAST Gene: Exon and intron region from a portion of the first repetitive domain of the ovine Calpastatin gene were amplified to a product of 622 bp using primers based on the sequence of the bovine and ovine Calpastatin genes [8]. DNA primers described by Palmer were used to PCR amplification [7]. Primers were obtained from Cinnagen Company in form of the lyophilized (non-sensitive to temperature).

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

The polymerase chain reaction (PCR) was performed using a buffer PCR 1X, 200 µM dNTPs, 1.5 µM MgCL2, 10 pmol each primer, 1.25 U taq DNA polymerase, 50 ng ovine gnomic DNA and H_2O up to a total volume of 25 µl. About 33 cycle of preliminary denaturation at 95°C (5 min), denaturation at 94°C (1 min), annealing at 60°C (1 min), extention at 72°C (2 min) and final extension at 72°C (8 min). The PCR products were separated by 1.2% (w/v) agarose gel electrophoresis. The amplified fragment of Calpastatin was digested with MspI. 15 µl of PCR production with 2 µl buffer, U (0.5) of MspI and 11.5 µl H_20 up to a total volume of 29 µl, following the manufacturers instruction for 12-16 h at 37°C. The digestion products were electrophoresed on 2% agarose gel in 1X TBE and visualized by Eithdium bromide staining for 1 h at 85 V. Estimates genotype and alleles frequencies and Hardy-Weinberg equilibrium was analysis with Pop Gene 32 package [9]. The relative frequency of particular allele in a population is called the allele frequency [10].

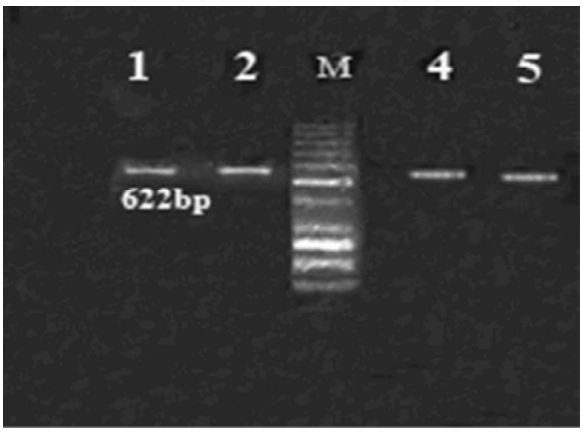

Fig. 2: PCR product analyzed by electrophoresis of the calpastatin (622 bp)

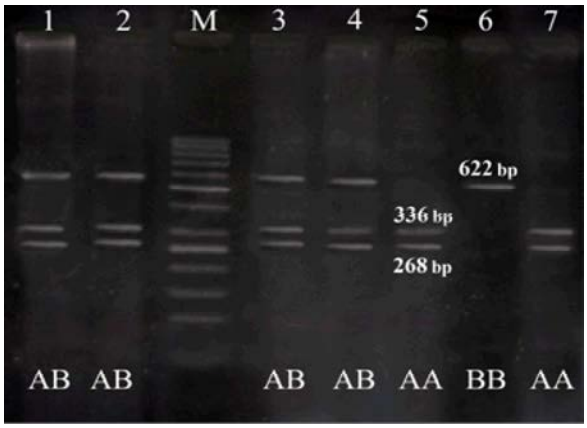

Fig. 3: Genotype AA, AB and BB of the calpastatin digestion with MspI

Description:
*x*2 = Hardy-Weinberg equilibrium test
O = observed number of genotype A11
E = expected number of genotype A11

RESULTS

The amplified Calpastatin resulted in a DNA fragment with 622 bp including the sequences of Exon and intron regions from a portion with PCR technique (Figure2). Due the digestion of 622 bp PCR product for CAST gene with restriction endonucleases MspI three different genotypes were observed (AA,BB,AB). In the first genotype (AA) showed the two band pattern (bands of approximately 336 and 286 bp). In the second genotype (AB), due to a mutation in one of the alleles, bands of 622, 336 and 286 bp were observed. In the third genotype (BB) one band pattern (approximately 622 were observed (Figure 3). After assessment of the samples the

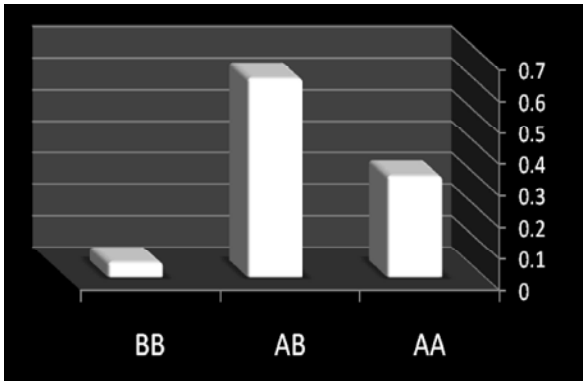

Fig. 4: Genotype frequencies of the calpastatin in lori sheep

Table 1: Chi-square test of the calpastatin gene in lori sheep

Genotype	Observed frequency	Expected frequency	p
AA	0.322	0.407	0.001281 **
AB	0.362	0.462	
BB	0.044	0.131	

frequencies of A and B alleles were calculated as 0.638 and 0.362, respectively. Also the frequencies of AA, AB and BB genotypes were calculated as 0.332 for AA, 0.046 for BB and 0.632 for AB respectively (Figure 4). According to the obtained data analysis, the results were significant in both tests used and sheep populations were not in Hardy Weinberg equilibrium (P<0.05) (Table.1).

DISCUSSION

Genetic polymorphism identification of the CAST gene could be used as a tool to predict meat tenderness in sheep allowing breeders to enhance the trait [10]. In the present study, we observed two alleles (A, B) and three genotypes (AA, AB, BB) for CAST gene in lori sheep breed. Two allelic systems of polymorphic variants (M and N) in the region of ovine CAST locus have been described by PCR-RFLP method [11]. Also frequency of the allele A in that the polymorphism was detected in CAST I segment, as previously reported in a variety of other sheep in the world. Such as, Polymorphism study on the same region of the CAST gene in Kurdi sheep by PCR-SSCP revealed three genotypes including aa, ab and ac genotype [12]. The polymorphism in the exon 1 of the CAST in sheep was also reported by other researchers using PCR-RFLP technique [11,13]. Also in present research, we reported the three fragments of 286 and 336 bp length and hence two alleles with three different genotypes, although the fragments size, the number of alleles and genotypes observed were similar to the results of other researchers on some other sheep breed. Two allelic systems of polymorphic variants (M and N) in the region of ovine CAST locus have been described by PCR-RFLP method [11]. According to Palmer, allelic frequencies were 77% and 12% for the M and N in Corriedale sheep, respectively Palmer *et al*.[6]. In goats and bovine the exon 6 of CAST gene were investigated for polymorphisms and a number of allelic variants were identified in these species [14]. Fortest reported higher frequencies of CAST gene's allele A compared to the allele B in Nellore (0.66), Rubia Gallega (0.72), Canchim (0.62), Brangus (0.78) and Pardo Suico (0.80) cattle [15]. According to our obtained data analysis, Lori sheep populations for calpastatin gene frequently were not in Hardy Weinberg equilibrium. It seems breeding programs to improve the frequency of the calpastatin gene in this strain is useful. In this regard, studies show that the Hardy-Weinberg equilibrium can be affected by inbreeding, assortative mating, natural selection and population subdivision[6]. Lack of Hardy Weinberg equilibrium for Calpastatin gene in other populations have been reported [7,10,11]. The results indicate that it could be useful to consider genetic diversity at Calpastatin locus in lori sheep. The present study was the first attempt for identification of CAST gene variation in Iranian lory sheep. Further studies are required to investigate the relationship between CAST gene polymorphisms and the performance traits in lori sheep strain.

ACKNOWLEDGEMENTS

Authors sincerely thank Dean of Animal Science Research Institute (ASRI) and Azad University Borougerd Branch for support this project.

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