Study of Calpastatin Gene Polymorphism in Holstein Cattle and Buffalo

Soheil Yousefi, Mojtaba Ahani Azari

Department of Animal Science, Gorgan University of Agricultural Sciences and Natural Resources, Basij Square, Gorgan, Golestan, Iran

Abstract
In this research for studying genetic polymorphism of calpastatin (*CAST*) gene as candidate gene for marker-assisted selection in different domestic livestock species, blood samples were collected from 50 Holstein cattle and 40 buffalo randomly. DNA was extracted from blood samples and a 622 bp fragment of the L region of exon and intron 1 of this gene were amplificated by polymerase chain reaction. PCR products were analyzed using restriction fragment of length polymorphism (RFLP) method and restriction reaction were done using *MspI* enzyme. Results indicate that there are two alleles (M and N) and three (MM, MN in Holstein cattle and MN, NN in Buffalo) genotypes for this genes. The chi-square test revealed that this locus was not at Hardy–Weinberg equilibrium in the Holstein cattle while buffalo herd showed equilibrium (P<0.05).

Keywords: Buffalo, Calpastatin gene, Holstein cattle, Polymorphism, PCR-RFLP.

1. Introduction

Today, 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 meat genes, thus providing a potentially more efficient selection tool [1]. Selection efficiency, however, depends on allelic frequencies in the breeds and on the effect of these polymorphisms on meat traits and technological properties of meat. In studies related to meat quality calpastatin (*CAST*) gene has been identified as a candidate gene [2]. This gene is located on cattle chromosome 7 and is effective on formation, lysis of muscle texture and meat tenderness after slaughtering animals [3]. The Calpain/Calpastatin system (CCS) comprises a family of calcium dependent neutral proteases [4]. Calpastatin is a protein inhibitor that acts specifically on calpains and plays a regulatory role in postmortem beef tenderization and muscle proteolysis [5]. Polymorphisms in the bovine *CAST* gene have been associated with meat tenderness [6]. The CCS is found in most animal tissue and influences many important processes including muscle development and degradation, meat tenderization post mortem, cataract formation and fertility [7]. Dinparast Djadid *et al*., [8] amplified and sequenced a fragment of approximately 1.5 kb of the L domain of calpastatin in Afshari sheep. They found the similarity of 89% between the Afshari sequences and the reported bovine sequences of this gene in NCBI website.

Calpastatin deserve special attention because of their major role in meat production and quality. Therefore, aim of the present study was to determine of allelic and genotypic frequencies of calpastatin gene in Holstein cattle and buffalo by RFLP method.

* Corresponding author: Soheil Yousefi
Tel: 00989112772633, soheil.yousefi@yahoo.com

2. Materials and methods

2.1. Blood samples and DNA extraction

The blood samples were collected randomly from 50 Holstein cows (Behin Talise farm in Gorgan, Iran) and 40 Buffalos (Miankale's Agricultural and Husbandry farm in Behshar, Iran). DNA was extracted, using the salting out extraction protocol [9], then dissolved in TE buffer and kept at −20°C. Quality and quantity of extracted DNA was measured spectrophotometically and by electrophoresis on 1% agarose gel.

2.2. DNA amplification

Polymerase Chain Reaction (PCR) was carried out, using Personal Cycler™ thermocycler (Biometra, Germany) and PCR Master Kit (Cinnaclon Inc., Iran). Master Mix consisted of 0.04 U/µl of Taq DNA polymerase, 10X PCR buffer, 3 mM MgCl2 and 0.04 mM dNTPs (each). Each reaction mixture consisted of 7.5 µl of the master mix, 1 µl of the DNA solution (50 to 100 ng/µl), 1 µl of each primer (5 pmol/µl) and some deionized water making up a final volume of 15 µl.

Amplification for a 622 bp fragment from the exon I of the bovine calpastatin gene was carried out using primers suggested by Killefer and Koohmaraie [11], (Gen Bank accession no. L14450) under following condition: CAST - F (5'-TGGGGCCCAATGACGCCATCGATG-3')

CAST - R (5'-GGTGGAGCAGCACTTCTGATCACC -3')

The PCR program included a preliminary denaturizing at 95^{o}C for 3 min, followed by 35 cycles, denaturing at 95^{o}C for 1 min, annealing at 59^{o}C for 1 min, extension at 72^{o}C for 2 min and 7 min at 72^{o}C as final extension.

Products of amplification were recognized by electrophoresis using 1.5% agarose gel, stained with ethidium bromide.

2.3. PCR-RFLP

Calpastatin gene variants were identified by PCR-RFLP method. Samples (10 µl) of each PCR product were incubated for 16 h at 37°C with 10U *Msp*I enzyme, according to manufacturer's instructions (Fermentas, Lithuania). Digestion products were separated by electrophoresis on 2.5% agarose gel in 1x TBE buffer. Gel was run at 80 V for one hour and visualized under UV light after staining with ethidium bromide.

3. Results and discussion

Determination of gene and genotype frequencies and the χ2 test were carried out using the Pop-Gene 32 software [11]. The *PCR-RFLP* for *CAST* gene was carried out on agarose gel and two alleles (*M* and *N*) and three genotypes (*MM, MN* and *NN*) were observed. But, we just observed genotypes *MM* and *MN* in Holstein cattle and genotypes *MN* and *NN* in buffalo (Fig. 1, 2). The frequencies of *CAST* alleles and genotypes are presented in Table 1.

Table 2 shows the expected, observed and Average heterozygosities, and the HW equilibrium value of *CAST* gene in current study. Results showed a significant difference between the observed and expected heterozygosity frequencies of *CAST* gene in Holstein cattle herd that confirm the Hardy-Weinberg disequilibrium while in buffalo herd observed Hardy-Weinberg equilibrium.

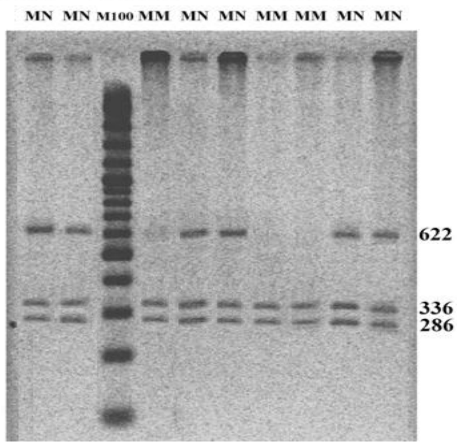

Figure 1. RFLP analysis of CAST gene on 2.5% agarose gel in Holstein cattle.

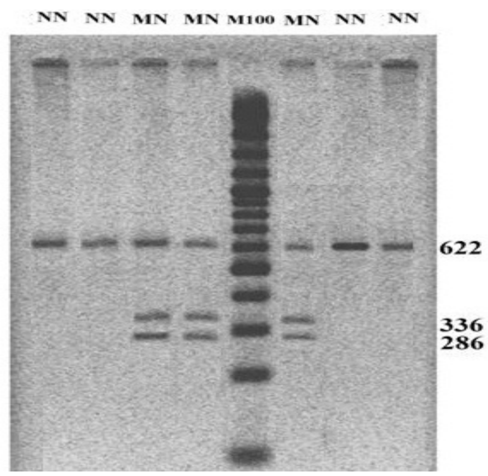

Figure 2. RFLP analysis of CAST gene on 2.5% agarose gel in Buffalo.

Table 1. Distribution of CAST alleles and genotypes frequency

Gene	Animal	Number	Allele Frequency (%)		Genotype Frequency (%)		
			M	N	MM	MN	NN
CAST	Holstein cattle	50	73	27	46	54	0
	Buffalo	40	22.5	77.5	0	45	55

Table 2: Expected (He), observed (Ho) and Average (Ave-He) Heterozygosities and HWE value obtained for *CAST*

Gene	Animal	He	Ho	Ave-He	HWE
CAST	Holstein cattle	0.4	0.54	0.39	6.54
	Buffalo	0.35	0.45	0.34	3.15*

*: P<0.05

The distribution of allele frequencies of *CAST* gene showed that the frequency of allele M was higher than allele N in Holstein cattle and buffalo. Similar results by Tahmoorespour *et al.,* [12] and Fakhr kazemi *et al.,* [13] reported in Sistani cattle that who observed allele M has higher frequency. In sheep, Eftekhar Shahroudi *et al.,* [14] detected two alleles M=0.79 and N=0.21 in Gharehgol sheep. Palmer *et al.,* [15]; Nassiri *et al.,* [16] and Nanekarani *et al.,* [17] also reported the same results in different sheep breeds. However, Mamaghani *et al.,* [18] detected three alleles A, B and C in Sarabi cows with frequencies 0.70, 0.26 and 0.04, respectively. Also, two single nucleotide polymorphisms (SNPs) have been identified in this gene, a G/C SNP in intron 5 [19] and an A/G SNP in the 3' UTR region [20, 21]. Several studies reported the association between this gene with meat tenderness [22, 23, 19].

In current study, the chi-square test revealed that this locus was not at Hardy–Weinberg equilibrium in the Holstein cattle while Buffalo herd showed equilibrium (P<0.05).

Also, because of very few studies on *CAST* polymorphism in cattle and especially in buffalo, the comparison of present results with other studies was not completely possible. This paper tried to give new and more information on polymorphism of *CAST* gene in buffalo and cattle, which eventually provide useful information for breeding program based on marker-assisted selection. The inconsistency of present results compare to other studies may be ascribed to breed differences, population size, sampling amount and environmental factors.

4. Conclusions

The goal of this study was to determine genetic polymorphism of calpastatin (*CAST*) in Holstein cattle and buffalo herds. These results revealed that polymorphism was detected in both species and showed that PCR-RFLP is an appropriate tool for detecting genetic polymorphism. This study also, opens interesting prospects for future selection programs, especially marker assisted selection.

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