Scientific Papers. Series D. Animal Science. Vol. LVI
ISSN 2285-5750; ISSN CD-ROM 2285-5769; ISSN-L 2285-5750

CALPASTATIN GENE POLYMORPHISM IN ÇINE ÇAPARI AND KARYA SHEEP

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Abstract

This study was carried out to determine Calpastatin gene polymorphism in native Çine Çapari and synthetic Karya sheep in Turkey. Calpastatin is an endogenous inhibitor of calpain. This gene has a key role on meat tenderness after slaughter, and also has been known as candidate gene in muscle growth efficiency. Calpastatin gene was located on 5th chromosome of sheep. Randomly taken blood samples were collected from 97 Çine Çapari and 90 Karya sheep raised in Western Anatolia. Intron I from L domain of the ovine calpastatin gene was amplified by PCR to produce a 565 bp fragment. Then, PCR products were digested with restriction endonuclease enzyme MspI. Digested products were separated by electrophoresis on agarose gel and visualized with gel documentation system. The digestion of the PCR products by MspI enzyme produced fragments of 306 and 259 bp. Data analysis was done using PopGen32 software. In Karya sheep population MM, MN and NN genotypes were identified with 0.296, 0.496 and 0.208 frequencies, M and N allele frequencies were identified with 0.544 and 0.456, respectively. In Çine Çapari sheep population MM, MN and NN genotypes were identified with 0.543, 0,388 and 0.069 frequencies, M and N allele frequencies were identified with 0.737 and 0.26, respectively.

Key words: *Sheep, Cine Capari, Karya, Calpastatin, PCR-RFLP.*

INTRODUCTION

Calpastatin (CAST) is an endogenous calpain-inhibitor protein (Takano et al., 1999). Page et al. (2002) demonstrated that calpain plays a leading role in the tenderness of meat by degrading myofibrillar proteins during the process of rigor mortis after slaughter. Many researchers working on the genetics of livestock raised for meat production investigated the calpastatin gene and its physiological role on meat tenderness (Boehm et al., 1998; Huff-Lonergan et al., 1996; Killefer and Koohmaraie, 1994; Lonergan et al., 1995). The differences between the levels of calpastatin was studied among various species (Koohmaraie et al., 1991), breeds (Shackelford et al., 1994; Shackelford, 1995), and muscles (Geesink and Koohmaraie, 1999).

Several studies have been conducted to identify the calpastatin gene polymorphisms in different animal species including mice (Hitomi et al., 2000), goats (Javanmard et al., 2010), swine (Choi et al., 2006), sheep (Palmer et al., 1998), and cattle (Juszczuk-Kubiak et al., 2004). The calpastatin gene, which plays a key role in regulating meat tenderness following slaughter, is also regarded as one of the potential gene affecting muscle development (Byun et al., 2008). The calpastatin gene is located on chromosome 5 in the sheep genome (Khederzadeh 2011; Palmer et al., 1998).

The first study to identify calpastatin gene in sheep genomes was conducted by Palmer et al. (1998). As a result of this PCR-RFLP based study, two distinct alleles of the calpastatin gene (M and N) were identified in Dorset sheep. Later on, the presence of these alleles was also confirmed in studies on different sheep breeds (Gábor et al., 2009; Gharahveysi et al., 2012; Khan et al., 2012; Khederzadeh, 2011; Mohammadi et al., 2008; Nanekarani et al., 2011a; Nanekarani et al., 2011b; Shahroudi et al., 2006; Suleman et al., 2012; Szkudlarek-Kowalczyk et al., 2011); moreover, certain single nucleotide polymorphisms (SNP) were identified as a result of DNA sequencing studies on calpastatin gene regions (Gregula-Kania, 2011). However, there are no reports of an attempt to identify this gene in Turkey's native sheep breeds.

This study was performed to identify calpastatin gene polymorphism in the native Cine Capari sheep breed, which is conserved as a genetic resource, and in Karya sheep, a synthetic genotype for which the breeding practices have become prevalent in Western Anatolia, at the DNA level by using PCR-RFLP.

MATERIALS AND METHODS

A total of 187 animals (97 Cine Capari sheep and 90 Karya sheep) were analyzed in the study. Blood samples of 4.5 ml were collected from the *vena jugularis* into anticoagulant K3 EDTA-containing vacuum tubes and DNA isolation was performed using a commercially available isolation kit (Invitrogen, Medsantek, Izmir). A PCR mixture containing PCR buffer (1X), $MgCl_2$ (2 mM), dNTP mixture (0.2 mM), forward and reverse primers (0.25 µM), *Taq* DNA polymerase (1U), genomic DNA (~100 ng), and sterile ddH_2O was prepared in a final volume of 25 µl. To amplify the suitable calpastatin gene region for a polymorphism analysis, the primer pair that was also used by Khederzadeh (2011) was synthesized. Forward and reverse primer sequences were;
CAST-F:CCTTGTCATCAGACTTCACC
CAST-R:ACTGAGCTTTTAAAGCCTCT,
respectively. The PCR conditions consisted of the following steps: 2 minutes of pre-denaturation at 95°C, followed by 35 cycles 1 minute of denaturation at 95°C, 1 minute of annealing at 65°C, 2 minute of extension at 72°C, and the last step that final extension was incubated for 10 minutes at 72°C. Amplified DNA regions were digested with *Msp*I restriction enzyme (Fermentas) for genotyping. For restriction digestion, 3 µl of 10X Buffer Tango, 1 µl of ddH_2O and 1 µl of *Msp*I (Fermantas) enzyme were added to the PCR products and this mix were incubated at 37°C for at least 6 hours. Digested PCR products were stained with SafeView Nucleic Acid Stain (NBS Biologicals), run in 2% agarose gel at 65 V for 2 hours, and visualized in a gel visualization system to determine the genotypes. Genotype and allele frequency analysis and Hardy-Weinberg equilibrium test were carried out using PopGene 32 software (Yeh et al., 1997).

RESULTS AND DISCUSSIONS

Resulting bands from PCR-RFLP were stained with SafeView, run in agarose gel electrophoresis, and visualized in a gel visualization system (Figure1). Following the digestion of the 565 bp PCR product, which was amplified using the primer pairs to discriminate between M and N alleles of the calpastatin gene, two bands with lengths of 306 bp and 259 bp were observed for the MM genotype, a single 565 bp band was observed for the NN genotype, and three bands with lengths of 259, 306 and 565 bp were observed for the MN genotype. Bands with different lengths were produced as a result of a single point mutation (CCGG à CCAG) in the calpastatin gene, which removes the MspI restriction cut site (...CCGG...) (Gregula-Kania and Monika, 2011).

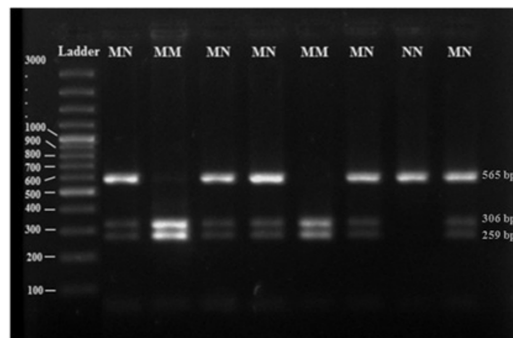

Figure 1. Gel picture of M and N alleles of the Calpastatin gene.

The allele and genotype frequencies are presented in Table 1. In the Karya samples, the allele frequencies were not significantly different, whereas the frequency of the M allele was significantly higher in Cine Capari sheep. This finding is in line with other studies (Gábor et al., 2009; Gharahveysi et al., 2012; Khan et al., 2012; Khederzadeh, 2011; Mohammadi et al., 2008; Nanekarani et al., 2011a; Nanekarani et al., 2011b; Nassiry et al., 2006; Suleman et al., 2012; Szkudlarek-Kowalczyk et al., 2011). When genotype frequencies are considered, the frequency of the NN genotype is significantly low in both sheep genotypes. In Karya sheep, the highest frequency was observed for the MM genotype, whereas the highest frequency was observed for the MN genotype in Cine Capari breed.

Table 1. The allele and genotype frequencies of the CAST gene in Karya and Cine Capari sheep.

		Allele Frequency		Genotype Frequency		
Breed/Genotype	**n**	***CAST* M**	***CAST* N**	**MM**	**MN**	**NN**
Karya	90	0.544	0.456	0.543	0.388	0.069
Cine Capari	97	0.737	0.263	0.296	0.496	0.208
Total	**187**	**0.644**	**0.356**	**0.415**	**0.458**	**0.127**

The observed and expected number of calpastatin genotypes in Karya and Cine Capari sheep and the result of the Hardy-Weinberg equilibrium tests with chi-square were showed in Table 2. Both genotypes were found to be in Hardy-Weinberg equilibrium.

Table 2. The observed and expected number of genotypes in Karya and Cine Capari sheep and the result of the Hardy-Weinberg equilibrium test

		Observed			Expected				
Breed	**n**	**MM**	**MN**	**NN**	**MM**	**MN**	**NN**	**2sd=1**	**P**
Karya	90	23	52	15	26.673	44.645	18.681	2.281	0.131
Cine Capari	97	51	41	5	52.702	37.594	6.704	0.713	0.399
Total	**187**	**74**	**93**	**20**	**77.661**	**85.702**	**23.656**	**1.273**	**0.259**

CONCLUSIONS

There are no previous studies to identify calpastatin gene polymorphism in Turkish sheep breeds. The aim of this study was to identify calpastatin gene polymorphisms in Cine Capari sheep, a native genetic resource facing danger of extinction, and in synthetic Karya sheep, which was obtained as a result of rotational crossbreeding practices of breeders in the Western Anatolian region, using the PCR-RFLP method. In conclusion, calpastatin gene polymorphims were identified in both of sheep studied in the present study. Two alleles, *CAST* M and *CAST* N, were determined in the calpastatin locus and the most frequent was the M allele. This study can be regarded as a reference study and it will be possible to perform future studies on other native sheep breeds of the country. Future studies to identify calpastatin polymorphisms in other native breeds and to determine the changes in development and meat quality with respect to calpastatin genotypes would be useful. Furthermore, this study detects a single nucleotide change only in the investigated genomic region. DNA sequence analysis of the calpastatin locus will prove if genomic differences exist in our breeds.

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