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Determination of Calpastatin Gene Polymorphism in Kıvırcık Crossbred Ewes by PCR-RFLP Method

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ABSTRACT

Calpastatin (CAST) is an endogenous and specific inhibitor of calpains found in meat. Because of this feature, CAST is a major gene that directly changes the toughness of meat. The objective of this study to determine the CAST gene polymorphisms in Kıvırcık crossbred ewes. Therefore, CAST gene polymorphisms were investigated in 100 Kıvırcık crossbred ewes grown in Thrace region. The PCR-RFLP method was used to determine the genetic variations of the CAST gene. In the current study, the estimated frequencies of three genotypes including MM, MN and NN at CAST/MspI polymorphism were 0.82, 0.16 and 0.02 and they were 0.90 and 0.10 for M and N alleles, respectively. There was no deviation from Hardy-Weinberg equilibrium (P>0.05) relative to CAST genotypes.

1. Introduction

In recent years, significant progress has been made in livestock breeding. Meat quality is an important economic trait for livestock breeding. However, water holding capacity, fat content and distribution, color, tenderness and texture one of the key features of meat quality (Glitsch 2000; Rosenvold and Andersen 2003). There are many genetic and environmental factors such as breeding, feeding, slaughtering processes and storage conditions have significant effect on meat quality. The discovery of the effects of these factors is an important scientific goal and is being studied extensively by scientists (Santos-Silva et al. 2002; Nuernberg et al. 2005; Koohmaraie and Geesink 2006; Ferguson and Warner 2008).

Meat quality is a quantitative trait which is controlled by many genes. The identification of effects of these genes is very important to improve meat quality traits. Thus, the recent developments in molecular genetics have made significant contributions to elucidate the gene and markers related to meat quality. In livestock animals, candidate gene identification and genomic sequence analysis are widely used in determining the loci related to meat quality. As a result of these analyzes, many of selected genes can be used in marker assisted selection for livestock breeding (Gao et al. 2007).

Toughness is an important feature that affects meat quality. Recently, low toughness or tenderness meat have preferred by consumers. Therefore, the level of meat tenderness is an important criteria for meat pricing in many countries. In this respect, meat tenderness is an important issue for meat industry (Koohmaraie 1996). There are many factors that influence meat tenderness such as breeding, genetic, protein composition of muscle fibers, before and after slaughtering conditions (Dinh 2006). However, genetic studies on genetic factors affecting the degree of toughness of meat have indicated that Calpastatin (CAST) gene is an important DNA marker for meat quality traits (Schenkel et al. 2006; Allais et al. 2011).

The calpaines play a key role in the process of meat tenderness with proteolytic activities in cold storage. CAST gene is located on the fifth chromosome of sheep and have significant effect on meat tenderness by inhibiting calpaines in post mortem process (Kawasaki and Kawashima 1996; Juszczuk-Kubiak et al. 2009; Khederzadeh 2011). Previous studies have also determined the relations of CAST gene and meat quality and carcass traits in livestock animals (Morris et al. 2006; Zhou and Hickford 2008; Pinto et al. 2010; Li et al. 2013). Morever, many studies reported the associations of CAST gene with average daily weight

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gain (Nassiry et al. 2006) live weight (Sutikno et al. 2011) birth weight (Byun et al. 2008) growth and carcass traits (Nikmard et al. 2012) in different sheep breed. Therefore, the aim of this study to investigate the genotype and allele frequencies of the CAST gene in Kıvırcık crossbred ewes by PCR-RFLP method.

2. Materials and Methods

Sample collection

A total of 100 Kıvırcık crossbred (Kıvırcık x Merino) ewes tissue samples were collected after slaughtering and stored at -20 °C in Genetics laboratory of Namık Kemal University Faculty of Veterinary Medicine. The PCR-RFLP method was used to determine for CAST gene polymorphisms. A 622 bp region of CAST gene was amplified using a pair of primers with the following nucleotide sequences: 5'-TGGGGCCCAATGACGCCATCGATG-3' and 5'-GGTGGAGCAGCACTTCTGATCACC-3' (Palmer et al. 1998).

Polymerase Chain Reaction and Enzyme Digestion

All PCR applications were performed with the Phire Tissue Direct Pcr Master Mix (ThermoFisher LSG-F170L) in accordance with the manufacturer's instructions. The PCR for CAST/MspI polymorphism was carried out in volumes of 50 µl using; 25 µl Phire Tissue Direct Pcr Master Mix, 0,3-0,5 mm tissue sample, 5 µM each primer, and the rest was ddH2O. The amplification was performed at 98 ºC for 5 sec, denaturation 98 ºC for 5 sec, annealing 63ºC for 5 sec, extension 72 ºC for 20 sec and a final extension of 72ºC for 1 min followed by 40 cycles. The PCR products were subjected to electrophoresis on 2 % agarose/ethidium bromide gel (Aga003R, Bioshop, Canada) in 1x TBE buffer (TBE-001, New Bioscience). Gels were visualized under UV light and documented in WGD30S Molecular Imager apparatus (Wisd). For CAST/MspI genotyping, 10 µl of PCR product were digested with 2 µl (20 U) of MspI (Fermentas - Kat.No: ER0541) restriction enzymes at 37°C for 5 hour. The restriction fragments were subjected to electrophoresis on 2 % agarose/ethidium bromide gel in 1× TBE buffer. Gels were visualized under UV light and documented in WGD30S Molecular Imager apparatus (Figure I).

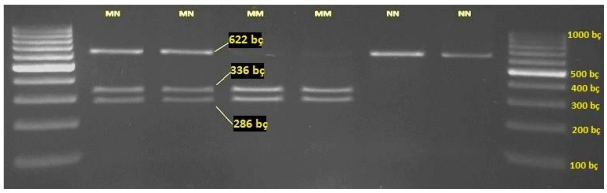
Statistical analysis

In this study, The Chi-square test whether genotype frequencies of CAST/MspI polymorphism were in Hardy Weinberg equilibrium estimated by PopGene Version 1.32 (Yeh *et al.* 1997).

3. Results and Discussion

In this study, polymorphisms of CAST/MspI gene in 100 Kıvırcık crossbred ewes grown in the Thrace region were investigated. The estimated frequencies of three genotypes including MM, MN and NN at CAST/MspI polymorphism were 0.82, 0.16 and 0.02 and they were 0.90 and 0.10 for M and N alleles, respectively. There was no deviation from Hardy-Weinberg equilibrium (P>0.05) relative to CAST genotypes. Table 1 shows the genotype and allele frequencies of CAST/MspI polymorphism in Kıvırcık crossbred ewes.

Figure 1 Gel image of the ovine CAST/MspI genotypes



In this study, M allele of CAST/MspI polymorphism was found a very higher frequency in Kıvırcık crossbred sheep population (0.90). This result is in agreement with the study reported by (Yılmaz et al. 2014) M (0.85) and (Avanus 2015) in M (0.70) in Kıvırcık sheep breed populations. However, there were many study about CAST/MspI polymorphism in different sheep breed populations. According to the literature, M allele frequency in the current study was higher than 0.63 in Lori sheep (Asadi et al. 2014), 0.34 in Sakız sheep (Yılmaz et al. 2014), 0.66 in Najdi sheep (Saleha and Alakilli 2015), (0.72) Sanjabi, (0.63) Afshari, (0.69) Ghezel, (0.48) Arkhamerino and (0.33) Mehraban sheep (Tohidi 2013), 0.76 in Polish Merino sheep (Szkudlarek-Kowalczyk et al. 2011), 0.74 in Bandur sheep (Sunilkumar et al. 2014), 0.50 in İvesi, 0.52 Güney Karaman and 0.52 Akkaraman sheep (Balcıoğlu et al. 2014), 0.73 in Karakul sheep (Avanus 2015). In contrast, M allele frequency was lower than 0.98 in Imroz sheep (Yılmaz et al. 2014), 0.92 in Berrichon du Cher and 0.95 Ile de France sheep (Szkudlarek-Kowalczyk et al. 2011), 0.99 in Gokceada sheep (Yılmaz et al. 2014), 0.92 in Shumen sheep (Georgieva et al. 2015). However, M allele frequency was similar with 0.87 in Harri sheep (Saleha 2015), 0.88 in Balkhi and 086 Kajli sheep (Riaz et al. 2012), 0.88 in Makui sheep (Tohidi 2013), 0.85 in Arabic Sheep (Mohammadi et al. 2008).

Table 1
The genotype and allele frequencies of CAST/MspI polymorphism in Kıvırcık crossbred ewes

CAST	N	Genotypes			Genotype Frequencies			Allel Frequencies		$(\chi^2)^1$
		MM	MN	NN	MM	MN	NN	M	N	1.39^{ns}
Observed	100	82	16	2	0.82	0.16	0.2	0.90	0.10	
Expected	100	81	18	1	0.81	0.18	0.01			

$^1\chi^2_{0.05;1;}$ 3.84 test of Hardy-Weingberg equlibrium, NS; not significant (P>0.05)

4. Conclusion

The present study provided basic information to understand the genetic diversity of Kıvırcık crossbred sheep in terms of CAST gene. The genetic improvement of economically important traits can be developed through marker assisted selection. CAST gene is playing pivotal role in in the process of meat tenderness. Moreover, this gene is well known marker for meat quality and carcass traits. In this study, CAST gene has found polymorphic in Kıvırcık crossbred ewes and provided valuable informations about sheep breeding. Taken together, it is very important to perform the further studies related to CAST gene polymorphism in different sheep and goat breed of Turkey for marker assisted selection programs.

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