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Determination of CAST/*Msp*I Polymorphism in Cattle by PCR-RFLP Method

Fatma İlhan[1*]

[1] Selcuk University, Faculty of Agriculture, Department of Animal Science, Konya, Türkiye

HIGHLIGHTS

- The calpastatin (CAST) gene is one of the genes associated with meat quality.
- In this study, the CAST/*Msp*I polymorphism was examined among cattle breeds.
- CAST/ *Msp*I polymorphism can be used as a molecular marker for meat quality

Abstract

The calpastatin (CAST) gene is one of the genes associated with meat quality. Studies have shown that this gene is associated with quality traits such as body weight gain, carcass yield, meat tenderness and fat content in meat. In this study, the polymorphism of the CAST gene was determined using the PCR-RFLP method in Holstein, Simmental and Brown Swiss cattle. As a result of genotyping, three genotypes (MM, MN and NN) and two alleles (M and N) were determined. It was found that the MM genotype and the M allele had the highest frequency in all breeds examined, while the NN genotype had the lowest frequency. The NN genotype was only found in the Brown Swiss breed. All Simmental animals used in the study belonged to the MM genotype.

Keywords: Calpastatin; cattle; PCR-RFLP; holstein; simmental; brown swiss

1. Introduction

Until a few years ago, most studies focused on meat yield. Today, meat quality has become an important criterion alongside meat yield. In the studies on meat quality, the taste, texture, nutritional value and shelf life of the meat are emphasized (Munekata et al. 2021). These characteristics can be improved through care and feeding, but the genotype of the animal can limit this improvement. Better meat quality can be obtained from animals with genotypically good meat quality. As a result of studies on meat quality, the calpastatin (CAST) gene has been identified as one of the genes associated with meat quality.

Calpastatin, first described in the 1960s, is a protease inhibitor and specifically inhibits calpain enzymes, calcium-dependent proteases that occur in cells (Guroff 1964; Kotova et al. 2023). Calpain enzymes are involved in many functions in cells, but their uncontrolled activity can cause cell stress and even apoptosis

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(programmed cell death). Therefore, calpastatin maintains the cellular balance by regulating calpain activity. In particular, calpastatin maintains the integrity of muscle fibers in muscle tissue. Increased calpain activity in muscle fibers can lead to the deterioration of muscle fibers and muscle breakdown (Bai et al. 2023; Huff-Lonergan et al. 1996). Therefore, calpastatin is important for the healthy function of muscle tissue. In particular recent studies have shown that the calpain system plays an important role in the initiation of proteolysis in the myofibril protein cycle; therefore, the calpain system acts as a regulator of muscle protein accumulation in domestic animals (Muroya et al. 2012). The CAST gene, which encodes calpastatin, is located on chromosome 7 in cattle and consists of 36 exons (Casas and Kehrli Jr 2016).

The CAST gene has been studied in more detail in sheep. Studies using RFLP (Bozhilova-Sakova and Dimitrova 2021; Kolosov et al. 2021; Ramadevi et al. 2020), SSCP (Esteves et al. 2020) and DNA sequencing (Machado et al. 2020; Muhana et al. 2021) techniques have shown that the CAST gene is associated with meat quality and growth. There are few studies on this gene region in cattle (Curi et al. 2010; Curi et al. 2008; Juszczuk-Kubiak et al. 2008; Wicińska and Szreder 2004). In studies conducted at the DNA level in cattle, the polymorphism of the CAST gene was found to be associated with meat tenderness (Curi et al. 2009), fat content (Schenkel et al. 2006), shear force (Chung and Davis 2012; Nattrass et al. 2014) in meat.

In this study, the polymorphisms of the CAST gene were determined in the Brown Swiss, Holstein and Simmental cattle breeds.

2. Materials and Methods

Blood samples from Brown Swiss (n=54), Holstein (n=50) and Simmental (n=52) cattle were used for this study. The blood samples were stored at -20 °C in the biotechnology laboratory of the Selcuk University Department of Animal Science. DNA isolation was performed according to the salting out procedure developed by Miller et al. (1988).

Genotyping of the obtained DNA samples was performed using the PCR-RFLP method. Primers with the sequences 5'-TGGGGGCCCAATGACGCCATCGATG-3' and 5'-GGGTGGAGCAGCAGCACTTCTGATCACC-3' were used for PCR (Juszczuk-Kubiak et al. 2008). For 10 µL PCR solution, 2 µL DNA (50-100 ng/µL), 0.25 µL (10 pmol/µL) of each primer, 5 µL Taq green PCR Master Mix and 2.5 µL ddH_2O were used. The PCR protocol was as fallows: initial denaturation at 94°C for 5 minutes, followed by 35 cycles of 94 °C for 45 seconds for denaturation, annealing at 60 °C for 45 seconds and an extension step at 72 °C for 1 minute. Finally, the PCR process was completed by holding at 72 °C for 10 minutes.

The obtained PCR products were then digested overnight at 37°C by adding *Msp*I enzymes, and the bands were detected on a 2% agarose gel. Allele and genotype frequencies were determined using the POPGEN 3.1 program.

3. Results and Discussion

In the study, exon 12 and 13 regions including intron 12 of the CAST gene (Juszczuk-Kubiak et al., 2008) were genotyped by PCR-RFLP method, using DNA obtained from Holstein, Simmental and Brown Swiss cattle breeds. The 622 bp region of the CAST gene was amplified and the *Msp*I enzyme was used to recognize the C^CGG sequence. As a result of the *Msp*I enzyme digestion, MM: 336, 286; MN: 622, 336, 286; NN: 622 bp, 3 genotypes were obtained (Figure 1).

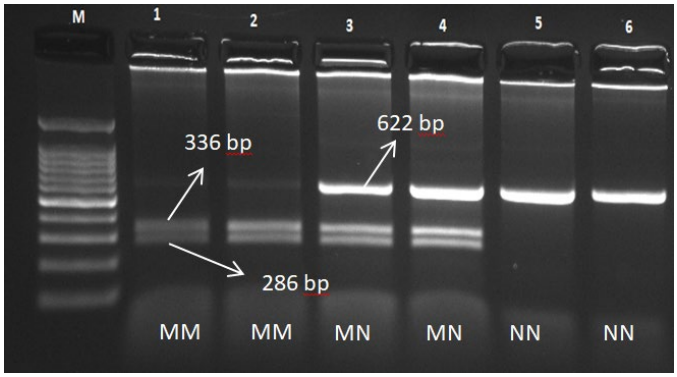

Figure 1. PCR-RFLP pattern of for CAST gene with *Msp*I enzyme Lane M: 100 bp DNA ladder marker.

Table 1. The genotype and allele frequencies obtained as a result of PCR-RFLP analysis, the expected and observed heterozygosity values, and the χ^2 values

Breeds		Genotype			He	Ho	Allele frequency		χ^2
		MM	MN	NN			M	N	
Brown Swiss	Frequency	0.80	0.13	0.07					12.3
	Obs.	43	7	4	0.24	0.13	0.86	0.14	p:0.00*
	Exp.	39.98	13.04	0.98					
Holstein	Frequency	0.76	0.24	0					0.84
	Obs.	38	12	0	0.21	0.24	0.88	0.12	p:0.36
	Exp.	38.67	10.67	0.67					
Simmental	Frequency	1	0	0					-
	Obs.	52	0	0	0	0	1	0	
	Exp.	-	-	-					

χ^2 =chi-square value; Obs.: observed frequencies; Exp.: expected frequencies He= expected heterozygosity; Ho= observed heterozygosity

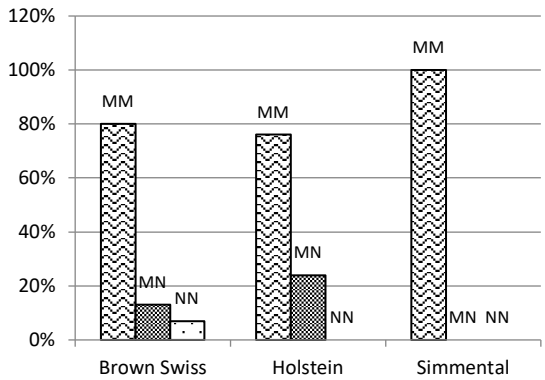

Figure 2. The genotype frequencies obtained as a result of PCR-RFLP analysis.

The genotype and allele frequencies obtained as a result of PCR-RFLP analysis, the expected and observed heterozygosity values, and the χ^2 values calculated to test whether the population is in Hardy-Weinberg equilibrium are shown in Table 1. The analysis of Figure 2 shows that the MM genotype and the M allele have the highest frequency in all breeds; the genotype with the lowest frequency is the NN genotype. Among the available breeds, the Simmental breed was found to be monomorphic for the CAST gene and all animals of this breed had the MM genotype. The NN genotype was only found in the Brown breed among these three breeds in this study. The observed heterozygosity value was higher in the Holstein breed (0.24) than in the Brown Swiss breed (0.13). When the chi-square results were analyzed, it was found that only the Brown Swiss breed was not in balance ($P < 0.05$) and the other breeds were in balance. These results are consistent with the literature. The CAST gene has been examined in more detail in sheep and as a result of the *Msp*I enzyme digest of the CAST gene, the NN genotype was not found or was found in very low frequency in the majority of the breeds examined (Asadi et al. 2014; Avanus 2015; Balcıoğlu et al. 2014; Bayraktar and Shoshin 2022; Gorlov et al. 2016; Ibrahim and Kali 2017; Saeed-ul-Hassan et al. 2012; Suleman et al. 2012). Similar results were obtained in several studies in cattle. In a study on Holstein cattle, the CAST gene was cut with the *Msp*1 enzyme and 44% MM and 56% MN genotypes were determined and no NN genotype was found(Yousefi and Mojtaba 2012). In another study on Sistani cattle, the proportion of CAST/*Msp*I MM, MN and NN genotypes was 62%, 29% and 9%, respectively(Tahmoorespur et al. 2007). For this gene, genotyping was performed in cattle using RFLP with various enzymes, in addition to the *Msp*I polymorphism, to determine the relationship of these genotypes with productivity. Studies conducted to determine the relationship between CAST/*Alu*I polymorphism and yield in cattle, it was found that the CAST gene was associated with traits such as live weight, daily live weight gain and fattening period (Ardicli et al. 2017; Ardıçlı et al. 2017).

4. Conclusions

In this investigation, the CAST/*Msp*I polymorphism was examined among Brown Swiss, Holstein, and Simmental cattle breeds. While both the Brown and Holstein breeds exhibited polymorphism in this gene, the Simmental breed was observed to be monomorphic. The CAST gene, known for its association with meat quality, emerges as a potential marker gene for marker-assisted selection studies targeting traits such as meat tenderness, fat content, shear force, as well as growth parameters including live weight and daily weight gain.

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