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Genotyping of Kappa-Casein Locus by PCR-RFLP in Brown Swiss Cattle Breed

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Abstract: A Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) test was performed on DNA samples extracted from blood samples of Brown Swiss cattle to detect genotype frequency of the bovine kappa-casein (CNS3) locus. A 351 bp fragment of CSN3 was amplified and digested with Hinf I restriction enzymes. Samples were loaded on agarose jel (3%) and genotyped under UV light. Three genotypes were observed, frequencies were 19.35, 20.43 and 60.22% for AA, BB and AB, respectively. Two genetic variants CSN3 A and CNS3 B were identified and the allelic frequencies were estimated as 0.495 and 0.505, respectively. It was concluded that the population was in Hardy-Weinberg equilibrum.

Key words: Kappa-casein, PCR-RFLP, genetic marker, polymorphism, brown swiss, cattle

INTRODUCTION

DNA polymorphic markers as candidate gene or quantitative trait loci allows the determination of indivudual genotypes at many loci and provides information on population parameters such as allele frequencies as well as improving selection by Marker-Assisted Selection (MAS). Drogemuller *et al.* (2001), suggested that MAS together with traditional selection methods can be most effective for complex traits, improving accuracy, reducing generation interval and accelerating genetic progress.

Candidate gene approaches provide tools for identifying and mapping genes affecting quantitative traits. A candidate gene can be defined as a gene with biological effects on the physiology of a trait of interest (functional) or as a gene closely linked to a functional gene (positional). Polymorphisms within selected candidate genes can be tested for their association with quantitative traits to better understand their effects and can be used in MAS programs (Wu *et al.*, 2005).

Exon IV of kappa-casein (CSN3) gene is very important as it contains most of the sequence coding for it's molecule. Though 9 variants of CSN3 have been described; A, B, C, E, F G, H, A (1) and J (Prinzenberg *et al.*, 1999), most diffused CSN3 alleles are A and B. Aallele A and B differ by 2 amino acid substitutions, Thr136/Ile and Asp148/Ala (Mercier *et al.*, 1973).

Milk protein genetic polymorphism has received considerable research interest in recent years because of possible associations between milk protein genotypes and economically important traits in dairy cattle (Kemenes *et al.*, 1999; Golijow *et al.*, 1999). Many research reports have indicated that certain milk protein variants may be associated with milk production (Ng-Kwai-Hang *et al.*, 1984; Bech and Kristiansen, 1990; Falaki *et al.*, 1997), milk composition (Lundén *et al.*, 1997; Robitaille *et al.*, 2002; Johnson *et al.*, 2007) and cheese production (Mclean and Schaar, 1989; Aleandri *et al.*, 1990; Lundén *et al.*, 1997; Comin *et al.*, 2008). Therefore, milk protein genes could be useful as genetic markers for additional selection criteria in dairy cattle breeding.

MATERIALS AND METHODS

Blood samples were collected from 93 Brown Swiss cattles reared at Research and Application Fram Collage of Agriculture, Ataturk University, in a 10 mL vacuum tube containing K_3EDTA, from the left jugular vein. The tubes were maintained at -20°C until used for DNA extraction. Genomic DNA was extracted from blood using the Purgane kit (Gentra Systems, Minnesota, USA) and stored at 4°C.

The kappa-casein specific primers (5′-ATT TAT GGC CAT TCC ACC AA-3′ and 5′-ATT AGC CCA TTT CGC CTT CT-3′) were used to amplify a 351 bp fragment in cattle. Amplification reactions were done in a final volume of 50 μL containing 300 ng DNA, 1 μM of each primer, 10X PCR buffer, 1.5 mM $MgCl_2$, 100 μM dNTPs and 0.5 U Taq DNA polymerase. The reactions were subjected to 94°C for 5 min (initial denaturation), 30 cycles of 94°C for 45 sec, 60°C for 45 sec and 72°C for 60 sec, followed by 72°C for 7 min.

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The PCR products were digested with Hinf I restriction endonuclase in a 20 μL of reaction mixture separately. The reaction mixture comprised of 15 μL PCR reaction, 2 μL of enzyme buffer, 5 U of restiriction enzyme and 2.8 μL of dH_2O. The digestion reaction were incubated at 37°C for overnight. After digestion, the digested products were resolved on 3% agarose gel at 45 V for 2.5 h. The gels were visualized under UV light after staining with ethidium bromide.

CSN3 allele frequencies were determined by gene counting. The chi-square (χ^2) test was used to check whether the population were in Hardy-Weinberg equilibrium or not (SPSS, 1996).

RESULTS AND DISCUSSION

A 351 bp CNS3 gene fragment was amplified in cattle using specific primers. After PCR amplification, enzymatic digestion with Hinf I and agarose jel electrophoresis the CNS3 3 restriction pattern were observed in Brown Swiss cattle. The enzymes cut the PCR product in 2 fragments of 261/89 bp for the BB genotype and in the 3 fragmants of 131/131/89 bp for the AA genotype. Heterozygotes AB are a combination of the two alleles A and B 4 fragments of 262/131/131/89 bp (Fig. 1).

The frequencies of A and B alleles were 0.495 and 0.505 and those of AA, BB and AB genotypes were 0.1935, 0.2043 and 0.6022, respectively (Table 1). The ratios between the observed genotype frequencies in our sample fit the Hardy-Weinberg equilibrium ($\chi^2 = 3.886$).

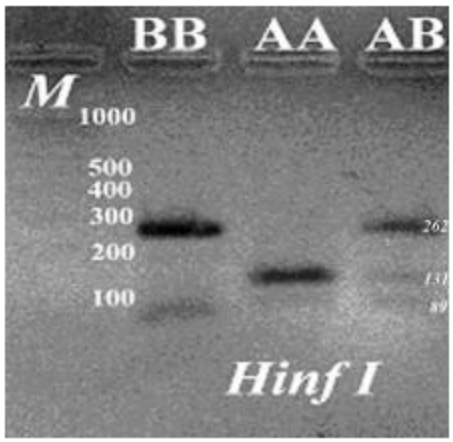

Fig. 1: Restriction pattrens of 351 bp CSN3 fragment after digesting with Hinf I

Table 1: Distribution of CSN3 genotypes and allele frequencies in Brown Swiss cattle

Genotype	Frequency	Allele (frequency)	χ^2
AA	0.1935	A (0.495)	3.886 ns
BB	0.2043	B (0.505)	
AB	0.6022		

ns: not significant

The frequency of B allele of CNS3 gene in different breeds ranges from 0.06-0.57 (Van Eenennaam and Medrano, 1991; Ron *et al*., 1994; Cowan *et al*., 1992; Golijow *et al*., 1999; Tsiaras *et al*., 2005; Sulimova *et al*., 2007). The highest frequency is observed in Brwon Swiss and Jersey with 0.67 and 0.86, respectively (Lein *et al*., 1999).

Allelic polymorphism at the CSN3 gene was originally studied at the protein level. At present, CSN3 alleles typing using DNA polymorphism analyses based on PCR-RFLP. The procedure of DNA typing animals may be used in agricultural practice for CNS3 allele genotyping of cattle in order to decrease spreading of alleles causing low quality or quantity of production in cattle population.

CONCLUSION

The results of PCR-RFLP analysis showed the 3 genotypes (AA, BB and AB) for CSN3. Using the PCR-RFLP technique based on molecular markers and allows direct genotyping for milk CSN3 with certainty and accuracy in bulls and females, we established an easy, low-cost and efficient method that can be use to determine the genotype of cattle.

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