IDENTIFICATION OF BOVINE KAPPA-CASEIN GENOTYPES IN TWO IRANIAN HOLSTEIN FARMS BY PCR-RFLP

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ABSTRACT
Milk protein polymorphisms have received considerable interest because of their potential use as an aid to genetic selection and genetic characterization of bovine breeds. In Holstein dairy cattle the B variant of Kappa-Casein (CSN3) is associated with a higher protein content, better quality of crude and increased yield of cheese. It had been suggested that identification of CSN3 genotypes could be an economically important selection critrion for dairy herds designed for industrial milk production. Identification of A and B alleles of K-Casein was performed by amplification of a 228 bp DNA fragment, located in exon IV, by means of PCR-RFLP method. Peripheral blood was sampled from 25 (Kenehbist farm) and 64 (Nemoneh farm) Holstein cattles. DNA was extracted and a 228 bp fragment of genomic DNA was amplified in PCR reactions by standard methods. The amplicon then was digested with *HinfI* or *HindIII* restriction endonucleases. Genotypic frequencies were 12.00% and 21.87% for AA, 88.00% and 56.25% for AB and 00.00% and 21.87% for BB in Kenehbist and Nemoneh farm rspectively. Frequencies of alleles A and B were 0.56 and 0.44 in Kenehbist farm and 0.50 and 0.50 in Nemoneh farm. The Nei's heterozygosity value of K-CN locus for Kenehbist farm was 49.28% and for Nemoneh farm was 50.00%. The Kenehbist farm was not in Harrdy-Weinberg equilibrium. Differences in the profile of allele and genotype frequencies of K-CN locus between farms studied were revealed.

KEYWORDS
Kappa-casein, genotyping, PCR, RFLP.

INTRODUCTION

In Holstein dairy cattle the B variant of kappa-casein (CSN3) is associated with a higher protein content, better quality of crud and increased yield of cheese. It has been suggested that identification of CSN3 genotypes could be an economically important selection criterion for dairy herds designated for industrial milk production (Pederson, J. 1991). Genotyping of milk proteins, such as CSN3, can be performed by electrophoresis, directly from milk samples, as the expression of the caseins occurs only during the lactation phase in mammary gland cells. Therefore, the use of electrophoresis for genotyping of milk proteins is strongly limited because it can only be used in cows in the lactation stage. With newly developed techniques based on DNA analysis, which include polymerase chain reaction and restriction fragment length polymorphisms (PCR-RFLP) methods, it is now possible to determine the CSN3 genotype of all individuals in a given population under selection, regardless of sex, age or physiological stage (Lara, M.A.C. et al. 2002).

MATERIALS AND METHODS

Samples were supplied from Kenehbist (25 samples) and Nemooneh (64 samples) farms, two iranian Holstein dairy herds located at mashhad. For each sample 2-5 ml of blood was collected in tubes containing 0.5-1 ml EDTA 10% as anticuagulant. Genomic DNA was exteracted from 100 microlitere of blood according to Boom et al.(198). A fragment of 228 bp from exone IV of CSN3 gene was amplified using 100 ng of genomic DNA in a PCR buffer solution containing 10 pmol of each primer (forward, 5'-TATCATTTATGGCCATTCCACCA-3'; reverse, 5'-CTTCTTTGATGTCTCCTTAGAGTT-3'), 2 mM of $MgCl_2$, 250 uM of each dNTPs and 2 U of *Taq* start antibody in a total volume of 25 ul. Samples were denatured at 94 ºC for 2 min. and then were subjected to 35 cycles of 94 ºC for 1 min., 56 ºC for 1 min. and 72 ºC for 1.5 min. with a final extension step of 72 ºC for 5 min. PCR products were electrophoresed on a 1.5% horisontal agarose gel containing 0.5 ug/ml of ethidium bromide and photographed under UV light. Five microliters of the PCR products was digested with 5 U each of *Hinf*I (recognition site, 5'-GANTC-3') and *Hind*III (recognition site, 5'-GGCC-3') in two separate reactions. samples were incubated at 37 ºC for 3h. The visualization of digestion products was in 8% nondenaturing polyacrylamide gel and staining with ethidium bromide solution. The frequency of genotypes in co-dominant locus was caculated by directly adding the genotype number. PopGen32 software (ver. 1.31) was used to estimating the alleles and genotypes frequencies.

RESULTS AND DISCUSSION

Identification of A and B alleles of CSN3 was performed by amplification of a DNA fragment of 228 bp, located in exone IV, by PCR-RFLP method (fig. 1). The DNA fragment amplified from allele A has no any restriction site for *Hind*III and remains undigested. Allele B was charachterized by the presence of two fragments, correponding to 135 and 95 bp. Genotypic frequencies were 22% and 12% for AA, 56% and 88% for AB and 22% and 0.0% for BB in Nemoneh and Kenehbist farms respectively. Frequencies of alleles A and B were 50% and 50% in Nemoneh and 56% and 44% in Kenehbist farm. The Nei's heterozygosity value of CSN3 locus for Nemoneh and Kenehbist farm was 0.50 and 0.49 respectively (Table 1). The Kenehbist farm was not in Harrdy-Weinberg equilibrium that may be due to indirect effects of selection for high milk production that affects on genotypes frequencies. another probable reason for this disequilibrium is use of A.I. sperms with specific genotype for CSN3 gene. Genotype AB has greatest value in two farms while genotype BB was not seen in the Kenehbist farm.

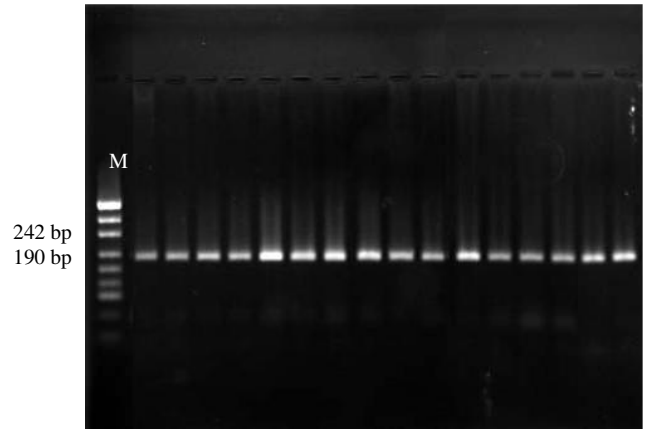

Figure 1.
PCR products from exone IV of CSN3 gene. M : pUC19 DNA/*Msp*I. The size of products is 228 bp.

Table 1. Gene and genotypic frequencies, Nei's heterozygosity and X^2 test for two farms.

Farm	No.	Frequencies					Nei's value	X^2
		A	B	AA	AB	BB		
Kenehbist	25	0.56	0.44	0.12	0.88	0.00	0.49	14.67^{*}
Nemooneh	64	0.50	0.50	0.22	0.56	0.22	0.50	0.87^{ns}

ns: Not-significance value ($P \leq 0.05$).
* : Significance value ($P \leq 0.05$).

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