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Full Length Research Paper

Kappa-casein gene polymorphism in Holstein and Iranian native cattle by polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP)

Abbas Doosti*, Asghr Arshi, Mahmood Vatankhah and Pouya Amjadi

Biotechnology Research Center, Islamic Azad University, Shahrekord Branch, Shahrekord, Iran.

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Caseins amount to nearly 80% of the protein output in cow milk. Caseins are biologically important proteins and they are also a raw material for the cheese making industry. The aim of this study was to identify kappa-casein genotype in Holstein and Iranian native cattle. DNA was extracted from 457 blood samples of 247 Holstein and 210 native cattle for identification and genotyping of kappa-casein gene by polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP) assay using *Hind*III and *Taq*I restriction endonucleases. The PCR product of the specific primer K-F and K-R gives the 379 bp specific band. Digestion of 379 bp fragment by restriction endonuclease *Hind*III generated two fragments of 156 and 223 bp. Result of the cut with this enzyme indicate there genotypes AA, AB and BB in the samples. Also, the amplified DNA (379 bp) from the samples remained undigested by *Taq*I restriction enzyme. These findings suggest that BB genotype could be a good factor for increase of fat and protein content of milk.

Key words: Polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP), kappa-casein gene, genotyping, Holstein, native cattle.

INTRODUCTION

Casein is a family of milk proteins that exists in several molecular forms and is the main protein present in the bovine milk (Alipanah et al., 2005). The bovine milk specific proteins include casein fractions: *α-s1*-casein (CSN1S1), *α-s2*-casein (CSN1S2), *β*-casein (CSN2) and κ –caseins (CSN3) as insoluble fractions, α-lactalbumin (LALBA) and β-lactogloulin (LGB) which are classified as soluble fractions (Galila and Darwish, 2008). Casein is made up of many components; the main ones are *α-s1*-casein, *α-s2*-casein, *β*-casein and κ-casein and they constitute about 78 to 82% of bovine milk proteins (Azevedo et al., 2008). Kappa-casein plays an important role in the formation, stabilization and aggregation of the casein micelles, thus altering the manufacturing properties and digestibility of milk (Jann et al., 2004). The casein proteins are encoded in a locus that comprises four casein genes: the evolutionary related calcium-sensitive casein encoding genes (*α-s1*, *α-s2* and *β*) and the functionally related κ-casein gene (Rijnkels et al., 1997). The casein genes are tightly linked and inherited as a cluster, so they have a potential value and can play an important role in marker-assisted selection for milk traits (Lien and Rogne, 1993). The gene is located on chromosome 6q31. The κ-casein gene comprises a 13 kb sequence divided into 5 exons (Alexander et al., 1988). Point mutations in exon IV of the bovine kappa-casein (CSN3) gene determine two allelic variants, A and B (Alipanah et al., 2007). The A and B variants differ in the amino acids 136 and 148. At position 136, threonine is replaced by isoleucine, while at position 148, aspartic acid is replaced by alanine, for A and B, respectively (Alexander et al., 1988). This variation associated with processing properties like cheese production technology (Alipanah et al., 2007) and physiological process such as cytotoxic and antibacterial effects, enhance the immunity (Hamza et al., 2010). The B allele was found to be associated with thermal resistance, shorter coagulation

*Corresponding author. E-mail: biotechshk@yahoo.com.Tel: +98-38-13361001. Fax: +98-38-13361001.

Abbreviations: κ-caseins, Kappa-casein; **RFLP,** restriction fragment length polymorphism.

time, better curdles and micelles of different sizes, which are preferable in cheese making (Azevedo et al., 2008). The cheese yield from cows with genotype BB is 10% higher when compared with *AA* cows (Azevedo et al., 2008). These variants were distinguished by polymerase chain reaction (PCR) and restriction fragment length polymorphism (PCR-RFLP) analysis in some areas of the world (Rachagani and Gupta, 2008).

Restriction fragment length polymorphism (RFLP) technology has been used extensive in similar studies and has several advantages, including being a relatively inexpensive method. However, RFLP methodology is often used in conjunction with another method to ensure maximum discrimination of alleles. Especially if funding is limited, researchers may use RFLP analysis for screening of the total sample set for polymorphism, after which, samples exhibiting different RFLP patterns are subjected to DNA sequencing analysis (Scheepers et al., 2010).

The aim of this study was to determine possible κ-casein gene polymorphism in Holstein and Iranian native cattle using PCR-RFLP technique.

MATERIALS AND METHODS

Samples collection and DNA extraction

Blood samples were collected from 224 Holstein and 210 native cattle into a vacutainer tube and stored in 10% 0.5 M EDTA-coated vacutainer tubes (BD Vacutainer Systems, Plymouth, UK). DNA extraction was performed using DNA isolation kit (Gentra Inc., Minneapolis, MN) according to manufacturer's instructions.

PCR-RFLP assay for kappa-casein genotypes

For detection of kappa-casein genotypes, a 379 bp DNA fragment was amplified by PCR, using primer K-F: 5´- CACGTCACCCAC ACCCACATTTATC- 3´ and K-R: 5´- TAATTAGCCCATTTCG CCTTCTCTGT- 3´. The PCR mixture contained 1X PCR buffer, 200 mM dNTPs, 1 U of Taq DNA polymerase and 0.2 µM each of sense and antisense primer, 2.5 µl of DNA, 1.5 µM $MgCl_2$ and sterilized distilled water to make a final volume of 25 µl. The PCR reaction included pre-denaturation for 5 min at 95°C followed by 30 cycles 94°C for 1 min, 62 °C for 1 min, 72 °C for 1 min and a final extension of 5 min at 72°C.

For genotyping, PCR product was digested with *Hind*III and *Taq*I which was used for the determination of kappa-casein A and B alleles. Gene fragments was subjected to digestion by restriction enzymes in a total volume of 20 µl (10 µl reaction solution, 2 µl enzyme buffers, 0.2 µl enzymes and 7.8 µl distilled water) and placed in the incubator at 37°C for 4 h. The restriction products were analyzed by electrophoresis on a 2% agarose gel.

Statistical analysis

The probability of Hardy-Weinberg equilibrium associated with the observed genotypic frequencies was obtained using the χ^2 test for each breed composition and the exact probability test. Allele frequency and their standard error were calculated as follows:

$$N_A = 2\times NAA + NAB \Rightarrow F_A = \frac{2NAA + 2NBB}{2N}$$

Also, standard error of mean allele frequencies in two populations using the following formula was calculated:

$$s.e = \sqrt{\frac{pq}{2n}}$$

To test deviation from Hardy-Weinberg equilibrium test of the two, x^2 and G^2 were used as follows:

$$x^2 = \frac{\sum (0-e)^2}{e} \qquad G^2 = -2(LnLo - LnL1)$$

RESULTS

The PCR product of the primer specific for kappa casein gene (K-F and K-R) gives the 379 bp DNA fragment. Digestion of 379 bp fragment of kappa-casein gene by *Hind*III restriction endonuclease generated two fragments at 156 and 223 bp sizes. The results were the 379 bp fragment of uncut PCR product representing homozygotes *A* allele, two fragments of 156 and 223 bp representing homozygotes B allele, and three fragments 379, 156 and 223 bp representing heterozygotes (A/B) for kappa casein gene. A representative Nested-PCR and RFLP pattern is depicted in Figure 1. On the other hand, the amplified DNA (379 bp) from blood samples remained undigested by *Taq*I restriction enzyme (Figure 2). The number and frequency of alleles in the two populations are shown in Table 1.

DISCUSSION

κ-Casein is of special interest as a milk protein polymorphism due to its known relationship with milk quality and composition (Scheepers et al., 2010). Caseins are milk proteins secreted by mammary gland cells (Azevedo et al., 2008). Kappa-casein constitutes approximately 12% of the casein and is a constituent of bovine milk (Azevedo et al., 2008). Normally, cow's milk contains 3 to 5% protein, of which 80% is casein and 20% is whey protein. These whey proteins and the caseins, a second major class of milk proteins, such as kappa-casein, are a source of minerals and amino acids for the young. These proteins play a crucial role in the coagulation and curdling of milk. This role in coagulation is also important to humans in which it is a required component in the production of cheese (Patel et al., 2007). The bovine casein genes (*αS1*, *αS2*, *β* and kappa) reside in a region of less than 200 kb on chromosome 6 and form a strong gene cluster (Shende et al., 2009). κ-Casein B allele was reported to have a favorable and significant effect on both milk and milk protein yield. Milk produced by BB genotype cows yielded significantly more cheese than that produced by AA-genotype cows (Patel et al., 2007).

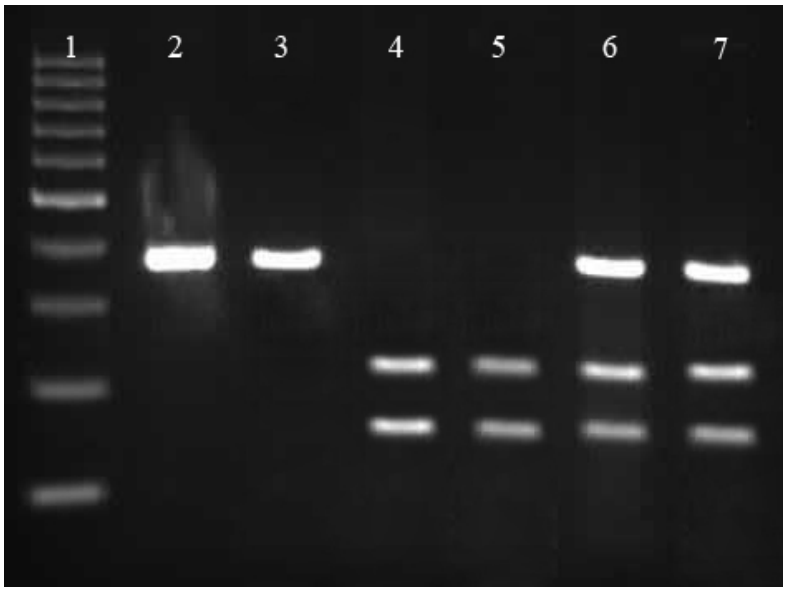

Figure 1. Analysis of amplified product of κ-CN gene on 2% agarose gel. Lane 1: 100 bp DNA ladder; Lanes 2 and 3: Not cutting products, AA genotype. Lanes 4 and 5: BB genotype; Lanes 6 and 7: AB genotype.

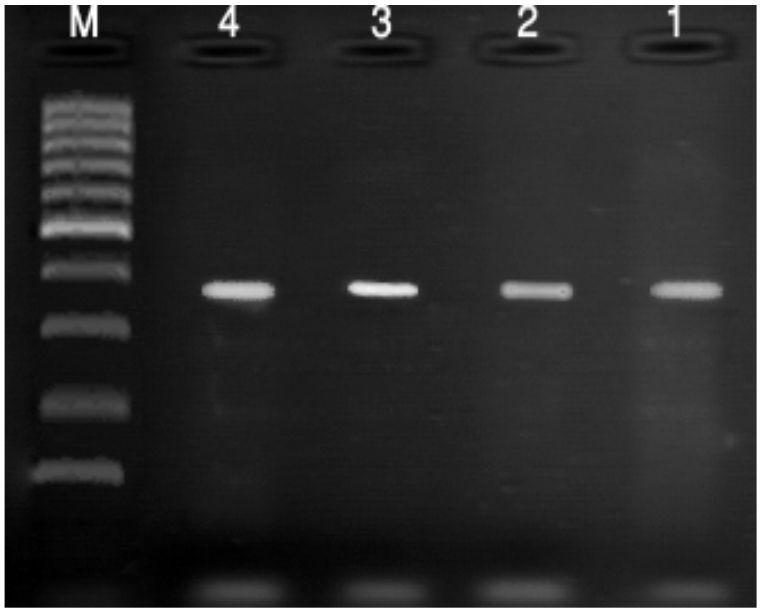

Figure 2. Undigested fragment by *Taq*I enzyme.

Due to differences in the interpretation between type and variant and a number of almost simultaneous publications of κ-casein variants, it was shown by Scheepers and et al. (2010) that inconsistencies in the nomenclature exist, and is caused by the assigning the same letter to different variants. Currently, two κ-casein types can be identified by iso-electric focusing (IEF), namely AIEF and BIEF, and all κ-casein variants or alleles can be categorized into these two groups (Scheepers et al., 2010).

Genotypic frequencies were 18 and 69% for AA, 82 and 26% for AB and 0.0 and 0.05% for BB in Holstein and Iranian native cattle, respectively. Frequencies of alleles A and B were 59 and 41% in Holstein and 81 and 19% in Iranian native cattle, respectively. The A allele was more frequent than the B allele. Cows of AB and BB genotypes showed a higher milk fat content when compared to the *AA* genotype (Botaro et al., 2009). Because of the effects of κ-casein genetic variants on cheese

Table 1. The number and frequency of alleles in two populations.

Population	Number of A	Number of B	Frequency of A (%)	Frequency of B (%)	S.e	Interval confidence of A	Interval confidence of B
Holstein	132	92	59	41	0.03	0.53 - 0.63	0.35 - 0.47
Native	171	39	81	19	0.03	0.75 - 0.87	0.13 - 0.25

yield, selection of animals with the favorable κ- casein B allele is considerable.

Future studies should concentrate on the association between cattle genotype and milk composition characteristics, in order for it to make a useful contribution in the upliftment of the rural communities. In addition, the fact that caseins are encoded by a tight 250 kb cluster of 4 genes in the order: CSN1S1, CSN2, CSN1S2 and CSN3, indicates that future research should concentrate on casein haplotypes and not on one casein gene alone (Scheepers et al., 2010). In conclusion, in Iran, milk producers have only recently started to pay attention on the manufacturing properties of milk, because some industries are beginning to differentially remunerate the milk according to its composition in terms of fat and protein percentage as well as cheese making properties. The wide variation found for κ-casein gene in the Iranian native cattle probably reflects the overall existing variation in milk constituents in these breeds. In addition, the increasing demand by the industry for milk constituents should greatly have impact on dairy cattle breeding strategies in Iran (Azevedo et al., 2008).

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