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Investigation of Kappa Casein Gene Polymorphism by PCR-RFLP in Najdi Cattle Breed Population in Khuzestan Province

Research Article

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ABSTRACT

More than 80% of the total milk protein contains caseins that are to forms α-s1, α-s2, β-casein and kappa casein. Kappa casein is smaller than other milk proteins but due to have a role in size and stability of micelles. The present study describes polymorphism of kappa casein gene. This is the first study of kappa casein gene polymorphism in Najdi cattle of Iran. We used the polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP) technique to screen DNA polymorphism in cattle. We amplified the 453 fragment consisting on part of exon 4. The amplified fragment was digested with HinfI restriction endonuclease and subjected to electrophoretic separation in 3% agarose gel. Two alleles were observed, A and B. It gives frequencies of 0.54 and 0.46 for A and B alleles. Also, genotypes AA, AB and BB with frequencies of 35%, 37.5% and 27.5% of the total study population were diagnosed. Existence of Hardy-Weinberg equilibrium indicated that there was not any choice in order to increase or decrease the frequency of genotypes in the kappa-casein population in this herd. Also, in this population kappa-casein gene has medium level of heterozygosity.

KEY WORDS kappa casein gene, Najdi cattle, PCR-RFLP, polymorphism.

INTRODUCTION

The genetic diversity of the world's livestock populations is decreasing, both within and across breeds. A wide variety of factors has contributed to the loss, replacement or genetic dilution of many local breeds. Genetic variability within the more common commercial breeds has been greatly decreased by selectively intense breeding programmes. Conservation of livestock genetic variability is thus important, especially when considering possible future changes in production environments (Boettcher *et al.* 2010). DNA polymorphic markers as candidate gene or quantitative trait loci allows the determination of individual genotypes at many loci and provides information on population parameters such as alleles frequencies as well as improving selection by marker assisted selection (MAS). Dromuller *et al.* (2001) suggested that MAS can be effective for complex trait, improving accuracy, reducing generation interval and accelerating genetic progress. Candidate gene approaches provide tools for identifying and mapping genes affecting quantitative traits. Polymorphisms within selected candidate genes can be tested for their association with quantitative trait to better understand their effects and can be used in MAS programs (Wu *et al.* 2005). The association of genetic polymorphism with milk production and composition has stimulated interest in using genetic polymorphism of casein genes in molecular marker assisted selection (MAS) to improve milk performance traits in farm animals (Kumar *et al.* 2006). Caseins are milk proteins secreted by mammary gland cells. They constitute about 78-82% of bovine milk proteins and are subdivided into four main fractions: α-s1-casein (CSN1S1), α-s2-casein (CSN1S2), β-casein

(CSN2) and κ-caseins (CSN3) as insoluble fractions and α-lactalbumin (LALBA) and β-lactogloulin (LGB) which are classified as soluble fractions (Galila and Darwish, 2008; Shende *et al.* 2009). Kappa-casein (κ-Cn) constitutes approximately 12% of the casein in bovine milk. This protein 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). These proteins and their genetic variants have been extensively studied and reported as an important factor associated with lactation performance, milk composition and cheese yield efficiency (Konovalova *et al.* 2004). 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). These proteins include casein, located on chromosome 6 within a 200-kb fragment in the order α-S1, β, α-S2 and κ. The α-S1-, β-, and α-S2-casein genes are the most closely linked and form an evolutionarily related family, whereas the κ-casein gene is at least 70 kb away from them (Ferretti *et al.* 1990). The kappa-casein gene is located on chromosome 6q31. The overall length of the κ-Cn gene is close to 13 kb, but most of the coding sequences for the mature κ-Cn protein are contained in the fourth exon. κ-Cn is considerably different from other caseins in structure and other properties. The κ-casein variants A and B differ in amino acid 136 and 148. In position 136, Thr (ACC) is changed for Ile (ATC) and in position 148 Asp (GTA) is changed for Ala (GCT) (Tinaev, 2003). Until now, researchers have reported 11 genetic variants (A, B, C, F1, F2, G1, G2, H, I and J) in cattle for κ-casein gene (Farrell *et al.* 2004). 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 *et al.* 2002). κ-Cn is likely QTL candidate that affects an economically important trait in dairy animal including milk yield and composition (Komisarek *et al.* 2006). The aim of the present investigation was to study polymorphism in κ-Cn gene of Najdi cattle.

MATERIALS AND METHODS

In this study, blood samples were randomly collected from 80 Najdi cattle in the Khuzestan province including 29, 18, 17 and 16 heads from Najdi cattle Research Center, Shoshtar; Shadegan, Mahshar and Dasht-e-Azadegan cities, respectively (in Southwestern of Iran). From each animal, about 5 cc of blood was collected from the jugular vein with vaccum tubes coated with ethylenediaminetetraacetic acid (EDTA) and transported to the Central Laboratory of the University× of Khuzestan Ramin Agriculture and Natural Resources and stored at 4 ˚C until DNA extruction. Genomic DNA was isolated by using DNA extraction kit DIAtom DNA Prep 100. Spectrophotometer was used investigating quality and quantity.

Samples show an optical density (OD) ratio (260 nm/280 nm) ranging from 1.6 to 1.8. The PCR amplification of specific fragment DNA included SNP in 453 bp region of fourth exon (Alexander *et al.* 1988). The sequence using PCR primers was designed by Azevodo *et al.* (2008). Forward primer was:

5'-TGTGCTGAGTAGGTATCCTAGTTATGG-3'

and reverse primer was:

5'-GCGTTGTCTTCTTTGATGTCTCCTTAG-3.

The PCR reaction volume of 25 µL contained approximately 33.3 ng of genomic DNA, 1.25 m*M* Taq DNA polymerase, 2.5 µL of 1X PCR buffer, 1.5 m*M* $MgCl_2$, 0.2 m*M* dNTP and 10 pM of each primer. Amplification conditions included an initial denaturation at 95 ˚C for 5 min, followed by 35 cycles at 95 ˚C for 30 s, 58 ˚C for 1 min and 72 ˚C for 1 min, followed by a final extension at 72 ˚C for 10 min. The PCR products were separated by 1% (w/v) agaros gel electrophoresis. The PCR products of 453 bp were digested with the HinfI restriction enzyme (Fermentas). 15 µL of PCR production with 1 µL buffer, 5 U (0.5) of HinfI, 13.5 µL H_2O up to a total volume of 30 µL, following the manufacturer's instruction for 20 min at 37 ˚C in a water bath. The digestion products were electrophoresed on 3% agarose gel in 1X TBE and visualized by for 1 h at 90 V. The banding was visualized and a documentation system was used. Gen Alex 6 / 3 software were applied to estimate the gene and genotype frequencies, the heterozygosity and effective number of alleles. Expected theoretical heterozygosity from Hardy-Weinberg assumption was calculated.

RESULTS AND DISCUSSION

The use of PCR-RFLP to detect new mutation is only feasible if such mutation creates or destroys a restriction target for the enzyme used. All samples were evaluated with PCR-restriction fragment length polymorphism (RFLP) technique using HinfI restriction enzymes. In this study, two alleles were observed, A and B. It gave frequencies of 0.54 and 0.46 for A and B alleles, respectively. For the 453 bp PCR kappa casein fragment, restriction sites were not found in the AA genotype but in the BB genotype two restriction sites as 326 and 100 bp fragments were found. Among the 80 Najdi cattle's examined four restriction sites as 426, 326, 100 and 27 bp (heterozygous type) were found in the AB genotype (Figure 1).

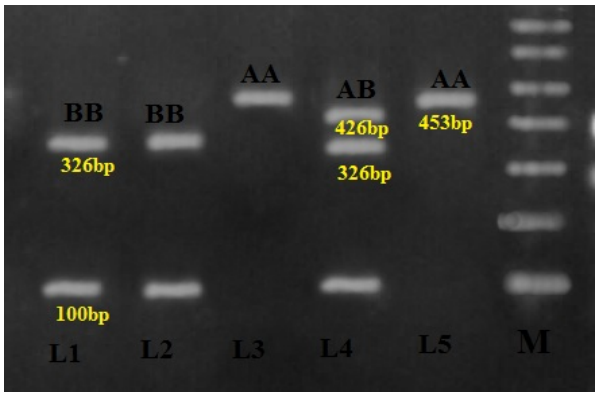

Figure 1 Kappa casein genotyping by PCR-RFLP method. M: DNA marker; Lanes 1 and 2: BB genotype; Lane 3 and 5: AA genotype undigested amplification product; Lanes 4: AB genotype (27 bp has not seen on the gel)

It should be noted that 27 bp fragments was not found due to have small size and it was removed from the gel. The frequencies of genotypes were 0.375, 0.35 and 0.275 for AB, AA and BB, respectively. Chi square χ^2 test was used to evaluate Hardy-Weinberg equilibrium (HWE). Deviations between observed genotypic frequencies and those expected under Hardy-Weinberg equilibrium were not significant. It suggested that the Najdi cattle population sampled is in equilibrium for the K-Cn locus (χ^2=4.832). Average genetic diversity in Najdi cattle breed population in Khuzestan province was observed. Effective allele and true allele are estimated 1.989 and 2.000, respectively. Our results show that kappa casein gene had approximately similar value for frequency mutant B and A allele in the population of Najdi cattle. Genotype and gene frequencies of four regions κ-Cn gene were average in this population. Our findings are the first report of kappa casein gene polymorphism in Najdi cattle and the results are presented in Table 1.

Table 1 Genotype and gene frequencies of the HinfI-RFLP in different regions cattle

Region	Genotype ferquencies			Gen ferquencies	
	AA	AB	BB	A	B
Shoshtar	0.24	0.35	0.41	0.414	0.586
Shadegan	0.39	0.45	0.16	0.611	0.389
Mahshar	0.35	0.41	0.24	0.559	0.441
Dasht-e-azadegan	0.50	0.31	0.19	0.656	0.344

Each four population follows Hardy-Weinberg equilibrium. Average heterozygosity for four regions was estimated. The results indicated that average heterozygosity of Mahshar was the highest and Dasht-e-azadegan was the least. The obtained results indicated that BB homozygote frequency was low. The kappa casein allele frequencies estimated for HinfI in the present study were approximately similar to the Brka *et al.* (2010).

CONCLUSION

The findings presented in this study indicate that the 453 bp kappa casein gene fragment is polymorphic in this population. Although nine alleles could be detected for the κ-Cn gene, we analyzed only the most common alleles (A and B). The above conclusion should be confirmed in future investigation, taking into consideration all possible genotype at different loci and using other restriction enzymes for recognizing the variants.

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