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Full Length Research Paper

Allele and genotype frequencies of β-lactoglobulin gene in Iranian Najdi cattle and buffalo populations using PCR-RFLP

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In modern programmes of animal breeding, the polymorphism of the milk proteins can be used as marker systems. β-Lactoglobulin is the major milk whey protein in the ruminants. Studies have indicated that this protein is polymorphic in the many breeds of cattle. This is the result of a single base pair substitution in the β-lactoglobulin gene that also give rise to the *Hae* III restriction fragment length polymorphism. The aim of this work was to analyze the genotype distribution of β-lactoglobulin in Iranian Najdi cattle and buffalo. Blood samples were supplied from 80 Najdi cattle and 80 buffalo from different cities of Khouzestan province. Polymerase chain reaction was applied to amplification of a 247 bp fragment of exon and intron IV of bovine β-lactoglobulin gene. The *Hae* III enzyme was used for restriction of the PCR products. The digested products were separated by electrophoresis on 2.5% agarose gel. The allele B of β-Lactoglobulin occurred at a higher frequency than the allele A in both Najdi cattle and buffalo. The genotype frequencies of AA, AB, and BB in Najdi cattle and buffalo were 0, 0.175, 0.825 and 0.04, 0.3, 0.66 respectively. Frequencies of A and B alleles were 0.0875 and 0.9125, and 0.1875 and 0.8125 in Najdi cattle and buffalo, respectively. The deviation from Hardy-Weinberg equilibrium was not detected in any of those animals. Detection of heterozygosity indicator evidence on low level of heterozygosity and genetic variability in both populations.

Key words: Polymorphism, β-lactoglobulin, PCR-RFLP, Najdi cattle, Buffalo.

INTRODUCTION

Genetic polymorphism of milk proteins has evoked considerable research interest in recent years because of possible associations between milk protein genotypes and economically important traits in dairy cattle. Therefore, milk protein genes could be useful as genetic markers for additional selection criteria in dairy cattle breeding. β-Lactoglobulin is amphiphatic and an extremely acid stable protein which exists at the normal pH of bovine milk as a dimer with a molecular weight of 36,000 Daltons. It is a single chain polypeptide of 18 kDa comprising of 162 amino acid residues. The complete amino acid sequence of β-lactoglobulin has been reported and genetic variation in amino acids sequence has been identified (Creamer et al., 1983). The biological functions of this protein are still not known. It could have a role in metabolism of phosphate in the mammary gland and the transport of retinol and fatty acids in the gut (Hill 1997).

The β-lactoglobulin gene is situated on bovine chromosome 11 and encodes the main protein of whey. β-lactoglobulin (LGB) loci affect the milk production parameters and quality of milk protein. Their polymorphisms explain a part of the genetic variance and improve the estimation of breeding value. Such loci can be taken into account as a suitable supplement to conventional breeding procedures (Pribyl, 1995). Polymorphism of this gene was discovered in 1955 and a total of 15 alleles are known (Matejicek et al., 2007). Common alleles are A, B, C and D, with alleles A and B being the most frequent. The bovine β-lactoglobulin A variant differs from B variant by two amino acids only, aspartate-64 and valine-118. These amino acids are substituted by glycine and alanine respectively in the B variant (Rachagani et al., 2006). The LGB locus affects mainly milk composition and milk quality and especially B

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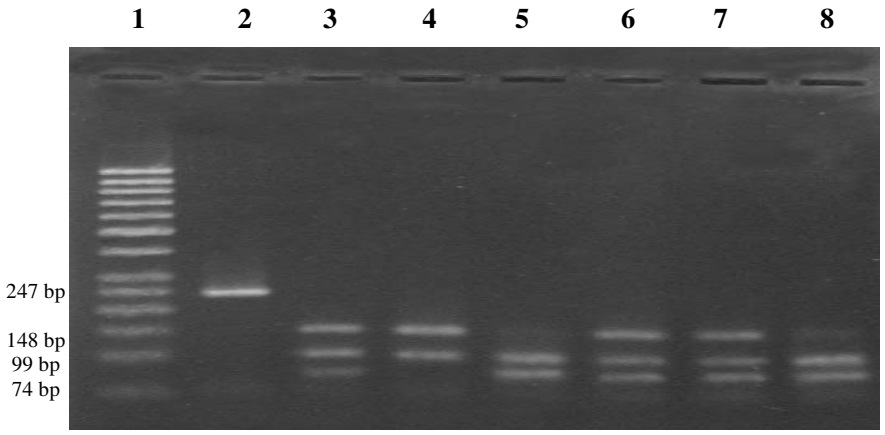

Figure 1. Agarose (2.5%) electrophoresis patterns of 247 bp PCR products of β-lactoglobulin gene digested with *Hae* III endonuclease. Lane 1: 50 bp DNA marker; lane 2: PCR product; lanes 5 and 8: BB genotype; lanes 3, 6 and 7: AB genotype; and lane 4: AA genotype. Banding pattern of lanes 3, 4 and 5 were observed in buffalo and banding pattern of lanes 6, 7 and 8 belong to Najdi cattle.

allele was recognized as superior for milk quality in European cattle breeds whereas allele A is associated rather with yield parameters (Strzalkowska et al., 2002).

The buffalo is considered to be a better converter of fibrous feeds into milk, and to be more resistant to diseases and local climatic conditions. In addition, very little work has been carried out on buffalo genetics that could serve as an additional tool in effective implementation of breeding programmes. Najdi cattle (*Bos indicus*) are native of Khouzestan province in southern of Iran and are considered for milk production. Breeding and genetic improvement in this animal is essential in order to have properties like resistance to diseases and adaptation to tropical climate.

MATERIALS AND METHODS

Blood samples were collected in vacutainers containing sodium EDTA as an anticoagulant from 80 Najdi cattle and 80 buffalo from various cities of Khouzestan province. The tubes were maintained at -20°C until used for DNA extraction. Genomic DNA was extracted from 100 µL blood sample according to Boom et al. (1990) method that modified by Shaikhayev (1995). The Gel monitoring and the spectrophotometeric methods were used for determination of the DNA quality and quantity. The sequences of primers used for amplification of exon IV of β-lactoglobulin gene containing polymorphic sites for A and B alleles were: 5´TGTGCTGGACACCGACTACAAAAAG-3´ (forward) and 5´-GCTCCCGGTATATGACCACCCTCT-3´ (reverse). Amplification reactions were done in a final volume of 25 µL, containing 100 ng DNA, 0.5 µM of each primer, 1X PCR buffer, 1.5 mM $MgCl_2$, 0.2 mM dNTPs and 1U Taq polymerase. Thermal cycling conditions included: an initial denaturation step at 95°C for 5 min followed by 30 cycles of 94°C for 45 s, 60°C for 1 min, 72°C for 1 min and a final extension at 72°C for 5 min. PCR products were recognized by electrophoresis on 1.2% agarose gel stained with ethidium bromide. The restriction digestion of the PCR products was carried out with *Hae* III enzyme. The PCR products were subjected to digestion by restriction enzymes in a total volume of 25 µl. The reaction was set up with 6.5 µl of ddH_20, 2.5 µl of restriction endonuclease buffer, 10 units of *Hae* III and 15 µl of PCR product, and incubated at 37°C for about 12 h. The digested product was loaded and visualized on 2.5% agarose gel after staining with ethidium bromide. The allele and genotype frequencies were estimated by direct counting. The heterozygosities (as gene variation indicates) were calculated using the POPGENE software version 1.31 (Yeh et al., 1999), according to Nei procedure (1978).

RESULTS

Electrophoretic analysis of isolated DNA using 0.8% agarose gel followed by observation on a UV Transilluminator revealed sharp high molecular weight bands of DNA that indicate that the DNA was of good quality and suitable for PCR-RFLP analysis. The visual estimation revealed that the concentration of DNA was about 100 ng/µl. The optical density (OD) readings obtained at 260 nm and 280 nm by using a UV spectrophotometer were used for estimating the quality and quantity of isolated DNA. The ratio of the reading at 260 nm and 280 nm ranged from 1.5 to 2.0, which indicates good quality DNA and no contamination. The restriction digestion analysis of the 247 bp PCR product of β-lactoglobulin gene indicated the presence of three types of restriction pattern; two fragments of 148 and 99 bp (AA-genotype), two fragments of 99 and 74 bp (BB-genotype) and three fragments of 148, 99 and 74 bp (AB-genotype) were observed. Each of three types of restriction pattern were found in buffalo, but in the case of Najdi cattle, restriction pattern of 148 and 99 bp (AA-genotype) was not found (Figure 1). The genotypic frequencies of AA, AB and BB were 0.04, 0.3 and 0.66 in buffalo and zero, 0.175, and 0.825 in Najdi cattle, respectively. Gene frequencies of A and B alleles were 0.1875 and 0.8125 in buffalo, and

Table 1. Gene and genotype frequencies of β-lactoglobulin gene determined by PCR-RFLP in buffalo and Najdi cattle.

Animal	No. of animals	Chi-square test	Gene frequency		Genotype frequency		
			A	B	AA	AB	BB
Buffalo	80	0.04 $^{n.s}$	0.1875	0.8125	0.04	0.3	0.66
Najdi cattle	80	0.68 $^{n.s}$	0.0875	0.9125	0	0.175	0.825

n.s: Not significant.

0.0875 and 0.9125 in Najdi cattle, respectively (Table 1). The frequency of an allele was found to be lower than that of the B allele in both groups of animals studied. Expected heterozygosity value (H_{exp} = 0.16) in Iranian Najdi cattle was slightly lower than observed heterozygosity (H_{obs} = 0.175). In Iranian buffalo, expected heterozygosity value (H_{exp} = 0.31) was similar to the observed heterozygosity (H_{obs} = 0.3). χ^2 test showed no significant difference between expected and observed genotype frequencies was detected. Therefore, the population sample was in Hardy-Weinberg equilibrium.

DISSCUSION

The frequency of an allele was found to be lower than that of the B allele in both groups of animals studied, and in close agreement to the results of earlier workers in *Bos taurus* (Kucerova et al., 2006; Matejicek et al., 2007) and *Bos indicus* (Kemenes et al., 1999; Patel et al., 2007). The genotyping results of Najdi cattle are similar to those reported in Gyr, Nelore, Sindi (Del lama and Zago 1996), Sahiwal and Tharparkar cattle (Rachagani et al., 2006). These results further confirm that *B. indicus* cattle are predominantly of β-lactoglobulin B type as compared to *B. taurus* cattle. However, in other studies, all of three genotypes are observed, but we did not find AA genotype in Najdi cattle. Our observations in Iranian buffalo are similar to the findings of Patel et al. (2007) who noted two alleles A and B in Murrah, Surti, Jaffarabadi and Pandharpuri breeds of buffaloes. In all of these breeds, the genotype frequencies of AB were the highest while in Iranian buffalo the BB genotype were more frequent. Because of paucity of literature on β-lactoglobulin in buffalo, it is difficult to compare with other observations; however, our observations are similar to those in cattle, where all three genotypes and two alleles have been reported. A heterozygosity of less than 0.5 indicated low variation for this gene in the studied populations. It is suggested that strategies such as migration, introduction of new diversity and crossbreeding for increasing gene diversity and its conservation besides exploration of this potential genetic diversity should be adapted.

Most of the reports that are available concern cattle and indicate that milk produced by β-lactoglobulin AA-genotype cows has been found to contain more lactoglobulin, less casein and less fat than that obtained from BB-genotype cows and this shows that milk pro-duced by BB-genotype cows yielded significantly more cheese than that by AA-genotype cows (Lunden et al., 1997; Van der Berg et al., 1992). Other studies indicated that the β-lactoglobulin genotype AB is associated with the highest milk and protein production (Tsiaras et al., 2005; Sabour et al., 1996). Therefore, these genotypes appear to be obvious candidates for selection aiming at improving milk production traits. Although the allele frequency of B is high in both the animals studied, the A allele (favorable allele) frequency is not too high. There-fore, it is suggested that crossbreeding should be done between these populations and/or with exotic breeds to increase the frequency of the favorable allele.

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