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Full Length Research Paper

Exploring β-Lactoglobulin Gene Diversity: A Comparative Study Between Holstein and Indigenous Iranian Cattle

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β-Lactoglobulin (β-LG) is the major protein in whey, making up about 50 to 60% of the total protein. A biological task for β-LG has been the subject of much speculation, but no specific function has been proven. About eleven protein variants of β-LG are known, of which variants A and B are common in most cattle breeds. The purpose of this study was to identify genotypes of the *β-LG* gene in Holstein and Iranian native cattle using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) technique. Blood samples were obtained from 278 Holstein and 210 Iranian native cattle. The primers β-LG F and β-LG R amplified a 247 bp fragment. The *Hae*III enzyme was used for restriction of the polymerase chain reaction (PCR) products. The frequency of allele *A* and *B* in Holstein and native cattle were 0.53, 0.47 and 0.23, 0.77, respectively. According to previous studies *AA* genotypes of β-LG gene is an important factor in increasing the milk production and the results of this study showed that high frequency of *AA* genotypes in Holstein and Iranian native cattle and could be use in creating the next generation for increasing the milk production.

Key words: Polymorphism, *β-lactoglobulin* gene, polymerase chain reaction-restriction fragment length polymorphism, Holstein, native cattle.

INTRODUCTION

β-Lactoglobulin (β-LG) is the major whey protein in the milk of ruminants, horses, pigs, cats, dogs, dolphins, whales, and kangaroos but not in the milk of humans, rodents, and lagomorphs (Perez et al., 1995). β-LG 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 (Karimi et al., 2009).

The quantitative effects of the variants on milk composition and cheese-making properties have been functions of this protein are still not known. It could have a role in metabolism of phosphate in the mammary gland reported frequently (Lum et al., 1997). The biological and the transport of retinol and fatty acids in the gut (Hill et al., 1997). The *β-LG* gene is situated on bovine chromosome 11 and encodes the main protein of whey. *β-LG* loci affect the milk production parameters and quality of milk protein. Their polymorphisms partly explain the genetic variance and improve the estimation of breeding value. Such loci can be taken into account as suitable supplements to conventional breeding procedures (Karimi et al., 2009). Polymorphism of this gene was discovered in 1955 and more than ten alleles are known so far (Matejicek et al., 2007). Two most common allelic variants of β-lactoglobulin (β-LG) are *β-LG A* and *β-LG B* (Lum et al., 1997). The point mutations in exon IV of the bovine *β-LG* gene determine two allelic variants A and B.

The bovine *β-LG A* variant differs from the B variant by two amino acids only, for example aspartate-64 and valine-118. These amino acids are substituted by glycine and alanine, respectively in the B variant (Rachagani et al., 2006). Heterozygous cattle have significantly more allelic production of *β-LG A* than of *β-LG B* (Elmaci et al., 2006). The *β-LG* locus affects mainly milk composition and milk quality and the *B* allele has been especially

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Abbreviations: *β-LG*, Beta-lactoglobulin; **RFLP,** restriction fragment length polymorphism.

recognized as superior for milk quality in cattle breeds, whereas allele *A* is associated rather with yield parameters (Strzalkowska et al., 2002). The aim of this study was to determine the frequencies of alleles and genotypes of *β-LG* gene in Holstein and Iranian native cattle using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method (Table 1).

MATERIALS AND METHODS

Samples collection and DNA extraction

528 blood samples were collected in vacutainers containing sodium EDTA as an anticoagulant from 278 Holstein and 250 native cattle. The tubes were maintained at -20°C until used for DNA extraction. Genomic DNA was extracted from 100 µl blood samples. The gel monitoring was used for determination of the DNA quality and quantity.

β-lactoglobulin gene amplification and genotyping analysis

Genotyping of *β-LG* was performed using PCR-RFLP. The 247 bp *β-LG* promoter fragment was PCR amplified from genomic DNA. The sequences of primers used for amplification of exon IV of *β-LG* gene containing polymorphic sites for *A* and *B* alleles were: *β-LG* F: 5´TGTGCTGGACACCGACTACAAAAAG-3´ and *β-LG* R: 5´ GCTCCCGGTATATGACCACCCTCT- 3´. Amplification reactions were done in a final volume of 25 µl, containing 100 µl DNA, 0.2 µM of each primer, 1X PCR buffer, 1.5 mM $MgCl_2$, 200 mM dNTPs and 1U of *Taq* polymerase. Thermal cycling conditions included: an initial denaturation step at 95°C for 5 min followed by 30 cycles of 94°C for 1 min, 60°C for 1 min, 72°C for 1 min and a final extension for 5 min at 72°C. PCR products were recognized by electrophoresis on 2% agarose gel. 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 20 µl (10 µl PCR product, 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 digested products were analyzed on 2% agarose gel.

Statistical analysis

The genotype and allele frequencies of the *β-LG* locus were estimated by direct counting. The χ^2 test was performed on the basis of the Hardy–Weinberg law for determining genetic equilibrium.

$$X^2 = \frac{\sum (0-E)^2}{E} \qquad G^2 = -2(L_N LO - L_N L1)$$

RESULTS

Digestion of 247 bp fragment of *β-LG* gene by *Hae*III restriction endonuclease generated two types of restriction pattern: two fragments at 99, 74 bp sizes (*BB*-genotype) and three fragments 148, 99 and 74 bp (*AB*-genotype). The restriction pattern that includes two fragments of 148, 99 bp was *AA*-genotype. In the analyzed popula-tions of cattle, the *BB* genotype was most frequent. Frequencies of *AA*, *BB* and *AB* genotypes were 0.280, 0.220, 0.498 and 0.052, 0.592, 0.354 in Holstein and native cattle, respectively. The frequencies of the *A* and *B* alleles were 0.53, 0.47 in Holstein and 0.23, 0.77 in native cattle, respectively. A representative PCR-RFLP pattern is depicted in Figure 1. Genotype frequencies were in accordance with the Hardy-Weinberg equilibrium (P-value >0.001).

DISCUSSION

β-Lactoglobulin, a globular protein of 162 amino acid residues, is the most abundant whey protein in bovine milk (Ariyaratnea et al., 2001). A large part of the variation in amount of *β-LG* protein is associated with *β-LG* protein variants (Ganai et al., 2008). Certain genetic variants of *β-LG* are correlated with the presence of a high percentage of milk fat. So far, 11 genetic variants which encode different forms of the *β-LG* protein have been discovered, thus influencing the quality of the milk: A, B, C, D, E, F, G, H, I, J and W. Out of these, the A and B forms are of the highest interest since they are associated with milk production performances. The *BB* homozygote individuals supply milk rich in fat and protein, very valuable in the process of cheese making, while the *AA* homozygote ones supply milk with a low percentage of fat (Balcan et al., 2007). Variant *A* has a higher *β-LG* protein concentration than variant *B* (Hill et al., 1997). It is likely that this difference in amount of *β-LG* protein is not caused by the amino acid substitutions, but rather by different levels of expression of the corresponding *A* and *B* alleles of the *β-LG* gene (Ganai et al., 2008). Variants *A* and *B* differ at two sites. Asp64 in *A* is changed to Gly in *B*, and Val118 in *A* is changed to Ala in *B* (Ariyaratnea et al., 2001). Milk from cows with the *β-LG BB* genotype had a greater fat percentage, while the milk yield and protein percentages of the *β-LG AB* genotype cows were higher, but this difference was not significant (Karimi et al., 2009).

The study of Kumar et al. (2009) on *β-LG* gene in Indian goats showed that the amplified product was of 426 bp and the restriction digestion with *Sac*II revealed three genotypes, namely S_1S_1, S_1S_2 and S_2S_2 at the *β-LG* locus (Kumar et al., 2006). Mele et al. (2007) on dairy ewes showed a significant relationship among hetero-zygous *β-LG* and TFA milk content that was more than 20% higher compared to the two homozygote *β-LG*. Patel et al. (2007) in India were showed that *B*-allele frequency (0.72) of beta-lactoglobulin was much higher compared to *A*-allele (0.27) in crossbred dairy bulls.

Karimi et al. (2009) showed that allele frequency of *β-LG* with regard to the *B*-allele (0.9125) was higher than that of the *A*-allele (0.0875). Frequencies of *AB* and *BB* genotypes were 0.175 and 0.825, respectively, while the

Table 1. The frequency of alleles in Holstein and Iranian native cattle.

Population	Frequency *A* (%)	Frequency *B* (%)	S.e	Interval confidence *A*	Interval confidence *B*
Holstein	0.53	0.47	0.03	0.75 - 0.87	0.13 - 0.25
Native	0.23	0.77	0.03	0.53 - 0.65	0.35 - 0.47

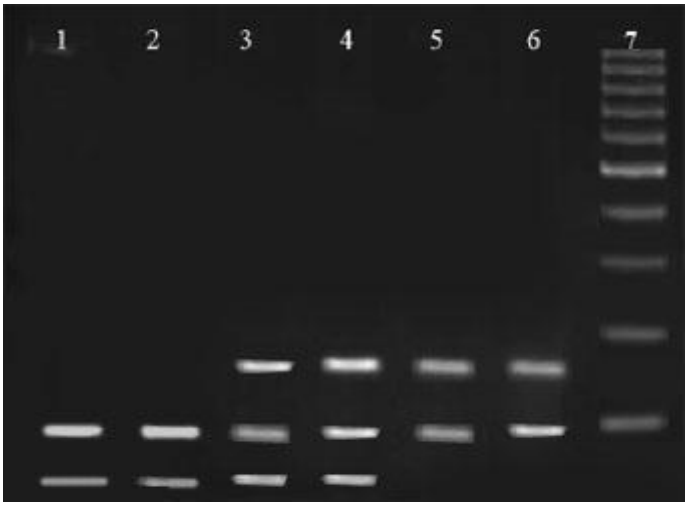

Figure 1. Gel electrophoresis of PCR product after digestion with *Hae*III restriction enzyme. Line 1 and 2: *BB* genotype, line 3 and 4: *AB* genotype, Line 5 and 6: *AA* genotype, and line 7: 100 bp DNA marker (Fermentas, Germany).

AA genotype was found to be absent (Karimi et al., 2009).

To test differences between observed and expected frequencies, $\chi 2$ analysis was performed on the basis of the Hardy-Weinberg law (Recio et al., 1997). In conclusion, these genotypes appear to be obvious candidates for selection aiming at improving milk production traits. Although the allele frequency of *B* is high in native population, the *A* allele (favorable allele) frequency is not too high. It is suggested that cross-breeding should be done between these populations or with exotic cattle to increase the frequency of the favorable allele.

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