

# Polymorphism of the $\beta$ -lactoglobulin gene and its association with milk production traits in Iranian Najdi cattle

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## Abstract

The objective of this study was to determine the polymorphism of bovine  $\beta$ -lactoglobulin ( $\beta$ -LG) gene and its association with milk production traits in Iranian Najdi cattle. Blood samples were collected from 80 Najdi cattle from the Najdi cattle breeding station located in Shoshtar, Khuzestan. Polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP) techniques were used for identification of various genotypes. The results indicated that allele frequency of  $\beta$ -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 AA genotype was found to be absent. Genotype frequencies were in accordance with the Hardy-Weinberg equilibrium. The effect of  $\beta$ -LG genotypes on milk production traits were analysed using a general linear model (GLM). There was no significant association between different genotypes of  $\beta$ -LG and milk production traits of the analysed cows.

**Key words:** Polymorphism;  $\beta$ -lactoglobulin; Milk production traits; Najdi cattle

## INTRODUCTION

The use of polymorphic genes as detectable molecular markers is a promising alternative to the current methods of trait selection once these genes are proven to be

associated with traits of interest in animals. Selection efficiency, however, depends on allelic frequencies in the breeds and on the effect of these polymorphisms on selected traits (e.g. dairy traits and technological properties of milk). Among specific genes that may affect economically important traits in cattle, the  $\beta$ -lactoglobulin ( $\beta$ -LG) locus has been extensively studied (Tsiaras *et al.*, 2005).  $\beta$ -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. The complete amino acid sequence of  $\beta$ -LG has been reported and genetic variation in amino acids sequence has been identified (Rachagani *et al.*, 2006; 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 (Formaggioni *et al.*, 1999; Hill, 1993).

The  $\beta$ -LG gene is situated on bovine chromosome 11 and encodes the main protein of whey.  $\beta$ -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. Polymorphism of this gene was discovered in 1955 and a total of 15 alleles are known so far (Matejcek *et al.*, 2007). Common alleles are A, B, C and D, with alleles A and B being the most frequent. The point

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mutations in exon IV of the bovine  $\beta$ -LG gene determine two allelic variants A and B. The bovine  $\beta$ -LG A variant differs from the B variant by two amino acids only, i.e., aspartate-64 and valine-118. These amino acids are substituted by glycine and alanine, respectively in the B variant (Rachagani *et al.*, 2006).

Effects of milk protein polymorphisms on milk production traits have been investigated during the past decades and, in some cases, results are still conflicting (Kucerova *et al.*, 2006; Prinzenberg *et al.*, 2003; Bovenhuis *et al.*, 1992; Aleandri *et al.*, 1990). Results of studies on the effect of  $\beta$ -LG genotypes on milk production traits have been rather more consistent. The AA genotype of  $\beta$ -LG has been shown to have a favorable effect on protein yield (Lunden *et al.*, 1997; Sabour *et al.*, 1996), whereas positive effects of the BB genotype on fat content have also been reported (Tsiaras *et al.*, 2005; Ron *et al.*, 1994; Hill, 1993). The  $\beta$ -LG locus affects mainly milk composition and milk quality and the B allele has been especially recognized as superior for milk quality in European cattle breeds, whereas allele A is associated rather with yield parameters (Strzalkowska *et al.*, 2002). However, significant association between production traits and different genotypes of milk protein has not been reported in other researches (Curi *et al.*, 2005; Ng-Kwai-Hang, 1998). Often alkaline and acidic polyacrylamide gel electrophoresis and PCR-based markers, especially PCR-RFLP, have been used for determination of different genotypes in such researches.

The Najdi breed of cattle (*Bos indicus*) represent native and low productive dairy cattle that are mainly bred in Khuzestan located in southern Iran, Resistance to diseases, adaptation to tropical climate and their role in the economy of rural families are important reasons for consideration of breeding and genetic improvement of this animal. The aim of this study was to identify genotypes of the  $\beta$ -LG gene and its effects on milk production traits in the Najdi cattle.

## MATERIALS AND METHODS

Blood samples were collected from 80 Najdi cattle at the Najdi cattle breeding station located in Shoshtar, Khuzestan. DNA was extracted from whole blood using the guanidin thiocyanate-silica gel method, as described by Boom *et al.* (1989). The gel monitoring and the spectrophotometric methods were used for

determination of DNA quality and quantity. The primers used for amplification of a 247 bp fragment of the  $\beta$ -LG gene were those described by Strzalkowska *et al.* (2002), with the following nucleotide sequence: 5'-TGTGCTGGACACCGACTACAAAAA-3' (forward); 5'-GCTCCCGGTATATGACCACCTCT-3' (reverse).

Amplification reactions were carried out in a final volume of 25  $\mu$ l, containing 100 ng of DNA, 0.5  $\mu$ M of each primer, 1X PCR buffer, 1.5 mM  $MgCl_2$ , 0.2 mM dNTPs and 1U of *Taq* DNA polymerase. The following cycles were applied: initial denaturation step at 95°C for 5 min followed by 30 cycles : denaturation at 94°C for 45 sec, primer annealing at 60°C for 60 sec, PCR product synthesis at 72°C for 60 sec and final synthesis step at 72°C for 5 min. PCR products were recognized by electrophoresis on 1.2% agarose gel (0.48 g agarose was dissolved in 40 ml TBE 1X buffer), stained with ethidium bromide. The restriction digestion of the PCR products was carried out with the *Hae*III enzyme. The digested products were loaded and visualized on 2.5% agarose gel (1 g agarose was dissolved in 40 ml TBE 1X buffer) agarose gel after staining with ethidium bromide. The allele and genotype frequencies were estimated by direct counting.

Records of milk yield (kg/day), fat and protein percentages were collected from the Najdi cattle station. Milk samples were analyzed monthly for fat and protein percentages. The time frame of all data collections is from 1997 to 2007. Detailed information was available for some reproductive events and was used to determine the traits of reproductive performance for each cow. Statistical analysis was carried out by means of the programme package SAS (SAS Inst., Inc., Cary, NC., U.S.A). The effect of the observed  $\beta$ -lactoglobulin genotypes on milk production traits were analysed using a general linear model (GLM). The following statistical model was used:

$$Y_{ijklm} = \bar{i} + G_i + L_j + S_k + Y_l + e_{ijklm}$$

Where:

$Y_{ijklm}$  = records of analysed traits (milk yield, fat and protein percentages).

$\mu$  = overall mean of population

$G_i$  = fixed effect of  $\beta$ -lactoglobulin genotypes ( $i = 1, 2$ ).

$L_j$  = fixed effect of lactation number ( $j = 1, 2, \dots, 5$ ; lactation numbers  $>5$  were pooled with lactation numbers of 5).

$S_k$  = fixed effect of calving season ( $k = 1, 2$  for hot

**Table 1.** Gene and genotype frequencies of the  $\beta$ -LG gene as determined by PCR and RFLP in Najdi cattle.

No. of animals	Chi-square ( $\chi^2$ ) test (Testing at the 0.05 level of significance)	Gene frequency		Genotype frequency		
		A	B	AA	AB	BB
80	0.68 n.s	0.0875	0.9125	0	0.175	0.825

n.s: not significant

**Table 2.** The means of the analysed traits for observed  $\beta$ -LG genotypes.

Analysed traits	Genotypes	
	BB	AB
Milk yield (kg/day)	2.58	2.75
Fat percentage (%)	4.02	3.57
Protein percentage (%)	3.58	3.71

and cool seasons, respectively).

$Y_l$  = fixed effect of calving year ( $l = 1, 2, \dots, 10$  for 1997, 1998, ....., 2006, respectively).

$e_{ijklm}$  = random residual error.

## RESULTS

The restriction digestion analysis of the 247 bp PCR product of the  $\beta$ -lactoglobulin gene indicated the presence of two types of restriction pattern; two fragments of 99, 74 bp (BB-genotype) and three fragments of 148, 99, 74 bp (AB-genotype), were observed. The restriction pattern that includes two fragments of 148, 99 bp (AA-genotype) was not found. In the analysed population of the Najdi cattle, the BB genotype was most frequent. The genotype frequencies of AA, AB and BB were 0, 0.175, and 0.825, respectively. Gene frequencies of the A and B alleles were 0.0875 and 0.9125, respectively. Expected frequency of the AA genotype was less than 5, therefore AA and AB genotypes were placed in the same category. Then chi-square ( $\chi^2$ ) test was performed on the basis of the Yates' correction (Stansfield, 2004). Genotype frequencies were in accordance with the Hardy-Weinberg equilibrium (Table 1). The above results reveal that there was polymorphism in the  $\beta$ -LG gene, but that the

B allele frequency was very much higher than the A allele thus indicating that genetic variability in this breed is low.

Milk from cows with the  $\beta$ -LG BB genotype had a greater fat percentage, while the milk yield and protein percentages of the  $\beta$ -LG AB genotype cows were higher, but this difference was not significant. There was no significant association between different genotypes of  $\beta$ -LG and milk production traits of the analysed cows. The means of the analysed traits for the observed  $\beta$ -LG genotypes are shown in Table 2.

## DISCUSSION

The frequency of the A allele was found to be lower than that of the B allele in Najdi cattle. The frequencies of  $\beta$ -LG alleles obtained in this study are similar to those reported for Gyr, Nelore, Sindi (Del Lama and Zago, 1996), Sahiwal and Tharparkar breeds (Rachagani *et al.*, 2006). These results mainly show that the frequency of A allele in the native breeds of cattle is lower than the European breeds of cattle. This study did not confirm the association between  $\beta$ -LG genotypes with milk production traits in Najdi cattle. In other studies of the  $\beta$ -LG genotypic effects on milk yield, several authors have reported no significant associations (Ghasemzadeh *et al.*, 2007; Kucerova *et al.*, 2006; Lunden *et al.*, 1997; Ojala *et al.*, 1997; Van Eenennaam and Medrano, 1991). However, reports exist where the  $\beta$ -LG genotypes AA (Kaminiski *et al.*, 2002; Ikonen *et al.*, 1999) or AB (Tsiaras *et al.*, 2005) have been positively associated with milk yield. Several authors have reported significant effects of  $\beta$ -LG on the milk fat percentage and in all of these studies, the  $\beta$ -LG BB genotype was associated with higher

milk fat content (Bobe *et al.*, 1999; Van der Berg *et al.*, 1992). This inconsistency is also found in sheep studies. According to Dario *et al.* (2005) the breed differences, population size, frequency distributions of genetic variants, the structure of data analysed, the model used for statistical analysis and a failure to consider the relationship among animals are possible reasons for this inconsistency. Population size and structure of data analysed may further affect our findings. Hence it is essential that we improve methods of the standard recording of data. The limited number of animals and restricted methods applied so far did not help us to draw firm conclusions. Hence it should be verified in future studies with the more balanced populations of dairy cattle.

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