Short Communication

Association of genetic variants of β-lactoglobulin gene with milk production in a herd and a superior family of Holstein cattle

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Abstract
Polymorphism of the β-lactoglobulin (*β-LG*) gene in 101 cows belonging to the Holstein herd and a superior cow, producing more than 150 Kg milk/day, together with four offsprings was investigated by the Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) method. In the Holstein herd, the alleles A and B of the *β-LG* gene had frequencies of 0.53 and 0.47, respectively. The genotypes AA, AB and BB of the *β-LG* gene were estimated to have frequencies of 0.257, 0.544 and 0.198, respectively. Genotypes were distributed according to the Hardy-Weinberg equilibrium. Results indicated that the *β-LG* genotypes significantly affected (P< 0.01) milk yield (genotype AA being more effective than genotype BB). The superior cow and her progenies were all heterozygotes (AB).
Key words: *β-LG*; Holstein; Milk production; Polymorphism; PCR-RFLP

β-lactoglobulin (*β-LG*) is the major whey protein of ruminant species and is also present in the milk of many, but not all mammalian species (Kontopidis *et al*., 2004). *β-LG* is located on chromosome 11 in the cow (Daniela *et al*., 2008) and has 12 known variants in which A and B variants are the most frequent ones (Rachagani *et al*., 2006). The *β-LG* binding affinity for retinol (Frapin *et al*., 1993) and fatty acids (Fugate and Song, 1980) has been demonstrated, which suggests a possible role of *β-LG* in the transport and metabolism of these components (Lum *et al*., 1997; Eigel *et al*., 1984). There are many contradictory reports on the association between genetic variants of *β-LG* and milk production and composition (Tsiaras *et al*., 2005; Aleandri *et al*., 1990; Ng-Kawi-Hang *et al*., 1986; Ng-Kawi-Hang *et al*., 1984). Several researches have shown that the BB genotype of *β-LG* is associated with higher fat and increased cheese yields (Wedholm *et al*., 2006; Aleandri *et el*., 1990; Ng-Kawi-Hang *et al*., 1986). An association of *β-LG* BB with higher protein yield has also been reported (Ng-Kawi-Hang *et al*., 1984). On the contrary, Bovenhuis *et al*. (1992) reported lower protein yield for this genotype. In various studies, the AA genotype of the *β-LG* gene has been associated with higher milk production (Ikonen *et al*., 1999; Bovenhuis *et al*., 1992; Ng-Kwai-Hang *et al*., 1990) and higher milk protein (Strazalkowska *et al*., 2002; Ng-Kwai-Hang *et al*., 1990; Ng-Kwai-Hang *et al*., 1984). In other reports, the AB genotype has been associated with higher milk production (Tsiaras *et al*., 2005; Kaygisiz and Douan, 1999), lactose and protein yield (Tsiaras *et al*., 2005).

The current study was carried out from July 2008 to

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November 2008 in the Molecular Genetics laboratory at the Department of Animal Sciences at Gorgan University of Agricultural Sciences and Natural Resources. The aim was to investigate *β-LG* polymorphism and its association with milk yield in a herd of Holstein cows. The blood samples were collected randomly from 101 Holstein cows (Behin Talise farm in Gorgan, Iran) having at least one lactation record. In the meantime, blood samples of a superior cow (producing more than 150 Kg milk/day) and her four female progenies from a farm near the region of the investigated herd were also collected. DNA was extracted, using the salting out extraction protocol (Miller *et al*., 1988). The 247 bp fragment, comprising a part of the IV exon and intron of the genomic DNA was amplified by using primers as suggested by Strazalkowska *et al*. (2002). The sequences of the primers were as follows:

forward: 5´-TGT GCT GGA CAC CGA CTA CAA AAA G-3´; and reverse: 3´-GCT CCC GGT ATA TGA CCA CCC TCT-5´.

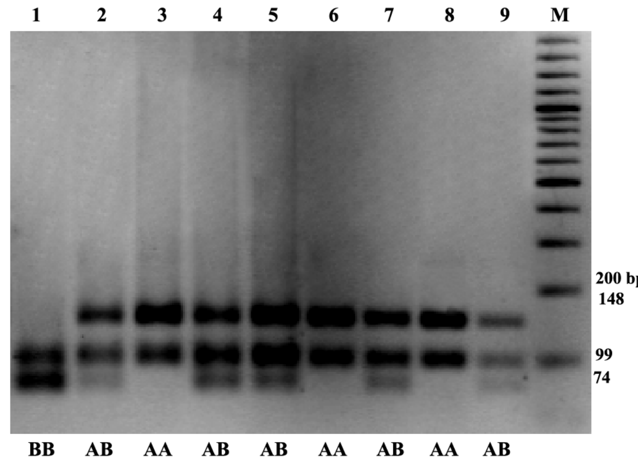

Figure 1. Electrophoresis of the digestion products of *Hae*III on 2.5% (w/v) agarose gel. Gel was stained with ethidium bromide, Lanes 1-9= digestion products of *Hae*III, Lane 1= BB genotype, Lanes 2, 4,5,7,9 = AB genotype, Lanes 3, 6= AA genotype, Lane M= Generuler™ 100-bp DNA Ladder Plus marker (Fermentas, European Union).

Polymerase Chain Reaction (PCR) was carried out, using the Personal Cycler™ thermocycler (Biometra, Germany) and the PCR Master Kit (CinnaGen Inc., Iran). The kit master mix consisted of 0.04 U/µl of *Taq* DNA polymerase, 10X PCR buffer, 3mM $MgCl_2$ and 0.04 mM dNTPs (each). Each reaction mixture consisted of 12.5 µl of the master mix, 1 µl of the DNA solution (50 to 100 ng/µl), 1 µl of each primer (5 pmol/µl) and some deionized water making up a final volume of 25 µl. The amplification program was as follows: initial denaturation at 94ºC for 5 min followed by 35 cycles of denaturation at 94°C for 1 min, annealing at 60ºC for 1 min, extension at 72°C for 1 min and a final extension at 72°C for 5 min. Digestion of PCR products was carried out, using the *Hae*III restriction endonuclease (Fermentas, European Union) at 37°C for 16 h and then analyzed by electrophoresis on a 2.5% (w/v) agarose gel (Fig.1). Determination of gene and genotype frequencies and the χ^2 test were carried out using the POPGene 32 software (Yeh *et al*., 1997).

The *β-LG* allele and genotype frequencies of the herd and those of other studies are summarized in

Table 1. *β-LG* gene and genotype frequencies in the Holstein breed and other breeds.

Breeds	Allele Frequency		Genotype Frequency			Reference
	A	B	AA	AB	BB	
Holstein	0.529	0.471	0.257	0.544	0.198	Current study
	0.270	0.730	0.060	0.525	0.414	Celik, (2003)
	0.516	0.484	-	-	-	Kaygisiz and Douan, (1999)
	0.420	0.580	-	-	-	Sabour *et al*. (1993)
	0.498	0.502	-	-	-	Lunden et al. (1997)
	0.520	0.480	0.284	0.471	0.245	Tsiaras et al. (2005)
	0.231	0.769	0.037	0.387	0.576	Lin and Mcallister, (1986)
	0.387	0.613	-	-	-	Ng-Kawi-Hang and Kim, (1984)
	0.520	0.480	-	-	-	Ron *et al*. (1994)
Brown Swiss	0.440	0.560	0.172	0.289	0.537	Celik, (2003)
Sahiwal	0.170	0.830	0.031	0.276	0.693	Rachagani *et al*. (2006)
Tharparkar	0.390	0.610	0.023	0.733	0.244	Rachagani *et al*. (2006)
Guernsey	0.210	0.790	-	-	-	Eenennaam and Medrano,(1991)
Milking Shorthorn	0.310	0.690	-	-	-	Eenennaam and Medrano,(1991)
Red-sindhi	0.250	0.750	-	-	-	Meignanalakshmi *et al*. (2001)
Ayrshire	0.158	0.842	0.190	0.279	0.702	Lin and Mcallister, (1986)

Table 1. In most of the studies, the frequency of the *β-LG* B allele was more than the A allele. The frequencies of the genetic variants A and B varied from 0.23 to 0.52 and 0.47 to 0.77, respectively in the Holstein breed. The results of the current study are in agreement with other studies, particularly with those of Ron *et al*. (1994) and Tsiaras *et al*. (2005). The maximum differences between A and B alleles' frequencies have been observed in the Ayrshire (Lin and Mcallister, 1986) and Sahiwal (Rachagani *et al*., 2006) breeds. It must be noted that differences between allele frequencies of the gene depend on economic strategies and animal breeding programs for every breed and herd. Genotype of the superior cow and her progenies were determined as AB by using the same method.

A single-trait fixed model was used to examine the effect of *β-LG* genotypes on milk production. The mature equivalent milk yield records, corrected for missing data, were used. Statistical analysis was performed using the Generalized Least squares Means (GLM) procedure of the SAS software (2002) and comparison of the least squares means was carried out using the Tukey-Kramer test (SAS software, 2002).
The following fixed model was used:
$y = Xb + e$,
with $E(y) = Xb$ and $var(y) = V = var(e)$
where;
X = known incidence matrix relating observation in y to the fixed effects in b,
b = vector of levels of fixed effects, e = vector of random residual terms,
E = the expectation of y and V = the variance of y

The fixed effects are represented by the calving years (2006, 2007 and 2008), seasons with 4 classes, parity with 8 classes and *β-LG* genotypes (AA, AB, BB).

Comparisons of the lactation milk yields of the *β-LG* genotypes showed that cows with the AA genotype produced more milk than animals with the BB genotype ($P< 0.006$) (Table 2). Comparison of expected and observed genotypic frequencies yielded a χ^2 value of 0.78, suggesting that the *β-LG* locus was in Hardy-Weinberg equilibrium ($P< 0.05$).

Table 2. Comparison of the Least Squares Means (LSM) ± Standard Errors (SE) of lactation milk yield of the genotypes*.

Genotype	LSM± SE (Kg)
AA	12979^{a} ± 837
AB	12032^{ab}± 796
BB	10788^{b}± 922

*Least squares means with different letters differed significantly ($P< 0.01$).

The significantly higher milk production yield of cows with the AA genotype was in agreement with the results of other researches (Ikonen *et al*., 1999; Bovenhuis *et al*., 1992; Aleandri *et el*., 1990; Ng-Kwai-Hang *et al*., 1990).

Although there was a difference between AA and AB genotypes, but it was statistically not significant (Table 2). The superior cow, because of having both the A and B alleles and the AB genotypes, apparently was superior in milk production and fat yields, as well as the quality of cheese. Some reports have confirmed significant association between the AB genotype and higher milk yield (Tsiaras *et al*., 2005; Kaygisiz and Douan, 1999). Therefore, these genotypes (AA, AB) seem to be suitable candidates for selection aiming at improving milk production.

It must be pointed out that other factors might also affect milk yield of the cow, such as physiological status and other major genes. Hence, sequencing the genome of this cow will be an attractive theme for future studies to understand the main factors affecting this high production level.

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