

Beta-Lactoglobulin and Kappa-Casein Gene Polymorphisms in Two Turkish Holstein Cattle Populations in Turkey[‡]

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Abstract

Milk protein polymorphisms have intensively been studied in order to determine the possible associations between milk protein genotypes and economically important traits in dairy cattle. The purpose of this study was to estimate the allele and genotype frequencies for β -lactoglobulin (β -LG) and κ -casein (κ -CN) genotypes in two Turkish Holstein cattle herds which have been raised in two different region in Turkey. Genomic DNA samples of 167 Holstein cattle (78 from Bala and 89 from Ceylanpinar) were genotyped for β -LG and κ -CN using by PCR-RFLP method. The frequencies of A allele at β -LG locus were calculated 0.51 ± 0.04 and 0.34 ± 0.03 in Bala and Ceylanpinar farms respectively. Chi-square analysis revealed that both populations were in Hardy-Weinberg equilibrium. The frequencies of A allele in κ -CN locus were calculated 0.80 ± 0.03 for Bala and 0.84 ± 0.02 for Ceylanpinar populations. Bala population was in Hardy-Weinberg equilibrium while Ceylanpinar was not.

Key words: PCR-RFLP, milk protein polymorphism, β -lactoglobulin, κ -casein

Türkiye'deki İki Siyah Alaca Populasyonunda Beta-Laktoglobulin ve Kappa-Kazein Gen Polimorfizmi

Özet

Süt sığırlarında ekonomik öneme sahip özellikler ile süt protein genotipleri arasındaki ilişki nedeniyle süt protein polimorfizmi yoğun olarak çalışılmaktadır. Bu çalışmanın amacı Türkiye'de farklı bölgelerdeki iki farklı Siyah Alaca sürüsünde β -laktoglobulin (β -LG) ve κ -kazein (κ -CN) genotiplerinin belirlenerek allel ve genotip frekanslarının tahmin edilmesidir. 167 Siyah Alaca sığına (78 Bala ve 89 Ceylanpinar) ait genomik DNA örnekleri PCR-RFLP yöntemi kullanılarak β -LG ve κ -CN genotipleri belirlenmiştir. β -LG A allelinin frekansı Bala populasyonunda 0.51 ± 0.04 ve Ceylanpinar populasyonunda 0.34 ± 0.03 olarak hesaplanmıştır. Ki-kare testleri her iki populasyonun da Hardy-Weinberg dengesinde olduğunu göstermiştir. κ -CN A allelinin frekansı Bala populasyonunda 0.80 ± 0.03 ve Ceylanpinar populasyonunda 0.84 ± 0.02 olarak hesaplanmıştır. Bala populasyonu Hardy-Weinberg dengesindeyken Ceylanpinar populasyonu dengede değildir.

Anahtar kelimeler: PCR-RFLP, süt protein polimorfizmi, β -laktoglobulin, κ -kazein

Introduction

Studies on milk protein genetic variability have been started almost 50 years ago since the detection of bovine β -lactoglobulin (β -LG) main variants A and B by paper electrophoresis (Ashaffenburg & Drewry 1955, 1957). Milk protein genetic polymorphism has received considerable

research interest because of possible associations between milk protein genotypes and economically important traits in dairy cattle. In the last 15 years, a new impulse has been given to investigations, not only for the well-known influence of milk protein variants on milk properties but also

[‡]: This study has produced from thesis of the first author.

because of the availability of new molecular techniques (Ceriotti et al. 2004).

Bovine milk contains six major proteins that can be classified into two groups; caseins (alpha s1-casein, alpha s2-casein, beta-casein, kappa-casein) and whey proteins (beta-lactoglobulin and alpha-lactalbumin). These proteins are controlled by codominant autosomal genes depending on presence different allelic forms according to Mendelian inheritance. All these proteins have been shown to display genetic polymorphisms caused by some mutations such as deletions or substitutions of one or more bases in the nucleotide sequence of the genes. There have been many reports in the literature describing the relations of these polymorphisms or genetic variants to milk yield, milk composition and cheese yield (Ikonen 2000; Martin et al 2002; Strzalkowska et al 2002; Tsiaras et al 2005; Michalcová & Krupová 2007; Hallén et al 2008).

β -LG, the major protein of bovine milk whey, is found in several genetic variants of which A and B are predominant. The gene encoding β -LG has been mapped on chromosome 11 (BTA 11) in cattle. Two amino acid substitutions, Aspartic acid 64 (GAT)→Glycine (G \underline{G} T) and Valine 118 (GTC)→Alanine (G \underline{C} C), distinguish the B from the A variant. At the DNA level both the A→G and the T→C substitutions give rise to restriction fragment length polymorphisms (RFLP) with *Hph*I and *Hae*III restriction sites occurring in DNA coding for the B variant. Earlier studies have shown that A and B variants of β -LG may affect milk composition and properties. β -LG AA genotype cow's milk contains more β -LG, less casein and fat than β -LG BB cow's milks. Some studies showed that milk produced by β -LG BB genotype cows yielded significantly more cheese than that by AA cows. Moreover some researchers reported that in the presence of β -LG B variant increase resistance to mastitis.

κ -casein (κ -CN) plays an important role in preserving the other caseins from precipitation. The treatment of milk with chymosin (rennin) cleaves κ -casein, resulting in curd formation. κ -casein gene has been mapped on chromosome 6 (BTA 6). Two major genetic variants of κ -CN, A and B, have been identified. Variant A has Threonine (A \underline{C} C) and Aspartic acid (GAT) at positions 136 and 148, respectively. In variant B Threonine is substituted by Isoleucine (A \underline{T} C) and Alanine (G \underline{C} T) substitutes into Aspartic acid. These differences result from a single base substitution in κ -CN gene and two alleles may be distinguished by the presence or absence of *Hind*III restriction site. Furthermore, the change in the GAT coding for Aspartic acid at amino acid position 148 abolishes a *Hinf*I site in the B allele. κ -CN B variant have a

favorable and significant effect on both milk and milk protein yield. Relationships between κ -CN B variant and technological properties have also been reported. κ -CN B variant is associated with shorter renneting time of the milk.

The purpose of this study was to determine genotypic and allelic frequencies of β -LG and κ -CN in Holstein cattle populations reared in Turkey.

Material and Method

A total 167 of blood samples were taken from Holstein cattle reared in Bala (78) and Ceylanpinar (89) Agricultural Enterprises were used as the material in the study. Blood samples were collected by puncture of jugular vein into sterile tubes containing Ethylenediaminetetraacetic acid (EDTA). Blood samples were stored in a refrigerator at +4 °C until DNA isolation. The DNA was extracted from white blood cells (leukocytes) by Salting out method. The quality of DNA was checked on 1% agarose gel electrophoresis, and the quantity was measured by using a spectrophotometer at A_{260} / A_{280} nm.

Analysis of β -LG genotype

The primers used for amplification of the β -LG gene were described by Wilkins and Kuys (1992), with the following nucleotide sequence: forward primer (5' ACC TGG AGA TCC TGC TGC AGA AAT G 3') and reverse primer (5' CAT CGA TCT TGA ACA CCG CAG GGA T 3').

In order to amplify 961 bp fragment, a total reaction mix of 25 μ l containing 10 X PCR buffer, 1.5 mM MgCl₂, 0.6 mM of each dNTP, 1pM of each primer, 1 U of taq polymerase and 100 ng DNA was subjected to 30 cycles of 94 °C for 1 min, 61 °C for 30 s and 72 °C for 2.5 min in a Thermal Cycler. The PCR product was restricted by adding 2 U of *Hph*I to 10 μ l of the mix and continuing incubation at 37 °C for 1 h. Then the products analyzed by electrophoresis on 2% agarose gels stained with ethidium bromide.

Analysis κ -CN genotype

κ -CN gene variants were identified according to Pinder et al. (1991) and κ -CN gene was amplified using the following primers: 5' GTG CTG AG(T/C) AGG TAT CCT AG 3' and 5' GTA GAG TGC AAC AAC ACT GG 3'.

In order to amplify 874 bp fragment, a total reaction mix of 20 μ l containing 10 X PCR buffer, 1.5 mM MgCl₂, 0.2 mM of each dNTP, 1pM of each primer, 1 U of taq polymerase and 100 ng DNA was subjected to 30 cycles of 95 °C for 1 min, 57 °C for 1 min and 74 °C for 3 min in a Thermal Cycler. The PCR product was restricted by adding 2 U of *Hind*III to 10 μ l of the mix and continuing incubation at 37

°C for 1 h. Then the products analyzed by electrophoresis on 2% agarose gels stained with ethidium bromide.

Results and Discussion

Detection of alleles at the β -LG locus by PCR-RFLP

We have amplified a 961 bp region of the β -LG gene from the junction of exon 2 – intron 2 through to the end of exon 3. In this region non-polymorphic *HphI* site is found within intron 2. The polymorphic site within exon 3 is cut by *HphI* in the B but not in the A allele.

All DNA samples gave the expected 961 bp fragment by amplification with no other DNA being visible under these PCR conditions. On digestion with *HphI*, all samples gave the 741 bp constant fragment plus either a 220 bp fragment (A allele) or a 166 and 54 bp (B allele) or a combination of the three (AB) (Figure 1).

Detection of alleles at the κ -CN locus by PCR-RFLP

We have amplified an 874 bp fragment of the exon 4 of κ -CN gene. In the A allele this contains no site for *HindIII*. The B allele is cleaved by *HindIII* into fragments of 521 and 353 bp. Figure 2 shows results of PCR-RFLP for 9 DNA samples from animals of identified genotype. AA homozygotes showed a single band at the same position as uncut PCR products while BB homozygotes two faster running bands and AB heterozygote animals gave all three bands after digestion with *HindIII*.

Statistical analysis

Gene counting method was used to estimate allele and genotype frequencies of β -LG and κ -CN (Nei 1987). The Chi-square (χ^2) test was used to check whether the populations were in Hardy-Weinberg equilibrium or not. Frequencies of genotypes and alleles of β -LG and κ -CN are shown in Table 1. Previous studies are supporting the findings for these allele and genotype frequencies of Holstein cattle populations (Aleandri et al 1990; Sabour et al 1993; Ron et al 1994; Bobe et al 1999; Bonvillani et al 2000; Öner & Elmacı 2006; Hallen et al 2008).

At the β -LG locus, the most common genotype was AB although AA and BB genotypes were determined in both populations in this study.

Approximately half of the cattle had the heterozygous genotype (AB) for the β -LG locus, with a predominance B allele in Ceylanpinar population. The frequencies of A allele at β -LG locus were calculated 0.51 ± 0.04 and 0.34 ± 0.03 in Bala and Ceylanpinar samples respectively. Expected genotypic frequencies were calculated and then compared with observed frequencies. χ^2 values were calculated as 2.54 and 0.009, suggesting that both populations were in Hardy-Weinberg equilibrium (testing at the 0.05 level of significance).

The most common allele was A at κ -CN locus, and AA genotype was more frequent than BB and AB. The frequencies of A allele in κ -CN locus were calculated 0.80 ± 0.03 for Bala and 0.84 ± 0.02 for Ceylanpinar populations. χ^2 value of 0.004 was calculated, suggesting Bala population was in Hardy-Weinberg equilibrium while χ^2 value of 4.372 showed Ceylanpinar population was not in Hardy-Weinberg equilibrium (testing at the 0.05 level of significance). This unexpected result might be, either due to the sampling error or due to the fact that bull gave his sperm to the cows was in a non-random way. However, even if the mating was non-random, one would still expect bias not only one but also both loci from Hardy-Weinberg equilibrium. Therefore, we could infer that the bias from Hardy-Weinberg equilibrium was due to the sampling error.

Conclusions

In this study we have detected the genotypes and polymorphism of β -lactoglobulin and κ -casein proteins for two Holstein populations from different regions of Turkey. Both populations were found to be polymorphic in two loci. PCR-RFLP technique can be used as a fast, accurate, low cost method independent of age and sex for genotyping of milk protein gene, hence, allowing the selection of animals with the favorable genotypes to improve quantitative and qualitative features of milk.

Further studies looking at the relation of the various yield and quality features of milk with the genetic variation in the milk proteins such as β -lactoglobulin and κ -casein could serve as a selection criterion for milk production in breeding programs.

Table 1. Genotype and allele frequencies

Herd	No. of animals	Genotypes						Allele frequencies			
		β -lactoglobulin			κ -casein			β -lactoglobulin		κ -casein	
		AA	BB	AB	AA	BB	AB	A	B	A	B
Bala	78	17	15	46	50	3	25	0.51	0.49	0.80	0.20
Ceylanpinar	89	10	39	40	65	5	19	0.34	0.66	0.84	0.16

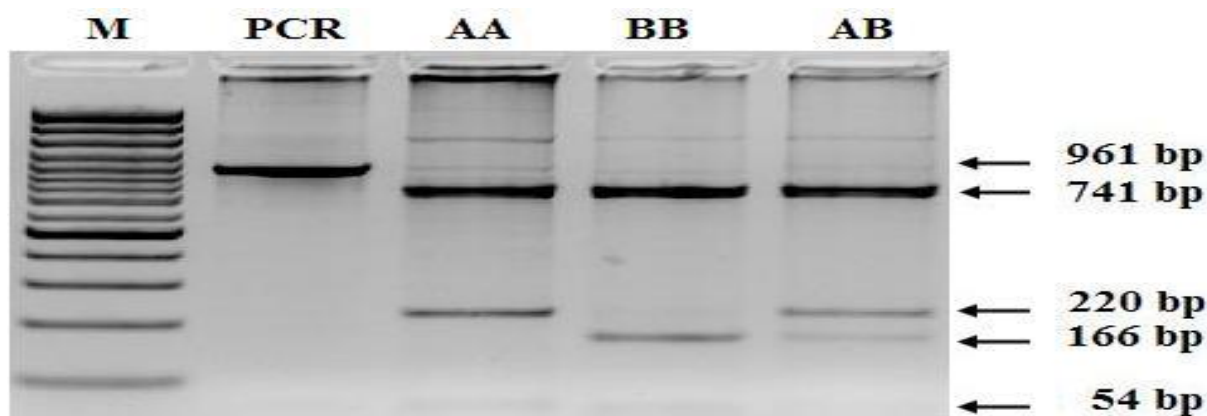


Figure 1. Identification of β -lactoglobulin genotypes on 2% agarose gels by PCR-RFLP (M 100 bp DNA marker)

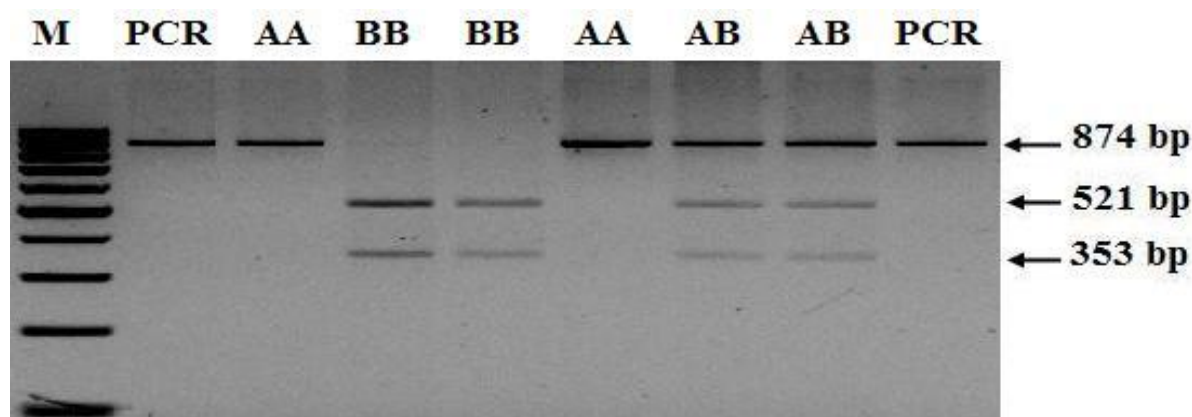


Figure 2. Identification of κ -casein genotypes on 2% agarose gels by PCR-RFLP (M 100 bp DNA marker)

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