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SHORT COMMUNICATION

Beta-Lactoglobulin Gene Types in Turkish Fat-Tailed Sheep Breeds [1]

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Summary

The genetic polymorphism of the β-lactoglobulin gene was investigated in 182 sheep of Turkish fat-tailed sheep breeds by using PCR-RFLP methods. In the study, two alleles (A and B) and three genotypes (AA, BB and AB) at β-lactoglobulin locus were observed. The most common genotype at the β-lactoglobulin locus was AB. The greatest frequency of allele A was detected in Tuj breed as 0.7188. Results of the present study show that genetic polymorphism exists in the Turkish fat-tailed sheep breed population used in this study.

Keywords: *Turkish sheep breeds, Genetic polymorphism, β-lactoglobulin gene, PCR-RFLP*

Türk Yağlı Kuyruklu Koyun Irklarındaki Beta-Laktoglobulin Gen Tipleri

Özet

β-laktoglobulin geninin genetik polimorfizmi Türkiye yağlı kuyruklu koyun ırklarının 182'sinde PCR-RFLP metodu kullanılarak incelenmiştir. Çalışmada, β-laktoglobulin lokusunun iki alleli (A ve B) ve üç genotipi (AA, AB ve BB) gözlenmiştir. β-laktoglobulin lokusundaki en yaygın genotip AB genotipidir. A allelinin en yüksek frekansı 0.7188 olarak Tuj ırkında belirlenmiştir. Mevcut çalışmanın sonuçları, kullanılan Türk yağlı kuyruklu koyun ırklarında genetik polimorfizmin varlığını göstermiştir.

Anahtar sözcükler: *Türkiye koyun ırkları, Genetik polimorfizm, β-laktoglobulin geni, PCR-RFLP*

INTRODUCTION

The Turkish fat-tailed sheep is an important genetic resource due to their special economic and ecological characteristics. The genetic polymorphism of milk proteins has great importance in animal breeding and has been made in the subject in recent years due to its relationships with production traits, milk composition and milk quality [1-8]. β-lactoglobulin (β-lg) is the major milk whey protein in ruminants, consists of 162 amino acids and forms stable dimmers in milk. Among specific genes that may effects economically important traits in sheep, the β-lg locus has been extensively studied [5,6,9-11].

Three genetic variants of this protein: A, B [11] and C [7] have been identified in sheep and variants A and B are also associated with milk production and milk composition. Variants A and B are present in all breeds and differ by a Tyr/His substitution in position 20 [11] corresponding to a single nucleotide substitution in the β-lg gene. The variant C is a subtype of ovine variant A with a single exchange of Arg-Gln at position 148 [7]. In earlier studies with Turkish sheep breeds; Kıvırcık, Gökçeada, Sakız [6] and Awassi and Mor Karaman [5] and Karacabey Merino [9], β-lg A and β-lg B genetic variants have been described.

The objective of this study was to reveal the genetic polymorphism of the β-lactoglobulin locus in native Turkish fat-tailed sheep breeds (Akkaraman, Dağlıç, Awassi, Tuj, Karakaş, Norduz, Güney Karaman and Kangal) by using PCR-RFLP methods.

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MATERIAL and METHODS

Animals and DNA Isolation

Blood samples used for isolation of DNA were obtained from 182 sheep belonging to 8 native Turkish sheep breeds taken from different regions of Turkey. The animals were treated according to the Animal Care and Use Regulation "European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purpose 1996". Genomic DNA was extracted from blood samples using methods described by Miller et al.[12]. To determine DNA quality and quantity, agarose gels and spectrophotometric methods were used for the analysis.

PCR and RFLP Procedure

Allele discrimination was based on size differentiation of β-lg gene. After optimizing PCR conditions, β-lg variants were amplified using primers on *Table 1*. PCR products were digested using *Rsa*I restriction enzymes to determined β-lg A and β-lg B variants and M*sp*I restriction enzymes to determined β-lg A and β-lg C variants for 2 h at 37°C. The digestion products were separated on 3% agarose gels and visualized under UV after staining with ethidium bromide. The restriction fragments in the gel used to establish the genotypes of sheep at β-lg locus.

Statistical Analysis

Direct counting methods was used to estimate phenotype and allele frequencies of β-lg gene. The chi-square statistic was used to check whether the populations were in Hardy-Weinberg equilibrium.

RESULTS

The ovine β-lg gene was amplified as a 120 bp fragment from exon II in 182 animals. The restriction digestion of these PCR products with R*sa*I enzyme revealed three genotypes AA, AB and BB. Variant A has two R*sa*I sites, digestion three fragments of 66, 37 and 17 bp are produced while the PCR product from variant B has just one R*sa*I site and digestion produces two fragments of 103 and 17 bp and heterozygotes have all four distinct bands. But 17 bp fragment is very weak or not visible on the gel *(Fig. 1)*. Also, in this study, β-lg C allele was not observed in Turkish sheep breeds investigated.

The distribution of β-lg phenotype and gene frequencies are presented in *Table 2*. The heterozygous genotype AB occurred most frequently (90 samples) while the other two homozygotes, AA and BB, appeared in 57 and 35 samples, respectively. BB genotype was not detected in the Norduz populations. Kangal breed has the most frequent AB genotype.

Frequencies of β-lg A and B alleles were almost equal in Dağlıç, Akkaraman, Karakaş and Kangal breeds. The greatest frequency of allele A belonged to the Tuj (0.7188 followed by Norduz (0.7059), while the lowest frequency of this allele was detected in the Karakaş (0.4286). The most common genotype at the β-lg locus was AB and was clearly predominant for six breeds *(Table 2)*. All samples were found to be in Hardy-Weinberg equilibrium at the β-lg locus.

Table 1. *Primer sequences and annealing temperatures for β-lg variants*
Tablo 1. *β-lg variantları için primer sekansları ve bağlanma sıcaklıkları*

β-lg Variants	**Primer Sequences**	**Restriction Enzymes**
β-lg A-B variants	F: 5′-CAA CTC AAG GTC CCT CTC CA-3′ R: 5′-CTT CAG CTC CTC CAC GTA CA-3′	*Rsa*I
β-lg A-C variants	F: 5′-TCA GGA CCC CGG AGG TGG ACA AC-3′ R: 5′-CCT CCA GCT GGG TCG GGT TGA AG-3′	M*sp*I

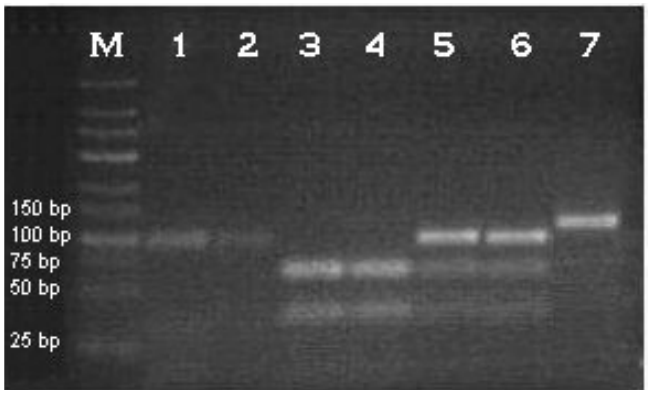

Fig 1. The illustration of β-lg genotypes on 2% agarose gel; M: 50 bp DNA marker; lane 1, 2: BB genotype; lane 3, 4: AA genotype; lane 5, 6: AB genotype and lane 7: PCR product

Şekil 1. 2%'lik agaroz jelde β-lg genotiplerinin örneklemesi. M: 50 bç DNA markeri; sıra 1, 2: BB genotipi; sıra 3, 4: AA genotipi; sıra 5, 6: AB genotipi ve sıra 7: PCR ürünü

DISCUSSION

So far, a lot of studies have shown that this protein is polymorphic in many breeds of sheep as a result of a single base pair substitution in the β-lg gene. The occurence of β-lg variants in this study is similar to other sheep breeds studied previously. β-Lg A and B alleles were widely reported in almost all breeds studied [5,6,10], on the other hands, β-Lg C was rare and identified in Merinoland [7] and Merino [8] sheep breeds. As in the study on Sakız, Gökçeada

Table 2. *Phenotype distribution and mean gene frequencies of β-lactoglobulin locus in 8 native Turkish sheep breeds*
Tablo 2. *Sekiz Yerli Türk koyun ırkındaki β-laktoglobulinin fenotip dağılımı ve ortalama gen frekansları*

Breed	N	β-lg phenotype[a]			Gene frequency		
		AA	AB	BB	β-lg A	β-lg B	$(\chi^2)^b$
Awassi	19	6	12	1	0.6316	0.3684	2.4265
		7.58	8.84	2.58			
Dağlıç	19	4	10	5	0.4737	0.5263	0.0573
		4.26	9.48	5.26			
Akkaraman	19	4	10	5	0.4737	0.5263	0.0573
		4.26	9.48	5.26			
Tuj	16	9	5	2	0.7188	0.2812	0.8144
		8.27	6.46	1.27			
Karakaş	21	4	10	7	0.4286	0.5714	0.0156
		3.86	10.28	6.86			
Norduz	17	7	10	-	0.7059	0.2941	1.4794
		8.47	7.06	1.47			
Güney Karaman	29	14	10	5	0.6552	0.3448	1.6230
		12.45	13.1	3.45			
Kangal	42	9	23	10	0.4881	0.5119	0.0106
		8.79	22.97	10.24			

[a] *Upper row. observed values; lower row. expected values*
[b] *Test of Hardy-Weinberg equilibrium; not significant*

and Kıvırcık native sheep breeds [6], β-lg C allele wasn't also observed in this study. Frequencies of β-lg A and B alleles were almost equal in Dağlıç, Akkaraman, Karakaş and Kangal breeds. Similar results for the allele of ovine β-lg in Iranian and Russian sheep breeds were reported by Mohammadi et al.[10]. The greatest frequency of allele A belonged to the Tuj (0.7188) followed by Norduz (0.7059), similar findings for allele A were obtained in Kıvırcık (0.7759), Gökçeada (0.7632) and Sakız (0.9756) sheep breeds by Elmaci et al.[6]. Allele A was also high in Awassi (0.6316) and Güney Karaman (0.6552). Similarly, Çelik and Özdemir reported that the frequency of allele A in Awassi and Morkaraman were 0.63 and 0.56, respectively [5]. Considering the researches, it can be concluded that allele A and genotype AB are prevalent in sheep populations worldwide. As results of this study are comparable to other Turkish sheep breeds, similarly the β-lg AB genotype and allele A were more common in sheep breeds [5,6]. On the other hand population size may further affect our findings.

This study shows that Turkish fat-tailed sheep breeds have a genetic variability in the β-lactoglobulin locus. It is determinate that milk protein polymorphism has been considered a potential tool for selection of Turkish sheep breed, especially marker assisted selection between different genotypes of milk and cheese characteristics. In addition, these results also show that the chosen primers are adequate to amplify the sequence of β-lg gene exon II in sheep.

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