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RESEARCH ARTICLE

Identification of β-Lactoglobulin Gene *Sac*II Polymorphism in Honamli, Hair and Saanen Goat Breeds Reared in Burdur Vicinity [1]

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Summary

This study was conducted to determine DNA-polymorphism of a *Sac*II RFLP at exon 7 of the β-lactoglobulin (β-LG) gene in Honamli (31), Hair (39) and Saanen (41) goat breeds. A total of 111 goats were genotyped for the β-lactoglobulin-*Sac*II polymorphism by polymerase chain reaction and restriction fragment length polymorphism (PCR-RFLP). In the studied breeds, digestion of amplification product with *Sac*II restriction enzyme revealed two alleles namely, A and B and three genotypes (AA, BB and AB). Allelic frequencies for Hair, Saanen and Honamli breeds were 0.42, 0.37 and 0.53 respectively for A allele; 0.58, 0.63 and 0.47 respectively for B allele, while genotypic frequencies were 0.13, 0.12 and 0.19 for AA, 0.28, 0.39 and 0.13 for BB, and 0.59, 0.49 and 0.68 for AB respectively. Deviation from Hardy-Weinberg equilibrium was not observed in the studied breeds. As a result, this study provided information on the polymorphism of β-lactoglobulin in three goat breeds. Additionally, this study reported the existence of a genetic polymorphism at β-LG gene in Honamli goat breed for the first time.

Keywords: *β-lactoglobulin, Goat, Honamli, Polymorphism*

Burdur İli ve Civarında Yetiştirilen Honamlı, Kıl ve Saanen Keçi Irklarında β-Lactoglobulin Geni *Sac*II Polimorfizminin Belirlenmesi

Özet

Bu çalışma Kıl (39), Saanen (41) ve Honamlı (31) keçi ırklarında β-lactoglobulin geninin 7. ekzonunun *Sac*II RFLP polimorfizminin incelenmesi amacıyla yapılmıştır. β-lactoglobulin-*Sac*II polimorfizmi için toplam 111 baş keçi PZR-RFLP (Polimeraz Zincir Reaksiyonu ve Restriksiyon Parça Uzunluk Polimorfizmi) ile genotiplendirilmiştir. İncelenen ırklarda *Sac*II enzim kesimi sonucu iki allel (A ve B) ve üç genotip (AA, BB ve AB) belirlenmiştir. Kıl, Saanen ve Honamlı keçi ırkları için allel frekansları; A alleli için sırasıyla 0.42, 0.37 ve 0.53; B alleli için sırasıyla 0.58, 0.63 ve 0.47 bulunmuştur. Genotipik frekanslar ise AA genotipi için sırasıyla 0.13, 0.12 ve 0.19; BB genotipi için 0.28, 0.39 ve 0.13; AB genotipi için ise; 0.59, 0.49 ve 0.68 bulunmuştur. Çalışılan ırklarda Hardy-Weinberg dengesinden sapma görülmemiştir. Sonuç olarak, bu çalışmada üç keçi ırkında β-lactoglobulin gen polimorfizmi hakkında bilgi verilmiştir. Ayrıca, bu çalışma ile Honamlı keçisinde β-lactoglobulin gen polimorfizmi varlığı ilk defa bildirilmiştir.

Anahtar sözcükler: *β-lactoglobulin, Keçi, Honamlı, Polimorfizm*

INTRODUCTION

Goat is a significant resource in developing countries like Turkey due to high quality meat, milk, hair and leather [1]. Goat breeding provides an important source of animal protein in Turkey, especially for those living in rural areas.

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Several goat breeds such as the Angora, Kilis, Honamli, Hair, Norduz, Saanen are reared in different regions of the Turkey. Hair goat, also known as black goat, is a major goat breed of the Turkey. This goat breed is a multipurpose animal in terms of meat, milk and hair [2]. Saanen goat is a breed of Switzerland and known worldwide for high milk yield [2]. Honamli goat breed is widespread throughout the slopes of Taurus Mountains, provinces of Burdur, Antalya, Isparta and Konya for both milk and meat yields [3].

Polymorphisms in milk protein genes are used as markers for milk yield. Milk protein polymorphisms have been studied intensively because of their effect on the yield and processing properties of milk and its products. Beta-lactoglobulin (β-LG) is one of the two major whey proteins identified in the milk of animals including cattle, sheep, goat, dogs, and pigs. On the other hand, this protein is not identified in human, mice, lagomorphs and other some mammalian species [4]. Genetic polymorphisms identified in β-LG gene lead to the formation of its different variants within and between various species [5]. The relationship between genetic variants of the β-LG and milk composition, milk yield and cheese-making ability has been reported in cow (Shiwal and Tharparkar cattle [6] and Najdi cattle [7]), goat (Indian goats) [4] and sheep (Manchega sheep [8] and Massese ewes [9]). β-LG was the first milk protein in which polymorphism was identified by protein electrophoresis of bovine milk [10]. Then, genetic polymorphisms in β-LG have been described both at protein level [11-13] and DNA level [14-16] in farm animals. The electrophoretic pattern of the β-LG gene in domestic goats has been analyzed for the first time by SDS-PAGE [4]. Additionally, two genetic variants (A and B) of β-LG were reported in goat milk [17] at protein level. Then, β-LG gene polymorphism has been analyzed by PCR-RFLP [4]. Polymorphisms in both exon 7 and the proximal promoter region of β-LG gene in Spanish and French goats were investigated, and two novel genetic variants were reported [18,19]. β-LG polymorphism was reported in different goat populations at protein [20-22] and DNA [23,24] level in Turkey. However, no information has been reported for especially Honamli goats regarding β-LG polimorphisms at the DNA level.

The aim of the present paper was to investigate genetic polymorphism of a *Sac*II RFLP at Exon 7 of the β-LG gene in Hair, Honamli and Saanen goat breeds by PCR-RFLP.

MATERIAL and METHODS

Samples and DNA isolation

A total of 111 blood samples were collected from three different goat breeds around Burdur region in their natural habitats. The animal material included 39 Hair goats, 41 Saanen goats and 31 Honamli goats. Blood samples from the goats were placed into an EDTA tubes for DNA isolation. Genomic DNA was isolated using Genomic DNA Isolation kit (Qiagen QIAamp DNA Blood Mini Kit) following the manufacturer's protocol. The quality of DNA was checked on 0.8% agarose gels and stained with ethidium bromide.

DNA Amplification and Genotyping

Genotyping for β-LG-*Sac*II polymorphism was performed by Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) as proposed by Pena et al.[18]. The sequences of the forward and reverse primers for the amplification of the β-lactoglobulin gene (accession number Z33881.1) were: forward 5'-CGG GAG CCT TGG CCC TCT GG-3'; reverse 5'-CCT TTG TCG AGT TTG GGT GT-3'. PCR for the β-lactoglobulin gene was performed in a 25 µl reaction mixture, containing 1.5 mM $MgCl_2$, 200 µM of each dNTPs, 200 µM of each primer, 1 X PCR buffer, 1U Taq polymerase and 100 ng of genomic DNA template. The reaction mixture was placed in a DNA Amplitronyx 6 thermal cycler. Thermal cycling conditions included: an initial denaturation step at 95°C for 5 min followed by 35 cycles of 95°C for 30 s, 65°C for 60 s, 72°C for 60 s and a final extension at 72°C for 5 min. The PCR products were digested with 10 U of *Sac*II restriction endonuclease (Fermentas) at 37°C for at least 3 h. PCR products and restriction fragments were electrophoresed on 2% and 3% agarose gels respectively and stained with ethidium bromide.

Statistical Analysis

Direct counting was used to estimate genotype and allele frequencies of β-LG gene *Sac*II genetic variants. Chi-square statistic (χ^2) was used to check whether the populations were Hardy-Weinberg equilibrium. All statistical analyses were performed using PopGene32 software [25].

RESULT

The β-LG gene (exon 7 to the 3'flanking region) of Hair, Honamli and Saanen goat was investigated by PCR-RFLP method. A fragment of 426 bp was successfully amplified *(Fig. 1)* and digested with *Sac*II restriction enzymes to detect the presence of A or B variants. PCR-RFLP with the *Sac*II enzyme revealed the polymorphic site, which was produced by a single nucleotide substitution in position +4601 [18]. As a result of amplification product with *Sac*II digestion, two alleles, A and B, were observed. Restriction digestion of 426bp PCR products with *Sac*II enzymes revealed three genotypes *(Fig. 2)* of AA (426 bp), BB (349 and 77 bp) and AB (426, 349 and 77 bp). The allelic and genotypic frequencies of the β-LG gene polymorphism for the Hair, Saanen and Honamli goats were given in *Table 1*. The results of Chi-square statistic reflected that breeds were in Hardy-Weinberg equilibrium.

Allelic frequencies for Hair, Saanen and Honamli breeds were determined as 0.42, 0.37 and 0.53 respectively for A allele; 0.58, 0.63 and 0.47 respectively for B allele, whereas genotypic frequencies were 0.13, 0.12 and 0.19 for AA,

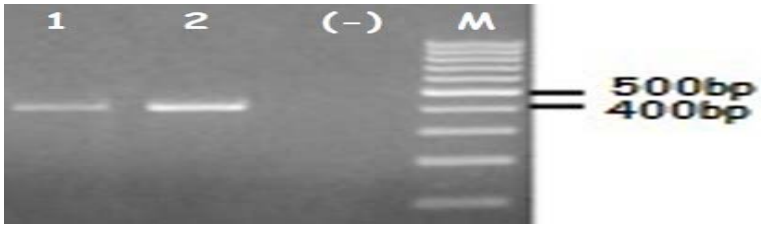

Fig 1. PCR amplifications of β-LG gene (426bp, lanes 1-2). Lane M, molecular size marker (100 bp DNA ladder)

Sekil 1. β-LG geninin PZR ürünleri (426bp, hat 1-2). Hat M, moleküler büyüklük belirteci (100 bç DNA ladder)

Fig 2. Electrophoresis of RFLP of caprine β-LG gene after digestion by *Sac*II of animals with AA (Lane 2,5; 426bp), AB (Lane 4,6; 426bp/349bp/77bp, BB (Lane 1,3; 349bp/77bp) genotypes. Lane M, molecular size marker (100 bp DNA ladder)

Sekil 2. Keçi β-LG geninin *Sac*II enzim kesim ürünlerinin agaroz jeldeki fotografı. Hat 2,5 AA (426bç); Hat 4,6 AB (426bç/349bç/77bç); Hat 1,3 BB (349bç/77bç) genotipleri. Hat M, molekülür büyüklük belirteçi (100 bç DNA merdiveni)

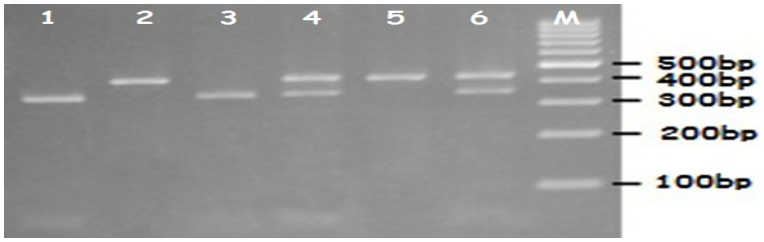

Table 1. *Allele and genotype frequencies of β-LG gene for SacII site in Hair, Saanen and Honamli goat breeds*
Tablo 1. *Kıl, Saanen ve Honamli keçi ırklarında β-LG geninin SacII bölgesinin allel ve genotip frekansları*

Breed	n	Genotype						Allele Frequency		χ^2(df=1)	p-value
		AA		AB		BB		A	B		
		Obs (Exp)	F.	Obs (Exp)	F.	Obs (Exp)	F.				
Hair	39	5 (6.857)	0.13	23 (19.286)	0.59	11 (12.857)	0.28	0.423	0.577	1.487	0.223 ^{NS}
Saanen	41	5 (5.370)	0.12	20 (19.259)	0.49	16 (16.370)	0.39	0.366	0.634	0.062	0.803 ^{NS}
Honamli	31	6 (8.656)	0.19	21 (15.689)	0.68	4 (6.656)	0.13	0.532	0.468	3.673	0.055 ^{NS}
All breeds	111	16 (20.634)	0.14	64 (54.733)	0.58	31 (35.634)	0.28	0.432	0.568	3.212	0.073 ^{NS}

F: *Frequency;* ^{NS}*: Non significant*

0.28, 0.39 and 0.13 for BB, and 0.59, 0.49 and 0.68 for AB respectively. A significant deviation from Hardy-Weinberg equilibrium was not observed in the investigated breeds.

DISCUSSION

In the present study, genetic polymorphism of the β-LG gene in Hair, Honamli and Saanen goat breeds were investigated. The 426 bp product of exon 7 to the 3' flanking region of the β-LG gene was digested with restriction endonucleas *Sac*II in three goat breeds. For *Sac*II site two alleles (A and B) were found and three genotypes (AA, AB and BB) were detected.

Regarding polymorphism of β-LG locus eight variants have been reported at the DNA level at the bovine β-LG locus, however, alleles A and B are the most frequent [26] and allele B was associated with a lower whey protein content and a higher casein content in milk [26]. In sheep, three variants (A, B and C) have been reported [27]. Polymorphisms in both exon 7 and the proximal promoter region of β-LG gene in Spanish and French goats were investigated, and two novel genetic variants were reported [18,19]. Two genetic variants (A and B) of β-LG were reported in goat milk at protein [17] and DNA [4,18,19] level. The presence of β-LGA and β-LGB at this locus was reported in Alpine and Saanen goats [28] and in Jamunapari, Sirohi, Barbari and Jakhrana breeds [11]. Similar results were observed in all studied breeds. Similarly, three genotypes were observed in Hair, Saanen and Honamli goats at the β-LG locus. AB genotype was the most frequent genotype in the entire studied sample.

Goat β-LG polymorphism has been investigated with 233 Hair goat breeds by Elmaci et al.[24] They revealed that the frequency of AA (S2S2, 0.11) genotype was found to be lower than BB (S1S1, 0.45) and AB (S1S2, 0.44) genotypes and then the genotypic frequencies of BB and AB genotypes were very close. Additionally Elmaci et al.[24] showed that the frequency of S2 (A) allele at β-LG locus was lower compared to the frequency of S1 (B) allele. This was consistent with the result of the present study in exception with Honamli goat. Previously, polymorphism studies about β-LG locus for Barki, Damascus and their cross breeds [14] showed lower frequencies of B allele in comparison with the present study (all breeds). Kumar et al.[4] reported the frequency of S2S2 (BB) genotype with a large range of 0.42 to 1.00 in the population and S2 (B) allele frequency higher than S1 (A) allele frequency [4]. This was consistent with Hair and Saanen goat breeds, but not Honamli goat. Garg et al.[12] reported the presence of two genetic variants at β-lactoglobulin locus (A and B) and the gene frequencies of β-LGA and β-LGB were 0.910 and 0.090, respectively. This

was consistent with Honamli goat, but not Hair and Saanen goat breeds. Kumar et al.[11] and Boulanger [28] were studied this locus and indicated that variant A was dominant over variant B, which is corroborated with Garg et al.[12] findings. But this was consistent with the result of the present study in exception with Honamli goat.

In the present study, it was reported that Hair, Honamli and Saanen goat breeds from Burdur have genetic polymorphism in β-LG gene. Especially, this study showed the existence of a genetic polymorphism at β-LG gene in Honamli goats for the first time. Further evaluation is required to confirm the correlation with milk yield. In addition, it is very crucial to investigate further goat breeds for determining the polymorphism at proximal promoter region of β-LG gene and other genes to generate results of the present study. Finally data showed that PCR-RFLP is an appropriate tool for investigating genetic polymorphism. Also, the results of β-LG gene polymorphism can be reliably used in genetic characterization of Turkish native goat breeds, determination of biodiversity and evolution in world goat breeds.

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