Kafkas Univ Vet Fak Derg
19 (3): 419-422, 2013
DOI: 10.9775/kvfd.2012.7961

Journal Home-Page: http://vetdergi.kafkas.edu.tr
Online Submission: http://vetdergikafkas.org

RESEARCH ARTICLE

Growth Hormone Gene Polymorphism in Four Cattle Breeds in Turkey

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Makale Kodu (Article Code): KVFD-2012-7961

Summary

This study was conducted to determine DNA-polymorphism of a *Alu*I RFLP at bovine growth hormone (bGH) gene in Zavot (n=48), East Anatolian Red (n=40), Simmental (n=94) and Brown Swiss (n=64) cattle breeds. A total of 246 cattle were genotyped for the bGH-*Alu*I polymorphism by polymerase chain reaction and restriction fragment length polymorphism (PCR-RFLP). In the study, two alleles (L and V) and three genotypes (LL, VV and LV) were revealed after than digestion of amplification product with *Alu*I restriction enzyme. Allelic frequencies for EAR, SIM, BS and Zavot breeds were determined as 0.775, 0.734, 0.781 and 0.760 respectively for L allele; 0.225, 0.266, 0.219 and 0.240 respectively for V allele. Otherwise, genotypic frequencies were 0.65, 0.57, 0.63 and 0.63 for LL, 0.10, 0.11, 0.06 and 0.10 for VV, and 0.25, 0.32, 0.31 and 0.27 for LV respectively. A significant deviation from Hardy-Weinberg equilibrium was not observed in the investigated breeds. As a result, this study provided information on the polymorphism of bGH in four cattle breeds. Additionally, this study reported the existence of a genetic polymorphism at bGH gene in Zavot cattle breed for the first time.

Keywords: *Growth Hormone, Cattle, Zavot, Polymorphism*

Türkiye'deki Dört Sığır Irkında Büyüme Hormonu Gen Polimorfizmi

Özet

Bu çalışma, Zavot (n=48), Doğu Anadolu Kırmızısı (n=40), Simental (n=94) ve İsviçre Esmeri (n=64) sığır ırklarında büyüme hormonu (bGH) geninin *Alu*I RFLP polimorfizminin incelenmesi amacıyla yapılmıştır. Büyüme hormonu-*Alu*I polimorfizmi için toplam 246 baş sığır polimeraz zincir raksiyonu-restriksiyon parça uzunluk polimorfizmi (PZR-RFLP) ile genotiplendirilmiştir. Bu çalışmada, *Alu*I enzim kesimi sonucu iki allel (L ve V) ve üç genotip (LL, VV ve LV) belirlenmiştir. DAK, SIM, İsviçre Esmeri ve Zavot sığır ırkları için allel frekansları; L alleli için sırasıyla 0.775, 0.734, 0.781 ve 0.760; V alleli için sırasıyla 0.225, 0.266, 0.219 ve 0.240 bulunmuştur. Diğer taraftan, genotipik frekanslar LL genotipi için sırasıyla 0.65, 0.57, 0.63 ve 0.63; VV genotipi için 0.10, 0.11, 0.06 ve 0.10; LV genotipi için ise; 0.25, 0.32, 0.31 ve 0.27 bulunmuştur. Çalışılan ırklarda Hardy-Weinberg dengesinden sapma görülmemiştir. Sonuç olarak, bu çalışmada dört sığır ırkında bGH gen polimorfizmi hakkında bilgi verilmiştir. Ayrıca, bu çalışma ile Zavot sığırında bGH gen polimorfizmi varlığı ilk defa bildirilmiştir.

Anahtar sözcükler: *Büyüme hormonu, Sığır, Zavot, Polimorfizm*

INTRODUCTION

The bovine growth hormone (bGH) gene has been intensively studied in livestock because of its effects on growth, body composition, metabolism regulation, lactation and mammary gland development [1-3]. Therefore, there is an interest in the growth hormone (GH) gene polymorphism to improve production traits in farm animals and GH gene has been suggested as a putative candidate for variability. bGH is a single copy gene spanning 1800bp on the chromosome region 19q26-qter and consists of five exons [4].

Growth hormone gene may an important candidate genetic marker for growth and milk yield traits in livestock [5]. Polymorphisms were detected in the fifth exon [6], the third intron [7,8] and the 3 'UTR region [9] of bGH gene. One of the most investigated is the *Alu*I restriction site situated in the fifth exon region. The *Alu*I restriction site polymorphisms of GH gene have been previously reported in dairy cattle [10,11], Bavarian Simmental [12], Indian native cattle breeds [13], Hereford and composite cattle breeds [14]. There are several studies on

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the relationship between bGH genotypes and milk yield traits in cattle [15,16], sheep [17] and goat [18]. The results of these studies have demonstrated that the *Alu*I polymorphism of bGH gene can be used as a potential marker for milk yield, protein and fat content in milk [19]. In addition, there are some other studies on the association between GH genotypes and growth traits in goat [20,21], in sheep [22] and in cattle [23].

Three different genotypes (LL, VV and LV) for *Alu*I restriction have been reported [24]. bGH was shown to be polymorphic in many cattle breeds, being that the distribution of GH variants (LL, LV and VV) and their frequencies differ among each breed [6,9,16,18]. Several studies have reported an association between allelic variants of this gene with high fat and high protein contents in milk [25]. In another study, the V allele was reported favorable for milk yield, fat yield and protein yield [26]. Yardibi et al.[27] reported an association between bGH-*Alu*I polymorphism and milk fat percentage in East Anatolian Red (EAR) and South Anatolian Red (SAR) cow two of the native cattle breeds of Turkey. Akyüz et al.[28] have reported that the highest frequency of the L allele has been found in the SAR breed (0.938), which has the highest milk yield in Turkish native cattle breeds, and the high L allele frequency has been found in EAR breed (0.898). Dario et al.[29] reported that daily milk yield in the LL genotype was higher than in the LV genotype.

Various cattle breeds are raised in different regions of Turkey. Nearly 50% of Turkey's cattle population is consisted of European originated cattle (Holstein, Brown Swiss, Simmental, Jersey) and their crosses [30]. Simmental has a special place among them with more meat producing capacity besides the milk yield on Anatolian highlands [30,31]. Brown Swiss have higher fattening performance [31]. East Anatolian Red (EAR) breed raised in the eastern Anatolian region. An important part of the meat requirement is provided from EAR breed in Turkey. EAR are well suited to the harsh climate and poor pasture [31]. Zavot cattle have been in Kars and Ardahan provinces in Northeast Anatolian region for more than 150 years. It is generally accepted that the Simmental and Brown Swiss genotypes have played an important role in the construction of the Zavot breed [32,33]. Zavot breed raised for milk and meat production [31].

The aim of the present study was to investigate of *Alu*I polymorphism at exon 5 of the bGH gene in Zavot, EAR, (Simmental) SIM, (Brown Swiss) BS cattle breeds by PCR-RFLP.

MATERIAL and METHODS

Samples and DNA Isolation

A total of 246 blood samples were collected from Zavot (n=48, Ardahan), SIM (n=94, Kayseri-Nevşehir), BS (n=64, Kayseri) and EAR (n=40, Erzurum and Kars) cattle breeds. The blood samples were placed into tubes containing EDTA for DNA isolation. Genomic DNA was isolated using the phenol-chloroform-isoamlyalcohol (25:24:1) method [34]. The quality of DNA was checked on 0.8% agarose gels and stained with ethidium bromide.

DNA Amplification and Genotyping

The genotyping for bGH-*Alu*I polymorphism was performed by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) according to the method proposed by Chrenek et al.[35]. PCR products were amplified using primers (gene accession number EF592534.1): forward 5'-GCT GCT CCT GAG GGC CCT TCG-3' and reverse 5'-GCG GCG GCA CTT CAT GAC CCT-3'. PCR for the bGH gene was performed in a 25 µl reaction mixture, containing 1.5mM $MgCl_2$, 200 µM of each dNTPs, 5 pmol of each primer, 1X PCR buffer, 1U Taq polymerase and 100 ng of genomic DNA template. Thermal cycling conditions included: an initial denaturation step at 95°C for 4 min followed by 35 cycles of 94°C, 60°C, 72°C each for 40s and a final extension step at 72°C for 5 min. The PCR products were digested with 10 U of *Alu*I restriction endonuclease (Fermentas) at 37°C for at least 3.5 h. The 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 bGH gene *Alu*I 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 [36].

RESULT

By using PCR a 223 bp fragment was successfully amplified *(Fig. 1)* and this fragment was digested with *Alu*I restriction enzymes to detect the presence of L or V variants. PCR-RFLP with the *Alu*I enzyme revealed the polymorphic site. As a result of digesting the amplification product with, two alleles, L and V, were observed. Restriction digestion of 223 bp PCR products with *Alu*I enzymes revealed three genotypes of VV (223 bp), LL (171 and 52 bp) and LV (223, 171 and 52 bp). An example of gel photograph showing the polymorphisms of the amplified product cut by *Alu*I restriction enzyme were shown in *Fig. 2*. The allelic and genotypic frequencies of the bGH gene polymorphism for the EAR, SIM, BS and Zavot cattle were given in *Table 1*. The results of Chi-square statistic reflected that breeds were in Hardy-Weinberg equilibrium.

DISCUSSION

The bovine growth hormone gene can be used as potential candidate genetic marker for growth, body composition, metabolism regulation, lactation and mammary gland

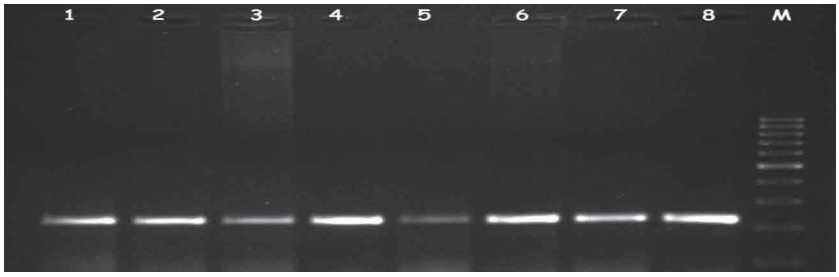

Fig 1. PCR amplifications of bGH gene (223bp, lanes 1-8). Lane M, molecular size marker (100 bp DNA ladder)

Şekil 1. bGH geninin PZR ürünleri (223bp, hat 1-2). Hat M, moleküler büyüklük belirteci (100 bç DNA ladder)

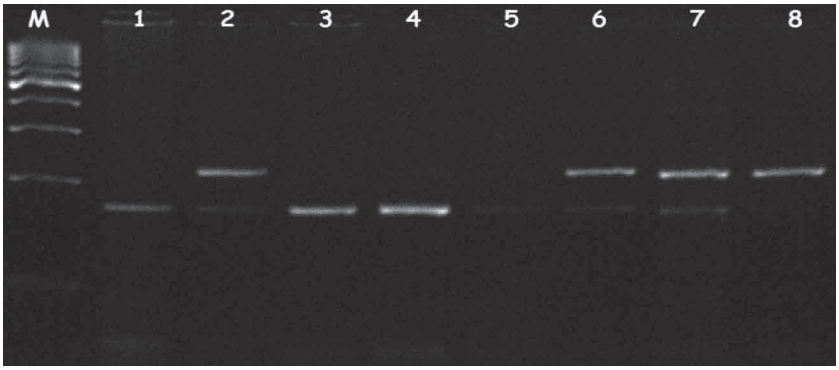

Fig 2. Photograph of *Alu*I enzyme digestion products of bovine GH gene on agarose gel. Lane 8; VV (223bp), Lane 2,6,7; LV (223bp/171bp/52bp, Lane 1,3,4,5; LL (171bp/52bp) genotypes. Lane M, molecular size marker (100 bp DNA ladder)

Şekil 2. Sığır GH geninin *Alu*I enzim kesim ürünlerinin agaroz jeldeki fotografı. Hat 8; VV (223bç), Hat 2,6,7; LV (223bç/171bç/52bç), Hat 1,3,4,5; LL (171bç/52bç) genotipleri. Hat M, moleküler büyüklük belirteci (100 bç DNA merdiveni)

Table 1.*Allele and genotype frequencies of bGH gene for AluI site in the EAR, SIM, BS and Zavot cattle breeds*
Tablo 1. *Zavot, DAK, Simental ve İsviçre Esmeri sığır ırklarında bGH geninin AluI bölgesinin allel ve genotip frekansları*

Breed	n	Genotype						Allele Frequency		χ2	χ2 P-Value	G²	G² P-Value
		LL		VV		LV		L	V				
		Obs (Exp)	F	Obs (Exp)	F	Obs (Exp)	F						
EAR	40	26 (23.937)	0.65	4 (1.937)	0.10	10 (14.126)	0.25	0.775	0.225	3.581	0.058^{NS}	3.192	0.074^{NS}
ZAV	48	30 (27.663)	0.63	5 (2.663)	0.10	13 (17.674)	0.27	0.760	0.240	3.484	0.062^{NS}	3.180	0.075^{NS}
SIM	94	54 (50.551)	0.57	10 (6.551)	0.11	30 (36.898)	0.32	0.734	0.266	3.341	0.068^{NS}	3.170	0.075^{NS}
BS	64	40 (38.977)	0.63	4 (2.976)	0.06	20 (22.047)	0.31	0.781	0.219	0.569	0.451^{NS}	0.540	0.462^{NS}

F: *Frequency;* ***NS:*** *Non significant*

development, milk yield traits in livestock. Therefore, studies were conducted genetic polymorphism of the bGH in different cattle breeds [19,28,29]. We investigated this genetic polymorphism of the bGH in EAR, SIM, BS and Zavot cattle breeds. Especially, this study showed the existence of a genetic polymorphism at bGH gene in Zavot breed for the first time. Digestion of amplification product with *Alu*I restriction enzyme for bGH gene revealed two alleles namely, L and V and three genotypes (LL, VV and LV).

The findings of the present study on allele and genotype frequencies were similar to those reported in the literature [37,38]. Jakaria et al.[39] reported that the L allele frequency of GH *Alu*I loci was higher for cattle with European origin [40]. bGH polymorphism has been investigated with 164 Jersey cows by Dario et al.[29]. They reported that the frequency of LL (0.22) genotype was found to be lower than LV (0.61) and VV (0.17) genotypes and then the genotypic frequencies of LL and VV genotypes were very close. Additionally Dario et al.[29] showed that the frequency of V (0.48) allele at bGH locus was lower compared to the frequency of L (0.52) allele. This was consistent with the result of the breeds reared in Turkey. A higher frequency of L allele (0.898 and 0.830) bGH gene was found for EAR and BS, respectively [28]. This finding on allele frequency was similar to that reported for EAR and BS (0.775 and 0.781) in this study. Ozdemir [41] has shown that L allele is predominant and its frequency is ranging from 0.893 to 0.976 in Turkish Grey Breeds and EAR, respectively. Similarly, Ozkan et al.[42] reported that L allele was predominant with frequencies 0.842 in EAR and 0.867 in Turkish Grey. However, Yardibi et al.[27] found that bGH V gene was predominant as 0.570 in EAR. Previously, polymorphism studies about bGH locus for EAR and BS breeds [28] showed higher frequencies of LL genotype in comparison with the present study. In a similar study, a higher freguency of L allele (0.976 and 0.905) bGH gene was reported for EAR and BS, respectively [41].

The revealing polymorphism of economically important genes is necessary to explain to the genetic structure of animal population and configuration of selection program. Therefore more comprehensive studies using further loci associated with economically important yields and including pedigree records are needed. Further investigation

is necessary to perform statistical analysis aiming the existence of association between bGH-*Alu*I genotypes and growth, milk traits in the Turkish breed of cattle.

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