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*Hae*III Polymorphism of Growth Hormone (GH-1) Gene in Some Goat Breeds Reared in Turkey by Using PCR-RFLP Method

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Abstract
The purpose of this study is to determine *Hae*III polymorphism in the exon 2 and 3 regions of the growth hormone -1 (GH-1) gene that regulates tissue growth and muscle development in six different goat breeds reared in Turkey. The *Hae*III polymorphism in GH-1 gene (422 bp) was investigated by Restriction Fragment Length Polymorphism (PCR-RFLP) methodology in 303 goats including 52 Hair, 51 Angora, 50 Honamlı, 50 Halep, 50 Saanen and 50 heads of Kilis breeds. Two alleles (A and B) and 3 genotypes (AA, BB and AB) were identified in the study. A and B allele frequencies in Hair, Angora, Honamlı, Halep, Saanen and Kilis breeds were found to be 0.4038 and 0.5962, 0.4314 and 0.5686, 0.4600 and 0.5400, 0.4500 and 0.5500, 0.3800 and 0.6200, 0.5400 and 0.4600, respectively. AA, AB and BB genotype frequencies were found to be 00.019, 0.769 and 0.212 ($P<0.05$), 0.235, 0.392 and 0.373 ($P>0.05$), 0.080, 0.760 and 0.160 ($P<0.05$), 0.060, 0.780 and 0.160 ($P<0.05$), 0.160, 0.440 and 0.400 ($P>0.05$), 0.160, 0.760 and 0.080 ($P<0.05$), respectively. When all breeds were taken into consideration, A and B allel frequencies were found to be 0.4439 and 0.5561, and AA, AB and BB genotype frequencies were found to be 0.119, 0.650 and 0.231 ($P>0.05$). As a result, it was determined that goat population had high heterozygosity (0.494) in general and was not in Hardy-Weinberg equilibrium in terms of *Hae*III polymorphism in the exon 2 and 3 regions of the GH-1 gene ($P>0.05$).

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1.Introduction

In the world, there are 1 034 406 504 goats are reared and statistically classified as 53.3% in Asia, 40.9% in Africa, 3.6% in the USA, 1.6% in EU and 0.4% in the Oceania, respectively. Goats are mostly reared in China, India, Nigeria, Pakistan, Bangladesh, Chad Republic, Sudan, Ethiopia, Mongolia, and Kenya countries, respectively (FAO, 2017). Turkey owns 10 992 427 goats and approximately 98% of the total goat is made up of Hair goats and hybrids and 2% of them is Angora goats. Its share in livestock is 17.35% (Anonim, 2018). When expressed in goat breeding in Turkey comes to mind Hair goat breeding. Small ruminant breeding, weak pastures in general, fallow, stubble and vegetable production by evaluating areas not suitable for meat, milk, wool, hair and leather products. Turkey's natural resources, generally meadow-pastures are more suitable for the breeding of sheep and goat species, reasons such as consumption habits of rural people; it is suitable for ovine breeding. Despite the importance of sheep and goat breeding, there are significant decreases in the number of sheep and goats in recent years, although it causes a decrease in production (Kaymakci et al., 2006). Recently, some improvement has been achieved through incentive and rehabilitation projects in this field. Goat breeding is a traditional animal production branch, which is generally performed in underdeveloped and developing countries. It is an important source of livelihood and food for families with low income in rural and forested regions. Another feature is the use of mountainous, heathland and stony lands that cannot be evaluated in any other way for the production of goats and products such as milk and meat (Kaymakci, 1997).

Since the increase in yield in farm animals will be possible by improving genotype and/or environment, having knowledge about the genetic structure of populations enhances the success of breeding. In particular, there is a high correlation between the polymorphic properties and the traits emphasized. The presence of criteria such as the feasibility of early detection and non-gender dependence makes a significant contribution to the breeders in selection. To date, many candidate genes have been studied in farm animals and the relationships between these genes and yield characteristics of economic importance have been investigated. Growth hormone (GH) gene is one of these genes in goats, is a protein hormone secreted from eosinophilic cells in the anterior lobe of the pituitary. The growth stopped in animals with pituitary gland removed and of growth hormone from the outside by detecting the continuity of the growth by administering GH through which determined the main task of growth. Growth hormone released from the pituitary gland except for postnatal growth, bone in the body, muscle, acting on adipose tissue; glucose, protein, lipid metabolisms, nitrogen, and mineral balance. Growth hormone, the product of the Growth Hormone (GH) gene, is the main regulator of postnatal growth and metabolism in mammals. Growth Hormone, growth rate, body composition, health, milk yield and the expression of several genes involved in these processes by regulating aging (Ge et al., 2003). Therefore, by the role of GH gene in milk production and growth regulation, it is thought to be a good candidate gene for identifying carcass and immune system related genetic markers in the livestock breeding (Yao et al., 1996; Ge et al., 2003). The entire sequence of the growth hormone (GH) gene for goats is reached in the GenBank database with access number NC_030826 and 1634 bp (Anonymous, 2017). The GH gene is 2.6 to 3.0 kbp in most mammals and consists of 5 exons and 4 introns on chromosome 19 (19q22) in goats (Fries, 1993; Wallis, 1998; Wickramaratne et al., 2009; Dettori et al.,

2013). The aim of this study was carried out to determine *Hae*III polymorphism in exon 2 and 3 of the GH-1 gene in 6 different goat breeds reared in Turkey.

2.Material and Methods

2.1. Blood sampling

In this study, a total of 303 blood samples were obtained from 52 heads Hair goats, 51 heads Angora goats, 50 heads Honamlı goats, 50 heads Halep goats, 50 heads Saanen goats and 50 heads Kilis goats. Blood sample was collected from jugular vein by using vacutainer tubes containing with 10 ml Ethylene Diamine Tetra Acetic (EDTA) tubes and brought to Selçuk University Faculty of Agriculture Department of Animal Science Biotechnology Laboratory and then stored in refrigerator (-20ºC) before DNA isolation.

2.2. DNA extraction

The DNA isolation was performed using the Quick Gene DNA (DB-S) kit (Fujifilm Corp., Tokyo, Japan). After DNA isolation, all samples were measured on a Nano drop spectrophotometer (ND1000; Nano Drop Technologies, USA) and DNA concentrations were determined.

2.3. Amplification and genotyping of DNA target (PCR-RFLP)

The PCR amplification of the 422 bp region in the exon 2 and 3 regions of the GH-1 gene was used. After DNA isolation, all samples were measured on a Nano drop spectrophotometer (ND1000; Nano Drop Technologies, USA) and DNA concentrations were determined. In PCR amplification of the 422 bp region in the exon 2 and 3 region of the GH-1 gene, primers with nucleotide sequences F: 5'-CTCTGCCTGCCCTGGACT-'3 and R: 5'-GGAGAAGCAGAAGGCAACC'-3 (Hua et al., 2009).

The PCR reaction was performed in 2 -µL DNA, 1X Master mix, 0.25 µM primers and 5.5 µL ddH_2O with a total concentration of 10 µL ul reaction volumes. The amplification was performed in a gradient thermal cycler (Techne TC-512) using the following program: an initial denaturation step at 94 °C for 5 min, followed by 35 cycles of 95 °C for 30 s, 62.7 °C for 30 s and 72 °C for 45 s. Final extension was at 72 °C for 7 min. To determine the polymorphism in the gene region of interest, 10 µL of PCR product, 1 µL 10 U *Hae*III restriction enzyme, 2 µL 10X Buffer and 7µL ddH_2O were added and treated for 30 min at 37°C. DNA samples amplified by RFLP method and cut with *Hae*III restriction enzyme were carried by electrophoresis on a 2% agarose gel and visualized on UV transiluminator. To identify the allele variation, PCR products obtained from the target gene was then analyzed by using RFLP with *Hae*III restriction enzymes with the cutting site.

2.4. Statistical analysis

PopGene32 (ver. 1.32) statistical program was used to analyze whether the distribution of the genotype frequencies was in the Hardy-Weinberg equilibrium (Yeh et al., 1997).

3.Results and Discussion

3.1. Amplification GH-1 gene

GH-1 gene region amplified by the method and the genotypes obtained by cutting enzyme *Hae*III were given in Figure 1.

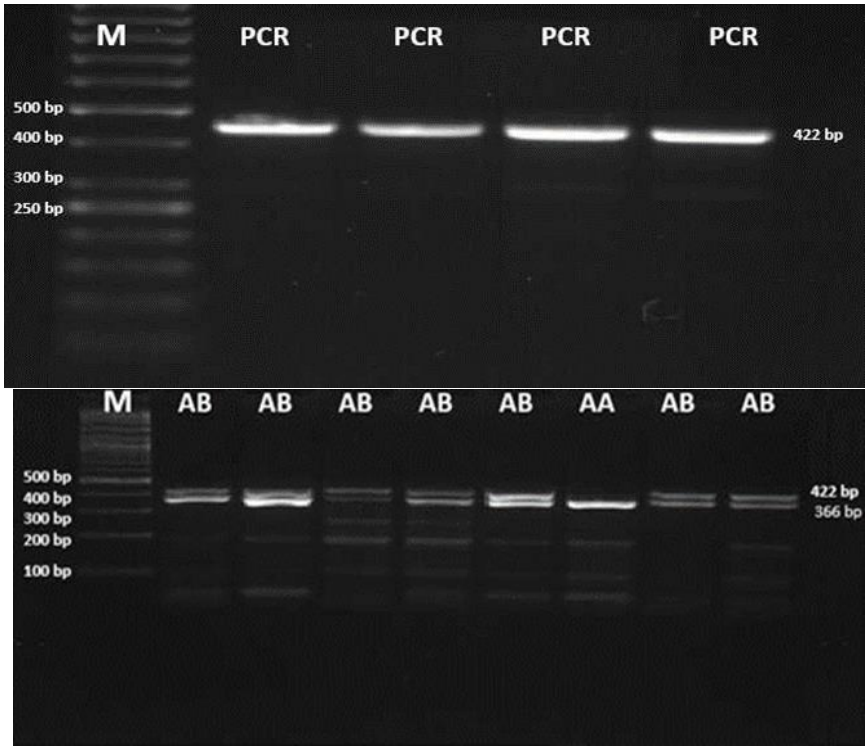

Figure 1. Agarose gel electrophoresis of PCR (left) and digested products (right), M = 100 bp DNA ladder, Uncut ting PCR product (422 bp), *Hae*III cutting size, AA genotype (422 bp), BB genotype (363bp) ve AB genotype (422, 363 ve 116 bp), M: 50 bp and 100 bp DNA Ladder

3.2. Genotype and allele's frequency and Hardy Weinberg equilibrium

The GH-1 / *Hae*III polymorphisms in terms of genotype and allel frequencies of some goat breeds of Turkey were given in Table 1.

In this study, obtained as a result of statistical analysis in which general breeds were evaluated together; 2 alleles (A and B), 3 genotypes (AA, AB and BB) were detected. At 303 heads of six goat breeds as a result of GH-1 / *Hae*III polymorphism were identified as 36 AA, 197 AB and 70 BB genotypes as the number of animals. A and B allele frequencies 0.4439 and 0.5561 respectively, the frequencies for AA, AB and BB genotypes were 0.119, 0.650 and 0.231, respectively. Also, Genotype and alleles frequencies distributions of the breeds except for Saanen and Angora breeds was not in Hardy-Weinberg equilibrium (HWE) ($P<0.05$).

Table 1. The GH-1/ *Hae*III polymorphisms in terms of genotype and allele frequencies in different 6 goat breeds of Turkey

		Genotype frequencies			Allele frequencies		He	χ^2
Breeds	**N**	**AA (n)**	**AB (n)**	**BB (n)**	**A**	**B**		
Hair	52	0.019 (1)	0.769 (40)	0.212(11)	0.4038	0.5962	0.482	18.567*
Angora	51	0.235 (12)	0.392 (20)	0.373(19)	0.4314	0.5686	0.491	2.053
Honamlı	50	0.080 (4)	0.760 (38)	0.160 (8)	0.4600	0.5400	0.497	14.034*
Halep	50	0.060 (3)	0.780 (39)	0.160(8)	0.4500	0.5500	0.495	16.575*
Saanen	50	0.160 (8)	0.440 (22)	0.400(20)	0.3800	0.6200	0.471	0.219
Kilis	50	0.160 (8)	0.760 (38)	0.080(4)	0.5400	0.4600	0.497	14.034*
Total	303	0.119 (36)	0.650 (197)	0.231(70)	0.4439	0.5561	0.494	30.431*

χ^2: Hardy-Weinberg equilibrium test; He: expected heterozigosity; *P<0.05

GH-I gene in terms of *Hae*III polymorphism, genotypes AA, AB and BB were determined in Hair, Angora, Honamlı, Halep, Saanen and Kilis goats (Table 1). The chi-square test showed agreement to Hardy-Weingberg equilibrium except Angora and Saanen goat breeds.

Hua et al. (2009) studied the polymorphism of growth hormone (GH) gene was analyzed as a genetic marker candidate for growth traits in Boer goat bucks at birth, weaning, at the age of 11 months. PCR products of the growth hormone gene (2064 bp) in their study were digested using the *Hae*III restriction enzyme. Because of restriction, four alleles (A, B, C and D) and only 4 genotypes (AA, AB, CC and CD) were observed. Researchers reported the genotype frequencies in order 0.1623, 0.8377, 0.8571 and 0.0974. According to the results obtained from the study AB and AA genotype, goats are reported to be 2 kg heavier than the other groups at weaning; it affects the growth rate that is why the growth hormone gene can be used as a marker gene. Zhang et al. (2011), in their study were investigated in both a high prolificacy (Matou, n=182) and a low prolificacy breed (Boer, n=352) by using the PCR-RFLP method. Genotypes (AA and AB, CC and CD) in each mutation were detected in these two goat breeds. Neither BB nor DD homozygous genotypes were observed. The genotypic frequencies of AB and CC were significantly higher than those of AA and CD. In the third parity, Matou dams with AB or CC genotypes had significantly larger litter sizes than those with AA and CD ($P<0.05$). On combining the 2 loci, both Matou and Boer dams with ABCD genotype had the largest litter sizes when compared to the other genotypes ($p< 0.05$). These results show that the 2 loci of GH gene are highly associated with abundant prolificacy and superovulation response in goat breeds. Alakilli et al. (2012), in this study, Egyptian and Saudi goat breeds (Barki, Zaribi, Ardi and Masri), to detect the genotype of Growth Hormone GH-1 gene, exons 2 and 3 which encloses a *Hae*III endonuclease restriction site show four unique PCR-RFLP banding patterns (genotypes AA, AB, CC and CD). The frequencies of the A allele in the samples from the goat breeds varied from 0.410 to 0.620. Othman et al. (2015), reported that the Growth Hormone GH gene, exon 2 and 3, was polymorphic in sheep and goat breeds in Egypt. The results obtained in this study; 2 GG and AG genotypes were found, GG and AG genotype frequencies; 43.56% and 56.44% respectively in 101 sheep and goat animals, 48 were tested, the total frequency of GG and AG genotypes in order; 12.5% and 87.5%. Increasing this heterozygous genotype in ovine breeds has the AG genotype of the GH gene through selection of animals and has been recommended to breeding programs of Egyptian ovine as a way of enhancing production properties. Singh et al. (2015), in the study, total of 80 kids involving forty each of Sirohi and Barbari breeds of goat were included in the study. *Hae*III polymorphism of exon 2 and exon 3 of Growth Hormone (GH-1) gene were introduced. The PCR product of genomic DNA isolated from kids of Sirohi and Barbari breeds of goat on digestion with the restriction enzyme *Hae*III revealed 2 genotypic variants as, AB and BB. None of the 2 breeds was in Hardy–Weinberg equilibrium for these variants. The least squares analysis of variance revealed non-significant effect of GH genotype and breed × genotype interaction on chest girth and paunch girth from birth to 180 days of age. The effect of breed was highly significant ($P<0.01$) at all ages Mete et al. (2016). This study was carried out on Hair goat kids to determine polymorphism of growth hormone (GH-1) gene, then; both PCR products were digested with same restriction endonucleases enzyme, *Hae*III, to determine nucleotide polymorphisms. As

a result of analyzed genotype data that obtained from images, genotype frequencies of 0.19, 0.65 and 0.16 were obtained for AA, AB and BB genotypes of GH-1 region and allele frequencies of 0.51 and 0.49 were obtained for A and B alleles, respectively. In the analysis of the growth characteristics of Capricorn, it was found that the genotypes obtained for the 2 gene regions examined had no significant effect on the growth characteristics Ilham et al. (2016). The purpose of this study is to identify the *Hae*III /GH-1 gene polymorphism in Kacang goat with Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) methods. 168 blood samples from Kacang goat in two regions of Indonesia (Gorontalo and South Sulawesi province) were used. The results obtained two kinds of genotypes, with the AA genotype frequencies (0.095) and AB (0.904). The frequency of allele A (0.547) and B (0.452) indicate a polymorphism in the GH-1 A781G locus in Kacang goat. The observed (Ho) and expected (He) heterozygosity value were respectively 0.0904 and 0.496. GH-1 allele's distribution in Kacang goat populations were not in hardy Weinberg equilibrium Mahrous et al. (2018). The objective of this study was to identify GH-1 gene variants in three goat breeds (Barki, Damascus, and Zaraibi), via polymerase chain reaction–restriction fragment length polymorphism (PCR–RFLP) and gene sequencing analyses. GH1-*Hae*III/RFLP showed only two genotypes (AB and BB) in all breeds, with absent AA genotype. Shareef et al. (2018) in current study, polymorphism in Growth Hormone (GH-1) and Insulin-like Growth Factor-1 (IGF-1) genes and their association with growth-related traits were studied in Beetal goat. (AA, AB and BB) genotypes were detected. Homozygous carrier genotype (BB), body weight higher (47.13±0.78kg) reported that significantly correlated ($P<0.05$). Bayan et al. (2018), planned to study GH gene exon 2-3 polymorphism using Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) in Surti and Mehsani goats. GH gene exon 2-3 region was found to be polymorphic on restriction digestion with *Hae*III which revealed 2 genotypes AA and AB with genotypic frequencies of 0.24 and 0.76 in Surti goats and 0.20 and 0.80 in Mehsani goats respectively. Both the population of Surti and Mehsani goats were not found to be in genetic equilibrium for GH locus exon 2-3 indicating selection pressure for growth. Rony et al. (2020), this study was identify of polymorphism GH gene of Lakor goat in Lakor island, DNA was extracted from hair follicles. A 422 bp specific DNA fragment was successfully amplified and genotyped by PCR-RFLP method using *Hae*III enzyme. 2 variant of genotypes (AA and AB) and 2 alleles (A and B). AB genotype was dominant in all of populations (93.7%) with A and B alleles were 0.53 and 0.47, respectively. Heterozygosity observed and expected value reached 0.502 and 0.498, respectively while Polymorphic Information Content was in moderate values (0.374). All of populations were in disequilibrium genetic.

The *Hae*III polymorphism of GH-1 gene of Hair, Angora, Honamlı, Halep, Saanen and Kilis goats as well as the genetic structure and allele frequencies of these polymorphisms were determined. The results obtained from this study conducted for the purpose of growth performance of the related polymorphism were evaluated by comparing with other studies on the subject.

4.Conclusion

As a result, AA, AB and BB genotypic frequency of goat breeds reared in Turkey in terms of GH-1/*Hae*III polymorphism was found to be as 0.019, 0.769 and 0.212 in Hair goats, 0.235, 0.392 and 0.32 in Angora goats, 0.080, 0.760 and 0.160 in Honamlı goats, 0.060, 0.780 and 0.160 in Halep

goats, 0.160, 0.440 and 0.460 in Saanen goats and 0.160, 0.760 and 0.080 in Kilis Goats in this study respectively. Polymorphism in GH-1 Exon 2 and 3, *Hae*III section in hair, Ankara goat, Honamlı, Aleppo, Saanen and Kilis goat breeds detect genetic polymorphism of PCR-RFLP method. The chi-square test showed that genotypes were not under the Hardy-Weinberg equilibrium except for Saanen and Angora breeds, indicating that GH-1/*Hae*III exon 2-3 shows that there are some disruptive facrs for some goat breeds (P>0.05).

Declaration of Author Contributions

The authors declare that they have contributed equally to the article. All authors declare that they have seen/read and approved the final version of the article ready for publication.

Declaration of Conflicts of Interest

All authors declare that there is no conflict of interest related to this article.

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