How to cite: Yılmaz, H.C., Bilgen G., IGF-1/CaC8I, LEP/NmuCI and PRLR/Hin1II Polymorphisms in Saanen Goats, J. Anim. Prod., 2022, 63 (1): 15-20, https://doi.org/10.29185/hayuretim.1018577

Research Article
(Araştırma Makalesi)

J. Anim. Prod., 2020, 63 (1): 15-20
https://doi.org/10.29185/hayuretim.1018577

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* Bu makale sorumlu yazarın doktora tezinden elde edilmiştir.

Key words:
DNA markers, PCR-RFLP, single nucleotide polymorphism, nasal swab

Anahtar Kelimeler:
DNA belirteçleri, PCR-RFLP, tek nükleotid polimorfizmi, nazal sürüntü

IGF-1/CaC8I, *LEP*/NmuCI and *PRLR*/Hin1II Polymorphisms in Saanen Goats*

Saanen Keçilerinde *IGF-1*/*Cac*8I, *LEP*/*Nmu*CI ve *PRLR*/*Hin*1II Polimorfizmleri

Alınış (Received): 03 11 2021 Kabul tarihi (Accepted): 28 02 2022

ABSTRACT

Objective: This study was aimed to identify *IGF-1*, *LEP* and *PRLR* polymorphisms by PCR-RFLP method in Saanen goats.

Material and Methods: In the study, nasal samples were collected from 72 Saanen goats via swabs and DNA was extracted. Polymorphisms of *IGF-1*, *LEP* and *PRLR* were determined by PCR amplification followed by RFLP method using restriction enzymes *Cac*8I, *Nmu*CI and *Hin*1II, respectively.

Results: *LEP*/*Nmu*CI produced three genotypes as TT, TC and CC with frequencies 90.3, 6.9 and 2.8%, respectively and two alleles (T=93.8%, C=6.2%). The frequencies of genotype for *PRLR* gene were CC (90.3%), CT (5.5%) and TT (4.2%) and allele frequencies were C (93.1%) and T (6.9%). The *IGF-1*/*Cac*8I locus was monomorphic in Saanen goat population.

Conclusion: The present results may be useful for future studies conducted on goat genome with respect to *IGF-1*, *LEP* and *PRLR* genes.

ÖZ

Amaç: Bu çalışmada Saanen keçilerinde *IGF-1*, *LEP* ve *PRLR* polimorfizmlerinin PCR-RFLP yöntemi ile saptanması amaçlanmıştır.

Materyal ve Yöntem: Çalışmada, 72 Saanen keçisinden sürüntü yoluyla burun içi epitel hücre örnekleri toplanmış ve DNA izolasyonu yapılmıştır. *IGF-1*, *LEP* ve *PRLR* polimorfizmleri, sırasıyla *Cac*8I, *Nmu*CI ve *Hin*1II restriksiyon enzimleri kullanılarak PCR amplifikasyonunu takiben RFLP yöntemi ile belirlenmiştir.

Bulgular: *LEP*/*Nmu*CI lokusunda genotip frekansları TT (%90.3), TC (%6.9) ve CC (%2.8) ve allel frekansları T (%93.8), C (%6.2) şeklinde tespit edilmiştir. *PRLR*/*Hin*1II için genotip frekansları CC (%90.3), CT (%5.5), CC (%4.2) ve allel frekansları C (%93.1) ve T (%6.9) olarak hesaplanmıştır. *IGF-1*/*Cac*8I lokusunun popülasyonumuzda monomorfik olduğu tespit edilmiştir.

Sonuç: Bu sonuçların *IGF-1*, *LEP* ve *PRLR* genleri ile ilgili olarak keçi genomu üzerinde yapılacak gelecekteki çalışmalar için faydalı olabileceği düşünülmektedir.

INTRODUCTION

Goats have been an important source of milk, meat, fur, and skins throughout human history in a very wide geography of the world. It is estimated that the world goat population is 1 billion heads, 218 million of which are dairy goats, %95 of this is Asia and Africa continents (FAO 2019). The goat breeding is widespread especially in Aegean, Mediterranean, and South-Eastern Anatolia regions of Türkiye. Saanen goats are one of the most milk-producing goat breeds all over the world and Saanen goats and their hybrids

are widespread in Türkiye (Tölü et al. 2010). The studies on DNA markers of candidate genes of production traits in goats have an important role in goat selection and breeding. Insulin-like growth factor 1 (*IGF-1*), Leptin (*LEP*) and Prolactin Receptor (*PRLR*) genes are some of these candidate genes related to growth, meat quality, milk and reproduction traits.

IGF-1 gene takes a key part in development and growth regulation (Werner and Bruchim, 2009). *IGF-1* stimulates protein metabolism and it is important for the function of some organs as a factor of cell differentiation and proliferation (Yoshimura, 1998, Yu et al. 2004). *IGF-1* gene is located on the 5th chromosome of the goat and consists of 6 exons and 5 introns. The associations of SNPs in *IGF-1* gene and production traits have been investigated in livestock species, such as chicken (Li et al. 2008; Abdalhag et al. 2016) sheep (Darwish at al., 2017; Dettori et al. 2018; Bayram et. al. 2019) and cattle (Mullen et al. 2011; Gui et al. 2018; Daş et al. 2019). There are studies examining the relationship between IGF-1 gene polymorphisms and growth characteristics in goats. (Supakorn and Pralomkarn, 2013; Naicy et al. 2017; Shareef et al. 2018). Also, associations between *IGF-1* gene polymorphisms and cashmere traits were investigated by Shanaz et al. (2020).

Leptin is an enzyme that has pleiotropic effects on the regulation of body weight, energy homeostasis, fat deposition, reproduction and lactation. In mammals, plasma leptin level is highly associated with the body fat and energy balance when the amount of body fat increases, the plasma leptin level also increases, signaling the receptors in the hypothalamus to adjust the body's energy balance and appetite is suppressed (Leifers et al. 2003). The leptin gene is located on the 4th chromosome in goats consists of 3 exons and 2 introns, nevertheless only 2 of its exons are translated to protein (Shojaei et al. 2010). Leptin, which contains 146 amino acids, is first secreted from the adipose tissue and then enters the blood circulation after the 21 amino acid signal peptide is detached from the molecule (Işık and Özdil 2020). Associations between *LEP* gene and growth, milk and meat production traits in cattle were investigated in various studies (Kulig and Kmiec 2009; Curi et al. 2011; Anton et al. 2011; 2012; Trakovická et al. 2013; Kawaguchi et al. 2017; Kök and Vapur, 2021). There were studies conducted on the relationships between LEP gene polymorphisms and growth and meat quality traits in sheep (Boucher et al. 2006; Shojaei et al. 2010; Saleem et al. 2018). It has been stated that there were associations between *LEP* gene polymorphisms and some growth traits in goats (Wang et al. 2015). Similar studies have been carried out in goats, although they are limited in number according to studies on livestock.

The prolactin receptor, encoded by the PRLR gene, is a member of the growth hormone/prolactin receptor gene family, which contains the same DNA sequence regions. The *PRLR* gene is located on the 20th chromosome in goats and consists of 10 exons and 9 introns. Prolactin exerts its effects by binding to PRL receptors on target cells. This structure contributes to the activation of JAK2 kinases (Janus kinase 2) and subsequent phosphorylation of STAT5 (signal transducer and activator of transcription 5) transcription factors that regulate intracellular transcription of milk protein genes. The JAK-STAT signaling pathway transfers information from extracellular chemical signals to the nucleus, and this mechanism results in DNA transcription and the expression of related genes. For this reason, it is stated that the *PRLR* gene is a crucial candidate gene associated with milk, protein and fat yield in livestock (Baran et al. 2002; Işık and Bilgen 2019). Polymorphisms in *PRLR* gene are associated with growth, meat, reproduction milk quality traits in livestock (Lu et al. 2011; Di et al. 2011; Hou et al. 2013; 2014; Li et al. 2011; Cosenza et al. 2018; Liang et al. 2019).

IGF-1, *LEP* and *PRLR* are candidate genes for various production traits which affect metabolism with different signal pathways. Considering these genes affect some similar traits in animals, evaluating these genes and their interactions with each other can provide a better knowledge of their effects. To the best of our knowledge, there is no polymorphism study with *IGF-1*, *LEP* and *PRLR* genes all together has been investigated in goats. The aim of the present study was to detect *IGF-1*, *LEP* and *PRLR* polymorphisms by using PCR-RFLP method in Saanen goats.

MATERIAL and METHOD

Collection of Epithelial Cells and DNA Isolation

In this study, nasal epithelial cell samples for the DNA isolation were obtained from a total of 72 Saanen goat raised in a private farm in Manisa province, Türkiye according to the animal experiments local ethics committee directive of Ege University. Nasal epithelial cells were collected via sterile nasal swabs by inserting a nasal swab to the animal's nostrils and rubbing firmly against the walls of nasal cavity for 30-40 seconds. Then swabs were put back into their sterile tube and

stored at +4 °C until DNA isolation process. The nasal swabs were swirled 30-60 s in 200 µL of 1x phosphate-buffered saline (PBS) prior to DNA isolation. Genomic DNA isolated from PBS solution with nasal epithelial cells samples by using Thermo Scientific™ GeneJET Genomic DNA Purification Kit. The isolated genomic DNA stored at -20 ℃ until use.

PCR-RFLP

A 294 bp fragment in 5′ flanking region of *IGF-1* were amplified by PCR according to Naicy et al. (2017). The primers of *IGF-1* gene (accession no: HQ731040) were; F: 5′TGAGGGGAGCCAATTACAAAGC 3′, R: 5′CCGGGCATGAAGACACACACAT 3′. For identification of polymorphisms in *LEP* gene, a 412 bp fragment in second exon of the gene was amplified by PCR according to Wang et al. 2015. The primer sequences for *LEP* gene (accession no: JQ739233) were; F: 5′ ATGTGGGTGGTAACGGAGCA 3′ and R: 5′ GGTGGGAGGCAAGGGAAGT. A 162 bp fragment in 5′ UTR region of *PRLR* gene was amplified by PCR according to Zhou et al. (2011). The primers of *PRLR* gene (accession no: EU678913.2) were; F: 5′ AGGGCTTCCATAGAGTCTCAG 3′, R: 5′ ACTGTAATC*CAC*TAGGCTCCTC 3′. The PCR mix was 25 µl total reaction volume including 100 ng genomic DNA, 2.5 µl 10X reaction buffer, 2.5 mM $MgCl^2$, 0.2 mM dNTP, 0.5 µM of each primer and 0.5 U of Taq DNA polymerase (Thermo Scientific). The PCR conditions were as follows: Initial denaturation at 95°C for 5 min, 38 cycles; denaturation at 94°C for 30 sec, annealing (*IGF-1*: 54.0 ℃, *LEP*: 58℃, *PRLR*: 59℃) for 60 sec and extension at 72°C for 90 sec, followed by a final extension at 72°C for 5 min.

Following to thermal cycling process, the PCR products were digested with different restriction enzymes for each gene. PCR products of *IGF-1* gene were digested with restriction enzyme *Cac*8I (NEB) using the protocol as follows: Digestion protocol was carried out in a 20 µl volume with 200 ng of the amplified DNA, 5 U enzyme, and 2 µl of manufacturer's buffer and incubated at 37°C for 3 hours. PCR products of *LEP* gene were digested with restriction FastDigest *Nmu*CI (Thermo Scientific) using the manufacturer's protocol as follows: A 15 µl volume with 200 ng of the amplified DNA, 0.5 U enzyme, and 1 µl of manufacturer's buffer and incubated at 37°C for one hour. PCR products *PRLR* gene were digested with 5U *Hin*1II (Thermo Scientific), 200 ng of the amplified DNA, 2 µl buffer in a total 20 µl volume. PCR and restriction fragments were sorted out by electrophoresis on a 3% agarose gel stained with SafeView™ Classic (abm).

Statistical Analysis

The number and frequency of alleles, the observed (Ho) and the expected (He) heterozygosities, and the Hardy-Weinberg equilibrium were calculated by using the PopGene (Yeh and Boyle 1999).

RESULTS and DISCUSSION

A 294 bp long PCR fragment of the *IGF-1* gene was amplified. The *Cac*8I digestion of the PCR products produced digestion fragments of 196 bp, 72 bp and 26 bp (26 is not visible) for GG homozygotes (Figure 1). The restriction site showed a monomorphic pattern. AG and AA alleles weren't detected in this population. Despite our results, the locus of *IGF-1* was investigated by Naicy et al. (2017) in Malabari and Attappady Black goats and detected 89 GG, 86 AG genotypes and 84 GG, 18 AA genotypes in Malabari and Attappady Black goats, respectively. It has been observed that growth traits (body height, body weight and chest circumference) in goats were significantly affected by *IGF-1* polymorphisms.

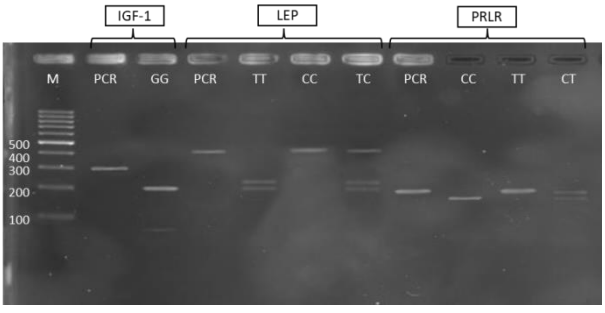

Figure 1. Genotypes of PCR-RFLP for the *IGF-1*, *LEP* and *PRLR* genes (M: Thermo Scientific™ GeneRuler 100 bp ladder. *IGF-1*: PCR product (294 bp), GG (196 bp, 72 bp and 26 bp (not visible)). *LEP*: PCR product (412 bp), TT (218 bp, 194 bp). CC (412 bp). TC (412 bp, 218bp, 194 bp). *PRLR*: PCR product (162 bp), CC (136 bp, 26 bp). TT (162 bp), CT (162 bp, 136 bp, 26 bp))

Şekil 1. *IGF-1, LEP ve PRLR genleri PCR-RFLP genotipleri (M: Thermo Scientific™ GeneRuler 100 bç merdiven. IGF-1: PCR ürünü (294 bç), GG (196 bç, 72 bç, 26 bç (jelde görünmüyor)). LEP: PCR ürünü (412 bç), TT (218 bç, 194 bç), CC (412 bç), TC (412 bç, 218, 194 bç). PRLR: PCR ürünü (162 bç), CC (136 bç, 26 bç), TT (162 bç), CT (162 bç, 136 bç, 26 bç))*

In the present study, a SNP in exon 2 (g.117 T>C) within the PCR product (412 bp) of the leptin gene was screened in Saanen goats. Digestion of the 412 bp PCR fragment with *Nmu*CI restriction enzyme generated two bands (218 bp and 194 bp) as homozygous TT, three bands (412 bp, 218 bp and 194 bp) as heterozygous TC and one band (412 bp) as

homozygous CC individuals (Figure 1). The genotypic frequencies of the TT, TC and CC genotypes were 90.3%, 6.9% and 2.8%, respectively and the allele frequencies of the T and C alleles were 93.8% and 6.2%, respectively (Table 1). A chi-squared ($\chi 2$) test showed that the population was not in Hardy-Weinberg equilibrium for this locus (P>0.05). The degree of heterozygosity obtained in the Saanen goat population for observed heterozygosity (Ho) value were 0.069, while for the expected heterozygosity (He) 0.118 (Table 1). *LEP* gene SNPs and their relations with growth traits were investigated by Wang et Al. (2015). *LEP* gene's same SNP locus was detected with PCR-RFLP method, and the major allele frequency (T) ranged between 0.583 and 0.987 within the five goat breeds. It has been stated that SNPs of the *LEP* gene were related with growth traits (body weight, length, height and chest circumference). Intron 2 of leptin gene polymorphism in goat breeds in Iran was revealed by using PCR-RFLP. Three genotypes including MM, Mm and mm were detected with genotype frequency of 94%, 1% and 5% in the goat population, respectively (Esmaeili et al. 2020). Furthermore, Genetic variations of the *LEP* gene and their associations with economic traits have been investigated in other farm animals. Significant associations were revealed between polymorphisms of the *LEP* gene and the dressed carcass weight and fatty acid compositions in Japanese Black Cattle (Kawaguchi et al. 2017). De Oliveira et al. (2013) revealed significant associations between the exon 2 polymorphisms of the *LEP* gene and various meat quality traits in Nellore cattle. Two SNPs in the exon 3 and intron 2 of *LEP* gene and their associations with milk traits were investigated by Kulig and Kmiec (2009) and it has been stated that *LEP* gene variations significantly affect milk, protein, and fat yield. Effects of *LEP* gene polymorphisms on reproductive efficiency in Awassi ewes was revealed by using DNA sequencing method and it was found significant associations between litter size and *LEP* polymorphisms (Younis et al. 2019).

Table 1. *LEP* and *PRLR* gene frequencies (chi-square ($\chi 2$), probability value (p-value), observed heterozygosity (Ho) and expected heterozygosity (He))

Çizelge 1. *LEP ve PRLR gen frekansları (ki-kare ($\chi 2$), olabilirlik değeri (p-value), gözlenen heterozigotluk (Ho) ve beklenen heterozigotluk (He))*

Locus		**Genotype Frequency**			**Allele Frequency**		**$\chi 2$**	**p-value**	**Heterozygosity**	
		TT	TC	CC	T	C			**Ho**	**He**
LEP	Observed	65 90.3%	5 6.9%	2 2.8%	0.938	0.062	13.63	0.000223	0.069	0.118
	Expected	63.25 87.8%	8.50 11.8%	0.25 0.4%						
		CC	CT	TT	C	T	**$\chi 2$**	**p-value**	**Ho**	**He**
PRLR	Observed	65 90.3%	4 %5.5	3 4.2%	0.931	0.069	26.11	0.000000	0.056	0.130
	Expected	62.31 86.6%	9.37 12.9%	0.32 0.5%						

Hardy-Weinberg equilibrium by the $\chi 2$- test, $\chi 2 = 3.81$ $P = 0.05$, $\chi 2 = 6.63$ $P = 0.01$

To identify the *PRLR* 5' UTR polymorphism, an SNP (g.970 C>T) within the PCR product (162 bp) of the leptin gene were screened in Saanen goats. Digestion of the 162 bp PCR fragment with *Hin*1II restriction enzyme generated two bands (136 bp and 26 bp) as homozygous CC, three bands (162 bp, 136 bp and 26 bp) as heterozygous CT, and one band (162 bp) as homozygous TT individuals (Figure 1). 26 bp is not visible on agarose gel. The genotypic frequencies of the CC, CT and TT genotype were 90.3%, 5.5% and 4.2%, respectively and the allele frequencies of the C and T alleles were 93.1% and 6.9%, respectively. The degree of heterozygosity for *PRLR* locus for Ho value was 0.056, while for He value 0.130 (Table 1). (Table 1). A chi-squared ($\chi 2$) test showed that the population was not in Hardy-Weinberg equilibrium for this locus (P>0.05) (Table 1). Zhou et al. (2011) investigated associations between the same *PRLR* 5′ UTR region SNP and fiber traits in cashmere goats. The genotypes were revealed CC genotype (509 goats), and CT genotype (81 goats) and also the allele frequencies were 93.14% for C and 6.86% for T. It has been stated

that *PRLR* gene variations significantly affect cashmere weight and diameter. The combined effects of four SNPs within *PRLR* gene on milk production traits were searched in goats by Hou et al. (2013) and it has been stated that *PRLR* gene variations might affect milk yield, fat and protein. Xiong et al. (2016) has searched *PRLR* gene SNPs and their relations with growth traits and litter size in goats and stated that *PRLR* gene variations significantly affect growth traits. It was also shown that the *PRLR* gene associates with litter size in goats in various studies (Li et al. 2011; Di et al. 2011; Hou et al. 2014; An et al. 2015).

CONCLUSION

IGF-1, *LEP* and *PRLR* genes are candidate genes for production traits such as growth, milk and reproduction traits. Various studies have been performed to reveal the relationships between *IGF-1*, *LEP* and, *PRLR* genotypes and production traits separately. The objective of current study was to investigate the polymorphisms in the *IGF-1*, *LEP* and *PRLR* genes in Saanen goats. In future studies, the possible relationships in goats between these identified single nucleotide polymorphisms and productions traits might be determined.

REFERENCES

Abdalhag MA, Li T, Duan L, Zhang T, Zhang G, Wang. J. 2016. Association analysis of IGF-I gene expression with growth and reproductive traits in Jinghai yellow chickens. Genetics and Molecular Research 15: 1-11.

An XP, Hou JX, Gao TY, Lei YN, Li G, Song YX, Wang JG, Cao BY. 2015. Single-nucleotide polymorphisms g. 151435C > T and g. 173057T > C in PRLR gene regulated by bta-miR-302a are associated with litter size in goats. Theriogenology 83: 1477-1483.

Anton I, Kovács K, Holló G, Farkas V, Lehel L, Hajda Z, Zsolnai A. 2011. Effect of leptin, DGAT1 and TG gene polymorphisms on the intramuscular fat of Angus cattle in Hungary. Livestock Science 135: 300-30.

Anton I, Kovács K, Holló G, Farkas V, Szabó F, Egerszegi I, Rátky J, Zsolnai A, Brüssow KP. 2012. Effect of DGAT1, Leptin and TG gene polymorphisms on some milk production traits in different dairy cattle breeds in Hungary. Archiv fur Tierzucht 55: 307-314.

Baran N, Kelly PA, Binart N. 2002. Characterization of a prolactin-regulated gene in repro ductive tissues using the prolactin receptor knockout mouse model. Biology of Reproduction 66: 1210-1218.

Bayram D, Akyüz B, Arslan K, Özdemir F, Aksel EG, Çınar MU. 2019. DGAT1, CAST and IGF-I gene polymorphisms in Akkaraman lambs and their effects on live weights up to weaning age. Kafkas Üniversitesi Veteriner Fakültesi Dergisi 25:9-15.

Boucher D, Palin MF, Castonguay F, Gariépy C, Pothier F. 2006. Detection of polymorphisms in the ovine leptin (LEP) gene: Association of a single nucleotide polymorphism with muscle growth and meat quality traits. The Canadian Journal Animal Science 86: 31-35.

Cosenza G, Iannaccone M, Auzino B, Macciotta NPP, Kovitvadhi A, Nicolae I, Pauciullo A. 2018. Remarkable genetic diversity detected at river buffalo prolactin receptor (PRLR) gene and association studies with milk fatty acid composition. Animal Genetics 49: 159-168.

Curi RA, Chardulo LAL, Arrigoni MDB, Silveira AC, de Oliveira HN. 2011. Associations between LEP. DGAT1 and FABP4 gene polymorphisms and carcass and meat traits in Nelore and crossbred beef cattle. Livestock Science 135: 244-250.

Daş A, Şahin T, Akbulut Ö, Bengü AŞ, Bozkaya F. 2019. Türkiye'de yetiştirilen et ırkı kültür sığırlarında leptin. ghrelin ve insülin benzeri büyüme faktörü-1(IGF-1) gen polimorfizmlerinin belirlenmesi. Harran Üniversitesi Veteriner Fakültesi Dergisi 8 (1): 108-115.

Darwish HR, El-Shorbagy HM, Abou-Eisha AM, El-Din AE, Farag IM. 2017. New polymorphism in the 5'flanking region of IGF-1 gene and its association with wool traits in Egyptian Barki sheep. Journal of Genetic Engineering and Biotechnology 15: 437-41.

de Oliveira JA, da Cunha CM, Crispim Bdo A, Seno L, de O, Fernandes AR, NogueiraGde P, Grisolia AB. 2013. Association of the leptin gene with carcass characteristicsin Nellore cattle. Animal Biotechnology 24: 229-242.

Dettori ML, Pazzola M, Paschino P, Amills M, Vacca GM. 2018. Association between the GHR. GHRHR. and IGF1 gene polymorphisms and milk yield and quality traits in Sarda sheep. Journal of Dairy Science 101(11): 9978-9986.

Di R, Yin J, Chu MX, Cao GL, Feng T, Fang L, Zhou ZX. 2011. DNA polymorphism of introns 1 and 2 of Prolactin Receptor Gene and its association with litter size in goats. Animal Science Papers and Reports 29: 343-350.

Esmaeili NAV, Esmaeilizadeh AK, Ayatolahi AM. 2020. Genetic diversity of leptin gene intron 2 in wild and native and exotic goat breeds in Iran. Journal of Microbiology, Biotechnology and Food Sciences 9: 1110-1113.

Food and Agriculture Organization of the United Nations (FAO) Food and Agriculture Organization of the United Nations statistical databases. http://faostat.fao.org/ (20 Ekim 2021)

Gui LS, Wang ZY, Jia JL, Zhang CT, Chen YZ, Hou SZ. 2018. IGF-1 gene polymorphisms influence bovine growth traits in Chinese Qinchuan cattle. Kafkas Üniversitesi Veteriner Fakültesi Dergisi 24(3).

Hou JX, An XP, Song YX, Wang JG, Ma T, Han P, Fang F, Cao BY. 2013. Combined effects of four SNPs within goat PRLR gene on milk production traits. Gene 529: 276–281.

Hou JX, Fang F, An XP, Yan Y, Ma T, Han P, Meng FX, Song YX, Wang JG, Cao BY. 2014. Polymorphisms of PRLR and FOLR1 genes and association with milk production traits in goats. Genetics and Molecular Research 13: 2555-2562.

Işık R, Bilgen G. 2019. Associations between genetic variants of the POU1F1 gene and production traits in Saanen goats, Archives of Animal Breeding 62; 249–255.

Işık R, Özdil F. 2020. Leptin receptor gene polymorphisms in some Turkish donkey populations, Journal of Equine Veterınary Science, 84: 102823-102826.

Kawaguchi F, Okura K, Oyama K, Mannen H, Sasazaki S. 2017. Identification of leptin gene polymorphisms associated with carcass traits and fatty acid composition in Japanese Black cattle. Animal Science Journal 88: 433-438.

Kök S, Vapur G. 2021. Effects of leptin and thyroglobulin gene polymorphisms on beef quality in Holstein breed bulls in Türkiye. Turkish Journal of Veterinary and Animal Sciences 45: 238-247.

Kulig H, Kmiec M. 2009. Association between leptin gene polymorphisms and growth traits in Limousin cattle. Genetika. 45: 838-41.

Leifers SC, Veekamp RF, Te Pas MF, Delvand C, Chiliard Y, Vander Lende T. 2003. Leptin concentration in relation to energy balance, milk yield, intake, live weight and estrus in dairy cattle. Journal of Dairy Science. 86: 799-807.

Li G, An XP, Fu MZ, Hou JX, Sun RP, Zhu GQ, Wang JG, Cao BY. 2011. Polymorphism of PRLR and LHβ genes by SSCP marker and their association with litter size in Boer goats. Livestock Science 136: 281-286.

Li HF, Zhu WQ, Chen KW, Wu X, Tang. Q, Gao Y. 2008. Associations between GHR and IGF-1 gene polymorphisms and reproductive traits in Wenchang chickens. Turkish Journal of Veterinary and Animal Sciences 32: 281-285.

Liang K, Wang X, Tian X, Geng R, Li W, Jing Z, Han R, Tian Y, Liu X, Kang X, Li Z. 2019. Molecular characterization and an 80-bp indel polymorphism within the prolactin receptor (PRLR) gene and its associations with chicken growth and carcass traits. 3 Biotech 9(8): 296.

Lu A, Hu X, Chen H, Dong Y, Pang Y. 2011. Single nucleotide polymorphisms of the prolactin receptor (*PRLR*) gene and its association with growth traits in Chinese cattle. Molecular Biology Reports 38: 261-266.

Mullen MP, Berry DP, Howard DJ, Diskin MG, Lynch CO, Giblin L, Kenny DA, Magee DA, Meade KG, Waters SM, David A. 2011. Single nucleotide polymorphisms in the insulin-like growth factor 1 (*IGF-1*) gene are associated with performance in Holstein-Friesian dairy cattle. Frontiers in Genetics 2: 1-9.

Naicy T, Venkatachalapathy T, Aravindakshan T, Raghavan KC, Mini M, Shyama K. 2017. Association of a novel single nucleotide polymorphism at the exon-2 of Insulin-Like Growth Factor 1 (IGF 1) gene with phenotypic variants in goats. Veterinarski arhiv 87: 457-472.

Saleem AH, Javed K, Babar ME, Hussain T, Ali A, Ahmad N, Farooq MZ and Dawood M. 2018. Association of Leptin Gene Polymorphism with Growth Rate in Lohi Sheep. Pakistan Journal of Zoology. 50(3): 1029-1033.

Shanaz S, Ganai TAS, Hussain I, Ahmad SF, Sheikh FD, Shabir N, Nabi N, Ganai NA. 2020. IGF-1 gene polymorphism and its association with cashmere fiber trait in Changthangi goats. Journal of Natural Fibers 17(12): 1819-1826.

Shareef M, Basheer A, Zahoor I, Anjum AA. 2018. Polymorphisms in growth hormone (GH) and insulin-like growth factor-1 (IGF-1) gene and their association with growth traits in beetal goat. Pak. Journal of Agricultural Sciences 55(3): 713-720.

Shojaei M, Mohammad Abadi M, Asadi Fozi M, Dayani O, Khezri A, Akhondi M. 2010. Association of growth trait and Leptin gene polymorphism in Kermani sheep. Journal of Cell and Molecular Research 2: 67-73.

Supakorn C and Pralomkarn W. 2013. Genetic polymorphisms of growth hormone (GH). insulin-like growth factor 1 (IGF-1) and diacylglycerol acyltransferase 2 (DGAT–2) genes and their effect on birth weight and weaning weight in goats. Philipp Agriculture Scientist 96: 18-25.

Tölü C, Yurtman İY, Savaş T. 2010. Gökçeada. Malta ve Türk Saanen Keçi Genotiplerinin Süt Verim Özellikleri Bakımından Karşılaştırılması. Hayvansal Üretim 51(1): 8-15.

Trakovická A, Moravčíková N, Kasarda R. 2013. Genetic polymorphisms of leptin and leptin receptor genes in relation with production and reproduction traits in cattle. Acta Biochimica Polonica 60: 783-787.

Wang C, Zhang H, Niu L, Guo J, Jia X, Wang L, Li L, Zhang H, Zhong T. 2015. The novel SNPs of leptin gene and their associations with growth traits in Chinese Nanjiang Yellow goat. Gene 572: 35-41.

Werner H, Bruchim I. 2009. The insulin-like growth factor-I receptor as an oncogene. Archives of Physiology and Biochemistry 115:58-71.

Xiong Q, Chai J, Li XF, Suo XJ, Zhang N, Tao H, Liu Y, Yang QP, Jiang SW, Chen MX. 2016. Two tagSNPs in the prolactin receptor gene are associated with growth and litter traits in Boer and Macheng Black crossbred goats. Livestock Science 193:71–77.

Yeh F, Boyle T. 1999. POPGENE version 1.3.2: Microsoft window-based freeware for population genetic analysis.

Yoshimura Y. 1998. Insulin-like Growth Factors and Ovarian Physiology. Journal of Obstetrics and Gynaecology Research 24(5): 305-323.

Younis LS,, Al-Mutar HAA, Abid AA. 2019. Effect of leptin gene polymorphism on reproductive efficiency in awassi ewes. Advances in Animal and Veterinary Sciences 7(1): 17-23.

Yu LM, Jiang ML, Qiang SG, Jie PQ, Wei S, Lian WG. 2004. Polymorphism analysis of goat growth hormone gene in the 5' regulatory sequence. Hereditas 6: 831-5.

Zhou JP, Zhu XP, Zhang W, Qin F, Zhang SW, Jia ZH. 2011. A novel single-nucleotide polymorphism in the 5' upstream region of the prolactin receptor gene is associated with fiber traits in Liaoning cashmere goats. Genetics and Molecular Research 10: 2511-2516.