jotaf **Journal of Tekirdag Agricultural Faculty**
Tekirdağ Ziraat Fakültesi Dergisi

Eylül/September 2025, 22(3)
Başvuru/Received: 22/01/25
Kabul/Accepted: 18/08/25
DOI: 10.33462/jotaf.1624772

http://dergipark.gov.tr/jotaf
http://jotaf.nku.edu.tr/

ARAŞTIRMA MAKALESİ **RESEARCH ARTICLE**

Combined Genotype Effects of PRLR and GH Polymorphisms on Litter Weight in Kilis Goats*

Kilis Keçilerinde PRLR ve GH Polimorfizmlerinin Kombine Genotip Etkilerinin Döl Ağırlığı Üzerine Etkisi

Zühal GÜNDÜZ[1*]

Abstract

This study investigates the relationship between polymorphisms in the PRLR, GH1, and GH2 genes and their impact on litter weight in Kilis goats, a native breed of significant economic importance in Türkiye. Within the scope of the research, a total of 198 goats were used under breeder conditions in Kilis Province. This study was conducted as part of the National Small Ruminant Breeding Project, coordinated by the Ministry of Agriculture and Forestry of the Republic of Türkiye and implemented by the General Directorate of Agricultural Research and Policies (TAGEM). Allele frequencies for the PRLR/RsaI, GH1/HaeIII, and GH2/HaeIII loci were analyzed, revealing that the C allele at PRLR/RsaI and the D allele at GH2/HaeIII were the most prevalent in this population. While no significant differences in litter weight were observed among PRLR genotypes ($P > 0.05$), significant associations were identified between the GH1 and GH2 loci and litter weight ($P \leq 0.05$). Specifically, Kilis goats with the AB genotype at GH1 and the CD genotype at GH2 exhibited significantly higher litter weights compared to other genotypes, highlight the potential influence of these genetic variations on reproductive performance. Furthermore, significant genotype interactions were observed, with the highest litter weight recorded in goats carrying the AB-CD genotype combination (7.613 ± 2.973 kg). These findings suggest that genetic variations at the GH1 and GH2 loci play a critical role in determining reproductive traits such as litter weight. Strategic breeding programs targeting these loci could enhance productivity in Kilis goats. Given the economic significance of litter weight in livestock production, these results provide a foundation for further research into the genetic basis of reproduction traits in goats. Additional studies across diverse breeds and environmental conditions are necessary to validate these findings and explore the broader implications for genetic selection in livestock breeding.

Keywords: Kilis goats, Litter weight, Polymorphism, Prolactin receptor, Growth hormone

[1]***Sorumlu Yazar/Corresponding Author:** Zühal Gündüz, Aydın Adnan Menderes University Faculty of Agriculture Department of Agricultural Biotechnology, Aydın, Türkiye. E-mail: zuhal.gunduz@adu.edu.tr OrcID: 0000-0002-1427-1501
Atıf: Gündüz, Z. (2025). Kilis keçilerinde PRLR ve GH polimorfizmlerinin kombine genotip etkilerinin döl ağırlığı üzerine etkisi. *Tekirdağ Ziraat Fakültesi Dergisi,* 22(3): 793-802.
Citation: Gündüz, Z. (2025). Combined genotype effects of PRLR and GH polymorphisms on litter weight in Kilis goats. *Journal of Tekirdag Agricultural Faculty,* 22(3): 793-802.

*This study was summarized from Zühal GÜNDÜZ's PhD thesis.

Öz

Bu çalışma, Türkiye'nin ekonomik açıdan önemli yerli ırklarından biri olan Kilis keçilerinde PRLR, GH1 ve GH2 genlerindeki polimorfizmler ile döl ağırlığı arasındaki ilişkiyi incelemeyi amaçlamaktadır. Araştırma kapsamında, Kilis ilinde yetiştirici koşullarında T.C. Tarım ve Orman Bakanlığı koordinatörlüğünde Tarımsal Araştırmalar ve Politikalar Genel Müdürlüğü (TAGEM) tarafından yürütülen Halk Elinde Küçükbaş Hayvan Islahı Ülkesel Projesi kapsamında elde edilen toplam 198 baş keçi kullanılmıştır. Bu hayvanlarda PRLR/RsaI, GH1/HaeIII ve GH2/HaeIII lokuslarına ait allel ve genotip frekansları hesaplanmış ve popülasyonun genetik yapısı analiz edilmiştir. Analiz sonuçları, PRLR/RsaI lokusunda C alelinin ve GH2/HaeIII lokusunda D alelinin popülasyonda en yaygın aleller olduğunu göstermiştir. PRLR genotipleri ile döl ağırlığı arasında anlamlı bir fark bulunmazken ($P > 0.05$), GH1ve GH2 lokuslarının döl ağırlığı üzerinde istatistiksel olarak önemli etkiler gösterdiği belirlenmiştir ($P \leq 0.05$). Özellikle, GH1 lokusunda AB genotipine ve GH2 lokusunda CD genotipine sahip Kilis keçilerinin, diğer genotiplere kıyasla istatistiksel olarak daha yüksek döl ağırlığına sahip olduğu tespit edilmiştir. Ayrıca, genotip kombinasyonları arasındaki etkileşimler incelenmiş ve AB-CD genotip kombinasyonuna sahip bireylerde en yüksek döl ağırlığı gözlenmiştir (7.613 ± 2.973 kg). Bu bulgular, GH1 ve GH2 lokuslarındaki genetik varyasyonların, Kilis keçilerinde döl ağırlığı gibi önemli üreme özelliklerinin belirlenmesinde kritik bir rol oynadığını ortaya koymaktadır. Ayrıca, bu genlerin stratejik olarak kullanılacağı genetik ıslah programlarının, Kilis keçilerinin ekonomik değerini artırma potansiyeline sahip olduğu vurgulanmıştır. Döl ağırlığının hayvansal üretimdeki ekonomik önemi göz önüne alındığında, bu çalışma, keçilerde üreme özelliklerinin genetik temellerine dair daha kapsamlı araştırmalar için bir temel oluşturmaktadır. Farklı ırklar ve çevresel koşullar altında yapılacak sonraki çalışmalar, bu bulguların doğrulanması ve genetik seleksiyonun etkilerinin daha detaylı incelenmesi açısından büyük önem taşımaktadır.

Anahtar Kelimeler: Kilis keçisi, Döl ağırlığı, Polimorfizm, Prolaktin reseptör, Büyüme hormonu

1. Introduction

With global distribution, goats are a vital livestock species, particularly in developing nations. They provide essential products such as meat, milk and fiber, which are crucial for communities living in arid and semi-arid regions (Gündüz and Biçer, 2023; Gül et al., 2018; Keskin et al., 2017). Goat farming in arid and semi-arid areas such as Türkiye requires a lower initial investment compared to other large livestock species. Their adaptability to harsh climatic conditions and remarkable resilience makes them an ideal choice for such ecosystems.

In Türkiye, goat farming plays a significant role in the agricultural economy, with an estimated population of approximately 10.3 million goats, as reported by the TUIK (Anonymous, 2023). Among the indigenous breeds, the Kilis goat is particularly significant due to its unique genetic identity, which has resulted from historical uncontrolled crossbreeding between Damascus and Hair goat populations (Karaköse, 2024; Gündüz and Biçer, 2023; Gül et al., 2020; Keskin et al., 2017). Well-adapted to the arid and semi-arid regions of Türkiye, the Kilis goat is renowned for its exceptional milk production and reproductive efficiency. Consequently, this breed offers promising opportunities for dairy farming and genetic improvement programs in the country. Moreover, previous studies have demonstrated that a combination of genetic and environmental factors—including age, lactation stage, parity, and management practices—significantly influences the reproductive and milk production traits of goats (Gündüz and Biçer, 2023; Daşkıran et al., 2022; Gül et al., 2022; Tilki and Keskin, 2021; Özdemir and Keskin, 2018; Keskin et al., 2016). These findings emphasizes the importance of genetic research in identifying valuable markers for improving goat productivity. Furthermore, investigating gene variations in native breeds through molecular markers is essential for preserving genetic diversity and developing sustainable utilization and improvement strategies (Togan et al., 2005).

Litter weight is a crucial trait in animal breeding, as it directly influences economic efficiency, reproductive performance, and offspring survival. Higher litter weights at birth and weaning are associated with increased neonatal viability, improved growth rates, and enhanced genetic selection for superior reproductive efficiency (Cobo et al., 2021). In pigs, greater litter weight correlates with higher weaning weights and reduced pre-weaning mortality, making it a key selection criterion for commercial breeding programs (Kiszlinger et al., 2015). Similarly, in sheep, genetic evaluations indicate that litter weight exhibits moderate heritability, making it a viable trait for improvement through selective breeding (Cobo et al., 2021). The ability to select for high litter weight using marker-assisted selection and genetic evaluation tools offers a powerful mechanism for improving herd efficiency, sustainability, and profitability in commercial livestock production.

Studies examining polymorphisms in the prolactin receptor (PRLR) and growth hormone (GH) genes suggest a significant impact on litter weight, primarily mediated by their effects on litter size, fetal growth, and milk production. PRLR is particularly relevant in swine and goats, where specific allelic variants are associated with higher litter weights and improved reproductive performance. For instance, research on Minpig and Landrace sows identified a Nae I polymorphism in PRLR, where sows with the BB genotype exhibited significantly higher litter weights at birth and greater piglet uniformity at weaning (Hu and Wang, 2006). Similarly, a study on Large White pigs revealed multiple single nucleotide polymorphisms (SNPs) in exon 10 of PRLR that correlated with an increased total number born and number born alive, ultimately leading to increased litter weight (Wu et al., 2023). Research on Mangalica pigs indicated that the AA genotype of the PRLR gene was associated with an increase of 1.11 piglets per litter compared to the BB genotype, suggesting a direct effect on litter weight (Tempfli et al., 2011). In goats, a specific single nucleotide polymorphism identified as g.173057T>C in PRLR was found to modify microRNA (miRNA) binding, thereby influencing PRLR expression and litter size (An et al., 2015). Regarding GH gene polymorphisms, although the literature on their direct influence on litter weight is limited, GH plays a crucial role in fetal growth, metabolic regulation, and milk yield, which indirectly affects litter weight (Hua et al., 2009). GH gene polymorphisms have been associated with improved growth traits and reproductive efficiency, suggesting that the selection of specific GH alleles may enhance PRLR-related improvements in litter weight.

The objective of this study was to identify polymorphisms in the PRLR and GH genes using restriction fragment length polymorphism (RFLP) analysis. Furthermore, the study aimed to investigate the potential association between the PRLR and GH genes, as well as their combined genotypes, and litter weight in Kilis goats.

This research enhances our understanding of the genetic mechanisms that influence litter weight and offers valuable insights for genetic selection programs aimed at improving reproductive efficiency in goats.

2. Materials and Methods

2.1. Procedure for Animal Selection and DNA Sampling Process

A total of 198 unrelated Kilis goats were obtained from the National Breeding Project for Kilis Goats, conducted under farm conditions. This project was support from by the General Directorate of Agricultural Research and Policy (TAGEM), under the Ministry of Agriculture and Forestry of the Republic of Türkiye and implemented in Kilis province. The study was conducted in accordance with the approval granted by the Hatay Mustafa Kemal University, the Animal Experiments Local Ethics Committee (approval date: 29/09/2016; approval number: 2016/8-1). Goats were selected from the 2nd and 3rd kidding season, matched for body weight at mating, and raised under identical management and feeding conditions. Approximately 9 mL of blood was collected from the jugular vein of each dairy goat using EDTA-coated vacutainer tubes (BD Vacutainer Systems, Plymouth, UK). Genomic DNA was extracted from white blood cells utilizing the high-salt technique described by Miller et al. (1988) and stored at -20°C for subsequent analysis.

2.2. PCR Reactions and Genotyping

For the targeted amplification of the PRLR, GH1, and GH2 genes, two sets of primers were designed specifically for this study. The nucleotide sequences of the primers and the fragment sizes of the PCR products are presented in *Table 1*.

Table 1. Primer sequences of target genes and PCR product sizes

Primers	Primer sequence	Gene Bank ID	Region	Product size
PRLR_F	5'-AGTGAGAGTTATGGAAGGATG-3'	KJ572972.1	3' UTR	443 bp
PRLR_R	5'-AAGGTTAAGCAACTGGTCTT-3'			
GH1_F	5'-CTCTGCCTGCCCTGGACT-3'	D00476.1	exon 2, intron 2, exon 3	422 bp
GH1_R	5'-GGAGAAGCAGAAGGCAACC-3'			
GH2_F	5'-TCAGCAGAGTCTTCACCA AC-3	D00476.1	exon 4	116 bp
GH2_R	5'-CAACAACGCCATCCTCAC-3'			

To amplify the target genes, PCR was performed using specific primers for PRLR, GH1, and GH2. The reaction mixture consisted of 1X PCR buffer, 2 mM $MgCl_2$, 200 µM of each deoxynucleotide triphosphate (dNTP), 1 µM of each forward and reverse primer, 1 unit of Taq DNA polymerase (Thermo Scientific, USA), and approximately 100 ng of genomic DNA in a total volume of 30 µL. PCR amplification was initiated with an initial denaturation step at 95°C for 5 minutes. This was followed by a specific number of cycles for each gene, consisting of denaturation at 94°C for 40 seconds, annealing at the optimal temperature (51.4°C for PRLR, 54°C for GH1, and 63°C for GH2) for 40 seconds, and extension at 72°C for 1 minute. A final extension step at 72°C for 5 minutes was included to ensure complete extension of the PCR products. PCR amplification was conducted using a Bio-Rad C1000 Touch™ Thermal Cycler. The PCR products of the PRLR and GH genes, 30 µL aliquots were digested with 10 units of RsaI (for PRLR) and HaeIII (for GH1 and GH2) restriction enzymes (Thermo Scientific, USA) for 2 hours at 37°C, following the manufacturer's protocol. The resulting restriction fragments were subsequently separated on a 2% agarose gel and visualized under ultraviolet (UV) light after being stained with ethidium bromide.

2.3. Statistical Analysis

The experimental animal population consisted of goats in their 2nd and 3rd parity. Additive correction factors were calculated using a general linear model to account for systematic variations due to parity and farm origin effects, ensuring a more accurate comparison across groups. Allelic and genotypic frequencies, and deviations from Hardy-Weinberg Equilibrium (HWE), were computed using GenAlex 6.5 (Peakall and Smouse, 2012) and PopGene 32 (Yeh et al., 2000) software packages. To examine the association between animal genotypes and litter weight, statistical analyses (Eq. 1) were performed using SPSS software (Kinnear and Gray, 1999). The following linear model was applied:

$$y_{ij} = \mu + a_i + e_{ij} \quad \text{(Eq. 1).}$$

Yij is the observed litter weight of the i j-th animal,
μ represents the overall population mean,
αi denotes the fixed effect of the ii-th genotype,
eij represents the random error associated with each observation.

3. Results and Discussion

3.1. Genotyping

Following the digestion of the goat PRLR gene with the RsaI restriction enzyme, the genotypes CC (383 bp, 60 bp), CT (443 bp, 383 bp, 60 bp), and TT (443 bp) were identified, using the GeneRuler 50 bp DNA Ladder (Thermo Scientific) as a reference (Hou et al., 2014). However, visualizing the 60 bp fragment on a 2% agarose gel posed a significant challenge. In contrast, the larger fragments, measuring 443 bp and 383 bp, displayed strong clarity, enabling reliable differentiation between the genotypes.

The digestion of the goat GH1 and GH2 genes with the HaeIII restriction enzyme revealed distinct genotypic patterns, using GeneRuler DNA Ladders (Thermo Scientific) as references. For the GH1 gene, the identified genotypes were AA (366 bp, 56 bp), AB (422 bp, 366 bp, 56 bp), and BB (422 bp). For the GH2 gene, the observed genotypes were CC (88 bp, 28 bp), CD (116 bp, 88 bp, 28 bp), and DD (116 bp) (Hua et al., 2009). In both cases, the smallest fragments—56 bp for GH1 and 28 bp for GH2—were difficult to resolve on a 2% agarose gel, rendering some fragments indistinguishable during visual inspection. Despite these limitations, the larger fragments—422 bp and 366 bp for GH1, and 116 bp and 88 bp for GH2—were clearly visible, enabling for accurate differentiation of the genotypes (*Figure 1*).

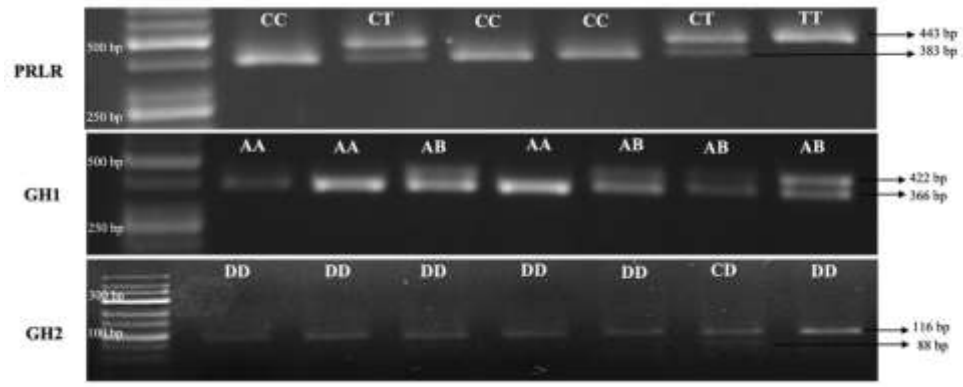

Figure 1. Electrophoretic profile for the PRLR, GH1 and GH2 fragments

3.2. Distribution of Allelic and Genotypic Fragments in Genes

In this study, the PCR-RFLP method was employed to investigate the allelic and genotypic frequencies of specific genes in goat genomic DNA. The findings, shown in *Table 2*, highlight the frequency distribution of alleles for each locus investigated.

Table 2. Allelic and genotyping frequencies of the PRLR, GH1 and GH2

Locus	Allele Frequencies		Genotype Frequencies			Ho	He	$\chi 2$
	C	**T**	**CC**	**CT**	**TT**	0.258	0.253	0.000286^{ns}
PRLR	0.851	0.149	0.722	0.258	0.020			
	A	**B**	**AA**	**AB**	**BB**	0.854	0.488	0.373^{ns}
GH1	0.573	0.427	0.146	0.854	-			
	C	**D**	**CC**	**CD**	**DD**	0.020	0.019	0.0001^{ns}
GH2	0.010	0.990	-	0.020	0.980			

Observed (Ho) and Expected (He) Heterozygosity and Chi-Square Statistic ($\chi 2$); ns: non-significant

The allele frequencies for the PRLR/RsaI locus were calculated to be 0.851 for the C allele and 0.149 for the T allele. Similarly, at the GH1/HaeIII locus, the frequencies of the A and B alleles were 0.573 and 0.427, respectively, with no occurrence of the BB genotype. For the GH2/HaeIII locus, the frequency of the C allele was

0.010, while the frequency of the D allele was 0.990, and the CC genotype was not detected. Additionally, the genotype distributions for all analyzed loci were consistent with Hardy-Weinberg equilibrium.

Nei's method was utilized to calculate the observed heterozygosity (Ho) and expected heterozygosity (He) for each locus. At the PRLR/RsaI locus, the Ho and He values were determined to be 0.258 and 0.253, respectively. For the GH1/HaeIII locus, the values were found to be 0.854 for Ho and 0.488 for He. Meanwhile, the GH2/HaeIII locus exhibited Ho and He values of 0.020 and 0.019, respectively.

3.2. The Relationship Between Genetic Variability and Litter Weight in Goats

As shown in *Table 3*, no statistically significant differences were observed in litter weight among the PRLR genotypes in Kilis goats ($P > 0.05$). The mean litter weights were 5.537 ± 2.238 kg for CC genotype, 4.954 ± 1.915 kg for CT genotype, and 6.900 ± 3.536 kg for TT genotype. In contrast, significant genotypic variations were detected at the GH1 and GH2 loci ($P \leq 0.05$). At the GH1 locus, the AB genotype demonstrated a significantly higher mean litter weight of 5.541 ± 2.232 kg compared to 4.677 ± 1.874 kg for the AA genotype. Similarly, at the GH2 locus, the CD genotype exhibited a significantly greater mean litter weight of 7.612 ± 2.972 kg compared to 5.369 ± 2.169 kg for the DD genotype.

Table 3. Effect of the PRLR, GH1 and GH2 polymorphisms on litter weight

Genotypes		**LW ± SD**
PRLR	**N**	**P=0.11**
CC	143	5.537 ± 2.238
CT	51	4.954 ± 1.915
TT	4	6.900 ± 3.536
Mean	198	5.415 ± 2.200
GH1	**N**	**P=0.05**
AA	29	4.677 ± 1.874[a]
AB	169	5.541 ± 2.232[b]
Mean	198	5.414 ± 2.200
GH2	**N**	**P=0.04**
CD	4	7.612 ± 2.972[a]
DD	194	5.369 ± 2.169[b]
Mean	198	5.415 ± 2.200

N: Number of animals; LW: Litter weight (kg); SD: Standard deviation.
Different letters in the same column indicate significant differences

Studies conducted across various species have demonstrated that polymorphisms in the PRLR gene significantly influence traits such as litter weight and birth weight (Yang et al., 2023; van Rens and van der Lende, 2002), and litter size (Wu et al., 2023; El-Shorbagy et al., 2022; Zhang et al., 2020; Guo et al., 2017; An et al., 2015; Xing-Ping et al., 2008). The absence of significant differences in litter weight among PRLR genotypes suggests that this gene may not directly influence litter weight in Kilis goats. This finding is consistent with previous studies, such as Sankhyan et al., (2019) and Terman (2005), which reported no significant association between PRLR polymorphisms and reproductive traits in goats. Furthermore, research conducted by An et al. (2015) suggests that while certain variants of the PRLR gene may be associated with reproductive efficiency in some goat breeds. Direct evidence supporting their influence on litter weight is still limited. Although PRLR is recognized for its role in lactogenesis and maternal behavior (Hou et al., 2014), its contribution to variations in litter weight is still not well established. Additional research involving larger sample sizes, diverse goat populations, and controlled environmental factors is required to clarify its precise role in reproductive performance. In studies conducted on goats, sheep, and cattle, the influence of GH polymorphism on birth weight has been reported as statistically insignificant (Kumar et al., 2024; Muniasamy et al., 2023; Sedykh et al., 2020; Gholamhoseinzadeh et al., 2018; Hua et al., 2009). However, other researches have demonstrated a significant effect of GH polymorphism on both birth weight and litter size (Buranakarl et al., 2024; Zhang et al., 2011). In the current study, the findings reveal that the two distinct GH loci examined exhibit a statistically significant influence on litter weight. These results contribute to the expanding body of evidence underscoring the potential role of genetic variation in growth hormone (GH) in modulating reproductive traits, particularly litter weight, in livestock. Further research is required

to investigate the underlying mechanisms and confirm these effects across diverse breeds and environmental conditions.

Table 4 and *Table 5* present an evaluation of the combined effects of PRLR and GH genotypes on litter weight (LW) in Kilis goats.

Table 4. Combined genotype effects of PRLR-GH1 and PRLR-GH2 on litter weight

PRLR-GH1			PRLR-GH2		
P=0.057			P=0.042		
Genotypes	**N**	**LW ± SD**	**Genotypes**	N	**LW ± SD**
CC-AA	26	4.752±1.969	CC-DD	139	5.478±2.199^{ab}
CC-AB	117	5.712±2.265	CC-CD	4	7.616±2.973^{b}
CT-AA	3	4.033±0.115	CT-DD	51	4.954±1.915^{a}
CT-AB	48	5.011±1.960	TT-DD	4	6.900±3.536^{ab}
TT-AB	4	6.900±3.536			
Mean = 5.415 ± 2.201 (N=198)					

N: Number of animals; LW: Litter weight (kg); SD: Standard deviation.
Different letters in the same column indicate significant differences.

No statistically significant differences in litter weight were observed among the combined PRLR-GH1 genotypes (P > 0.05). However, the highest mean litter weight was observed in goats with the TT-AB genotype (6.900±3.536 kg), whereas the lowest mean litter weight was recorded for the CT-AA genotype (4.033±0.115 kg). Significant differences in litter weight were observed among the combined PRLR-GH2 genotypes (P<0.05). The CC-CD genotype demonstrated the highest litter weight (7.616±2.973 kg), significantly exceeding that of the CC-DD genotype (5.478±2.199 kg), which exhibited the lowest mean value.The combined GH1 and GH2 genotypes exhibited statistically significant effects on litter weight (P<0.05). The AB-CD genotype showed the highest mean litter weight (7.613±2.973 kg), significantly exceeding that of the AA-DD genotype, which had the lowest mean litter weight of 4.678±1.874 kg. Our findings align with those of Hua et al. (2009), who reported that while the individual effects of polymorphisms at two distinct growth hormone loci were statistically insignificant for birth weight, their combined effect was significant. The observed increase in litter weight associated with specific GH1 and GH2 genotypes suggests a potential genetic advantage that could be exploited in breeding programs to enhance reproductive efficiency in Kilis goats. This observation supports the hypothesis that gene-gene interactions at multiple loci can influence complex traits, such as litter weight. Similar results have been reported in other livestock species, where interactions between growth-related genes contribute to variations in reproductive and production traits (Chen et al., 2001; He et al., 2014; Hou et al., 2013).

Table 5. Combined genotype effects of GH1-GH2 and PRLR-GH1-GH2 on litter weight

GH1-GH2			PRLR-GH1-GH2		
P=0.023			P=0.030		
Genotypes	**N**	**LW ± SD**	**Genotypes**	N	**LW ± SD**
AA-DD	29	4.678±1.874^{a}	CC-AA-DD	26	4.752±1.969^{ab}
AB-DD	165	5.491±2.199^{a}	CC-AB-DD	113	5.645±2.223^{abc}
AB-CD	4	7.613±2.973^{b}	CC-AB-CD	4	7.613±2.973^{c}
			CT-AA-DD	3	4.033±0.115^{a}
			CT-AB-DD	48	5.011±1.960^{ab}
			TT-AB-DD	4	6.900±3.536^{bc}
Mean = 5.415 ± 2.201 (N=198)					

N: Number of animals; LW: Litter weight (kg); SD: Standard deviation.
Different letters in the same column indicate significant differences.

The interaction among all three loci (PRLR-GH1-GH2) revealed statistically significant differences in litter weight (P<0.05). The CC-AB-CD genotype exhibited the highest mean litter weight (7.613±2.973 kg), whereas the CT-AA-DD genotype displayed the lowest mean value (4.033±0.115 kg). Intermediate values were observed for genotypes such as CC-AB-DD (5.645±2.223 kg) and TT-AB-DD (6.900±3.536 kg).The findings demonstrate

that specific combinations of PRLR, GH1, and GH2 genotypes exert a significant influence on litter weight in Kilis goats. Notably, the most favorable genotype combinations associated with enhanced litter weight include the CD allele at the GH2 locus and the AB genotype at the GH1 locus. These results highlight the potential role of genetic interaction in modulating this economically important trait.

4. Conclusions

This study provides genetic variations at the GH1 and GH2 loci significantly influence litter weight in Kilis goats, highlighting their potential as key genetic determinants of this economically important trait. Although no direct association was found between the PRLR gene and litter weight, the findings emphasize the complex genetic interactions between GH1 and GH2, which could have important implications for breeding strategies aimed at enhancing reproductive efficiency. Notably, the identification of specific genotype combinations, such as AB-CD, associated with increased litter weight offers valuable insights for targeted genetic selection. These results contribute to a deeper understanding of the genetic architecture underlying reproductive traits in Kilis goats and may facilitate the development of precision breeding approaches to optimize productivity in small ruminant populations. Further studies with larger sample sizes, diverse goat populations, and controlled environmental conditions are necessary to better define its exact role in reproductive performance.

Acknowledgment

This work supported supported by the Hatay Mustafa Kemal University Scientific Research Projects Institutional Coordinator (Project No: 16460), Türkiye. The author acknowledges the Republic of Türkiye's Ministry of Agriculture and Forestry for providing animal materials from "The National Breeding Project of Kilis Goats under Farm Conditions", which was backed by the General Directorate of Agricultural Research and Policy. The author also extends their appreciation to the Aydin Adnan Menderes University Agricultural Biotechnology and Food Safety Application and Research Center (ADU-TARBIYOMER) for providing laboratory facilities to conduct molecular genetic analysis. This article is derived from the author's doctoral thesis at Hatay Mustafa Kemal University. In accordance with the principles of scientific transparency and reproducibility, the data used and analyzed in this study are available from the corresponding author upon reasonable request.

Ethical Statement

This study was prepared under the permission numbered 2016/8-1, dated 29/09/2016, from the Ethics Committee of Hatay Mustafa Kemal University.

Conflicts of Interest

There is no conflict of interest between the article authors.

Authorship Contribution Statement

Concept: Zühal, G.; Design: Zühal, G.; Data Collection or Processing: Zühal, G.; Statistical Analyses: Zühal, G.; Literature Search: Zühal, G.; Writing, Review and Editing: Zühal, G.

References

An, X., Hou, J., Gao, T., Lei, Y., Li, G., Song, Y., Wang, J. and Cao, B. (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(9): 1477-1483.

Anonymous (2023). Turkish Statistical Institute (TUIK). https://www.tuik.gov.tr (Accessed Date: 15.10.2024)

Buranakarl, C., Chamsuwan, S., Thammacharoen, S., Ratchakom, P. and Chuaypen, N. (2024). Single-nucleotide polymorphisms of growth hormone gene and its relationship with growth traits in Black Bengal goats. *Animals,* 14(6): 834.

Chen, K., Li, N., Huang, L., Zhang, Q., Zhang, J., Sun, S., Luo, M. and Wu, C. (2001). The combined genotypes effect of ESR and FSHβ genes on litter size traits in five different pig breeds. *Chinese Science Bulletin*, 46: 140-143.

Cobo, E., Raoul, J. and Bodin, L. (2021). Genetic parameters of litter weight, an alternative criterion to prolificacy and pre-weaning weight for selection of French meat sheep. *Livestock Science*, 250: 104596.

Daşkıran, İ., Yılmaz, A. and Günbey, V. S. (2022). A study on lactation milk yield and milk qualtiy characteristics of Kilis goats in farm conditions. *Journal of Tekirdag Agricultural Faculty*, 19(3): 678-687. (In Turkish)

El-Shorbagy, H. M., Abdel-Aal, E. S., Mohamed, S. A. and El-Ghor, A. A. (2022). Association of PRLR, IGF1, and LEP genes polymorphism with milk production and litter size in Egyptian Zaraibi goat. *Tropical Animal Health and Production*, 54(5): 321.

Gholamhoseinzadeh Gooki, F., Mohammadabadi, M. R. and Asadi Fozi, M. (2018). Polymorphism of the growth hormone gene and its effect on production and reproduction traits in goat. *Iranian Journal of Applied Animal Science*, 8(4): 653-659.

Gül, S., Keskin, M. and Kaya, Ş. (2022). Effects of environmental factors on growth performance of Kilis goat in Gaziantep province. *Livestock Studies*, 62(1): 16-20.

Gül, S., Keskin, M., Güler, Z., Dursun, A., Gündüz, Z., Önel, S. E. and Bebek, D. T. (2018). Effects of pre-milking resting on some lactation characteristics of Damascus (Shami) and Kilis goats. *Journal of Animal Production*, 59(1): 17-24.

Gül, S., Yilmaz, O., Gündüz, Z., Keskin, M., Cemal, I., Ata, N. and Önel, S. E. (2020). The genetic structure of the goat breeds belonging to Northwest part of Fertile Crescent. *Small Ruminant Research,* 182: 22-28.

Gündüz, Z. and Biçer, O. (2023). Milk-y Way: the impact of single-nucleotide polymorphisms on milk production traits in Kilis dairy goats. *Archives Animal Breeding*, 66(4): 369-378.

Guo, H. Y., Jin, Y., Li, Y. J., Chen, G. H. and Zhang, H. (2017). The relationship between PRLR gene polymorphism and lambing performance of Hu sheep. *Journal of Yangzhou University (Agriculture and Life Sciences Edition),* 38: 57-61.

He, H., Zhang, H. L., Li, Z. X., Liu, Y., and Liu, X. L. (2014). Expression, SNV identification, linkage disequilibrium, and combined genotype association analysis of the muscle-specific gene CSRP3 in Chinese cattle. *Gene*, 535(1): 17-23.

Hou, J. X., An, X. P., Song, Y. X., Wang, J. G., Ma, T., Han, P., Fang, F. and Cao, B. Y. (2013). Combined effects of four SNPs within goat PRLR gene on milk production traits. *Gene*, 529(2): 276-281.

Hou, J., Fang, F., An, X., Yan, Y., Ma, T., Han, P., Meng, F., Song, Y., Wang, J. and Cao, B. (2014). Polymorphisms of PRLR and FOLR1 genes and association with milk production traits in goats. *Genetics and Molecular Research*, 13(2): 2555-2562.

Hu, X. and Wang, X. (2006). Nae 1 Polymorphism of PRLR gene and its effect on reproductive traits in Minpig, Landrace and their F~ 1 crossbreed sows. *Acta Veterinaria Et Zootechnica Sinica*, 37(11): 1135.

Hua, G. H., Chen, S. L., Yu, J. N., Cai, K. L., Wu, C. J., Li, Q. L., Zhang, C. Y., Liang, A. X., Han, L., Geng, L. Y., Shen, Z., Xu, D. Q. and Yang, L. G. (2009). Polymorphism of the growth hormone gene and its association with growth traits in Boer goat bucks. *Meat Science,* 81(2): 391-395.

Karaköse, İ. (2024). *Determination of genetic characterization of Damascus, Kilis, Hatay and kil goat breeds by mtDNA-D loop sequence analysis*. (MSc. Thesis) Aydın Adnan Menderes University, Natural and Applied Sciences Institute, Aydın, Türkiye. (In Turkish).

Keskin, M., Gül, S., Biçer, O. and Gündüz, Z. (2017). Kıl keçisi yetiştiriciliğinin organik üretim bakımından uygunluğu. *Turkish Journal of Agriculture-Food Science and Technology*, 5(13): 1700-1704. (In Turkish)

Keskin, M., Gül, S., Can, E. and Gündüz, Z. (2016). Milk yield and reproductive traits of Shami and KilisxHair goat crossbred genotypes under semi-intensive conditions. *Lalahan Hayvancılık Araştırma Enstitüsü Dergisi*, 56(1): 20-24. (In Turkish)

Kinnear, P. R. and Gray, C. D. (1999). SPSS for Windows Made Simple. 3rd Edn. Department of Psychology University of Aberdeen, UK.

Kiszlinger, H. N., Farkas, J., Kövér, G., Nguyen, N. T. and Nagy, I. (2015). Genetic parameters and breeding value stability estimated from a joint evaluation of purebred and crossbred sows for litter weight at weaning. *Acta Agraria Kaposváriensis,* 19(1): 1-7.

Kumar, S., Yadav, A. S., Magotra, A., Bangar, Y. C., Garg, A. R. and Kumar, N. (2024). Polymorphism of growth hormone (GH) gene and its association with performance and body conformation of Harnali sheep. *Tropical Animal Health and Production*, 56(3): 116.

Miller, S. A., Dykes, D. D. and Polesky, H. F. (1988). A simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic Acids Research,* 16: 1215.

Muniasamy, S., Veerasamy, J., Ramanujam, R. and Gopalan Tirumurugaan, K. (2023). Polymorphism of the growth hormone (GH) gene and its association with growth traits in the Kilakarsal breed of sheep. *Veterinarski Arhiv,* 93(4): 427-434.

Özdemir, F. and Keskin, M. (2018). Comparison of Kilis Goats raised in Gaziantep and Kilis Provinces due to some morphological and physiological characteristics. *Journal of Agricultural Faculty of Mustafa Kemal University*,23(1): 115-123. (In Turkish)

Peakall, R. and Smouse, P. E. (2012). GenAlEx 6.5: Genetic analysis in Excel. Population genetic software for teaching and research-an update. *Bioinformatics,* 28: 2537-2539.

Sankhyan, V., Thakur, Y. P. and Dogra, P. K. (2019). No association of Prolactin Receptor (PRLR) gene and litter size in Gaddi goat breed reared under migratory system in Western Himalayan ranges of Himachal Pradesh. *Journal of Animal Research,* 9(2): 237-242.

Sedykh, T. A., Dolmatova, I. Y., Valitov, F. R., Gizatullin, R. S. and Kalashnikova, L. A. (2020). The influence of growth hormone gene polymorphism on growth rate of young cattle. *Iranian Journal of Applied Animal Science,* 10(3): 445-451.

Tempfli, K., Farkas, G., Simon, Z. and Bali Papp, Á. (2011). Effects of prolactin receptor genotype on the litter size of Mangalica. *Acta Veterinaria Hungarica*, 59(2): 269-277.

Terman, A. (2005). Effect of the polymorphism of prolactin receptor (PRLR) and leptin (LEP) genes on litter size in Polish pigs. *Journal of Animal Breeding and Genetics,* 122(6): 400-404.

Tilki, H. Y. and Keskin, M. (2021). Relationships between different body characteristics and milk yield traits in Kilis goats. *Journal of Agricultural Faculty of Mustafa Kemal University*, 26(2): 272-277.

Togan, İ., Soysal, İ., Berkman, C. C. and Koban, E. (2005). Molecular Markers in Conservation of the Breeds. *Journal of Tekirdağ Agricultural Faculty,* 2(1): 44-49. (In Turkish)

van Rens, B. T. and van der Lende, T. (2002). Litter size and piglet traits of gilts with different prolactin receptor genotypes. *Theriogenology,* 57(2): 883-893.

Wu, Y., Xie, J., Zhong, T., Shen, L., Zhao, Y., Chen, L., Gan, M., Zhang, S., Zhu, L. and Niu, L. (2023). Genetic diversity of porcine PRLR gene and its relationship to litter size in Large White pigs. *Folia Biologica (Kraków),* 71(1): 28-36.

Xing-ping, W., Li-xian, W., Zhuo-ma, L. R. and Shi-duo, S. U. N. (2008). Analysis of PRLR and BF genotypes associated with litter size in Beijing black pig population. *Agricultural Sciences in China,* 7(11): 1374-1378.

Yang, S., Guo, X. J., Yang, H. W., Xiong, L., Li, C., Tan, Y. C., Wang, C. and Zhang, Y. Y. (2023). Correlation of PRLR gene polymorphisms and reproductive traits in Kele pig. *Acta Agriculturae Zhejiangensis,* 35(3): 556-564.

Yeh, F., Yang, R. C. and Boyle, T. (2000). Popgene Microsoft Windows Based Freeware for Population Genetic Analysis. Https://Sites.Ualberta.Ca/~Fyeh/ (Accessed Date: 5.10.2024).

Zhang, C., Liu, Y., Huang, K., Zeng, W., Xu, D., Wen, Q. and Yang, L. (2011). The association of two single nucleotide polymorphisms (SNPs) in growth hormone (GH) gene with litter size and superovulation response in goat-breeds. *Genetics and Molecular Biology,* 34: 49-55.

Zhang, Y., Yuan, J., Gao, Y. S., Kang, B. N., Li, Z. K., Zhao, Y., and Min, L. J. (2020). Polymorphism of PRLR gene in Jining Green goat and its association analysis with main economic traits. *Anhui Agricultural Sciences,* 48: 94-97.