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An Investigation of Polymorphism on the FASN, SCD1, and SLC27A3 Genes in Sheep

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ABSTRACT

Milk traits of sheep are affected by many environmental and genetic factors. These traits are quantitative traits and are determined by many genes. This study aimed to investigate polymorphisms of milk-related Fatty-acid synthase (FASN), Stearoyl-CoA desaturase (SCD1), and solute carrier 27A3 (SLC27A3) genes in cross-bred Hamdani sheep. Blood samples were collected from 100 healthy cross-bred Hamdani ewes from the jugular vein into K3-EDTA-containing tubes. Genomic DNA was extracted using a commercial DNA isolation kit. PCR products of FASN (275 bp), SCD1 (225 bp), SLC27A3-P1 (341 bp), and SLC27A3-P2 (319 bp) were subjected to restriction fragment length polymorphism (RFLP) analysis using *SSil (AciI), Cfr13I, AluI,* and *Hpy188III* the restriction enzyme, respectively. However, all gene regions were found to be monomorphic. In the study, only TT, AA, AA, and GG genotypes were detected for FASN, SCD1, SLC27A3-P1, and SLC27A3-P2, respectively. Allele and genotype frequencies were 1.00 for all genotypes and alleles. Although this study did not reveal favorable genotypes in FASN, SCD1, and SLC27A3 genes that can be used for milk traits, more comprehensive studies with larger sample sizes should be conducted to investigate polymorphisms in cross-bred Hamdani sheep.

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Introduction

Sheep is one of the important livestock species and is a significant source of animal products such as milk, meat, leather, and wool. Sheep milk, in particular, is highly valued for its rich nutritional content (Koca et al., 2023a; Ocak et al., 2009; Turgut et al., 2023). Milk traits are vital for sheep production and are influenced by various environmental factors and genetic structures (Bayraktar & Shoshin, 2021; García -Fernandez et al., 2010a; García-Fernandez et al., 2010b; Koca et al., 2023a; Pecka-Kiełb et al., 2021; Turgut et al., 2023). Many factors such as management, genetics, birth type, season, environment, lactation period, and diseases significantly impact the composition of sheep milk (Abd Allah et al., 2011; Koca et al., 2023a; Ocak et al., 2009; Turgut et al., 2023; Turgut et al., 2024). Genetic structure is a major factor affecting milk traits, which are controlled by multiple gene regions. Research shows that genetic polymorphisms in milk-related genes can influence the nutritional content of milk, including fat, protein, and lactose (Bayraktar & Shoshin, 2021; Pecka-Kiełb et al., 2021; Turgut et al., 2024).

Fatty-acid synthase (FASN) is an enzyme crucial for the synthesis of fatty acids, playing a role in lipid production in milk during lactation and converting carbohydrates into lipids in the liver and adipose tissue (Anderson et al., 2007). In mammals, FASN is a complex protein of about 250 kDa with around seven enzymatic functions. This protein is encoded by FASN gene which is considered a candidate gene affecting the fatty acid composition of milk in sheep and cattle (Garcia-Fernandez et al., 2010a).

Stearoyl-CoA desaturase 1 (SCD1) is another important enzyme in fatty acid biosynthesis, converting saturated fatty acids into unsaturated ones (Czerniawska-Piątkowska et al., 2021). SCD1 protein is encoded by SCD1 gene and shows high similarity across mammals (Bernard et al., 2001). In ruminants, the SCD gene is responsible for synthesizing 80% of conjugated linoleic acid (CLA) in milk (Corl et al., 2001). Studies have found polymorphisms in the SCD1 gene in different sheep breeds, linking these polymorphisms to milk traits (Czerniawska-Piątkowska et al., 2021; García-Fernández et al., 2010a; Miari et al., 2009; Pecka-Kiełb et al., 2021).

The solute carrier 27A (SLC27A) gene family, also known as fatty acid transport proteins (FATPs), is involved in the uptake of fatty acids into cells. This gene family, expressed in all tissues, consists of six members, with SLC27A3 (FATP3) being one of them (Doege & Stahl, 2006; Houten & Wanders, 2010). The SLC27A3 gene is an important candidate gene affecting milk traits in ruminants (Calvo et al., 2006; Kowalewska-Łuczak et al., 2017; Kulig et al., 2010; Pecka-Kiełb et al., 2020). Polymorphisms in this gene have been found in various sheep breeds (Bayraktar & Shoshin, 2021; Kowalewska-Łuczak et al., 2017; Pecka-Kiełb et al., 2020).

Cross-bred Hamdani sheep, raised in Siirt province, Türkiye, are preferred by breeders due to their adaptation ability to the region (Bakır & Mikail, 2019; Turgut et al., 2023). This study aims to investigate milk-related FASN, SCD1, and SLC27A3 gene polymorphisms in cross-bred Hamdani sheep raised in Siirt province.

Material and Methods

Animals and Sampling

In the study, blood samples were collected from 100 healthy cross-bred Hamdani ewes. Blood samples were collected from the *vena jugularis* into 9 mL K3EDTA-containing tubes (BD Vacutainer®, Becton Dickinson). Collected blood samples were immediately transferred to the lab on ice (0℃) and stored at -20℃ for further analyses. The study was approved by Siirt University Animal Experiments Local Ethics Committee (Approval no: 2023-01-10).

DNA Isolation and Quality Control

Genomic DNA was extracted from blood using a genomic DNA isolation kit from (Hibrigen, Hydra Biotechnology, Türkiye) following the manufacturer's instructions. The purity and concentration of the genomic DNA were assessed by measuring the optical density (OD) at 260/280 nm with a spectrophotometer (Allsheng, Hangzhou, China). The integrity of the DNA was checked using 0.8% agarose gel electrophoresis under UV. OD values of all DNA samples were between 1.70 and 1.90. In addition, genomic DNA bands were intact on the agarose gel for all samples.

Polymerase Chain Reaction (PCR)

Primer pairs of FASN, SCD1, and SLC27A3 genes were presented in Table 1. PCR reactions were carried out in 25 µl total volume; 50-100 ng genomic DNA, 12.5 µl 2X Taq PCR Mix (Hibrigen, Hydra Biotechnology, Türkiye), 5 pmol of each primer, and water up to 25 µL for FASN, SCD1, and SLC27A3-P1 and SLC27A3-P2. PCR was carried out on a Kyratec SC300G thermal cycler (Kyratec, Queensland, Australia). PCR were performed as follows: initial denaturation at 95℃ for 5 minutes, denaturation at 94℃ for 30 seconds, annealing, extension at 72℃ for 30 and final extension at 72℃ for 7 minutes. PCR annealing temperatures were 64.6℃, 68.3℃, 58.6℃, and 63.3℃ for FASN, SCD1, SLC27A3-P1, and SLC27A3-P2 gene regions, respectively. Negative controls were used to evaluate specificity of PCR reactions.

Restriction Fragment Length Polymorphism (RFLP)

Following PCR reactions, PCR products were treated by restriction enzymes. PCR products of FASN and SCD1 gene regions were treated by *SSil (AciI)* (Thermo Scientific™, USA) and *Cfr13I* (Thermo Scientific™, USA) restriction enzymes as follows, respectively: 0.5 µg PCR product, 10 units restriction enzyme, 2 µl 10X Tango Buffer and nuclease-free water up to 30 µL at 37 ℃ for ten hours. On the other hand, the RFLP reaction was carried out for 15 min at 37 ℃ in 50 µL of total volume for SLC27A3-P1 and SLC27A3-P2 PCR products as follow; 1 µg PCR product, 10 units *Alu1* and Hpy188III restriction enzymes respectively (NEB, UK), 5 µL 10X rCutSmart buffer (NEB, UK), and nuclease-free water up to 50 µL. Then, 5 µL RFLP products were loaded on 4% agarose gel electrophoresis with 110V for 30 minutes and fragments were analyzed in under UV. Then, genotypes were evaluated.

Results

PCR reactions were carried out and target regions of FASN (275 bp), SCD1 (225 bp), SLC27A3-P1 (341 bp), and SLC27A3-P2 (319 bp) genes were amplified successfully. However, In the RFLP reaction, all gene regions were monomorphic. For the FASN gene, *the SSil (AciI)* enzyme revealed only AA genotype (168 bp and 107 bp) by cutting 275 bp PCR products into two fragments for all samples (Figure 1).

Table 1. Primer pairs and PCR-RFLP conditions

Gene	Location	Primer (5'→3')	AS	AT	PC	Restriction enzyme	References
FASN	Exon 32 257 C>T	F: TGAGATGGGGCAGCAGGCCT R: GGAACACTGTTCGCTTGCGGG	275	64.6℃	35	*SSil (AciI)*	Pecka-Kiełb et al., (2021)
SCD1	Promoter 31 C>A	F: CAGGGGCAGGGGCAGAGGCA R: CGCTGGCAGCCGGTGACTGTG	225	68.3℃	40	*Cfr13I*	
SLC27A3-P1	Exon 7 1517 T>A	F: CTCCAGGTTTGTGTCCAGGT R: TTTGGGTCCCAGAGATTCAG	341	58.6℃	35	*AluI*	Pecka-Kiełb et al., (2020)
SLC27A3-P2	Exon 2 754 G>T	F: GTAGAACTGCGGGGCTGTG R: AGGAGGTCATAGTTCCTGTTCC	319	63.3℃	35	*Hpy188III*	

AS: Amplicon size (bp); AT: Annealing temperature; PC: PCR Cycle

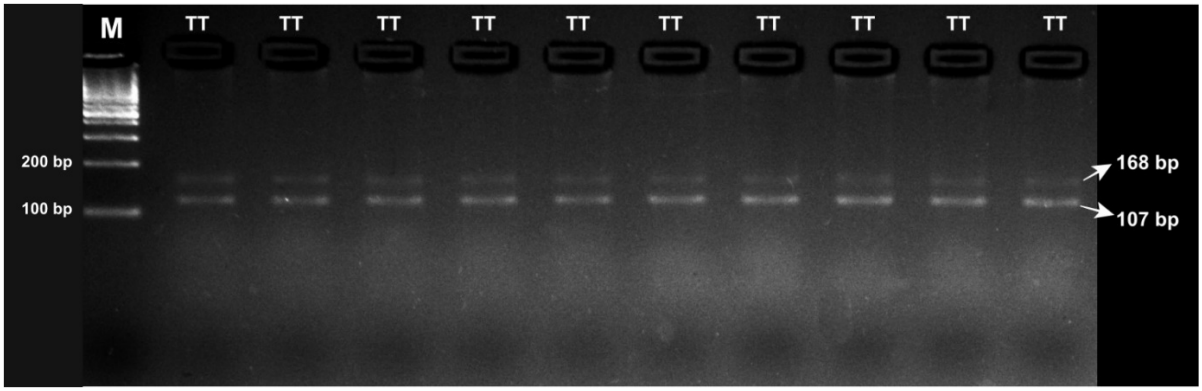

Figure 1. *SSil (AciI)* RFLP restriction pattern of FASN gene. TT genotype (168, 107 bp). M: 100 bp DNA ladder.

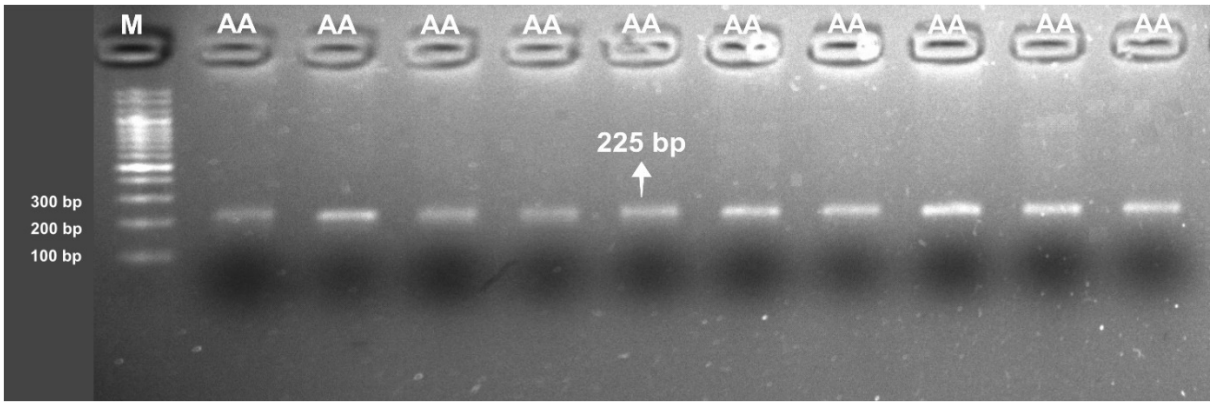

Figure 2. *Cfr13I* RFLP restriction pattern of SCD1 gene. AA genotype (225 bp). M: 100 bp DNA ladder.

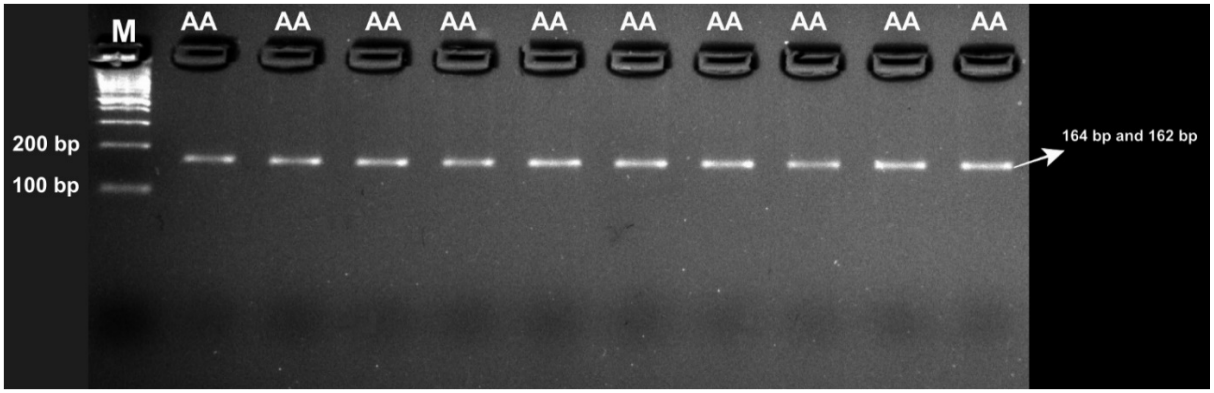

Figure 3. *AluI* RFLP restriction pattern of SLC27A3-P1 gene region. AA genotype (164, 162, and 15 bp). The smallest fragment (15 bp) is not visible on the gel. M: 100 bp DNA ladder.

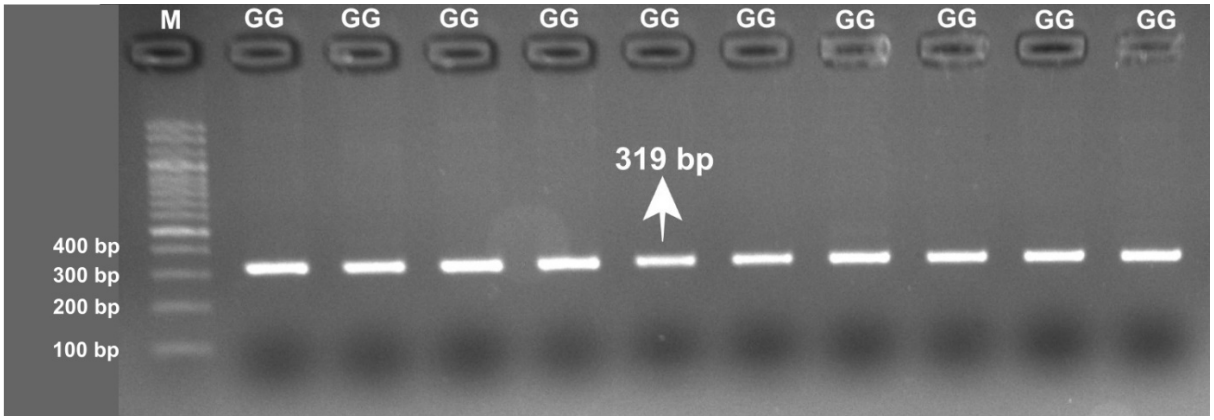

Figure 4. *Hpy188III* RFLP restriction pattern of SLC27A3-P2 gene region. GG genotype (319 bp). M: 100 bp DNA ladder.

For SCD1 gene, Cfr13I enzyme did not cut 225 bp PCR products. Therefore, only AA genotype was detected (Figure 2).

For SLC27A3-P1 gene region, *AluI* enzyme cut 341 bp PCR products into three fragments in all samples and only AA genotype (164 bp, 162 bp, and 15 bp) was detected (Figure 3). RFLP bands, 164 bp and 162 bp, did not well discriminate on the gel due to the size of fragments.

Hpy188III enzyme did not cut 319 bp PCR products of SLC27A3-P2 gene region and only GG genotype was detected (Figure 4).

Discussion

Production traits of animals are affected by different factors such as diseases (Koca et al., 2023b, Koca et al., 2024), genetic structure (Bayraktar & Shoshin, 2021; Turgut et al., 2024, Turgut & Koca, 2024a; 2024b) and environmental factors (Ocak et al., 2009; Abd Allah et al., 2011). In this context, polymorphisms in milk-related genes may influence milk composition in sheep (Pecka-Kiełb et al., 2021; Bayraktar & Shoshin, 2021; Turgut et al., 2024b). The cross-bred Hamdani sheep are predominantly raised in the Siirt province of the Southeastern Anatolian region, Türkiye, and its surroundings (Mikail and Bakır, 2019; Turgut et al., 2023; Turgut et al., 2024). Limited research has been conducted on milk traits and polymorphisms of milk-related genes in cross-bred Hamdani sheep (Turgut et al., 2023; Turgut et al., 2024).

In this study, no polymorphisms were detected in the FASN, SCD1, SLC27A3-P1, and SLC27A3-P2 gene regions of cross-bred Hamdani sheep. The allele and genotype frequencies were all 1.00 for FASN (TT), SCD1 (AA), SLC27A3-P1 (AA), and SLC27A3-P2 (GG) genotypes and the T, A, A, and G alleles, respectively. Pecka-Kiełb et al., (2021) identified only CC and CT genotypes in the FASN gene, with a higher frequency of the CC genotype and C allele in the Zošľachtená valaška sheep breed. Similarly, Crisà et al., (2010) reported that a polymorphism in exon 32 of the FASN gene. They detected C and T allele frequencies as 0.93 and 0.7, respectively. However, contrary to our results, Symeou et al., (2020) reported C and T allele frequencies as 0.67 and 0.33, respectively. Sztankoova et al., (2018) also detected that the same polymorphism (exon 32) in the FASN gene with higher C allele frequencies in East Friesian, Lacaune, Romanov, and Valachian sheep breeds.

Our results indicated that SCD1 gene region was monomorphic for relevant single nucleotide polymorphism (SNP) andonly AA genotype was observed. Pecka-Kiełb et al., (2021) and Czerniawska-Piątkowska et al., (2021) also detected polymorphisms in the SCD1 gene in Zošľachtená valaška sheep. They reported AA, CA, and CC genotypes in the SCD1 gene, with the AC genotype frequency higher than AA and CC genotype frequencies for relevant SNP. The C and A allele frequencies were reported as 0.63 and 0.37, respectively. contrary to our results. Additionally, C20:1 fatty acid composition was higher in the CC and CA genotypes compared to the AA genotype. However, SCD1 genotypes were not related to milk fat, protein, or lactose (Pecka-Kiełb et al., 2021; Czerniawska-Piątkowska et al., 2021). Naeemah & Al-Anbari, (2023) reported SCD1 gene polymorphisms associated with milk yield and composition in Iraqi Awassi sheep. Matar et al., (2023) found that SCD1 gene polymorphism is related to milk fatty-acid composition in Al-Khalidiyah sheep.

In this study, the SLC27A3 gene was found to be monomorphic for two different regions. Pecka-Kiełb et al., (2020) identified AA, AT, and TT genotypes for the SLC27A3-P1 region in Zošľachtená valaška sheep, with genotype frequencies of 0.30, 0.44, and 0.26, respectively. The A and T allele frequencies were 0.48 and 0.52, respectively. Milk fat and protein content was higher in the TT genotype compared to the AA and AT genotypes. They also detected GG, GT, and TT genotypes for the SLC27A3-P2 region, with genotype frequencies of 0.38, 0.30, and 0.32, respectively. The G and T allele frequencies were 0.53 and 0.47, respectively. However, no significant effect of these genotypes on milk composition traits was detected. In another study, Bayraktar & Shoshin (2021) found the SLC27A3-P2 gene region to be polymorphic in Hamdani sheep, with GG, GT, and TT genotype frequencies of 0.38, 0.42, and 0.20, respectively. The G and T allele frequencies were 0.59 and 0.41. Additionally, milk protein and fat content were higher in GT genotype-carrying ewes compared to GG and TT.

In conclusion, no favorable genotypes were detected in the FASN, SCD1, and SLC27A3 genes in this study, and all genes were found to be monomorphic for relevant SNPs. The findings of this study were not consistent with previous reports on polymorphisms in these genes in different sheep breeds. In this study, sampling was conducted on a small cross-bred Hamdani sheep herd, which may have limited the detection of polymorphisms in the FASN, SCD1, and SLC27A3 genes. Therefore, more comprehensive studies with larger sample sizes should be conducted to investigate polymorphisms related to milk traits in cross-bred Hamdani sheep.

Declarations

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