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# DGAT1 GENE POLYMORPHISM IN MORKARAMAN AND TUSHIN

**İremnur AYDIN[1], Sinan KOPUZLU[2]***

*[1]Atatürk University, Graduate School of Natural and Applied Sciences, Department of Animal Science, 25240, Erzurum, Türkiye*
*[2]Atatürk University, Faculty of Agriculture, Department of Animal Science, 25240, Erzurum, Türkiye*

**Abstract:** This study aims to investigate the polymorphism of the Diacylglycerol acyltransferase1 (DGAT1) gene locus in 105 Morkaraman and 65 Tushin lambs to determine the distribution of genotype and allele frequencies of lambs in terms of related genes. DGAT1/Alu1 gene polymorphism was defined by using the PCR-RFLP method in the DNAs isolated from hair samples taken from Morkaraman and Tushin lambs used in this study. PCR- RFLP products were run in an electrophoresis medium and the results were visualized on an ultraviolet (UV) transluminator. When the population was examined in terms of allele frequencies, it was defined that the C allele and the T allele were 0.72% and 0.28% for the Morkaraman, and 0.71% and 0.29% for Tushin, respectively. The CC, CT, and TT genotype frequencies of the DGAT1 gene in the population were found to be 53.3%, 38.1%, and 8.6% for the Morkaraman and 50.8%, 40.0%, and 9.2% for the Tushin, respectively. In the Hardy-Weinberg genetic equilibrium test, it was observed that the distribution of genotype frequencies was in balance (P>0.05) in the population. It has been defined that the genotype and allele frequencies determined in terms of DGAT1 gene polymorphism may be found to be sufficient to reveal the genotype diversity of the breeds. The genotype and allele frequencies determined in terms of DGAT1 gene polymorphism were sufficient to reveal the genotype diversity of the breed, the sheep with CC genotype are economically advantageous in the herd, and therefore DGAT1 gene can be used for marker-assisted selection (MAS).

**Keywords:** Polymorphism, DGAT1 gene, PCR-RFLP method, Sheep

***Corresponding author:** Atatürk University, Faculty of Agriculture, Department of Animal Science, 25240, Erzurum, Türkiye
**E mail:** srt2569@hotmail.com (S. KOPUZLU)
İremnur AYDIN https://orcid.org/0000-0003-3374-4586
Sinan KOPUZLU https://orcid.org/0000-0002-1582-3929

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## 1. Introduction

The most valuable product in human nutrition is products of animal origin. Animal proteins obtained from these products, which have a great contribution to the healthy and balanced diet of humans, can't be replaced by a different agricultural product (Gürer and Ören, 2013). Animal products are very important in the adequate and balanced nutrition of humans. In a healthy diet, nutrients such as carbohydrates, proteins and fats, which are the basic needs of the body, should be consumed in proportion. 40% of the amount of protein consumed by humans should be of animal origin (Turhan et al., 2010).

Today, the level of awareness of the need for animal products is taken into account as a development indicator of countries. The reason for this is that animal protein foods such as meat, milk and eggs are of great importance in human nutrition. In parallel with the economic and social development in developing countries, it is seen that the consumption amounts of animal products are increasing day by day with the change in consumption patterns (Yılmaz and Yılmaz, 2012).

The two basic methods to increase productivity in sheep breeding are to provide good environmental conditions for sheep and to increase their genetic value, in other words, to improve the genotype. Improving the genotype is important in animal breeding because it is permanent and continuous (Sönmez et al., 2009).

Quantitative genetic studies conducted on sheep show that some genetic factors are effective in the birth weight of lambs. It is reported that the heritability of lamb birth weight in various sheep breeds is between 0.15 and 0.24 (Safari, et al., 2005). The application of genomic selection in sheep is a successful approach to improve the studied trait since the heritability of the yield traits of these animals has been determined in the studies on this subject. Therefore, the application of genomic selection is considered a successful approach for improving the studied traits. For example, despite the small impact on livestock breeding, some candidate genes are reported to help improve some polygenic traits, such as growth, to help accurately predict the genetic value of different livestock species, including sheep (Dekkers, 2004; Ranjbari et al., 2012).

Thanks to the molecular genetic technologies developed in farm animals, markers, which are markers used at the molecular level to define the genetic structure that affects yield, provided an advantage for the determination, identification and conservation programs of populations that can be used as genetic resources (Öner et al., 2011). It has been stated in the studies that the rate of genetic progress in livestock with MAS can increase by around

15-30% (Bal and Akyüz, 2014). The genetic progress aimed to be achieved in the desired breed with MAS is faster than classical selection methods (Reis et al., 2001).

Molecular genetics techniques allow the identification of relationships between yield traits and diversity in the Quantitative Trait (QTL), and the identification of genetic variation at various loci. Selection aims to estimate the genetic value of the animal with greater accuracy and thus increase the genetic gain resulting from the selection. It has been reported in studies that variations in genes that affect physiological events related to the phenotype may be effective on quantitative variations in the related phenotype (Tambasco et al., 2003).

Phenotypic traits and polymorphisms in marker genes are also used in the characterization of races. In addition, genetic polymorphisms in candidate genes have been an important research topic in genetic selection and specifying evolutionary relationships between different races. One of these genes is the Diacylglycerol acyltransferase1 (DGAT1) gene (Bal and Akyüz, 2014).

DGAT1 is a microsomal enzyme involved in the synthesis of triglycerides in adipocytes (Winter et al., 2002). DGAT1 also plays a fundamental role in intestinal fat absorption, lipoprotein assembly, regulation of plasma triacylglycerol concentrations, fat storage in adipocytes, energy metabolism in muscles, and milk production, including mammalian oocytes. It catalyzes the terminal and only stable step in the synthesis of triacylglycerol by using diacylglycerol and fatty acyl-coenzyme A as substrates. Acyl CoA catalyzes the terminal and only stable step in the synthesis of triacylglycerol using diacylglycerol and fatty acyl-coenzyme A as substrates. (Cases et al., 1998). The DGAT1 gene encoding this enzyme is found in many tissues. However, it is predominantly found in adipose tissue and the small intestine (Buhman et al., 2002). Studies have shown that there is a relationship between DGAT1 gene and fat accumulation in sheep and cattle carcasses. The DGAT1 gene is a putative alternative gene for milk fat content in sheep (Curi et al., 2011; Mohammadi et al. 2013). However, studies investigating the relationship between SNPs in the DGAT1 gene and mutton productivity are scarce. In one of the studies conducted with the Mogan Iranian sheep breed, it was reported that there is a relation between the polymorphism in exon 7 of the DGAT1 gene and the carcass weight (Noshahr and Rafat, 2014). DGAT1 is a candidate gene due to its important role in fat metabolism, milk fat content and carcass characteristics in dairy sheep and goats (Sadeghi et al., 2020).

Morkaraman breed is generally raised in many provinces of Türkiye, especially in Erzurum, Kars, Ağrı, Muş and Van provinces located in the Eastern Anatolia Region. In the environmental conditions of the regions where it is grown, the race has characteristics such as being well-adapted, walking long distances, resistance, and high viability. Morkaraman comes after Akkaraman sheep breed in terms of breeding density in Türkiye. The average birth weight of lambs was 3-4 kg, average live weight of lambs weaned in 90 days was 20 kg. While the average mature live weight is between 50 and 60 kg in rams, it was between 40 and 60 kg in sheep. Morkaraman lambs were found to have an average daily live weight gain of 200 gr, an average hot carcass weight of 21 kg and an average hot carcass yield of 49% under the current pasture conditions in the region (Köprücü, 1975; Geliyi and İlaslan, 1978; Ulusan and Aksoy, 1996; Macit and Aksoy, 1996; Esenbuğa et al., 1998).

Tushin sheep is a breed that is mostly bred in northeastern Türkiye in Kars (Çıldır district), Ardahan and Iğdır provinces. This breed is usually small in size, the body fleece is bright and white. It is known as a breed that makes good use of pastures because it can be cultivated in regions with mountainous, high altitude and rough terrain conditions. The average birth weight of lambs in this breed is 3.7 kg, 18-month live weight is between 45 and 50 kg, daily live weight gain is 190 g and carcass weight is around 20 kg (Anonymous, 2009).

This study aims to investigate the presence of polymorphism in terms of DGAT1 gene locus using PCR-RFLP methods in Morkaraman and Tushin sheep raised on the mentioned farm and to reveal the distribution of genotypes and allele frequencies of sheep in terms of this gene locus.

## 2. Materials and Methods

### 2.1. Materials

The hair samples were taken from 105 Morkaraman breed and 65 Tushin breed sheep and genomic DNA was obtained from them, which were raised at Atatürk University, Food and Livestock Application and Research Center, Sheep Breeding Branch. Birth weights were taken within the first 24 hours of birth and ear-number earings were attached to the ears of each animal. Birth and weaning weights were measured with a 100 g precision scale. Depending on the pasture conditions, the average age of the lambs on the day they went out to graze was calculated as 49 days for Morkaraman and 60 days for Tushin.

Hair samples were taken from the sheep and taken into 10 ml Eppendorf tubes, the label numbers were recorded on the tubes, and the samples were transported to the Genetics Laboratory of the Department of Animal Science, Faculty of Agriculture, Atatürk University, with sample carrying bags containing.

### 2.2. Methods

Genomic DNA isolation was conducted using a commercial DNA isolation kit (Purgene DNA kit, Gentra Systems, Minnesota). The qualitative and quantitative controls of the obtained DNAs were determined by using the NanoDrop ND-1000 (NanoDrop Technologies Inch.) spectrophotometric methods.

In the PCR, the 309 bp DNA region was amplified using primers F: 5'-GCA TGT TCC GCC TCC TGG-3' and R: 5'-GGA GTC CAA CAC CCC TGA-3'. For PCR, 3 µl of genomic DNA samples were taken into 0.2ml tubes, and 3.75 µl of

10x Buffer, 1 µl of Primer R, 1 µl of Primer F, 1 µl of MgCl2, 0.5 µl of DNTPS, 2.4 and 20 µl of the 12.5 µl dH2O mixture was added and centrifuged. Afterwards, the tubes were placed in the PCR device and the PCR program was applied. The PCR program was set to 35 cycles with an initial denaturation at 96 °C for 5 minutes, denaturation at 96ºC for 50 seconds, bonding at 58ºC for 50 seconds, elongation at 72 °C for 1 minute, and final elongation at 72 °C for 5 minutes.

10 µl of each amplified DGAT1 PCR product was taken and placed in 0.2 ml sterile Eppendorf tubes, and 5 µl of Alu1 enzyme, 5 µl of Buffer R and 2.4 µl of Buffer Tango were added. Then, it was centrifuged by covering it with approximately 5-10 µl of mineral oil. Incubation was carried out at 37 °C for 12 hours. The incubated products were carried out on a 2% agarose gel at 45 volts for 90 minutes and electrophoresis was applied. After the electrophoresis, the gel was taken and examined under UV light for genotyping.

**2.3. Statistics Analyses**

Whether the DGAT1 genotype frequencies are in Hardy-Weinberg equilibrium was investigated by the Chi-square test. A correlation study was conducted using the birth and weaning weight records of Morkaraman (105) and Tushin (65) sheep raised at Atatürk University Food and Livestock Application and Research Center Farm. The data obtained from the records of these breeds were analyzed for variance separately for each breed, and the SPSS 20.0 statistical package program was used for these analyses.

## 3. Results and Discussion

This section may be divided into subheadings. It should provide a concise and precise description of the experimental results, their interpretation, as well as the experimental conclusions that can be drawn.

**3.1. Observation of PCR Results**

Each of the DNA samples obtained from Morkaraman and Tushin sheep hairs was PCR performed and run on a 1% agarose gel and DNA bands were obtained. The agarose gel image of the PCR products under UV light is shown in Figure 1.

**3.2. PCR-RFLP Results**

DNA samples obtained from Morkaraman and Tushin breed sheep were amplified in PCR device and cut with Alu1 Restriction Endonuclease enzyme and DGAT1 gene polymorphic regions were determined. In theory; It gives bands of CC: 309 bp, CT: 309/272/37 bp and TT: 272/37 bp in length. An exemplary agarose gel image of the PCR-RFLP results under UV light is presented in Figure 2.

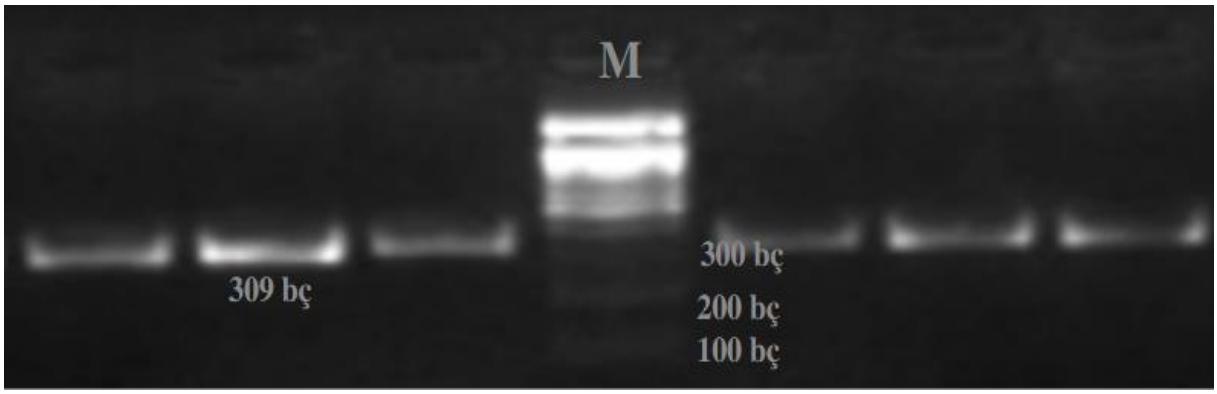


**Figure 1.** DGAT1 gene PCR products treated with ethidium bromide 1.5% agarose gel DNA band views (M: Marker, Other lines amplification products: 309 bp).

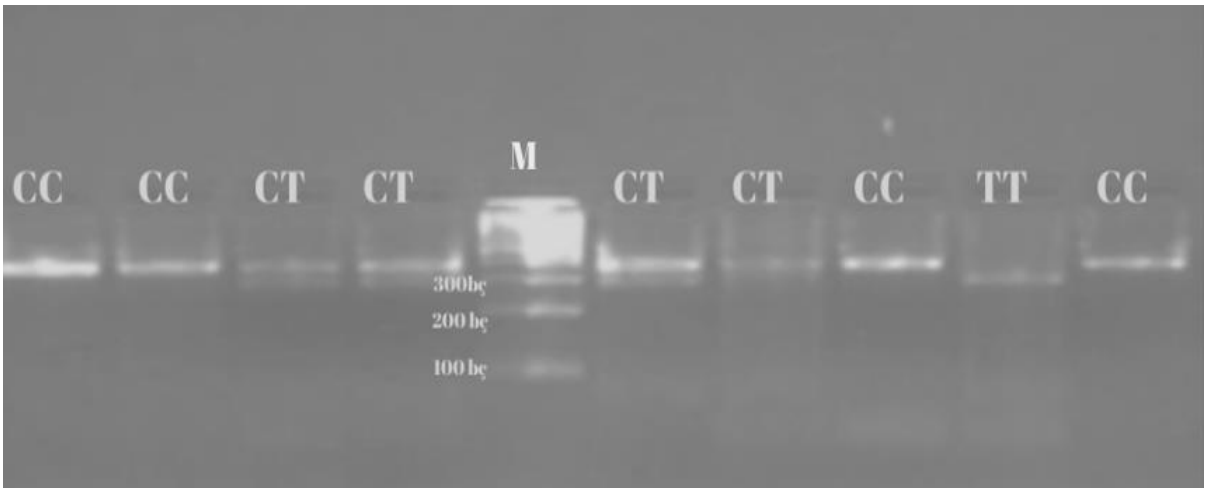


**Figure 2.** Electrophoretogram of *Alu*I digested PCR product generated by amplification of genomic DNA, using DGAT-specific primers. Lane: M: 100-bp DNA ladder; Lanes 1.5: CT, Lines 2.4: CC, Line 3: TT.

### 3.3. Genotype Frequencies and Genetic Equilibrium Test Results

According to the Hardy-Weinberg genetic equilibrium test, the distribution of genotype frequencies belonging to Morkaraman and Tushin sheep was found to be in equilibrium (P>0.05) in the distribution of genotype frequencies belonging to 105-head Morkaraman and 65-head Tushin sheep.

The Hardy-Weinberg genetic equilibrium test results of the breed DGAT1 gene Alu-1 polymorphism are presented in Table 1.

In the study, 3 different genotypes belonging to the Alu1 enzyme cutting region on the DGAT1 gene polymorphic region were defined as TT, TC and CC. Detected genotypes and allele gene frequencies are presented in Table 2.

When the population was examined in terms of allele frequencies, it was determined that the C allele was 76 (72%) and the T allele was 29 (28%) in Morkaraman sheep, while the C allele was 46 (71%) and the T allele was 19 (29%) in the Tushin sheep breed (Table 2). It was observed that the C allele was at a higher frequency than the other allele in both breeds.

Animals with 56 CC genotypes, 40 CT genotypes and 9 TT genotypes were determined in the Morkaraman sheep breed, 33 CC genotypes, 26 CT genotypes and 6 TT genotypes were determined in Tushin sheep breed. The CC, CT and TT genotype frequencies were obtained as 53.3%, 38.1% and 8.6% in Morkaraman sheep, 50.8%, 40% and 9.2% in Tushin sheep, respectively. It was observed that the CC genotype frequencies were the highest in the population in both breeds, while the TT genotypes had the lowest frequency.

Unlike this study, the result that the DGAT1 gene is polymorphic with two genotypes, CC and CT, obtained from other similar studies on DGAT1 gene with Barki, Rahmani and Osseimi (Mahrous et al., 2015), Deccani, Mandya and Ganjam (Kumar et al., 2016), Akkaraman (Bayram et al., 2019), Barki, Najdi and Harri (Altwaty et al., 2020) sheep breeds was determined differently from this study, and when the frequencies of these genotypes were taken into consideration, it was determined that the frequency of the CC genotype had the highest value compared to the frequencies of other genotypes in both breeds in this study as in other studies.

Among the previous studies on the DGAT1 gene with different sheep breeds, Barki, Rahmani and Osseimi sheep breeds (Mahrous et al., 2015), Deccani, Mandya and Ganjam sheep breeds (Kumar et al., 2016), Akkaraman sheep breed (Bayram et al., 2019), Barki, Najdi and Harri sheep breeds (Altwaty et al., 2020) were found to be polymorphic with two genotypes, CC and CT, unlike this study, and when the frequencies of these genotypes were considered, it was observed that the frequency of the CC genotype had the highest value, which was consistent with the results of the study. Among the studies conducted with other sheep breeds, Tan, Ganjia, Oula and Qiaoke sheep breeds (Yang et al., 2011), Moghani sheep breed (Noshahr and Rafat, 2014), Mehraban sheep breed (Sajad et al., 2014), Turcana breed (Tăbăran et al., 2014), Jaisalmeri, Muzzafarnagri, Nali, Nellore and Magra sheep breeds (Kumar et al., 2016), Malpura sheep breed (Meena et al., 2016), Jaisalmeri, Muzaffarnagri, Nali, Nellore and Magra sheep breeds (Kumar et al., 2016), Lori sheep breed (Nanekarani et al., 2016), Egyptian Barki sheep breed (Abousliman et al., 2020), and Awassi sheep breed (Bayraktar and Shoshin, 2021) were found to be polymorphic with three genotypes (CC, CT and TT), which was consistent with this study. At the same time, in these studies indicating DGAT1 gene polymorphism, it was reported that the CC genotype was at a higher frequency than the CT and TT genotypes, and it was found to be similar to the findings of this study.

The C allele gene frequency was found to be higher than the T allele gene frequency in both breeds. This result is consistent with the results published by Yang et al., 2011; Ala Noshahr and Rafat, 2014; Tăbăran et al., 2014; Mahrous et al., 2015; Meena et al., 2016; Kumar et al., 2016; Nanekarani et al., 2016; Bayram et al., 2019; Altwaty et al., 2020; Abousoliman et al., 2020; Bayraktar and Shoshin, 2021. However, in some other studies (Xu et al., 2008; Mohammadi et al., 2013; Noshahr and Rafat, 2014; Özmen and Kul, 2016), contrary to this study, T allele gene frequency was found to be higher than the C allele gene frequency.

**Table 1.** Genotype frequencies and Hardy-Weinberg genetic equilibrium test results

| | N | Observed | | | Expected | | | $\chi^2$ | P |
|---|---|---|---|---|---|---|---|---|---|
| | | CC (%) | CT (%) | TT (%) | CC (%) | CT (%) | TT (%) | | |
| Morkaraman | 105 | 56 (53.3) | 40 (38.1) | 9 (8.6) | 55 | 42 | 8 | 0.234 | 0.629 |
| Tushin | 65 | 33 (50.8) | 26 (40.0) | 6 (9.2) | 32.5 | 26.9 | 5.6 | 0.072 | 0.789 |

**Table 2.** DGAT1 Allele gene frequencies of Morkaraman and Tushin

| Allel Gene Frequency (%) | Morkaraman | | Tushin | |
|---|---|---|---|---|
| | C | T | C | T |
| | 76(%72) | 29(%28) | 46(%71) | 19(%29) |

## 5. Conclusion

Thanks to molecular techniques such as PCR and RFLP based on DNA, provide the opportunity to determine genotypes more easily and accurately at very early ages, regardless of gender. These techniques are used as a tool for the early identification of animals and the association between genotypes and performance traits. In addition to polymorphism studies, studies on associating polymorphism with various performance characteristics should also be conducted. It is thought that working with different breeds and larger populations of these breeds in demonstrating the usability of the polymorphism determined by these studies in animal breeding may reveal new possibilities in the definition and development of animal husbandry in Türkiye.

**Author Contributions**

The percentage of the author(s) contributions is presented below. All authors reviewed and approved the final version of the manuscript.

| | İ.A. | S.K. |
|---|---|---|
| C | 50 | 50 |
| D | 40 | 60 |
| S | 30 | 70 |
| DCP | 80 | 20 |
| DAI | 20 | 80 |
| L | 80 | 20 |
| W | 20 | 80 |
| CR | 20 | 80 |
| SR | | 100 |
| PM | 20 | 80 |
| FA | | 100 |

C=Concept, D= design, S= supervision, DCP= data collection and/or processing, DAI= data analysis and/or interpretation, L= literature search, W= writing, CR= critical review, SR= submission and revision, PM= project management, FA= funding acquisition.

**Conflict of Interest**

The authors declared that there is no conflict of interest.

**Ethical Consideration**

The experimental procedures were approved by the Ethics Committee of the Faculty of Agriculture of Atatürk University, (protocol code: 2023/05 and date: May 15, 2023).

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