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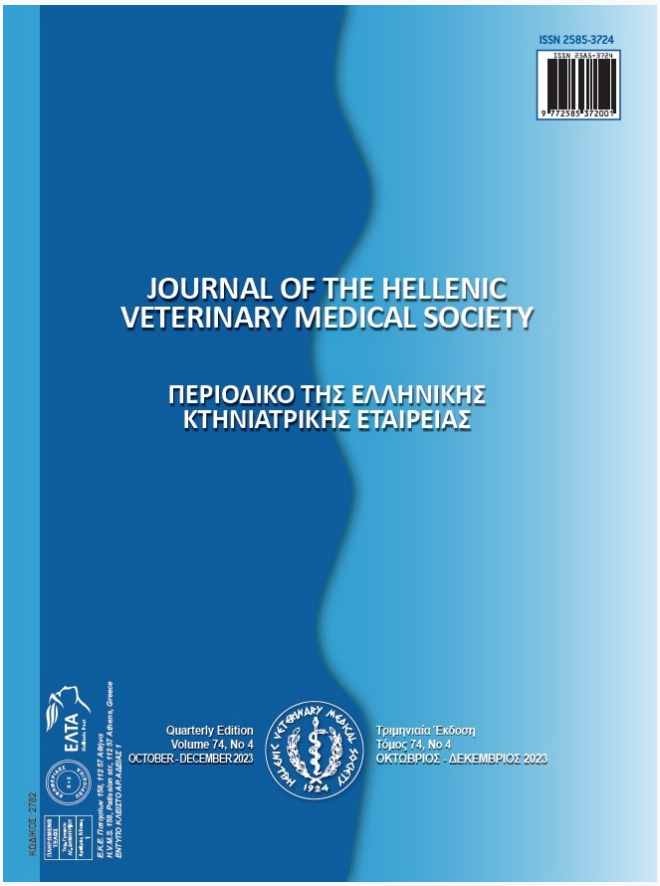

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Associations Between DGAT1/Alu1 Gene Polymorphismand Some Performance in Morkaraman andTushin Sheep

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ABSTRACT: DGAT is a microsomal enzyme that plays a central role in the biosynthesis of cellular triacylglycerols. The DGAT1 gene, which is involved in fatty acid metabolism, is accepted as a candidate gene for improving meat quality characteristics in sheep. In Morkaraman and Tujsheep, The objective of the current research was to examine the genotypes of the myostatin gene and reveal the associations between the genotypes and some performance traits such as birth weight, weaning weight, weaning age, average daily weight gain, and litter size. Genotypes were determined by the PCR-RFLP method using the Alu1 restriction enzyme, and the genotype frequencies of Morkaraman and Tushin sheep were found to be 50% and 53%; 38.7% and 38%; 11.3% and 9% for CC, CT, and TT in the DGAT1 locus, respectively. It was defined that the C and the Tallele frequencie were determined as 69% and 31% for Morkaramans and 72% and 28% for Tuj sheep, respectively. It was observed that the distribution of the DGAT1 gene locus examined in both breed populations was in balance according to the HW genetic equilibrium test. The association analysis revealed no statistically significant impact of the DGAT1 gene polymorphism in exon 3 on birth weight, weaning weight, weaning age, litter size, and daily live weight gain(P>0.05). DGAT1 gene showed polymorphisms in Morkaraman and Tushin sheep and can be regarded as a genetic marker for both sheep breed selection according to the association analysis results.

***Keywords*:** DGAT1; Morkaraman; Tushin; polymorphism; PCR-RFLP.

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INTRODUCTION

Sheep breeding is a preferred animal production branch in the World and especially in Turkey, due to its feature of being a low-input production branch in rural areas with scarce resources. Sheep make good use of the natural vegetation of such areas and transform the min to foods such as meat and milk needed for human nutrition. Turkey is a rich country in terms of domestic sheep breeds raised in different regions and altitudes. According to 2022 statistics, 91,1% of the country's total 46.123 million sheep (TUİK, 2022) assets consist of domestic sheep breeds. Morkaraman reared primarily for meat yield and Tushin sheep reared for meat, fleece, and milk yield breeds area mong the domestic sheep breeds in Turkey (Anonymous, 2009).

To increase productivity and yield potential in sheep breeding, It has been emphasized to provide better environmental conditions for sheep and to increase the genetic value of sheep or to improve their genotype. The improvement of the genotype from these two main ways gains importance in animal breeding as it is permanent and continuous (Sönmez et al., 2009). With the identification of genes belonging to various traits, it will be possible to predict the future phenotypes of individuals in animal husbandry by looking at their genotypes at an earlier period. Thus, it will be possible to shorten the generation period by making a direct selection in terms of the desired trait. By keeping individuals with the desired characteristics in herds by direct selection, animal breeders will be provided with an increase in productivity and, accordingly, economic benefits.

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). 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(Tambasco et al., 2003).

The production and quality of sheep meat can be increased even further by researching the impacts of the DGAT1 gene on sheep meat production and quality and making the said gene available for breeding studies in sheep breeding.

The yield obtained from animals occurs concerning the joint effect of phenotype, environment, and genotype. Therefore, improving both the environment and the genotype leads to an increase in yield. The phenotypic value in these quantitative traits often does not reflect the genotypic value because the characteristics of various yields (such as milk, fleece, egg, and meat) obtained from farm animals are under the control of a large number of genes and are greatly affected by various environmental factors. For these reasons, it is of great importance to estimate the phenotypic level of such characters (Özdemir, 2005).

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. DGAT1 also plays a fundamental role in intestinal fat absorption, lipoprotein assembly, and 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 CoA as substrates (Cases et al., 1998). 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 the 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 link between the polymorphism in the 17th exon 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 Turkey, 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, resisting, and having high viability. Morkaraman comes after Akkaraman sheep breed in terms of breeding density in Turkey. 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 (Geliyi and İlaslan 1978, Macit and Aksoy 1996, Esenbuğa et al. 1998).

Tuj sheep is a breed that is mostly bred in northeastern Turkey 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 altitudes, 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 aimed to investigate the genotypic structures of the DGAT1 gene locus AluI polymorphism and research the relationships between DGAT1 genotypes and some traits such as birth weight, weaning weight, weaning age, litter size and average daily weight gain between birth and weaning in Morkaraman and Tushin sheep.

MATERIALS AND METHODS

Materials

In the current research, hair samples were collected from unrelated 124 Morkaraman sheep raised as recorded in the pedigree and growth data in the Food and Livestock Application and Research Center (GHUAM), Sheep Breeding Branch at Ataturk University, Erzurum province.

The animal material of this study consisted of hair samples taken from 124 Morkaraman breed and 100 Tushin breed sheep and genomic DNA obtained from them, which were raised at Atatürk University, Food, and Livestock Application and Research Center (GHUAM), Sheep Breeding Branch. Various laboratory stages of the study were carried out Molecular Genetics Laboratory and Agricultural Biotechnology Laboratory, Faculty of Agriculture, Department of Animal Science, at Atatürk University.

Birth weights were taken within the first 24 hours of birth and ear tags earings were attached to the ears of each animal. Birth and weaning weights were measured with a 100 g precision scale. The average weaning age was determined as 49 days for Morkaraman sheep and 60 days for Tushin sheep.

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 +4° ice trays.

Methods

Genomic DNA isolation was obtained 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.) spectrophotometer device.

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 96ºC for 5 minutes, denaturation at 96ºC for 50 seconds, bonding at 58ºC for 50 seconds, elongation at 72ºC for 1 minute, and final elongation at 72ºC 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.

Statistical analysis

The allele gene and genotype frequencies and HardyWeinberg genetic equilibrium test for the examined population were computed in the GenAlEx 6.5 software (Peakall and Smouse 2012).

The Morkaraman aged 4 years and Tushin aged 2 years sheep were held under semi-extensive conditions. The associations between some yield traits and genotypic structures of Morkaraman sheep were studied. To this end, primarily their birth weight, weaning weights, weaning ages, average daily weight gains and litter size were determined as the production and reproduction traits of both sheep breeds. As reproductive traits, the lambing rates (litter size) in birth for each ewe mated were calculated. But due to insufficient data, the weaning age and litter size traits of Tushin sheep were not analyzed. Feeding and management practices were administered in an equal manner to all lambs. While the data were analyzed statistically, the race factor was not included in the model and the model for data analysis separately per breed. In the analysis of the data, SPSS statistical software (IBM SPSS 25.0 Corp. Inc.) was utilized based on the general linear model. The birth weight would be preferrably included in the model as a covariate. The association analyses separately examined the impact of genotype on birth weight, weaning weight, weaning age, litter size, and average daily weight gain. Whether the DGAT1 genotype frequencies are in Hardy-Weinberg equilibrium was investigated by the Chi-square test.

The following statistical model was used according to the yield characteristics in the study.

Y_i:$\mu+a_i+e_{ij}$

Yi: Value of any sheep for the considered performance (birth weight, weaning weight, litter size, weaning age and average daily weight gain) traits

µ: population mean;

a_i: effect of genotype (i: 3; CC; CT; TT);

e_{ij}: margina lerror

Inthe model used, the genotype effects were accepted as constant, while the error was accepted as random.

RESULTS AND DISCUSSION

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.

DNA samples obtained from Morkaraman and

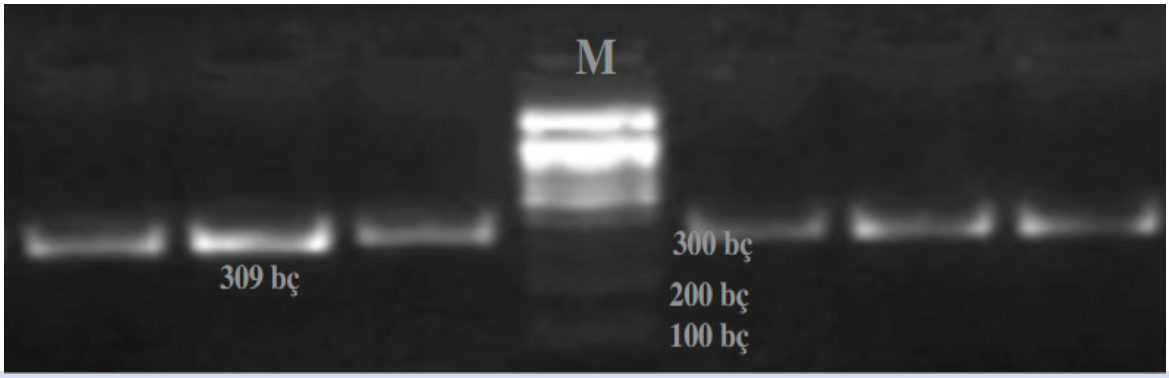

Figure 1. Agarose gel image of PCR products (M: marker, DGAT1:309 bp)

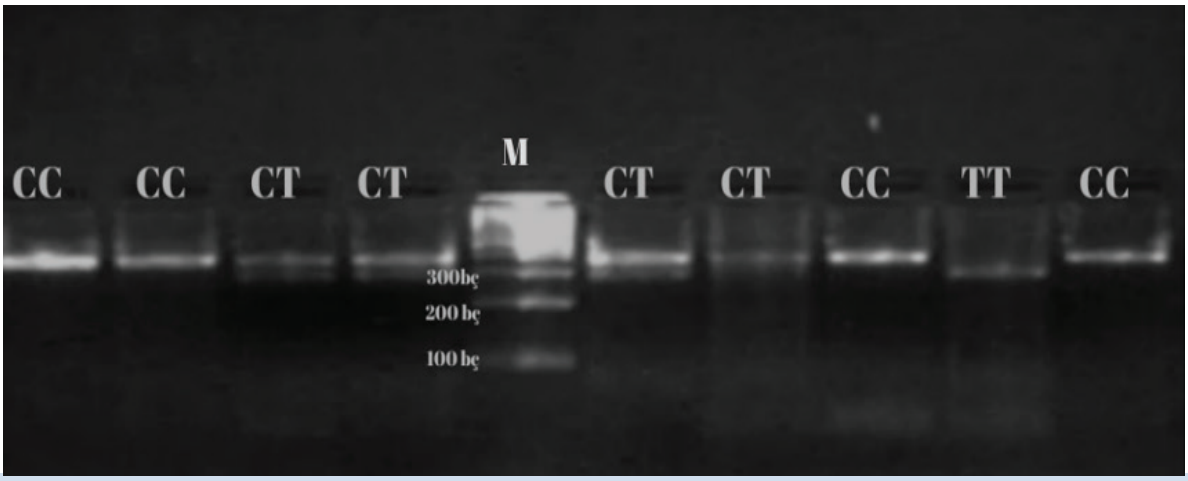

Figure 2. PCR-RFLP gel image of the DGAT1 gene (CC; 309 bp, CT; 309/272/37 bp, TT; 272/37 bp)

Tushin breed sheep were amplified in a 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.

In the study, 3 different genotypes belonging to the Alu1 enzyme cutting region on the DGAT1 gene polymorphic region were defined as CC, TC, and TT Detected genotypes and allele gene frequencies are presented in Table 1.

When the population was examined in terms of allele frequencies, it was determined that the C allele was 0.69 and the T allele was 0.31 in Morkaraman sheep, while the C allele was 0.72 and the T allele was 0.28 in the Tushin sheep breed (Table 1). It was observed that the C allele was at a higher frequency than the other allele in both breeds.

Animals with 62 CC genotypes, 48 CT genotypes and 14 TT genotypes were determined in Morkaraman sheep breed, and 53 CC genotypes, 38 CT genotypes, and 28 TT genotypes were determined in Tushin sheep breed. The CC, CT, and TT genotype frequencies were obtained as 50%, 38.7%, and 11.3% in Morkaraman sheep, 53%, 38%, and 9% 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.

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 our study, and when the frequencies of these genotypes were considered, it

Table 1. DGAT1 Genotype and Allele Gene Frequencies of Morkaraman and Tushin Sheep

Genotype	Morkaraman		Tushin	
	N	%	N	%
CC	62	50.0	53	53
CT	48	38.7	38	38
TT	14	11.3	9	9
Allele Gene	**C**	**T**	**C**	**T**
Frequency (%)	69	31	72	28

Table 2. Hardy-Weinberg Genetic Equilibrium Test Results

Breed	N	Observed			Expected			X^2	P
		CC	CT	TT	CC	CT	TT		
Morkaraman	124	62	48	14	59.6	52.7	11.6	0.99	0.32
Tushin	100	53	38	9	51.8	40.3	7.8	0.33	0.57

was observed that the frequency of the CC genotype had the highest value, which was consistent with our 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), Lori sheep breed (Nanekarani et al., 2016), Egyptian Barki sheep breed (Abousliman et al., 2020), and Awassi sheep breed (Bayraktar and Shoshin, 2022) 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 our study.

In studies, the C allele gene frequency was found to be higher than the T allele gene frequency (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; Kumar et al., 2016; Bayram et al., 2019; Altwaty et al., 2020; Abousoliman et al., 2020; Bayraktar and Shoshin, 2022) and it was determined to be compatible with allele gene frequencies as in our study. However, in other studies (Xu, et al., 2008; Mohammadi et al., 2013; Noshahr and Rafat, 2014; Özmen and Kul, 2016) it was concluded that the T allele frequency was found to be higher than the C allele frequency, and this study differs from the study we conducted.

The Hardy-Weinberg genetic equilibrium test results of the breed DGAT1 gene Alu1 polymorphism are presented in Table 2.

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 Morkaraman and Tushin sheep.

Associations between DGAT1/AluI genotypes and some performance traits, including birth weight, weaning weight, weaning age, litter size, and weight gain, were researched. Table 3 demonstrates the least squares mean and standard errors of the DGAT1/AluI genotypes concerning several yield traits.

The mean birth weights of Morkaraman and Tushin sheep and their standard deviations are given in Table 3. The mean birth weight of the Morkaraman and Tushin sheep was determined as 4.40 kg and 3.94 kg, respectively. As a result of the evaluation of the data obtained, the highest mean birth weight value among DGAT1 genotypes was determined in the CC genotype (4.43 kg) for Morkaraman and in the CT genotype (4.26 kg). while it was determined as 4.28 kg and 4.25 ± 0.10 kg in TT and Ct genotypes in Morkaraman and 4.11 kg and 3.96 kg in CC and TT genotypes in Tushin, respectively. When the results of the analysis of variance were examined, it was observed that the effect of genotype on birth weight for both breeds was insignificant (P>0.05). Among the studies conducted in terms of the relationship between the DGAT1 gene and birth weight in sheep, different from this study; the average birth weight was found higher in TC genotype lambs in the Akkaraman breed (Bayram et al., 2018) and TT genotype lambs in Egyptian Barki breed (Abousoliman, et al., 2020) compared to lambs with other genotypes. In both studies, it was reported that the effect of genotype on birth weight was insignificant in parallel with the results of both breeds in this study.

In the study, the general average weaning weight of sheep raised under operating conditions was found to be 14.36 kg in Morkaraman sheep and 11.52 kg in Tushin sheep. When the genotypes were examined by taking into account the weaning weight, in both Morkaraman and Tushin, the TT genotype has the lowest value with 13.87 kg and 11.94 kg, respectively. In Morkaraman, Ct (14.67 kg) and CC (14.54 kg) genotypes have close values. In Tushin, the average values of this feature were found to be 16.67 kg in the CT genotype and 12.98 kg in the TT genotype. As a result of statistical analysis, weaning weight average differences between genotypes in both breeds were found to be insignificant (P>005). No significant difference was observed between the means. In the association studies between DGAT1 gene polymorphism and growth trait in sheep, the highest value in terms of weaning weight was determined in Morkaraman and Tushin lambs with CT genotype lambs, In a study conducted by Bayram et al (2018), they found that Akkaraman lambs with CC genotype had the highest weaning weighting value (9.12) compared to other genotypes. This result was different from the result we found in our study. Similar to our findings, Abousoliman et al. (2020) stated that Egyptian Barki lambs with the CT genotype had the highest weaning

weighting value (13.73kg) compared to lambs with the other two genotypes. As in this study, the differences between the weaning weight averages determined for the DGAT1 genotypes in both studies were found to be insignificant (Table 3). The mean weaning age in the CC, CT, and TT genotypes was determined to be 48.20 days, 52.04 days, and 47.15 days, respectively, and the order was CT>CC>TT in Morkaraman. The weaning age of the TT genotype was revealed to be shorter than that of the sheep with the CT and CC genotypes, and the said difference was statistically insignificant (P>0.05).

The impact of genotype on litter size in both breeds was determined to be insignificant (P>0.05). Whereas the mean litter size of CC, CT, and TT genotype groups was acquired as 1.03 and 1.06, 1.07 and 1.47, and 1.03 and 1.00 in Morkaraman and Tuj sheep, respectively, the order for Morkaraman and Tushin was CT>CC>TT and CT>CC>TT, respectively. The means of the genotype groups in both breeds did not differ significantly (Table 3).

The mean daily live weight gain from birth-toweaning in Morkaraman lambswas 204.11g. The mean daily live weight gain from birth to weaning in Morkaraman lambs was 204.11g. These values were determined as 209.24 g in CC genotype199.78 g in CT genotype, and 203.32 g in TT genotype. The order of genotypes was CC>TT>CT. As a result of the statistical analysis, There was no significant difference between genotypes in terms of mean daily live weight gain between birth and weaning (P>0.05).

CONCLUSIONS

PCR-RFLP method was used on the hair samples of Morkaraman and Tushin sheep breeds raised in Atatürk University, Food and Livestock Application and Research Center, Sheep Branch, and three genotypes (CC, CT. and TT) belonging to the DGAT1 gene Alu1 polymorphism were determined. As a result, three genotypes, CC, TT, and CT were determined in the current research at a rate of 50.0%, 11.3%, and 38.7%, for Morkaraman, at a rate of 53%, 9%, and 38% for Tushin respectively. The C and T allele frequency was found to be 69% and 31% in Morkaraman, respectively, whereas the C and t frequency was revealed to be 72% and 28% in Tushin, respectively. In both breeds, No statistical association was found between the impact of the DGAT1/AluI polymorphism and birth weight, weaning weight, weaning age, litter size, and average daily live weight gain.

DGAT1 genotypes of each sheep breed were determined using molecular techniques such as PCR and RFLP based on DNA. Thanks to these techniques, it is used as a tool for identifying animals in early periods and associating genotypes with performance characteristics. The DGAT1 gene exhibits polymorphisms in Morkaraman and Tushin sheep. However, for it to be regarded as an important genetic marker, further investigations on the DGAT1 gene polymorphisms are suggested in all sheep breeds all around the world to assess potential sheep breeds and use it as a genetic marker in improving growth traits.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

Table 3. The least squares mean and standard errors of DGAT1 genotypes in terms of some yield traits in Morkaraman andTushin sheep

Breed	Genotype	N	Birth Weight (kg)		Weaning Weight (kg)		Weaning Age (Days)		LitterSize		Daily Live Weight Gain (g)	
			Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Morkaraman	CC	60	4.43	0.084	14.54	0.466	48.20	1.525	1.03	0.018	209.24	6.881
	CT	48	4.28	4.281	14.67	0.521	52.04	1.705	1.07	0.020	199.78	7.693
	TT	13	4.48	4.477	13.87	1.002	47.15	3.276	1.03	0.038	203.32	14.782
	Total	121	4.40	0.047	14.36	0.407	49.13	1.322	1.04	0.016	204.11	6.010
Tushin	CC	24	4.11	0.121	12.98	0.712	-	-	1.06	0.267	-	-
	CT	20	4.26	0.132	16.67	0.779	-	-	1.47	0.293	-	-
	TT	4	3.96	0.295	11.94	1.743	-	-	1.00	0.655	-	-
	Total	48	3.94	0.171	11.52	1.009	-	-	1.13	0.379	-	-

SE: Standard Error

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