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The Effects of DGATI-K232A Gene Polymorphisms on Milk Performance Traits in Simmental Cattle

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ABSTRACT

Growing world population, scientists aim to achieve high-yielding products by using new techniques and methods in the fields of food, agriculture, and livestock. The aim of this study is to identify the DGAT/K232A gene polymorphism for Enhancing Performance Characteristics in Simmental cattle and to use it in breeding programs. DGAT/K232A gene polymorphism was analyzed by RFLP method in 70 Simmental cattle using CfrI restriction enzyme. The frequency of the K allele was found 0.77, while A allele was 0.23. The distribution of DGAT1-K232A genotype frequencies in the breed was not balanced ($p<0.05$). No significant relationship was found between DGAT1/K232Agene polymorphisms and milk yield due to the small number of samples.

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Introduction

The presence of multiple genes controlling economically significant traits could pose limitations to contemporary breeding methodologies in animal breeding.Therefore, new molecular techniques and statistical methods need to be used to understand the effects of specific genes and non-genetic factors that influence the incidence of a trait in populations. (Akbay & Bilgiç, 2023).

DGAT1 is an enzyme found in the microsome and is closely related to cholesterol acyltransferases 1 and 2, which are involved in the metabolism of fatty acids and acyl-CoA. Its primary role is to facilitate the synthesis of triglycerides by catalyzing the acylation of diacylglycerol at the sn-3 position (Farese et al. 2000). DGAT1 may impact various physiological processes, including the creation of adipose tissue, lipoprotein assembly, and intestinal fat absorption, and the regulation of lactation plasma Triacylglycerol concentrations, as well as influence lactation and production (Mohammed et al.2015; Anggraeni 2019). The DGAT1 gene is responsible for encoding the DGAT1 enzyme, which is essential in the final step of triglyceride synthesis in the mammary gland (Schennink et al. 2007; Leskova et al. 2013).

The variation in the Bos taurus cattle breeds, found at positions 10433 and 10434 on exon 8 of the DGAT1 gene (rs AJ318490.1), leads to the substitution of alanine for lysine amino acid at position 232. This variation may have an impact on milk production, growth, and reproductive traits in cattle breeds (Winter et al. 2002; Grisart et al. 2002; Gautier et al. 2007). This genetic variation has been found to have a significant impact on both milk yield and milk composition in dairy cattle.(Gautier et al. 2007; Naslund et al. 2008). The K232 Lysine variant, has been found to be linked to higher protein and fat contents, as well as fat yield, whereas the A232 Alanine variant, is linked to higher milk and protein yields (Winter et al. 2002). This genetic variation is responsible for a significant quantitative trait locus (QTL) that affects milk production traits not only in Holstein dairy cattle but also in other breeds of cattle (Gautier et al. 2007). The frequency of these two alleles varies in different breeds of cattle, and it is possible that there may be additional mutations that contribute to the observed QTL effect on milk production yields (Bijl et al. 2014; Boichard et al. 2014; Vanbergue et al.2016).

The DGAT K232A gene polymorphism is known to have an effect on milk and meat yield in cattle, and it is considered to have high potential as a marker for improving performance traits of Simmental cattle and for use in breeding programs. Therefore, knowledge of this gene polymorphism is an important source of information for breeders aiming to increase cattle productivity. The purpose of this research was to investigate the DGATI/K232A gene variation as a potential marker in Simmental cattle and to assess its impact on milk production characteristics.

Material and Methods

Animal material and DNA Extraction

Blood samples taken from 66 Simmental cattle breed raised in semi-instantive environment in Erzurum were used as DNA material. All of the cattle were subjected to the same feeding system and had low levels of inbreeding. Blood samples were collected from the tail vein of the animals using vacutainers that contained K3EDTA as an anticoagulant. DNA was isolated from the gathered blood samples with the QIAGEN Genomic DNA Purification kit (Gentra Puregene, USA) following the provided guidelines. Primers were designed based on the DGAT1 sequence (AA/GC) at positions 10433 and 10434 (rs AJ318490.1) GenBank acc. ARS-UCD1.2 (GCF_002263795.1)) and used Primer3web version 4.1.0 (https://bioinfo.ut.ee/primer3-0.4.0). The primer sequences used and the relevant PCR programs are presented in Table 1.

PCR Conditions and RFLP Analysis

The PCR reaction mixture (20 mL) contained; 2,5 µL genomic DNA, 0.5 µL F –Primer, 0.5µL R-Primer, 0.5 µL of dNTPmix (D7595: Sigma, St. Louis, MO, USA, 0.25mM), 0.5 units of Taq DNA polymerase (D1806: Sigma), 10 µL of 10x PCR Buffer, 2.5 µL of 0.25 mM MgCl2 and ddH2O making the total volume of 20 µL were used for PCR amplification (Table1).

The PCR amplified DGAT/K232A gene region was digested using CfrI(EaeI) restriction enzyme and incubated at 37 °C for 2 hours. The digested products were then separated on a 3.0% agarose gel for 3.0 hours at 45 Volts and viewed under UV light. The allele frequency of the base mutation of each gene was determined using the PopGen 32 software developed by Yeh et al. (1999) to verify whether they were in Hardy-Weinberg (HW) equilibrium.

Statistical Analysis

The effects of DGATI/K232A gene polymorphisms on lactation and some other milk yield traits were investigated in Simmental cattle raised in a private enterprise in Erzurum. A correlation analysis was performed between the milk yield records of animals in different lactations whose yield records were systematically kept between 2017-2020 and the relevant polymorphic regions. The statistical analysis used real milk yield, 305-day milk yield, lactation period, and daily milk yield as parameters to evaluate the association between genotype and milk yield traits. The general linear model in the SPSS 25.0 software program was used to examine the data (IBM SPSS 25.0 Corp. Inc.). Environmental factors such as lactation order, genotype, calving seasons and were considered to have an impact on the relevant yield trait and were taken into account in the analysis. According to the yield traits in the research, the following statistical model was employed (Equation 1).

$$yijk=: \mu+ai+bj+ck+eijk \quad (1)$$

Yijk is any of the milk yield traits (305-day milk yield, lactation milk yield and daily milk yield); µ is the population average; ai is the ith genotype effect; bj is the effect of the jth lactation order (j: 3; 1st Lactation: 1, 2nd Lactation: 2, 3rd Lactation: 3…. 7 Lactation: 7th); ck is the effect of the kth calving season (k: 2; 1: winter-spring, 2: summer-autumn); eijk is the margin of error.

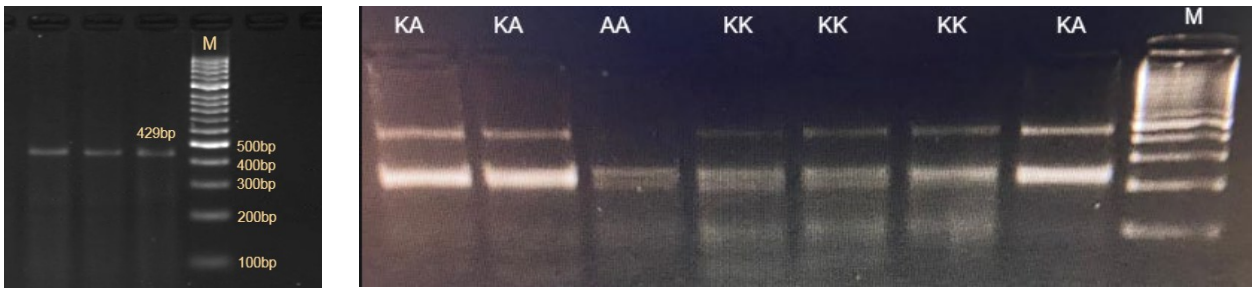
Results and Discussion

PCR and RFLP results and band sizes of 66 samples, excluding 70 samples with no PCR bands observed after PCR amplification, are shown in Figure 1. The DGAT1/K232A polymorphisms were analyzed using PCR-RFLP, and the expected band sizes for each genotype were determined. Following digestion with the restriction enzyme, DGAT1/K232A PCR products revealed KK genotype as bands of 429 bp and 209 bp, KA genotype as bands of 429 bp, 219 bp, and 209 bp, and AA genotype as bands of 219 bp and 209 bp (Figure 1).

The DGATI gene in Simmental cattle was found to have genotype frequencies of 0.64, 0.26, and 0.10 for KK, KA, and AA, respectively. The corresponding allele frequencies for KK and AA were determined to be 0.77 and 0.23, respectively. The Hardy-Weinberg genetic equilibrium test showed that the distributions of DGATI genotype distributions not in balance ($P<0.05$) in the studied breeds (Table 2).

Table 1. Table 1. DGATI gene primers and PCR conditions

PCR Primers				
Gene region	Reference	Primer sequences	PCR product	Softwares
DGATI/ CfrI	ARS-UCD1.2 (GCF_002263795.1)	F: 5-TGGCCCTGATGGTCTACA-3 R: 5-AGGAAGCGCTTTCGGATG-3	429	https://www.ncbi.nlm.nih.gov/tools/primer-blast/
PCR Conditions				
Initial denaturation	Denaturation Annealing	Extension	Number of cycles	Final extension
95 C/45 sec	60 C /50 sec	72 C/2 min	35	72 C/5 min



(a) (b)

Figure 1. a.PCR results b. DGAT1/K232ARFLP results
(KK, KA, and AA genotypes of the DGAT1 gene with Standard 100 bp DNA marker images, respectively)

Table 2. The genotype and allele frequencies and H-W equilibrium DGATI/K232A genes

Genotype	N	allele K	allele A	Observed Frequency	%	H-W Expected Frequency	H-Wa (p value)
KK	42	84	0	42	63.64	38.64	
KA	17	17	17	17	25.76	23.72	
AA	7	0	14	7	10.61	3.64	0.021*
Total	66	101	31	66	100.00	66.00	
Allel Frequences	132	0.77	0.23	132			

* P<0.05 indicates that the sampled population is imbalanced Hardy-Weinberg equilibriums, a. p-value for the test for Hardy-Weinberg equilibrium.

Table 3. Effects of DGATI/K232A genotypes on milk yields in Simmental cattle

Variation Sources		N	Lactation milk yield	305-day milk yield	Daily milk yield	Lactation period
			X ± Sx	X ± Sx	X ± Sx	X ± Sx
DGATI/CfrI	AA	9	5439.632±611.955	5385.193±472.945	17.660±1.551	312.760±29.857
	KA	23	5533.781±373.474	5743.017±288.636	18.868±.947	289.876±18.222
	KK	69	5142.732±249.400	5340.576±192.747	17.516±.632	297.560±12.168
		P	0.449	0.432	0.789	0.589
Calving season	1	62	5267.697±308.177	5387.134±238.172	17.672±.781	294.581±15.036
	2	39	5476.400±371.123	5592.056±286.820	18.357±.941	305.551±18.107
		P	0.589	0.493	0.484	0.561
Lactation order	1	3	5138.145±1021.702	5352.121±789.615	17.554±2.589	299.218±49.849
	2	8	4899.464±654.154	4946.878±505.558	16.258±1.658	305.915±31.916
	3	18	5670.094±440.776	5769.680±340.650	18.943±1.117	301.813±21.506
	4	22	5509.839±410.575	5573.682±317.310	18.309±1.041	308.043±20.032
	5	23	5230.101±387.050	5641.915±299.128	18.506±.981	268.256±18.884
	6	18	5141.044±430.314	5169.697±332.565	16.951±1.091	304.638±20.995
	7	9	6015.651±611.740	5973.192±472.778	19.580±1.550	312.575±29.847
		P	0.810	0.580	0.582	0.731
Total		101	5372.048±281.461	5489.595±217.525	18.015±.713	300.066±13.733

* X: General Means, Sx: Standard deviation.

The DGATI gene in Simmental cattle was found to have genotype frequencies of 0.64, 0.26, and 0.10 for KK, KA, and AA, respectively. The corresponding allele frequencies for KK and AA were determined to be 0.77 and 0.23, respectively. The Hardy-Weinberg genetic equilibrium test showed that the distributions of DGATI genotype distributions not in balance (P<0.05) in the studied breeds.

Based on the analysis of the DGATI/K232A fragment differences in 66 Simmental cattle over 7 lactations, there were no significant differences in milk yield averages based on genotype, calving season, or lactation order factors (p>0.05). Table 3 shows the values for daily milk yields, corrected milk yields, and lactation periods for the breed (Table 3).

In the study, the general averages for milk yield, 305-day milk yield, daily milk yield and lactation period of 66 Simmental cattle in different lactations were 5372.048 kg, 5489.595 kg, 18.015 kg and 300.066 days, respectively. The KA genotype had the highest average milk yield, while the KK genotype had the lowest average. The second season range in the DGATI genotype had the highest overall averages for all yield values, while the first season range had the lowest averages. The highest average daily milk yield was 19,580 kg in the seventh highest lactation, and the second lowest was 16,258 kg. The highest average real milk yield was 6015.651 kg in the seventh highest lactation, and the second lowest was 5093.744 kg. The highest average 305-day milk yield was 5973.192 kg in individuals in the seventh lactation, and the lowest average was 4946.878 kg in animals in the second lactation. The seventh highest lactation period was 312.575 days, while the fifth lowest was 268.256 days.

Table 4. Allele frequency distributions of DGAT1 K232A gene obtained from the present study and other literatures

DGAT1/CfrI	Allele frequencies			
Breeds	N	K	A	References
Borgou	83	0.77*	0.23	Houaga et al.2018
White Fulani	96	0.92*	0.08	Houaga et al.2018
Hardhenu (Holstein Friesian cross breed)	181	0.60*	0.40	Gothwal et al.2022
Sahiwal	83	0.96 *	0.04	Gothwal et al.2022
Polish Holstein- Fresian	144	0.28	0.72	(Kęsek-Woźniak et al. 2020)
Holstein	53	0.43	0.57	(Anggraeni, 2019)
IItalian Simmental	95	0.01	0.99	(Scotti et al. 2016)
Holstein Friesian	53	0.57*	0.43	Bhat et al.2017
Jersey	200	0.60*	0.40	Bhat et al.2017
Jersey cross (Local Kashmiri)	200	0.58*	0.42	Özkan Ünal et al. 2015
Anatolian Black (AB)	42	0.58 *	0.42	Özkan Ünal et al. 2015
South Anatolian Red (SAR)	47	0.80*	0.20	Özkan Ünal et al. 2015
Holstein Friesian (HF) bulls	281	0.59*	0.41	(Patel et al. 2009)
Angus, Charolais, Simmental	243	0.09	0.91	(Li et al. 2013)
Croatian beef cattle	175	0.70*	0.30	(Kelava et al. 2013)
Iran Holstein	398	0.37	0.63	(Koopaei et al. 2012)
Holstein cows	1061	0.14	0.86	(Mao et al. 2012)
Simmental beef cattle	26	0.17	0.83	Karolyi et al. 2012
Iranian Holstein Bulls	103	0.80*	0.20	(Mashhadi et al. 2012)

We conducted a study on Simmental cattle, focusing on the allele frequencies for the DGAT1/K232Agenes. For the DGAT1/K232A gene polymorphism, the dominant K allele frequency was 0.77 and the A allele frequency was 0.23. The allele frequencies of previous studies on DGATI/K232A gene polymorphism in different cattle breeds are presented in Table 4. Those similar to the allele frequencies we determined in the Simmental breed are indicated with * symbol.

Hardy-Weinberg equilibrium test was conducted to evaluate the balance of genotype frequencies in the breed for the DGAT1/K232A gene. The test results revealed that the genotype frequencies were not balanced and did not meet the criteria for Hardy-Weinberg equilibrium, which could be attributed to a high rate of inbreeding and the limited sample size of the cattle analyzed ($P<0.05$). In studies conducted on different cattle breeds, it is observed that genetic distribution is not in HW equilibrium in Czech Simmental and Holstein breeds, Hardhenu and Sahiwal, and Simmental beef cattle breeds due to the low number of cattles (Karolyi et al. 2012; Cıtek et al. 2021; Gothwal et al. 2022). On the other hand, in Holstein, purebred Holstein cows, Fleckvieh, and Croatian (Simmental, Hereford, Charolais) cattle breeds, it has been observed that DGAT1/K232A genotype frequencies are balanced in distribution (Kelava et al. 2013; Bartoň et al. 2016; Ardicli et al. 2018; Anggraeni, 2019), and in different cattle breeds such as 351 Italian Brown cows and 1061 cows sampled from 2 Chinese Holstein cattle, allele distributions are in Hardy Weinberg equilibrium equilibrium, which consisted of a large number of breeds (Conte et al. 2010; Mao et al. 2012a).

Due to the limited size of the Simmental breed sample and the available milk yield records, In the study did not find any significant correlation between the DGAT1/K232Agenotype polymorphisms and milk yield ($P<0.05$). A parallel study conducted on Holstein cattle using microarray analysis on mammary tissue also found that the gene expression of DGAT1 had negligible effects on milk yield (Mach et al. 2012). Similarly, studies investigating the association between the DGAT1 K232A gene polymorphism and milk yield have reported no significant associations with milk yield or milk components in Holstein cattle and daily milk yield in Dutch Holstein Friesian cattle (Mach et al. 2012).

There are studies available on different breeds with large population sizes investigating the K232A gene polymorphisms and their associations with 305-day milk yield, total milk yield, milk protein and fat yield, milk composition, daily milk yield, and milk quality parameters (pH, milk fat, milk density, and milk acidity) including Holstein (n=1236), Holstein Friesian (n=415), Jersey (n=340) and Hungarian Simmental (n=481) (Smaragdov, 2011; Anton et al. 2012; Mao et al. 2012; Cerit et al.2014; Ardicli et al. 2018; Cıtek et al. 2021).

In the study we conducted, milk yield was found to be higher in individuals with the KA and lowest KK genotype of DGAT1 K232A, but the differences in milk yield averages were not statistically significant ($P>0.05$). The DGAT1 polymorphism, especially the allele of Lysine (K), showed the strongest association with milk fatty acid, milk fat yield and fat content, protein content, and saturated fatty acid composition, which confirms the crucial role of DGAT1 in the lipid metabolism of the mammary gland (Conte et al. 2010; Vanbergue et al. 2016; Bhat et al. 2017; Houaga et al. 2018; Palombo et al. 2018; Kęsek-Woźniak et al. 2020). Additionally, membrane arrangement or cell structure of mammary gland epithelial cells is impacted by the DGAT1 KK and AA gene polymorphism (Lu et al. 2015).

The DGAT1 K232A gene polymorphism is widely used as a marker in beef cattle breeds, as it affects not only milk production in dairy breeds but also various performance traits in beef cattle. In beef cattle breeds, it has been shown to affect backfat thickness, backfat retention rate, meat yield, intramuscular fat content, fatty acid composition, and types (Anton et al. 2011; Curi et al. 2011; Karolyi et al. 2012b; Barton et al. 2016). It also has

significant effects on important meat quality traits such as marbling score, backfat thickness, fat color, Warner-Bratzler shear force and longissimus muscle area, as well as on meat tenderness and marbling in beef cattle and saturated fatty acid content in milk (Wu et al. 2012; Karolyi et al. 2012b; Li et al. 2013; Babii et al. 2018).

Conclusions

Several association analysis studies have been published that investigate the potential of DGAT1 genes polymorphisms as markers in cattle breeding, with significant implications found for body weight, meat yield, and reproductive performance in general. However, there is still much to learn about the specific effects of DGATI gene polymorphisms on performance traits in Simmental cattle. Future research should aim to expand sample sizes to improve the statistical power of the study, as well as to examine other genetic factors that may interact with the gene to influence performance traits. The DGATI K232A polymorphic region has a high potential to be used as a marker in Simmental cattle breeding.

Declarations

Acknowledgment

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Authors' Contributions

The project idea, design, and study implementation were aided by ZS, HU, and SK. The lab analyses were under the supervision of ZS and HM. The finalization of the paper and scientific editing fell to ZS and SK.

Conflict of Interest

The authors declare no conflict of interest

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