Original Article

Identification of Novel SNPs and 10 bp Deletion of Ovine *dgat1* Gene and Their Association with Milk Production Traits

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Abstract

Acyl coenzyme A: diacylglycerol acyltransferase 1 (*dgat1*) plays a key role in triacylglycerol synthesis by catalyzing the final committed step in the formation of triglycerides. Moreover, it could be used as a positional marker gene associated with milk production and composition traits. The purpose of the study was to identify genotype frequencies of single nucleotide polymorphisms (SNPs) in ovine *dgat1* gene and its possible association with milk traits in dairy sheep breeds. The genetic structures of SNPs were examined by PCR-RFLP and DNA sequencing in three sheep populations. Significant association of *dgat1* gene of the intron 16 g.8384T>C loci with milk fat percentage and milk yield in Sakiz sheep breed was demonstrated. The present study reported for the first time eight SNPs and a 10 bp deletion (g.8213_8223delTGGGGCGGGG) in the *dgat1* gene and its association with milk traits in dairy sheep breeds. These preliminary results indicate that the identified SNPs and deletion lend themselves readily for further research regarding physiological impacts such as milk production and reproductive traits in other dairy sheep populations.

Keywords: dairy sheep breeds, *dgat1* gene, milk traits, SNPs

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Introduction

Sheep milk has a higher solid content than cow and goat milk, which means that it is particularly suited to cheese processing (Barillet, 1997; Gutiérrez-Gil et al., 2014). In Turkey, sheep breeds are generally multi-purpose, reared for meat and milk production, and dairy sheep have been farmed traditionally (Unal et al., 2008). As all milk is used for cheese production, milk content traits are very important (Carta et al., 2009).

According to FAO, the total sheep milk production of Turkey was 1.101.013 tons in 2013 and the number of sheep was 27,425,233 heads in the same year (FAO, 2014).

Milk fat contains approximately 98% triglycerides (TGs) (Marshall and Nudsen, 1997). Triglycerides are the major storage molecules of metabolic energy in most living organisms. The final and the only committed step in the biosynthesis of TGs is catalysed by acyl- CoA: diacylglycerol acyltransferase (*dgat1*) enzymes. *Dgat1* is part of a large family of membrane-bound O-acyltransferases (MBOAT) and membrane-associated enzymes catalysing O-acylation reactions, transferring fatty acyl moieties onto the hydroxyl or thiol groups of lipid and protein acceptors. Its members are involved in lipid metabolism, signal transduction, and hormone processing. A common feature of the MBOAT family is a long hydrophobic region that contains asparagine and histidine residues of the putative active site (corresponding to amino acids 378 and 415, respectively, of human *dgat1*; 389 and 426 of mouse *dgat1*). In sheep, the *dgat1* gene comprises 17 exons and spans 8.67 kb on chromosome 9 and the gene encodes a protein of about 489 amino acids. In sheep *dgat1* gene sequence, exon 15-17 regions have been covered the MOBAT domain (Yen et al., 2008). Based on the above considerations, the objective of this study was to detect novel variants of the exon 8 and exon 15-17 regions in the ovine *dgat1* genotypes and its possible association with milk production traits in dairy sheep breeds.

Materials and Methods

Animal Resources and DNA Isolation: Sakiz is a high milk yield local dairy sheep breed in Turkey and is well known for its early sexual maturity and outstanding prolificacy. Milk production varies from 120-250 kg for 175-day lactation length depending on management and husbandry conditions. Akkaraman is the largest local sheep breed in Turkey. It is fat tailed sheep and bred as a dual purpose breed with milk production varying from 50-60 kg per 140-day lactation. Awassi is principally a dairy breed reared in south-eastern Turkey. Its milk production varies from 120-160 kg per 165-day lactation (Akcapinar, 2000).

Four hundred and fifty blood and milk samples were used in the study, which included 150 samples from each of Sakiz, Akkaraman and Awassi ewes. The samples randomly selected consisted of animals that were 4 years old, multiparous and lactating. Akkaraman-Awassi and Sakiz breeds were reared, respectively, in Elazig and Balikesir counties at commercial herds of three farms. The animals were fed 250 g/head/day concentrate commercial feed (crude protein 20% and 2500 ME kcal/kg) as supplement and raised in semi-intensive conditions. Measurement of milk yield was initiated on the 14th day of lactation to exclude the risk of contamination with colostrum. The animals were milked twice a day at constant milking intervals and 20 ml milk sample was collected for milk analysis. Individual milk yield was recorded every day during lactation for each individual and each breed. The milk sample was analysed for milk composition (fat, protein, density and non-fat solids) using Lactoscan milk analyser (Milktronic Company, Nova Zagora, Bulgaria). Jugular blood samples (2 ml/ewe) were collected aseptically from each of the animals, using EDTA as an anticoagulant. Genomic DNA was extracted from whole blood using the phenol chloroform method (Sambrook et al., 1989). All samples were delivered to the laboratory in ice.

The study was approved by the Ethical Committee of Laboratory Animals; 2012/06.65, 22/05/2012.

Table 1 Primers and restriction enzymes used for PCR amplification and RFLP analysis of sheep *dgat1* gene

Primer Pair	Forward Primer 5′-3′	Reverse Primer 5′-3′	Restriction enzyme	Annealing Temparature (°C)	Amplicon Size (bp)	Covered Region
Primer 1 Region	CCACCATTCTC TGCTTCCCA	GAAGTTGAGCTCGT AGCACAG	*Eae*I	60	541	Partial exon 6, İntron 6, Exon 7, İntron 7, Exon 8, İntron 8
Primer 2 Region	CCCAGACACTT CTACAAGCC	TGCCCAATGATGAG TGACAG	*Nla*III	55	401	Exon 15, İntron 15, Exon 16, İntron 16, partial exon 17
Primer 3 Region	CTTCTTCCATG AGGTCAGTGCA	GAGGCAAAGCAGT CCAACAC	*Alu*I	54	429	Exon 15, İntron 16, Exon 16, İntron 16, exon 17, partial 3' UTR

DNA Amplification and Genotyping: According to the ovine (EU178818) sequence of *dgat1* gene, three pairs of primers were designed to amplify for exon 8 and exons 15-17, respectively, in *dgat1* gene. Primer pairs and their annealing temperatures are shown in Table 1. For primer design, Primer3 software (Rozen and Skaletsky, 2000) was used. PCR reaction was carried out for forward and reverse primers in 25 µL of total volume,

containing 10 X PCR buffer (50 mM/L KCl, 10 mM/L Tris-HCl (pH 8.0), 0.1% Triton X-100), X mM $MgCl_2$, 0.2 mM of each dNTP, 10 pM/L of each primer, 50 ng ovine genomic DNA and 1U Taq DNA polymerase. PCR conditions were as follows: denaturation at 94°C for 4 min, followed by 34 cycles of denaturation at 94°C for 45 sec, annealing at X°C for 30 sec, extension at 72°C for 40 sec and final extension at 72°C for 10 min (Table 1). The ewes were genotyped by using the polymerase chain reaction and restriction fragment length polymorphism (PCR-RFLP) and by direct sequencing. PCR product of 20 µL were digested separately with 10U *Eae*I (BioLabs, New England), 10U *Nla*III (Promega, USA) and 10U *AIu*I (Thermo Scientific) for exon 8 (primer 1 region), exon 16 (primer 2 region) and exon 17 (primer 3 region), respectively, at 37°C for 6 hours. The exon 8 and exon 17 PCR products were separated by electrophoresis on 2% agarose gel, while the exon 16 PCR products were separated by 3% MetaPhor agarose gel in 1X TBE buffer. Gels were stained with ethidium bromide and the results of electrophoretic separation were analysed in UV light using a transilluminator. Genotypes were identified by differential migration according to fragment size. PCR products showing different band patterns on RFLP gel were selected for sequencing. To confirm RFLP results, 20 randomly chosen PCR samples of each genotype were sequenced from both directions. Direct sequencing was performed by commercial services using 3100 ABI PRISM sequencer (Applied Biosystems, USA). Sequences were obtained with the same primers used for PCR amplification.

Bioinformatics Analysis: Sequences were analysed using the BIOEDIT version 7.2.5 software (Hall, 2014). All chromatogram peaks were confirmed by visual inspection and compared to ovine *dgat1* reference sequence (GenBank accession number EU178818). Genotype, allelic frequencies and observed and expected heterozygosity and Hardy-Weinberg equilibrium were calculated using Arlequin version 3.5.1.3 package program (Excoffier et al., 2006).

The DnaSP software 5.10.01 (Rozas et al., 2003) was used to calculate polymorphic sites, average number of nucleotide differences (k), number of haplotypes (h), nucleotide diversity (π) haplotype diversity (H_d) within and among breeds; Watterson's theta estimator for the studied species separately using a haplotype sequence was obtained. Pi is based on the average number of nucleotide differences between the sequence, and theta is based on the total number of segregating sites in the sequence (Iso-Touru et al., 2009) (data not shown).

Association analysis: Statistical analysis was performed using R-Project software (R Core Team, 2013) and general linear mixed model (GLMM) was applied to analyse association between *dgat1* variants and milk yield, fat and protein percentages. Dependent variables in the analysis were milk yield, fat and protein percentage, whereas the *dgat1* genotypes, breed and gene region were considered as fixed effects in the model. The model used was as follows:

$$Y_{ijklm} = \mu + B_i + G_j + E_k + GxB_l + A_m + e$$

where Y is test day milk yield, fat percentage and protein percentage; B_i is the fixed effect of the breed; G_j is the fixed effect of the genotype; E_k is the fixed effect of the gene region; GxB_l is the fixed interaction effect of breed and genotype; A_m is the random effect of the animal and e is the residual effect. P value <0.05 was considered statistically significant.

Results

Genotypic and Allelic Frequencies: The results of this study indicated the presence of three *Eae*I cleavage sites (443, 70 and 28 bp) within the *dgat1* gene exon 8 region, however no SNPs were detected in exon 8, and all sheep breeds were homozygous for allele 'AA', which encodes lysine.

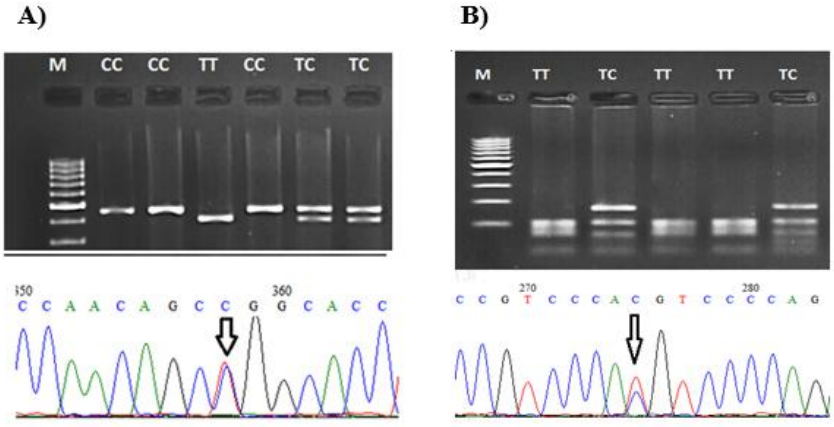

Figure 1 *Dgat1* PCR-RFLP analysis. **A)** Agarose gel electrophoresis band and electropherogram patterns after digestion with *Alu*I endonuclease within exon 17 sequence. Lane M: 100 bp DNA marker; lanes 2, 3, 5: CC genotype (429 bp); lane 4: TT genotype (372 and 57 bp); lanes 6-7: TC genotype (429, 372 and 57 bp). As one small bands (57 bp) were invisible on 2% agarose gel, only two bands were visible for TC genotype. **B)** Agarose gel electrophoresis band and electropherogram patterns after digestion with *Nla*III endonuclease within exon 16 and its flanking region sequence. Lane M: 25 bp low range DNA marker; lanes 2, 4-5: TT genotype (106, 100, 78, 67, 27 and 22 bp); lanes 3, 6: genotype TC (178, 106, 67, 27 and 22 bp). As one small bands (27 and 22 bp) were invisible on 3% Metaphor agarose gel, only three bands were visible for TC genotype and four bands were visible for genotype TT.

Restriction fragment length polymorphism reflected differences in nucleotide sequences resulting from mutations or variations within a gene. This mutation created a restriction site for the *AIu*I enzyme, which cuts DNA into two fragments of 372 and 57 base pairs. During electrophoresis, two bands of 372 and 57 base pairs were obtained for the TT genotype, three bands of 429, 372 and 57 base pairs were obtained for the TC genotype and a band of 429 base pairs for the CC genotype (Fig. 1). The results for genotypic and allelic frequencies of the examined populations are reported in Table 2. The allele frequency was different between the Sakiz and Akkaraman-Awassi breeds. As a result, genotype TT and TC were not shown in the Sakiz sheep breed and it was found as monomorphic for *dgat1* gene in the exon 17 region. The genotype frequency of CC, which was the predominant genotype, was 76% and 80% in the Akkaraman and Awassi ewes, respectively. The allele distribution of *dgat1* gene exon 17 region in all sampled sheep breeds was in agreement with Hardy-Weinberg equilibrium by the Chi-square test (Table 2).

The results of RFLP assay presented six cleavage sites (21-24 (exon 15), 88-91 (exon 15), 194-197 (exon 16), 221-224 (exon 16) and 297-300 (intron 16)) within primer 2 sequence region, but only one (297-300) was shown to be two allelic forms. After digestion with *Nla*III endonuclease two genotypes were detected: TT and TC. Allele T contained the restriction site for *Nla*III and resulted in 100 and 78 bp fragments, whereas the absence of the restriction site in the C allele resulted in a single 178 bp fragment. Allele T restriction site resulted in 106 bp, 100 bp, 78 bp, 64 bp, 27 bp and 22 bp fragments, whereas C allele occurred the absence of the restriction site in the 297-300 position resulted in five fragments 178 bp, 106 bp, 67, 27 bp and 22 bp (Fig 1). Therefore, genotypes TT and TC demonstrated six and five bands, respectively (Table 2). There were no significant differences among the populations in the distribution of genotypes.

Table 2 Distribution of observed allele frequencies for *dgat1* gene, expected genotype frequencies in accordance with Hardy-Weinberg equilibrium, P values for the fitness to Hardy-Weinberg equilibrium, in Sakiz (SAK), Akkaraman (AKK) and Awassi (AWS) sheep breeds

Locus	Population	n	Allele Frequency		Observed Genotypes			P value
			(T)	(C)	(TT)	(TC)	(CC)	
***dgat1*-Exon 16**	SAK	150	0.87	0.13	111	39	0	NS
	AKK	150	1	0	150	0	0	monomorphic
	AWS	150	1	0	150	0	0	monomorphic
	TOTAL	450			411	39	0	
			(T)	**(C)**	**(TT)**	**(TC)**	**(CC)**	
***dgat1*-Exon 17**	SAK	150	0	1	0	0	150	monomorphic
	AKK	150	0.13	0.87	4	31	115	NS
	AWS	150	0.11	0.89	2	29	119	NS
	TOTAL	450			6	60	384	

NS; Not significant

Table 3 SNP position at exon 15, intron 15, exon 17 (primer 3 region) in *dgat1* gene in Sakiz (SAK), Akkaraman (AKK) and Awassi (AWS) sheep breeds. Nucleotides are numbered according to reference sequence EU178818.

Haplotypes (GenBank no.)	Haplotype Frequency			Nucleotide Sites							
				8134	8184	8187	8231	8236	8384	8413	8431
				Exon 15	Intron 15	Intron 15	Intron 15	Intron 15	Intron 16	Exon 17	Exon 17
	SAK	AKK	AWS								
H1 (EU178818)				**G**	**T**	**G**	**T**	**A**	**T**	**C**	**C**
H2 (KP215640)	n:8	-	-	A	C	A	C	G	C	G	T
H3 (KP215641)	n:12	-	-	A	C	A	C	G	.	G	T

Haplotype Frequencies: In this study, the sequencing analysis and alignment of exon 15, intron 15, exon 16, intron 16 and exon 17 of the *dgat1* gene showed the presence of eight polymorphic sites in the Sakiz breed (Table 3). For exon 15, intron 15 and exon 17 regions, except for reference sequences (EU178818), two

different haplotypes were obtained (Table 3). One variation point was determined in exon 15, which is synonymous mutation: g.8134G>A; four variation points were determined in intron 15: g.8184T>C, g.8187G>A, g.8231T>C, g.8236A>G; one variation point was determined in intron 16: g.8384T>C; and two variation points were determined in partial exon 17, of which is synonymous mutations: g.8413C>G, g.8431C>T. Furthermore, by comparing sequences from the Sakiz breed, a novel 10 bp deletion (g.8213_8223delTGGGGCGGGG) in the intron 15 region was identified (Fig 2). The *dgat1* haplotype sequences from the Sakiz sheep breed were deposited in the GenBank database under the access number: KP215640-KP215641.

Table 4 Least Squares Means (± SEM) of milk traits in sampled sheep breeds

Traits	**Sheep Breeds**			
	Sakiz	Awassi	Akkaraman	P value
Fat (%)	7,066^{a} ±0,586	5,171^{b}±0,455	4,121^{c} ±0,381	***
Protein (%)	6,435^{a} ±0,571	3,919^{c} ±0,327	4,029^{b} ±0,335	***
Milk yield (ml/day)	1,102^{a} ±0,083	1,051^{a} ±0,078	0,804^{b} ±0,063	***

^{a,b,c} Different letters indicate significant difference between breeds and related milk production traits.

*** Confidence level of the predicted factors (P<0,001)

Table 5 Associations of *dgat1* exon 17 and intron 16 genotypes (primer 2 region) with milk traits in Akkaraman, Awassi and Sakiz sheep breeds, respectively (mean ± SEM)

Locus	**Milk Traits**		
Exon 17	**Fat**	**Protein**	**Milk Yield**
TT	4,5151^{A}±0,256	5,8424^{A}±0,042	1,0209^{A}±0,014
TC	5,6749^{A}±0,188	5,8689^{A}±0,029	0,8850^{A}±0,018
CC	5,9096^{A}±0,211	5,7836^{A}±0,034	0,8911^{A}±0,035
Intron 16			
TT	6,965^{A}±0,056	5,477^{A}±0,023	1,069^{B}±0,017
TC	4,940^{B}±0,179	5,458^{A}±0,043	1,606^{A}±0,019

^{A,B,C} Different letters indicate significant difference between genotypes and milk traits (P<0,001).

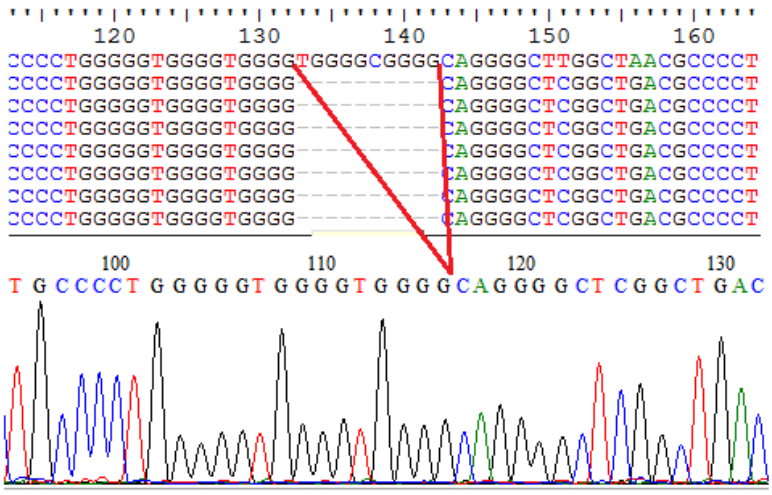

Figure 2 Sequencing results of exon 16 region of ovine *dgat1* gene. The 10 bp deletion is indicated by red lines.

Genotype effects on milk production traits: Least squared means of milk traits are represented in Table 4. As for the milk analysis results, milk yield, fat and protein percentage were found statistically significant among the sheep breeds (P<0,001). The Sakiz sheep breed showed the highest milk yield, protein and fat percentage values when compared to the other sheep breeds.

Results of the association study of *dgat1* genotypes with milk production traits are shown in Table 5. For exon 17 region, there was no significant effect of the genotype on milk production traits in the Akkaraman and Awassi breeds. The *dgat1* intron 16 locus was found polymorphic only in the Sakiz

populations. For exon 16 region, the Sakiz breed carrying TC genotype had a greater milk yield than the TT genotype (P<0,001), while the TT genotype had a greater milk fat percentage than the TC genotype (P<0,001). However, there was no statistically significant difference in protein percentage according to the genotypes in the Sakiz ewes (Table 5).

A total of two haplotypes and 10 bp deletion were detected in the ovine *dgat1* gene, exon 15, intron 15, exon 16, intron 16 and exon 17 regions.

Discussion

Dgat1 is a candidate gene for milk production traits in bovine and other dairy ruminants. Many studies have demonstrated the association between the *dgat1* gene and milk yield, milk fat and protein percentages in cattle (Grisart et al., 2002; Winter et al., 2002; Furbass et al., 2006; Spelman et al., 2002). The results showed that polymorphisms in exon 8 of the *dgat1* gene in Bos taurus, AA-GC exchange resulted in a non-conservative substitution of amino acid 232 Lysine (K) to Alanine (A). Allele 'AA' is considered wild type and responsible for increased fat yield in milk, fat and protein content, whereas allele 'GC' increases both milk and protein yield in cattle (Grisart et al., 2002; Winter et al., 2002). In dairy sheep, only two studies were carried out of the *dgat1* gene and demonstrated their associations with production traits. Scata et al. (2009) characterized the complete region of the ovine *dgat1* gene in a population of Sarda, Altamurana and Gentile di Puglia. No SNPs were detected in exon 8; all studied sheep breeds were homozygous for the alleles encoding Lysine. The researchers did not detect presence of the K232A polymorphism in three Italian breeds, in agreement with this study. It was confirmed that the non-synonymous mutation in exon 17 was not significantly associated with fat content.

Garcia-Fernandez et al. (2010) found three mutation points in the ovine *dgat1* gene. These mutations, which were g.7255C>A, g.7486C>T and g.8539C>T, were found in exon 9, intron 10 and exon 17, respectively, whereas no significant association was found for the *dgat1* gene. In this research similar observations were found for the exon 17 region in the Akkaraman and Awassi ewes.

In the present study the K232A variant of the cattle, located in *dgat1* gene of the exon 8 region and affecting milk production traits and milk fat in cow, was not found in any of the sheep breeds tested. In agreement with other studies (Garcia-Fernandez et al., 2010; Staiger et al., 2010), the K232A mutation in sheep breeds tested was not found in this study. According to Garcia-Fernandez et al. (2010), despite the close phylogenetic relationship between cattle and sheep, the genetic architecture of milk production in these two ruminant species may involve different underlying factors.

This study for the first time demonstrated that the TC genotype (g.8384T>C) of ovine *dgat1* gene of intron 16 was positively associated with milk yield (P<0,001), but it influenced fat percentage negatively (P<0,001). Different alleles associated with high milk production in different breeds could be the result of different linkage phases between the *Nla*III polymorphism and the functional mutation (Staiger et al., 2010). However, there was no statistically significant difference in milk production traits according to genotypes for the *dgat1* gene of the exon 17 region.

The classical view is that intronic mutations do not alter the composition or function of the protein produced by a gene (Staiger et al., 2010). Nevertheless, introns and the act of their removal by the spliceosome can affect gene expression at many different levels, including transcription, polyadenylation, mRNA export, translational efficiency, and the rate of mRNA decay (Nott et al., 2003).

Alignment results of the obtained sequences revealed the presence of 8 SNPs in the *dgat1* gene exon 15, intron 15, exon 16, intron 16 and exon 17 regions. The number of polymorphisms identified showed high variability within the Sakiz breed. All of these haplotypes were specific for the Sakiz breed. Interestingly, Akkaraman and Awassi sheep did not show variation in the haplotypic level, whereas the Sakiz breed had different variations. This study proposes that this difference is probably the consequence of selection and possibly even QTL or gene(s) regulating milk production traits.

This study firstly revealed the significant association of *dgat1* gene g.310T>C loci with milk fat percentage (P<0,001) and milk yield (P<0,001) in Sakiz sheep breed. The TT genotype was positively associated with fat percentage (P<0,001). It is determined that this mutation can be detected by restriction enzyme *Nla*III. As our results were limited to Sakiz sheep breeds, further research is needed to determine whether this correlation exists in other dairy sheep breeds. SNP in intron 16 might be used as a marker in association studies to determine whether genetic variation at the sheep *dgat1* intron 16 locus has any quantitative effect on mammary gland and milk yield. Even though it is not expected to have any functional effects, this T-to-C SNP could be useful as a genetic marker in association studies to detect influence milk fat content and milk traits.

This study identified for the first time a 10 bp deletion in *dgat1* gene of the intron 15 region in Sakiz sheep breed. It is concluded that the identified SNPs and deletion lend themselves readily for further research regarding physiological impacts such as milk production and reproductive traits in other dairy sheep populations. In addition, our findings might be of significance for dairy industry, particularly those producing local products such as Gouda cheese, which requires milk with high fat level. In this case, genotype TT might be recommended for cheeses high in fat.

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Conflict of interest

The authors declare that they have no conflict of interest.

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บทคัดย่อ

การตรวจหา SNPs และ 10 bp Deletion ในยีน *dgat1* และความสัมพันธ์ของพันธุกรรมการผลิตน้ำนมในแกะ

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เอ็นไซม์ Acyl coenzyme A หรือ diacylglycerol acyltransferase 1 (*dgat1*) มีบทบาทสำคัญในการสังเคราะห์ triglycerides นอกจากนี้ยีน dgat1 ยังใช้เป็นยีน marker ที่เกี่ยวข้องกับพันธุกรรมการผลิตน้ำนมและส่วนประกอบน้ำนม การศึกษาครั้งนี้มีวัตถุประสงค์เพื่อหาความถี่ของ single nucleotide polymorphisms (SNPs) ในยีน ovine *dgat1* และความสัมพันธ์ของพันธุกรรมการผลิตน้ำนมในพันธุ์แกะนม โดยศึกษา SNPs ด้วยวิธี PCR-RFLP และ DNA sequencing ในกลุ่มประชากรแกะ 3 กลุ่มประชากร พบว่าบริเวณ intron 16 g.8384T>C ของยีน *dgat1* มีความสัมพันธ์กับเปอร์เซนต์ไขมันในน้ำนมและปริมาณน้ำนมในแกะพันธุ์ Sakiz อย่างมีนัยสำคัญ นอกจากนี้การศึกษาครั้งนี้เป็นรายงานแรก ที่พบ SNPs จำนวน 8 แห่ง และ 10 bp deletion จำนวน 1 แห่ง (g.8213_8223delTGGGGCGGGG) ในยีน *dgat1* และมีความสัมพันธ์กับลักษณะทางพันธุกรรมการผลิตน้ำนมในแกะ ผลการศึกษานำร่องในครั้งนี้แสดงให้เห็นว่า การหา SNPs และ deletion สามารถนำไปใช้ต่อยอดในการศึกษาวิจัยอื่นๆ เช่น การศึกษาที่เกี่ยวข้องกับพันธุกรรมการผลิตน้ำนมหรือการสืบพันธุ์ ในแกะนม

คำสำคัญ: พันธุ์แกะนม ยีน *dgat1* ลักษณะการผลิตน้ำนม SNPs

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