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Research Article

Detection of Diacylglycerol Acyltransferase 1 (DGAT1) Gene Polymorphism in Imroz and Chios Sheep Breeds in Turkey Using PCR-RFLP Method [1]

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Abstract

DGAT1 which catalyzes the last step of triacylglycerol synthesis has two alleles of the in animals (DGAT1^{A} and DGAT1^{K}), the allele carrying the amino acid Lysine (K) is associated with milk with high fat content and low milk yield, whereas the allele carrying the amino acid Alanine (A) is the contrary. These alleles are known as candidate marker genes in choosing the animals for breeding. The aim of this study was to examine the genetic structures to the DGAT1 gene by PCR-RFLP method in Imroz (n=60) and Chios (n=52) sheep breeds. In this study, it was found that DGAT1 gene's allelic frequencies varied significantly between the two sheep breeds. The AA genotypic frequency was found the highest in Chios sheep breed (11.538%); the KK genotypic frequency was found the highest in Imroz sheep breed (68.333%) and the KA genotypic frequency was found the highest likewise in Imroz sheep breed (36.538%) in DGAT1 gene. In this study, the A allele frequency (0.702) was found higher than the K allele frequency in Imroz sheep breed. But, the K allele frequency (0.817) was found higher than the A allele in Chios sheep breed. It is believed that by increasing the DGAT1-like loci in order to ameliorate the milk yield capacity of native sheep breeds and merging the results obtained from these loci with the data and pedigree records of animals will prove especially useful to make better deductions and discoveries.

Keywords: *Sheep, Imroz, Chios, DGAT1*

Türkiye'deki Gökçeada ve Sakız Koyun Irklarında Diacylglycerol Acyltransferase 1 (DGAT1) Gen Polimorfizminin PCR-RFLP Yöntemi İle Belirlenmesi

Özet

Triasilgliserol sentezinin son basamağının katalizini yapan DGAT1'in hayvanlarda iki alleli (DGAT1^{A}ve DGAT1^{K})var olmakla birlikte, bunlardan Lysin aminoasiti (K) taşıyan varyant süt içinde yüksek yağ oranına ve düşük süt verimine sahip iken, Alanin (A) taşıyan ise tam tersidir. Bu alleller, marker yoluyla seçilim için elverişli olarak bilinmektedirler. Bu çalışmada, Gökçeada (n=60) ve Sakız (n=52) ırkı koyunlarda DGAT1 gen yapısının PCR-RFLP yöntemiyle incelenmesi amaçlanmıştır. Yapılan çalışmada incelenen iki koyun ırkında DGAT1 geninin allel frekanslarının önemli derecede farklı olduğu belirlenmiştir. DGAT1 geninin; AA genotip frekansının en yüksek (%11.538) Sakız koyun ırkında, KK genotip frekansının en yüksek (%68.333) Gökçeada koyun ırkında ve KA genotip frekansının ise benzer şekilde en yüksek (%36.538) Gökçeada koyun ırkında görüldüğü belirlenmiştir. Bu çalışmada incelenen koyun ırklarından Gökçeada koyunlarında A allel frekansı (0.702), K allel frekansından yüksek bulunmuştur. Sakız koyunda ise K allel frekansı (0.817), A allel frekansından yüksek bulunmuştur. Yerli koyun ırklarında süt verim kabiliyetinin arttırılması için DGAT1 benzeri lokus sayısının da arttırılması ile bu lokuslardan elde edilecek sonuçların hayvanlara ait verim ve pedigri kayıtları ile birleştirilmesi daha verimli çıkarımlar sağlayacağına inanılmaktadır.

Anahtar sözcükler: *Koyun, Gökçeada koyunu, Sakız koyunu, DGAT1*

INTRODUCTION

Humans, for their well-being, should consume a sufficient quantity of vegetal and animal based food products. However, even though all around such food products are produced in sufficient quantities, their consumption shows an uneven distribution. In the future, with the increase of the world's population along with

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global warming, this imbalance is going to grow worse and worse as time goes by.

It is a known fact that between developed countries and countries in development there is a significant difference in consumption of animal based food products per person. While in the future, no increase in animal based food demand is expected within developed countries, it is being reported that by 2020s, the demand for meat and milk is going to be doubled within developing countries, population of which is rapidly increasing [1]. This means that milk production which is limited to bovines is going to be less and less sufficient therefore requiring alternative ways for milk production. This, as a result, shows us that agriculture and animal husbandry is a sector which needs to be ameliorated at a national scale [2].

It is a known fact that there are lots of similarities between cow, goat and sheep milk and that sheep milk is preferred for the production of milk with alternative proteins because of its nutritional contents. Since sheep milk is more concentrated and has twice the fat percentage and proteins, it is preferred over cow and goat milk. It has been reported that sheep milk contains 40% more proteins than cow and goat milk [3]. As a result, relationships between milk protein/fat polymorphism and milk yield have become more and more important in time.

Diacylglycerol acyltransferase (DGAT) uses Diacylglycerol and Acyl-CoA as substrates and therefore catalyzes the last step of triacylglycerol synthesis [4]. This enzyme also has an important role in adipose tissue and intestinal fat absorption [5]. In animals, there are two variants of DGAT1 gene (them being DGAT1^{A} and DGAT1^{K}). Of these variants, the one carrying the Lysine amino acid is associated with milk with high triglyceride content and low milk yield, whereas the variant carrying the Alanine amino acid is associated with high milk yield and low triglyceride content. Both of these variants code for integral membrane proteins between which there is no sequence homology [6]. DGAT1 is the first gene that is known to code for a protein that has a DGAT activity [7]. Mice without DGAT1, *i.e.* carrying the mutant allele, are viable, fertile and resistant to diet-induced obesity [8]. Absence of DGAT1 on one hand, causes an increase in saturated fat levels while decreasing unsaturated fat levels thereby modifying the fatty acid compositions in adipose tissues and skeletal muscles on the other hand alters the triglyceride metabolism in tissues such as mammalian gland tissues and as a result causes an absence of milk production [9]. Among European cattle breeds, Lysine to Alanine substitution at the loci 10433 and 10434 on exon 8 (DGAT1 K232A) is reported to possess several polymorphic characteristics [10]. Mutation of the allele "K" can increase the milk fat content [11,12]. The medical literature also reports that the allele K is responsible for the saturated fat levels in milk [13,14]. Individual characteristics in the aforementioned loci in cattle breeds can affect the DGAT1 K232A polymorphism and thus have different effects on milk fat [10]. Allele frequencies can even vary among the aforementioned cattle breeds [15]. As a result, studies on K232A polymorphism in cattle is highly important for the milk industry and for the consumer since it improves the milk quality.

DGAT1 which is located under the centromeric region of the 14th Cattle Chromosome, is also defined as one of the Quantitative Trait Loci (QTL) which influence the milk production properties [16,17]. Within animals possessing the known QTL genotypes, the DGAT1 gene was sequenced and a non-conserved K>A mutation was detected which in certain dairy cattle breeds had a significant effect on milk yield and content [12,16,17]. In cows DGAT1 has become a strong candidate gene for milk yield, milk fat and intra-muscular fat content. In dairy sheep breeds, the importance of role of DGAT1 in fat metabolism has made it an intriguing candidate gene in explaining the variations of the influence of DGAT on the milk content. Recently, Scata et al.[18] have found that in sheep breeds with high milk fat had an SNP which was present at 5´UTR of DGAT1 whereas in breeds containing low milk fat (Sarda sheep breed) this SNP was negatively correlated with milk fat content. With the advancement of methods in molecular genetics and biotechnology, these two areas found widespread use in livestock husbandry and as a result, several links were found between structural gene loci and milk yield, fat and protein levels in cattle. Thus these genes were reported as being possible marker genes in choosing the animals for breeding [11,19]. Geneticists performed selection studies on these genes in order to increase the frequency of alleles which have a positive effect on a chosen trait [20]. The variations which are economically important among these genes are used for markers to be used in marker based selections [21]. That's why, the data obtained in this study on the relationship between milk yield and milk fat percentage is going to be important when it comes to choosing the candidate animals to breed and therefore reveal the genetic value of them. The main purpose in selection is to guess the genetic value of the animal as best as possible and thus increase the genetic gain.

In livestock husbandry, in studies in which the relationships between polymorphisms in molecular markers and yield are investigated, the possibility of there being a relationship between milk proteins and milk fat content has been becoming more and more important [22,23]. In the light of studies in recent years, a relationship between certain genes and their allelic composition and milk yield was found and these genes were reported to have the potential to be used as a genetic marker [19,24].

The aim of this study was to determine the polymorphism of the DGAT1 gene in Imroz and Chios sheep breeds using the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method.

MATERIAL and METHODS

Genomic DNA Extraction

Peripheral blood samples collected from Imroz sheep (n=60) and Chios sheep (n=52) breeds which were raised in various farms within the Marmara Region. The blood samples were kept within blood collection vials containing anticoagulants. Genomic DNA was extracted using Genox Genome Extraction Kit. The quantity of acquired DNA as well as its purity was determined using a spectrophotometer with a ratio of $O.D._{260/280}$.

Detection of the DGAT1 Gene

Following the DNA isolation, the alleles Lysine (K) and Alanine (A) of the DGAT1 gene were identified using PCR amplification and *AluI* (EURx) endonuclease digestion. Simple PCR amplification was made with a total volume of 26 µL. For each primer, 12.5 µL of 2X PCR master mix, 0.5 µM of (forward primer: 5′-GCATGTTCCGCCCTCTGG-3′, reverse primer: 5′-GGAGTCCAACACCCCTGA-3′) [11] and 50 ng of DNA sample were used. For the PCR optimization, 5% DMSO was added to the solution. These two alleles were added in order to ameliorate the amplification and thus get better results. Next, the following the PCR amplification cycle was executed:

The PCR condition consisted of initial denaturing at 95°C for 15 min followed by 35 cycles at 94°C for 1 min, 60°C for 1 min, 72°C for 1 min, and after the last cycle the samples were kept at 72°C for 3 min. About 10 µL of the PCR product was digested with 5U of *AluI* restriction endonuclease enzymes. After the cutting process of the prepared mixture kept for 4.5 h at 37°C the tubes for the enzyme inactivation was performed by keeping 65°C for 20 min. The DNA bands were dyed with ethidium bromide and detected in 2% agarose gel. A 2.5 µL 100bp standard DNA ladder (Analitika Lab.) containing all the locus specific alleles was used.

Statistical Analyses

The allelic and genotypic frequencies were calculated according to Cerit et al.[25]. Standard χ^2 test was performed in order to calculate the statistical significance between the observed and expected frequencies. For each genotype, expected and observed heterozygosity were also calculated.

Genotypic distribution for the Hardy-Weinberg equality was tested via the statistical application GENEPOP (Version 1.2) [26], PIC (Polymorphic Information Content) value for each genotype and effective allele count were also calculated [27,28].

Ethics Approval

Following the application to the Local Ethics Committee for Animal Experimentations of University of Istanbul, it has been concluded that this study did not require any ethics committee approval.

RESULTS

The purity of the obtained DNA was calculated with the ratio OD_{260}/OD_{280} for the DNA obtained from 112 samples and was found to be 1.9273 on average which is 19.28 ng/µL. Wild type and mutant bands were detected under 2% agarose gel electrophoresis. *AluI* enzyme was identified and it cut the mutant variant A into two strands of DNA; 272 and 37 bp. Because *AluI* enzyme was unable to recognize the allele K, wild type allele K was seen as a 309 bp single band. KA genotype appeared as three bands at 309, 272 and 37 bp. KK homozygote, AA homozygote and KA heterozygote individuals must show single, double and triple bands, respectively *(Fig. 1, Fig. 2)*.

Genotypic and allelic frequencies, expected and observed heterozygosity and Hardy-Weinberg Equilibrium (HWE) values were shown on *Table 1* and *2*. PCR analysis has shown us that DGAT1 gene was present in both sheep breeds. In Imroz sheep breeds, the allelic frequencies of the alleles K and A were0.817 and 0.183 whereas in Chios sheep breeds, the alleles K and A presented allelic frequencies of 0.298 and 0.702 respectively *(Table 1)*.

Effective allele number shows us how much of the alleles within a population contributes to the genetic diversity, which is the expected heterozygosity *(Table 2)*. It has been found that this number is 1.72 and 1.43 in Chios and Imroz breeds respectively. This means that since the expected heterozygosity in Chios breeds was higher than Imroz breeds, the former breeds therefore have higher

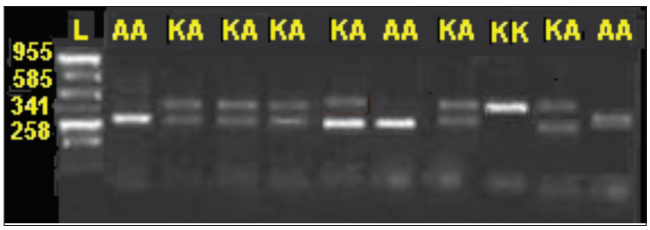

Fig 1. DNA band images obtained from Chios sheep breeds
Şekil 1. Sakız koyun ırkından elde edilen DNA bant görüntüleri

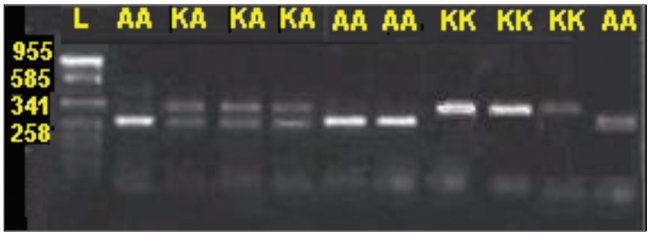

Fig 2. DNA band imagesobtained from Imroz sheep breeds
Şekil 2. Gökçeada koyun ırkından elde edilen DNA bant görüntüleri

Table 1. *Genotypic and allelic frequencies in Imroz and Chios sheep breeds*
Tablo 1. *Gökçeada ve Sakız koyun ırklarında genotipik ve allelik frekanslar*

Breeds	n	Genotypic Frequency (%)			Allelic Frequency (%)	
		KK	KA	AA	K*	A
Imroz	60	68.333 (n= 41)	26.667 (n= 16)	5.000 (n= 3)	0.817	0.183
Chios	52	51.923 (n= 27)	36.538 (n= 19)	11.538 (n= 6)	0.298	0.702

** (P<0.01)*

Table 2. *Genetic polymorphism parameters of the DGAT1 gene in Imroz and Chios sheep breeds*
Tablo 2. *DGAT1 geninin Gökçeada ve Sakız koyun ırkları arasındaki genetik polimorfizmi parametreleri*

Breeds	n	Heterozigosity		HWE[a]	PIC[b]	Effective Allele Number
		Observed	Expected			
Imroz	60	0.267	0.299	0.397	0.255	1.427
Chios	52	0.365	0.418	0.360	0.331	1.720

[a] Hardy-Weinberg Equilibrium, [b] Polymorphic Information Content

allelic contribution. In Imroz and Chios breeds, PIC is 0.255 and 0.331, respectively which indicates low genetic variation.

DISCUSSION

In cattle husbandry, for a long time, milk yield and milk fat content has been of great importance. In this study, it was found that DGAT1 gene's allelic frequencies varied significantly between the two sheep breeds, Chios and Imroz. Between these two breeds, the allele K which was observed the most in Imroz breed (0.817) was dominant whereas in Chios sheep breed, it was the complete opposite, with the allele A being more prominent and possessing the dominant characteristic (0.298). However, since it was observed that the allele A was responsible for the significant increase of the average milk yield in both of the breeds, it can be deduced from the findings in this study that when it comes to the production of milk with high yield and low fat ratio, genetic selection highly favors the Chios sheep breed. Since for the production of dairy products which require milk with high fat content, such as butter, buttermilk and cheese, allele K is the most favorable choice, Imroz sheep breed would be the best choice for such products since it has the highest allelic frequency when compared to the Chios breed. When it comes to the low-fat dairy products, choosing the allele A which promotes the production of low-fat milk and therefore choosing the Chios sheep breed where this allele is the most frequent.

The allele K variant of DGAT1 gene is involved in the significant decrease of protein and milk yield as well as the significant increase of fat content while, on the contrary, the allele A is associated with the significant increase of milk and protein yield and the significant decrease of fat content [29-31]. Kaupe et al.[32], in their study where they investigated the K232A polymorphism on DGAT1 locus among 1748 belonging to 38 different cattle breeds have found that beef cattle possessed a higher allele A frequency while it was the opposite in dairy cattle where the frequency was lower. DGAT1 is identified as being the most important gene when it comes to milk fat content and milk yield and it is known to increase the milk fat content by around 55% by encoding the Lysine amino acid. Schennink et al.[33], in one of their studies, have reported that in Holstein-Friesian cattle, DGAT1 gene had affected the milk fat content by 50%. According to the findings in this study, sheep with high allele K frequency (Imroz breeds) had significantly higher milk fat content than sheep with low allele K frequency (Chios breeds). Sun et al.[34] reported that theallele K of the DGAT1 gene increased the fat concentration in milk while decreasing the milk yield, which is in accord with the findings.

Scata et al.[18] in their studies have found that in sheep breeds indigenous to Italy (Sarda, Altamurana and Gentile di Puglia) the mutation g.5553C-T in the DGAT1 gene caused a decrease in milk fat percentage which corresponds to the findings in this study related to the allele A. However, contrary to their findings, genotypic frequencies of the alleles A and K found in this study are significantly different between the two breeds. Again, in a study on the same mutation done by Nanekarani et al.[35], the allelic frequencies of the studied alleles C and T were 0.562 and 0.438 respectively. They concluded that the allelic frequencies were almost the same, which is in contrast to the findings of this study. Which means that in Chios and Imroz sheep breeds, there exists a specific preference for the alleles A and K respectively which as a result influences the milk content of these breeds.

Hardy-Weinberg Equilibrium for Chios and Imroz sheep breeds was calculated to be 0.360 and 0.397 respectively which means that both of the breeds are in Hardy-Weinberg

Equilibrium (P<0.05). This would mean that the DGAT1 locus of these breeds is not undergoing any kinds of mutations, selections or migrations. In the aforementioned study done by Nanekarani et al.[35], they found that Lori sheep breed was not in HWE, in contrast to the findings in this study.

Bal and Akyuz [19], in their studies, have investigated the allelic frequencies of A and K in DGAT1 among three different cattle breeds (Holstein, Eastern Anatolian Red and Native Black). In Holstein and Eastern Anatolian Red breeds, allele A was found to be of higher frequency (0.75 and 0.62 respectively) while in Native Black breed, allele K was slightly more frequent (0.51). In the molecular marker study done by Ozdemir and Dogru [36] it was stated that this variant could be used as a genetic marker in selection studies. Also, the results obtained in this study is in accord with the findings coming from another study of Ozdemir [37] where the allelic frequency of the DGAT1 variants A and K in Native Black breeds was in the range of 0.35-0.60 (N=25). The values of HWE for the both sheep breeds indicate stability of the variants A and K. In a study done by Nanekarani et al.[35] where the exon 16-17 of DGAT1 gene in Lori sheep breed was studied. The alleles C and T of this gene had an allelic frequency of 0.562 and 0.438 respectively (n=118).

PIC value denotes the relationship between genotypic variation and alleles. If PIC is zero, then there's no genetic variation, if it's 1, then it means that each variation corresponds to an allele. In this study, the PIC value was found to be low in both sheep breeds, which indicates low allelic variation. According to the findings by Yang et al.[11] in their study done on four different sheep breeds (Tan, Oula, Ganjia and Qiaoke), the PIC value was also low, indicating low allelic variation, which is in accord with this study.

In conclusion, we believe that DNA typing is an effective method in identifying the alleles K and A of DGAT1 gene. Also, according to the findings in this study, there is a statistically significant difference in relation to the milk yield and fat content parameters between the sheep breeds with high allele K frequency (Imroz breed) and low allele K frequency (Chios breed). In selection studies which will be done according to the milk yield parameter, the use of Chios sheep breed which carry the allele K232A of DGAT1 gene which in turn corresponds to a high milk yield is believed to be favorable. It has been reported that DGAT1 and its allele K appeared in European sheep breeds long ago, having been inherited to sheep breeds from their ancestral species [29]. However, it has been reported that the allelic frequencies of the native breeds are lower than those of pure blood European breeds [38]. Correct usage of crossbreeding and artificial insemination techniques on native breeds in order to increase the allelic frequencies will be beneficial on the amelioration of milk quality parameters which in turn make it possible to provide the consumer with higher quality milk [39]. For example, cattle carrying the allele A of the gene ABCG2 (which codes the Breast Cancer Resistant Protein, Bcrp) have been reported to be selected since they are economically favourable [40]. In sheep husbandry, using DGAT1 gene's polymorphism will be useful for selection studies although more studies similar to this one need to be done in order to find the exact relationships between milk quality parameters and DGAT1's genetic variance. Polymorphism data acquired in this study can be used in certain areas related to animal husbandry in order to develop correct selection methods. It is believed that by increasing the DGAT1-like loci in order to ameliorate the milk yield capacity of native sheep breeds and merging the results obtained from these loci with the data and pedigree records of animals will prove especially useful to make proper deductions and discoveries. Finally, further studies are needed in order to elucidate the mechanisms of action of genetic polymorphisms of DGAT1 genes which have an impact on economically important production traits in sheep such as milk yield and milk fat content.

REFERENCES

1. Anonymous: Devlet Planlama Teşkilatı, Süt Mamülleri Sanayii Yayın No: 2239, ÖİK: 367, Ankara, 1990.

2. Anonymous: Tetrapak 2010 Raporu. www.tetrapak.com, 2010. *Accessed:* 23.11.2014.

3. Wendorff B: Milk composition and cheese yield. *Proc. 7th Great Lakes Dairy Sheep Symposium Ithaca, NY, USA,* 104-117, 2003.

4. Cases S, Smith SJ, Zheng Y-W, Myers HM, Lear SR, Sande E, Farese RV: Identification of a gene encoding an acyl CoA:diacylglycerol acyltransferase, a key enzyme in triacylglycerol synthesis. *Proc Natl Acad Sci U S A,* 95, 13018-13023, 1998. DOI: 10.1073/pnas.95.22.13018

5. Demirci M: İçme Sütü. İhlas Matbaacılık, İstanbul, 1998.

6. Giannoulia K, Haralampidis K, Poghosyan Z, Murphy DJ, Hatzopoulos P: Differential expression of diacylglycerol acyltransferase (DGAT) genes in olive tissues. *Biochem Soc Trans*, 28, 695-697, 2000. DOI: 101042/0300-5127

7. Cases S, Stone SJ, Zhou P, Yen E, Tow B, Lardizabal KD, Farese RV: Cloning of DGAT2, a second mammalian diacylglycerol acyltransferase, and related family members. *J Biol Chem*, 276, 38870-38876, 2001. DOI: 10.1074/jbc.M106219200

8. Smith SJ, Cases S, Jensen DR, Chen HC, Sande E, Tow B, Farese RV: Obesity resistance and multiple mechanisms of triglyceride synthesis in mice lacking Dgat.*Nat Genet*, 25, 87-90, 2000. DOI: 10.1038/75651

9. Chen HC, Smith SJ, Ladha Z, Jensen DR, Ferreira L D, Pulawa LK, Farese Jr RV: Increased insulin and leptin sensitivity in mice lacking acyl CoA: Diacylglycerol acyltransferase 1. *J Clin Invest*, 109, 1049-1056, 2002. DOI: 10.1172/JCI14672

10. Schennink A, Heck JM, Bovenhuis H, Visker MH, van Valenberg HJ, van Arendonk JA: Milk fatty acid unsaturation: Genetic parameters and effects of ateroyil-CoA desaturase (SCD1) and acyl CoA: diacylglycerol acyltransferase 1 (DGAT1). *J Dairy Sci,* 91, 2135-2143, 2008. DOI: 10.3168/jds.2007-0825

11. Yang JT, Zang RX, Liu WJ, Xu HW, Bai JL, Lu JX, Wu JP: Polymorphism of a mutation of DGAT1 gene in four Chinese indigenous sheep breeds. *Asian J Anim Vet Adv*, 6, 460-468, 2011.

12. Spelman RJ, Ford CA, McElhinney P, Gregory GC, Snell RG: Characterization of the DGAT1 gene in the New Zealand dairy population. *J Dairy Sci,* 85, 3514-3517, 2002. DOI: 10.3168/jds.S0022-0302(02)74440-8

13. Pinto B, Chenoll E, Aznar R: Identification and typing of food-borne *Staphylococcus aureus* by PCR-based techniques. *Sys Appl Microbiol,* 28, 340-352, 2005. DOI: 10.1016/j.syapm.2005.01.002

14. Reis C, Navas D, Pereira M, Cravador A: Growth hormone AluI polymorphism analysis in eight Portuguese bovine breeds. *Arch Zootec,* 50, 41-48, 2001.

15. Pareek CS, Czarnik U, Zabolewicz T, Pareek RS, Walawski K: DGAT1 K232A quantitative trait nucleotide polymorphism in Polish Black- and White cattle. *J Appl Genet*, 46, 85-87, 2005.

16. Grisart, B, Coppieters W, Farnir F, Karim L, Ford C, Berzi P, Cambisano N, Mni M, Reid S, Simon P, Spelman R, Georges M, Snell R: Positional candidate cloning of a QTL in dairy cattle: Identification of a missense mutation in the bovine DGAT1 gene with major effect on milk yield and composition. *Genome Res*, 12, 222-231, 2002. DOI: 10.1101/gr.224202

17. Thaller G, Kühn C, Winter A, Ewald G, Bellmann O, Wegner J, Zühlke H, Fries R: DGAT1 a new positional and functional candidate gene for intramuscular fat deposition in cattle. *Anim Genet*, 34, 354-357, 2003. DOI: 10.1046/j.1365-2052.2003.01011.x

18. Scata MC, Napolitano F, Casu S, Carta A, De Matteis G, Signorelli F, Moioli B: Ovine acyl CoA: Diacylglycerol acyltransferase 1-molecular characterization, polymorphisms and association with milk traits. *Anim Genet*, 40, 737-742, 2009. DOI: 10.1111/j.1365-2052.2009.01909.x

19. Bal O, Akyüz B: Halk elinde yetiştirilen Holştayn, Doğu Anadolu Kırmızısı ve Yerli Kara sığır ırklarında Diacylglycerol-O-Acyltransferase 1 (DGAT1) gen polimorfizminin PCR-RFLP yöntemi ile belirlenmesi. *Erciyes Üniv Vet Fak Derg*, 11, 7-13, 2014.

20. Dekkers JC: Commercial application of marker-and gene-assisted selection in livestock: strategies and lessons. *J Anim Sci*, 82 (13 Suppl.): E313-E328, 2004.

21. Parmentier I, Portetelle D, Gengler N, Prandi A, Bertozzi C, Vleurick L, Renaville R: Candidate gene markers associated with somatotropic axis and milk selection. *Domest Anim Endocrinol,* 17, 139-148, 1999. DOI: 10.1016/S0739-7240(99)00031-4

22. Dybus A: Associations between Leu/Val polymorphism of growth hormone gene and milk production traits in Black-and-White cattle. *Arch Tierz*, 45, 421-428, 2002.

23. Dybus A, Grzesiak W, Szatkowska I, Błaszczyk P: Association between the growth hormone combined genotypes and dairy traits in Polish Black-and-White cows. *Anim Sci Pap Rep,* 22, 185-194, 2004.

24. Bennewitz J, Reinsch N, Paul S, Looft C, Weimann C, Erhardt G, Thaller G, Kühn C, Schwerin M, Thomsen H, Reinhardt F, Reents R, Kalm E: The DGAT1 K232A mutation is not solely responsible for the milk production quantitative trait locus on the bovine chromosome 14. *J Dairy Sci,* 87, 431-442, 2004. DOI: 10.3168/jds.S0022-0302(04)73182-3

25. Cerit H, Altınel A, Elmaz O, Avanus K: Polymorphism evaluation of various genomic loci in Kivircik sheep breed of Turkey. *Turk J Vet Anim Sci*, 28, 415-425, 2004.

26. Raymond M, Rousset F: GENEPOP (version 12): population genetics software for exact tests and ecumenicism. *J Heredity*, 86, 248-249, 1995.

27. Nagy S, Poczai P, Cernák I: PICcalc: An online program to calculate polymorphic information content for molecular genetic studies. *Biochem Genet,* 50, 670-672, 2012. DOI: 10.1007/s10528-012-9509-1

28. University of Wyoming: Assessing genetic structure using codominant, allelic markers within and among populations (WAAP analysis), meaning of AE and tips on software-based analyses, 2008. http://www.uwyo.edu/dbmcd/molmark/waap.html, *Accessed:* 18.07.2015.

29. Thaller G, Kramer W, Winter A, Kaupe B, Erhardt G, Fries R: Effects of DGAT1 variants on milk production traits in German cattle breeds. *J Anim Sci*, 81, 1911-1918, 2003.

30. Weller JI, Golik M, Seroussi E, Ezra E: Population wide analysis of a QTL affecting milk-fat production in the Israeli Holstein population. *J Dairy Sci*, 86, 2219-2227, 2003. DOI: 10.3168/jds.S0022-0302(03)73812-0

31. Spelman RJ, Ford CA, McElhinney P, Gregory GC, Snell RG: Characterization of the DGAT1 gene in the New Zealand dairy population. *J Dairy Sci,* 85, 3514-3517, 2002. DOI: 10.3168/jds.S0022-0302(02)74440-8

32. Kaupe B, Winter A, Fries R, Erhardt G: DGAT1 polymorphism in *Bos indicus* and *Bos taurus* cattle breeds. *J Dairy Res,* 71, 182-187, 2004. DOI: 10.1017/S0022029904000032

33. Schennink A, Stoop WM, Visker MH, Heck JM, Bovenhuis H, van der Poel JJ, van Valenberg HJ, van Arendonk JA: DGAT1 underlies large genetic variation in milk-fat composition of dairy cows. *Anim Genet,* 38, 467-473, 2007. DOI: 10.1111/j.1365-2052.2007.01635.x

34. Sun D, Jia J, Ma Y, Zhang Y, Wang Y, Yu Y, Zhang Y: Effects of DGAT1 and GHR on milk yield and milk composition in the Chinese diary population. *Anim Genet*, 40, 997-1000, 2009. DOI: 10.1111/j.1365-2052.2009.01945.x

35. Nanekarani S, Kolivand M, Goodarzi M: Polymorphism of a mutation of DGAT1 gene in Lori sheep breed. *J Adv Agr Technol*, 3, 38-41, 2016. DOI: 10.18178/joaat.3.1.38-41

36. Özdemir M, Doğru Ü: Sığırların verim özellikleri üzerine etkili önemli moleküler markörler. *Atatürk Üniv Ziraat Fak Derg,* 39, 127-35, 2008.

37. Özdemir, M: Çeşitli sığır ırklarında süt protein polimorfizmi ve verim özellikleri ile ilişkisi. *MSc Thesis.* Atatürk Üniv. Fen Bil. Enst., 2001.

38. Liron JP, Ripoli MV, De Luca JC, Peral-Garcia P, Giovambattista G: Analysis of genetic diversity and population structure in Argentine and Bolivian Creole cattle using five loci related to milk production. *Genet Mol Biol,* 25, 413-419, 2002. DOI: 10.1590/S1415-47572002000400010

39. Ağaoğlu ÖK, Akyüz B, Kul BÇ, Bilgen N, Ertuğrul O: Genetic Polymorphism of Five Genes Associated with Meat Production Traits in Five Cattle Breeds in Turkey. *Kafkas Univ Vet Fak Derg,* 21, 489-497, 2015. DOI: 10.9775/kvfd.2014.12722

40. Yıldırım M, Şahin E: ABCG2 Gene Polymorphism in Holstein Cows of Turkey. *Kafkas Univ Vet Fak Derg*, 16, 473-476, 2010. DOI: 10.9775/kvfd.2009.1047