Eid et al. The Journal of Basic and Applied Zoology (2020) 81:38
https://doi.org/10.1186/s41936-020-00176-w

The Journal of Basic and Applied Zoology

RESEARCH Open Access

DGAT1 polymorphism in Egyptian Zaraibi goat breed and their association with milk yield and composition

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Abstract

Background: The triacylglycerols in milk fat determine the physical and functional properties of dairy products rich in milk fat. Therefore, understanding the variability of genes related to fat synthesis is important for food production. We investigated the effect of diacylglycerol O-acyltransferase 1 (DGAT1) gene polymorphism on milk production parameters of the Zaraibi goat. Milk components were estimated by infrared spectroscopy. Moreover, Restriction Fragment Length Polymorphisms (RFLP) were used to detect genetic variants in DGAT1 genes. The amplified products were sequenced and aligned to the caprine reference sequence of this gene.

Results: Two alleles (T and C) were identified in Zaraibi goats. The T allele resulted in one silent mutation while the C allele specified two-point mutations: one was located within a non-coding region (T703C) and the other (T713C) causing a Ile → Thr substitution in the deduced amino acid sequence. Moreover, the DGAT1 polymorphism significantly ($p < 0.05$) affected total solid content of milk, wherein harboring CC genotype had significantly higher amount of total solid than those with TC genotype. Milk contents and yields did not differ significantly between goats with CC and TC genotype.

Conclusion: These results advance our understanding of the genetic architecture of Zaraibi milk composition and will help to improve the management and breeding program of the Egyptian dairy goat.

Keywords: DGAT1, Zaraibi, Polymorphism, Milk, RFLP

Introduction

Zaraibi goats (Egyptian Nubian) are the most promising dairy goat among the local Egyptian breeds characterized by high genetic potential for milk production (Dowidar, El-Sayed, Elrefy, & Shoura, 2018). Goat milk is of particular economic interest in many countries of the world and is desirable for many reasons, including its richness in various nutrients and its health benefits (Selvaggi, Laudadio, Dario, & Tufarelli, 2014; Chen et al., 2019). It is highly digestible, absorbable, and tolerated by people with allergies to cow's milk (Clark & Mora Garcia, 2017).

The quantity and composition of milk from farm goats have been manipulated for quantity by human-managed systems across different regions of the world. Programs that depend on genetic selection for elevation of milk production have led to improvements in milk yield and composition (Narayana et al., 2017; Heimes et al., 2019). Genetic selection programs for dairy goats are still uncommon in many developing countries, but it has succeeded remarkably in enhancement of breeding strategies for commercial production of milk and meat from goats (Shrestha and Fahmy, 2007; Vacca et al., 2018).

Milk fatty acids play an essential technical role in the production of cheese. Milk fat contains approximately 98% triglycerides (TGs) (Mansson, 2008). The DGAT1 gene codes for an enzyme that is involved in the final and only committed step in the biosynthesis of TGs exported to the milk (Naserkheil et al., 2019). The essential role of DGAT1 in milk fat metabolism makes the

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DGAT1 gene an interesting candidate for explaining the genetic variation of milk traits in dairy goats (Dixit et al., 2015). It also plays an important role in physiologic processes involving triacylglycerol metabolism such as intestinal fat absorption, adipose tissue formation, and lactation in mammals.

In addition to being an underlying quantitative trait locus for milk production, the DGAT1 gene is located in the centromeric region of the bovine chromosome 14 (Mohammed et al., 2015). The Spanish goat (*Capra hircus*) DGAT1 gene includes 8265 bp, organized into 17 exons, and located on chromosome 14 (Martin, Palhiere, Maroteau, Rupp, & Tosser-Klopp, 2016) (GenBank accession number LT221856). This gene encodes an enzyme, of 457 amino acids, involved in the biosynthesis of triglycerides and is expressed in many tissues, particularly in the liver, mammary gland, and adipose tissues (Angiolillo et al., 2007). Polymorphisms in DGAT1 could explain variations in traits that could assist in the selection of dairy animals and offer a better idea of the physiology behind the expression of desired traits (Naserkheil et al., 2019).

Defining the genetic contribution of single nucleotide polymorphisms (SNPs) located in candidate genes associated with milk production traits can be used to enhance the efficiency of selective breeding programs (Naserkheil et al., 2019). Angiolillo et al. (2007) reported a silent SNP within the Caprio DGAT1 gene. Another silent (Ala → Ala) SNP in the exon 17 of DGAT1 genes was observed in the Turcana goat breed (Tăbăran et al., 2014). They proposed that silent SNPs could be used as a marker in association studies to determine if hereditary variety at the goat DGAT1 locus affects milk fat quantity. They also reported that the frequencies for CC, TT, and CT genotypes were 86%, 2%, and 12%, respectively. Moreover, no significant differences were observed in fat percent in milk collected from goats carrying CC and CT.

Here, we identify SNPs in the region between nucleotides 405 and 805 (exon 16 including the whole exon 15, intron 15, exon 16, intron 16, and partial exon 17) of DGAT1 gene and their influence on Zaraibi goat milk fat composition to improve goat milk production.

Materials and methods

Resource population

The data for the DGAT1 SNP came from blood samples from a total of 165 Egyptian dairy Zaraibi goats raised at Sakha experimental station farm—Animal Production Research Institute (APRI) after kidding season February/March 2013. The goats included 105 kids, 7 bucks, and 53 does. Does weighed between 28 and 42 kg and aged from 24 to 108 months. Kids and bucks were selected randomly from the same farm to be representative of the genetic diversity of the whole population.

Ethics statement

Animals were fed according to the Nutrient Requirements of Goat (NRC, 2007). Experimental procedures, animal management, and care followed the recommendations of European Union directive 86/609/EEC. Blood and milk samples were taken at the experimental farm. The animals were not part of any experimental design. They were sampled by veterinarians and/or under veterinarian supervision as part of routine management practices.

Milk and blood samples

Milk samples (150 ml) were collected in sterile tubes, directly from the udder, during the suckling period at days 7, 14, 30, 60, and 90 and the lactation period at days 120 and 210 after kidding. Samples were refrigerated (0–4 °C) until analysis. During suckling, milk yield was estimated using an oxytocin technique according to Banda, Ayoade, Karua, and Kamwanja (1993). Animals were machine milked during the lactation period. Total milk yields during days 14, 30, 60, 90, 120, and 210 were calculated by multiplying daily milk records by the number of the days. Peripheral whole blood samples (10 ml) were collected from jugular veins into tubes containing K3-EDTA as an anticoagulant and stored at − 20 °C.

DNA extraction

White cells from the blood were collected by centrifugation, and genomic DNA was extracted from all collected blood samples using the salting out method as described by Miller, Dykes, and Polesky (1988). Quantity and purity of extracted DNA were measured using spectrophotometer (Eppendorf Biophotometer plus).

PCR-RFLP genotyping

Zaraibi goats were studied for the presence of possible DGAT1 polymorphisms by amplifying the region between nucleotides 405 and 805 of the DGAT1 gene (accession no. LT221856.1).

The amplification was done according to the protocol previously published by Ozmen and Kul (2014), including primers F 5′CCCAGACACTTCTACAAGCC3′ and R 5′ TGCCCGATGATGAGTGACAG3′, deduced from the *Capra hircus* sequence. PCR was carried out in 25 μl reaction mixtures containing ~ 150 ng genomic DNA, 12.5 μl of 2 × Dream Taq Green PCR Master Mix (K1081; Promega), and 10 pmol of each primer. PCR was performed under the following conditions: 94 °C for 4 min followed by 34 cycles of 94 °C for 1 min, 53.5 °C for 1 min, 72 °C for 1 min, and a final extension step of 72 °C for 10 min.

Amplified products (10 μl) were digested with 5U Fast Digest NlaIII (FD1834; Thermo Fisher Scientific) enzyme for 4 h at 37 °C and were separated by gel electrophoresis (1% agarose) in 1× TBE running buffer, set at 200 V for 45 min. The gel was then analyzed with the

Gel-Pro Analyzer program (Media Cybernetics, Silver Spring, MD, USA).

PCR cleanup and DNA sequencing

The PCR products showing different RFLP banding patterns on the gel were selected for sequencing. Amplified PCR products were enzymatically purified using the QIA quick PCR purification kit (Qiagen). To identify SNPs in the DGAT1 gene, the purified samples were sequenced in both strands on an ABI Prism 3130 Genetic Analyzer according to the dideoxynucleotide chain-termination technique (Sanger, Nicklen, & Coulson, 1977) using a BigDye® Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems) with a Bio-Rad C1000 Touch Thermal cycler.

Sequence analysis

A homology search of the obtained sequences was performed by BLAST with the *Capra hircus* genome sequence on the NCBI (National Center for Biotechnology Information) GenBank (http://www.ncbi.nlm.nih.gov/). Multiple sequence alignment analysis was performed with BioEdit sequence alignment editor program v7.2.5.

Milk analysis

Milk fat, protein, lactose, total solid, and solid not fat percentages were estimated in milk samples using Milkoscan 133B analyzer (Foss-Electric DK 3400, Hillord, Denmark) calibrated for goat's milk at dairy cattle breeder unit in Sakha, Kafr El-Sheikh, Egypt.

Statistical analysis

Genotypes were estimated by counting the electrophoretic pattern of DGAT1 gene NlaIII genetic variants. Allele and genotype frequencies were calculated according to the following formulas:

$$f(\mathrm{A}) = \left[(\mathrm{AA} \times 2) + \mathrm{AC} + \mathrm{AD}\right] \Big/ 2N \quad \text{for allele frequencies}$$

$f(\mathrm{AA}) = f(\mathrm{A}) \times f(\mathrm{A}) \times N$ for homozygous genotypes frequency

$f(\mathrm{AC}) = f(\mathrm{A}) \times f(\mathrm{C}) \times N$ for heterozygous genotypes frequency

where A is the allele; AA, AC, and AD were the counted genotypes for DGAT1; and N is the number of total individuals. The calculated frequencies for alleles and genotypes should follow the Hardy-Weinberg equilibrium, where $[f(\mathrm{A}) + f(\mathrm{B}) + f(\mathrm{C}) + f(\mathrm{D})]2 = 1$. A chi-square test ($\chi^2$) was used to analyze the Hardy-Weinberg equilibrium and performed by the PopGene32 software (Yeh, Yang, Boyle, Ye, & Xiyan, 2000).

All collected data were statistically analyzed using General Linear Model (SAS, 2000). Results were expressed as least square means (LSM ± SE). The effect of DGAT1 genotypes on daily milk yield and composition was assessed using the following linear model:

$$Yj = \mu + \mathrm{GD}j + ej$$

where μ = the overall mean, Y = the observed records on daily milk yield and milk composition, GDj = the fixed effect of jth DGAT1 genotype (j = CC, TT, TC), and ej = the random error.

Results

Diacylglycerol O-acyltransferase 1 polymorphism

PCR-RFLP analysis revealed that *DGAT1* demonstrated two alleles (T and C) in the total population, exhibiting an amplicon size of 401 bp (Fig. 1). T allele exhibited 4 bands (Fig. 1) whereas C allele exhibited 2 bands after NlaIII digestion (Fig. 2). Three genotypes were identified in Zaraibi goats: CC (at 178 and 173 bp), TT (at 106, 100, 78, and 67 bp), and TC (at 178, 173, 106, 100, 78, and 67 bp). The frequencies of alleles and genotypes observed in all Zaraibi goats (n = 165) are shown in Table 1. Genotypic frequencies corresponding to CC, TT, and TC were 57.58%, 32.73%, and 9.7%, respectively, in the total population. Allelic frequencies of C and T alleles were 62.4% and 37.6%, respectively, thereby demonstrating the dominant nature of C allele in the whole population. Furthermore, the most common estimated genotype in Zaraibi does was CC (90%), while TC occurred at low frequency (10%). No homozygous TT genotypes were detected.

Sequence analysis revealed that the tested Zaraibi goat sequences (T and C alleles) aligned with *Capra hircus* DGAT1 sequence (Fig. 3). We detected one transition in allele T and two in allele C. In allele T, a transition from C to T occurred at position 416, causing a silent (Phe → Phe) substitution of TTC to TTT. Moreover, allele C showed a transition of T to C at positions 703 and 713 (Fig. 3).

The SNP sustituation for allele C (T713C) caused a non-synonymous substitution of ATC to ACC (Thr → Ile) in the mature protein. Meanwhile, the T 703 C SNP was located in the non-coding sequence. All these SNP have been submitted to The Single Nucleotide Polymorphism Database (dbSNP) with the submitted SNP ID number (#ss2137543765).

Effect of diacylglycerol O-acyltransferase 1 genotypes on milk yield and composition

Alterations in daily milk yield and composition associated with DGAT1 genotypes are shown in Table 2. No significant changes were observed in daily milk yield between does with CC and those carrying TC genotype. Moreover, results showed that genotypes significantly ($p < 0.05$) affected total solids, with does harboring CC having significantly ($p < 0.05$) higher total solid (11.12 ± 0.23%) than those with the TC genotype (9.96 ± 0.55%). No significant

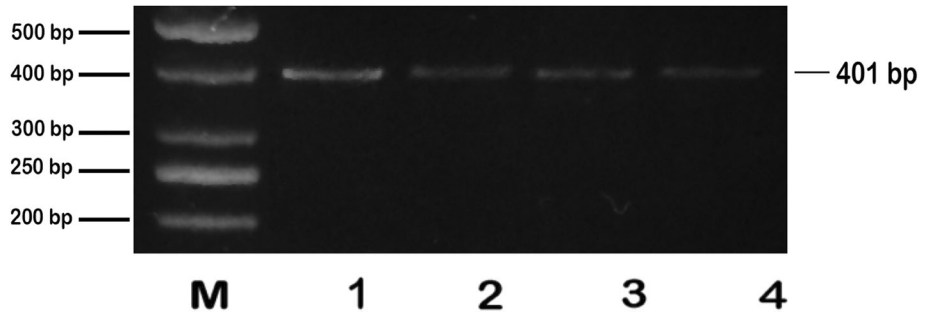

Fig. 1 The electrophoretic pattern of PCR amplification of DGAT1 gene fragment. M, 50 bp ladder marker. Lanes 1–4, 401 bp PCR product of DGAT1

effect of DGAT1 genotypes on fat, protein, lactose, and solid not fat levels was observed.

Discussion

In the present study, three genotypes were detected in the DGAT1 locus (CC, TT, and TC) in Zaraibi goats, where CC was the most common genotype followed by TT and TC. These genotypes derived from two alleles C and T, as allele C was more frequent than T, which agrees with Dixit et al. (2015). Yang et al. (2011) recorded similar results in sheep, where the CC allele of the DGAT1 gene was the predominant genotype in Tan, Ganjia, Oula, and Qiaoke sheep in China followed by TC and TT genotypes.

On the other hand, Angiolillo et al. (2007) estimated allele C with very low frequencies and T variant with high frequencies in Murciano, Malaguen, Saanen, and Girgentana goat breeds. Also, Xu, Chen, Ma, and Xue (2008) detected CC and TC genotypes with lower frequencies than TT in Small-tailed Han, Tan, and Inner Mongolia sheep. They reported that allele T was more frequent than C allele in the corresponding sheep breeds. In addition, Ozmen and Kul (2014) recorded high frequencies of allele T in Saanen, Maltase, Alpine, Damascus, Halep, and Kilis goats, while allele C present in low frequencies and absent in Alpine goats. They also reported that TT genotype was the more frequent in the same goat breeds, while TC was the lowest and absent in Alpine goats.

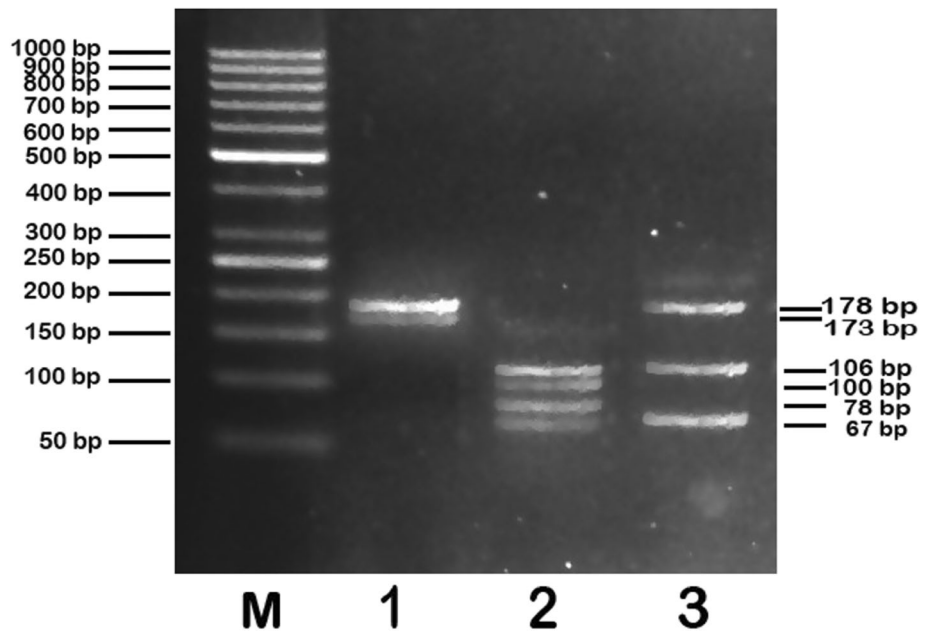

Fig. 2 The electrophoretic pattern after digestion of the goat DGAT1 gene fragment with *NlaIII* endonuclease. M, 50 bp ladder marker. Lanes 1 and 2, homozygous genotypes (CC and TT, respectively). Lane 3, heterozygous genotype TC

Table 1 Alleles and genotype frequencies for the DGAT1 gene in Zaraibi goat

Allele		Genotype		
Type	Frequencies (%)	Type	No. of animals	Frequencies (%)
C	62.4	CC	95	57.58
T	37.6	TT	54	32.73
		TC	16	9.70
		Total	165	100

No significant changes were observed in daily milk yield between Zaraibi goats carrying CC and TC genotypes. Does carrying CC genotypes had significantly higher total solid than those carrying TC genotype. Meanwhile, fat, protein, lactose, and solid not fat levels were not significantly different among DGAT1 genotypes. Similarly, Ozmen and Kul (2014) detected no significant effect of TT and TC genotypes in milk yield, fat, and lactose values in Saanen, Maltase, Alpine, Damascus, Halep, and Kilis goat breeds. Moreover, Tăbăran et al. (2014) recorded no significant differences between fat percent in milk collected from Turcana ewes and Carpatian goats carrying CC and those with CT genotype. They detected one silent mutation that did not affect milk fat percent.

An et al. (2012) recorded that the frequencies of C3 and T3 allele were 0.70–0.72 and 0.30–0.28, respectively at the g.6852C → T locus, while no homozygote was observed at the g.6798 C → T locus. They also reported that C1C2 and C3T3 genotypes had significant effect on fat percentage and milk yield, respectively. Meanwhile, goats with the C1C2 genotype showed significantly higher milk fat than those carrying C1C1 genotype, while goats with C3T3 genotype had greater milk yield than those with C3C3 genotype. Ozmen and Kul (2014) recorded that frequencies for TT/TC in the Saanen, Maltase, Alpine, Damascus, Halep, and Kilis populations were 0.85/0.15, 0.83/0.17, 1/0, 0.73/0.27, 0.70/0.30, and 0.76/0.24, respectively. Moreover, allele frequencies for these breeds were 0.92, 0.93, 1, 0.86, 0.85, and 0.88 respectively for allele T and 0.08, 0.07, 0, 0.13, 0.15, and 0.12, respectively for allele C. They detected no significant effect for TT and TC genotypes on milk yield, fat, and lactose. Angiolillo et al. (2007) observed a SNP (T to C) transitional position 703 in intron 16 when sequencing a 1 kb genomic fragment (from exons 12–17) to detect a higher level of variability in Spanish goats. They suggested the use of this as a marker in association studies with milk traits in this breed. They also detected a C variant with very low frequencies, while T variant was present in high frequencies in different goat breeds.

Scata et al. (2009) reported that the transversion of C to A in the DGAT1 gene at position 127 had a significant negative association with milk fat and protein content and a positive association with milk yield in the Sarda sheep. They suggested that the low frequency of the detected (C127A) in the higher milk fat breeds (Altamurana and Gentile) may be the reason for the negative effect of it on fat content.

Yang et al. (2011) recorded a SNP (C → T) in exon 17 of DGAT1 gene, which is a non-synonymous mutation (GCC (Ala) → GCT (Ala)). Moreover, Ozmen and Kul

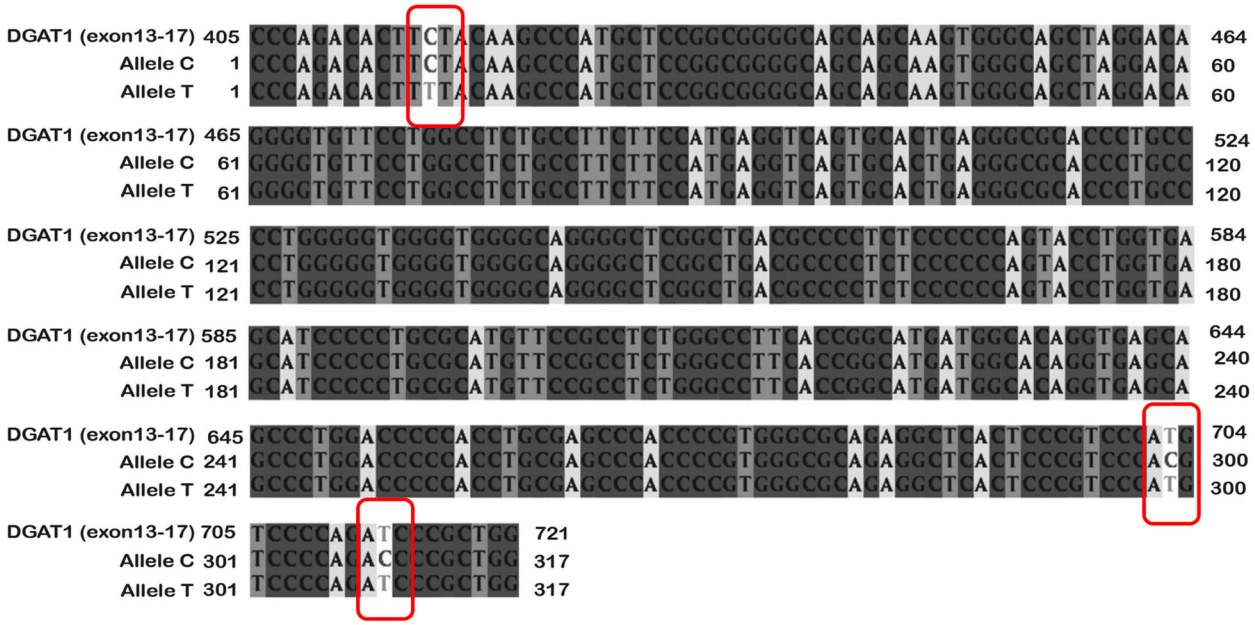

Fig. 3 Alignment of DGAT1 sequence in Zaraibi goats and their corresponding region from GenBank

Table 2 LSM (± SE) of daily milk yield and composition in Zaraibi goats as affected by DGAT1 genotypes

Genotype	Daily milk yield	Milk composition (%)				
		Fat	Protein	Lactose	Total solid	Solid not fat
CC	1.34 ± 0.04[a]	3.44 ± 0.10[a]	2.92 ± 0.07[a]	4.04 ± 0.09[a]	11.12 ± 0.23[a]	7.67 ± 0.11[a]
TC	1.42 ± 0.10[a]	3.28 ± 0.23[a]	2.89 ± 0.18[a]	3.94 ± 0.21[a]	9.69 ± 0.55[b]	7.53 ± 0.25[a]
Significance	**NS	NS	NS	NS	*S ($p < 0.05$)	NS

Superscript a and b = means with the different letters are significantly different
*S significantly different
**NS non-significantly different

(2014) detected a variation in intron 16 heterozygous point (g.273T → C) using NlaIII PCR-RFLP method, which gave rise to substitution of CATG-to-CACG in Saanen, Maltase, Damascus, Halep, and Kilis goats. Tăbăran et al. (2014) observed one SNP in the exon 17 of DGAT1 genes (C to T transition) after restriction digestion with AluI enzyme. They reported that this SNP did not cause any substitution of the amino acid (Ala → Ala) in Turcana ewes and Carpatian does. In sheep, Scata et al. (2009) detected a transversion of C to A in DGAT1 gene at position 127 in Sarda sheep. An et al. (2012) detected different points of mutation in Xinong Saanen and Guanzhong goat breeds (g.407–408insC (in intron 14), g.6852C → T, and g.6798C → T (in exon 7)).

In cattle, some studies showed that SNPs were associated with increased milk fat content in Holstein cows (Spelman, Ford, McElhinney, Gregory, & Snell, 2002) and decreased protein content and milk yield in Jersey cows (Weller, Golik, Seroussi, Ezra, & Ron, 2003). Cardoso et al. (2015) found a variable nucleotide repeat (VNRT) in the promoter region of DGAT1 that explains 32% of additive genetic variance of fat percentage. Meanwhile, Yuan, Zhou, Deng, Hu, and Li (2007) found a non-synonymous nucleotide substitution in the DGAT1 gene (Ala484Val) in Murrah buffaloes.

Kong et al. (2007) investigated the SNPs of the DGAT1 exons, as well as intron numbers 8 and 17 in Hanwoo cattle. They recorded two polymorphic sites 10, 433 (A → G) and 10,434 (A → C) in the exon 8 and one polymorphic site in the 3′UTR at 11,993 (T → C). They reported that the polymorphism occurred at the exon 8 changed the amino acid Lys (K = AAG) to Ala (A = GCC) at position 232 of the protein sequence denoted as K232A. They also found T and A alleles with frequencies 0.773 and 0.227, respectively, while frequencies for TT, TC, and CC genotypes were 0.546, 0.454, and 0, respectively. Moreover, they reported no significant effects on economic traits in Hanwoo cattle in the separate analysis of K232A and T11993C polymorphisms, while the interaction between both showed a significant effect.

Conclusions

In general, polymorphisms of the goat DGAT1 gene are a valuable indicator for selecting favorable genotypes associated with the highest milk yield and desired composition to improve yield and milk quality as it affected the total solid content of milk in our study. Further studies of genetic variations of the DGAT1 gene using more Zaraibi goats reared in different regions in Egypt are recommended for additional genetic characterization, which could be utilized in selective breeding schemes aiming to improve the quality of processed milk and cheese in this breed.

Abbreviations

DGAT1: Diacylglycerol O-acyltransferase 1; RFLP: Restriction Fragment Length Polymorphisms; TGs: Triglycerides; SNPs: Single nucleotide polymorphisms; bp: Base pair

Acknowledgements

We would like to thank the staff member of the Sheep and Goat Research Department, Animal Production Research Institute, Cairo University, for their assistance.

Authors' contributions

Conceptualization, J.E. and S.M.; methodology, S.M.; software, D.T.; validation, D.T. and S.M.; formal analysis, J.E. and A.E; investigation, S.M.; resources, J.E and D.T.; data curation, S.M.; writing—original draft preparation, J.E.; writing—review and editing, D.T. and A.E.; visualization, S.M.; supervision, J.E., D.T., and A.E. The authors read and approved the final manuscript.

Funding

This research received no external funding.

Availability of data and materials

Not applicable

Ethics approval and consent to participate

Experimental procedures, animal management, and care followed the recommendations of European Union directive 86/609/EEC. The animals were not part of any experimental design. They were sampled by veterinarians and/or under veterinarian supervision as part of routine management practices.

Consent for publication

Not applicable

Competing interests

The authors declare no conflict of interest.

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Received: 25 February 2020 Accepted: 9 June 2020
Published online: 22 June 2020

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