Identification of Polymorphism in Goat and Sheep *DGAT1* Gene Associated with Milk Production Traits

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Abstract

Diacylglycerol acyltransferase 1 (DGAT1) is considered a key marker in milk production traits at bovines. Recently, several studies were conducted on sheeps and goats to reveal possible single nucleotide polymorpshims (SNPs) that might influence the production and could help in individual selection. The study was conducted on 50 ewes from the Turcana breed and 60 goats from the Carpatian breed rasied in Transylvania mountain area. The method applied was PCR-RFLP with *Alu*I enzyme. The results revealed no polymorphism in exon 14-16 at Carpatian goats, leading us to the conclusion that sequencying should be applied as method for further SNP detection. In exon 17 a previously reported mutation (C-T) was identified and three corresponding genotypes (CC, CT and TT). No statistically significant differences were found among these three genotypes in what concerns the fat percent in milk. We concluded that further investigations are needed in DGAT1 gene to detect possible SNP associated with milk production traits in these species.

Keywords: *DGAT1, genotypes, milk, sheep, SNP*

INTRODUCTION

The worldwide concern on healthier and more nutritious food products has lead the researchers to the investigation of possible markers that could improve the quality of our animal products. It is a known fact that milk and dairy products are of fundamental importance in the livestock production and the subsequent economy (An et al., 2011; Erhardt et al., 2010). Various genes have been investigated in the direction of improving the cattle dairy products (Dekkers, 2004; Parmentier *et al.*, 1999) but still there are few studies on genetic marker assisted selection of goat and sheep population in our country.

This study was meant to investigate Diacylglycerol acyltransferase 1 (*DGAT1*) gene polymorphism in goats and sheep populations found in Transylvania area. Diacylglycerol acyltransferases (*DGAT*s) are involved in the process of catalyzation of the final step of the triacylglycerol (*TAG*) biosynthesis (Hatzopoulos et al., 2011). This enzyme has been found to be encoded by two genes (*DGAT1* and *DGAT2*), of which the most studied and important one reavealed to be *DGAT1*. This gene is responsible for the codification of the protein related to *DGATs* activity (Cases *et al.*, 2001).

In bovine, it has been established to be located on chromosome 14, where quantitative trait locus (QTL) for milk production have been located in the centromic region of this area (Grisart *et al.*, 2002; Winter *et al.*, 2002). It is for this reason that investigations continued in relation to the possible influence of the QTL found with the quality of the bovine milk. Studies have shown that in several dairy cattle breeds the nonconservative lysine to alanine substitution at position 232, is associated with a major effect on milk yield and fat composition (Grisart *et al.*, 2002; Spelman *et al.*, 2002; Winter *et al.*, 2002).

In sheeps and goats the DGAT1 K232A polymorphism has not been so extensively studied, although the essential role of DGAT1 in milk fat content genetic variation makes this

marker an important factor for the variety of milk traits found in individuals (Scata et al., 2009; Xu *et al.*, 2008). The previous work done by Scata et al. (2009) on possible DGAT1 polymorphism revealed a single nucleotide polymorphism (SNP) in the 5'UTR of DGAT1 woth possible influence on milk fat percent.

Our study aimed at identifying possible DGAT1 polymorphisms in sheep and goat breeds raised in the mountain areas of Transylvania and investigate the possible influence on milk yield and fat percent.

MATERIALS AND METHODS

For this study 50 ewes from the Turcana breed and 60 goats from the Carpatian breed were selected from different herds located in the center of Romania (Transylvanian region). All the animals were kept under the same feeding conditions and examined during spring season.

Milk samples (150 mL) were collected during the begining of the lactation by hand directly from the udder in sterile tubes and stored under refrigeration conditions until analysis (0...4^{0}C).

Peripheral whole blood samples (2 ml) were collected from jugular veins into tubes containing K3-EDTA as an anticoagulant and stored at 4^{0}C.

DNA extraction

White cells from blood were collected by centrifugation, and genomic DNA was extracted using the Isolate II genomic DNA kit according to the manufacturer's instructions (Bioline, England). DNA quantity and purity of each sample were assessed on a Nanodrop ND-1000 spectrophotometer analyzer (NanoDrop Technologies, Inc., Wilmington, DE, USA).

PCR-RFLP genotyping

Both species were studied for the presence of possible DGAT1 polymorphisms by amplifying the exon 14 – 17 of sheep and goat DGAT1 gene according to the protocol previously published by An et al. (2013), respectively Yang et al. (2011). Briefly, primers were acquired according to those mentioned in the reference studies. The PCR amplification protocol used was the following: 1X PCR green Buffer, 2.5 mM MgCl2, 5 pmol of each primer, dNTPs each at 200 µM, 2.5 U of Taq DNA Polymerase (Promega, Madison, WI, USA) and 100 ng of genomic DNA. PCR was performed under the following conditions: 94^{0}C for 3 min followed by 35 cycles of 94^{0}C for 30 sec, 58^{0}C for 30sec, 72^{0}C for 1 min and a final extension step of 72^{0}C for 7 min.

Amplified products (15 µl) were digested with 10U *Alu*I (Fermentas, Lithuania), 4 h at 37^{0}C and were separated in 2.5% agarose gel containing 1X SybrSafe (Invitrogen, Eugene, OR, USA). Electrophoresis was performed in a TBE buffer (pH= 8.5) at 60 V constant current for 2.5 hours. The gel was then analyzed with a Molecular Imager Gel Doc XR System (BioRad Laboratories, Hercules, CA, USA). Unfortunetly we were not able to perform the PAGE electrophoresis as mentioned in the reference protocols and indicated in such single nucleotide polymorphisms.

Milk fat percent evaluation

The milk samples were analysed for fat percent with a Milkoscan 134 analyzer (Foss-Electric A/C, Hillord, Denmark) (IDF standard 141 B:1996).

Statistical methods

For the interpretation of our results we used the OriginPro (Software version 8.5, Origin Lab Institute, USA) and applied the ANOVA one-way and the least significant difference test. Differences were considered significant at a P value lower than 0.05 marked.

RESULTS AND DISCUSSION

The DNA extraction protocol applied in this study was suitable, given the high quantity of DNA extracted which exceeded 50 ng/µl in each of the sample investigated. The purities in the DNA samples ranged in between 1.8 – 2 wavelengths 260/280, which is suitable for a PCR protocol application.

The primers used in this study successfully amplified the 328 base pair phragments in goats, respectively the 309 base pair phragments in sheep. Although the breeds were different in the reference studies, these sets of primers showed specificity for the Turcana sheep breed and Carpatian goat breed also.

After the amplification we applied restriction and found no SNP in the Carpatian goat breeds raised in Transylvania area. Also, the K232A polymorphism that was extensively studied in cattles was found by us to be lacking in the population of goats investigated. The studies need to be developed further in order to identify by fully sequencing the gene in this particular goat breed and possibly find other SNPs related to dairy production.

After the restriction with *Alu*I enzyme in the Turcana breeds we found one SNP that was previously revealed also in other four chinese indigenous breeds by Yang *et al.* (2011). Although the interpretation was not so accurate given the lack of PAGE application and sequencing, we strongly incline that the varience found is similar to that revealed earlier by other authors.

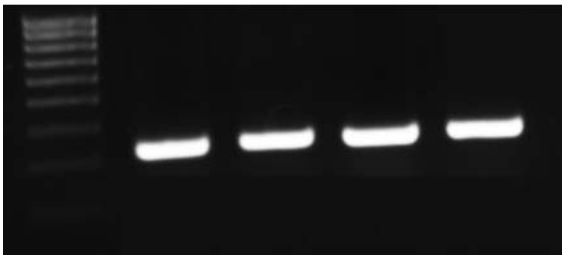

Fig.1. Electrophoresis profile of the 309 bp phragment that *Alu*I enzyme did not cut corresponding to C allele

The size of the non-restricted product was of 309 bp. The amplicon studied included exon 16 and exon 17. The mutation was found in exon 17, where a single nucleotide polymorphism represented by a C to T transition created a restriction site for the enzyme used (*Alu*I). At the electrophoresis we found two alleles: one allele C (309 bp – the non-restricted phragment) and one allele T (272 bp – restricted phragment). The phragment of 37 bp was not visible in the gel given the few base pairs. We were able to identify in the population studied three genotypes: TT (272 bp), CC (309 bp) and CT (309 and 272 bp).

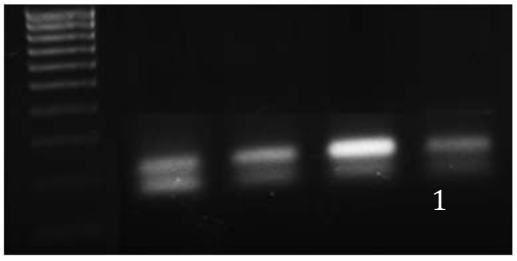

Fig.2. Electrophoresis profile of the 309 bp and 272 phragments after *Alu*I enzyme, corresponding to the presence of both alleles (C and T)

The genotype frequency and allelic frequency has revealed significant differences ($p<0.05$). In the population of Turcana sheep the C to T transition found in exon 17 is not so high. The CC genotype was found in a percent of 86% (n=43) (Fig.1). The TT genotype was identified only in one sample (2%) which was sent also for sequencying to make sure that our result was correct. The rest of the samples (n=6) were represented by the heterozygous genotype (CT) which showed the presence of both allele (C and T) in the same individual (Fig. 2)

After the genotyping of all the samples we compared the values found with the fat percent in milk collected from these individuals. No statistically different results ($p>0.05$) were found when comparing the CC with the CT genotype. The accurate comparison between CC and TT genotype was not possible given the fact that only one individual was found positive for the TT genotype.

The lack of effect of this mutation on the fat percent in milk might be due to the fact that this is a silent mutation, which does not cause an effect on the amino acid substitution (Ala → Ala).

The SNP identified in our study did not have any functional effect. It has been previously reported in other studies at various sheep breeds by Xu *et al.* (2008) in three Chinese indigenous sheeps, Scata *et al.* (2009) in three Italian sheep breeds and Yang et al. (2011) in four chinese breeds. These studies were performed on various individuals and several mutations were found besides the one revealed in our study in exon 17. These author concluded also that there is no significant association with the milk fat content. However, Xu *et al.* (2008) has revealed a significant association of this polymorphism with the IMF content, muscle marbling score and meat tenderness. The authors have concluded that the T allele, which was found in a lesser frequency in our study, had a positive effect on sheep meat quality traits.

CONCLUSION

Our study revealed that the identified SNP in the exon 17 of DGAT1 genes in Turcana breed does not have any effect on the milk fat percent. Even though it is a silent mutation, it migh be useful as a molecular marker in association studies to detect additional DGAT1 polymorphisms which might influene the milk and meat quality traits. Further investigations need to be made on goat and sheep DGAT1 gene to detect possible SNP associated with milk production traits.

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