Polymorphism of a Mutation of DGAT1 Gene in Lori Sheep Breed

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***Abstract*—The objective of this study was to analyze exon 16 - 17 of DGAT1 gene polymorphism in Lori sheep breed. This Gene which is a key acyltransferase in triglyceride biosynthesis, has been located to chromosome 9 of sheep. A total of 118 blood samples have been collected from Lori sheep breed and genotypes were determined by PCR-RFLP assay. The amplified product was observed as 309 bp and the restriction digestion with AluI revealed three genotypes, TT (272 and 37 bp), TC (309, 272 and 37 bp) and CC (309 bp). The results showed that allele frequencies of the DGAT1 gene were 0.562 and 0.438 for C and T allele, respectively. Average heterozygosity for this locus was suitable (0.49). The genotype of SNP in population was not in consent with Hardy-Weinberg equilibrium (p<0.01). The results indicated that this indigenous sheep breed is proper polymorphism and could be used to guide association studies between this polymorphism and Carcass traits.**

***Index Terms*—lori sheep, PCR-RFLP, DGAT1 gene, polymorphism**

I. Introduction

Over the past decade there has been an abundance of Animal Breeding. Using phenotypic data along with the molecular genetics have been able to improve the performance of the animals. Quantitative traits in domestic animals is controlled by many genes, that each minor effects, but some genes have major effects, which have significant effects on a special performance (trait). Mutations in these genes can sometimes lead to a change in the expression of the trait (phenotype). Therefore, identification of candidate genes and mutations in the population of animals can be a key step in improving its performance characteristics.

DGAT (Diacylglycerol O-acyltransferase) is a microsomal enzyme that plays a central role in the biosynthesis of cellular triacylglycerols. It catalyzes the terminal and only committed step in triacylglycerol synthesis, by using diacylglycerol and fatty acyl CoA as substrates [1]. DGAT1 is one of two known DGAT enzymes that catalyze the final step in mammalian triglyceride synthesis [2]. The ovine DGAT1 gene is located on chromosome 9, and one of the candidate genes to improve meat quality traits in sheep.

In dairy sheep, the essential role of DGAT1 in milk fat metabolism makes the DGAT gene an interesting candidate for explaining the genetic variation of milk traits [3], [4]. Scata *et al*. (2009) showed that the novel detected SNP in the 5´UTR of the DGAT1 gene might explain a component of the variation of milk fat content in the Sarda sheep. They also reported that in other SNP (g.5553C>T SNP), a significantly positive effect (0.45%) of the mutated allele on fat content is evident in the Altamura breed, but no effect was found on either milk yield or protein content [3]. In another study that was conducted by Alexander *et al*. (2014), the identified SNP in the exon 17 of DGAT1 genes in Turcana breed did not had any effect on the milk fat percent [4].

Xu *et al*. (2009) showed that, there is a significant association between genotypes and meat quality traits in sheep, as the TT genotype possessed significantly higher muscle marbling score, intramuscular fat (IMF) content, lower shear force and drip loss rate. In addition, the T allele had a positive effect on tenderness, IMF content and marbling score in sheep meat [5]. A SNP (C→T) was diagnosed in 4 Chinese sheep breeds, which creates a non-synonymous single base mutation (GCC (Ala) → GCT (Ala)) at restriction site for endonuclease AluI (AGCT) and the studied breeds had moderate polymorphism [6].

SNPs at 16– 17 exon of DGAT1 gene in Lori–Bakhtiari and Zel sheep breeds investigated and At the DGAT1 locus, CC sheep showed the significantly Greater fat-tail weight ($P < 0.05$) and backfat thickness ($P < 0.01$) [7]. Recently, the results indicated that polymorphism of exon 17 of DGAT1 gene in Moghani sheep had a significant effect on carcass weight [8].

In IRAN, Meat production is the most important economy trait and so the identification of candidate genes for meat quality and quantity be useful. Lori sheep is a fat-tailed meat breed with good traveling ability and suitable conformation for Fattening. The main objective of this work was to identifying polymorphism that might be used to evaluate the association of DGAT1 genotypes with meat quality traits in this breed.

II. Materials and Methods

A. Blood Samples

Lori sheep examined in this study were fat-tailed sheep, with large size and meat type. This sheep have strong constitution, good traveling ability with suitable conformation as a mountain sheep. Blood samples were

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collected randomly from 118 Lori sheep from jugular vein, using vacuum blood collection Tubes containing EDTA and stored at 4 ℃.

B. Genomic DNA Extraction

Genomic DNA was extracted by salting out procedure according to (Miller *et al*. 1988) with minor modifications [9]. DNA quantity and purity of each sample were assessed by spectrophotometer and agarose gel electrophoresis, which were proper for a PCR protocol application.

C. PCR-RFLP

The primers used in this study successfully amplified the 309 bp fragments in sheep (partial exon 16, intron 16 and exon 17 of DGAT1 gene). The Primer sequences used for the DGAT1 AluI site: Forward primer: 5′-GCATGTTCCGCCCTCTGG-3′ and Reverse primer: 5′-GGAGTCCAACACCCCTGA-3′. The PCR amplification protocol used was the following: 1X PCR green Buffer, 2.5 mM $MgCl_2$, 5 pmol of each primer, dNTPs each at 200 µM, 2 U of Taq DNA Polymerase (Promega, Madison, WI, USA) and 100 ng of genomic DNA. The amplification was performed using: 95°C for 4 min; 35 cycles of 30 sec at 94 ℃, 30 sec at 58 ℃ and 30 sec at 72°C; followed by a final extension at 72°C for 10 min. Amplified products (10 µl) were digested with 5 U AluI (Fermentas, Lithuania), for 4 h at 37 ℃. Digested products were separated in 3% agarose gel containing 1X SybrSafe (Invitrogen, Eugene, OR, USA) and visualized by an UV Gel Documentation System (UVitec, UK). Digestion of the 309 bp fragment in DGAT1 gene with AluI restriction enzyme can reveal three genotypes. CC Genotype should produce an un-cut segment of 309 bp, TT genotype should produce segments of 272 and 37 bp and heterozygotes should beget fragments of 309, 272, and 37 bp.

D. Statistical Analysis

Genetic diversity parameters, genotype and allele frequencies of DGAT1 gene were calculated using POPGEN software (version 1.32). Hardy-Weinberg equilibrium for the population was also analyzed using Chi-square (X^2) test [10].

III. Results and Discussion

A. PCR and RFLP Analysis of 16-17 Exon of DGAT1 Gene

The primers used in this study successfully amplified the 309 base pair fragment. The mutation was confirmed by AluI PCR-RFLP assay. The results showed that the SNP represented by a C to T transition which provided a restriction site for the enzyme used (AGCT). This SNP was identified as a silent allele (GCC (Ala) → GCT (Ala)), which creates a no substitution change for the amino acid sequence of DGAT1 protein. As expected, at the electrophoresis we found two alleles: one allele C (309 bp – the non-restricted fragment) and one allele T (272 bp – restricted fragment). The fragment of 37 bp was not visible in the gel given the few base pairs. It is worth noting that in the studied population, all three genotypes were observed. "Fig. 1,"

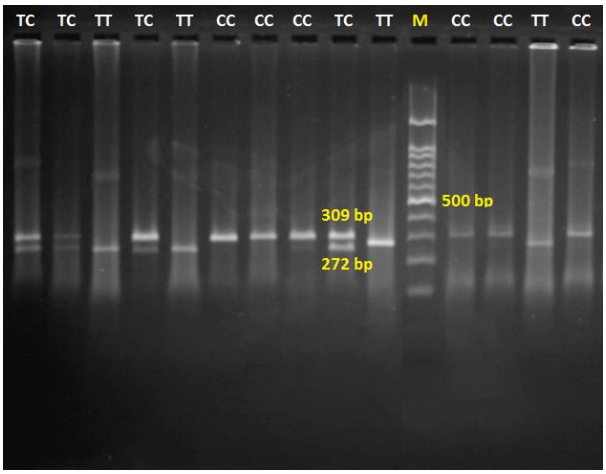

Figure 1. PCR-RFLP analysis of 309 bp fragment of DGTA1 gene by AluI enzyme on 3 % agarose gel. Lane M: 100 bp DNA; Lane CC: (309 bp); Lane TC: Heterozygous genotype (309 bp, 272 bp and 37 bp) and Lane TT (309 bp, 272 bp) Polymorphism parameters of DGAT1 gene

In the population under study, estimation of the parameters of genetic diversity and polymorphism for candidate genes is imperative. So, to study this candidate gene in Lori sheep population these parameters were measured.

The frequencies of TT, TC and CC genotypes were 0.309, 0.258 and 0.433 respectively. The calculated allelic frequencies were 0.438 and 0.562 for T and C respectively. "Fig. 2,"

CC genotype frequency was higher than that of TC and TT genotypes. Similar results in this gene region reported in four Chinese indigenous breeds and Turcana breed of Romania [4], [6]. While, in three Iranian native Breeds (Lori-Bakhtiari, Zel and Moghani) TT genotype was dominant [7], [8].

The H_o (observed) and H_e (expected) heterozygosity, N_e (Effective number of alleles) and Chi-Square value for Lori sheep breed are shown in Table I.

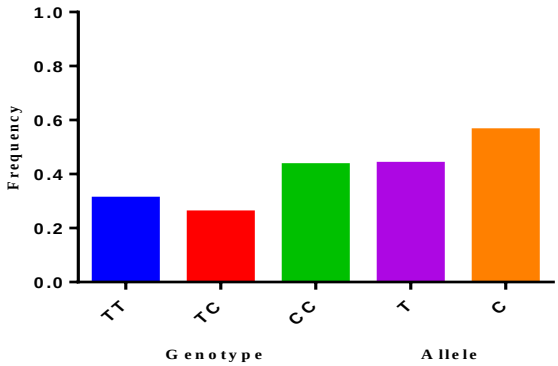

Figure 2. Genotype and allele frequencies of DGTA1 gene in Lori sheep breed

It should be noted that the level of heterozygosity is one of the most important indexes of genetic diversity in the population. In this study, rate of observed heterozygosity was low but due to the allelic frequencies were almost the same, expected heterozygosity to be high

predicted. It also the observed and effective number of alleles to be identical. The results genetic diversity demonstrated that Lori sheep breed have suitable polymorphism. The X^2 test confirmed that the population was not in HWE (Hardy-Weinberg equilibrium) for the tested locus (P<0.01). Therefore, it can be concluded the study population is probably undergoing selection, migration, or mutation at the DGTA1 locus. This is accordant with the result of Xu *et al*. (2009) that the allele distribution of the three Chinese sheep populations including Tan sheep was not in agreement with Hardy-Weinberg equilibrium (P<0.01) [5]. Yang *et al*. (2011) reported that the allele distribution of exon 17 locus of DGAT1 gene in Tan and Oulu sheep breeds was in agreement with HWE, Versus Ganjia and Qiaoke Sheep breeds was not in agreement with HWE [6]. Similarly, the allele distribution of the three Iranian sheep populations including Lori-Bakhtiari, Zel and Moghani breeds was not in assent with Hardy-Weinberg equilibrium [7], [8].

TABLE I. GENETIC DIVERSITY PARAMETERS AND CHI- SQUARE VALUES

Breed	N	HO	HE	Ne	X^2
Lori	118	0.258	0.495	1.97	22.5 and (P<0.01)

The effect of this SNP of DGAT1 on meat quality traits or other traits in Lori sheep breed should be carried out further investigation. This point has been formerly presented in other studies at various sheep breeds by researchers. Scata *et al*. (2008) showed the found SNP in the 5' UTR (g.127C>A) significant negative relationship with milk fat content in the Sarda sheep [3]. However Xu *et al*. (2009) stated the T allele had a positive effect on tenderness, IMF content and marbling score in three Chinese sheep breeds [5]. Beside Tabaran *et al*. (2014) revealed that the identified SNP in the exon 17 of DGAT1 genes in Turcana breed did not have any effect on the milk fat percent [4]. Mohammadi *et al*. (2013) in the two Iranian sheep breeds (Lori-Bakhtiari and Zel) demonstrated novel associations in which the C allele had a positive effect on fat-tail Weight and backfat thickness in fat-tailed sheep [7]. Ala Noshahr and Rafat, (2014) represented the effect of different genotypes on hot carcass weight was significant and CC genotype had the Most hot carcass weight in the Moghani sheep [8].

IV. CONCLUSION

The results obtained in this study indicate that this native sheep breed is substantially polymorphism at the DGAT1 locus. The identified SNP in the exon 17 of DGAT1 genes in Lori sheep breed it might be useful as a molecular marker in association studies. Accordingly, association between SNP in the Ovine DGAT1 Gene and traits of economic interest should be performed subsequent research.

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