The diacylglycerol acyltransferase 1 gene (DGAT1) was identified as a strong candidate gene affecting mutton quality traits in sheep. Single nucleotide polymorphism creates a single base mutation (C to T) in AGCT site of endonuclease AluI. DGAT1 is one of the candidate genes to improve carcass characteristics in feedlot animals. In order to study area T487C in exon 17 of the DGAT1 polymorphism, Iranian Moghani sheep breeds randomly slaughtered in the abattoir were recorded. DNA was extracted from 150 samples of Moghani sheep. Polymerase chain reaction to amplify 309 bp of exon 17 DGAT1 gene using a pair of specific primers was performed. Genotypes obtained from method PCR-RFLP and directly from agarose gel. Two alleles T and C with frequencies of 0.829 and 0.171 were observed respectively. Statistical analysis showed polymorphism in exon 17 region of the gene significantly correlated with carcass weight and dressing percentage (P<0.05). So that the CC genotypes of the significant mean carcass weight and dressing percentage heavier than had TT genotypes (P<0.05). Of polymorphism can be observed that improvement in breeding programs to improve carcass weight and dressing percentage through selection in favor of superior genotypes be used.

**KEY WORDS** carcass weight, DGAT1 gene, dressing percentage, Moghani sheep, polymorphism.
higher muscle marbling score and intramuscular fat (IMF) content and lower shear force and drip loss rate (Xu et al. 2009; Moioli et al. 2007; Barillet et al. 2005).

Found on the Moghan steppe of northwestern Iran the Moghani are a fat-tailed meat breed with carpet quality wool. Given the importance of Moghani sheep for meat production, this research was necessary. Therefore, the aim of this research was to determine the genotype and allelic frequencies and study of the relationship between genotypes of exon 17 of DGAT1 gene site with carcass traits in Moghani sheep.

**MATERIALS AND METHODS**

**Blood samples**

Blood samples were collected randomly from 150 Moghani sheep from jugular vein (in slaughter house in Ardabil province from November to December 2012), using vacuum blood collection Tubes containing 0.25% ethylene diamine tetra acetic acid (EDTA). After slaughtering sheep and dump all the contents of the abdominal, hot carcass weights were recorded.

**DNA extraction**

Genomic DNA was extracted from 100 µL using a Tiangen genome extraction kit according to the manufacturer’s instructions (Tiangen Biotech (Beijing) Co. Ltd., China). Concentrations, purities and integrity of genomic DNA were measured by spectrophotometer and agarose gel electrophoresis. Primers used for amplification of 309 bp fragment including:

Forward primer: 5-GCATGTTCCGCCCTCTGG-3′
Reverse primer: 5-GGAGTCCAACACCCCTGA-3′

PCR was carried out with a total 25 µL reaction volume, containing 100 ng genomic DNA, 2.5 µL 10X PCR standard reaction buffer, 6 pmol dNTPs, 12 pmol each forward and reverse primer, 1.5 U Taq DNA polymerase, then add additional distilled water to 25 µL. The amplification was performed using: 95 °C for 5 min; 35 cycles of 30 sec at 94 °C, 30 sec at 60 °C and 30 sec at 72 °C; followed by a final extension at 72 °C for 10 min. PCR results were identified by 2% agarose gel electrophoresis. The PCR products were then directly genotyped by RFLP gel. Briefly, PCR products were digested by *Alu I* at 37 °C water bath for 4 h. The PCR products showing different band patterns on RFLP gel were selected for sequencing.

**Statistical analysis**

Effective information content of population and genotype distribution for Hardy Weinberg equilibrium was tested by using general linear model (GLM) procedure of the SAS, (2004) program and least squares means of the banding patterns were compared using the Tukey test. The following statistical model was used:

\[ Y_{ijkl} = \mu + A_i + S_j + G_k + B(W_{ijkl} - \bar{W}) + (AG)_{ik} + (SG)_{jk} + e_{ijkl} \]

Where:
- \( Y_{ijkl} \): the dependent variable.
- \( \mu \): the overall mean.
- \( A_i \): the \( i^{th} \) animal age effect in blood sampling.
- \( S_j \): \( j^{th} \) animal sex.
- \( G_k \): the \( k^{th} \) genotype of DGAT1.
- \( B \): the regression coefficient on weight.
- \( W_{ijkl} \): the animal weight in blood sampling period.
- \( \bar{W} \): the mean of animal weight in blood sampling period.
- \( (AG)_{ik} \): the interaction effect between age and genotype.
- \( (SG)_{jk} \): the interaction effect between sex and genotype.
- \( e_{ijkl} \): the random residual error.

Note that interactions have no significant effect and were removed from the model. Average genotype frequencies, allelic diversity and population heterozygosity were calculated by statistical software Pop Gen 32.

**RESULTS AND DISCUSSION**

**PCR amplification of 17 exon of DGAT1 gene**

The size of expected PCR amplicon of DGAT1 gene in Moghani sheep breed was about 309 bp (Figure 1a). From the DGAT1 amplicon a SNP was found in exon 17 (C → T) which creates a restriction site for endonuclease *Alu I* (AGCT). As expected, the electrophoresis results showed the SNP had two alleles of C (309 bp) and T (272 and 27 bp) and three genotypes of TT (272 and 37 bp), TC (309, 272 and 37 bp) and CC (309 bp) (Figure 1b), by means of the PCR-RFLP technique. This SNP is a non-synonymous mutation (GCC (Ala) → GCT (Ala)), which creates no substitution change for the amino acid sequence of DGAT1 protein.

**Figure 1** Agarose gel electrophoresis examination of amplification product 309 bp of DGAT1 (a); agarose gel electrophoresis digestion products with *Alu I* endonuclease (b)
Polymorphisms of sheep DGAT1 gene

The genotype frequency, allelic frequency and results of Hardy Weinberg test in exon 17 of DGAT1 gene in experimental animals are shown in Table 1. The allele distribution of exon 17 locus of DGAT1 gene due to some factors, including selection and sample size, was not in agreement with Hardy Weinberg equilibrium by the Chi-square test (P>0.01). This is compliant 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). This is compliant 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).

Table 1: Genetic diversity of DGAT1 in Moghani sheep population

<table>
<thead>
<tr>
<th>Index</th>
<th>Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Animals</td>
<td>150</td>
</tr>
<tr>
<td>Number of animals with TT genotype</td>
<td>105(0.698)</td>
</tr>
<tr>
<td>Number of animals with TC genotype</td>
<td>39(0.262)</td>
</tr>
<tr>
<td>Number of animals with CC genotype</td>
<td>6(0.04)</td>
</tr>
<tr>
<td>Allele frequency of T</td>
<td>0.829</td>
</tr>
<tr>
<td>Allele frequency of C</td>
<td>0.171</td>
</tr>
</tbody>
</table>

Least squares means (LSM) of different traits in Moghani sheep are shown in Table 2. The fixed effects of sex and age on hot carcass weight and hot dressing percentage were significant (P<0.05).

Table 2: Least square mean (LSM) different traits in Moghani sheep

<table>
<thead>
<tr>
<th>Trait</th>
<th>Sex</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hot carcass weight (kg)</td>
<td>Male 24.19±2.01, Female 20.63±1.89</td>
</tr>
<tr>
<td>Hot carcass weight free fat-tail (kg)</td>
<td>Male 20.15±0.29, Female 20.41±0.41</td>
</tr>
<tr>
<td>Hot dressing percentage (%)</td>
<td>Male 46.43±0.88, Female 47.09±1.32</td>
</tr>
<tr>
<td>Hot dressing percentage free fat-tail (%)</td>
<td>Male 40.17±0.84, Female 41.41±1.09</td>
</tr>
</tbody>
</table>

The allele frequency of C at present study is the predominant allele and this allele may be an ancient allele of Moghani sheep breed.

CONCLUSION

The results indicated that polymorphism of exon 17 of DGAT1 gene has a significant effect on carcass weight. Whether, the non-synonymous SNP in exon 17 is directly related to DGAT1 functional variations needs to be confirmed. The effect of this SNP of DGAT1 on meat quality traits or milk quality traits in sheep breeds should be carried out further investigation. The mapping and linkage characterization of DGAT1 gene of sheep need to be studied in more detail and the exact mechanism of DGAT1 gene polymorphism contributing to sheep traits of economic interest also requires further investigation. The results of this research can be used in marker assisted selection (MAS) and breeding program of carcass weight.

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REFERENCES


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