Study of DGAT1 Gene Polymorphisms with Carcass Traits in Iranian Zel and Lori-Bakhtiari Sheep Breeds

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ABSTRACT

Diacylglycerol acyltransferase1 (DGAT1) plays an important role in the metabolism of triglycerides which catalyze the final step of triglyceride synthesis in animals. The objective of this study was to investigate the SNPs in 5'UTR, exon-1 and exon-2 of *DGAT1* in two Iranian indigenous sheep breeds. A total of 309 animals including fat-tailed Lori-Bakhtiari (n=152) and thin-tailed Zel (n=157) were used in this study. The genotypic patterns were detected by polymerase chain reaction-single strand conformation polymorphism (PCR-SSCP). Five SSCP patterns were detected for 5'UTR and exon-1 fragment by PCR-SSCP and subsequently confirmed by sequencing PCR products. The sequencing results revealed that there are three novel polymorphisms in 5'UTR and exon-1 fragment of *DGAT1* at the studied breeds. Out of the detected polymorphisms only A277G substitution in exon-1 of *DGAT1* leads to the changes in amino acids (p.Arg26Gly). There was significant correlation ($P<0.05$) between fat-tail weight (FTW) and back-fat thickness (BFT) and the observed genotypes in Lori-Bakhtiari breed; therefore, animals with G5 pattern had higher FTW and BFT compared to G1 pattern. The G1 and G5 genotypic patterns or haplotypes were different at their position 101 of 5'UTR region. No significant relationship ($P<0.05$) was found between the detected genotypes of 5'UTR and exon-1 fragment of *DGAT1* in Zel breed and carcass traits. These results revealed that detected *DGAT1* novel SNPs had significant effects on carcass traits and they can be used as a marker for these traits.

Keywords: PCR-SSCP, DGAT1 gene, Lori-Bakhtiari, Zel, sheep, Iran.

INTRODUCTION

The main goal of sheep breeding programs is to improve meat production and carcass traits (Kosgey *et al.* 2006). During the last forty years, the genetic improvement of livestock has been achieved by selection based on phenotypic information, but getting fast genetic improvement is difficult by this method. However marker-assisted selection (MAS) can increase the genetic improvement of polygenic traits which results from the presence of major genes and their effect on the performance

of these traits. Therefore, this method has provided opportunities to increase response to selection, especially for traits with small heritability (Dekkers, 2004).

Lori-Bakhtiari breed, with more than 1.7 million heads, is one of the most common indigenous sheep breed in Iran. This breed adapted to the hilly and mountainous regions in the west of Iran and has been raised mostly in villages under semi-intensive systems. The Lori-Bakhtiari breed has high body weight and the largest fat tail compared to other Iranian Indigenous breeds (Kianzad *et al.* 2003; Vatankhah and Talebi, 2008). Zel breed, with about 1.5 million heads, is the only thin-tailed Iranian breed. Zel is raised in the north part of Iran located between the northern slopes of the Alburz mountain and the Caspian Sea (Kamalzadeh *et al.* 2008).

Diacylglycerol acyltransferase1 (DGAT1) gene is involved in the metabolism of triglycerides and catalyzes the last step of the triacylglycerol synthesis (Hatzopoulos *et al.* 2011; Li *et al.* 2013). *DGAT1* is extensively expressed in almost all tissues including the mammary glands, small intestine, adipose tissue, and skeletal muscle, with the highest expression level in adipose tissue and small intestine (Xu *et al.* 2008).

The DGAT1 gene spans approximately 8.5 kbp and comprises 17 exon 16 introns, located on the chromosome 14 of bovine (Grisart *et al.* 2002). Homologous sequence was found on the chromosome 9 of sheep (*Ovis aries*). This gene contains about 1470 nucleotides that code a polypeptide with 489 amino acids (Xu *et al.* 2009). Specific polymorphisms in *DGAT1* were detected and their association with some production traits were confirmed in farm animals, especially cattle. Then, *DGAT1* was introduced as a candidate gene for milk production traits in dairy (Banos *et al.* 2008; Li *et al.* 2009; Signorelli *et al.* 2009; Cerit *et al.* 2014) and carcass traits (Anton *et al.* 2010; Souza *et al.* 2010; Pannier *et al.* 2010; Li *et al.* 2013; Tait *et al.* 2014; Ardicli *et al.* 2017) in cattle. Moreover, DGAT1 due to its key role in fat metabolism (Scata *et al.* 2009) is a candidate gene for milk fat content (Xu *et al.* 2009; Scata *et al.* 2009; Yang *et al.* 2011: An *et al.* 2013; Tabaran *et al.* 2014; Martin *et al.* 2017) and carcass traits (Xu *et al.* 2009; Mohammadi *et al.* 2013) in dairy sheep and goat.

However, there are only few studies on the association between *DGAT1* polymorphisms and carcass traits in sheep (Xu *et al.* 2009; Mohammedi *et al.* 2013; Noshahr and Rafat, 2014; Nanekarani *et al.* 2016). Xu *et al.* (2009) sequenced the whole coding region of sheep DGAT1 and surveyed the associations between polymorphism of *DGAT1* and 11 meat production traits. They reported the positive effect of the T allele in exon-17 of *DGAT1* on meat quality and quantity in three Chinese sheep breeds. Mohammadi *et al.* (2013) reported that there is a significant correlation between exon-17 of *DGAT1* polymorphisms (T487C) and carcass weight and dressing percentage, and concluded that the CC genotypes had higher carcass weight and dressing percentage.

Few studies have been carried out on the association of *DGAT1* polymorphisms with carcass traits in sheep breeds. All of these studies suggested that further investigations are necessary to make a definitive statement about the association of this gene polymorphisms with carcass traits in sheep (Xu *et al.* 2009, Noshahr and Rafat, 2014 and Mohammadi *et al.* 2013). The aim of this study is to investigate 5'UTR, exon-1 and exon-2 regions of *DGAT1* polymorphisms and their association with carcass traits in Lori-Bakhtiari and Zel Iranian indigenous breeds.

MATERIALS AND METHODS

Animals and data collection

A total of 309 blood samples were collected from Lori-Bakhtiari (n=152) and Zel (n=157) sheep breeds. Lori-Bakhtiari and Zel samples were taken from two industrial slaughterhouses located in

٨٠ the Qom and Mazandaran provinces of Iran, respectively. Before slaughter, body weight (SBW) of
٨١ lambs was measured. After slaughter, carcass weight (CW), fat-tail weight (FTW), carcass weight
٨٢ without fat-tail (CWFT), and back fat thickness (BFT) immediately were measured and fat-tail
٨٣ percentage (FTP), dressing percentage (DP) and dressing percentage without fat-tail (DPWFT) were
٨٤ calculated. Genomic DNA was extracted by the Salting-out procedure (Miller *et al.* 1998). The
٨٥ quality and concentration of extracted DNA were evaluated by visualizing on 0.8% agarose gel
٨٦ under UV light and spectrophotometer (Thermo, NanoDrop 1000).

٨٧ **DNA amplification with PCR**

٨٨ Two primer pairs *DGAT1* (5'UTR and exon-1) F: 5'-GGAACTACGCTTCCCAGGAC-3'; R: 5'-
٨٩ ACGTCTCCGTCCTTGTCTGT-3'; and (exon-2) F: 5'-GTCTTGCATCACCAGCTCCT-3'; R: 5'-
٩٠ CAGGCATCTACTGGGATTCAG-3' were designed based on the published sheep sequences
٩١ (GenBank accession no. EU178818) to amplify the determined fragment by Primer-BLAST (Ye *et*
٩٢ *al.* 2012). The length of the amplified fragments were 360bp (5'UTR and partial exon-1) and 445bp
٩٣ (exon-2) in both breeds. PCR was carried out on 25 µL volume of the following ingredients: 1× PCR
٩٤ buffer (16 mM $(NH4)_2SO_4$, 67 mM Tris-HCl pH 8.8, 0.1% Tween-20), 2 mM $MgCl_2$, 200 µM dNTP,
٩٥ 6 pmol of each primer, 1.5 unit Taq polymerase (Genet Bio, Korea) and 50–100 ng of genomic
٩٦ DNA. PCR reactions were performed with a Genecycler (Bio-Rad, My Cycler, USA) with the
٩٧ amplification program consisted of an initial denaturation at 96°C for 2 mins, then 32 cycles of 96°C
٩٨ for 1 min, 57°C for 45 seconds and 72°C for 1 min, and a final extension of 72 °C was maintained
٩٩ for 10 mins for the exon-1 of DGAT1 gene. For exon-2 fragment, the thermal cycling conditions
١٠٠ consisted of initial denaturation at 96°C for 5 mins, followed by 30 cycles of denaturation at 96°C
١٠١ for 1 min, annealing at 60°C for 45 seconds, extension at 72°C for 1 min, with a final extension at
١٠٢ 72°C for 10 mins. The PCR products were identified by 2% agarose gel electrophorese and
١٠٣ visualized using UV light.

١٠٤ **Single -Strand Conformation Polymorphism**

١٠٥ SSCP method was applied to detect SNPs in 5'UTR, partial exon-1 and exon-2 of the DGAT1 gene.
١٠٦ For SSCP, 5µl PCR products were diluted in 5µl denaturing solution; the mixture was centrifuged
١٠٧ and denatured at 98°C for 10 mins, then rapidly chilled on ice for 5 mins and detected on 12%
١٠٨ polyacrylamide gel. The electrophoresis was performed in a vertical unit (Bio-rad), in 1x TBE buffer
١٠٩ at 4°C. The gel running conditions for the 360bp fragment of DGAT1 was 350 V for 18 hours and
١١٠ 445bp fragment of DGAT1 was 380V for 21 hours, respectively. Then, DNA fragments were stained
١١١ with 0.1% silver nitrate and visualized in 3% NaOH solution (Zhang *et al.* 2007). Two samples from
١١٢ each SSCP patterns were sequenced in both directions (Bioneer, Seoul, South Korea) and were
١١٣ determined by Bio Edit (Version 7.2.0) and Vector NT1 software. Different SSCP pattern sequences
١١٤ were aligned according to Genbank (EU178818.1) by BLAST algorithm to find homologous
١١٥ sequences in NCBI databases (HTTP:// www.ncbi.nlm.hih.gov/).

١١٦ **Statistical analysis**

١١٧ Genotypes patterns or haplotypes frequencies were calculated using GENALEX6.4 software
١١٨ (Peakall and Smouse, 2006). Two fixed models were used to make an association between 360bp
١١٩ fragment of DGAT1 variants and carcass traits using generalized linear model (GLM) procedure of
١٢٠ SAS software (SAS Institute Inc, 2007). Exon-2 of DGAT1 was eliminated from association
١٢١ analysis due to monomorphic state. Tukey–Kramer's multiple range tests were used for least squares

means (LSM) comparison. The statistical model used for determining CW, DP, BFT and DPWFT traits in two breeds was as follows:

$$Y_{ijklm} = \mu + A_i + B_k + G_l + \beta(W_{ijklm} - \overline{W}) + S_j(B_k) + (AG)_{il} + (SG)_{jl} + (BG)_{kl} + e_{ijklm}$$

Another model used for FTW, FTP and DP traits in Lori-Bakhtiari sheep breed was as follows:

$$Y_{ijlm} = \mu + A_i + S_j + G_l + \beta(W_{ijlm} - \overline{W}) + (AG)_{il} + (SG)_{jl} + e_{ijlm}$$

where y_{ijklm} is phenotypic value of carcass traits; μ is the overall population mean for each trait; A_i is the fixed effect of the ith age; S_j is the fixed effect of the jth sex; B_k is the fixed effect of the kth breed; G_l is the fixed effect of the lth genotype; $\beta(W_{ijklm} - \overline{W})$ is the covariate fixed effect of weight at slaughter; $S_j(B_k)$ is the effect of the jth sex in k^{th} breed; $(AG)_{il}$ is the interaction between the ith age and the lth genotype; $(SG)_{jl}$ is the interaction between the jth sex and the l_{th} genotype; $(BG)_{kl}$ is the interaction between the kth breed and the lth genotype and e_{ijklm} is the residual random effect.

RESULTS

Single Strand Confirmation Polymorphism analysis

The results showed that both PCR products including 360bp and 445bp fragments, successfully amplified by the used primers. Five SSCP patterns were detected for exon-1 and its 5′UTR (Figure 1). Furthermore, one SSCP pattern was observed for exon-2 and this segment was monomorphic in both breeds (Figure 2). The frequency of the observed genotypic patterns of 360bp fragment for each breed is shown in table 1. The G5 and G2 had higher frequency in Lori-Bakhtiari and Zel breeds, respectively. The G3 had the lowest frequency in both breeds. Also, the frequency of G2, G4 and G5 was different between Lori-Bakhtiari and Zel breeds

DNA sequence analysis

In this study, sequences of PCR amplicons from five different SSCP genotypic patterns of 360bp fragment variants were analyzed and compared with the NCBI reference sequences (Table 2). For 360bp fragment (including 5′UTR and Exon-1), three novel single nucleotide polymorphisms (SNPs) were detected in the studied breeds. One of the discovered SNPs is located in the coding region (g A277G) and two of them located in the 5′UTR region (g. T101- and C129A) of DGAT1 (Table 2). Moreover, the protein sequences of exon-1 region were aligned to NCBI reference sequence (Figure 3). Only substitution in exon-1 of G3 pattern (A 277G) leads to the changes in amino acids (p.Arg54Gly) (Figure 3).

Correlation between DGAT1 polymorphisms and carcass traits in sheep

In the present study, exon-2 of DGAT1 was monomorphic and omitted from association studies. Association of the 360bp fragment of DGAT1 polymorphisms with carcass traits was analyzed (Table 3). Significant relationship ($P<0.05$) was found between FTW and BFT traits in the Lori-Bakhtiari breed; and animals with G5 had higher FTW and BFT compared to G1 pattern. Although no significant differences ($P>0.05$) were observed for DP and FTP among five identified genotypic patterns in Lori-Bakhtiari, but animals with G3 pattern for DP and G5 pattern for FTP had the highest performance compared to other genotypes. No significant association ($p<0.05$) was found between observed genotypes of exon-1 of *DGAT1* in Zel breed and the studied carcass traits.

DISCUSSION

DGAT1 was selected as a candidate gene due to its effects on carcass traits that had been confirmed in previous studies on cattle (Souza *et al.* 2010; Pannier *et al.* 2010; Li *et al.* 2013; Tait *et al.* 2014) and sheep (Xu *et al.* 2009; Mohammedi *et al.* 2013; Noshahr and Rafat, 2014). The detection of SNPs in 360bp fragment was consistent with results of Scata *et al.* (2009) who amplified and sequenced whole of theDGAT1gene using 18 primer pairs and reported novel SNPs at 5'UTR (g. C127A) in Sarda, Altamurana and Gentile di Puglia sheep breeds. But their result was not consistent with present study for exon-1. The detection of SNPs in 360bp fragment was consistent with the results of Scata *et al.* (2009) who identified novel SNP in Sarda, Altamurana and Gentile di Puglia sheep breeds. Furthermore, results indicated that *DGAT1* had significant genetic effects on fat content in Lori-Bakhtiari breed are consistent with the findings of Xu *et al.* (2009), Mohammadi *et al.* (2013) and Noshahr and Rafat (2014). Based on the sequence analysis, two differences, including one deletion and one substitution, were found in 5'UTR region between detected genotypes (Table 2). Regulation of *DGAT1* expression in adipocytes mostly happens at the transcriptional and post-transcriptional steps (Yu *et al.* 2002), therefore mutations in 5'UTR region may influence *DGAT1* expression and consequently fat content. Therefore, identified mutations in the 5'UTR region of *DGAT1* could affect the function of DGAT1 enzyme in lipid metabolism. For instance, *DGAT1* has considerable effect on fat deposition and fatty acid composition in sheep meat and milk (Scata *et al.* 2009). In the beginning codon (ATG) has started from position 202, mutations have occurred before it was not able to change the protein sequence and only the mutation occurred in the exon-1 region of the DGAT1 gene which was able to change the amino acid sequence of translated protein derived from this gene. Therefore, mutation only occurred in position 277 of genotypic pattern G3 and led to the transformation of amino acid Arginine to Glycine (AGG to GGG). However, there was no significant difference (P>0.05) between this genotypic pattern and other genotypes. This result indicated that despite of the change in the sequence of the enzyme, performance did not change significantly. Therefore, this SNP could not be introduced as a marker for the studied traits. By the way, future investigations can be useful to detect possible effects of this SNP on the production traits of sheep.

Only CW, DP and BFT traits were analyzed in Zel breed, because this breed does not have any fat-tail for measuring other traits. In Zel breed, no significant difference (P> 0.05) was found among the observed five genotypes in carcass traits, but G3 pattern showed higher DP and lower BFT compared to others. The lack of significant differences between the most of the identified genotypic patterns may be due to the high standard errors found for some genotypic patterns, resulting from the low number of animals for these genotypes. Therefore, increasing the number of animals will probably change the results. The effects of different genotypes on common carcass traits in Zel and Lori-Bakhtiari breeds were similar, when analyzed separately or with each other. Possible contradictory results can be due to the influences of different genomic background and different genotypic frequencies.

CONCLUSION

In this study, five different PCR–SSCP genotypic patterns were detected for 360bp fragment (including 5′UTR and Exon-1) and three novel SNPs were detected for this region. There was significant correlation (P<0.05) between FTW and BFT traits and observed genotypes in Lori-

Bakhtiari breed, but there was no significant correlation ($P<0.05$) between detected genotypes of exon-1 of *DGAT1* in Zel breed and carcass traits. These results indicated that *DGAT1* is a potential candidate gene for carcass traits in MAS and breeding programs to improve FTW and BFT traits by selection in Lori-Bakhtiari breed. Although significant correlation ($P<0.05$) was found between carcass fat content and DGAT1 gene, but it can be due to the effect of other mutations in regions that were not investigated in present study (Xu *et al.* 2008) and also another QTLs close to the *DGAT1* (Coppieters *et al.* 1998). Further investigation is necessary to make a conclusive decision about using the detected polymorphisms of this gene in breeding programs for carcass traits in sheep.

ACKNOWLEDGMENT

The authors thank all the teams who worked on the experiments and provided technical assistance in the laboratory during this study.

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Table **1**. Genotypic patterns frequencies of SSCP variants of 5′ UTR and exon-1 of DGAT1 gene in Zel and Lori-Bakhtiari breeds

Genotypic patterns	No.	frequencies
Lori-Bakhtiari sheep breed(n=152)		
1	30	0.195
2	36	0.239
3	16	0.108
4	21	0.141
5	48	0.315
Zel sheep breed (n=157)		
1	30	0.189
2	81	0.515
3	13	0.084
4	15	0.094
5	18	0.115

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Table **2**. Nucleotide substitutions and types of SNPs for exon-1 of DGAT1 gene in Zel and Lori-Bakhtiari breeds.

Accession no. EU178818				Genotypic patterns or haplotypes				
Allele	Location	Nucleotide position	Codon No.	1	2	3	4	5
T	5' UTR	(101)	-	-	-	T	T	T
C	5' UTR	(129)	-	C	A	C	A	C
A	Exon-1	(277)	26	A	A	G	A	A

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Table **3**. Association of DGAT1 genotypes with carcass traits in Zel and Lori- Bakhtiari breeds

Lori- Bakhtiari sheep breed	DGAT1 genotypes (LSE±SE)				
Traits	1(30)	2(36)	3(16)	4(21)	5(48)
Carcass weight (Kg)	18.25±0.67	18.03±0.64	17.70±081	17.07±0.79	16.95±0.57
Fat-tail weight (Kg)	2.63±0.38^{a}	3.22±0.36^{ab}	3.66±0.45^{ab}	3.23±0.44^{ab}	3.81±0.32^{b}
Fat-tail percentage (%)	14.93±2.41	15.80±2.29	15.38±2.88	17.34±2.82	20.68±2.03
Dressing percentage (%)	47.46±0.89	48.66±0.85	49.28±1.07	47.29±1.04	47.80±0.75
Dressing percentage without fat-tail (%)	41.15±1.48	41.30±1.41	41.16±1.78	39.96±1.74	38.88±1.25
Back fat thickness (mm)	3.37±0.31^{a}	3.83±0.29^{ab}	3.98±0.37^{ab}	3.83±0.36^{ab}	4.27±0.26^{b}
Zel sheep breed	DGAT1 genotypes (LSE±SE)				
Traits	1(30)	2(81)	3(13)	4(15)	5(18)
Carcass weight (Kg)	11.13±0.19	11.24±0.13	11.68±0.29	11.50±0.28	11.38±0.25
Dressing percentage (%)	45.87±0.86	46.23±0.58	48.02±1.28	47.53±1.22	46.11±1.09
Back fat thickness (mm)	4.34±0.41	4.27±0.28	3.39±0.62	4.16±0.28	4.37±0.53
Zel and Lori- Bakhtiari breeds	DGAT1 genotypes (LSE±SE)				
Traits (Pooled data)	1(60)	2(117)	3(29)	4(36)	5(66)
Carcass weight (Kg)	15.86±0.21	16.07±0.17	16.58±0.28	15.87±0.27	15.85±0.21
Dressing percentage (%)	46.82±0.60	47.53±0.47	49.17±0.79	47.80±0.75	47.00±0.58
Back fat thickness (mm)	3.90±0.25	4.11±0.20	3.74±0.34	4.05±0.32	4.44±0.25

Lsmeans with a different superscript letter were significantly different ($P < 0.05$)

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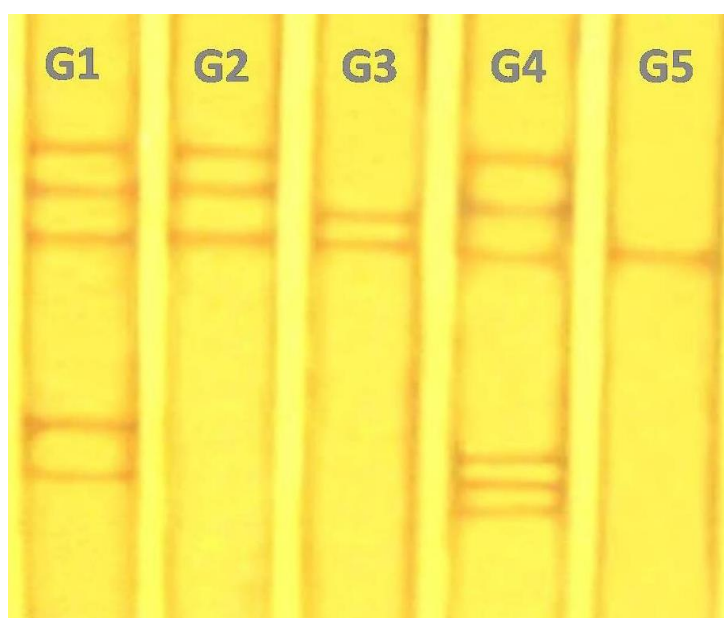

٣٩١ Figure 1: five PCR–SSCP genotypes of exon-1 and its 5'UTR fragment (360bp) of DGAT1 gene
٣٩٢ for Lori-Bakhtiari and Zel breeds.

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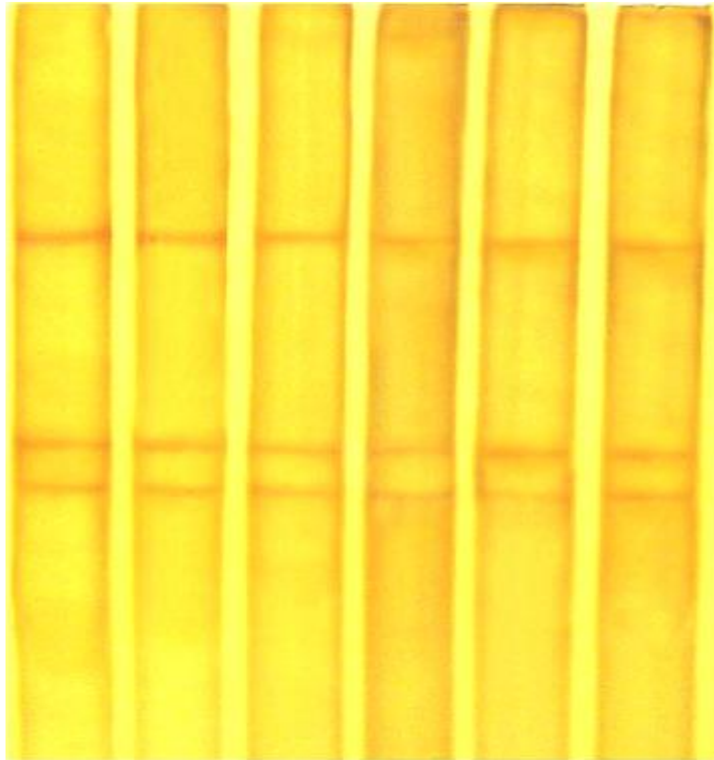

٤٠٠ Figure 2: PCR–SSCP genotype of exon-2 fragment (445-bp) of DGAT1 gene for Lori-Bakhtiari
٤٠١ and Zel breeds.

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Group	Sequence	Amino acids (1–54)
	Majority	MGDRGGAGGSRRRRTGSRPSIQGGSRPAAAEEEVRDVGAGGDAPVRDTDKDGDV
		10 20 30 40 50
Templet	EU178818-1P.seq	MGDRGGAGGSRRRRTGSRPSIQGGSRPAAAEEEVRDVGAGGDAPVRDTDKDGDV
G1	EDIT-31-1p.seq	MGDRGGAGGSRRRRTGSRPSIQGGSRPAAAEEEVRDVGAGGDAPVRDTDKDGDV
G2	EDIT56-1p.seq	MGDRGGAGGSRRRRTGSRPSIQGGSRPAAAEEEVRDVGAGGDAPVRDTDKDGDV
G3	EDIT-11-1p.seq	MGDRGGAGGSRRRRTGSRPSIQGGSGPAAAEEEVRDVGAGGDAPVRDTDKDGDV
G4	EDIT33-1p.seq	MGDRGGAGGSRRRRTGSRPSIQGGSRPAAAEEEVRDVGAGGDAPVRDTDKDGDV
G5	EDIT11-1p.seq	MGDRGGAGGSRRRRTGSRPSIQGGSRPAAAEEEVRDVGAGGDAPVRDTDKDGDV

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٤١٧ Figure 3: The amino acid sequence of exon-1 of DGAT1 gene for Lori-Bakhtiari and Zel breeds.

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