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Polymorphism of DGAT1 Gene and Its Relationship with Carcass Weight and Dressing Percentage in Moghani Sheep Breed

Research Article

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ABSTRACT

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.

INTRODUCTION

Nowadays because of very rapid advances in molecular genetics, accurate and rapid identification of genes controlling traits prepared. Using molecular markers in animal breeding, a combination of quantitative and molecular genetic methods was applied to identify genes controlling meat quality (Meuwissen *et al*. 2001).

Studies to find genes affecting carcass traits in beef cattle led to the discovery of the DGAT1 gene as a candidate gene for meat quality and quantity (Pannier *et al*. 2010; Souza*et al*. 2010). Diacylglycerol o-acyltransferase 1 (DGAT1) is a microsomal enzyme that catalyzes the final step of triglyceride synthesis (Winter *et al*. 2002). Because of its role in triacylglycerol synthesis and energy storage, DGAT1 also may be involved in intestinal fat absorption, lipoprotein assembly and the regulation of plasma triacylglycerol concentrations, fat storage in adipocytes, energy metabolism in muscle and milk production including mammalian oocytes (Cases *et al*. 1998). DGAT1 gene is widely expressed in many tissues, with the highest expression levels in adipose tissue and small intestine (Chen and Farese, 2000; Buhman *et al*. 2002). Over expression of DGAT1 in white adipose tissue of transgenic mice increases adipocyte size and adipose mass and the level of DGAT1 over-expression correlate with the degree of adiposity (Chen *et al*. 2002). A silent mutation of GCC (Ala 487) to GCT (Ala 487) was found in exon 17 at the 8539 base pair in 286 lambs of three Chinese breeds by polymerase chain reaction single nucleotide conformation polymorphism assay. The TT genotype conferred

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 150Moghani 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 extaction

Genomic DNA was extracted from 100µL 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.
µ: 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.

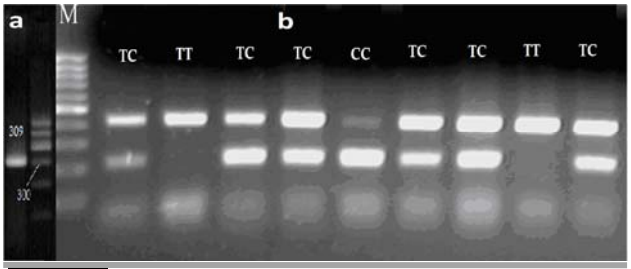

Figure 1 Agarose gel electrophoresis examination of amplification product 309 bp of DGAT1 (a); agarose gel electrophoresis digestion products with *AluI* endonuclease (b)

Polymorphisms of sheep DGAT1 gene

The genotype frequency, allelic frequency and results of Hardy Wineberg 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 the 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). CC genotype frequency was higher than that of TC and TT genotypes. The allelic frequency of C, which was the predominant allele, was 0.171. Similar results in this gene region reported in two Zell and Lori Bakhtiari sheep breeds in Iran and four Chinese indigenous breeds (Mohammadi *et al*. 2011; Xu *et al*. 2009).

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 1 Genetic diversity of DGAT1 in Moghani sheep population

Index	Number
Animals	150
Number of animals with TT genotype	105(0.698)
Number of animals with TC genotype	39(0.262)
Number of animals with CC genotype	6(0.04)
Allele frequency of T	0.829
Allele frequency of C	0.171

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

Trait	Sex	
	Male	Female
Animals	100	50
Hot carcass weight (kg)	24.19±2.01	20.63±1.89
Fat-tail weight (kg)	5.63±0.98	4.01±0.82
Hot dressing percentage (%)	50.39±3.14	48.44±2.85
Hot dressing percentage free fat-tail (%)	42.18±2.71	40.95±3.17

Genotype and carcass weights

Least square mean (LSM) of different genotypes and standard deviation of them are shown in Table 3. The effect of different genotypes on hot carcass weight was significant (P<0.05).

Most hot carcass weight observed in CC genotype. Also different between hemozygote genotypes of TT and CC were significant, but between TT and TC was not significant (P<0.05).

DGAT1 gene has been mapped to bovine chromosome 14 (BTA14) and contains a substitution of lysine-to-alanine polymorphism in exon 8 (K232A) that explains 50% of the genetic variation in milk fat percentage. Therefore, the bovine DGAT1 gene was proposed to be a causal mutation underlying a QTL with effect on milk composition (Grisart *et al*. 2002; Schennink *et al*. 2007).

Table 3 Associations of DGAT1 genotypes with studies traits in Moghani sheep

Trait	Genotype		
	TT	TC	CC
Hot carcass weight (kg)	22.75±0.89^{a}	23.61±0.52^{ab}	24.37±0.77^{b}
Hot carcass weight free fat-tail (kg)	20.15±0.29^{a}	20.41±0.41^{a}	20.74±0.28^{a}
Hot dressing percentage with fat-tail (%)	46.43±0.88^{a}	47.09±1.32^{ab}	48.74±2.19^{b}
Hot dressing percentage free fat tail (%)	40.25±0.84^{a}	41.41±1.09^{a}	42.87±1.96^{a}

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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