Research

Relationship of *TNF-α* Gene Polymorphism with fat-tail Measurements (fat-tail dimensions) and wool weights in fat-tail Makooei breed of Iranian sheep

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ABSTRACT

The assay of applicant genes, based on physiological results, is a notable tool to detect genes to be effected in marker-assisted choice methods. The current assay was planned to determine relation TNF-α gene single nucleotide polymorphisms (SNP) and with fat-tail Measurements (fat-tail dimensions) and wool weights in makooei sheep. DNA was extracted from whole blood samples collected from 100 sheep. PCR products were subjected to SSCP denaturation and polyacrylamide gel electrophoresis. In the measured makooei sheep populace, definitive calculational outcomes were determined in Fat thickness as well as wool weight in one year characteristics. Estimate of associations genotypes with fat-tail calculations also wool weights were appeared with 100 samples Individuals with the T1 and T3 genotype of TNF-α gene Individuals with the T1and T3 genotype of *TNF-α* gene had dominance Fat thickness (The thick rump) and wool1 when contrasted to those of individuals with difference genotypes respectively (P <0.05). These consequences displayed that TNF-α gene could be a genetic position, or bound to a major gene that definitively influences growth as well as the fore-mentioned economic characteristics in sheep.

Key words: *TNF-α*, Polymorphism, fat-tail dimensions, wool weights, Makooei breed

1. INTRODUCTION

Makooei is a breed of sheep arranged as fat-tailed, alike to Turkish White Karaman as well as illustrates a notable multi-purpose sheep for formation in the East and West Azerbaijan provinces of Iran. The flock was managed under a semi-migratory system. The breeding period extended from late August to late October (20-25 ewes randomly assigned randomly to everyone ram) and consequently, lambing being started in late January. Young ewes are mated to lamb for the first time at ap-

proximately 1.5 years of age. Ewes are supplemented, depending upon the ewes' requirements, for a few days after lambing. The lambs are identified at birth day with birth weights, as well as sex, birth type and pedigree information recorded. During the suckling period, lambs are fed with their mothers' milk while being allowed dry alfalfa after 3 weeks of age. Lambs are weaned at approximately 100 days of age. Animals are kept on natural pasture during spring, summer and autumn seasons. Since environ-

mental conditions are adverse during the winter, therefore the animals are kept indoors during the three winter months (1).The domestic sheep Ovisariesis an organism of worldwide notability to horticulture. Polymorphisms have been presented in lower than 5%of ovine genes to age, from a contemporary amount of 1410 described genes. Most of these polymorphisms were detected while determining methods ovine productivity (2).The TNF gene encodes a multifunctional cytokine that belongs to the tumor necrosis agent (TNF) super family. This cytokine is included in the regulation of a broad spectrum of natural mechanisms, composing lipid metabolism (3). Tumor necrosis factor alpha (TNF-α) plays an important role in development of mammary gland (4). The possible function of TNF-α may be to boost death signaling in order to kill the embryo if initial damages triggered by detrimental stimuli may culminate in structural anomalies, and stimulate protective mechanisms if the repair of these damages may prevent mal development (5).The TNF-α gene is present as a single copy gene 2,773 base pairs (bp) in length on bovine chromosome 23.The gene consists of four exons and three introns. There are 28 single nucleotide polymorphisms (SNP) of bovine TNF-α gene in the dbSNP database, but validation status for most of them is unknown (6). TNF-α is known to induce a normal mammary gland development, but at the same time it is also able to induce apoptotic cell death (7).An association of immune system genes with productive traits of livestock animals may be considered in two respects. First, there may be a positive correlation between immune answers also these traits, due to of animal breeding in the existence of pathogens that would favor correcting creative traits mostly in the genetically resistant animals. Secondly, the correlation may be negative if a trade-off between immune function and productive efforts is involved. There are only few studies analyzing the associations of polymorphisms in immune genes with productive and reproductive traits in livestock animals. For example, the association of lymphoid enhancer-binding factor-1 (LEF1) gene with the number of functional and inverted teats in pigs was described (8). The association of SNPs in the chemokine CCL2 and caspase recruitment domain CARD15 genes with milk production traits was found in Canadian Holstein cattle (9). Other authors reported the associations of SNPs in Toll-like Receptor 4 (TLR-4) gene, chemokine receptor 1 (CXCR1) gene, CD14 gene, and serum protease inhibitor (SERPINA1) gene with milk production in dairy cattle (10).Some evidences have linked tumor necrosis factor alpha (TNF alpha) to the metabolic abnormalities of obesity and adipose tissue has been shown to be a site for TNF-alpha synthesis, with a direct correlation between adipokines, adipose tissue, TNF-alpha and insulin levels (11). The tumor necrosis agent (TNF) is the prototype part of a great family of proteins with different acts, involving influence of apoptosis also regulation of lymphocyte proliferation. In sheep, there is few evidences about polymorphism of the TNF gene. In the 1990s, ovine TNF was cloned by three various classes. There is unique copy of TNF-α in the sheep genome. Past assays to discover polymorphism in this gene were useless (12). The title wool is commonly approved as the general definition of the naive fiber of domesticated sheep (Ovisaries), although it is also used as the generic name of hair from animals such as goat, camel, vicuna, alpaca, Angora rabbit and yak. Almost all Iranian sheep breeds have large fat tails Fat tail and other adipose depots; negatively affect the sale of sheep by sheep industries in some country like Iran. Fat-tail plays an important role as a source of energy for adult ewe during periods of food shortage (Falland especially winter).The aim of this study was to investigate the relationship between TNF-α conformational patterns with fat-tail Measurements and wool weights traits using SSCP method in Makooei sheep.

2. MATERIALS AND METHODS

2.1. Data collection and animal genomic screening

The Makooei breed of sheep were examined in this study, they are fat-tailed sheep with medium body size, white in color with black spots on face and feet. They are farmed in the east and west Azerbaijan provinces of Iran for meat and wool. Blood samples were collected into a 5 ml EDTA vacutainer tube and moved to the laboratory within 2 hours for DNA extraction. entire DNA extractions were made with a modified salting out procedure (13). From complete fresh blood. Quality and quantity of DNA was checked using NanoDrop Spectrophotometer (ND-1000) and the quantity and was diluted to a final concentration of 25 ng/µl. Amplification was verified by electrophoresis on 1.5% (w/v) agarose gel in 1 x TBE buffer (2 mM of EDTA, 90 mM of Tris-Borate, pH 8.3), using a 100bp ladder as a molecular weight marker for confirmation of the

length of the PCR products. Gels were stained with ethidium bromide (1 μg/mL).

2.2. Amplification of the exon 4 and 3' UTR of TNF-α gene

The DNA amplification of the TNF-α gene was achieved by PCR. Two PCR primers, TNF-α-up (5-CTGCCGGAATACCTGGACTA-3) and TNF-α-dn (5´-TCCAGTCCTTGGTGATGGTT-3´), targeting a fragment of (273) bp were employed as described (12). The PCRs were carried out in 50 μl volumes using PCR mastermix kit (Cinnagen, Iran) containing 2.5 units Taq DNA Polymerase in reaction buffer, 4 mM MgCl2, 50 μM each of dATP, dCTP, dGTP and dTTP, 0.5 μM of each primer and about 100 ng of extracted DNA as a template. The thermal outline consisted of 5 min at 94°C, approached by 35 cycles of 45 s at 94°C, 45 s at 56°C as well as 45 s at 72°C, with a last development of 10 min at 72°C. Broadening was carried out in a Mastercycler (Eppendorf, Germany).

2.3. Single strand confirmation polymorphism (SSCP)

For single-strand conformation polymorphism (SSCP) analysis, several factors were tested to optimize the methodology: Amount of PCR product (4 - 15 μL), dilution in denaturing solution (20 - 85%), denaturing solution (A: 95% of formamide, 10mM NaOH, 0.05% xylene-cyanol and 0.05% bromophenol blue; B: same as A, plus 20mM of EDTA), acrylamide concentration (6 - 14%), percentage of cross linking (1.5 to 5%), presence (10%) or absence of glycerol, voltage (100 - 350 V), running time (2-12 h) and running temperatures (4, 6, 10 and 15 °C). Each PCR reaction was diluted in denaturing solution, denatured at 95 °C for 5 min, chilled on ice and resolved on non-denaturing polyacrylamide gel.

2.4. Statistical analysis

The characteristics accounted were tail length (Rump length), Fat thickness (The thick rump), Tail width (Rump width) wool1 (A fleece weight at age), wool2 (Wool weight two years of age), wool3 (Fleece weight at three years of age), wool4 (Fleece weight at four years of age). Greasy fleece weight (GFW) which was experimented at decreasing, and clean fleece weight (CFW) which was measured product of GFW also yield, wool features were calculated by the experiment Centre for Animal Breeding as well as determination Maku, Western Azerbaijan. For the relation assays, the characteristics of caution were analyzed using the general linear model (GLM) procedure of the SAS program (14), according to the following statistical model:

$$Y_{ijklm} = \mu + G_i + S_j + e_{ijklm}$$

Where:

Y_{ijklm} = growth traits, μ = the overall mean, G_i = the fixed effect of the ith genotype for TNF-α, S_j = = the fixed effect of sex (j = 1, 2), e_{ijklm} = the random residual error.

3. RESULTS AND DISCUSSION

Polymerase chain reaction-single strand conformation polymorphism (PCR-SSCP) analysis of the exon 4 and 3' UTR of TNF-α gene revealed three EE (T1), OE (T2), RE (T3) banding patterns in "Makooei" sheep in west Azerbaijan. The frequencies of the observed genotypes were 0.4667, 0.3556, 0.1777 for EE (T1), OE (T2), RE (T3), respectively. Allele frequencies were 0.7333, 0.1778 and 0.0889 for E, O and R respectively. The Observed heterozygosity (Hobs) value for TNF-α gene was 0.4227. In the present assay, we accounted the relation between various TNF-α genotypes also with fat-tail calculations(fat-tail dimensions) as well as additionally wool weights including the Tail length, Tail width, Fat thickness, wool1, wool2, wool3, wool4 in fat-tail Makooei breed of Iranian sheep. The acts of various genotypes were measured. In the assayed Makooei sheep populace, definitive statistical consequences were determined in Fat thickness also wool weight in one year characteristics. In other words, the measurement of a relation between these SSCP patterns with wool1, wool2, wool3, wool4 detected a positive effect of the all patterns with Fat thickness also wool weight in one year. Individuals with the T1 and T3 genotype of TNF-α gene had superiority Fat thickness (The thick rump) and wool1 when contrasted to those of individuals with other genotypes respectively (P <0.05). Measurement of associations between genotypes also wool weights and fat-tail calculations were accomplished with 100 samples. Steps of significance, least squares means, as well as standard defects are described in Table 1.

Table 1. Least square means and standard errors of the wool weights and fat-tail Measurements of Makooei sheep according to the different TNF-α pattern

Tn alpha	fat-tail dimensions		
	Tail length	Tail width	Fat thickness
T1	28.61±0.418	36.78±0.51	0.483a± 0.015
T2	29.47±0.482	37.51±0.65	0.468a±0.018
T3	28.29±1.25	38.21±1.76	0.364b±0.043
P value	0.71^{ns}	1.97^{ns}	2.82^{*}

Tn alpha	wool1	wool 2	wool 3	wool 4
T1	0.450±0.078b	1.46±0.047	1.54±0.045	1.62±0.051
T2	0.534±0.014ab	1.43±0.054	1.42±0.033	1.78±0.059
T3	0.652±0.073a	1.39±0.299	1.33±0.22	1.61±0.301
P value	3.16^{*}	0.65^{ns}	0.89^{ns}	0.96^{ns}

Equal letters in column demonstrate no significant difference (P> 0.05); Different letters in column demonstrate significant difference (P< 0.05).

Wool1 (A fleece weight at age), wool2 (Wool weight two years of age), wool3 (Fleece weight at three years of age), wool4 (Fleece weight at four years of age), tail length (Rump length), Fat thickness (The thick rump), Tail width (Rump width). Until now, a few polymorphisms of TNF-α gene have been showed in small ruminants and sheep have been much less. Alvarez-Busto et al determined five dissimilar single-strand conformational polymorphism (SSCP) prototypes in a character of independent animals also three various alleles were identified as well as sequenced. These alleles varied in one deletion also one unique nucleotide polymorphism (SNP) as well as were identified TNF*01, TNF*02 also TNF*03.There was no significant difference between genotypic and allelic frequencies in these breeds (P > 0.05 in both samples) (12).There is restricted evidence about connection between gene polymorphisms with fat-tail estimates in sheep. It has been described that the association between band originals observed with tail length as well as tail down circumference was close to the significant level in fourth exon of growth hormone gene in kermanian sheep And the statistical analysis were displayed definitive association between the band patterns (exon 3 Ovine Leptin gene) observed definitive association with the tail, chest, abdomen as well as neck circumference (p<0.05) and body length (p <0.01), in the Zel breed also

in the Bakhtiari breed the models were related with character, gap tail length also middle as well as down tail Width (p <0.05). And Statistical analysis demonstrated definitive relation between band models 16–17 exon of DGAT1 gene in Lori-Bakhtiari sheep (LB) also Zel sheep (Z) breeds. At the DGAT1 locus, CC sheep demonstrated the significantly lower fat-tail weight ($P < 0.05$) also backfat thickness ($P < 0.01$). The results of this research manifest modern relations in which the C allele had a positive cause on fat-tail weight also backfat thickness in fat-tailed sheep (16). Two SNP polymorphisms of TNF alpha gene in the exon as well as promoter regions were found to have a strong association with the early first ovulation after parturition in the high-producing dairy cows (17). So far only one SNP (-824A/G) of TNF-α gene was found may affect the functionality of the gene, since it influences the transcriptional promoter activity in vitro. There are studies association between polymorphism in TNF gene with Sex of Calf with Lactation Performance in Cattle. These results suggest that the TNF-α -824A/G gene polymorphism may have an influence on the reproductive efforts of cows over the course of lactation events depending on the sex of progeny. Allocation of resources according to sex of the calf allows optimizing the energy cost of lactation. This may be a probable reason for high G allele frequency in Yakut cattle breeding in extreme environmental conditions 18)(.There are also studies claiming association between polymorphism in TNF gene and pig fatness. The most significant results were breed-specific, but the failure to replicate associations could be due to the indirect nature of the associations or in sufficient sample size to detect modest direct effects. As far as BFT (backfat thickness) and the SNP at the promoter region of TNF are concerned, the inconsistent estimates of genotypic values suggest an indirect effect of this polymorphism. Also a significant association occurred between haplotype TNF variants (g.6464C N T and g.8653A N G) and BFT (point C1) in PLW (P = 0.003).There are also studies claiming relation between polymorphism inIL6 also TNF as well as affinity to human obesity. For example, a meta-analysis of the G-308A SNP in the TNF gene manifested its relation with such an affinity (15). These authors demonstrated that a definite haplotype, included of 6 SNPs, is joined with body mass index (BMI) also waist circuit. These assays demonstrate that dual genes (IL6 also TNF) are interesting applicants for fat addition characteristics and detected in difference mammals

(19), that described that the IGFBP-3 gene Different geno-types slightly affected several wool traits of Chinese Merino sheep. The individuals of genotype AA, AB, also BB had no definitive change in post-shearing weight also clean wool measure. Staple length (SL) was diminished with the genotype of AA, AB, (BMI) also waist circumference. These assays de-note that dual genes (IL6 also TNF) are interesting applicants for fat accumulation characteristics and found in other mam-mals (19). That genotype AB (P<0.01) and BB (P<0.05); Av-erage fiber diameter (AFD) in individuals of genotype AA was definitively higher than that in individuals of genotype AB (P<0.01) also BB (P<0.05).In the existing assay three geno-types (EE, OE, RE) were determined for the exon 4 as well as 3' UTR of TNF-α gene in "Makooei " sheep in west Azerbai-jan. The evaluation of an association between these SSCP pat-terns with wool1, wool2, wool3, wool4 showed a positive ef-fect of the all patterns with Fatthickness also wool weight in one year. Individuals with the T1and T3 genotype of TNF-α gene had superiority Fat thickness (The thick rump) and wool1 when compared to those of individuals with other genotypes respectively (P <0.05).This is the doubly report of allelic change in the ovine TNF-α gene. Several assays reported only the polymorphism, not the relation analyses.

4. CONCLUSION

To date, this was the initially assay that attempted to detect allele difference in the ovine TNF-α gene as well as its rela-tion with fat-tail calculations in Iranian sheep breeds. It is ad-visable to serve additional markers, besides the affected mark-ers, in this breed as well as some other native breeds such as Ghezel and Moghani in order to uncover their genetic rela-tionship. Lastly, the aim of this examine was to determine the association between TNF-α conformational patterns with fat-tail calculations (fat-tail dimensions) also fat-tail Measure-ments traits operating SSCP procedure in Makooei sheep.

AUTHORS CONTRIBUTION

This work was carried out in collaboration between all authors.

CONFLICT OF INTEREST

Authors have declared that no conflict interests exist.

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