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HAPLOTYPE ASSOCIATION OF OVINE LEPTIN GENE ON BREEDING VALUE OF BODY MEASUREMENTS IN MAKOOEI SHEEP BREED

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Abstract: The research was undertaken to find association of genetic variation in the exon 3 of the leptin gene and breeding value of body weight traits in Makooei sheep breed using single strand conformation polymorphism (SSCP). The PCR product was obtained to encompass exon 3 of leptin gene corresponding to ovine leptin gene. The PCR fragments were subjected to electrophoresis to reveal the SSCP patterns. Among the total of 130 sheep, five SSCP patterns (haplotypes) were identified for amplified fragment. The frequencies of SSCP patterns of polymorphic fragment were 0.09, 0.17, 0.37, 0.14 and 0.23.The relation between the different haplotypes and body measurements including body length (BL), heart girth (HG), height at withers (HW), height at back (HB), rump length (RL) and scrotal circumference (SC) were ascertained in all of the analyzed animals. According to our results, there is significant association between the different haplotypes of this fragment with additive estimated breeding value for the HG and RL traits. These results confirmed the potential usefulness of leptin gene in marker-assisted selection programs for sheep breeding in Makooei sheep breed.

Key words: Body measurements, leptin, polymorphism, breeding value, Makooei sheep

Introduction

Leptin is a 16 kDa polypeptide hormone and secreted mostly by adipose tissue. Leptin acts as a satiety factor by inhibiting neuropeptide Y in the hypothalamus, provides a satiety signal with subsequent increase of energy expenditure and metabolic processes intensification. Hence, the leptin gene has gained much attention as a key regulator of biological processes such as appetite and metabolism that are related to very important productive traits, such as feed intake, fat content and meat quality in farm animal (*Houseknecht et al., 1998;*

Geary et al., 2003; Van der Lende et al., 2005). However, some evidences indicate that Leptin also functions as acytokine, mediating thymichomestasis and both the innate and adaptive immune systems (*La Cava and Matarese, 2004*). Leptin is also known to play a role in different parts of the body, such as the male and female reproductive organs, the mammary gland, bone mineral density, the gut, the kidney, and the lung (*Baratta, 2002*).In turn, polymorphisms in this gene have been proposed as predictors of relative differences among individuals for those traits (*Nkrumah et al., 2004; Schenkel et al., 2005*).

Several polymorphisms have been found in leptin gene that some of them may affect either activity or expression of leptin. While polymorphism in the leptin gene has been thoroughly investigated in human, pig and bovine, but limited information is available on genetic variation in the ovine leptin gene. Polymorphism in the human leptin gene is reported to be associated with low leptin levels (*Hager et al., 1998*), overweight or obesity and non-insulin-dependent diabetes mellitus (*Van der Lende et al., 2005*). *Kuryl et al. (2003)* studied the relationship of leptin genotype and the quality of fatness of various pig breeds and indicated significant differences in dressing percentage, content of meat in ham, and mass of loin in different leptin genotypes. Genetic variations in the bovine leptin gene have been extremely described and the associations were reported with feed intake (*Lagonigro et al., 2003; Zwierchowskiet al., 2001*), milk production (*Liefers et al., 2002; Buchanan et al., 2003*), somatic cell count (*Kulig et al., 2009*) and carcass and meat quality traits (*Schenkel et al., 2005; Zwierchowski et al., 2001*). Genes encoding leptin was mapped to ovine chromosome 4 (*Perucatti et al., 2006*) and contains three exons. *Zhou et al. (2009)* reported four single nucleotide polymorphisms (SNPs) in exon 3 of the ovine leptin gene that three of these SNPs were non-synonymous and resulted in amino acid changes at codon positions 105, 120 and 144.Therefore, the objective of this study was to estimate the association of different genotypes of ovine exon3 with breeding value of body measurements in Makooei sheep breed.

Materials and Methods

Blood samples were obtained from 130 unrelated Makooei sheep stored in EDTA-coated tubes. Genomic DNA was extracted from 0.3 ml blood using the genomic DNA purification kit (Cat. No 0512, Fermentas, EU) according to manufacturer's instructions.

Two PCR primers, LEP-up (5-AGGAAGCACCTCTACGCTC-3) and LEP-dn (5´-CTTCAAGGCTTCAGCACC-3´), targeting a fragment of 471 bp were employed as described (Zhou et al., 2009). The PCR were carried out in 50 µl volumes using PCR master mix kit (Cinnagen, Iran) containing 2.5 units Taq DNA polymerase in reaction buffer, 4 mM $MgCl_2$, 50 µM each of dNTP, 0.5 µM of each primer and 100 ng of extracted DNA as a template. The thermal profile consisted

of 2 min at 94°C, followed by 35 cycles of 30 s at 94°C, 30 s at 59°C and 30 s at 72°C, with a final extension of 5 min at 72°C.

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. The gels were subsequently fixed in 10% ethanol, stained with 0.15% AgNO3 and revealed with 1.5% Na2CO3.

Statistical Analysis

The analyzed traits were yearling weight (*YW*) and six body dimensions measured at yearling age: body length (*BL*), heart girth (*HG*), height at withers (*HW*), height at back (*HB*), rump length (*RL*) and scrotal circumference (*SC*). The following fixed effects model was employed to estimate breeding value (*BV*) with *DFREMEL* software.

Model: $y_{ijklmn} = \mu + YR_i + SX_j + BT_k + AD_l + AN_m + e_{ijklmn}$

Where:

y_{ijklmn}= dependent variable evaluated on the i^{th} level of the random factor; μ = overall mean for each trait; year (YR_i, i=1, 2, 3,…, 21), the j^{th} level of the fixed factor; sex (SX_j , j=1 and 2), the k^{th} level of the fixed factor; number of offspring in each birth (BT_k , k= 1,2 and 3), the i^{th} level of the fixed factor; mother age (AD_l , l=1,2,…,7), m^{th} level of the random additive genetic effect (AN_m, m= number of animal for each trait) and e_{ijklmn} is the random error effect. The estimated parameters according to model were: phenotypic variance (σ_p^2), direct additive genetic variance (σ_a^2), residual variance (σ_e^2) and direct heritability (h_a^2) was equal to $\frac{\sigma_a^2}{\sigma_p^2}$.

For the association studies, the traits of interest were analyzed using the general linear model (*GLM*) procedure of the *SAS 9.1 (2002)*, program according to the following statistical model:

$y_{ijk} = \mu + G_i + S_j + e_{ijk}$

y_{ijk}= breeding value of body measurements, μ = the overall mean, G_i= the fixed effect of the i^{th} genotype for leptin gene (i= 1, 2… 5), S_j= the fixed effect of sex (j = 1, 2), e_{ijk} is the random error. Effect of sex was removed for the *SC* trait (scrotal circumference).

Results

The PCR-SSCP analysis of amplified fragment from ovine leptin gene revealed five distinct patterns. Our study confirmed polymorphic nature of ovine leptin exon3 as revealed by *Zhou et al., (2009)*.The frequencies of the observed genotypes were 0.09, 0.17, 0.37, 0.14 and 0.23 for L1, L2, L3, L4 and L5, respectively (Figure 1).

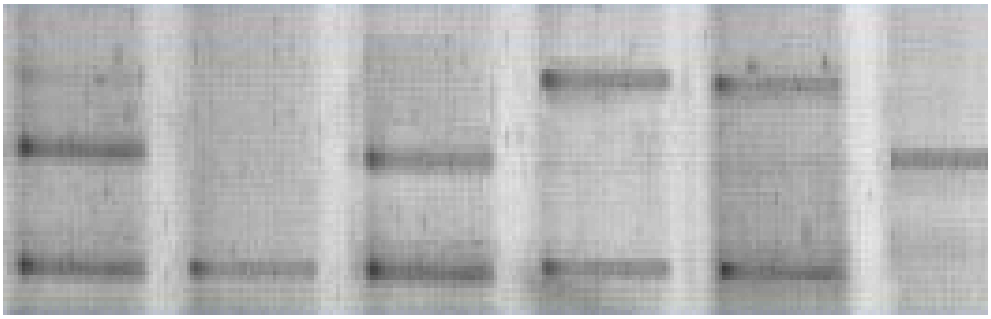

Figure 1. Different SSCP patterns of amplified fragment from ovine exon 3 leptin gene.

Variance and covariance components were estimated based on animal model with the restricted maximum likelihood (*REM*L) approach using a derivate-free (*DF*) algorithm (*Meyer, 1989*). Estimates of variance components and genetic parameters for yearling weight, body length, heart girth, height at withers height at back and scrotal circumference from the single-trait analyses are indicated in Table 1. The direct heritability estimates varied between 0.10 and 0.27 for development traits.

The relationship between these identified haplotypes with body length, heart girth, height at withers, and height at back, scrotal circumference and yearling weight were evaluated. Results indicated that different patterns in this fragment had a significant association with additive estimated breeding value for the HG ($P<0.01$) and RL ($P< 0.05$) traits (Table 2).

Table 1. Parameter estimated of analyzed traits

Traits	σ_a^2	σ^2_e	σ^2_p	h^2
BL	1.235	9.537	10.772	0.105
HG	8.071	29.643	37.714	0.214
HW	2.981	9.563	12.544	0.238
HB	3.452	9.323	12.775	0.270
SC	1.612	4.559	6.171	0.261
RL	2.137	7.429	9.566	0.223
YW	3.228	11.63	14.95	0.221

Body length (BL), heart girth (HG), height at withers (HW), height at back (HB), scrotal circumference (SC), rump length (RL) and yearling weight (YW).

Animal expressing L4genotype had significantly lower breeding value for *GH* (-0.43) relative to the others. Also, Makooei sheep expressing L3 haplotype showed higher breeding value for RL. There was no significant difference between detected genotypes and HB, HW, BL, SC and YW traits, but the combined genotypes of amplified fragment revealed to tend significant differences (P=0.073) between observed genotypes and the breeding value for YW trait (Table 3).

Table 2. Least square means of the EBVs of Makooei sheep according to the different leptin pattern

Leptin	Body measurements estimated breeding values						
	HB	HW	HG	BL	SC	RL	YW
L1	0.3050	0.0001	0.2505^{ab}	0.8827	0.2102	-0.0146^{a}	0.783
L2	0.0560	-0.0004	0.0192^{ab}	0.3734	0.1262	0.2439^{ab}	0.927
L3	0.5947	0.0007	1.1172^{a}	0.3965	0.6247	0.6111^{b}	0.195
L4	0.0087	-0.0004	-0.4352^{b}	0.7740	0.2983	0.1556^{a}	0.965
L5	0.2786	-0.0010	0.1960^{ab}	0.8502	0.2462	0.3617^{ab}	0.544
SEM	0.174	0.0021	0.249	0.183	0.126	0.119	0.292
Prob.	0.317	0.972	0.0068	0.482	0.264	0.0281	0.073

[abc] Means in a column with different superscripts differ statistically (P< 0.05),
Height at back (HB), height at withers (HW), heart girth (HG), Body length (BL), scrotal circumference (SC), rump length (RL) and yearling weight (YW).

Discussion

Considerable interest exists in determining the relationship between SNP for different genes with productive and health traits of economic importance for the livestock industry. Genetic differences in the leptin gene were first reported in mice and humans (*Ohshiro, 2000; Halaas et al., 1995*). In recent years studies have been performed on the association between leptin gene polymorphisms and production traits in farm animal (*Yazdani et al., 2010; Hajihosseinlo et al., 2012*). Most association studies involving leptin gene have focused on carcass composition and beef quality traits (*Zwierchowski et al., 2001*). Our finding for ovine genetic

variation of exon3 are similar to *Zhou et al. (2009)*, who reported five SSCP patterns for this fragment and indicated the high potential of ovine exon3 as an effective DNA marker for marker assisted selection (*MAS*) in sheep.

To date, this was the first study that attempted to detect allele variation in the ovine exon3 of leptin gene and its association with development traits in Iranian sheep breeds. In the analyzed population significant statistical results were found in additive estimated breeding value for the *GH* and *RL* traits. The L3 genotype was associated with low breeding value for HG, and L3 haplotype was associated with high breeding value for *RL*. The observed association between the ovine exon 3 genotypes and sheep body measurement traits in the present study has not been previously reported. It is, however, unclear why and how exon 3 haplotypes would affect sheep body measurement traits, but sequence analysis of these haplotypes revealed four SNPs in ovine exon 3 that three of these SNPs were non-synonymous and resulted in amino acid changes at codon positions 105, 120 and 144. Based on the results for the overall effects of the ovine exon 3 haplotypes in the present study, we can assume that leptin locus or genes linked to it affect body measurement traits in sheep. Few studies have been undertaken to evaluate the association of leptin gene polymorphism with performance traits in sheep. For example, significant associations were found with same haplotypes of this study and additive estimated breeding value for weaning weight and six month weight in Makooei sheep (*Hajihosseinlo et al., 2012*). In addition, in the Suffolk breed, the leptin genotypes were associated with reduced muscle thickness and loin eye area and with increased shear forces, pH and cross-sectional area of the slow-twitch oxidative fibers of the L muscle (*Boucher et al., 2006*). *Tahmoorespur et al. (2009)* analyzed the association of leptin polymorphism with average daily gain (*ADG*) in Baluchi sheep breed. They reported a significant association between leptin polymorphism and *ADG* at birth to 3 months of age. The association of different leptin genotypes with the growth traits in Kermani sheep indicates that the growth traits are significantly affected by the genotypes (*Shojaei et al., 2010*).

However, research on associations between leptin gene polymorphism and performance traits in sheep is rather scanty, but several studies confirmed association between bovine leptin gene polymorphism and performance traits in cattle. For example, significant associations were reported between the polymorphism in exon 2 of bovine leptin gene with grade fat and average fat, with the *T* allele associated with higher fat, but with no significant association with carcass marbling score (*Buchanan et al., 2002*). *Schenkel et al. (2005)* also reported that the two SNPs in the exon 2 of the bovine leptin gene were associated with fat, lean yield and grade fat. Three single nucleotide polymorphisms (the R4C polymorphism in exon 2, the Sau3AI polymorphism in intron 2 and the A59V polymorphism in exon 3) were genotyped in Jeresy cows and significant associations were found between the R4C and Sau3AI polymorphisms with somatic cell count (*Kulig et al., 2009*). An association of the bovine leptin

polymorphism increased chest girth in Anatolian Black cattle breed (*Kaygisiz et al., 2011*).

Conclusion

In conclusion, these results indicate that different genotypes of ovine exon 3 contributed to variation in the traits analyzed and may lead to the use of these haplotypes as genetic marker in marker-assisted selection and sheep breeding programs. However, to prove this, additional investigations are required with bigger sheep populations of different breeds. In addition, there are no data on regulation of ovine leptin expression. Therefore, this raises the questions of what influence these haplotypes might have on the regulation of ovine leptin expression. Hence, gene expression analysis also needs to investigate the functional role of these identified haplotypes in the exon 3 of ovine leptin gene.

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Haplotipska veza leptin gena ovce i priplodne vrednosti telesnih mera ovaca rase makooei

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Rezime

Istraživanje je imalo za cilj da se pronađu veze između genetskih varijacija u egzonu 3 leptin gena i priplodne vrednosti osobine telesne težine ovaca rase Makooei, koristeći jedinstveni lanac usaglašenosti polimorfizma (Single strand conformation polymorphism - SSCP). PCR proizvod koji je dobijen obuhvata egzon 3 leptin gena koji odgovara ovčijem leptin genu. PCR fragmenti su podvrgnuti elektroforezi kako bi se otkrili SSCP obrasci. Od ukupno 130 ovaca, pet SSCP obrazaca (haplotipova) je identifikovano za amplifikovane fragmente. Učestalosti SSCP obrazaca polimorfnog fragmenta su bile 0,09, 0,17, 0,37, 0,14 i 0,23. Odnos između različitih haplotipova i telesnih mera, uključujući dužinu tela (BL), obim (HG), visinu grebena (HV), visinu krsta (HB), dužinu krsta (RL) i obim testisa (SB) je utvrđen kod svih analiziranih životinja. Prema našim rezultatima,

postoji značajna povezanost između različitih haplotipova ovog fragmenta sa aditivima procenjene priplodne vrednosti za HG i RL osobine. Ovi rezultati potvrdili su potencijalnu korisnost leptin gena u programima marker asistirane selekcije u ovčarstvu za odgoj Makooei rase ovaca.

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