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Effects of genetic polymorphismat the growth hormone gene on growth traits in Makooei sheep

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ABSTRACT

To explore the relevance between sheep Growth hormone(GH)gene polymorphism and growth traits ,so as to improve the overall growth rate and economic benefits. Growth hormone (GH) a single polypeptide hormone produced in the anterior pituitary gland is a promising candidate gene marker for improving milk and meat production in farm animals. Genomic DNA was extracted from whole blood samples collected from 100 sheep. A 200bp GH exon 4segment was amplified by standard PCR, using the locus specific primers. PCR products were subjected to SSCP denaturation and polyacrylamide gel electrophoresis. SSCP bands were visualized with silver staining. Five SSCP patterns, representing five different genotypes, were identified. The evaluation of an association effect between these SSCP patterns with birth weight (BW), weaning weight (WW), six month weight (SW), nine month weight (9W), W12(yearling weight)suggest a positive effect of pattern G2 with weaning weight (WW), six month weight (SW), nine month weight (9W)and additive estimated breeding value for the weaning weight (WW), six month weight (SW). This result may be related to the GH large role in post weaning weight.

Key words: Growth Traits, Growth hormone, Polymorphism, Breeding Value, Makooei Sheep

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INTRODUCTION

The native breeds, because of their natural selection against harsh environment and adaptation to regional conditions are important to resource-poor farmers and pastoralists. 'Makuie' sheep breed is a native breed of Iran and also could found in Turkey (called as AkKaraman). The total number of its population is estimated about 2.7 millions [1]. It has been adapted to cold and highland environments [2]. They are fat-tailed sheep with medium body size, white color with black rings around eyes, nose and feet [3].

In animal industry, growth traits of animal are always of primary concern during breeding for its determinant economic value [4]. Main application and potential for use of markers to enhance genetic improvement in livestock is through within-breed selection [5].
The genes of the growth hormone axis affect a wide variety of physiological parameters such as appetite control, growth, body composition, ageing and reproduction [6](Byatt et al., 1993) as well as immune responsiveness [7].

The structure of the ovine growth hormone gene is similar to that found for other growth hormone genes and, as expected, is very homologous to the bovine gene [8].

Growth hormone (GH) a single polypeptide hormone produced in the anterior pituitary gland is a promising candidate gene marker for improving milk and meat production in goats and other farm animals [9]. Growth hormone gene is encoded by 1800 base pairs (bp), consisting of five exons, separated by four intervening sequences [8].The complete oGH DNA sequence and its predicted amino acid sequence have been established by Orianet al.[10] from an ovine pituitary genomic library. In the ovine, two alleles of the GH gene have been described. The Gh1 allele contains a single gene copy (GH1), whereas in the Gh2 allele the gene is duplicated (copies GH2-N and GH2-Z) with the two copies being located 3.5 kb apart [11]. Sequence differences between the GH2-N and GH2-Z copies have been demonstrated and polymorphisms have been found in oGH coding and non-coding regions [12].SSCP is a simple and reliable technique, based on the assumption that changes in the nucleotide sequence of a PCR product affect its single strand conformation. Molecules differing by as little as a single base substitution should have different conformers under non-denaturing conditions and migrate differently. Therefore, those differences can be detected as a shift in the electrophoretic mobility [13].To simplify and make SSCP analysis more efficient, alternative staining methods with ethidium bromide [14]and with silver [15]were described.

Genetic polymorphisms of GH have been reported in various domestic livestock, mainly in cattle, and several studies have related association effects between bovine GH (bGH) polymorphisms and milkyield traits. The aim of this study is the evaluation of genetic variability of growth hormone gene using a nonradioactive SSCP protocol and its association with growth traits in Makooei Sheep.

MATERIALS AND METHODS

Animals and DNA samples

The Makui 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. A live female sheep weight about 47 kg. Birth weight of the lambs is 3.7–3.5 (male/females) kg. Maximum milk yield per lactation is 100 kg. (6%fat) and maximum of yearly fleece weight reaches to 3 kg per sheep [16].Blood samples were collected into a 5 ml EDTA vacutainer tube and transferred to the laboratory within 2 hours for DNA extraction. Total DNA extractions were made with a modified salting out method Miller[17]from whole fresh blood.Quality and quantity of DNA was checked using Nano Drop 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).

DNA amplification by PCR

Polymerase chain reaction (PCR) was carried out on about 25 ng genomic DNA in a 25 µl reaction volume. PCR reactions were performed in an UNO II thermocycler (Biometra) using Ready-To-Go PCR Beads (Amersham Biosciences) according to the following conditions: 25ng of genomic DNA; 10 pmol of each primer (Table 1); 1.5U *Taq*DNA polymerase; 10mM Tris–HCl, pH 9; 50mM KCl; 1.5mM MgCl2; 200_M of each dNTP and stabilisers including BSA, for a final volume of 25 _l. The amplification included a initial denaturation at 95 ◦C for 5 min, 30 cycles of denaturation at 95 ◦C for 30 s; annealing at 57–62 ◦C (Table 1) for 30 s and extension at 72 ◦C for 30 s followed by a final extension at 72 ◦C for 5 min. Each amplification product was analysed by electrophoresis on a 2% agarose gel (5 V/cm), using ethidium bromide staining. Two pairs of oligonucleotide primers were designed and a standard PCR protocol was used to amplify three fragments.

SSCP analysis

For SSCP analysis, 5 or 10 µl of each amplification product was added to, respectively, 15 or 10 µl of Stock Solution (95% formamide, 10mM NaOH, 0.05% xylenecyanol and 0.05% bromophenol blue). The samples were heat-denatured at 95 ◦C for 5 min, chilled at 0 ◦C, and the total volume was loaded onto an 8–12% polyacrylamide/TBE gel. The electrophoresis was performed in 0.5 × TBE buffer (Tris 100 mM, boric acid 9 mM, EDTA 1mM) at room temperature (18°C) and constant 200 V for 3 h. Polyacrylamide gels were stained with silver according to the protocol described by Herring et al.[18].

Statistical analyses

The allelic and genotypic frequencies, expected means, observed and expected Nei'sheterozygosities (HE=1-Σ P_i^2 , where P_i is the frequency of allele i) and Hardy-Weinberg equilibrium were calculated using PopGene32 program (ver 1.31, Canada).

The following fixed effects model was employed for calculate of BV (breeding value) with DFRIMEL software [19].

Yijklm= μ+ YRi + SXj + BTk + ADl + ANm + Eijklm

Where: Yijklm= dependent variable evaluated on the ith level of the random factor; year (YRi , i=1, 2, 3,…, 21), the jth level of the fixed factor; sex (SXj , j=1 and 2), the kth level of the fixed factor; number of offspring in each birth (BTK , K= 1,2 and 3), the lth level of the fixed factor; mother age (ADl , l=1,2,…,7), mth level of the random additive genetic effect (ANm, m= number of animal for each trait), μ = overall mean for each trait and Eijklm is the random error effect.

For the association studies, the traits of interest were analyzed using the general linear model (GLM) procedure of the SAS program [20] according to the following statistical model:

Yijklm= μ + Gi+Sj+lsk

Yijklm= production trait, μ = the overall mean, Gi= the fixed effect of the ith genotype for growth hormone, Sj= = the fixed effect of sex (j = 1, 2), lsk= the fixed effect of litter size (k=1, 2), eijklm=the random residual error.

RESULTS

Effect of GH SSCP Variants on the Growth Traits

Research shows that a new gene identification technology allows investigating the genotype of the animal and determining the genes encoding the productivity and quality traits and using them in the selection process as genetic markers. Use of genetic markers in selection can greatly accelerate the breeding process, improve the quality of agricultural production, reduce its production costs and make production more competitive in foreign markets [21].

Our data show that PCR-SSCP is a simple and efficient technique for the detection of single base substitutions and can be employed for evaluating genetic variability in large populations. The identified gene variants, however, need large population studies in order to establish a breeding program me for marker assisted selection/improvement in productivity of the sheep resources of Iran.

The growth traits are complex quantitative traits involving multiple genes, loci and interactions. Some studies described only the polymorphism, not the association analyses [22].

In the present study, we assessed the association between different GH genotypes and growth traits including the birth weight (BW), weaning weight (WW), six month weight (SW), nine month weight (9W), W12 (yearling weight) of' Makooei' sheep. The effects of different genotypes were estimated. The quantified results showed that GH is an important growth-regulating gene in sheep.

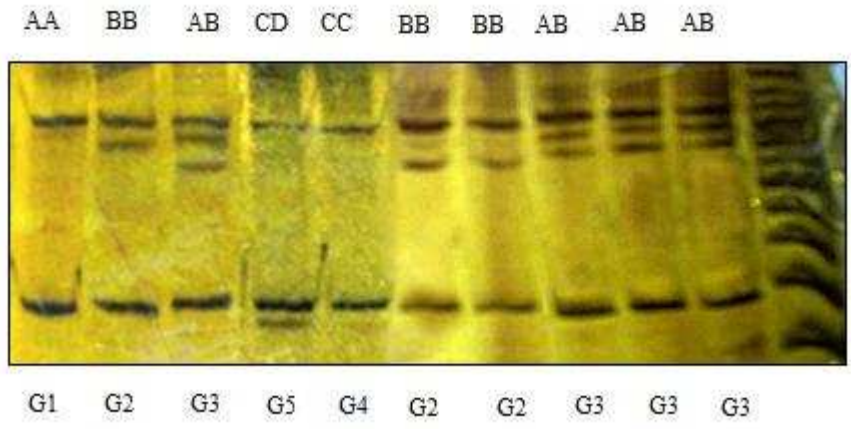

Fig1. The PCR-single-strand conformation Polymorphism (SSCP)-based DNA test for GH(a part of intron3, exon4 and a part of intron4)polymorphism

Until now, a few polymorphisms of GH gene have been detected in small ruminants and sheep have been much less studied. Gupta et al[23] have used the PCR-SSCP analysis to investigate GH polymorphism in Indian Black Banegal goats. Bastos et al. [24] detected two and five SSCP patterns for exon 4 and 5, respectively, in Portuguese

indigenous sheep breed ‘‘Churra da Terra Quente,’’ not the association analyses. Marques et al., [25] showed associations between SSCP polymorphisms at the GH gene and milk yield in ‘‘Serra da Estrela’’ sheep.

In this study, variation in the exon 4 coding sequence of the ovine GH gene was investigated using PCR–SSCP analysis. In the present study three alleles (A, B, C,and D) and five genotypes (AA, AB, BB, CD, and CC) were identified for exon IV of GH gene in "Makooei " sheep in west Azerbaijan, Iran**(Fig1).**

The most frequent allele and genotype in the "Makooei " sheep were allele A and genotype AB with frequencies of 63% and 64.6%, respectively**(Table1).**

Table1. Observed allele and genotypic frequencies for GH locus in 'Makoei' sheep breed

A	B	C	D	AA	AB	BB	CC	CD
0.63	0.327	0.028	0.015	31.3%	64.6%	1%	1%	2.1%

In the tested Makooei sheep population, significant statistical results were found in weaning weight (WW), six month weight (SW), nine month weight (9W), additive and maternal estimated breeding value for the weaning weight(WW), six month weight (SW).(**Table2**)

Table 2: Least square means (± S.E) of the growth traits of Makooei sheep according to the SNP genotype in GH

	Weight estimated breeding values (means ± SE, kg)				
GH	BW	W3	W6	W9	W12
G1	-0.009±0.042	1.38±0.30^{ab}	0.72±0.52^{b}	0.50±0.27	0.39±0.35
G2	0.017±0.043	2.22±0.31^{a}	2.33±0.54^{a}	1.15±0.27	0.68±0.36
G3	0.050±0.041	1.80±0.29^{ab}	1.88±0.51^{ab}	0.85±0.26	0.59±0.34
G4	0.035±0.053	0.99±0.38^{b}	1.93±0.67^{ab}	0.50±0.34	-0.14±0.44
G5	0.018±0.045	1.78±0.32^{ab}	1.28±0.56^{ab}	0.66±0.28	0.47±0.37
P value	0.54^{ns}	2.87*	2.41*	1.25^{ns}	1.85^{ns}

	Growth traits				
GH	BW	W3	W6	W9	W12
G1	3.70±0.114	18.61±0.618^{ab}	26.85±0.922 ^{b}	28.08±0.872^{ab}	36.43±1.108
G2	3.88±0.125	21.50±0.678^{a}	30.83±1.012^{a}	31.24±0.957^{a}	39.17±1.216
G3	3.77±0.105	19.18±0.566^{ab}	28.47±0.846^{ab}	29.08±0.799^{ab}	37.17±1.016
G4	3.86±0.151	18.63±0.817^{ab}	28.19±1.22^{ab}	27.93±1.153^{b}	36.11±1.465
G5	3.89±0.127	18.02±0.688^{b}	27.38±1.02^{ab}	28.19±0.^{971ab}	35.24±1.234
P value	0.65	5.25**	6.93**	2.75*	1.81^{ns}

CONCLUSION

Results of this study partly are in accordance with the results of Tahmoorespur et al., [26] that effect of the GH gene was significant with six month weight (SW). To date, this was the (secondly) study that attempted to detect allele variation in the ovine GH gene and its association with growth traits in Iranian sheep breeds.

Our findings suggest that polymorphisms in GH gene might be one of the important genetic factors that influence growth traits and maybe explain partial source of genetic variation. Finally our results indicate that further study is needed to explain the DNA polymorphism and to obtain more definite conclusions about effects on growth traits. It is advisable to apply more markers, in addition to the applied markers, in this breed and some other native breeds such as Ghezel and Moghani in order to uncover their genetic relationship.

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