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Genetic effect of growth hormone gene on yearling weight and wool traits in Zel sheep (Brief Report)

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Background

The growth hormone (GH) gene is a candidate for growth in sheep, since plays an important role in growth regulation and development (Boyd & Bauman 1989). Most genetic studies on the growth of sheep have concentrated on birth weight, weaning weight and yearling weight (Bathaei & Leroy 1998). Pereira *et al.* (2005) found significant effect for bovine growth hormone (bGH) genotype on yearling weight. Tambasco *et al.* (2003) observed a positive association between genotype LV and daily body weight gain from weaning to yearling in *Bos Taurus* × *Bos indicus* crosses.

Wool traits like greasy fleece weight, clean yield, fiber diameter and its coefficient of variation are very important selection goals in sheep breeding programs, however new traits such as staple strength and staple length are of increasing importance in the wool industry (Fogarty 2006). Initial observations using daily injections of crude pituitary extracts showed that wool growth decreased by 17 % during the second treatment period (Ferguson 1954). Allain *et al.* (1998) found segregation for coefficient of variation of fiber diameter and staple length on chromosomes 3 and 4 in a composite sheepline (INRA401). Zel sheep is raised in North of Iran. This sheep is a native non-fat tailed breed with small-sized (Saadat-Noori & Siah-Mansoor 1990). The aim of this study was to investigate the relationship between GH genotypes and wool traits and yearling weight using single strand conformation polymorphism (SSCP) method in Zel sheep.

Procedures

Animals

Zel sheep were randomly selected from flock of Zel sheep (Shirang Research Station, located in Golestan, Iran). Data used in this study were wool traits and yearling weight (YW) that gathered in one year. Wool characteristics including staple strength (SS), staple length (SL) and sulfur were measured in laboratory of Research Center of Animal Sciences, Karaj, Iran.

DNA extraction

The blood samples were collected randomly from 100 Zel sheep (92 ewes and 8 rams). DNA was extracted from 100 µl of blood, using a commercial kit (Diatom DNA Prep100, ISO Gene, Moscow) following the manufacturer's protocol.

PCR conditions

Polymerase chain reaction (PCR) was carried out, using the TPersonal thermocycler (Biometra, Göttingen, Germany) and the PCR Master Kit (Cinna Gen Inc., Tehran, Iran). Each reaction mixture consisted of 12.5 µl of the master mix, 1 µl of the DNA solution (50 to 100 ng/µl), 1 µl of each primer (5 pmol/µl) and some deionised water making up a final volume of 25 µl.

For amplifying a 365 bp fragment from the exon V of the ovine growth hormone gene primers described by Barracosa (1996) and following PCR protocol were used:

GH-F (5′-GAA ACC TCC TTC CTC GCC C-3′)

GH-R (5′-CCA GGG TCT AGG AAG GCA CA-3′)

An initial denaturation step at 95 °C for 5 min followed by 35 cycles of denaturation at 95 °C for 1 min, annealing at 62 °C for 50 s and extension at 72 °C for 90 s, and a final extension of 72 °C for 10 min.

Single-strand conformation polymorphism analysis

For SSCP analysis, 5 µL of each amplification product was added to 15 µL of denaturising solution. The samples were heat-denatured at 95 °C for 5 min, immediately chilled on ice and loaded onto 8 % polyacrylamide gel (39:1). The gels were run at 240-280 V for 6-8 h, at 4 °C. The electrophoresis was carried out in a vertical unit in 1 × TBE buffer. The gels were stained with silver nitrate to observe the conformational patterns.

Statistical analysis

Allele and genotype frequencies were calculated with Pop-Gene software v1.31 (Yeh *et al.* 1997). One hundred samples were used for statistical analysis of yearling weight and wool traits.Yearling weight and wool traits analysis were performed using general linear model (GLM) procedure of the SAS program v8 (SAS Institute Inc., Cary, NC, USA) and least squares means of the genotypes were compared by the Tukey-Kramer test. The following model was used:

$$Y_{ijklm} = \mu + S_i + D_j + A_k + G_l + e_{ijklm} \quad (1)$$

where Y_{ijklm} is the dependent variable considered (wool parameters consisting staple strength, staple length, sulfur and yearling weight); μ is the overall mean; S_i is the fixed effect of the i-th of sex (i=1 and 2); D_j is the fixed effect of the j-th type of birth (j=1, single and 2, twin); A_k is the fixed effect of the k-th age (K=5, 6 and 7); G_l is the fixed effect of the l-th genotype (l=1, 2 and 3) and e_{ijklm} is the random residual error.

Results

Genotype frequencies

The PCR-SSCP for GH gene was carried out on 8 % polyacrylamide gel. We obtained three different conformational patterns. The frequencies were 19 % for pattern 1 (G1), 51 % for pattern 2 (G2) and 30 % for pattern 3 (G3).

Effect of GH genotypes on wool traits and yearling weight

Evaluation of relationships between genotypes and wool traits and yearling weight were done with 100 samples. According to statistical analysis no significant effect ($P>0.05$) was found between GH genotypes and yearling weight in present population (Table 1). Although, yearling weight was influenced significantly by type of birth effect ($P<0.05$).

Table 1
Least square means and standard error for yearling weight in kg

Pattern	Yearling weight, kg
G1	23.696±0.968
G2	25.066±0.732
G3	25.946±0.805

$P<0.05$

Also, the analysis of wool traits showed that G1 conformational pattern of growth hormone gene have a significant effect on staple strength ($P<0.05$). However, growth hormone gene patterns revealed no effects on staple length and sulfur in current study (Table 2).

Table 2
Comparison of the least squares means±standard errors of wool traits

Pattern	Staple length, mm	Staple strength, N/ktex*	Sulfur, %
G1	34.25±5.86	12.36^{a}±1.03	3.44±0.29
G2	30.43±4.04	9.84^{b}±0.71	3.49±0.19
G3	30.03±5.13	8.91^{b}±0.9	3.62±0.25

^{ab}Least squares means are significantly different ($P<0.05$), *Newtons/kilo tex

Finally, the aim of this study was to investigate the relationship between GH conformational patterns with wool traits and yearling weight using SSCP method in Zel sheep. The results of the present study indicated significant effect between G1 pattern of GH gene and staple strength ($P<0.05$). Although, no effects between GH genotypes and staple length, sulfur and yearling weight were observed, but further studies with larger numbers of animals are necessary to confirm the effects between this gene and wool traits and yearling weight. Furthermore, Results showed that PCR-SSCP is appropriate tool for detecting polymorphism and evaluating genetic variability.

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