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EVALUATION OF K-CASEIN AND GROWTH HORMONE GENE POLYMORPHISM IN NATIVE DALAGH SHEEP

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ABSTRACT

In this research for studying genetic polymorphism of κ-casein (*CSN3*) and growth hormone (*GH*) genes as candidate genes for marker-assisted selection in different domestic livestock species, blood samples (120 samples) were collected from Dalagh sheep randomly. DNA was extracted from blood samples and a 416 bp fragment from exon 4 of the *CSN3* gene and a 365 bp region from exon 5 of the *GH* gene were amplificated by polymerase chain reaction. PCR products were analyzed using single strand conformation polymorphism (SSCP) method on a polyacrilamide gel. Results indicate that there are two (K1=0.92 and K2=0.08) and three (G1=0.84, G2=0.13 and G3=0.03) conformational patterns for *CSN3* and *GH* genes, respectively.

Key words: CSN3; GH; Dalagh sheep; PCR-SSCP

INTRODUCTION

Casein genes are organized as a tightly linked cluster on ovine chromosome 6 in a 250 kb DNA segment (Thread gill and Womack, 1990; Lévéziel *et al*. 1991; Bevilacqua *et al.* 2006). The performance of technological processes of cheese production depends on the structure of Kappa-casein gene (Zaton *et al*., 1999). Among small ruminants, goats have been thoroughly investigated for milk protein genes and noticeable genetic variation has been identified, whereas the knowledge of milk protein genetic variants is more fragmentary in ovine species (Amigo *et al*. 2000; Moioli *et al*. 2007). Within ovine *CSN3* at protein level no variation could be identified (Moioli *et al.* 1998; Chessa *et al*. 2003). This is in contrast to the high degree polymorphisms described at *CSN3* in goat (Jann *et al*. 2004; Prinzenberg *et al*. 2005) and cattle (Prinzenberg *et al.*, 2008).

Growth hormone (*GH*) influences animal processes such as growth (Breier, 1999), lactation (Baldi, 1999), reproduction (Scaramuzzi *et al*., 1999) and metabolism (Bauman, 1999). In the ovine growth hormone (*OGH*) gene, restriction fragment length polymorphisms (*RFLP*) using restriction endonucleases *TaqI* and *PvuII* (Parsons *et al*. 1992; Gootwine *et al*. 1993, 1996) and *EcoRI* (Barracosa, 1996; Gootwine *et al*. 1998) and *PCR-SSCP* polymorphisms (Bastos *et al*. 2001; Marques *et al*. 2001; Santos *et al.* 2004) have been reported. In ovine, two alleles of the *GH* gene have been described. *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 (Valinsky *et al.* 1990). Sequence differences between the *GH2-N* and *GH2-Z* copies have been demonstrated and polymorphisms have been found in o*GH*coding and non-coding regions (Ofir and Gootwine, 1997).

Determination of the genetic polymorphism of κ-casein (*CSN3*) and growth hormone (*GH*) genes in Iranian indigenous Dalagh sheep were the aims of present study. This intends to be the first step for a deeper study of Dalagh breed in order to establish a breeding programme based on marker-assisted selection.

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MATERIAL AND METHODS

Dalagh sheep

Dalagh is a kind of fat-tail sheep and also known as Semi fat-tailed Turkmen or Atabai. This breed is found mainly in the northeast region of the Turkmen plain, located in Golestan Province. The sheep is resistant to humid environment and parasites (Saadatnoori and Siahmansoor, 1990).

Blood samples and DNA extraction

The blood samples were collected randomly from 120 Dalagh sheep by jugular vein puncture, using vacuum tubes treated with 0.25% *EDTA*. *DNA* was extracted using modified salting out method (Miller et al., 1988), then dissolved in TE buffer and kept at −20°C. Quality of *DNA* was evaluated by a spectrophotometer and visual methods.

DNA amplification

Polymerase Chain Reaction (PCR) was carried out, using Personal Cycler™ thermocycler (Biometra, Germany) and PCR Master Kit (Cinnaclon Inc., Iran). Master Mix consisted of 0.04 U/µl of Taq DNA polymerase, 10X PCR buffer, 3mM MgCl2 and 0.04 mMdNTPs (each). 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 deionized water making up a final volume of 25 µl.

Amplification for a 416 bp fragment from the exon IV of the ovine κ-casein gene was carried out using primers suggested by Barracosa (1996):
κ-CSN- F (5′-GAG AAAGAT GAAAGATTC TTC G-3′)
κ-CSN- R (5′-GCTTCT GGATTA TCTACAGTG-3′)

The amplification program consisted of: an initial denaturation at 95°C for 5 min followed by 32 cycles of denaturation at 95°C for 30 sec, annealing at 58°C for 1 min, extension at 72°C for 1 min and a final extension at 72°C for 8 min.

PCR for amplifying a 365bp fragment from the exon V of the ovine growth hormone gene was performed using primers described by Barracosa (1996):
GH-F (5′-GAAACCTCCTTCCTCGCC C-3′)
GH-R (5′-CCAGGGTCTAGGAAGGCACA-3′)

The amplification reaction was carried out in the following conditions: 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 sec and extension at 72°C for 90 sec, and a final extension of 72°C for 10 min.

Correctness of PCR was assessed by electrophoresis on 1.3% (w/v) agarose gel.

PCR-SSCP

CSN3 and *GH* variants were identified by *PCR-SSCP* method. For *SSCP* analysis, 5µL of PCR product was added to15 µL of denaturizing solution (95% formamide, 10mM *NaOH*, 0.05% xylene cyanol and 0.05% bromophenol blue). The samples were heat-denatured at 95°C for 5min, immediately chilled on ice and loaded onto 8% and 12% polyacrylamide gel (39:1) for *GH* and *CSN3* genes, respectively. The gels were run at (240-300 V) for (6–14 h), at 4°C. The electrophoresis was carried out in a vertical unit in 1x *TBE* buffer. The gels were stained using silver nitrate method for observing the conformational patterns.

RESULTS AND DISCUSSION

Allele and genotype frequencies were calculated with Pop-Gene software (V 1.31) (Yeh *et al*., 1997). The *PCR-SSCP* for *CSN3* and *GH* genes were carried out on a polyacrylamide gel and two (*K1* and *K2*) and three (*G1, G2* and *G3)* different conformational patterns were observed for *CSN3* and *GH*, respectively (Fig. 1, 2).The distributions of banding patterns and their frequency for *CSN3* and *GH* are presented in Table 1.

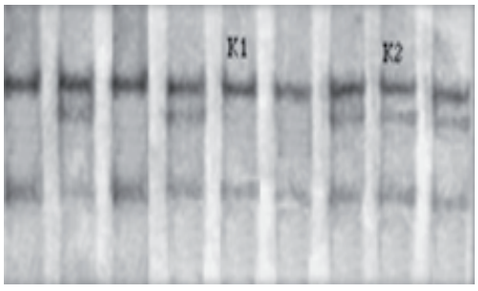

Fig 1: SSCP analysis of the 416 bp fragment of κ-Cn gene on 12% polyacrylamide gel

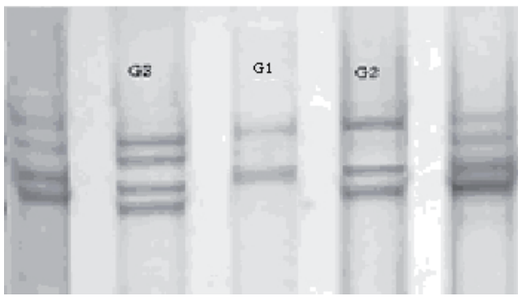

Fig. 2: SSCP analysis of the365 bp fragment of the GH gene on 8% polyacrylamide gel

Table 1: Distribution of banding pattern frequency of CSN3 and GH gene

Gene	Allele or Pattern	Frequency
KCN	K1	0.92
	K2	0.08
GH	G1	0.84
	G2	0.13
	G3	0.03

CSN3

Two conformational patterns with different frequencies (K1=0.92 and K2=0.08) were identified. These results are in agreement with previous studies by Ceriotti *et al*., (2004) who reported that the *PCR-SSCP* analysis of exon 4 showed 2 different patterns in 3 Italian sheep breeds. Barracosa (1996) also found two conformational patterns in Serra da Estrela ewes. In contrast, in Serra da Estrela breed (Bastos *et al*., 2001), Muzaffamagari breed (Mandal *et al*., 2008) and German sheep breeds (Giambra *et al.*, 2010) no polymorphic patterns were observed for *CSN3* gene. Also, Feligini *et al.* (2005) detected two alleles in three sheep breeds (Pag, T=0.12, C=0.88; Sarda, T=0.27, C=0.73 and Pramenka, T= 0.45, C= 0.55). Corral *et al*., (2010) showed five alleles by microsatellite analysis of CSN3 gene, in Merino sheep breed. Staiger *et al*., (2010) analyzed a *RsaI* polymorphism and reported gene frequencies of *CSN3* as C=0.51 and T=0.49.

GH

Three (G1=0.84, G2=0.13 and G3=0.03) different conformational patterns for the *GH* gene were observed. The frequency of pattern *G1* (0.84) was higher than other patterns. Current results are in agreement with previous studies by Tahmorespoor *et al*., (2011) who detected three conformational patterns using the *SSCP* analysis of exon 5 of the *GH* gene in Baluochi sheep. Shiri *et al.*, (2006) also observed three conformational patterns for exon 4 of this gene in Kordian sheep. However, Bastos *et al*., (2001) detected two conformational patterns using the *SSCP* analysis of exon 4 of the *GH* gene. The frequencies were 72.5% for pattern 1 and 22.5% for pattern 2. They also observed five different conformational patterns in exon 5 for the *GH* gene. The frequencies were 47.5% for pattern 1, 5% for pattern 2, 22.5% for pattern 3, 12.5% for pattern 4 and 5% for pattern 5. Marques *et al*., (2001) analyzed five ovine *GH* exons by *PCR-SSCP* in 200 Portuguese Serra da Estrela ewes and revealed that all exons except exon 1 are polymorphic. Ofir and Yossefi (1996) found that *GH2* allele of the ovine growth hormone locus contains two *GH* gene copies, *GH2-N* and *GH2-Z*. They described a *GH2* allele in which the two gene copies are polymorphic for *PvuII* sites at the second exon and intron. Barracosa (1996) detected a *RFLP-EcoRI* polymorphism in GH gene in Serra da Estrela breed. Gootwine *et al*., (1993) reported three *PvuII-RFLPs* related to the *oGH* copy number.

The inconsistency of present results camper to other studies may be ascribed to breed differences, sampling amount and/or environmental factors.

CONCLUSIONS

The goal of this study was to determine genetic polymorphism of κ-casein (*CSN3*) and growth hormone (*GH*) genes in Dalagh sheep. These results revealed that polymorphism was detected in all the studied loci and showed that *PCR-SSCP* is an appropriate tool for evaluating genetic polymorphism. This study also, opens interesting prospects for future selection programs; especially marker assisted selection.

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