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Analysis of Growth Hormone Gene in Alpine and Saanen Goats Using PCR-SSCP Method

Short Communication

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ABSTRACT

In this study the genetic polymorphism of growth hormone (GH) gene as a candidate gene in livestock was investigated. Blood samples were randomly collected from 34 Alpine and 42 Saanen goats. DNA was extracted from blood samples and a 365 bp region of exon 5 of the GH gene was amplified by polymerase chain reaction (PCR). PCR products were analyzed using single strand conformation polymorphism (SSCP) method on polyacrylamide gel. The results indicated that there are four (G1, G2, G3 and G4) conformational patterns with frequencies 0.38, 0.21, 0.23 and 0.18 for Alpine goat and 0.48, 0.21, 0.17 and 0.14 for Saanen goat, respectively. These results revealed that GH gene was polymorph in this study and showed that PCR-SSCP is an appropriate tool for detecting polymorphism and evaluating genetic variability.

KEY WORDS caprine GH gene, genetic polymorphism, goat.

INTRODUCTION

Growth hormone (GH) is an anabolic hormone synthesized and secreted by the somatotroph cells of the anterior lobe of the pituitary in a circadian and pulsatile manner (Ayuk and Sheppard, 2006). GH affects a wide variety of physiological processes such as lactation (Baldi, 1999), reproduction (Scaramuzzi *et al*. 1999), growth (Breier, 1999) and metabolism (Bauman, 1999). Lagziel *et al*. (1996) found 14 different haplotypes for the entire bovine GH gene using the SSCP technique and have reported favorable milk protein percentages with a specific haplotype. In goats, a study on GH gene polymorphism was performed by Malveiro *et al*. (2001) who reported several SSCP patterns in the Portuguese Algarvia goat. Genetic polymorphism of GH gene has also been reported in several studies (Ofir and Yossefi, 1996; Barracosa, 1996; Gootwine *et al*. 1998; Bastos *et al*. 2001; Marques *et al*. 2001; Ahani Azari *et al*. 2011) in different sheep breeds. Alpine is a breed of domestic goat known for its good milking ability. This breed originated from the French Alps. Mature weight is around 57 kg. Alpine goats can range in color from white or gray to brown and black. These are hardy, adaptable animals that thrive in any climate while maintaining good health and excellent production (American Dairy Goat Association, 2012). Saanens derived their name from the Saanen valley in the south of Canton Berne, Switzerland. In 1893 several thousand heads were taken out of the valley and spread throughout Europe. Saanen is a white or cream colored breed. Saanen is the most spread goat breed of the world. Mature weight is around 68 kg or more, with bucks weighing over 91 kg. The Saanen breed also produces the most milk on average, and tends to have lower butterfat content, about 2.5 to 3.0%. A Saanen goat produces an average of 3.8 liters of milk a day. This breed has a calm and mild temperament. They typically produce one or two

offspring. This breed is known as the "queen of the dairy goats" (American Dairy Goat Association, 2012). The aim of the present study was to describe sequence variability in the caprine GH gene in Alpine and Saanen goats using single strand conformation polymorphism (SSCP) as a powerful method for identifying sequence variation in amplified DNA as a first step for studying the relationship between this gene and economically important traits.

MATERIALS AND METHODS

Animals

34 Alpine (4 male and 30 female) and 42 Saanen (2 male and 40 female) were randomly selected from 46 Alpine and 68 Saanen goats that kept in a private farm, located in Golestan province, Iran. All animals reared at the same environmental and nutritional conditions.

Blood samples and DNA extraction

The blood samples were collected and DNA was extracted using modified salting out method (Miller *et al*. 1988), then dissolved in TE buffer and kept at -20 ˚C. The quality and quantity of extracted DNA were measured spectrophotometrically and by electrophoresis on 1% agarose gel.

DNA amplification by PCR

Polymerase chain reaction (PCR) was carried out, using the personal cycler™ thermocycler (Biometra, Germany) and the PCR master kit (Cinnagen Inc., 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 deionized water making up a final volume of 25 µL. For amplifying a 365 bp fragment from the exon 5 of the growth hormone gene primers described by Barracosa (1996) were used:

GH-F (5'-GAAACCTCCTTCCTCGCCC-3')
GH-R (5'-CCAGGGTCTAGGAAGGCACA-3')

The thermal cycling conditions consisted of 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, extension at 72 ˚C for 90 sec and a final extension of 72 ˚C for 10 min. The specificity of the PCR was assessed by electrophoresis of each sample (4 µL) on 1.3% (w/v) agarose gel.

PCR-SSCP

GH gene variants were identified by PCR-SSCP method. For SSCP analysis, 5 µL of each amplification product was added to 15 µL of denaturizing solution (95% formamide, 10 m*M* NaOH, 0.05% xylene cyanol and 0.05% bromophenol blue). The samples were heat denatured at 95 ˚C for 5 min, immediately chilled on ice and loaded onto 8% polyacrylamide gel (39:1 acrylamide:Bisacrylamide). 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 1 x TBE buffer. The gels were stained with silver nitrate to observe the conformational patterns. Allele and genotype frequencies were calculated using Pop Gene software (V 1.31).

RESULTS AND DISCUSSION

GH gene variants were identified by PCR-SSCP method and four (G1, G2, G3 and G4) different conformational patterns of this gene were observed (Figure 1).

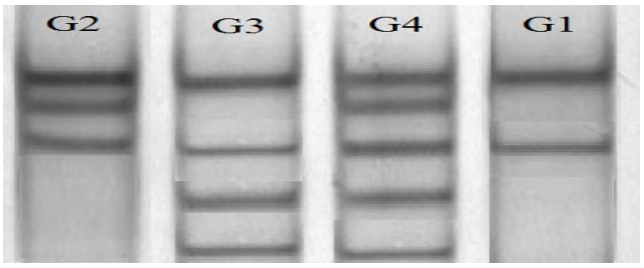

Figure 1 SSCP analysis of the 365 bp fragment of GH gene on 8% polyacrylamide gel after silver staining

The frequencies of banding patterns of GH gene are presented in Table 1. In the present study, four conformational patterns consisting G1, G2, G3 and G4 were determined for GH gene. The frequency of G1 pattern was higher than the other patterns in both goats.

Table 1 Frequencies of banding patterns of GH gene in Alpine and Saanen goats

Breed	Alpine	Saanen
Banding patterns	Frequency	Frequency
G1	0.38	0.48
G2	0.21	0.21
G3	0.23	0.17
G4	0.18	0.14

Marques *et al*. (2003) studied exons (1-5) of the GH gene in Serrana goats and observed two conformation patterns in exons 1 and 2, six in exon 3, ten in exon 4 and five in exon 5. Malveiro *et al*. (2001) also identified two conformational patterns in both exon 1 and 2, four patterns in exon 3, six patterns in exon 4 and five patterns in exon 5 of the GH gene in Algarvia goats. Furthermore, Mousavizadeh *et al*. (2009) detected nine conformational patterns for exon 4 of this gene in Iranian Talli goats. In sheep, Tahmorespoor *et al*. (2011) and Ahani Azari *et al*. (2011) also detected three conformational patterns using the SSCP method in exon 5 of this gene in Baluochi and Dalagh breeds. In addition, genetic polymorphisms of GH gene have also been reported

in several studies (Gupta *et al*. 2007; Ling Jiang *et al*. 2005; Zhang *et al*. 2011) in goats and (Shiri *et al*. 2006; Bastos *et al*. 2001; Marques *et al*. 2001) in sheep breeds.

CONCLUSION

Our results revealed that growth hormone gene was polymorphic in the two goats studied. Current study is a first step to study the effects of this gene on economical important traits, such as: milk production, daily weight gian, meat quality and so on by other researchers. Further studies among different goat populations are necessary to establish the distribution of these alleles.

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