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Polymorphism of the Growth Hormone Gene and Its Effect on Production and Reproduction Traits in Goat

Research Article

F. Gholamhoseinzadeh Gooki[1], M.R. Mohammadabadi[1*] and M. Asadi Fozi[1]

[1] Department of Animal Science, Faculty of Agriculture, Shahid Bahonar University of Kerman, Kerman, Iran

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*Correspondence E-mail: mrm@uk.ac.ir

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ABSTRACT

Growth hormone (GH) has an effect on a broad variety of physiological parameters such as lactation, reproduction, growth and metabolism. Thus, the aim of this study was to detect GH gene polymorphism and its association with breeding values of production and reproduction traits in Raini Cashmere goat. Breeding values were estimated using records on 26731 Raini Cashmere goats. To study GH gene polymorphism, 300 animals were selected based on their estimated breeding values (EBVs) for these traits. Then the animal's genotype was determined using PCR-RFLP. The genotype frequencies for AA, AB and BB were 0.15, 0.85 and 0, respectively. The number of observed alleles, number of effective alleles, expected heterozygosity, observed heterozygosity, mean of heterozygosity, expected homozygosity, observed homozygosity, Nei's index, Shanon's index and Fixation index (Fis) were 2, 1.96, 0.49, 0.85, 0.49, 0.51, 0.15, 0.49, 0.69 and -0.74, respectively. Results showed that mean estimated breeding values for birth type, fleece weight and birth weight traits in different genotypes varies, of course these differences were not statistically significant (P>0.05). However, for fleece weight and birth type traits AB genotype had higher EBV. Due to the relatively high diversity of growth hormone gene in Raini Cashmere goat and its association with important economic traits, using growth hormone gene in breeding programs of this breed can lead to acceptable genetic progress and applying AA genotype for birth weight trait and AB genotype for fleece weight and birth type traits can be used as an indirect marker for selection of superior animals.

KEY WORDS birth and fleece weight, birth type, growth hormone, PCR-RFLP, Raini Cashmere goat.

INTRODUCTION

Goat farming is operated globally, with goat products having a favorable perspective. The number of goats has increased globally, even in countries with high and intermediate incomes, despite major changes in agriculture due to industrial combination, globalization, and technological improvements in developed countries (Shamsalddini *et al.* 2016). Goat is mainly used for meat production. It is also used in dairy production, but to less extent. Most of the goats worldwide are found in Asia and Africa. In developing countries, 96% of the milk and meat producing goat populations are found and 4% are found in developed countries (FAO, 2008). There are 30 million heads of cashmere goats around the world and 4.5-5 million heads of them are in Iran that is 20% of all in the world (Baghizadeh *et al.* 2009). Goat production is one of the key elements contributing to the economy of farmers living in the arid and semi-arid regions including most areas of Iran. Raini goat is one of the most important Iranian native goats that spread in the southeast of Iran where these animals are kept for both meat and cashmere production (Moghadaszadeh *et al.* 2015). One of the most important purposes of the genetic improvement of this breed is enhancing the meat produc-

tion via programmed and accurate selection. On the other hand, determination of gene polymorphism is important in farm animal breeding (Ruzina *et al*. 2010; Mohammadabadi *et al*. 2010b; Soufy *et al*. 2009) in order to define genotypes of animals and their associations with productivity and reproductive traits. On the basis of studies on a restriction fragment length polymorphism (RFLP), Valinsky *et al*. (1990) reported that there are two alleles at the GH gene locus in sheep and goats. In sheep, in one allele (Gh1), the GH gene is represented by a single copy (GH1 gene), while in the other (GH2) the GH gene is duplicated (GH2-N (5*) and GH2-Z (3*) genes). Restriction maps of the sheep GH1 and GH2 loci indicated that the GH1, GH2-N, and GH2-Z genes are all very similar. Growth hormone (GH) has an effect on a broad variety of physiological parameters such as lactation, reproduction, growth and metabolism (Mousavizadeh *et al*. 2009).

This gene has 2544 bp length and is composed of 4 introns and 5 exons (Lan *et al*. 2007), and its polymorphisms have been reported in cattle (Mohammadabadi *et al*. 2010a; Schlee *et al*. 1994), sheep (Valinsky *et al*. 1990) and goat (Mousavizadeh *et al*. 2009). Yamano *et al*. (1988) and Yato *et al*. (1988) have described the sequence of goat pituitary growth hormone cDNA and Kioka *et al*. (1989) have introduced the GH gene sequence.

The researchers have reported the association among genetic polymorphism of GH and milk production (Falaki *et al*. 1997; Malveiro *et al*. 2001; Marques *et al*. 2003), litter size and weight (Lan *et al*. 2007; Zhang *et al*. 2011) and superovulation (Zhang *et al*. 1992).

Malveiro *et al*. (2001) have analyzed exons 1-5 of the goat growth hormone (gGH) gene by the PCR-SSCP method in Algarvia goats and have identified conformational patterns. Their results showed that patterns F/F of exon 4 and A/A of exon 5 are positively associated with milk production ($P<0.05$).

Marques *et al*. (2003) have also studied exons 1-5 of gGH gene and have found an association between patterns of exons 2 and 4 with milk yield in two ecotypes of Serrana goats. It has been suggested by Malveiro *et al*. (2001) and Marques *et al*. (2003) that the exon 4 is more polymorphic than other exons of gGH gene.

Although many studies have been performed on Raini goat (Askari *et al*. 2009; Askari *et al*. 2010; Askari *et al*. 2008; Hassani *et al*. 2010; Mohammadabadi, 2012; Tohidi Nezhad *et al*. 2015), but yet so far, no study concerning the polymorphisms of GH gene in Raini cashmere goat and its association with breeding values of triats has been published. Hence, the aim of this study was to describe for the first time association of the GH gene polymorphism with breeding values of production and reproduction triats of Raini Cashmere goat.

MATERIALS AND METHODS

In this study, the data of 26731 Raini Cashmere goat, recorded during 1991-2011 correspond to birth type, birth weight and fleece weight was obtained from the breeding station of Raini goat in Baft City (middle of Kerman Province, Iran). The data structure is given in the Table 1.

Obtained data considered firstly and out of range (3 standard errors bigger and smaller than mean) deleted. Excel, Linux and Pedigree softwares were used to prepare fix effects data with appropriate format and pedigree file data. Then breeding values of birth weight, birth type and fleece weight were estimated using ASReml software (Glimur *et al*. 2009) and below univariate animal model:

$$y= Xb + Z_1u + Z_2m + Z_3m_{pe} + Z_4pe + e \quad [1]$$

Where:
y: observation vector.
b: fixed effects vector.
u: animal random effects vector.
m: maternal genetics effects vector.
m_{pe}: environmental permanent maternal effects vector.
pe: environmental permanent animal effects vector.
e: error random effects vector.
X, Z_1, Z_2, Z_3 and Z_4: coefficients matrix for fixed effects, animal genetic effects, maternal genetic effects, environmental permanent maternal effects and environmental permanent animal effects, respectively.

As regards for birth type and fleece weight traits were used records from different years (repeated records), for genetic analysis of these traits repeatability model was used. Hence, in this model was used environmental permanent animal effects, but for genetic analysis of birth weight this effects was deleted from model. In used genetic analysis model, importance of any additional fixed effects including maternal genetic effects and environmental permanent maternal effects was applied log likelihood ratio test.

Based on this test, important effects were included in the model and non significant effects were deleted from genetic analysis model. In genetic analysis model of birth type, herd effect, kidding year, kidding season and kidding pregnancy of mother were considered as fixed effects. In genetic analysis model of fleece weight, herd effect, sex, dam (mother) age, birth type and year and month of shearing were also considered as fixed effects. Used fixed effects for genetic analysis model of birth weight were herd effect, sex, birth type, birth year and month and age of dam (mother). After estimating breeding values of animals based on birth weight, birth type and fleece weight, 300 animals were selected from 3 different groups.

Table 1 Description of data structure

Trait	NAP	NA	NR	NS	ND	NFs	Minimum	Maximum	SD
Birth weight	26731	13020	13020	336	6317	13	0.5	3.8	0.39
Birth type	26731	5498	10986	307	2017	13	1	2	0.33
Fleece weight	26731	7651	19948	380	3296	10	0.35	0.97	0.13

NAP: number of animals in the used pedigree; NA: number of animals; NR: number of records; NS: number of sire; ND: number of dam and NFs: number of flocks. SD: standard error.

100 animals with high breeding value (1 standard error bigger than mean), 100 animals with medium breeding value (between 1 standard error bigger and smaller than mean) and 100 animals with low breeding value (1 standard error smaller than mean). Blood samples were collected from the jugular vein of 300 animals into vacutainers with ethylenediaminetetraacetic acid (EDTA) as anticoagulant. Samples were kept at -20 ˚C until use. DNA was extracted from the whole blood using an optimized and modified salting-out method (Miller *et al*. 1988).

A 422 bp fragment encoding exon 2 and 3 within the goat *GH* gene was amplified using PCR primers 5'-CTCTGCCTGCCCTGGACT-3' and 5'-GGAGAAGCAGAAGGCAACC-3' (Hua *et al*. 2009). The PCR amplification was performed in a 25 µL reaction volume, containing negative controls, using CinnaGen PCR Master Kit according to the instructions by the manufacturer (CinnaGen Co., Iran). Initial denaturation for 5 min at 94°C was followed by 35 cycles of 30 s at 95 ˚C, 30 s at 64 ˚C, 45 s at 72 ˚C and a 7 min final extension step at 72 ˚C.

Amplification products were electrophoresed on 2% agarose gel at constant voltage and 1X TBE for approximately 2 h. The gels were visualized by staining with ethidium bromide and photographed under ultraviolet light and then all PCR products were digested with 10 U of *Hae*III enzyme (Fermentas) at 37 ˚C overnight, and the resulting products were separated by the 3.5% agarose gel and visualized by ethidium bromide staining. Measurement of diversity including gene diversity (H), observed number of alleles (Ne), Shannon's information index etc., were estimated by POPGEN 3.2 software (Yeh *et al*. 1999). Association analyses of the GH gene polymorphism with breeding values of production and reproduction triats were performed in 300 Raini Cashmere goats using ASReml software (Glimur *et al*. 2009).

RESULTS AND DISCUSSION

The extracted DNA had a good quality (Figure 1). The tested DNA of the Raini Cashmere goats in the Province of Kerman used in the present study was amplified using the specific primers and yielded PCR products at the expected size, 422 bp (Figure 2). Amplification of the exon 2 and 3 produced 422 bp fragments; when these fragments were digested with the restriction enzyme, the AA genotype produced two bands: 366 and 56 bp (one restriction site in the B allele), the BB genotype produced one band: 422 bp (no restriction sites in the B allele), and the AB produced three bands: 422, 366 and 56 bp (heterozygote genotype). The different alleles resulted from digestion of the PCR products with the *Hae*III restriction enzyme after running on the agarose gel electrophoresis are presented in Figure 3.

The genotypic and allelic frequencies of exon 2 and 3 within the goat GH gene in Raini Cashmere goats in Kerman have been shown in Table 2.

Mousavizadeh *et al*. (2009) genotyped 90 Talli goat breeds and observed nine conformational patterns in exon 4 of the gGH gene, with frequencies of 27.7% for the homozygous pattern (AA) and 72.2% for all of other heterozygous patterns (A/B, A/C, A/B/C, A/B/D/E, A/B/C/F, A/C/F, A/B/E, A/B/F). Their results showed that exon 4 of the GH gene in Talli goats is highly polymorphic. In other study Singh *et al*. (2015) studied Sirohi and Barbari goat breeds and the genotypic frequencies of AB and BB were 0.82 and 0.18 in Sirohi and 0.90 and 0.10 in Barbari goats, respectively.

The respective allelic frequencies of A and B were 0.41 and 0.59 in Sirohi and 0.45 and 0.55 in Barbari. The frequency of AB genotype obtained in the present study is in consonance with the findings of Singh *et al*. (2015) who reported genotypic frequency of AB as 0.82 and 0.90 in Sirohi and Barbari goat breeds respectively. However, they reported the presence of genotypes BB and AB; Genotype AA was missing in the sample of goats studied by them. This inconsistency may be due to breed difference and may also be the consequence of sampling fluctuations of populations under study.

In this study the Hardy Weinberg equilibrium was estimated with chi-square test and likelihood ratio test. The value of chi-square was 163.18, greater than the critical value. The likelihood ratio test was estimated as 210.35, greater than the critical value.

The population under this study was not found to be in a Hardy-Weinberg equilibrium, as for years it has been under selection for production and reproduction traits. In investigation of Singh *et al*. (2015) all two studied goat populations were not in Hardy-Weinberg equilibrium that are in agreement of our study.

The values of the population genetics parameters in Raini Cashmere goat population for GH gene were as follow: Numbers of observed alleles; 2, number of effective alleles; 1.96, expected heterozygosity; 0.49, observed heterozygos-

ity; 0.85, mean of heterozygosity 0.49, expected homozygosity; 0.51, observed homozygosity; 0.15, Nei's index; 0.49 and Shanon's index; 0.69. Fixation index (Fis) as a measure of heterozygote deficiency for A and B alleles was -0.74.

In this research association between GH gene polymorphism and breeding values of birth type, birth weight and fleece weight triats using univariate animal model and firstly importance of fixed effects were studied in genetic analysis model. On this basis, herd and birth year had significant effect on birth type, birth weight and fleece weight triats (P<0.001).

Effect of dam (mother) age on birth type and birth weight was significant (P<0.01) that can be caused because of increasing the size of the animal, thereby improving the conditions of the uterus in old age (Chungyan *et al*. 2008). Also sex showed a significant effect on birth weight and fleece weight (P<0.001). So that males had higher birth weight and fleece weight than females that can be caused by differences in physiologic and genetic reactions, such as hormones in males and females. Results of other researches is similar with our results. For example, Boujenane and Hazzab, (2008) showed that birth weight in Draa goat breed for males was 19% higher than females.

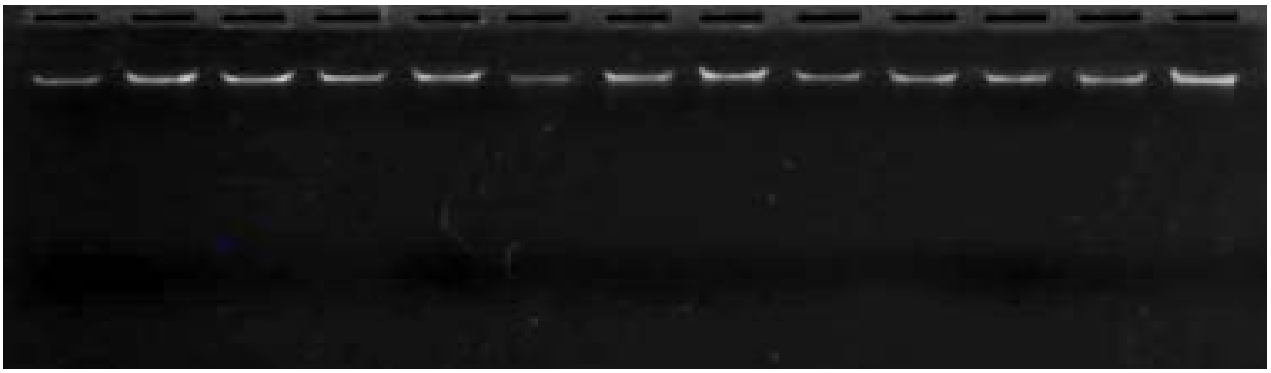

Figure 1 Some samples of the extracted DNA from the studied animals on the 1% agarose gel

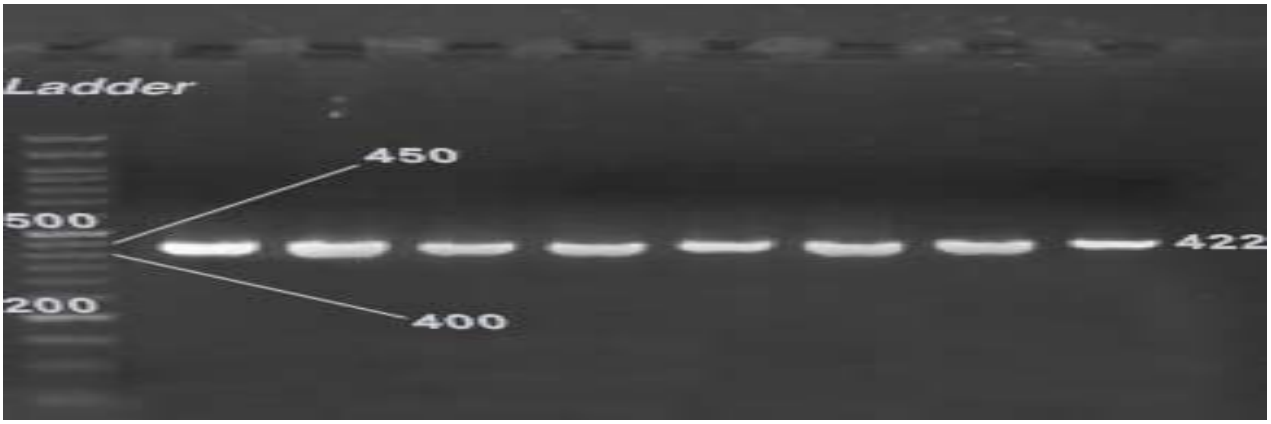

Figure 2 Ethidium bromide-stained agarose gel of amplified PCR products representing amplification of exon 2 and 3 within the goat GH gene in Raini Cashmere goats in Kerman, Iran. The ladder is the 50 bp size Marker. Other lanes are 422 bp PCR products amplified from the DNA of the studied goats

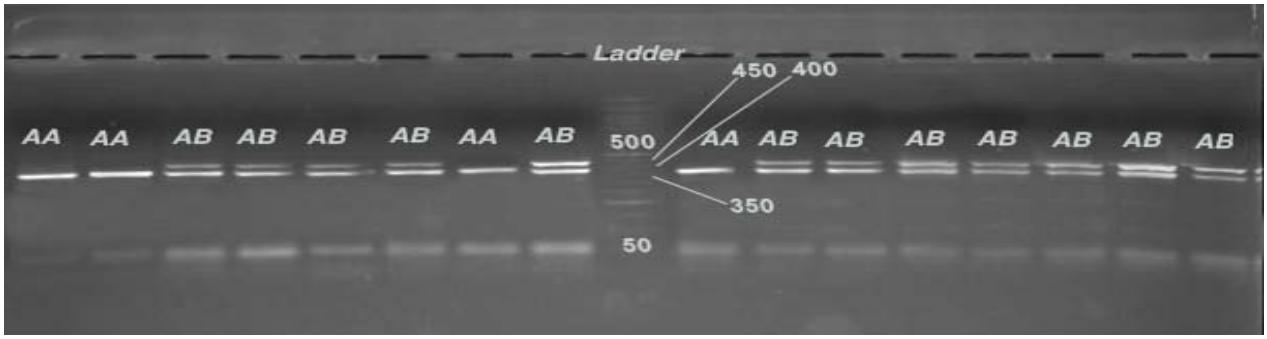

Figure 3 PCR amplified products of exon 2 and 3 within the goat GH gene in Raini Cashmere goats in Kerman, Iran digested with *Hae*III. The ladder is the DNA size marker and other lanes are AA and AB genotypes

In the Boer goats, Barbari goats and Iraq native goats also males had higher birth weight (Chungyan *et al*. 2008; Bharathidhasan *et al*. 2009; Hermiz *et al*. 2009). Fleece weight diferrence between males and females in one-year Australian goats was reported 51 grams (Restall and Pattie, 1989).

Walkden-brown *et al*. (2008) demonstrated that least square means of fleece weight in male Australian Angora goat in different ages is higher than females. Allain and Roguet (2003) also reported that male French Angora goats produce 250 grams fleece more than females. In other studies, Emami Meybodi (1993) and Mohebi Nejad and Asadi Fozi (2012) have reported difference between male and females fleece weight in Raini Cashmere goats 66.62 and 40 grams, respectively.

In genetic analysis model for every studied trait, beside of mentioned significant fixed effects, important random effects were also included. In genetic analysis model of birth type and fleece weight were considered only genetic animal effects and environmental permanent animal effects as important random effects and other additional random effects including maternal genetic effects and environmental permanent maternal effects were not considered. In genetic analysis model of birth weight, in addition to genetic animal effects, maternal genetic effects was also considered. In this model, environmental permanent maternal effects were not important and were deleted.

In this study association analyses of the GH gene polymorphism with breeding values of production and reproduction triats were also performed in 300 Raini Cashmere goats (Table 3). Results showed that mean estimated breeding values for birth type, fleece weight and birth weight traits in different genotypes varies, of course these differences were not statistically significant (P>0.05).

However, for fleece weight and birth type traits AB genotype had higher EBV that can be due to heterosis phenomenon. For birth weight trait, AA genotype had higher EBV than AB genotype.

Though, as for standard error amounts these differences can be due to small size of studied population.

Hua *et al*. (2009) also detected AA and AB genotypes in Boer goat bucks and showed that there was a tendency that AB genotype individuals had slightly greater performance although no significant differences appeared (P>0.05). Also studying exons 1-5 of growth hormone in Serrana goats by Marques *et al*. (2003) has introduced 10 genotypes from which 96% were heterozygote, that confirmed our results. Saleha *et al*. (2012) studied exon 2 of growth hormone gene in 4 Egyptian and Swedish goat breeds and showed that the frequency of allele A is higher than allele B in Zaribi and Masri breeds that is similar to our results and in Barki and Ardi breeds the frequency of allele B is higher than allele A that is not compatible with our results.

It should be noted that allele frequencies of a gene based on the breeding strategies and programs used in different flocks and breeds, which in turn associate with the tastes and eating habits of each region of the world, and this may be changed by economic conditions and other factors in the future.

According to the results of Table 3, it can be conclouded that allele A of growth hormone is a suitable allele for birth weight trait and allele B is a worthy allele for fleece weight and birth type traits.

Hence, the breeding programs of these traits can benefit from this information along with other phenotypic records. In the studied population, Shanon's index and expected heterozygosity were estimated 0.69 and 0.49, respectively, that represents a relatively high diversity in this population.

Due to the relatively high diversity of growth hormone gene in Raini Cashmere goat and its association with important economic traits, using growth hormone gene in breeding programs of this breeds can lead to acceptable genetic progress and applying AA genotype for birth weight trait and AB genotype for fleece weight and birth type traits can be used as an indirect marker for selection of superior animals.

Table 2 The genotype and allele frequencies of GH gene in Raini Cashmere goat in Kerman province of Iran

Genotype	Number of genotypes	Genotype frequencies	Allele	Allele frequencies
AA	45	0.15	A	0.575
AB	255	0.85	B	0.425
Total	300	1	-	-

Table 3 Least-square mean and standard error of estimated breeding values of the different genotypes for the studied traits

Genotype	Fleece weight	Birth weight	Birth type
AA	-0.012±0.011	0.22±0.047	-0.008±0.005
AB	0.009±0.022	0.025±0.097	-0.001±0.011
P-value	0.0663	0.920	0.228

CONCLUSION

Whereas studied traits are the polygenic traits and is controlled by many loci, however, the results of this study indicated the effect of growth hormone gene on productivity and reproductive traits. Thus, using the obtained information in the above locus in the optimal selection parameters, can increase incidentally the accuracy of selection, genetic progress and response to selection for these traits. Although polymorphism data achieved from a locus is important for studying its effect on desired traits, but can not alone be used as a selection criterion in practical condition and it is essential to use data from studied locus along with information from other examined loci. Our future study will be focused on the functional differences between genotypes of this locus within the goat GH gene.

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