

Association of Biometric Traits with Growth Hormone Gene Diversity in Raini Cashmere Goats

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Abstract

In recent years, the important effects of growth hormone on a broad variety of physiological parameters, such as lactation, reproduction, growth, and metabolism, have attracted many researchers' attentions. To detect GH gene polymorphism and its association with the biometric traits of Raini Cashmere goats, 300 animals were selected and the animals' genotype was determined using PCR-RFLP. Two different genotypes (AA and AB) were observed in exons 2 and 3 within the goat *GH* gene. The genotype frequencies for AA, AB, and BB were 0.15, 0.85, and 0, and frequencies of the A and B alleles were 0.575 and 0.425, respectively. The number of observed alleles, number of effective alleles, expected heterozygosity, observed heterozygosity, mean of heterozygosity, expected homozygosity, observed homozygosity, Nei's index, Shannon's index, and Fixation index (*F_{is}*) were 2, 1.96, 0.49, 0.85, 0.49, 0.51, 0.15, 0.49, 0.69, and -0.74, respectively. Birth type had a significant effect on the Chest height to earth (T26) trait (*P*-value = 0.032304). Furthermore, a significant effect of age on some biometric traits were observed in this study. The frequency of the AB genotype for most of the traits were higher in comparison to the AA genotype, although some traits did not show a significant effect, which might be explained by the heterosis phenomenon. According to the results, it can be concluded that allele A of growth hormone is a suitable allele for the THH (Toe height of hand hoof), THF (Toe height of foot hoof), HHF (Heel height of foot hoof), and CHE (Chest height to earth) traits, and allele B is a worthy allele for the NI (Nasal interval), LCI (Lip corner interval), NL (Neck length), CC (Chest circumference), AC (Abdominal circumference), LLF (Leg length of foot), WHC (Wither height to under the chest), and DAD (Dorsal angle distance of scapula to Hansch point) traits. Therefore, better results in the breeding programs for these traits can be achieved from this information, along with other phenotypic records.

Keywords: PCR-RFLP, Raini Cashmere goat, birth weight, fleece weight, birth type

Introduction

Goat farming is a common and popular practice in the world, with goat products having a favorable image. The number of goats has increased globally, despite major changes in agriculture due to industrial mergers, globalization, and technological advances in developed countries [1]. There are 30 million heads of Cashmere goats around the world, with 4.5 - 5 million heads of them in Iran, which is 20 % of all Cashmere goats in the world [2]. Goat production is one of the key elements contributing to the economy of farmers living in arid and semi-arid regions, including most areas of Iran. The Raini goat is one of the most important Iranian native breeds that are distributed in the southeast of Iran, where these animals are kept for milk, meat, and Cashmere production [3]. One of the most important purposes of the genetic

improvement of this breed is to enhance meat production through programmed and accurate selection. On the other hands, determination of gene polymorphism is important in farm animal breeding [4-6] in order to define genotypes of animals and their associations with productive, reproductive, and economic traits. Growth hormone (GH) has an effect on a broad variety of physiological parameters, such as lactation, reproduction, growth, and metabolism [7]. The GH gene has 2544 bp length, including 4 introns and 5 exons [8], and its polymorphisms have been reported in cattle [9,10], sheep [11], and goats [7]. Yamano *et al.* [12] and Yato *et al.* [13] described the sequence of goat pituitary growth hormone cDNA, and Kioka *et al.* [14] introduced the GH gene sequence. In some other studies, the association among genetic polymorphism of GH and plasma levels [10,15], milk production [16-18], milk protein percentages [19], litter size and weight [8,20], superovulation [20], and chest and paunch girth [21] have been reported. Although many studies have been done on Raini Cashmere goats [22-27], no studies concerning the polymorphisms of GH gene in Raini Cashmere goats and its association with biometric traits has been published. Therefore, the goal of this study was to describe the association of the GH gene diversity with the biometric traits of Raini Cashmere goats for the first time.

Materials and methods

The study was done using 300 female Raini Cashmere goats, age between 38 to 92 months, from a breeding station for Raini goats in Baft City (middle of Kerman Province, Iran). The animals' birth types had 2 levels, single and twin. Blood samples were collected from the jugular vein of the animals into vacutainers, with EDTA as the anticoagulant. The samples were kept at -20°C until analysis. DNA was extracted from the whole blood using an optimized and modified salting-out method [28].

A 422-bp fragment encoding exons 2 and 3 within the goat *GH* gene was amplified using PCR primers 5'-CTCTGCCTGCCCTGGACT-3' and 5'-GGAGAAGCAGAAGGCAACC-3' [29]. The PCR amplification was performed in a 25 μl reaction volume, containing negative controls, using CinnaGen PCR Master Kit according to the instructions of 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; then, all PCR products were digested with 10 U of *HaeIII* 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), the observed number of alleles (Ne), Shannon's information index, etc., were estimated by the POPGEN 3.2 software [30]. Association analyses of the GH gene polymorphism, with 29 biometric traits (**Table 1**), were performed for the 300 Raini Cashmere goats, using the following univariate animal model;

$$y = Xb + Z_1u + Z_2m + Z_3mpe + Z_4pe + e \quad (1)$$

where y is a vector of animal records, b is a vector of fixed effects, u is a vector of additive genetics effects, m is a vector of maternal genetics effects, mpe is a vector of permanent environmental effects of dams, pe is a vector of animal permanent environmental effects, and e is a vector of residual effects. X , Z_1 , Z_2 , Z_3 , and Z_4 are incidence matrices. ASReml [31] was used for the analysis.

Table 1 Characteristics of biometric traits.

Trait			Trait		
Number	Name	Abbreviation	Number	Trait name	Abbreviation
T1	Ear length	EL	T16	Wrist length of hand	WLH
T2	Ears interval	EI	T17	Toe height of hand hoof	THH
T3	Face length	FL	T18	Heel height of hand hoof	HHH
T4	Eyes interval	YI	T19	Leg length of foot	LLF
T5	Face width	FW	T20	Leg circumference of foot	LCF
T6	Jaws interval	JI	T21	Wrist length of foot	WLF
T7	Nasal interval: Interval from the front of nozzle to behind the ears, from the face side	NI	T22	Toe height of foot hoof	THF
T8	Lip corner interval: Lip corner interval to behind the ears, from the face side	LCI	T23	Heel height of foot hoof	HHF
T9	Neck length	NL	T24	Wither height to earth	WHE
T10	Neck circumference	NC	T25	Wither height to under the chest	WHC
T11	Neck width	NW	T26	Chest height to earth	CHE
T12	Chest circumference	CC	T27	Chest distance from back of thigh	CDT
T13	Abdominal circumference	AC	T28	Dorsal angle distance of scapula to Hänsch point	DAD
T14	Leg length of hand	LLH	T29	Hänsch distance to end of Hamstring	HDH
T15	Leg circumference of hand	LCH			

Results and discussion

The extracted DNA had good quality (**Figure 1**). The tested DNA was amplified using the specific primers and yielded PCR products at the expected size, 422 bp (**Figure 1**).

Amplification of exons 2 and 3 produced 422 bp fragments. When these fragments were digested with the restriction enzyme, the AA genotype produced 2 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 3 bands: 422, 366, and 56 bp (heterozygote genotype). The different alleles resulting from digestion of the PCR products with the *HaeIII* restriction enzyme after running on the agarose gel electrophoresis are presented in **Figure 2**.

The genotypic and allelic frequencies of exons 2 and 3 within the goat GH gene in Raini Cashmere goats in Kerman are shown in **Table 2**. Sampling fluctuations might also have contributed to the differences in allelic frequencies in the population.

In this study, the Hardy Weinberg equilibrium was estimated with the chi-square test and the 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 (**Table 3**), as for years it has been under selection for production and reproduction traits.

The values of the population genetics parameters in the Raini Cashmere goat population for the GH gene were as follow: Numbers of observed alleles, 2; number of effective alleles, 1.96; expected heterozygosity, 0.49; observed heterozygosity, 0.85; mean of heterozygosity, 0.49; expected homozygosity, 0.51; observed homozygosity, 0.15; Nei’s index, 0.49, and Shannon’s index, 0.69. Fixation index (Fis) as a measure of heterozygote deficiency for the A and B alleles was -0.74.

In this research, the association between GH gene polymorphism and biometric traits was studied using a univariate animal model. Birth type had a significant effect only on the Chest height to earth (T26) trait (P-value = 0.032304). The age also had a significant effect on some biometric traits (**Table 4**). Face length (T3), Nasal interval: Interval from the front of nozzle to behind the ears, from the face side (T7), Lip corner interval: Lip corner interval to behind the ears, from the face side (T8), Neck length (T9), Chest circumference (T12), Abdominal circumference (T13), Wither height to under the chest (T25), and Dorsal angle distance of scapula to Hänsch point (T28) were higher in the older animals, which can be caused by differences in physiologic and genetic reactions, such as hormone secretions at different ages. Therefore, it can be concluded that age is an important factor affecting the biometric traits of animals. Each of the studied traits are differentiated and develop at different ages. Some of the traits are matured and stop growing at early ages, and the rest at later ages. These results are in agreement with the results of Wiener *et al.* [32] and Yakubu *et al.* [33], who showed that the evolution of body traits occurs in mammals before puberty, and that growth continues to pre-puberty over a specific pattern and then stops.

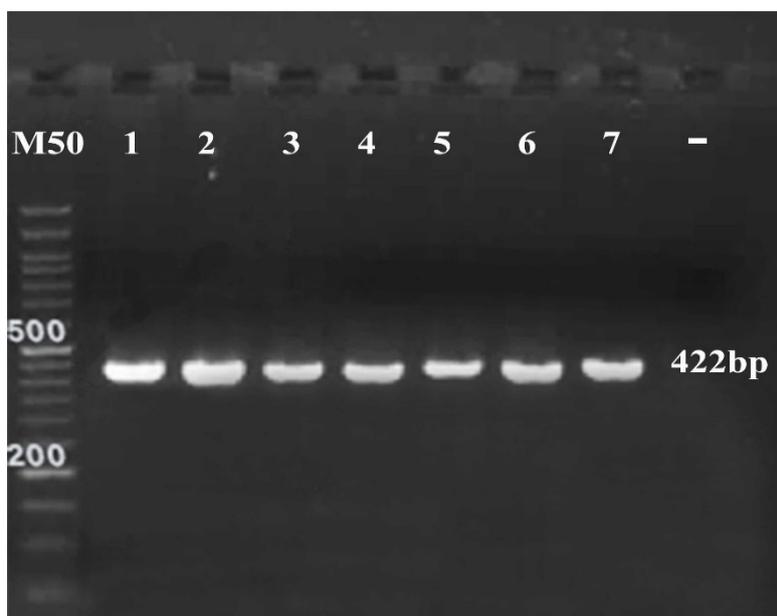


Figure 1 Ethidium bromide-stained agarose gel of amplified PCR products representing amplification of exons 2 and 3 within the goat GH gene in Raini Cashmere goats in Kerman, Iran. The size marker is the M50. Lanes 1 - 7 are 422 bp PCR products amplified from the DNA of the studied goats, and – is a negative control.

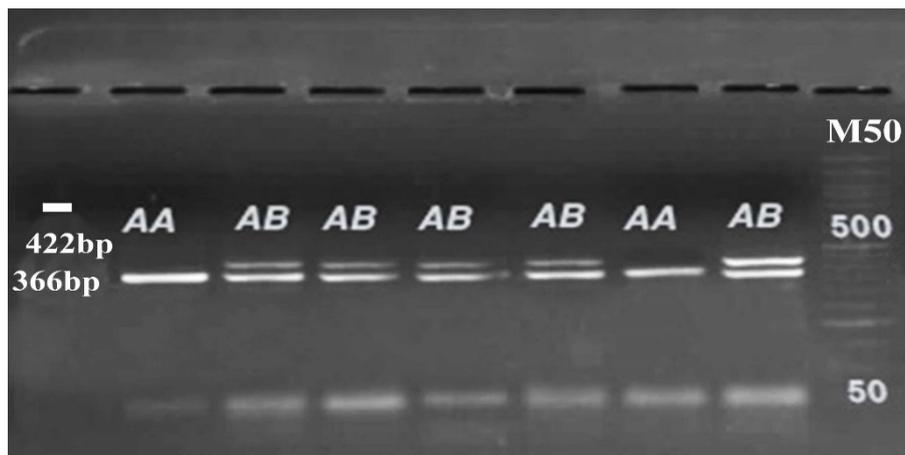


Figure 2 PCR amplified products of exons 2 and 3 within the goat GH gene in Raini Cashmere goats in Kerman, Iran, digested with *HaeIII*. The size marker is M50, – is a negative control, and other lanes are AA and AB genotypes.

Table 2 The genotype and allele frequencies of the GH gene in Raini Cashmere goats in the 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 K-square and G-square tests values in Raini Cashmere goats in the Kerman province of Iran for the Hardy-Weinberg equilibrium.

K-square value	G-square value	Degree of freedom	0.05 probability level
10.471	10.733	1	0.001

Table 4 Biometric traits affected by age.

Number	Name	Abbreviation	P-value
T3	Face length	FL	0.046698
T7	Nasal interval: Interval from the front of nozzle to behind the ears, from the face side	NI	3.32588E-05
T8	Lip corner interval: Lip corner interval to behind the ears, from the face side	LCI	8.48167E-05
T9	Neck length	NL	0.026687
T12	Chest circumference	CC	0.048584
T13	Abdominal circumference	AC	0.031969
T25	Wither height to under the chest	WHC	0.034156
T28	Dorsal angle distance of scapula to Hansch point	DAD	0.046698

The results of the current study are in agreement with some other studies. For example, Boujenane and Hazzabs [34] showed that the birth weight in the Draa goat breed for males was 19 % higher than for females. Boer goats, Barbari goats, and Iraq native goat males also had higher birth weights than females [35-37]. Fleece weight difference between males and females in one-year-old Australian goats was reported as 51 grams [38]. Walkden-Brown *et al.* [39] demonstrated that the least square means of fleece weight in male Australian Angora goats of different ages is higher than in females. Allain and Roguet [40] also reported that male French Angora goats produced 250 grams more fleece than females. In other studies, Meybodi [41] and Nejad and Fozi [42] reported the difference between male and female fleece weights in Raini Cashmere goats as 66.62 and 40 grams, respectively.

The results of our study showed that growth hormone genotypes had different effects on biometric traits in different genotypes (**Table 5**).

Generally, the AB genotype had a higher amount than the AA genotype, but for some traits had not shown significant effects (**Table 5**), which could be due to the heterosis phenomenon. It can be demonstrated that using different animals in breeding programs could result in heterotic achievement.

Only for traits T17 (Toe height of hand hoof), T22 (Toe height of foot hoof), T23 (Heel height of foot hoof), and T26 (Chest height to earth) did the AA genotype have higher amounts than the AB genotype; this difference was very low, and was not significant ($P > 0.05$). More efficient traits are used in breeding programs [43]. Accordingly, T17 (Toe height of hand hoof), T22 (Toe height of foot hoof), T23 (Heel height of foot hoof) and T26 (Chest height to earth) traits can be considered in breeding programs in order to produce goats with higher heights at early ages economically. These results are consistent with the results of other researchers [44-46].

The studies of Singh *et al.* [21] on the association of GH gene polymorphism with chest and paunch girth in Sirohi and Barbari breeds of goats revealed significant breed differences for the least squares means of chest girth at each age, so that the measured traits for Sirohi kids were significantly higher than those for Barbari kids. In this study, the least square means for genotype AB was numerically higher than BB for chest girth at all ages, which is in agreement with the present findings on Raini Cashmere goats.

The least square analysis of variance conducted to analyze the association of polymorphic variants of the GH gene with paunch girth at birth to 180 days of age showed a highly significant effect ($P < 0.01$) of breed on paunch girth at each stage. The least square means of paunch girth revealed significant breed differences at each age; Sirohi kids measuring significantly higher than Barbari kids. Although the least square means for the AB and BB genotypes did not differ significantly at any stage, the genotype AB was constantly superior over all the intervals for paunch girth [21].

In another study on Nanjiang Huang goats, Deng *et al.* [47] found significant effects of genotypes on chest girth at 2 months of age. Furthermore, in the study by Zhang *et al.* [48] on the IGF-I gene of Guanzhong goats (a gene similar to the GH gene in its effect), polymorphism, a significant ($P < 0.05$) effect of genotype on chest circumference, was reported.

Wickramaratne *et al.* [49] reported non-significant differences for chest girth at 2 months of age between different genotypes involving single nucleotide polymorphisms at different regions of GH genes in the Sangamneri breed of goat.

Hua *et al.* [29] also detected AA and AB genotypes in Boer goat bucks and showed that AB genotype individuals tended to have a better performance, although no significant differences were observed ($P > 0.05$). Furthermore, in another study on exons 1 - 5 of the growth hormone in Serrana goats, Marques *et al.* [18] introduced 10 genotypes, from which 96 % were heterozygote, which confirmed our results. Saleha *et al.* [50] studied exon 2 of the growth hormone gene in 4 Egyptian and Swedish goat breeds and showed that the frequency of allele A was higher than allele B in the Zaribi and Masri breeds, which agrees with our results, but not with the results from the study on the Barki and Ardi breeds, in which the frequency of allele B was higher than of allele A. 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, may be changed by economic conditions and other factors in the future.

According to the data presented in **Table 4**, it can be concluded that allele A of the growth hormone is a suitable allele for T17 (Toe height of hand hoof), T22 (Toe height of foot hoof), T23 (Heel height of

foot hoof), and T26 (Chest height to earth) traits, and allele B is a worthy allele for T7 (Nasal interval), T8 (Lip corner interval), T9 (Neck length), T12 (Chest circumference), T13 (Abdominal circumference), T19 (Leg length of foot), T25 (Wither height to under the chest), and T28 (Dorsal angle distance of scapula to Hänsch point) traits.

Table 5 Effects of growth hormone genotypes on the studied biometric traits.

Biometric traits (cm)	Genotype	<u>AA</u>	<u>AB</u>
		LSM ± SE	LSM ± SE
T1	NS	17.17 ± 0.89	18.04 ± 1.83
T2	NS	11.44 ± 0.55	11.63 ± 1.13
T3	NS	29.03 ± 0.15	29.30 ± 0.31
T4	NS	10.11 ± 0.22	10.22 ± 0.45
T5	NS	9.56 ± 0.23	9.94 ± 0.48
T6	NS	8.17 ± 0.27	8.29 ± 0.56
T7	*	24.90 ± 0.18	25.35 ± 0.37
T8	*	19.23 ± 0.16	19.59 ± 0.32
T9	**	29.09 ± 0.25	29.80 ± 0.51
T10	NS	32.22 ± 0.95	32.62 ± 1.95
T11	NS	27.33 ± 1.27	28.22 ± 2.7
T12	**	76.91 ± 0.44	78.33 ± 0.91
T13	*	91.97 ± 0.72	93.87 ± 1.48
T14	NS	12.11 ± 0.26	12.41 ± 0.54
T15	NS	7.44 ± 0.14	7.44 ± 0.28
T16	NS	5.17 ± 0.23	5.21 ± 0.47
T17	NS	4.22 ± 0.22	4.02 ± 0.45
T18	NS	2.11 ± 0.13	2.19 ± 0.27
T19	*	13.06 ± 0.26	13.72 ± 0.54
T20	NS	7.78 ± 0.27	8.13 ± 0.55
T21	NS	4.83 ± 0.20	4.93 ± 0.41
T22	NS	3.61 ± 0.17	3.59 ± 0.35
T23	NS	1.94 ± 0.14	1.87 ± 0.28
T24	NS	61.83 ± 1.15	62.33 ± 2.36
T25	**	33.05 ± 0.19	33.75 ± 0.39
T26	NS	33.13 ± 0.33	33.13 ± 0.68
T27	NS	64.28 ± 1.52	85.60 ± 3.12
T28	*	47.09 ± 0.41	48.00 ± 0.85
T29	NS	14.33 ± 0.43	14.90 ± 0.88

Hence, breeding programs of these traits can benefit from this information, along with other phenotypic records. In the studied population, Shannon's index and expected heterozygosity were estimated at 0.69 and 0.49, respectively, which represents a relatively high diversity in this population. Due to the relatively high diversity of the growth hormone gene in Raini Cashmere goats and its association with important economic traits, using growth the hormone gene in breeding programs of these breeds can lead to acceptable genetic progress. Furthermore, applying the AA genotype for traits including T17 (Nasal interval: Interval from the front of nozzle to behind the ears, from the face side), T22 (Toe height of foot hoof), T23 (Heel height of foot hoof), and T26 (Chest height to earth) traits, and the AB genotype for T7 (Nasal interval), T8 (Lip corner interval), T9 (Neck length), T12 (Chest circumference), T13 (Abdominal circumference), T19 (Leg length of foot), T25 (Wither height to under

the chest), and T28 (Dorsal angle distance of scapula to Hänsch point) can be used as an indirect marker for the selection of superior animals. These results might be explained by the genetic structure of the animals or the different expression patterns and levels of genes under various environments, where interactions of genotype and environment affect the physiology of animals, which results in different phenotypes.

Conclusions

The PCR restriction fragment length polymorphism technique used in this experiment proved to be an appropriate technique for screening samples for polymorphism with respect to the exon 2 and exon 3 regions of the GH gene. Moreover, the studied traits are polygenic traits and are controlled by many loci. However, the results of this study indicated that the growth hormone gene had effects on biometric traits. Thus, the polymorphism of the studied locus can be used to increase incidentally the accuracy of selection, genetic progress, and response to selection for these traits. Furthermore, although polymorphism data achieved from a locus is important for studying its effect on desired traits, it cannot be used alone as a selection criterion in practical conditions, and it is essential to use data from the studied locus along with information from other examined loci. Also, as the study was based on a limited sample size, further study on a larger sample is suggested. Our further study will be focused on the functional differences between the genotypes of this locus within the goat *GH* gene.

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