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Genetic Polymorphisms and Association of Growth Hormone Gene with Growth Traits in Beetal Goat

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ABSTRACT

Background: This investigation was aimed to determine the association of growth hormone *GH1* (A781G) and *GH2* (A1575G) locus with growth traits in Beetal goats by using PCR-RFLP method.

Methods: A total of 60 blood samples from Beetal goat were collected along with growth records (body weight in kg) at 0, 3, 6, 9 and 12 month of age maintained at Goat Dairy Farm Rajbagh, Jammu. DNA was extracted from blood samples by using DNA extraction kit. The target segment of *GH* 1 and 2 locus was amplified and digested with *HaeIII* restriction enzyme for scoring of genotypes.

Result: The amplified PCR product obtained for *GH*1 (422 bp) and *GH*2 (116 bp) locus were digested with *HaeIII* restriction enzyme at 37°C for 15 min. The resultant digested products revealed three genotypes for *GH*1: AA (422 bp), BB (366 bp, 56 bp) and AB (422 bp, 366 bp and 56 bp) whereas, one genotype was observed for *GH*2 *i.e.,* BB (88bp and 28bp). The genotypic frequencies in Beetal goat for *GH*1 were found to be 0.05 for AA genotype, 0.80 for AB genotype, 0.15 for BB genotype, whereas gene frequencies was 0.45 for A allele and 0.55 for B allele. *GH*2 locus was found to be monomorphic. The highly significant (P<0.01) Chi-square (χ^2)-test value for *GH*1 locus showed that the population was not in HWE. BB genotype has a significantly higher (P<0.01) body weight followed by AA and AB genotypes at 9 and 12 month of age. Thus, *GH*1 locus can be used as a candidate gene to improve growth traits and selection of superior animals at an early age of 9 months for phenotypic selection programmes in Beetal goats.

Key words: Beetal goats, *GH* 1 and 2 locus, Growth traits, PCR-RFLP Polymorphism.

INTRODUCTION

Beetal goats are vastly preferred for farming due to their superior body weight, milking strength and higher fecundity rate in tropical and sub-tropical regions of the world (Khan and Ashfaq 2012). It is recommended in J&K either in pure form or for up-gradation of non-descript goats for improving milk and chevon production to narrow down the gap between demand and supply.

Growth is primarily regulated by the growth hormone (*GH*), an anabolic hormone synthesized and secreted by the somatotroph cells of the anterior lobe of the pituitary (Bayan *et al.,* 2018). It is a single polypeptide hormone of 191 amino acids with a molecular size of 22kDa (An *et al.,* 2010). *GH* influences physiological processes such as growth, reproduction, lactation and metabolism (Bayan *et al.,* 2018 and Gitanjli *et al.,* 2020).

GH belongs to a family of somato-lactogenic hormones which is encoded by a single gene present on the short arm of chromosome 19q22 with 2.5 kb in length, consisting of five exons and four intervening introns. *GH*1 (A781G locus) and *GH*2 (A1575G locus) located on exon (2 and 3) and exon 4 having size of 422 bp and 116 bp, respectively (Hua *et al.* 2009). Polymorphism was reported for *GH* 1 and 2 locus by Aradhana *et al.,* 2021, Pandya *et al.,* 2021, Abbas *et al.,* 2022 and Rashijane *et al.,* 2022 in various goat breeds.

Association study of *GH*1 locus has been confirmed with different traits in goats such as body weight and chest girth at birth (Singh *et al.,* 2015), birth and weaning weight and weaning chest girth (Hua *et al.,* 2009), body weight (Gitanjli *et al.,* 2020, Rashijane *et al.,* 2022). Association between variants of *GH*2 locus has been reported with birth body weight (Hua *et al.,* 2009 and Gitanjli *et al.,* 2020), litter size and superovulation response (Zhang *et al.,* 2011). To assess genetic variation in goats, *GH* gene is frequently explored for its biological significance. With this background,

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current study was undertaken to investigate the *GH* 1 and 2 locus polymorphism and its association with growth traits in Beetal goats.

MATERIALS AND METHODS

Source population

The study comprised 60 Beetal goats maintained at Goat Dairy Farm (latitude 32.427828° N and longitude 75.402926° E), Rajbagh, Jammu, along with their growth traits at 0, 3, 6, 9 and 12 months of age. The study was carried out in the Division of Animal Genetics and Breeding, Faculty of Veterinary Sciences and Animal Husbandry, SKUAST-Jammu.

DNA isolation

Under sterile conditions, blood sample (5 ml) was taken from the jugular vein aseptically in a sterile EDTA-coated vacutainer. DNA was isolated from blood samples as per the protocol of the genomic DNA Blood mini Kit. Nano-drop spectrophotometer was used to determine the purity and concentration of genomic DNA by taking the ratio of optical densities at 260 nm and 280 nm. The quality of the DNA was checked on 0.8% horizontal submarine agarose by electrophoresis. The gel was then examined on a UV trans-illuminator for quality assessment.

Polymerase chain reaction (PCR) amplification

A pair of primers (forward: 5′CTCTGCCTGCCCTGGACT 3′ reverse: 5′GGAGAAGCAGAAGGCAACC3′) and (forward: 5′TCAGCAGAGTCTTCACCAAC3′reverse:5′ CAACAACGCCATCCTCAC 3′) were used to amplify a 422 bp and 116 bp PCR product for *GH* 1 and 2 locus respectively (Hua *et al.,* 2009). For *GH* 1 and 2 locus; PCR reaction was carried out in PCR tubes containing 1 µl of genomic DNA, 0.5 µl of (10 pm/ml) forward and reverse primers, 1 µl of $MgCl_2$ (4mM), 12.5 µl of 2X PCR master-mix and 9.5 µl of distilled water to make final reaction mixture of 25 µl. Amplification was accomplished in Eppendorf thermal cycler using Touch- down program having 13 cycles of denaturation at 95°C for 30 sec, annealing temperature at 65°C (-1°C per cycle) for 30 sec and extension at 72°C for 45 sec followed by constant annealing temperature of 52°C for 30 sec for the remaining 23 cycles. Initial denaturation was carried out at 94°C for 5 min, while the final extension was performed at 72°C for 7 min. The PCR product (5 µl) from each tube was methodically mixed with 1 µl of 6× loading dye buffer and loaded on 2% agarose gel containing ethidium bromide (1% solution @ 5 µl/100 ml) along with 100 bp DNA ladder at a constant voltage of 80 V for 45 min in 0.5 × TBE buffer. For confirmation of the amplification of the targeted gene, the amplified PCR product was visualized as a single compact band under a UV transilluminator and documented by gel photography.

Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method

PCR products of *GH* 1 and 2 locus were further digested with restriction enzyme *HaeIII* (GG↓CC) for perceiving the PCR-RFLP band patterns. The total reaction mixture of 30 µl consisting of 10 µl PCR product and the rest 20 µl consisting of *HaeIII*, 10x buffer and nuclease-free water was taken into a microcentrifuge tube. For uniform mixing, the reaction mixture was spun for a few seconds and then kept for 15 min at room temperature. The digested products were electrophoresis on 2.5% agarose gel comprised of 1% ethidium bromide (5 µl/100 ml) at a constant voltage of 80V for 60 min using 0.5× TBE buffer till the dye front reached the last third of the gel. For visualization of DNA ladder (molecular size marker) and digested products UV transilluminator was used afterward photographed with gel documentation system. Genotyping was carried out according to the band pattern of respective genotypes.

Statistical analysis

Based on the outcome of genotyping: genotypic frequencies, allelic frequencies, Hardy-Weinberg equilibrium (HWE) as well as population genetic indexes such as gene homozygosity (Ho), gene heterozygosity (He), effective allele numbers (ne), Shannon's Information index (I) and fixation index (Fis) were analyzed by POPGENE 32 version 1.32 software (Yeh *et al.* 1999).

The polymorphism information content (PIC) was calculated as per Botstein *et al.* (1980). The data on growth traits (0, 3, 6, 9 and 12 months of age) were analyzed with the help of the Statistical Package for Animal Breeding (SPAB2) programme (Sethi, 2006). The following model was used for this purpose:

$$Y_{ij} = \mu + G_i + e_{ij}$$

Where,

Y_{ij} = Performance traits of ith genotype;

μ = Overall performance;

G_i = Effect of ith genotype

e_{ij} = Random effect of error.

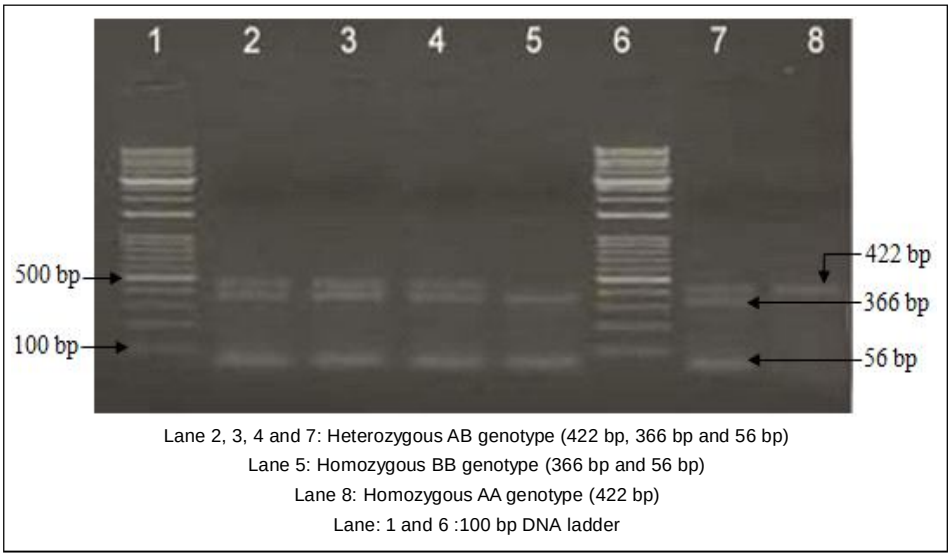
RESULTS AND DISCUSSION

The amplified PCR product of *GH* 1 and 2 locus revealed a single compact band of 422 bp and 116 bp size respectively in Beetal goat. The PCR product of the 422 bp size was also reported for *GH*1 locus by Hua *et al.* (2009) in Boer goats, Marini *et al.* (2015) in Savanna goats, Gitanjli *et al.* (2020) in Gaddi goats, Aradhana *et al.* (2021) in Ganjam and Baigani goats and Pandya *et al.* (2021) in Surti goats. Whereas, for *GH* 2 locus PCR product of 116 bp size was obtained, the same was reported by Hua *et al.* (2009) in Boer goats, Bayan *et al.* (2018) in Surti and Mehsani goats, Mahrous *et al.* (2018) in three goat breeds of Egypt, Gitanjli *et al.* (2020) in Gaddi goats and Abbas *et al.* (2022) in five goat breeds of Iraq.

Restriction digestion of PCR product of *GH*1 locus with *HaeIII* enzyme revealed three types of genotypic band patterns as shown in Fig 1. The RFLP patterns were AA (422 bp), BB (366 bp, 56 bp) and AB (422 bp, 366 bp, 56 bp) genotypes. Out of the total products (n=60) for *GH*1 locus, 03 homozygous (AA), 48 heterozygous (AB) and 09 homozygous (BB) genotypes were found. Similar RFLP patterns were reported in native goat breed of Kerala (Radhika *et al.,* 2016), Gaddi goat (Gitanjli *et al.,* 2020) and Surti goats (Pandya *et al.,* 2021). On the contrary, AA genotype of 422 bp was not found in Kacang goat (Ilham *et al.,* 2016), Surti and Mehsana goat (Bayan *et al.,* 2018), Lakor goat (Kunda *et al.* 2020) and female goats from different herds *i.e.,* native, Shami, Meriz, Kamori and wild mountain goat (Abbas *et al.,* 2022).

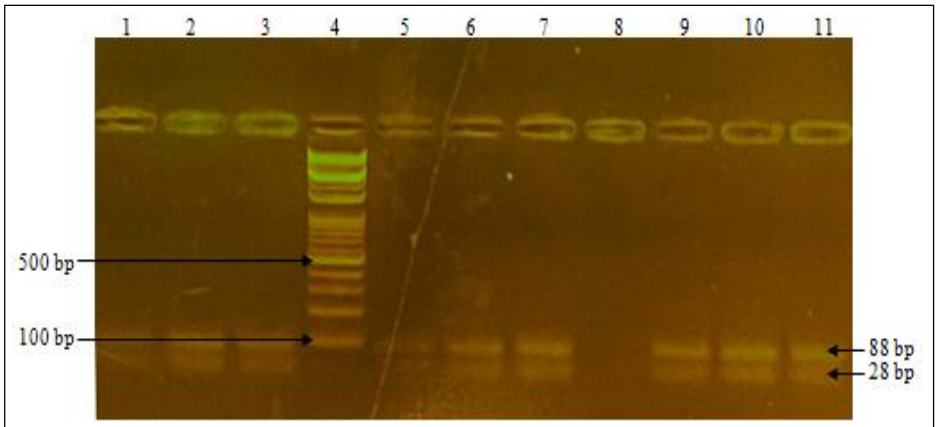
Restriction digestion of PCR product of *GH* 2 locus with *HaeIII* restriction enzyme revealed only one type of genotype band pattern as shown in Fig 2. The RFLP pattern (88 bp, 28 bp) was symbolized as BB type whereas; genotypes AA and AB were absent. Similar, RFLP patterns were revealed by Bayan *et al.* (2018) in Surti and Mehsani goat, Shareef *et al.* (2018) in Beetal goat and Abbas *et al.* (2022) in female goats from different herds. Contrary to the present findings, Mahrous *et al.* (2018) in three goat breeds of Egypt reported two homozygous genotypes AA (116 bp) and BB (88 bp and 28bp) and Gitanjli *et al.* (2020) in Gaddi goats of Western Himalayas reported AB (116 bp, 88 bp and 28 bp) and BB (88 bp and 28bp) genotype. Likewise, Susilorini *et al.* (2017) in Etawah goats reported two genotypes CC (116 bp and 88 bp) and CD (116 bp and 88 bp and 28 bp) however; they have used different nomenclature.

The genotypic frequencies for *GH*1 locus were found to be 0.05 for AA genotype, 0.80 for AB genotype, 0.15 for BB genotype, the gene frequencies were found to be 0.45 for A



Lane 2, 3, 4 and 7: Heterozygous AB genotype (422 bp, 366 bp and 56 bp)
Lane 5: Homozygous BB genotype (366 bp and 56 bp)
Lane 8: Homozygous AA genotype (422 bp)
Lane: 1 and 6 :100 bp DNA ladder

Fig 1: PCR-RFLP patterns of *GH* 1 locus in Beetal goat at 2.5 % agarose gel electrophoresis.



Lane 1-3, 5-7, 9-11: Homozygous BB genotype (88bp and 28bp)
Lane 4: 100bp DNA ladder.

Fig 2: PCR-RFLP patterns of *GH* 2 locus in Beetal goat at 2.5% agarose gel electrophoresis.

Table 1: Genotype distribution and allelic frequencies at *GH* 1 locus along with population genetic indexes in Beetal Goat.

Locus	Genotypes			Gene/Allele		χ^2-Test	Population genetic indexes							
	AA	AB	BB	A	B	HWE	Ho	He	na	ne	I	Fis	SE	PIC
GH 1	0.05 (03)	0.80 (48)	0.15 (09)	0.45	0.55	22.17**	0.501	0.499	2.000	1.980	0.688	-0.616	0.026	37.250

**Highly significant (P<0.01), Figures in parentheses are number of observations, HWE = Hardy-Weinberg Equilibrium, H_o = Expected homozygosity, H_e = Expected heterozygosity, n_a= Observed number of alleles, n_e= Effective number of alleles, I= Shannon's Information index, Fis= Fixation index of individual with sub-population, SE= Standard error and PIC= Polymorphic information content.

Table 2. Least- squares means along with standard error for various growth traits for *GH*1 locus in Beetal goat.

Genotype	Body weight (kg) for different month of age				
	Zero^{NS}	Three^{NS}	Six^{NS}	Nine**	Twelve**
AA (3)	3.067±0.305	8.733±0.696	12.567±0.943	16.500^{ab}±0.850	21.667^{b}±0.961
AB (48)	3.006±0.076	8.167±0.174	12.433±0.235	15.110^{b}±0.212	19.785^{c}±0.240
BB (9)	3.133±0.176	9.078±0.402	13.611±0.544	17.178^{a}±0.491	23.667^{a}±0.555

**(P<0.01), NS: Non-significant; values bearing different superscripts in a column differ significantly and figures in parentheses are number of observations.

allele, 0.55 for B allele. The highly significant (P<0.01) Chi-square (χ^2)-test value for *GH*1 locus showed that the population was not in HWE (Table 1) which might be due to selection or small population. For *GH* 2 locus, only one genotype BB was observed. Similar results for *GH* 1 locus were reported by Bayan *et al.* (2018) in Surti and Mehsana goats, Gooki *et al.* (2018) in Raini Cash mere goats and Pandya *et al.* (2021) in Surti goats. Contradicted findings are reported by Gitanjli *et al.* (2020) in Gaddi goats of Himalayas and Rahijane *et al.* (2022) in Boer goat.

Population genetic indexes

Population genetic indexes such as Ho, He, ne, I, Fis and PIC values for *GH*1 locus are shown in Table 1. It was observed that Ho (0.501) value was more than He (0.499) value. It indicates that the homozygosity was more in the Beetal goat population. Similar finding was reported by Gooki *et al.* (2018) in Raini Cashmere goat. In contrast the results obtained in the study were not in concord with the result of Gitanjli *et al.* (2020) in Gaddi goats and Abbas *et al.* (2022) in female goats from different herds in which Ho was less than He. Effective number of alleles (ne) for *GH*1 locus was found to be 1.980. Similar finding was reported by Gooki *et al.* (2018) in Raini Cashmere goat and Gitanjli *et al.* (2020) in Gaddi goats. Shannon index (I) is an information statistic index, was found to be 0.688. FIS were found to be -0.616. The result obtained in this study was in agreement with those reported by Gooki *et al.* (2018) in Raini Cashmere goat and Gitanjli *et al.* (2020) in Gaddi goats. The estimated PIC value showed median level polymorphism of 0.37. Similar result was reported by Kunda *et al.* 2020 in Lakor goat. PIC for co-dominant markers, the estimated value was median polymorphism (0.37) for *GH*1 locus. Thus, 37 per cent of the off spring should be informative**.**

Association of *GH* 1 and 2 locus polymorphic variants with growth traits

Least squares analysis of variance among the growth traits for different genotypes of *GH*1 locus revealed non-significant differences at 0, 3 and 6 month of age whereas, highly significant (P<0.01) differences were observed at 9 and 12 month of age in Beetal goat (Table 2). The values of least square means of growth traits at 9 month for *GH*1 locus were found significantly (P<0.01) higher in BB genotype (17.178±0.491 kg) and was least in AB genotype (15.110±0.212 kg) with intermediate value for AA genotype (16.500±0.850 kg). But at 12 month, values of least square means of growth traits were found significantly (P<0.01) higher in BB genotype (23.667±0.555 kg) followed by AA genotype (21.667±0.961 kg) and lowest in AB genotype (19.785±0.240 kg) for *GH*1 locus. Similar finding was reported by Gitanjli *et al.* (2020) and Pandya *et al.* (2021) reported superiority of BB genotypes at 9 month of age (Gaddi goats) and 6 months of age (Surti goats), respectively. Non-significant association among genotypes with body weight, growth and morphometric traits was reported by Radhika *et al.* (2016) in native goat breed of Kerala, Aradhana *et al.* (2021) in Ganjam and Baigani goats and Rahijane *et al.* (2022) in Boer goat respectively. Deviation in the results obtained in the study may be due to population size, breed, environmental condition, complex genotype-environment interactions, difference in the managemental practices and area specific breeding polices.

CONCLUSION

In this study, PCR-RFLP investigation revealed that *GH*1 was found to be polymorphic and *GH*2 was monomorphic in Beetal goat by using restriction enzyme *HaeIII*. The BB genotype has significant association with higher body

weight followed by AA and AB genotype at 9 and 12 month of age that might be a putative genetic marker for early selection of goat for improving body weight through marker-assisted selection.

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Conflict of interest

All authors declare that they have no conflicts of interest.

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