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Effect of *GH* gene polymorphism on growth traits in goats of Meghalaya's subtropical hilly region

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ABSTRACT

Growth hormone (*GH*) gene regulates milk production, reproduction, and growth in animals. Therefore, the present study was performed to investigate the genetic variations in the *GH* gene and their relationship with biometric features in the Sirohi and Assam Hill goats. PCR-RFLP was used to detect polymorphic loci (GH1-HaeIII and GH2-HaeIII) associated with growth parameters including body weight (kg), body length (cm), height at the withers (cm), and chest girth (cm) in the Assam Hill (n=114) and Sirohi (n=47) goats breeds. According to PCR-RFLP results, the *GH* gene's GH1-HaeIII locus contains two alleles (A and B) and two genotypes (AA and AB), whereas the GH2-HaeIII locus contains two alleles (C and D) and two genotypes (CC and CD). In both the goat breeds, A and B allele frequencies were 0.36-0.28 and 0.64-0.72, respectively, while the frequencies of alleles C and D were 0.71-0.70 and 0.29-0.30. The polymorphic loci were demonstrated to be in a state of Hardy-Weinberg disequilibrium in both breeds of goats. The growth characteristics of the Assam Hill goat breed were shown to be correlated with *GH* gene variations. In both of these breeds, AB and CD genotypes displayed slightly higher values of certain biometric traits than the AA and CC genotypes; however, only in Assam Hill goats, body length and chest girth were significantly correlated. Hence, the AB and CD genotypes have been proposed as potential markers for improved growth parameters in the examined goat breeds; however, more research with a larger sample size must be conducted to substantiate this finding.

Keywords: *GH* gene, Goat, Growth trait, Polymorphism

Raising goats is a common and widespread practice across many parts of the world, and products made from goats are trendy (Gooki *et al.* 2019, Khorshidi *et al.* 2019). Despite significant agricultural shifts caused by corporate mergers, globalization, and technological advancements in developed countries, the worldwide goat population has surged (Gooki *et al.* 2019). According to the 20th Livestock Census (2019), the number of goats in the nation are 148.88 million. This is an increase of 10.14% compared to the last Livestock Census (2012) findings and accounts for about 27.8% of the overall livestock population (Livestock Census 2019). The small to medium-sized Assam Hill goat (AHG) is a native goat breed in India's Hill-mountain ecosystems, known for its prolificacy, early sexual maturity, and high-quality meat (Sarmah *et al.* 2020a, 2020b). The Sirohi goat (SG) breed is the most significant and renowned goat breed due to its disease resistance, adaptability to challenging weather conditions, production and growth performance despite insufficient diet and management, and so on.

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Linear body measures and development are crucial for the production of meat from animals (Mohammed and Amin 1997, Khan *et al.* 2006). Growth characteristics have a clear correlation with the productive lifespan of animals (Mendonca *et al.* 2019). These attributes are influenced by a combination of genetic factors and various environmental variables (Favero *et al.* 2019). Comprehending the genes and associated chromosomal regions related to desirable growth characteristics may greatly assist in determining the breeding worth of progeny (Ceccobelli *et al.* 2022). To assess the breeding value of offspring, understanding the genes and chromosomal locations associated with desirable growth traits might be beneficial (Ceccobelli *et al.* 2022).

Growth hormone (*GH*) is a member of somato-lactogenic hormones. It is encoded by a single gene located on the short arm of chromosome 19q22. The gene is 2.5 kb long and is made up of five exons and four intervening introns. GH is produced and secreted by somatotroph cells in the anterior lobe of the pituitary gland in a circadian and pulsatile cycle (Ayuk and Sheppard 2006). It is a single polypeptide hormone with a molecular weight of 22kDa and 191 amino acids (An *et al.* 2010). *GH* encompasses an impact on several physiological processes like growth, reproduction, lactation, and metabolism (Bayan *et al.* 2018, Gitanjli *et al.* 2020).

GH is a well-studied candidate gene that regulates growth rates and lactation yield traits in several livestock species (Thomas *et al.* 2007) including goats (Amiri *et al.* 2018, Kour *et al.* 2018). Several researchers have studied SNP markers related to the growth and morphometric features of goats (Memon *et al.* 2021, Pandya *et al.* 2021). There have been reports of GH gene polymorphism in a few goat and sheep breeds from both India and globally. These breeds include: Sangamneri and Osmanabadi goats (Sheevagan *et al.* 2015), Black Bengal goats (Buranakarl *et al.* 2024), Beetal goats (Dhillon *et al.* 2024), Gaddi goats of Western Himalayas (Gitanjli *et al*. 2020), Surti and Mehsani Goats (Bayan *et al.* 2018), Harnali sheep (Kumar *et al.* 2024), Dorper sheep (Madikadike *et al.* 2024), and Merino cross rams (Putra *et al.* 2024).

Association study of *GH* locus has been confirmed with different traits in Jamunapari goats such as body weight and chest girth at birth (Singh *et al.* 2018), birth and weaning weight and weaning chest girth in Boer goat bucks (Hua *et al.* 2009), body weight at different months of age in Gaddi goats of western Himalayas (Gitanjli *et al.* 2020), Boer goat does (Rashijane *et al.* 2022) and Beetal goats (Dhillon *et al.* 2024). The *GH* gene is often studied in goats to understand genetic variations and its physiological significance. However, there has been a dearth of research on how various *GH* gene variants affect goats' growth rates and other traits, especially in Assam Hill and Sirohi goat breeds. Hence, this research intends to determine the *GH* gene polymorphisms and explore their relationship with growth attributes in Assam Hill and Sirohi goats, to obtain valuable information for goat genetic resources and breeding programs.

MATERIALS AND METHODS

Location and sampling of animals: A total of 161 goats from two well-known Indian native goat breeds (Assam Hill goat [AHG] n=114; Sirohi goats [SG] n=47) were used for the study. All selected individuals were healthy and unrelated. The animals were selected from different goat breeding farms in the North Eastern Hilly (NEH) region of Meghalaya (India). The elevation of Meghalaya ranges from 150 m to 1961 m above sea level and is located between 25.1°N and 26.7°N latitude and 89.50°E and 92.48°E longitude.

The animals were raised using semi-intensive methods that let them graze for 6-7 h/day. Individual measurements of growth characteristics, including body weight (kg), body length (cm), height at the withers (cm), and chest girth (cm), were taken from the resource population.

Isolation of genomic DNA: Blood samples (3 ml) were taken from the tested goat breeds through jugular vein piercing and stored at -20°C until DNA isolation. Qiagen DNeasy Blood & Tissue kit was used to extract genomic DNA from blood samples. Quantification of extracted DNA was assessed by Biospec-nano spectrophotometer. The purity of DNA was evaluated by determining the absorbance ratio at 260 nm and 280 nm. A range of 1.8-2.0 was considered acceptable, and then the sample was diluted to 50 ng/µL for further analysis.

Primer design, PCR, and sequencing: According to Mahrous *et al.* (2018), three primers were employed to amplify the GH1-GH2-GH6 loci of the goat growth hormone gene (Table 1). The cycling protocol was: 94°C for 4 min; 35 cycles of 45 s at 94°C; annealing for 45 s at 58.4-64°C, 72°C for 30 s, with a final extension at 72°C for 7 min. Each amplicon was electrophoresed on 1.8% agarose gels with 1× TAE buffer (Figs. 1 A-C).

Restriction fragment length polymorphism (RFLP): For restriction digestion, 20 µL of reaction mixture was prepared, including 10 µL of PCR product, 7.5 µL of nuclease-free water, 2.0 µL of 10×buffer, and 0.5 µL of Takara Bio HaeIII restriction enzyme. It was incubated at 37°C for 45 min. A UV transilluminator showed the digested fragments separated by electrophoresis in a 3.5% agarose matrix. Gels were photographed using the GeNeiTM imaging system.

Statistical analysis: Pop Gene version 1.32 was used for the Hardy-Weinberg test ($P \leq 0{\cdot}05$) for estimated values of gene and genotype frequency, Shannon index, predicted heterozygosity, effective allele number, and level of polymorphism (Yeh *et al.* 1999). The SPSS Version 16.0 (https://spss.software.informer.com/16.0/) general linear model (GLM) technique was utilized to analyze the associations between genotypes and growth attributes, including body weight (kg), body length (cm), height at the withers (cm), and chest circumference (cm). An analysis of variance was conducted using the statistical model below:

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

where, Y_{ij}, adjusted value of type traits of j^{th} animal to i^{th} genotype; μ, overall mean; G_i, fixed effect of i^{th} genotype; e_{ij}, random error associated with Y_{ij} observation and supposed

Table 1. Growth hormone (GH) primer sequences, PCR product size and their amplified location

Gene	Primers sequence (5′-3′)	Amplified region	Size of PCR products (bp)	Ta (°C)	Reference
GH1	F: CTCTGCCTGCCCTGGACT	Exons 2 and 3	422		Mahrous
	R: GGAGAAGCAGAAGGCAACC			64.0	*et al.* (2018)
GH2	F: TCAGCAGAGTCTTCACCAAC	Exon 4	116	64.0	
	R: CAACAACGCCATCCTCAC				
GH6	F: CCATCCAGAACACCCAGGT	Exon 3	405	58.4	
	R: CCAAGCTGTTGGTGAAGACTC				

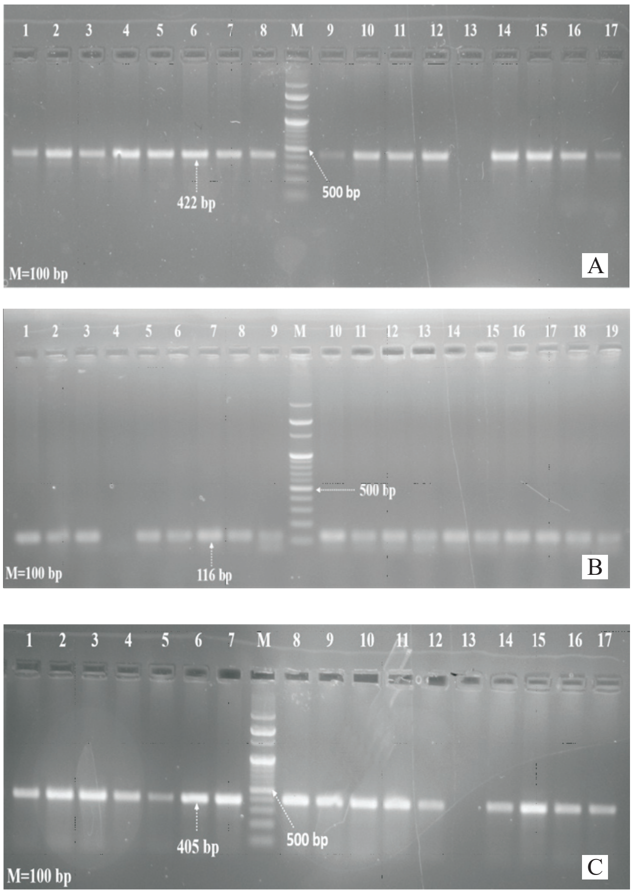

Fig.1. PCR amplification product of GH gene in Assam Hill and Sirohi goats: (A) *GH1* (422 bp); (B) *GH2* (116 bp); (C) *GH3* (405 bp) [Lane M = 100 bp Marker].

to be NID (0, σ^2e). The MEGA X and iTOL programs were used to construct a phylogenetic tree (neighbor-joining method).

RESULTS AND DISCUSSION

SNPs identification and allele frequencies: The *GH1*, *GH2*, and *GH6* polymorphisms were examined at three loci within the *GH* gene. The HaeIII restriction enzyme was responsible for the digestion of the SNP located at *GH1-HaeIII*. In both Assam Hill and Sirohi goats, restriction digestion yielded two alleles, designated A (an uncut segment of 422 bp) and B (366 bp and 56 bp segments). Genotypes BB (366 bp & 56 bp) and AB (422 bp, 366 bp & 56 bp) were identified with no homozygous genotype AA detected in the studied breeds (Fig. 2A).

Concerning the *GH2* gene, the SNP at *GH2-HaeIII* was digested by the HaeIII restriction enzyme. At this locus, the homozygous genotype CC (116 bp) and heterozygous genotype CD (116, 88, and 28 bp) were detected without the homozygous genotype DD in both goat breeds (Fig. 2B).

For the *GH6* gene, the restriction site was revealed in the *GH6-HaeIII* site, and following restriction digestion with HaeIII, only one allele (B) was generated, while allele A was absent. Among the investigated breeds, only genotype BB was found (108, 78, 70, 48, 44, 40, and 17 bp) (Fig. 2C), suggesting a state of monomorphism.

Genetic parameters of populations and Hardy Weinberg equilibrium (HWE): Two polymorphic sites were found in the studied goat breeds. Assam Hill and Sirohi goat have a heterozygous and homozygous genotype at the *GH1-HaeIII* and *GH2-HaeIII* loci and only one genotype at the *GH6-HaeIII* locus. Allele A of locus *GH1-HaeIII* was shown to have the lowest frequency (0.36), whereas allele B of the same locus was revealed to have the highest frequency (0.64) in the Assam Hill goat. Similarly, it was found that allele A of locus *GH1-HaeIII* was present at a minimum frequency (0.28), whereas allele B was present at a maximum frequency (0.72) in Sirohi goats (Table 2). Hardy Weinberg Equilibrium significantly deviated in all polymorphic loci among studied breeds ($P<0.05$). The loci's polymorphic information content (PIC) values in the current investigation were moderate, ranging between 0.32 to 0.35 and 0.32 to 0.36 in Assam Hill and Sirohi goats, respectively.

Genetic divergence and phylogenetic analysis: The phylogenetic relationship of the *GH* gene of Assam Hill

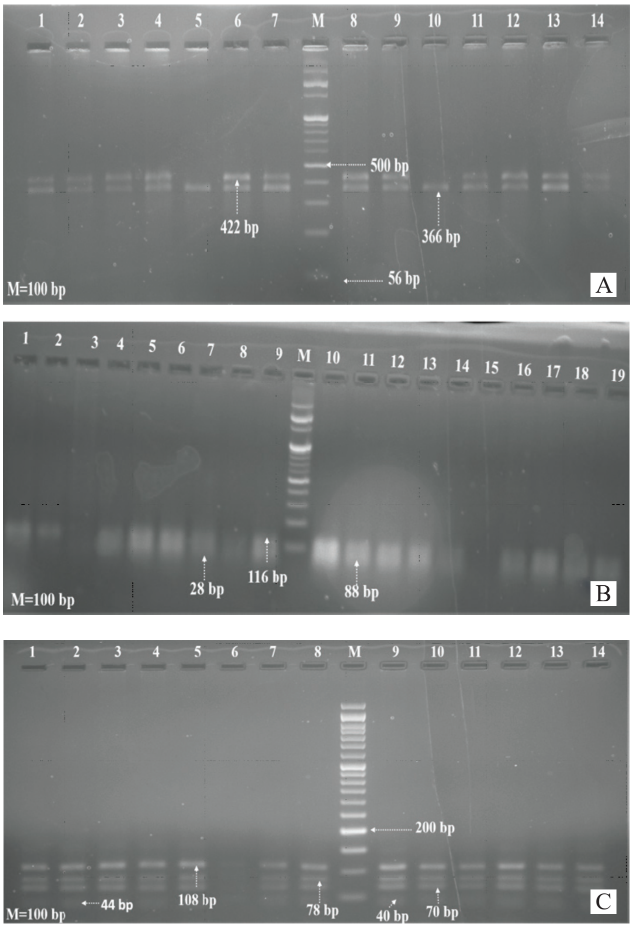

Fig. 2. Representative genotyping of *GH* gene at different loci in Assam Hill and Sirohi goats: (A) *GH1-HaeIIII* locus (422 bp, 366 bp & 56 bp); (B) *GH2-HaeIIII* locus (116 bp, 88 bp, 28 bp); (C) *GH3-HaeIIII* locus (108 bp, 78 bp, 70 bp, 44 bp & 40 bp).

Table 2. Genetic parameters of the *GH1*, *GH2*, and *GH6* genes in Assam Hill and Sirohi goats

Gene	Genotype frequency			Allele frequency		Ne*	I*	Nei*	Obs_Hom	Obs_Het	Exp_Hom*	Exp_Het*	Ave_Het	PIC*	χ^2*
Assam Hill goat (AHG)															
GH1-HaeIII	AA 0.32	AB 0.82	BB 0.00	A 0.36	B 0.64	1.85	0.65	0.46	0.28	0.71	0.5374	0.46	0.46	0.35	35.45
GH2-HaeIII	CC 0.48	CD 0.66	DD 0.00	C 0.71	D 0.29	1.69	0.60	0.41	0.42	0.57	0.58	0.41	0.41	0.32	18.58
GH6-HaeIII	AA 0.00	AB 0.00	BB 1.00	A 0.0	B 1.0	-	-	-	-	-	-	-	-	-	-
Sirohi goat (SG)															
GH1-HaeIII	AA 0.10	AB 0.37	BB 0.00	A 0.28	B 0.72	1.91	0.67	0.47	0.21	0.78	0.51	0.48	0.47	0.36	19.19
GH2-HaeIII	CC 0.18	CD 0.28	DD 0.00	C 0.70	D 0.30	1.74	0.61	0.42	0.38	0.61	0.56	0.43	0.42	0.32	8.97
GH6-HaeIII	AA 0.00	AB 0.00	BB 1.00	A 0.0	B 1.0	-	-	-	-	-	-	-	-	-	-

ne*, effective number of alleles; I*, Shannon's information value; Nei*, probable heterozygosity; Obs Hom*, observed homozygosity; Exp Het*, expected heterozygosity; Ave Het*, average heterozygosity; PIC*, Polymorphic information content and χ^2, chi-square value.

and Sirohi goats was established with *Mus musculus*: NC_000077.7.1, *Gallus gallus*: NC_052558.1, *Ovis aries*: NC_056064.1, *Bubalus bubalis*: NC_059159.1, *Homo sapiens*: NC_000017.11, *Rattus norvegicus*: NC_051345.1, *Bos taurus*: NC_037346.1); *Sus scrofa*: NC_010454.4, *Capra hircus*: NC_030826.1 and *Equus caballus*: NC_009154.3 by obtaining the sequence from NCBI using BLAST program (Fig. 3). Based on a phylogenetic tree, it was found that the GH genes of studied breeds are grouped differently from the genes of other breeds and species.

Analyzing the linkage disequilibrium (LD) between polymorphic loci in the Assam Hill and Sirohi goat populations: It was determined that polymorphic loci in the studied population had exceptionally high pair-wise LD. This was demonstrated by more than 0.50 and 0.20 being found for pair-wise Lewontin's D' and r^2 values, respectively. The D' and r^2 values in both breeds were similar (Fig. 4).

Relationship between genotypes and growth traits in Assam Hill and Sirohi goats: Associations of different variant loci with the four growth characteristics such as body weight, height at withers, body length, and chest girth of examined goat breeds are given in Tables 3 and 4. At *GH1-HaeIII* locus, Assam Hill goat with the AB genotype (34.08±1.23) demonstrated significantly (P<0.05) superior body length compared to those with the AA genotype (32.35±1.45) at 6 months of age (Table 3). Likewise, the AB genotype revealed slightly higher values for 1-month body length (24.18±0.65), 3-month body length (27.31±0.24), and 6-month body weight (8.14±0.08) when compared to the AA genotype. However, these differences did not reach statistical significance (P<0.5) in the AHG. In addition, the AB genotype of the *GH1-HaeIII* locus in Sirohi goats performed better than the AA genotype for certain growth traits, including 1-month body weight (4.13±0.23 kg), 1-month height at withers (37.11±0.31 cm), 3-month body weight (11.12±0.07 kg), 3-month body length (46.51±1.33), 3-month chest girth (51.07±1.62) and 6-month body weight (16.11±0.53). However, none of these differences were statistically significant (P>0.05), as shown in Table 4.

In relation to the *GH2-HaeIII* locus, it was found that Assam Hill goats with the CD genotype possessed a significantly (P<0.05) higher chest girth (43.09±1.51)

Table 3. Association of *GH1-HaeIII* and *GH2-HaeIII* genotypes of *GH* gene with growth attributes (Mean±S.E.) in Assam Hill goat

Growth trait	*GH1-HaeIII*			*GH2-HaeIII*		
	Genotype AA	Genotype AB	P-value	Genotype CC	Genotype CD	P-value
1-month body weight (kg)	1.92±0.43	2.01 ±0.07	0.827	1.87 ±0.11	1.93 ±0.25	0.612
1-month body length (cm)	23.13±0.89	24.18±0.65	0.316	22.01 ±0.66	23.20 ±0.06	0.210
1-month height at withers (cm)	28.49±0.73	28.16±0.54	0.289	28.11±0.82	27.84±0.43	0.139
1-month chest girth (cm)	33.45±1.19	33.57±1.26	0.956	34.21±1.07	34.07±1.40	0.671
3-month body weight (kg)	5.37±0.11	4.78 ± 0.06	0.312	5.10±0.79	5.22 ± 0.11	0.393
3-month body length (cm)	26.77±0.53	27.31±0.24	0.411	26.03±0.07	27.18±0.60	0.508
3-month height at withers (cm)	33.60±0.76	33.12±0.27	0.203	35.48±0.23	34.28±0.46	0.189
3-month chest girth (cm)	42.11±1.09	42.19±1.17	0.178	41.66±1.23[a]	43.09±1.5[b]	0.049
6-month body weight (kg)	7.50±0.12	8.14±0.08	0.266	8.07 ± 0.37	9.12 ± 0.47	0.451
6-month body length (cm)	32.35±1.45[a]	34.08±1.23[b]	0.043	34.07±1.68	34.12±1.10	0.638

Dissimilar superscripts show significant differences: P<0.05.

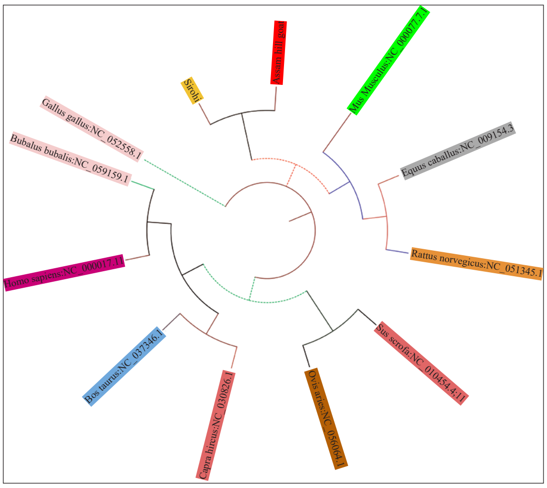

Fig. 3. Phylogenetic tree shows evolutionary relation among different animal breeds/species (using MEGA X and iTOL program).

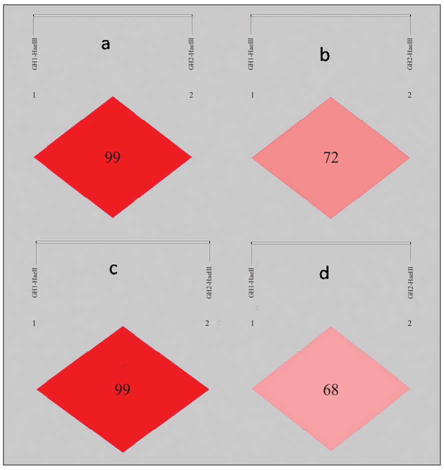

Fig. 4. LD analysis: (a) r^2 values in Assam Hill goat; (b) D′ values in Assam Hill goat; (c) r^2 values in Sirohi goat; (d) D′ values in Sirohi goat.

compared to those with the CC genotype (41.66±1.23) at three months of age. However, no significant differences were observed between different genotypes in relation to other growth traits (Table 3). Similarly, in the context of Sirohi goats at the *GH2-HaeIII* locus, individuals with the CD genotype demonstrated superior at one-month body weight (4.34±0.10), body length (35.04±0.27), wither height (37.08±0.82) and certain other biometric attributes at different ages compared to those with the CC genotype. However, no statistically significant differences were detected (Table 4). These outcomes align with Singh *et al.* (2015) in Sirohi and Barbari goats regarding body length across the lifespan.

The current study investigated the AB and CD genotypes, which exhibited slightly higher values of certain growth traits compared to AA and CC genotypes in the examined breeds. However, this superiority was statistically significant ($P<0.05$) only for body length and chest girth in the Assam Hill goat. The results of Hua *et al.* (2009) indicated that genotypes did not significantly affect birth weight, height, length, chest circumference, or body weight at 11 months of age; however, they reported a significant effect on chest circumference at birth and weight during weaning. Sarmah *et al.* (2020b) found no significant influence of GH polymorphism on body weights among the various genotypes in Assam Hill goats, which is consistent with the findings of the present investigation. Analogously, Aradhana *et al.* (2021) found that the AB genotype was connected to higher means on morphometric features in Odisha's Ganjam and Baigani goat populations; however, the differences were not statistically significant. Pandya *et al.* (2021) found that at six months of age, goats with BB genotypes had a superior body mass ($P<0.05$) than those with AB genotypes in Surti goats.

Numerous observations indicate that genetic polymorphisms in the *GH1* gene influence livestock economic characteristics (Carter-su *et al.* 2016, Abdelhafez *et al.* 2022, Wang *et al.* 2022). It was recently demonstrated by Yan *et al.* (2022) that two indel polymorphism loci, P49-bp indel (ID) and P1410-bp indel (II), were correlated with chest girth and chest width in Shaanbei white cashmere goats (SBWC). The same findings were reported by Esen and Elmac (2022), who found that yearling lambs with the P1 variant of the growth Hormone Exon-5 (*GHE5*) gene have a higher body length (BL), wider leg girth, and lean cannon bone periphery (CBP) ($P>0.05$), in comparison

Table 4. Association of *GH1-HaeIII* and *GH2-HaeIII* genotypes of *GH* gene with growth attributes (Mean±S.E.) in Sirohi goat

Growth trait	*GH1-HaeIII*			*GH2-HaeIII*		
	Genotype AA	Genotype AB	P-value	Genotype CC	Genotype CD	P-value
1-month body weight (kg)	4.06±0.14	4.13±0.23	0.152	4.10±0.32	4.34±0.10	0.134
1-month body length (cm)	35.10±0.58	35.37±0.41	0.434	34.98±0.13	35.04±0.27	0.367
1-month height at withers (cm)	36.08±0.23	37.11±0.31	0.170	36.11±0.64	37.08±0.82	0.210
1-month chest girth (cm)	36.21±0.09	35.04±0.72	0.542	35.82±0.16	35.01±0.90	0.340
3-month body weight (kg)	10.81±0.18	11.12±0.07	0.319	10.13±0.55	10.84±0.21	0.312
3-month body length (cm)	44.32±1.41	46.51±1.33	0.110	44.05±1.70	45.98±1.01	0.215
3-month height at withers (cm)	50.66±1.18	49.05±1.84	0.270	50.12±1.07	49.66±1.12	0.210
3-month chest girth (cm)	50.12±1.30	51.07±1.62	0.188	50.34±1.12	51.02±1.50	0.171
6-month body weight (kg)	15.07±0.42	16.11±0.53	0.506	14.99±0.16	15.28±0.69	0.423
6-month body length (cm)	52.07±1.17	51.09±1.08	0.741	52.13±1.99	52.02±1.07	0.599

to lambs with the other variant (P2), whose body length is shorter and CBP is thicker. Furthermore, Selionova *et al.* (2022) revealed a favourable connection between the molecular marker among *MSTN*, *HEG1*, *FGF10*, *FGF14*, *GHRH*, and *SLAIN* genes linked to body mass at four months old in Karachai goats. It was also demonstrated by SNP genotyping and their use for discovering selection signatures and applying them as part of GWAS for identifying polymorphic loci associated with various biological aspects and body size in sheep and goats (Gu *et al.* 2022, Saleh *et al.* 2022). As a result, this research led to the finding that the polymorphisms in the *GH* gene in goat populations might be responsible for the superior growth traits.

In conclusion, the HaeIII restriction digestion revealed polymorphism at the *GH1* and *GH2* loci in the examined breed, but the *GH6* locus indicated monomorphism. The association analysis found that the AB and CD genotypes demonstrated slightly greater values in certain growth traits compared to the AA and CC genotypes. However, this superiority was statistically significant only for body length and chest girth in the Assam Hill goat. The current results emphasize the importance of the *GH* gene to improve growth attributes in goat breeding plans. Nevertheless, additional research employing large sample sizes and a diverse range of breeds will be required to verify and subsequently implement these findings.

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