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Polymorphism of insulin-like growth factor-i gene and their association with weight at different age in Assam hill goats

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Abstract
The present investigation was carried out to study the occurrence of polymorphism in Insulin-Like Growth Factor- I (*IGF-I*) gene in Assam Hill Goat, a meat type variety of Assam, India and to study the association of polymorphic genes with body weight at different ages. A total of 256 blood samples, collected from male goats of different field units and Goat Research Station, Assam Agricultural University (AAU), Burnihat under the ICAR sponsored project entitled "ICAR-All India Coordinated Research Project on Goat Improvement" were utilized for the present study. Three genotypes, *viz.* AA (28.91%), AB (27.34%) and BB (43.75%) were detected with frequencies of 0.19, 0.49 and 0.33 respectively. The allele frequency for A and B were observed to be 0.43 and 0.57. Chi-square test revealed that the population was not in Hardy-Weinberg Equilibrium for the gene. The mean body weights (kg) along with SE of the AA, AB and BB genotypes detected in *IGF-I* gene were observed to be 1.16 ± 0.03, 1.21 ± 0.03 and 1.14 ± 0.02; 5.03 ± 0.11, 4.79 ± 0.09 and 4.95 ± 0.08; 6.90 ± 0.13, 7.17 ± 0.13 and 7.50 ± 0.09; 9.93 ± 0.17, 9.88 ± 0.17 and 9.99 ± 0.13 and 13.42 ± 0.18, 12.97 ± 0.18 and 13.16 ± 0.15, respectively. However, statistically there was no significant ($p>0.05$) difference in body weights among the different genotypes.

Keywords: Assam hill goat, polymorphism, growth, insulin-like growth factor

1. Introduction
Eastern and North-Eastern part of India is bestowed with very good quality meat type goats like Black Bengal, Assam Hill goat, Sumi-Ne, Ganjam etc. Chevon contains low cholesterol and high level of iron and potassium as compared to other types of meat [1]. Assam Hill Goat, a native goat variety of the state is mainly reared for meat purpose and the meat is of high quality with good flavour. The goat variety is known for its prolific nature of kidding, early sexual maturity and early kidding age. However, they are smaller in size with low birth weight, weaning weight and matured body weight compared to other Indian breeds of goat. So, in spite of having good quality genetic resources, there is a wide gap between the demand and the supply of meat in Assam. This gap could be bridged by augmenting the production of goat by enhancing growth performance as well as reproductive efficiency of the animals. For the last decade, molecular genetics has lead to the discovery of individual genes or candidate genes with substantial effects on the traits of economic importance. A total of 271 candidate genes have been detected in goats [2]. *IGF-I* gene is considered as a candidate gene for growth rate and meat production traits. In the goat, IGF-I is encoded by a single gene located on chromosome 5 [3], consisting of three leader exons (1W, 1 and 2) and three exons (3, 4 and 6), in which exon 3 and exon 4 encode the mature IGF-I peptide. *IGF-I* is an important component of the somatotrophic axis that plays a key role in postnatal growth and metabolism in mammals including farm animals [4, 5] and is being considered as a promising candidate gene for marker-assisted selection of growth traits. It has been reported that there is association of genetic polymorphisms of the *IGF-I* gene with growth traits in the chicken [6, 7, 8, 9], in swine [10], and in the bovine [11, 12, 13]. There are very few reports on polymorphisms of the goat *IGF-I* gene and the present study was undertaken to identify polymorphisms of the *IGF-I* gene and thereby to investigate association of these polymorphisms with growth traits in the Assam hill goat.

2. Materials and Methods

2.1 Body weight

Body weight of the goats under study was recorded at birth, 3, 6, 9 and 12 months of age with the help of weighing balance. After PCR-RFLP analysis of the amplicons, the goats were divided according to the genotypes.

2.2 Blood collection and DNA extraction

A total of 256 blood samples were collected from bucks maintained at four field units in three different districts of Assam, *viz*., Kamrup (Metro), Kamrup (Rural) and Darrang as well as bucks at the head quarter located at Goat Research Station, AAU, Burnihat, Assam under the project "ICAR-AICRP on goat Improvement". The average temperature and relative humidity of the area ranged from 14.7 to 32.2^{0}C and 51.2 and 93.7% respectively and the average annual rainfall was recorded as 2818.0 millimetres during the study period.

Five ml of blood was collected aseptically from the jugular vein in a vacutainer tube containing 2.7% EDTA as an anticoagulant and then genomic DNA was isolated from the whole blood by following standard protocol [14] with slight modification. The quality of DNA was checked on 1.5% agarose gels stained with ethidium bromide.

2.3 Amplification of *IGF-I* Gene by PCR

The sequences of the forward and reverse primers for the amplification of the *GH* gene in the present study were:

Forward: 5'- CTGCTGGAGATATACTGG -3' (Designed)
Reverse: 5'- GACACTATGAGCCAGAAG -3' (Designed)

PCR was performed in a 25 µl reaction mixture containing 10.7 µl NFW; 0.4 µl Primer F; 0.4 µl Primer R; 12.5 µl master mix and 1µl of genomic DNA template. Thermal cycling conditions included an initial denaturation step at 94°C for 4 min followed by 35 cycles of 94°C for 30s, 48°C for 30s, 72°C for 45s and a final extension at 72°C for 5 min. PCR products were electrophoresed on 1.5% agarose.

2.4 Restriction fragment length polymorphism (RFLP) analysis

The polymorphism was performed by Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP). The PCR products were digested with 0.4 µl *HaeIII* restriction endonuclease (Fermentas) at 37 ^{0}C for 120 min followed by deactivation at 80 ^{0}C for 20 min and were subjected to 12% Polymerase Agarose Gel Electrophoresis (PAGE) respectively and stained with ethidium bromide. Electrophoresis was carried out at 110 V for 1 hour and 15 minutes and the bands were visualized and documented using gel documentation system by comparing with 50 bp DNA ladder.

2.5 Sequence analysis

The PCR amplicons of *IGF-I* gene from goats with different polymorphic fragments were sequenced at first base DNA sequencing division, Malaysia by automated DNa sequencer following Sanger's dideoxy chain termination method [15]. Clustal W method of DNASTAR Software (Lasergene, USA) was used to analyze the sequences generate sequence alignment reports and residue substitution.

3. Statistical analysis

Statistical analysis to find the association between the polymorphic genes with the body weights at different ages were done by using SPSS software version 11.5. Chi-square statistic (χ2) was used to check whether the populations were Hardy-Weinberg equilibrium.

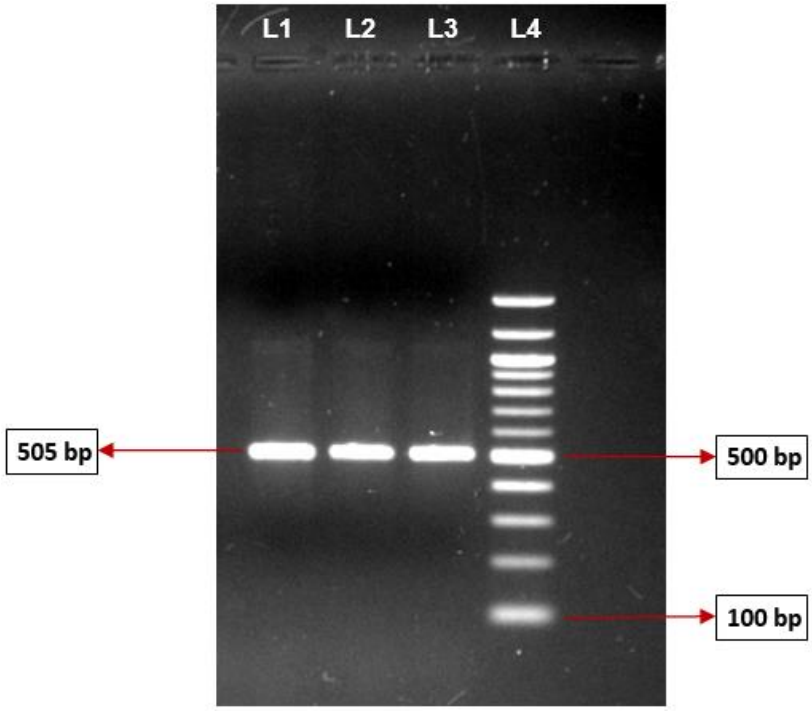
4. Results and Discussion

Amplification of *IGF-I* gene with the primers resulted in generation of 505 bp DNA fragment (Fig 1) which is consistent with the expected size as determined from their gene sequence information. PCR-RFLP analysis of the amplicons revealed three banding patterns (Fig 2). Out of these three patterns, one showed four fragments of 172 bp, 333 bp, 99 bp and 234 bp, another pattern produced three fragments of 99 bp, 172 bp and 234 bp and the last banding pattern formed two fragments with 172 bp and 333 bp in 70 (27.34%), 112 (43.75%) and 74 (2 8.91%) number of samples respectively. The representative PCR amplicons of the samples showing different banding patterns were sent for sequencing and the results of sequencing detected an SNP at 5752th position with nucleotide transversion from G to C.

The allele A (*IGF-I*-C) produced two bands, while allele B (*IGF-I*-G) produced three bands. The present study revealed three genotypes, referred to as AA with two bands, AB with four bands and BB with three bands. The frequency for A & B alleles was observed to be 0.43 & 0.57 respectively. The frequency for AA, AB & BB genotypes was recorded as 0.19, 0.49 & 0.33, respectively. The findings of the present study were comparable to the two alleles and three genotypes in *IGF-I* gene in goats [16, 17, 18, 19, 20, 21, 22]. However, Grochowska *et al.* (2017) and Naicy *et al.* (2017) [23, 24]. Detected two genotypes for the same gene.

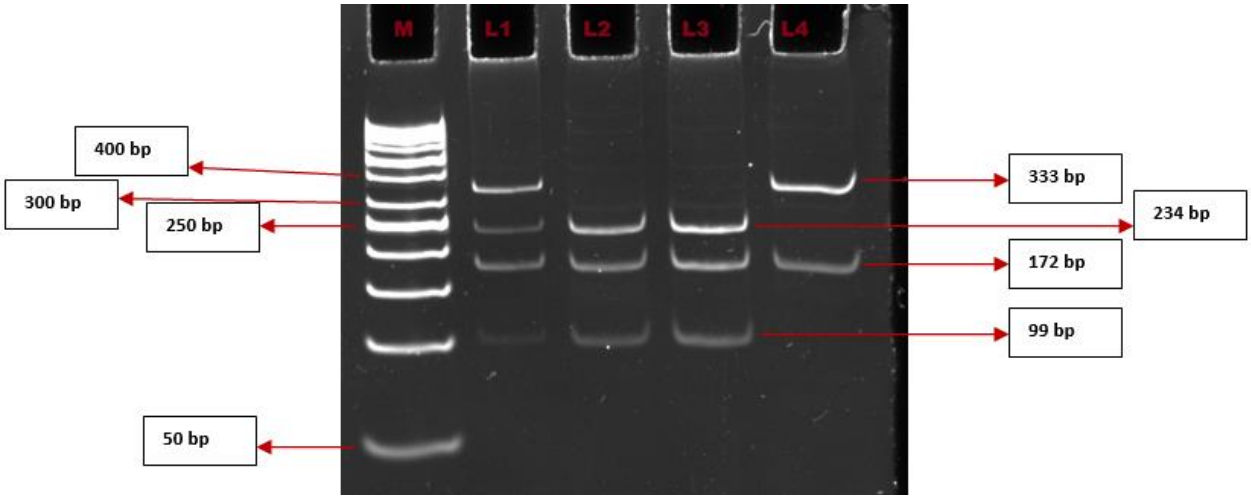
The body weights (mean ± SE) at birth, 3, 6, 9 and 12 months of age for goats with AA genotype were found to be 1.16 ± 0.03, 5.03 ± 0.11, 6.90 ± 0.13, 9.93 ± 0.17 and 13.42 ± 0.18 kg, for AB genotype 1.21 ± 0.03, 4.79 ± 0.09, 7.17 ± 0.13, 9.88 ± 0.17 and 12.97 ± 0.18 kg and for BB genotypes of *IGF-I* gene 1.14 ± 0.02, 4.95 ± 0.08, 7.50 ± 0.09, 9.99 ± 0.13 and 13.16 ± 0.15 kgs respectively.

The body weight of the goats at the same age group among the different genotypes was found to vary. However, statistically, no significant (P>0.05) difference could be observed between them. This might be due to the reason that the mutation (G5752C) occurred in the intron part of the gene which in turn led to no change in the regulation of protein. This finding corroborated with other studies made in Pomeranian Coarsewool sheep [25], female goats [6] and Markhoz goats [21], who also could not find any significant difference between the genotypes detected and the respective body weights. However, significant difference was detected between the genotypes and body weights in nanjiang huang goat, commercial goats of China, Sirohi goats, Markhoz and Kurdi goats and Attappady Black goats [16, 26, 27, 28, 29] where higher performance of one genotype over others was reported.



L1 to L3 = Amplified *IGF-I* gene; M= 100 bp Ladder

Fig 1: Amplified product of *IGF-I* gene



L1 = AB Genotype; L2 and L3= BB Genotype & L4= AA Genotype; M= 50 bp ladder

Fig 2: PCR-RFLP of *IGF-I* gene (12% PAGE)

5. Conclusion

In the present study, PCR-FRLP analysis revealed three genotypes, *viz.*, AA (28.91%), AB (27.34%) and BB (43.75%) in 505 bp of *IGF-I* gene in Assam Hill Goats. The frequency of A & B alleles was observed to be 0.43 & 0.57 respectively. The frequency for AA, AB & BB genotypes was recorded as 0.19, 0.49 & 0.33, respectively. Though Single Nucleotide Polymorphism (SNP) could be detected in the gene, no association, however, could be established between the different genotypes and the respective body weight at birth, 3, 6, 9 and 12 months of age. This might be due to occurrence of the mutation (G5752C) in the intron part of the gene which led to no change in the regulation of protein. Further investigation on other regions of the *IGF-I* to detect SNP and association of the SNP with productive and other traits of economic importance is desirable. Moreover, in the present study, the numbers of samples sequenced were less in number and hence, further studies using a greater number of individuals may be carried out in Assam hill goats.

6. Acknowledge

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7. References

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