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SINGLE NUCLEOTIDE POLYMORPHISMS IN *GH* (GROWTH HORMONE) GENE ASSOCIATED WITH GROWTH TRAITS IN NILAGIRI SHEEP OF TAMIL NADU

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Abstract: The Nilagiri sheep, native to the Nilgiris of Tamil Nadu are medium-sized animals having white coat colour with a convex face line giving them a typical Roman nose. Nilagiri is a dual purpose breed used for meat and fine wool production. Characterization of *GH* gene in Nilagiri sheep revealed two SNPs, 480 G>A (SNP-G1) and 871 G>A (SNP-G2) in Intron 1 and Intron 2 regions respectively. The SNPs of *GH* gene were genotyped in 60 animals using tetra-primer ARMS-PCR. The frequency of GG, GA and AA genotypes of SNP-G1were 0.48, 0.43 and 0.09 respectively. The *G* allele had a frequency of 0.70 and *A* allele had a frequency of 0.30. In SNP-G2 the *G* allele was absent and all the animals screened were of AA genotypes. Data pertaining to growth traits *viz*., birth weight, weaning weight, 6-months weight, 9-months weight and yearling weight were collected and pre-weaning and post-weaning ADG calculated. Using the least-squares analysis of variance, it was found that the SNP-G1 was associated significantly ($P<0.05$) with weaning weight and pre-weaning ADG in Nilagiri breed of sheep. Of the three genotypes, the animals with AA genotype had a significantly ($P<0.01$) higher weaning weight of 13.49 ± 1.15 kg and pre-weaning ADG of 120.89 ± 10.76 g and showed better growth traits and ADG at all ages. Therefore, *A* allele of SNP-G1 could be favourably considered as a marker for selection of weight gain in Nilagiri sheep as this allele is present with a moderate frequency (0.30).

Keywords: Nilagiri sheep, GH gene, SNP, Growth traits.

Introduction

Sheep make a valuable contribution to the livelihood of economically weaker sections of the society and sheep with its utility for meat, wool, skin and manure form an important component of rural economy. As per the 18th Livestock Census (2007), the sheep population in India is 71.6 million and India has a rich diversity of sheep genetic resources with 42 recognized breeds of sheep out of which Tamil Nadu has eight breeds. Many important traits like milk production, wool production, litter size etc. are multi-factorial - both genes and environment influence them. Despite impressive achievements in the understanding of gene structure and expression, molecular genetics had made little direct contribution to animal

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breeding and even in more advanced countries animal breeding relies heavily on the principles of quantitative genetics. The application of molecular genetics in identification of polymorphism in growth candidate genes that show association with specific economically relevant traits provide useful information to enhance genetic improvement programme in livestock and validation of genetic markers of growth traits is the initial and crucial step to establish a Marker Assisted Selection system (MAS). The genes that operate in the somatotropic axis mainly Growth Hormone (GH) is responsible for post-natal growth and development, tissue growth, lactation, reproduction as well as protein, lipid and carbohydrate metabolism. GH is an anabolic hormone synthesized in the anterior lobe of the pituitary gland and aid in body's immune response, wound healing and haematopoiesis. Current knowledge in production biology indicates that genetically superior animals differ from inferior animals mainly in their regulation of nutrient utilization and that GH exerts a key control in nutrient use. With this information, the present study was conceptualized and carried out to study the polymorphism in *GH* gene and its association with growth traits in Nilagiri sheep of Tamil Nadu and to analyse the potential of this gene to act as markers for growth traits.

Materials and Methods

The complete sequence of the ovine *GH* gene was obtained from the online database, the National Centre for Biotechnological Information (NCBI) (www.ncbi.nlm.nih.gov) (Gene accession number NC_019468 and GeneID: 443329). The entire *GH* gene (2162 bp) including five exons and four introns was amplified by designing four sets of primers using an online software tool "Primer3" input version 0.4.0 (http://frodo.wi.mit.edu/primer3/). The exon sequences were selected along with some intronic sequences for proper amplification. After PCR amplification and verification in agarose gel, the amplicons were sequenced by using automated ABI PRISM 3730XL Genetic Analyzer (Applied Biosystems, USA. Sequence data were analysed using the SeqMan program of LASERGENE software (DNASTAR Inc., USA) for identification of the SNPs. The SNPs detected in the *GH* gene were genotyped using tetra-primer ARMS-PCR in the population. The phenotypic data pertaining to the growth traits *viz*. weight in kilograms at birth, weaning, six months, nine months and 12 months of age for the Nilagiri sheep were collected from Sheep Breeding Research Station, Sandynallah of the Tamil Nadu Veterinary and Animal Sciences University. The ADG in g/day is calculated as

$ADG = (y_{t2} - y_{t1}) / (t_2 - t_1)$

Where, y_{t1} and y_{t2} refer to body weights at t1 and t2 ages in days respectively[1] [1].

Statistical Analysis

The least-squares analysis of variance[2] [2] was performed to study the association between SNPs and the phenotypic measures on growth traits. The statistical model used for the effect of different SNPs on the growth traits was as follows:

$$Y_{ijk} = \mu + S_j + G_k + e_{ijk}$$

Where, Y_{ijk} = Body weight of the i^{th} animal with k^{th} genotype and of the j^{th} sex

μ = Overall mean

S_j = Effect of j^{th} sex

G_k = Effect of k^{th} genotype

e_{ijk} = Random error associated with each observation, NID (0, $\sigma^2 e$)

Results and Discussion

GH gene has five exons and four introns. On analyzing the complete gene using four primers it was found that all the exons were highly conserved and did not show any polymorphism. In the Nilagiri sheep two SNPs, both transitions, 480 G>A (SNP-G1) and 871 G>A (SNP-G2) were identified in Intron 1 and Intron 2 regions respectively (Fig. 1 and 2). For the SNP-G2, all the animals had only A allele with respect to the G allele in the reference sequence and neither GA nor GG genotypes were identified. For SNP-G1, all three genotypes were identified in the screened population and tetra-primer ARMS protocol (Fig. 3) was used to genotype all the animals. The observed genotype frequencies were 0.48, 0.43 and 0.09 respectively for GG, GA and AA genotypes. The allelic frequencies of G and A were 0.70 and 0.30 respectively.

Until now, only a few polymorphism of *GH* gene have been detected in small ruminants and sheep has been a less studied species. Exon 1 of *GH* gene was found to be monomorphic in Portuguese ewes[3] [3] and in Gansu sheep[4] [4]. The Exons 2 and 3 were found to be polymorphic in Portuguese ewes[3] [3]. In Exon 4, two SSCP patterns in Portuguese indigenous sheep [5], three conformational patterns in Kordian sheep[6] [6] and five patterns in Makooei sheep[7] [7] were reported. The Exon 5 showed five SSCP patterns in Portuguese indigenous sheep[5] [5], three patterns in Iranian Dalagh sheep[8] [8], three patterns in Baluchi sheep[9] [9] and three patterns in Zel sheep[10] [10].

SNP-G1was found significantly ($P<0.05$) associated with weaning weight in Nilagiri breed of sheep. Of the three genotypes, the AA genotype had a higher weaning weight of 13.49 ± 1.15 kg, when compared to GA and GG genotypes, the difference being significant ($P<0.05$) statistically. However, this SNP did not have any effect on body weights recorded at later ages

(six months, nine months and yearling weights) in Nilagiri sheep. The pre-weaning ADG was also significantly ($P<0.05$) higher for AA genotypes in Nilagiri breed of sheep (120.89 ±10.76 g) (Table 1 and 2). While studying the SSCP patterns of various exons of *GH* gene, milk yield in Serra de Estrela sheep was found to be influenced by SSCP polymorphism[11] [11] and a positive effect of pattern G4 of Exon 4 with heart girth, scrotal circumference and wool traits was seen. There was no association found between the SSCP patterns of Exon 5 and wool traits or yearling weight[10] [10] or lambing rate[12] [12] in Zel sheep.

Conclusion

From the present study it was seen that the exons of *GH* gene were highly conserved and did not show any polymorphism. The animals with *A* allele of SNP-G1 performed consistently better for all traits in all age groups. This shows that the *A* allele of this SNP could be favourably considered as marker for selection for weight gain in Nilagiri sheep as this allele is present with a moderate frequency (0.30).

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Table 1: Least-squares means ± S.E. (kg) for the effect of SNP-G1 on body weights of Nilagiri sheep

	Body weight (kg)				
Genotypes	Birth	Weaning	6-months	9-months	Yearling
Overall	2.52 ± 0.13 (58)	11.48 ± 0.49 (57)	15.86 ± 0.48 (57)	19.06 ± 0.74 (56)	22.84 ± 0.86 (57)
GG	2.26 ± 0.16 (28)	10.19[b] ± 0.57 (28)	15.30 ± 0.56 (28)	18.78 ± 0.85 (28)	21.96 ± 0.99 (28)
GA	2.68 ± 0.13 (25)	10.77 [b] ± 0.44 (25)	15.55 ± 0.43 (25)	19.04 ± 0.66 (25)	22.69 ± 0.76 (25)
AA	2.62 ± 0.31 (5)	13.49 [a] ± 1.15 (4)	16.74 ± 1.12 (4)	19.36 ± 1.78 (3)	23.86 ± 2.07 (4)

Table 2: Least-squares means ± S.E. (g) for the effect of SNP-G1 on ADG of Nilagiri sheep

Genotypes	Pre-weaning	Post-weaning
Overall	99.71 ± 4.62 (57)	41.75 ± 2.23 (56)
GG	88.26[b] ± 5.34 (28)	42.44 ± 2.55 (28)
GA	89.97[b] ± 4.14 (25)	43.26 ± 1.97 (25)
AA	120.89[a] ±10.76 (4)	39.57 ± 5.34 (3)

Figure 1. Chromatogram showing 480 G>A transition in Intron 1 of *GH* gene in Nilagiri sheep (all three genotypes could be identified)

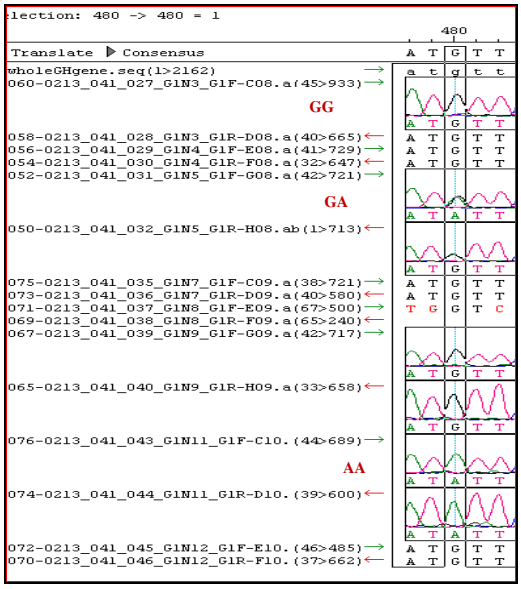

Figure 2. Chromatogram showing 871 G>A transition in Intron 2 of *GH* gene in Nilagiri sheep (only AA genotypes could be identified)

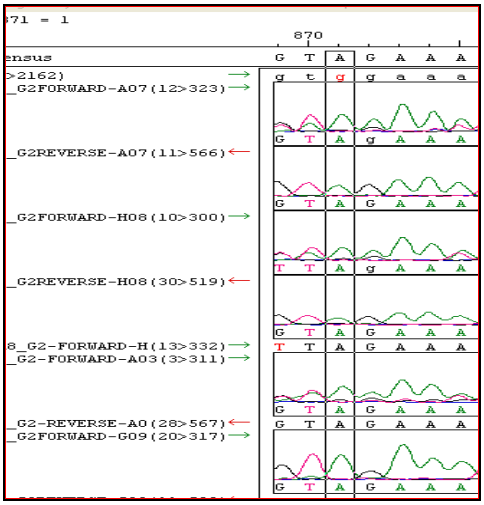

Figure 3. Tetra-primer ARMS-PCR genotyping of SNP-G1
M = Marker 50 bp ladder