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PCR-SSCP of growth hormone gene and its association with body weight in Black Bengal goat

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ABSTRACT

Present study was undertaken to investigate the single nucleotide polymorphism within growth hormone gene and its correlation with body weight in Black Bengal goat. Two fragments of growth hormone gene, 245 bp fragment (partial intron 1, exon 2 and partial intron 2) and 472 bp fragment (partial intron 2, exon 3 and intron 3 and partial exon 4) were analyzed for detection of polymorphism expected to be present at this locus. SSCP of 245 bp and 472 bp fragment revealed 4 and 5 genotypes, respectively. Sequencing revealed substitution at 5 places in 245 bp fragment whereas at 6 places in 472 bp fragment of growth hormone gene. Least square analysis revealed that only 472 bp fragment genotypes had significant effect on body weight at 6 and 9 month of age. Animals having AC genotype had the highest birth weight, whereas animals having CC genotype had lowest birth weight.

Key words: Goat, Growth hormone, Polymorphism, Sequencing

Growth hormone (GH), either directly or indirectly, is the main regulator of postnatal somatic growth, stimulating anabolic process and protein synthesis and its deposition in tissues and organs. Black Bengal, one of the most prolific breeds of goat in eastern India (Bihar, Bengal, Odisha, Asom etc), is known for its delicacy in meat, superior hide quality and fetches high prices even in the local market. Genetic improvement in livestock species, relies on spreading the germplasm of most superior male. Unfortunately, owing to market conditions, almost the opposite is happening with Black Bengal as fastest growing male reaches the market weight fast and sold prior to having any genetic impact for meat production as they fetch good money. In fact often the poorest males are used for breeding, leading to a negative selection response. This problem can be overcome by identification of molecular marker for growth traits so that the superior animal can be identified at the time of birth itself and reared specifically for breeding purposes. Growth hormone gene was widely investigated and used as marker for milk production in cattle (Khatami *et al*. 2005), sheep (Marques *et al*. 2006) and goats (Malveiro *et al*. 2001). However, association of GH gene with growth traits has been mainly carried out in cattle. bGH genotype has significant effect on yearling weight with positive effect associated with LV genotype in the Canchim beef cattle (Pereira *et al*. 2005). A heterozygous GH-MSP I RFLP genotype had greater daily average gain and carcass measure, then the homozygous genotype in Brangus cattle (Thomas *et al*. 2007). In goat, studies mainly focused on association between GH polymorphism with milk production traits. Workers had reported positive correlation of GH genotype GH2-N and GH2-Z with milk production in Portuguese Algravia breed (Malveiro *et al*. 2001). A few reports are available in literature for association of GH gene with caprine growth traits. PCR-SSCP analysis of gGh 5' region revealed that AA genotype had significantly higher weight at birth and yearling, compared to BB and AB genotype in Boer goat breed (Min *et al*. 2005). Hua *et al*. (2008) studied the GH gene in Boer goat population and reported 2 SNPs located in exon 2 (A781G) and 4 (A1575G). They found that goat with AB genotype weighed about 2 kg heavier than those with AA genotype at weaning and measured 1.4 cm greater than those with AA genotype in chest girth at birth. Dayal *et al*. (2014) reported that growth hormone genotypes significantly affected on birth weight in Black Bengal goat. Despite the important role of GH gene on growth performance traits, it is not explored in Black Bengal goat to study its association with growth performance traits. A few reports are available in which exon 4 and 5 was studied only for SNP identification (Gupta *et al.* 2007). Therefore, the present study was undertaken to identify polymorphism of growth hormone gene and to study its association with body weight in Black Bengal goat.

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MATERIALS AND METHODS

Sample: Blood samples were collected randomly from 100 Black Bengal goats from the organized herds. Genomic

DNA was extracted from 5 ml of blood by phenol-chloroform extraction method.

PCR amplification: Two fragments of growth hormone gene, first fragment comprising 245 bp fragment (partial intron 1, exon 2 and partial intron 2) and second fragment comprising 472 bp fragment (partial intron 2, exon 3 and intron 3 and partial exon 4) of growth hormone gene were analyzed for detection of polymorphism expected to be present at these loci. The primers used for amplification were designed on the basis of sequence available publicly at NCBI. Primers used for amplification of first fragment were forward, 5' ATCAGGCGTCTAGCTCTCTGG3' and reverse 5'CTCTAGGACACATCTCTGGGG3' where as for second fragment, primers were forward, 5'GGGGAGGG-TTCCGAATAAGG3' and reverse, 5'CCCAAGCC-ACGACTGGATAA3'. PCR cycling conditions were standardized with different concentrations of $MgCl_2$, *Taq* polymerase, dNTPs and primers. PCR reaction is performed in 25 µl with 100 ng of genomic DNA, 15 pmoles of each primer, 2 mM of $MgCl_2$, 100 µM of each dNTP, 1× PCR reaction buffer and 1 U of *taq* DNA polymerase. PCR programme followed for amplification of gene fragments was initial denaturation for 95°C for 2 min then 30 cycles of denaturation at 95°C for 30 sec, annealing at 60°C for 45 sec for first fragment and 58°C for second fragment, extension at 72°C for 45 sec and then final extension of 72°C at 5 min. Subsequently, the SSCP study was carried out to identify different allelic patterns and genotypes of the animal included in the study.

Single strand conformation polymorphism: PCR product (3 µl) was properly mixed with 15 µl formamide dye (95% formamide, 0.025% xylene cyanol, 0.025% bromophenol blue, 0.5 M EDTA). The mixture was denatured at 95°C for 5 min and snapped cool on ice for 15 min. Finally mixture was run on 10% native PAGE (30:1, acrylamide and bis-acrylamide) with 5% glycerol. The electrophoresis was performed at 4°C temperature at 250 V for 12 h (245 bp fragment) and 300 V for 18 h (472 bp fragment). Gel was stained with silver nitrate staining with slight modification to visualize the banding pattern (Dayal *et al.* 2005).

Sequencing: PCR products were run on 1% low melting agarose gel and the desired product was eluted from the gel using gel elution kit for purification. The purified PCR products were cloned by using TA cloning strategy in pGEMT easy vector. Cloned product was identified by blue white screening. Positive clones were sequenced by the automated dye-terminator cycle sequencing method.

Statistical analysis: A general linear model incorporating sire, genotype and season of calving as fixed effect was employed to estimate the effect of genotype on body weight at 3, 6 and 9 months of age in goat. Model:

$$Y_{ikl} = \mu + G_i + A_j + S_k + e_{ikl}$$

where, Y_{ikl}, l^{th} body weight for k^{th} season, for j^{th} sire, i^{th} genotype; µ, overall mean; G_i, effect of i^{th} genotype body weight; A_j, effect of j^{th} sire on body weight; S_k, effect of k^{th} season on body weight; e_{ikl}, random error associated with each observation.

RESULTS AND DISCUSSION

Polymorphism: SSCP revealed that growth hormone gene is polymorphic in nature at both 245 bp and 472 bp fragments. Sequencing of 245 fragment revealed 4 genotypes AA, AB, AC and CC and consequently, three alleles A, B and C at this locus. The frequencies of AA, AB, AC and CC genotypes were obtained as 0.28, 0.34, 0.23 and 0.15, respectively, while the frequencies of A, B and C allele were 0.565, 0.17 and 0.265, respectively. Sequencing of 472 bp fragment also revealed 5 genotypes AA, AB, AC, BB and CC and consequently, only 3 alleles A, B and C at this locus. The frequencies of AA, AB, AC, BB and CC genotypes and A, B and C alleles were estimated as 0.12, 0.40, 0.36, 0.07 and 0.05 and 0.50, 0.27 and 0.23, respectively. AB genotype and A allele was predominant in both the fragments of Black Bengal goat. This is in agreement with Malveiro *et al*. (2001) and Boutinaud *et al*. (2003). They also found that growth hormone is polymorphic in goat. Gupta *et al.* (2007) also reported 7 and 5 genotype in exon 4 and exon 5 fragments of growth hormone gene, respectively in Black Bengal goat.

Nucleotide sequence analysis: Sequence of all the alleles detected through sequencing is submitted to NCBI and accession numbers were obtained. Accession no. of various allelic variants of 245 bp fragment were KJ666532 (A allele), KJ666533 (B allele) and KJ666534 (C allele) whereas for 472bp fragment were KJ782050 (A allele), KJ782051 (B allele) and KJ782052 (C allele). Sequences of all the 3 allele A, B and C of both the fragment i.e. 245

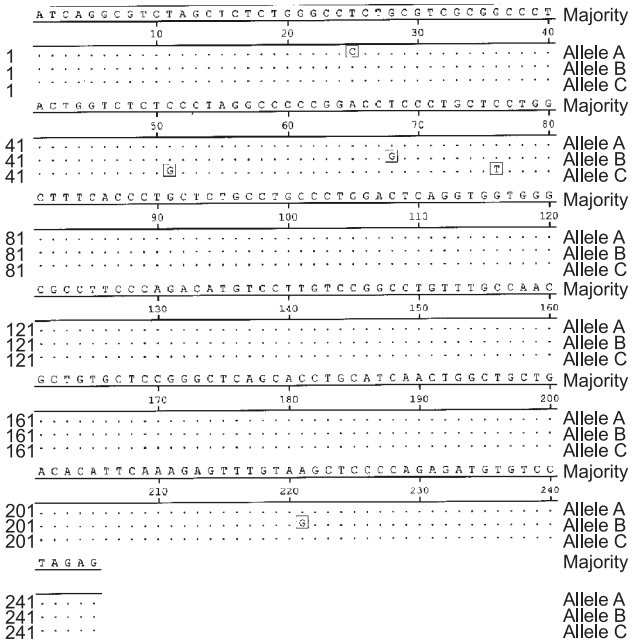

Fig. 1. Sequence alignment of allelic variants of 245 bp fragment of growth hormone gene.

bp and 472 bp fragment were aligned using MEGALIGN programme of DNASTAR software.

Alignment of allelic variants of 245 bp fragment (Fig.1) revealed that there are differences at 5 positions i.e. 25th, 51st, 68th , 76th and 221st among the alleles, out of which 3 i.e. 25th, 51st and 221st were found in intronic region whereas 2 i.e. 68th and 76th were found in exonic region. Out of 2 differences present in exonic region, nucleotide substitution present at 76th position (C→T) of allele C is silent mutation whereas nucleotide substitution at 68th position (T→G) leads to change in corresponding polypeptide sequence in allele B where serine get substituted by alanine. Silent mutation present in allele C is also important since this mutated site may be relatively more prone to future mutation or indirectly effect the process of transcription and translation during the expression of protein/polypeptide. Hua *et al*. (2008) also studied the GH gene in Boer goat population and reported 2 SNPs located in exon 2 (A781G) and 4 (A1575G).

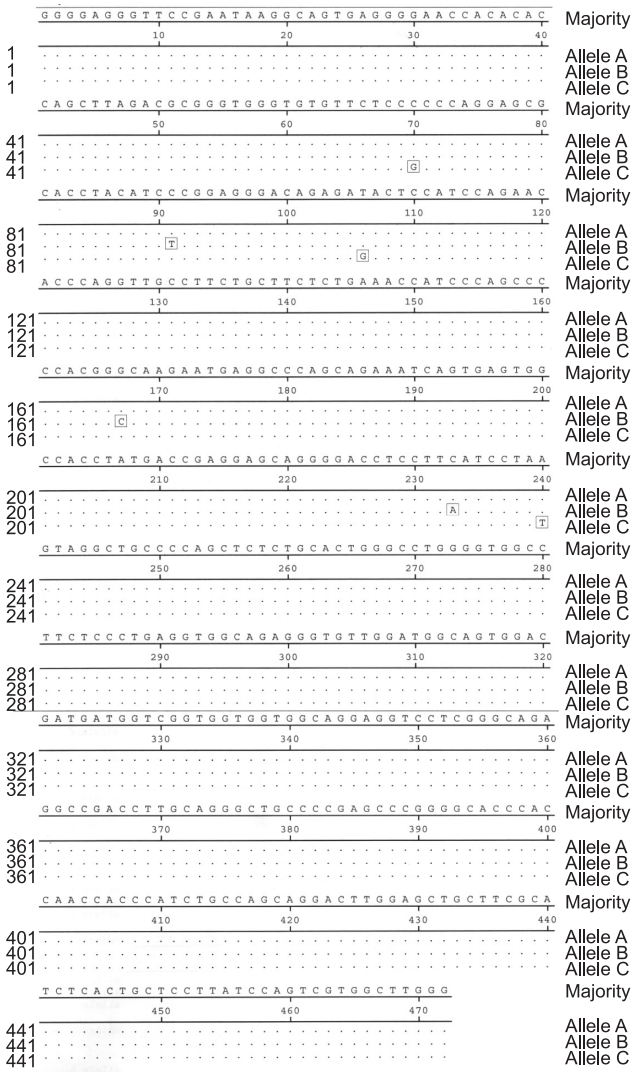

Fig. 2. Sequence alignment of allelic variants of 472 bp fragment of growth hormone gene.

Similarly, alignment of allelic variants of 472 bp fragment (Fig. 2) revealed that there are differences at 6 positions i.e. 70th, 91st, 106th, 167th , 233rd and 240th among the alleles, out of which 3 i.e. 70th, 233rd and 240th were found in intronic region, whereas 3 i.e 91st, 106th and 167th were found in exonic region. Mutation in exonic region lead to variation in polypeptide sequences of 3 alleles. Substitution at 91st position (C→T) and 167th position (G→C) leads to substitution of proline into serine and glycine to alanine, respectively in B allele. Similarly, substitution at 106th position of nucleotide sequence (T'!G) leads to substitution of tyrosine to aspartic acid in allele C. Gupta *et al.* (2007) found substitution at 7 places at exon 4 of growth hormone gene in Black Bengal goat.

Effect of growth hormone genotype on body weight of Black Bengal goat: Least square analysis revealed that genotypes of 245 bp fragments had no significant effect on body weight of Black Bengal goat at both 6 and 9 month of age. However, genotypes of 472 bp fragment had significant effect (P≤0.05) on body weight at both 6 and 9 month of age in Black Bengal goat (Table 1). Animals having AC genotype had highest body weight at both 6 and 9 month of age whereas animals having CC genotype had lowest body weight at both 6 and 9 month of age.

Animals having AC genotype had 65% more weight than the animal having CC genotype at both 6 and 9 month of age. The order of performance for body weight at 6 month of age was CC < AA, AB, BB < AC whereas order of performance at 9 month of age was CC < BB < AA, AB < AC . One interesting finding in this study is that both the homozygote AA and CC are having lowest body weight. However, heterozygous condition i.e. AC genotype is having highest body weight. This may be due to heterosis where heterozygous performs better than the both homozygote.

Some other workers also found that goat with AB genotype weighed about 2 kg heavier than those with AA genotype at weaning and measured 1.4 cm greater than those with AA genotype in chest girth at birth (Hua *et al*. 2008). AA genotype had significant higher birth weight and weight of one year old than BB and AB genotypes in Boer goats (Min *et al*. 2005). In Sirohi goat, various genotype of growth hormone had significant association with body weight at 9 month of age (Kumar *et al*. 2011). They revealed that BE

Table 1. Genotype wise (472 bp fragment) least-square means of Body Weight (kg) at different age in Black Bengal goat

Age	Genotype				
	AA	AB	AC	BB	CC
6 month	6.13 ±0.72^{b}	6.80 ±1.15^{b}	7.97 ±1.39^{c}	5.65 ±0.64^{b}	4.83 ±1.04^{a}
9 month	8.98 ±1.74^{c}	8.88 ±1.41^{c}	9.90 ±1.40^{d}	6.82 ±0.52^{b}	5.95 ±0.35^{a}

Different superscripts indicate significant difference at 5% level.

variant had significantly highest (20.042 kg) body weight compared to BA variant (18.558 kg) at the age of 9 months. RFLP of GH-Taq I was found to be associated with body weight at 7 and 13 month of age in Belgian white blue bulls (Sneyers *et al.* 1994).

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