Indian Journal of Animal Sciences **84** (9): 962–964, September 2014/Review Article

SSCP typing of growth hormone gene and its association with birth weight in Black Bengal goat

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Received: 15 November 2013; Accepted: 9 May 2014

ABSTRACT

Growth hormone either directly or indirectly is the main regulator of postnatal somatic growth, stimulating anabolic process such as skeletal growth and protein synthesis and its deposition in tissues and organs. Therefore, genetic variation of growth hormone gene and its association with birth weight as an indicator of growth performance was investigated in Black Bengal goat. A 245 bp fragment (partial intron 1, exon 2 and partial intron 2) of growth hormone gene was analyzed for detection of polymorphism expected to be present at this locus. SSCP typing revealed 5 genotypes AA, AB, AC, AD and CC and consequently, 4 alleles A, B, C and D were identified. Least square analysis revealed that genotypes had significant effect on birth weight. Animals having AC genotype had highest birth weight whereas animals having CC genotype had lowest birth weight.

Key words: Body weight, Goat, Growth hormone, Polymorphism

Growth hormone (GH), an anabolic hormone, is released by exocytosis in response to appropriate stimuli. Growth hormone either directly or indirectly is the main regulator of postnatal somatic growth, stimulating anabolic process such as skeletal growth and protein synthesis and its deposition in tissues and organs (Gluckman *et al*. 1987). Growth hormone gene has been widely investigated and used as marker for milk production in cattle (Khatami *et al*. 2005), sheep (Marques *et al*. 2006) and goats (Boutinaud *et al*. 2003, Malveiro *et al*. 2001). However, association of GH gene with growth traits in cattle bGH genotype showed 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. Malveiro *et al*. (2001) reported positive correlation of GH genotype GH2-N and GH2-Z with milk production in Portuguese Algravia breed. Boutinaud *et al*. (2003) found positive correlation of GH gene with milk fat and protein yield. A few reports are available on 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*. (2009, studied the GH gene in Boer goat population and reported that 2 SNPs are 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. Despite of the important role of GH gene on growth performance traits, it has not been explored in Black Bengal goat to study its association with growth performance traits. A few reports are available in literature on exon 4 and 5 (Gupta *et al.* 2007). Therefore present study was undertaken to identify the polymorphism of growth hormone gene and to study its association with birth weight in Black Bengal goat.

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 (Sambrook and Russel 2001).

PCR amplification: A 245 bp fragment (partial intron 1, exon 2 and partial intron 2) of growth hormone gene was analyzed for detection of polymorphism expected to be present at this locus. The primers used for amplification were designed on the basis of sequence available publicly at NCBI. Primers used for amplification were forward, 52 ATCAGGCGTCTAGCTCTCTGG 3' and reverse

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5'CTCTAGGACACATCTCTGGGG 3'. PCR cycling conditions were standardized with different concentrations of $MgCl_2$, *Taq* polymerase, dNTPs and primers. PCR reaction is performed in in a total volume of 25 µl with 100 ng of genomic DNA, 15 pmoles of each primer, 2 mM of $MgCl_2$, 100 µM of each dNTP, 1X PCR reaction buffer and 1 U of *taq* DNA polymerase. PCR programme followed for amplification of gene fragment was initial denaturation for 95°C for 2 min than 30 cycles of denaturation at 95°C for 30 sec, annealing at 60°C for 45 sec, 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 (SSCP): PCR product (2.5 µl) was properly mixed with 17.5 µl formamide dye (95% formamide, 0.025% xylen cyanol and 0.025% bromophenol blue and 4.5% 0.5M EDTA). The mixture was denatured at 95°C for 5 min and snapped cool on ice for 15 min. Finally mixture was run on 12% native PAGE (50:1, acrylamide and bis-acrylamide) with 5% glycerol. The electrophoresis was performed at 4°C temperature for 12 h at 200V. After electrophoresis gel was stained with silver nitrate staining to visualize the banding patterns.

Statistical analysis: A general linear model (Harvey 1987) incorporating sire, genotype and season of calving as fixed effect was employed to estimate the effect of genotype on birth weight in goat. All the animals included in the study have litter size 2.

Model: $Yikl = \mu + Gi + Aj + Sk + eikl$

where, Yikl, lth birth weight for kth season, for jth sire, ith genotype; µ, overall mean; Gi, effect of ith genotype birth weight; Aj, effect of jth sire on birth weight; Sk, effect of kth season on birth weight; eikl, random error associated with each observation.

RESULTS AND DISCUSSION

Polymorphism: SSCP typing in Black Bengal goat revealed that growth hormone gene is polymorphic in nature. Five genotypes AA, AB, AC, AD and CC (Fig.1) and consequently, 4 alleles A, B, C and D were identified at this locus. Genotype and gene frequency of studied population are given in Table 1.

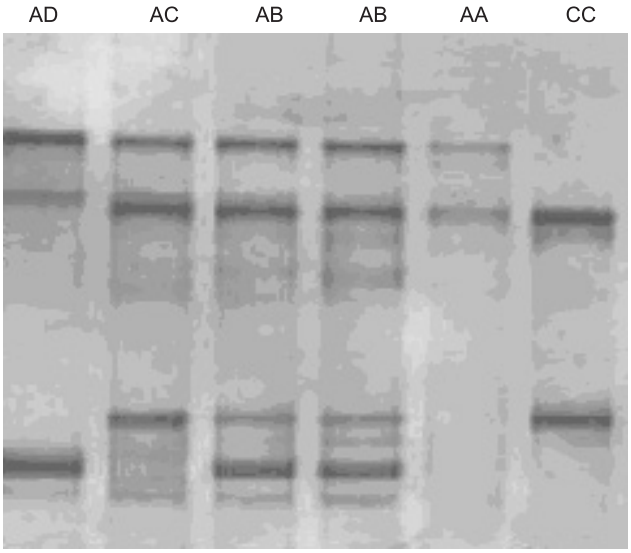

Fig.1. SSCP typing of Growth hormone gene.

Table 1. Genotype and gene frequency for 245bp fragment of growth hormone gene

Species	Genotype frequency				
	AA	AB	AC	AD	CC
Black Bengal	0.28 (n=28)	0.34 (n=34)	0.19 (n=19)	0.04 (n=4)	0.15 (n=15)
	Gene Frequency				
	A	B	C	D	
	0.565	0.170	0.245	0.020	

Table 2. Genotype wise least-square means of birth weight (kg)

Species	Genotype frequency				
	AA	AB	AC	AD	CC
Birth weight (kg)	0.98 ±0.06[a]	1.15 ±0.14[b]	1.40 ±0.20[c]	1.12 ±0.08[b]	0.95 ±0.08[a]

Different superscripts indicate significant difference at 5% level.

AB genotype and A allele was predominant in 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. Hua *et al.* (2009) also identified 2 SNP each at exon 2 and exon 4 in Boer 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.

Effect of growth hormone genotype on Black Bengal goat: Least square analysis revealed that genotypes had significant effect (Pdd0.05) on birth weight (Table 2) in Black Bengal goat. Animals having AC genotype had the highest birth weight whereas animals having CC genotype had lowest birth weight. Animals having AC genotype had 47% more weight than the animal having CC genotype. The order of performance for birth weight was AA, CC < AB, AD < AC. One interesting finding in this study is that both the homozygote AA and CC are having lowest birth weight. However, heterozygous condition i.e. AC genotype is having highest birth weight. This may be due to heterosis where heterozygous performs better than the both homozygote. Supakorn and Pralomkarn (2013) also found positive association with SSCP patterns of growth hormone and birth weight in various breeds of goat.

Min *et al*. (2005) also reported significant association of growth hormone gene with birth weight in Boer goat. They found that AA genotype had significantly higher body weight than the BB and AB genotype. Hua *et al*. (2009) also detected a significant and positive association of growth hormone gene in Boer goat. Ishida *et al.* (2010) also found significant association of growth hormone gene with birth

weight in Japanese Black cattle. They found that animals having haplotype G-C-A had significantly lower birth weight. The association analysis of variants of promoter region revealed that animals having AE variant had significantly lowest (2.227 kg) birth weight (P<0.05) while AH variants had highest (2.567 kg) birth weight with an increase of 13.25% in Sirohi goats (Kumar *et al*. 2011). Therefore, this study indicated that the growth hormone can possibly be used as marker for improving the birth weight in Black Bengal goat.

ACKNOWLEDGMENTS

The authors are thankful to the Director, ICAR Research Complex for Eastern Region, Patna, Bihar, India for providing necessary facilities and financial support to carry out this work.

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