Tropical Agricultural Research Vol. 21(4): 398 - 411 (2010)

Use of Growth Hormone Gene Polymorphism in Selecting *Osmanabadi* and *Sangamneri* Goats

S.H.G. Wickramaratne, B.R. Ulmek[1], S.P. Dixit[2], S. Kumar[3] and M.K. Vyas[3]

Veterinary Research Institute
P.O. Box 28, Peradeniya
Sri Lanka

ABSTRACT. *Goat meat holds tremendous demand irrespective of cultural and religious barriers. Therefore selection of goats for improved growth is advantageous. Growth hormone (GH) is a polypeptide hormone which is the major regulator of growth of animals, encoded by growth hormone gene. Polymorphic GH gene confers more active variants of the hormone enhancing growth. The objectives of the present study were to identify single nucleotide polymorphisms (SNPs) of GH gene and their association with growth traits of goats. DNA samples of 240 goats from two Indian breeds, namely Osmanabadi and Sangamneri were subjected to single strand confirmation polymorphism (SSCP) followed by DNA sequencing techniques with the objectives of studing single nucleotide polymorphisms (SNPs) in GH gene and their association with growth traits.*

Twenty three and eighteen nucleotide changes were observed in Osmanabadi and Sangamneri goats, respectively in comparison to goat sequence of accession No. 00476. Of them, twelve were seen unique to both goat breeds indicating considerable deviation from exotic goat sequence. The study revealed point mutations of G200T, A815G, A1753, C1763T and A1789G in GH gene sequence of both goat breeds. In contrast, A497G, A499G C500G C501-2 C730T C781T and C2055T were observed specific to Osmanabadi and Sangamneri breeds respectively indicating the possibility of using them as breed specific markers. G200T resulted GG, GT and TT genotypes and of them, GT was associated with heavy body weight and GG with low body weight ($P \leq 0.05$) in both the breeds. Sangamneri GT genotypes were 6.5% taller ($P \leq 0.05$) than GG. Similarly, AG of A815G revealed low body weight. AA of A1753 insertion revealed heavy body weight in both the breeds and 7% longer body in Sangamneri breed. Heterozygote counterparts for C1763T and A1780G SNPs exhibited heavy body weights (($P \leq 0.05$). It was concluded that SNPs and their association with body weight may be useful in selecting goats for higher growth traits.

INTRODUCTION

Animals exhibiting high genetic merits in weight gain, body measurements and carcass characteristics receive high priority in breeding programmes for meat purpose. Among the small ruminants, goat and sheep have immense contribution to the resource poor communities for their livelihood in the Asian context. *Osmanabadi* and *Sangamneri* are two popular indigenous goat breeds in Maharastra because of their high prolificacy and

[1] Pune Agriculture Collage, Pune, India

[2] National Bureau of Animal Genetic Resources, Karnal, India

adaptability to harsh environmental and poor management conditions. The *Osmanabadi* goat is mainly reared for mutton while the *Sangamneri* goat is reared for both milk and meat purposes. Lack of proper breeding materials and inherently slow growth rate are two alarming problem with *Osmanabadi* goats. Similarly, *Sangamneri* breed has been identified as a threatened breed due to its diminishing population size, genetic dilution due to cross breeding with other migratory goat breeds and inbreeding due to lack of a proper breeding system. Therefore, a sustainable breeding approach is an urgent need for the genetic improvement of these two goat breeds. Considerable achievements have been made in the past, through conventional breeding methods focusing on the breeding of individuals with superior phenotypes. However, poor expression of the phenotypes in the case of less heritable traits, (*i.e.* disease resistance) and in sex limited characters limit the use of these techniques. Moreover, their high time consuming nature and high cost prompted animal breeders to search for alternative strategies. In this context, use of molecular based techniques permit to investigate variations in the original gene structure more precisely, facilitating to approach selection decisions in a challenging way.

Growth is a complex process that involves the regulated coordination of a wide diversity of neuro-endocrine pathways that involve coordinated action of several hormones. Different polypeptide hormones secreted by the key organs and tissues are generally regulated by gene action. There are several genes that influence the growth and body mass of the animal. Growth hormone gene (GH), growth hormone receptor (GHR) and Insulin-like growth factor-1 (IGF-1) genes have been identified as the major genes in this biological process (Ge *et al.*, 2000; Ge *et al.*, 2003). Growth hormone is a peptide encoded by a single gene about 2.5kb in length and consists of five exons and four intervening introns. It is well established that animals with high level of these hormones exhibit enhanced growth performance. Therefore, selection of animals based on the high level of endogenous growth hormone helps to explore superior growth traits. This superiority could be a result of more active variants in the genes. Establishment of significant correlation of the quantitative traits and such polymorphic sites at the gene level could lead to the discovery of molecular markers which are useful in assisting selection programmes. Several polymorphisms have been identified in the GH gene of goats (Yao *et al*., 1996; Ofir and Gootwine, 1997, Malverio *et al*., 2001, Marque *et al*., 2003) but only a very few of them have been precisely characterized for nucleotide changes and position in the DNA sequence. Moreover, studies reported the association with growth traits of goats is very scanty. Therefore, the objectives of the present study were to identify polymorphism of the growth hormone gene and to investigate association of these polymorphisms with growth traits in *Osmanabadi* and *Sangamner* goats.

MATERIALS AND METHODS

Experimental location

Present investigation was conducted at the Biotechnology center of Mahatma Phule Krishi Vidyapeeth (an agricultural university), Rahuri, Maharastra and National Bureau of Animal Genetic Resources (NBAGR), Karnal, Haryana, India.

Animals

Osmanabadi goats were maintained at *Mahatma phule Krishi Vidyapeeth (MPKV), Rahuri*. The *Sangamneri* goats were maintained by smallholder farming community in the extension area of the University in *Ahmednagar* district. Blood samples of 248 goats progeny in the

ages of three to twelve months (120 *Osmanabadi* and 150 *Sangamneri*) and their sires (06 *Osmanabadi* and 16 *Sangamneri*) were collected. Both breeds of goats were grazed extensively on natural vegetation all year round. *Osmanabadi* goats were housed in the university farms under semi-intensive management while *Sangamneri* goats were reared by the smallholder goat keepers under similar semi-intensive management. Mating was usually performed by sire goats and the kidding period was about 12 weeks in winter, starting in mid October. Kids were weaned at the age of two months.

Phenotypic data

The data were collected from the All-India coordinated research project on goat at Mahatma Phule Krishi Vidyapeeth, Rahuri during the period of 2005 to 2008. Data collected include identification of progeny and their respective sires, year of birth, sex, birth weight and body weight at three, six, nine, and twelve month ages. In addition; primary data on body measurement (length, height, chest girth, pouch girth) were also recorded in different age categories of *Sangamneri* goats at the time of blood sample collection. The body weight data recorded on the same *Sangamneri* goat flocks by the extension staff on their monthly visits were utilized in the analysis. Data on birth weight of *Sangamneri* was not recorded instead, weight at one month age were used. Body weights were obtained by weighing each animal with an electronic scale and subsequently, a flat scale was used for other weight recodes. All the weighings were recorded in the morning before taking them for grazing. Body measurements were taken from the right side of each animal by two persons using a flexible tape and then averaged.

DNA isolation and PCR amplification

Genomic DNA was isolated using Phenol-chloroform extraction technique (Sambrook *et al.*, 2001) and was diluted to obtain final concentration of 50-100 ng/µl for PCR amplification. Whole growth hormone gene was amplified in eight fragments by PCR using eight primer pairs shown in the Table 1. The length of each amplified fragment varied from 181bp to 429bp. The primers were designed by PRIMER3 software (http://www.-genomewi.mit.edu) as per the published goat GH gene sequence (D 00476) of NCBI database (Kioka *et al.*, 1989).

Fragment length

The Figure 1 illustrates the structure of the GH gene and the length and location of each amplified fragment. The PCR reaction was performed in a total volume of 25 µl containing 2.5 µl (100 ng) template DNA, 0.6 µl (200 ng) of each forward and reverse primers, 0.5 µl (200 µM) of dNTP, 0.3 µl (1 unit) of *Taq* DNA polymerase (Bangalore Genei) and 2.5 µl of 10x PCR buffer (200 nmol Tris-HCl, 100 nmol Ammonium Sulphate, 100 mmol KCl, 1% Triton X-100, 20 mmol $MgCl_2$, pH 8.8), 18 µl of nuclease free water in a PTC-200 Thermal cycler (MJ Research Inc, Hercules, CA, USA). Samples were initially denatured at 94ºC for 5 min, followed by 29 cycles of annealing at 56 ºC -64 ºC (ranging as per the primer) for 30 sec, extension at 72 ºC for 30 sec. Final extension was at 72 ºC for 10 min.

Genotyping and sequencing

The PCR products were genotyped by single stranded conformation polymorphism (SSCP). Five micro liters of each PCR product was added to 8 µl of denaturing buffer (98 % formamide, 10 mmol EDTA, 0.05 % xylene cyanol and 0.05 % bromophenol blue). The

mixture was denatured at 95 ºC for 10 min, snap cooled in the crushed ice for 5 min and then loaded onto 12 % polyacrylamide:TBE gels (29:1 Acrylamide to N,N'-methylene bisacrylamide) PAGE and electrophoresed at 340 V for 6-10 hrs maintaining temperature at 6-10 ºC by circulating ice water around the jacket. After the electrophoresis, the gel was removed from the apparatus and stained with silver nitrate to visualize the banding pattern.

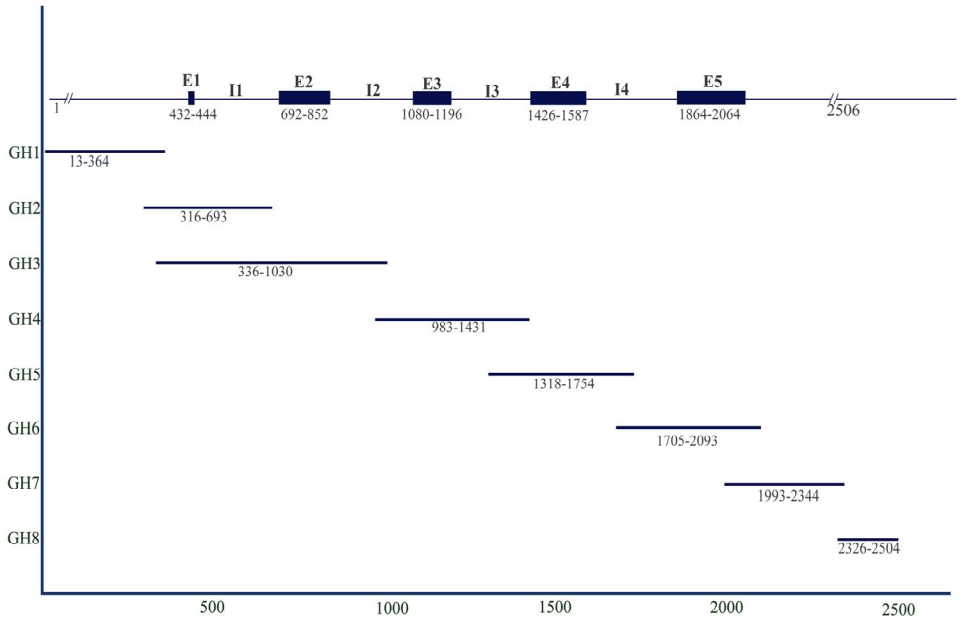


Fig. 1. Schematic representation of GH gene with exons (E) represented by black boxes, introns (I) represent the line connecting two boxes and numbers represent the localization of amplified fragments

Scoring of SSCP band patterns and sequencing

The most common band pattern identified and named as A. If there were more bands, in addition to the common bands, they were marked as B, C, D *etc.*, depending on the position of the individual bands. PCR products of representative samples showing unique band pattern in comparison to common patterns were purified by adding exo-Sap digestion using 1 µl of enzyme Exonuclease-1 and Alkaline phospotase master mix to each sample of 10 µl PCR product (50-100 ng /µl DNA) and exposing the thermal profile of 37ºC for 120 min in the thermal cycler. After checking the purity of the products on 1.5 % agarose, the samples were prepared for sequencing by adding reaction mix of ABI PRISM® BigDye® Terminator v3.1 cycle sequencing kit (Applied Bio systems, USA). The samples were then subjected to direct sequence analysis by dideoxy sequencing in ABI 3100 automated DNA sequencer (Applied Bio System).

Table1. Fragment location, primer sequence and annealing temperature details of primers used in amplifying growth hormone gene

Location*	Primer	Primer sequence (5′-3′)	bp	length	Anneal temp
Promoter 5´ (13-363 bp)	GH1-F	cccagggattaaacctgagtc	21	352	63.0
	GH1-R	ctctgctgggccctttttat	20		
5´-U-E1- I1 (345-693 bp)	GH2-F	gggggaaagggagagagaag	20	378	64.0
	GH2-R	ccctagggagagaccaggag	20		
I1-E2- I2 (674-1030bp)	GH3-F	gatcaggcatccagctctct	20	395	60.0
	GH3-R	tcactgccttattcggaacc	20		
I2-E3- I3 (1011-1344bp)	GH4-F	ggttccgaataaggcagtga	20	449	63.5
	GH4-R	caccaccaccaaccatcat	20		
I3-E4- I4 (1326-1667bp)	GH5-F	ccaccaaccacccatctgcc	20	437	60.0
	GH5-R	gaagggacccaagaacgcc	19		
I4-E5-D (1648-2012bp)	GH6-F	ctagcagcccagtcttgacc	20	389	56.0
	GH6-R	ggggaggggtaacaacagat	20		
E5-3´-D (1993-2344bp)	GH7-F	ctgcacaagacggagacgta	20	352	58.0
	GH7-R	tcacagagaaggggatgtgc	20		
3´-D (2325-2506bp)	GH8-F	gcacatccccttctctgtga	20	181	56.0
	GH8-R	cttcccactcttggaggcta	20		

*Location numbering is as per the NCBI GH gene accession no. D 00476 (Kioka *et al*, 1989) U= up stream E=exon, I=intron, D=down stream, Anneal = annealing

Sequence analysis

Sequencing was performed with forward primers only. Results were analyzed with Chromas Pro 1.49 (http:/www.technelysium.com.au) and Blast 2.0 (Altschul *et al.*, 1990) software. The "Edit sequence" software of DNA star was used to interchange data format between software. The sequences were aligned and the position of the identified SNPs was numbered as per the complete goat reference sequence of accession No. 00460 (Kioka *et al.*, 1989) by using ClustalW software (Thompson *et al.*, 1997).

The exact genotypes of the representative DNA samples revealed by sequencing were used to genotype the corresponding samples which was grouped based on the similarity of SSCP band pattern. Abbreviation of A, C, G, T were used to identify alleles of adenine, cytosine, guanine and thiamine respectively. The sequence variants were named by writing original nucleotide in the left side and its substituted nucleotide in the right side and the position of the nucleotide change in between.

Statistical analysis

Allele frequencies were estimated by the gene counting method while association between observed polymorphisms of the growth hormone gene and growth traits was analyzed using mixed model least squares and maximum likelihood computer software PC-2 (Harvey, 1990). The linear model used for body weight was as follows.

$$Y_{ijkl}= \mu + S_i + B_j + X_k + G_l + \beta_{age} + e_{ijkl}$$

Where Y_{ijkl} = Phenotypic observations (Birth weight, 3month weight, 6 month weight, 9 month weight, weight gain between birth to 3 month, weight gain between 6month to 9month and weight gain between 3month to nine month). μ=overall mean, S_i = fixed effect of sire, B_j = fixed effect of breed, X_k = fixed effect of sex, G_l = fixed effect of marker (SNP), β_{age} = age of goat as covariate, e_{ijkl} = random error

RESULTS AND DISCUSSION

The length of the PCR products of eight fragments of growth hormone gene, determined by agarose gel electrophoresis were as expected. Thirty and 24 unique alleles were defined by SSCP band pattern in *Osmanabadi* and *Sangamneri* goats, respectively. The allelic frequencies are given in the Table 2. Twenty four and 19 SNPs were detected in *Osmanabadi* and *Sangamneri* respectively in comparison to the NCBI goat GH sequence (accession no. D-00476) Of them, twelve variations were common to all the individuals of both the breeds indicating the deviation in the GH sequence to its exotic counterparts (Table 3). Among the total common variants, there were ten substitutions, one deletion and one insertion. In considering the locations of these variants, two, four, three and one variants were located in promoter, exon 2 intron 2, exon 5 and 3′ down stream region respectively. There were six G→C, one G→A, one A→G, one T→C (Figure 1&2), one C→T substitutions, one G insertion and one G deletion (Table 3). These variants might have been conserved in these two breeds.

The study revealed five SNPs and they were located at promoter (G200T), exon 2 (A815G), intron 4 (C1763T and A1780G), and exon 5 (C2055T) respectively. Of the total substitutions, two transitions (A497G and A499G), two transversions (A500C and T730A) and three insertion/deletions (501C2, 781T2 and 1753A54) were observed. In addition, six SNPs were observed, shared by both breeds of goats while six in *Osmanabadi* and one in *Sangamneri* were found specific or private to the respective breeds (Table 3). G200T (G/T substitution) at upstream promoter region (Table 3, Figures 2 and 3) had three genotypes (TT, TG and GG) with the frequencies of 0.24, 0.20 and 0.56 respectively in *Osmanabadi* goats while TT genotype was not observed among *Sangamneri* individuals. Absence of TT genotype may be due to the limited population size used in the study. The corresponding frequencies for TG and GG were 0.39 and 0.61 respectively. The novel T allele was observed in the sequence as a result of polymorphism. A1753 was an insertion/deletion of A at 1753rd nucleotide position in both *Osmanabadi* and *Sangamneri* goat breeds.

C1763T SNP detected at 1763 nucleotide position in the intron 4 region (Figure 3&4) revealed presence of CC and TT homozygote and TC heterozygote individuals with the frequencies of 0.17, 0.30 and 0.52, respectively. A1780 SNP detected at 1780th nucleotide position of intron 4 region in both goat breeds. Individuals were observed with AA, GG and AG genotypes with the frequency of 0.62, 0.10 and 0.28.

Table 2. The frequencies of SSCP variants observed in growth hormone gene of *Osmanabadi* and *Sangamneri* goats

Fragment	Breed	SSCP variants					
		A	B	C	D	E	F
GHP 1	OS	0.37 (37)	0.16 (16)	0.13 (13)	0.70 (7)	0.27 (27)	-
	SG	0.80 (7)	0.20 (18)	0.13 (12)	0.18 (16)	0.40 (36)	
GHP 2	OS	0.24 (18)	0.57 (43)	0.60 (4)	0.90 (8)	-	-
	SG	0.70 (4)	0.49 (27)	0.44 (24)	-	-	-
GHP 3	OS	0.60(4)	0.60 (41)	0.34 (23)	-	-	-
	SG	0.90 (10)	0.22 (24)	0.69 (76)	-	-	-
GHP 4	OS	0.15 (10)	0.65 (43)	0.20 (13)	-	-	-
	SG	0.32 (24)	0.51 (38)	0.16 (12)	-	-	-
GHP 5	OS	0.71 (71)	0.16 (16)	0.13 (13)	-	-	-
	SG	0.12 (6)	0.42 (21)	0.16 (8)	0.30 (15)	-	-
GHP 6	OS	0.43 (46)	0.33 (35)	0.50 (5)	0.14 (15)	0.20 (2)	0.30 (3)
	SG	0.53 (47)	0.14 (12)	0.17 (15)	0.20 (2)	0.30 (3)	-
GHP 7	OS	0.18 (13)	0.10 (7)	0.44 (31)	0.14 (10)	0.60 (5)	0.80 (6)
	SG	0.54 (36)	0.30 (20)	0.90 (6)	0.70(5)	-	-
GHP 8	OS	0.79(38)	0.15 (7)	0.40 (2)	0.20 (10)	-	-
	SG	0.14 (12)	0.25(22)	0.14 (12)	0.10 (1)	0.45 (10)	-

*Numbers within bracket are number of animals, OS = *Osmanabadi*, SG = *Sangamneri*
SSCP=Single strand confirmation polymorphism

The SNPs of A497G, A499G C500G C501-2 C730T C781T were detected only in the *Osmanabadi* breed while C2055T SNP was observed only in *Sangamneri* individuals. These mutations have not been reported elsewhere and these potential SNPs can be used as genetic markers for confirmation of the breed identity. The C2055T SNP was located in the exon-5 region showed a potential amino acid change of Ala /Val. Statistical analysis of growth traits with the novel genotype could not establish any significant association. The reason for non-significant results needs to be further investigated with higher number of population. On the other hand, effect of this polymorphism may be correlated with other phenotypic factors. Yao *et al.* (1996) also reported A to C transversion in exon 5 in goat growth hormone gene but its association with any phenotypic factor had not been reported. Luci *et al.*, 1993 reported Leu/Val polymorphism in exon 5 of bovine GH gene due to single base change of C to G. GH sequence with Leu variant has been associated with a higher GH release in German black and white cattle (Schlee *et al*., 1994) and a lower GH release in Polish Friesian cattle (Grochowska *et al.*, 2001). There were no previously reported studies on SNPs of similar locations in the goat growth hormone gene and their associations with growth traits. Few studies have reported polymorphic sites based on the SSCP or RFLP band patterns. But exact location and the nature of the polymorphism were not reported. Therefore, findings of this study would serve as the first of this kind.

Association analysis

The results of the association analysis are given in Table 4 and 5 for body weight and in Table 6 for other body measurements. Associations of different SNPs with body weight at different ages irrespective of the breed are given in the Table 4. Genotyping revealed that GG, TT and TG variants with respect to the G200T SNP at 200 nucleotide position (Table 3 and Figures 2 and 3) in *Osmanabadi* GH upstream promoter region. However, TT variant

was not observed among individuals of *Sangamneri* goat population. Their genotype frequencies were 0.58, 0.14 and 0.28, respectively.

Table 3. The details of the polymorphic sites detected in growth hormone gene of *Osmanabadi* and *Sangamneri* goats

Location	Nucleotide Position*	Sequence change	Name of SNP	Flanking sequence	Reference sequence	OS*	SG*
Promoter	164	G/C	G164C	CA**C**GC	CA**G**GC	D	D
	193	T/C	T193C	GA**C**GA	GA**T**GA	D	D
	200	G/T	G200T	GT**G**/**T**GG	CT**G**GG	D	D
Intron1	497	A/G	A497G	GG**G**GG	GG**A**GA	D	ND
	499	A/G	A499G	AG**A**AC	AG**G**AC	D	ND
	500	A/C	A500C	GG**C**CC	GA**A**CT	D	ND
	501-502	-/C	501C02	CC**C**TG	AC-TG	D	ND
Exon 2	730	T/A	T730A	TC**A**GC	TC**T**GC	D	ND
	815	A/G	A815G	GC**A**/**G**CC	GC**A**CC	D	D
	781	-/T	781T82	GGTCC	GG-CC	D	ND
Exon 4	1533	A/G	A1533G	GG**G**CC	GG**A**CC	D	D
	1551	G/A	G1551A	CT**A**AA	CT**G**AA	D	D
	1578	G/C	G1578C	GC**C**CT	GC**G**CT	D	D
	1585	C/T	C1585T	TG**T**GG	TG**C**GG	D	D
Intron 4	1602	G/C	G1602C	TT**C**TT	TTGTT	D	D
	1744	-G/	1744G45	CC-TT	CCGTT	D	D
	1752-1753	-/A	1752A1753	AA-CC	AA**A**CC	D	D
	1763	C/T	C1763T	CT**C**/**T**GC	CT**C**GC	D	D
	1780	A/G	A1780G	CTA/GTA	CTATA	D	D
Exon 5	1938	G/C	G1938C	CG**C**AG	CG**G**AG	D	D
	1956	G/C	G1956C	CT**C**AA	CTGAA	D	D
	2049	G/C	G2049C	GC**C**AG	GC**G**AG	D	D
	2055	C/T	C2055T	TG**C**/**T**GC	TG**C**GC	ND	D
3´Down stream	2451-52	-G/	2451G52	CG**G**TT	CG-TT	D	D

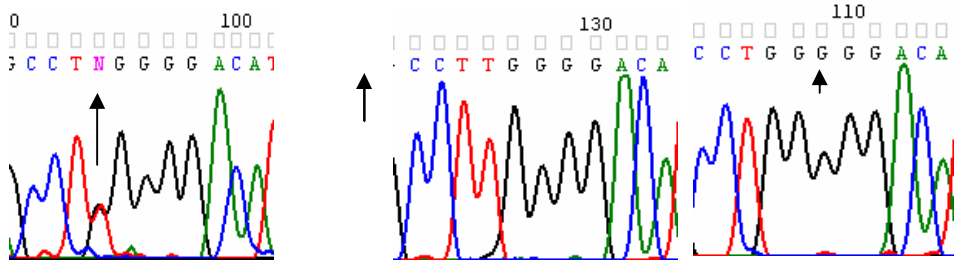
* OS= *Osmanabadi*; SG = *Sangamneri*; D= Detected; ND= Not detected;

Genotypes with heterozygote TG individuals had a significantly higher body weight ($P \leq 0.05$) than GG individuals at three month age. The highest dominance effect on body weight (580g) was observed at three month body weight while the highest additive effect was at six month of age (Table 5). The weight difference at six month was not significant at P=0.05). The actual weight may have been masked due to the nutritional deficiencies with the exposure of the animals for outdoor grazing after weaning at three month age. However, individuals with TT homozygote genotype did not show such significant association at any age group with respect to GG or TG. The additive effect of weight gain varied in between 6 to 20 g per day. Min *et al.* (2005) also reported polymorphism at the 5′ region of growth hormone gene of LuBei white goat, Boer goat, their first hybrid and first backcross generation.

Table 4. Association between genotypes of different SNPs and body weight at different ages across the breed difference

SNP	Geno type	Body weight (kg)			Average daily gain (kg)		
		3 m	6 m	9 m	0-3 m	3-6 m	6-9 m
G200T	GG	7.64±0.12^{a*}	13.22±0.44^{a}	16.03±0.12^{a}	0.057±0.002^{a*}	0.044±0.001^{a*}	0.056±0.001^{a*}
	TG	8.44±0.18^{b*}	12.34±0.66^{a}	15.56±0.19^{b}	0.043±0.003^{b*}	0.037±0.001^{b*}	0.047±0.002^{b*}
	TT	8.08±0.29^{ab}	12.60±1.08^{a}	16.01±0.30^{ab}	0.051±0.004^{ab}	0.042±0.003^{ab}	0.040±0.002^{ab}
A1752	AA	7.93±0.19^{a}	12.20±0.69^{a}	15.26±0.19^{a}	0.049±0.003^{a}	0.039±0.002^{a}	0.041±0.001^{a}
	--	7.65±0.15^{a}	12.83±0.55^{a}	15.85±0.15^{b}	0.055±0.002^{a}	0.042±0.001^{a}	0.046±0.001^{a}
C1763T	CC	8.01±0.22^{a}	12.54±0.82^{a}	15.96±0.23^{a}	0.053±0.003^{a}	0.042±0.002^{a}	0.045±0.002^{a}
	TC	8.16±0.13^{a}	13.03±0.47^{a}	15.80±0.13^{a}	0.048±0.002^{a}	0.040±0.001^{a}	0.043±0.001^{a}
	TT	7.39±0.17^{b*}	12.62±0.63^{a}	15.97±0.17^{a}	0.060±0.003^{b*}	0.044±0.002^{b}	0.048±0.001^{b}
A1780G	AA	7.94±0.27^{b*}	11.87±1.00^{a}	15.04±0.27^{a}	0.047±0.004^{b*}	0.037±0.002^{a}	0.040±0.002^{a}
	AG	8.32±0.14^{b}	13.00±0.53^{a}	15.95±0.14^{a}	0.048±0.002^{b}	0.040±0.001^{b}	0.043±0.001^{b}
	GG	7.51±0.25^{a}	12.31±0.94^{a}	15.90±0.25^{a}	0.060±0.004^{a}	0.040±0.002^{b}	0.047±0.002^{b}

* Different letters in the same column are significantly different at P≤0.05.



A). (G/T) allele **B). (T/T) allele** **C). (G/G) allele**

Fig. 2. The heterozygote (graph A) and homozygote (graph B and C) status of alleles at 200th nucleotide position at promoter region of growth hormone gene

Fig. 3. The sequence alignment showing T to C transition, and T/G heterozygote, T and G homozygote alleles in 193 and 200 nucleotide positions in promoter region of GH gene

Table 5. Estimates of the effects associated with the different variants/SNPs in the GH gene on growth traits of *Osmanabadi* and *Sangamneri* goats

Variant/ SNP	Trait name	Age category (month)	Number	LS Mean ± SE	Additive effect*	Dominance effect**
					GG-TT	**TG**
G200T	Body weight	3m	214	8.05±0.12	-0.44	0.58
		6m	214	12.72±0.45	0.62	-0.57
		9m	214	15.86±0.13	0.02	-0.47
	Av. daily gain	0-3	214	0.05±0.002	0.006	-0.011
		3-6	214	0.040±0.001	0.02	-0.006
		6-9	214	0.044±0.001	0.016	-0.001
A1753					**AA- --**	-
	Body weight	3m	214	7.79±0.12	0.28	-
		6m	214	12.51 ±0.44	-0.63	-
		9m	214	15.55 ±0.12	-0.59	-
	Av. daily gain	0-3	214	0.052 ±0.001	-0.006	-
		3-6	214	0.040 ± 0.001	-0.003	-
		6-9	214	0.043 ±0.001	-0.005	-
C1763T					**CC-TT**	**TC**
	Body weight	3m	214	7.93±0.13	-0.15	0.46
		6m	214	12.74±0.47	-0.49	0.45
		9m	214	15.49±0.12	-0.01	-0.17
	Av. daily gain	0-3	214	0.050±0.002	-0.007	-0.0085
		3-6	214	0.039±0.001	-0.002	-0.03
		6-9	214	0.042±0.001	-0.003	-0.0035
A1780G					**AA-GG**	**AG**
	Body weight	3m	214	7.92± 0.15	-0.43	0.60
		6m	214	12.39±0.56	0.44	0.91
		9m	214	15.63±0.15	0.86	0.48
	Av. daily gain	0-3	214	0.051±0.002	0.13	-0.006
		3-6	214	0.039±0.001	0.003	0.002
		6-9	214	0.043±0.001	0.007	0.005

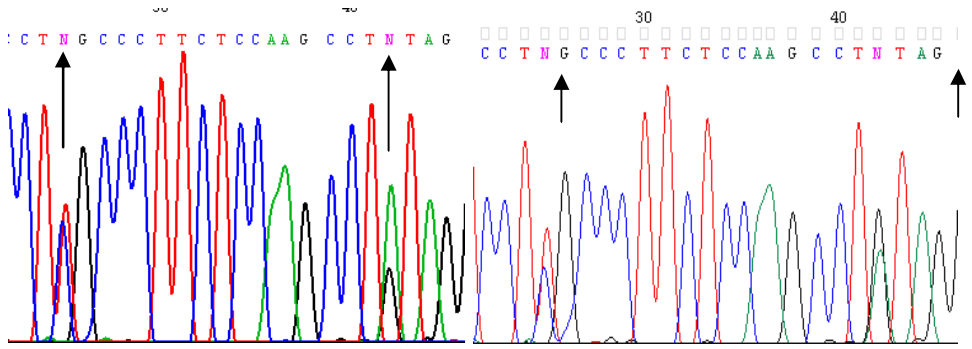
* Estimated by the difference between the solutions for two homozygote genotypes.

** Estimated by subtracting the average of solutions for homozygous genotypes from that for corresponding heterozygous genotype.

Individuals with AA genotype having A 1753 insertion, had significantly lower body weight at 9 month age over the counterparts without AA genotype. However, individual with AA genotype were less frequent (0.14). The C1763T SNP was detected at the 1763 nucleotide position in the intron 4 of GH gene in both goat breeds. Genotyping revealed CC and TT and TC genotypes with the frequencies of 0.17, 0.30 and 0.52, respectively. Although homozygote CC and TT individuals showed no difference in body weight at any age considered, significant weight difference was observed between TG and TT genotypes at three month of age and average daily gain at all ages (Figure 5 and 6)

A1780 in intron 4 in both goat breeds revealed AA, GG and AG genotypes with the frequency of 0.62, 0.10 and 0.28. Individuals with GG and AA genotypes were 9.7 % and 4.6 % respectively less weight than its heterozygote AG counterparts at the age of three month. However this weight difference was not observed in the subsequent ages. The positive

weight in AG genotype was also reflected in the average daily gain from birth to three month age (Figure 5 and 6).



Graph A *Sangamneri* **Graph B *Osmanabadi***

Fig. 4. Chromatograph (A and B) showing two heterozygote polymorphic sites of C/T in 1763bp and A/G in 1780bp positions located at intron 4

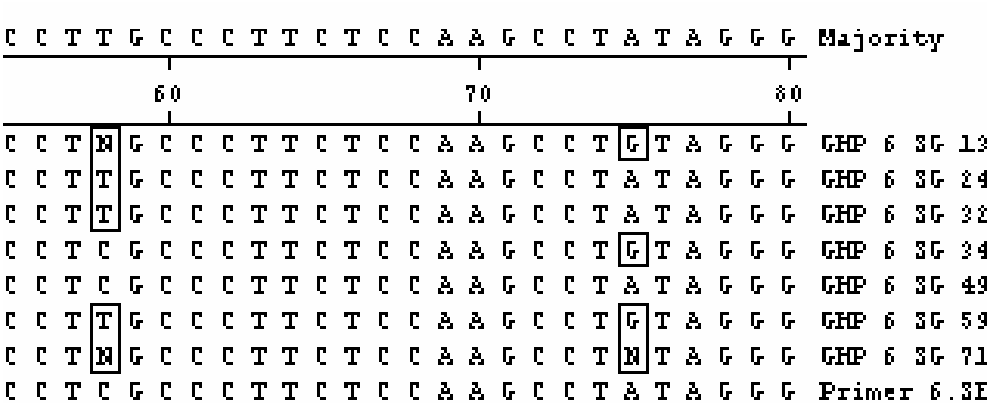


Fig. 5. Nucleotide sequences alignment showing TT, CC or T/C alleles at 1763bp position and AA, GG and A/G alleles at 1780bp position in the intron 4 of GH gene

Association of SNPs with other body measurements of S*angamneri* goats

Relationship of SNPs was studied with the primary data recorded on the body measurements (Table 6). The SNP G200T, at the promoter region of *Sangamneri* goat revealed GG and TG genotypes only. The absence of TT genotype may be due to the small population size used in the study. G200T and A815G SNPs did not show any association with body length, chest girth, paunch girth and body weight. However, both SNPs had positive relationship with body height of the animals. Estimation of the association of SNP G200T revealed animals with GG genotypes were 6% taller than their heterozygote GT counterparts (Table 6). A815G SNP revealed AG and GG genotypes and heterozygote AG individuals showed 1.1% superiority in height. A1753-54 SNP is an insertion of AA between 1753 and 1754 nucleotide position in the exon 4 region. Individuals with the presence of additional allele in

the sequence had 6.5% longer body and the difference was significantly higher at P=0.05 than the counterparts with absence of A allele. No significant difference was observed among the other body parameters. The SNP C1763T had TC and TT genotypes but CC genotype was not observed. Heterozygote counterparts were 6.1% longer body and the difference was significantly higher at P=0.05 than their TT homozygote counterparts. The difference observed in other body measurements had no significant difference. The A1780G SNP detected at the intron 4 region of the GH gene revealed AA, AG and GG genotypes with the frequencies of 16%, 73% and 10% respectively. There was no significant relationship between the three kinds of genotypes and body measurements. Absence of uniform distribution of genotypes in the population may be the reason for lack of such relationship

Table. 6. The significance of SNPs on different growth parameters in *Sangamneri* goats

SNP	Genotype	Length (in)	Height(in)	Chest girth(in)	Pouch girth (in)	Adult Wt (kg)
G200T	GG	47.78±1.12^{a}	55.25±1.53^{a}	54.17±1.22^{a}	57.26±1.66^{a}	10.92±0.59^{a}
	GT	48.48±1.25^{a}	51.89±1.71^{b}	52.89±1.37^{a}	57.86±1.86^{a}	10.78±0.66^{a}
A815G	AG	48.75±0.97^{a}	53.88±1.33^{a}	53.64±1.06^{a}	58.15±1.44^{a}	11.09±0.51^{a}
	GG	47.51±1.36^{a}	53.27±1.89^{b}	53.43±1.48^{a}	56.98±2.02^{a}	10.61±0.72^{a}
A1753-54	AA	49.75±1.10^{a}	53.83±1.46^{a}	54.26±1.17^{a}	59.15±1.59^{a}	11.13±0.56^{a}
	--	46.51±1.42^{b}	53.31±1.95^{a}	52.81±1.56^{a}	55.98±2.10^{a}	10.58±0.75^{a}
C1763T	TC	49.94±0.95^{a}	54.64±1.31^{a}	54.57±1.03^{a}	59.34±1.40^{a}	11.28±0.50^{a}
	TT	46.90±1.38^{b}	52.98±1.91^{a}	52.68±1.50^{a}	56.34±2.03^{a}	10.67±0.72^{a}
A1780G	AA	47.37±1.56^{a}	54.10±2.15^{a}	53.27±1.69^{a}	56.80±2.30^{a}	10.42±0.82^{a}
	AG	50.09±0.98^{b}	55.00±1.35^{a}	54.76±1.06^{a}	59.49±1.45^{a}	11.20±0.51^{a}
	GG	45.31±2.77^{ab}	49.23±3.82^{ab}	50.69±3.00^{ab}	54.82±4.09^{ab}	11.53±1.45^{ab}
C2055T	CC	45.11±2.11^{a}	54.43±2.95^{a}	52.51±2.32^{a}	54.46±3.18^{a}	10.70±1.10^{a}
	CT	49.56±2.06^{a}	54.22±2.89^{a}	54.22±2.27^{a}	59.36±3.11^{a}	10.34±1.07^{a}

* Different letters in the are significantly different at P≥0.05.

CONCLUSIONS

The SSCP technique used in this experiment proved to be an appropriate technique in screening samples for polymorphism. Considerable deviations were observed in the *Osmanabadi* and *Sangamneri* growth hormone gene sequence from that of exotic goats suggesting needs for further analysis of breed diversity. The SNPs reported in this study may be useful markers in selection of goats for high growth. However, as the study was based on a limited sample size, reconfirmation of findings is needed with an adequate sample size. The nucleotide deviations from exotic sequence which were commonly detected in both the breeds, need to be investigated with other Indian goat breeds as well to confirm whether they are specific only to Indian goat breeds. As the growth hormone gene is exceptionally a versatile gene responsible for promoting several beneficial functions including quantity and quality of milk and carcass characteristics, it is suggested to test the association of detected SNPs with those traits.

ACKNOWLEDGEMENTS

Authors are grateful to Sri Lanka Council of Agricultural Research Policy (CARP) for sponsoring Ph. D. scholarship. The research expenditure sponsored by the "Threaten Breed Conservation Project" of the central government Department of Animal Husbandry, India is also appreciated. Numerous support extended by the staff of the Department of Animal Science & Dairy Science., MPKV, Rahuri (MS) and DNA Bank, NBAGR, Karnal (Haryana) is gratefully appreciated.

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