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Genetic Polymorphism of Growth Hormone Gene Exon-4 in Surti and Mehsani Goats by PCR-RFLP

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Abstract

The present investigation was planned to study growth hormone (GH) gene exon-4 polymorphism using Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) in Surti and Mehsani goats. GH gene exon-4 region was found to be monomorphic on restriction digestion with *HaeIII,* which revealed only one genotype CC in both Surti and Mehsani goat breeds. The allelic frequency of C was 1.00 in both the breeds of goats with absence of D allele.

Introduction

Goat has distinct social, economical, managerial and biological advantages over other livestock species and often termed as the “Poor man’s cow. Out of the 28 defined goat breeds in India (NBAGR), 5 breeds namely, Surti, Mehsani, Kutchi, Zalawadi and Gohilwadi are native to Gujarat. The Surti and Mehsani goat breeds are found in two different parts of Gujarat, Surat and Mehsana respectively, kept for both milk and meat purpose.

Animals with high milk yield reveal superior Growth Hormone (GH) average levels than animals with lower production, mainely during peak lactation (Reinecke *et al*., 1993). GH gene, with its functional and positional potential, has been widely used as a molecular marker in several livestock species including the cattle, sheep and goat. GH gene may be used as a candidate gene for studying its polymorphism

and association in relation to growth due to its vital role of GH in animal growth and development (Supakorn, 2009).

GH is an anabolic hormone synthesized and secreted by the somatotroph cells of the anterior lobe of the pituitary in a circadian and pulsatile manner. GH affects a wide variety of physiological processes such as lactation, reproduction, growth and metabolism (Ayuk and Sheppard, 2006). GH is associated with both animal's growth (Hua *et al*., 2009) and milk yield traits (Malveiro *et al*., 2001; Marques *et al*., 2003) in many livestock animals. GH gene is physically located on goat chromosome 19q22 (Pinton *et al*., 2000). It is a peptide and encoded by 1,800 base pairs consisting of five exons and four intervening introns (Wickramaratne *et al*., 2010). This gene produces the GH from the anterior pituitary. This hormone is necessary for postnatal growth and metabolism in vertebrates (Ayuk and Sheppard, 2006).

GH gene polymorphism has been reported in some of the Indian goat and sheep breeds by various researchers including Malabari goats (Chitra and Aravindrakshan, 2004), Black Bengal goats (Gupta *et al*., 2007; Shankar *et al*., 2014), Jhakrana goats (Gupta *et al*., 2009), Sangamneri and Osmanabadi goats (Wickramaratne *et al*., 2010), Sirohi and Barbari goats (Kumar *et al*., 2011; Singh *et al*., 2015), Atapaddy Black, Malabari and Malabari crossbreds (Radhika *et al*., 2016), Chokla, Magra, Malpura, Nellore, Patanwadi, Sonadi, Garole, Bharat Merino, Avikalin sheep (Kumari *et al*., 2014) and Vembur sheep (Sheevagan *et al*., 2015).

The present investigation was undertaken to study the genetic polymorphism in the regions of exon-4 in GH gene in Surti and Mehsani goats.

Materials and Methods

Blood sampling

The study included 50 Surti goats maintained at Livestock Research Station, NAU, Navsari, Gujarat and 50 Mehsani goats maintained at Livestock Research Station, SDAU, Sardarkrushinagar, Dantiwada, Gujarat. About 5-7 ml of the blood was collected from the jugular vein of each animal into sterile 10 ml EDTA coated vacutainers and stored at -4ºC till further processing.

Genomic DNA extraction

The genomic DNA from whole blood samples of goats were extracted by Qiagen DNeasy Blood Kit. Purity and concentration of genomic DNA were estimated by Nanodrop 2000c spectrophotometric reading at OD_{260} and OD_{280}. The quality of genomic DNA was checked by 0.8 % agarose gel electrophoresis at a constant voltage of 80V for 60 minutes.

Plate 1: Genomic DNA of Surti and Mehsani goats.

Lane: L1 to L6: Genomic DNA in Surti goat, L7-L13: Genomic DNA in Mehsani goat.

Polymerase Chain Reaction (PCR)

Oligo primers [Table 1] specific to caprine GH gene locus as specified by Hua *et al*. (2009) were custom synthesized at Eurofins Genomics India Pvt. Ltd. Bengaluru and utilized to amplify the desired fragments in the present study.

Table 1: Sequence of Growth Hormone (GH) gene primers, PCR product size and amplified region (Hua *et al*., 2009)

Primers	Primer sequence	Product size	Amplified region
GH 2 F	TCA GCA GAG TCT TCA CCA AC	116 bp	Exon 4
GH 2 R	CAA CAA CGC CAT CCT CAC		

GH= Growth Hormone, F= Forward, R= Reverse

PCR was performed in a final reaction volume of 20 µl consisting of 10 µl of 2X master mix (Puregene), 3 µl genomic DNA (60 ng), 0.8 µl (8 pmole) of each forward and reverse primer and 5.4 µl of nuclease free water to amplify exon 4 of GH gene.

PCR amplification of exon 4 region of GH gene was carried out by using initial denaturation at 94^{0}C for 5 minutes followed by 35 cycles of denaturation at 95^{0}C for 30 seconds, annealing at 56^{0}C for 30 seconds and extension at 72^{0}C for 45 seconds and final extension at 72^{0}C for 10 minutes.

The PCR products were analysed by electrophoresis on 2% agarose gel and stained with ethidium bromide for 60 minutes at 80V. 100 bp DNA ladder was used as a molecular size marker. The bands were visualized under UV light and documented by gel documentation system (Alpha Imager HP).

Restriction Fragment Length Polymorphism (RFLP)

Restriction digestion of the amplified PCR products were carried out in a total volume of 30 µl having 10 µl PCR product, 3 µl 10X buffer, 16.5 µl nuclease free water and 0.5 µl restriction enzyme, *Hae III* (NEB). The restriction mixtures were incubated at 37^{0}C for 60 minutes and enzyme inactivation was done at 80^{0}C for 20 minutes. The digested PCR products were run on 4.0 % agarose gel at a constant voltage 80 V for 90 to 120 min in 0.5X TBE buffer. 50bp DNA ladder was used as a molecular size marker. The restriction patterns were visualized under UV light and photographed by gel documentation system.

Upon electrophoresis, the segments resolved in the gel and the genotypes were recorded after observing the PCR-RFLP patterns and the genotypic and allelic frequencies were calculated for Surti and Mehsani goat populations under study.

Results and Discussion

On amplification of exon 4 region of GH gene, amplified PCR product of 116 bp size was observed in both the breeds [Plates 2,3]. The PCR product of similar size has also been reported in various breeds of goats and sheep like Boer bucks (Hua *et al*., 2009), Boer and Matou dams (Zhang *et al*., 2011), Barki, Zaribi, Ardi and Masri goats (Alakilli *et al*., 2012), Savanna and Kalahari goats (Amie *et al*., 2012), Chokla, Magra, Malpura, Nellore, Patanwadi, Sonadi, Garole, Bharat Merino, Avikalin sheep breeds (Kumari *et al*., 2014) by amplification of these exons of the GH gene.

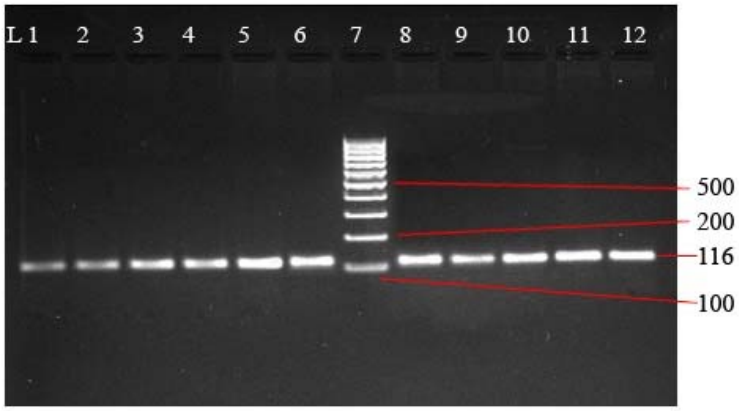
The present study on restriction digestion of exon 4 region of GH gene using *Hae III* revealed only one genotype, *i.e*., CC (88 and 28 bp), [Plate 4] in both Surti and Mehsani breeds of goats. The genotypes CD and DD were absent in both the breeds of goats and so the allelic frequency of C and D allele is 1.00 and 0.00, respectively, in both Surti and Mehsani goats [Table 2]. Therefore, exon-4 region of GH gene was found to be monomorphic in both the breeds of goats.

The RFLP pattern of our study for exon-4 of GH gene is in agreement with the studies of Amie *et al*. (2012) in Savanna and Kalahai goats, who reported single genotype CC in these breeds of

Table 2: Genotypic and allelic frequencies for GH2 locus in Surti and Mehsani goats with *HaeIII*

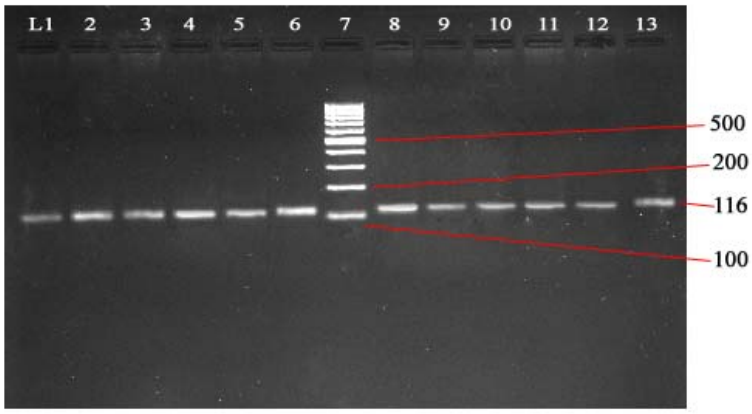
Goats	Locus	Genotype	Observed number of genotype	Genotypic frequency	Allele frequency	
					C	D
Surti	GH2 116	CC	50	1.0	1.0	0.0
		CD	0	0.0		
		DD	0	0.0		
Mehsani	GH2 116	CC	50	1.0	1.0	0.0
		CD	0	0.0		
		DD	0	0.0		

Plate 2: PCR products of exon 4 (116 bp) of GH gene in Surti goat.



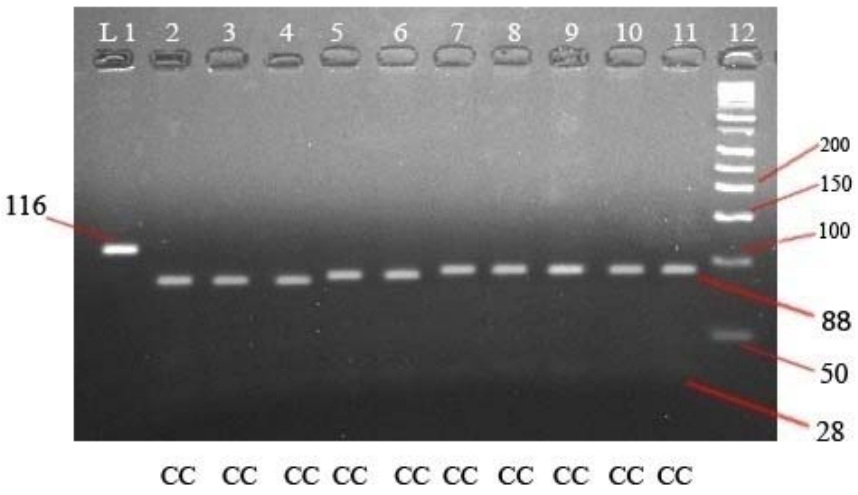
Lane: L7: 100 bp DNA ladder, L1-L6 and L8-L12: 116 bp PCR products.

Plate 3: PCR products of exon 4 (116 bp) of GH gene in Mehsani goat.



Lane: L7 : 100 bp DNA ladder, L1-L6 and L8-L13: 116 bp PCR products.

Plate 4: RE (*HaeIII*) digestion of GH gene exon 4 (116 bp) PCR products in Surti and Mehsani goats.



Lane: L1 : undigested PCR product, L2-L6: RFLP products of Surti goat, L7-L11: RFLP products of Mehsani goat, L12: 50 bp DNA ladder.

goats. Kumari *et al*. (2014) also reported similar results in nine different native sheep breeds of India namely Chokla, Magra, Malpura, Nellore, Patanwadi, Sonadi, Garole, Bharat Merino, Avikalin with a single genotype CC.

In contrast to the present findings, Hua *et al*. (2009) reported two genotypes CC (88 and 28 bp) and CD (116, 88 and 28 bp) in Boer bucks with the frequency of C allele to be 0.93. Similar results with two genotypes CC and CD were also reported by Zhang *et al*. (2011) in Matou and Boer dams and Alakilli *et al*. (2012) in four Egyptian and Saudi goat breeds, namely, Barki, Zaribi, Ardi and Masri.

Conclusion

GH gene exon-4 region amplified with caprine specific primers was found to be monomorphic on restriction digestion with *Hae III,* which revealed a snigle genotype CC with genotypic frequency 1.00 in both Surti and Mehsani goats. The allelic frequency of C allele was 1.00 with absence of D allele. Since the growth hormone locus studied was monomorphic in both the goat breeds, indicating monomorphism at this locus may be a species characteristic of goats probably due to absence of any mutation and high degree of sequence conservation. As goat GH exon 4 studied is monomorphic, it cannot be used as genetic marker for selection purpose.

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ANNOUNCEMENT: ISSAR-2018 CONFERENCE

XXXIV Annual Convention of the Indian Society for Study of Animal Reproduction (ISSAR) and International Symposium on "Productivity Enhancement through Augmenting Reproductive Efficiency of Livestock for Sustainable Rural Economy" will be hosted in collaboration with the ISSAR Gujarat Chapter by the College of Veterinary Science & Animal Husbandry, ***Anand Agricultural University, Anand - 388 001,*** Gujarat, India ***during December 28-30, 2018***. Brochure containing details of Awards, Sessions, Deadlines, Bank details for NEFT/RTGS, E-mail IDs etc is available on the website of the Society. The date for early bird registration and submission of abstracts is up to **30th November 2018** through e-mail.

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