POLYMORPHISM OF GROWTH HORMONE GENE IN MALABARI GOATS (*Capra hircus*)

By

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THESIS

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DECLARATION

I hereby declare that this thesis entitled "**Polymorphism of growth hormone gene in Malabari goats (*Capra hircus*)**" is a bonafide record of research work done by me during the course of research and that this thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title of any other University of Society.

Place: Mannuthy
Date : 1-11-2002

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CERTIFICATE

Certified that this thesis entitled "**Polymorphism of growth hormone gene in Malabari goats (*Capra hircus*)**" is a record of research work done independently by **Dr. R. Chitra,** under my guidance and supervision and that it has not previously formed the basis for the award of any degree, fellowship or associateship to her.

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R. Chitra

Dedicated to

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Introduction

1. INTRODUCTION

India is a vast reservoir for livestock bio-diversity in the form of species, breeds and strains. Goat is the earliest ruminant and probably the first, after dog, to be domesticated by man. Goats constitute a very important species of livestock in India, because of their short generation interval, high rate of prolificacy and easy marketing of goats and goat products. The indigenous breeds of goat are more efficient in converting coarse feed and fodder and are better adapted to withstand the harsh agro-climatic conditions prevailing in the country.

In India, mainly the landless farmers and small size holders maintain goats, which provide a dependable source of income to about 40% of the rural population who are below the poverty line. Goats produce a variety of products, mainly meat and skin, and to some extent milk, fleece and manure. Goat meat is the most preferred one in India and hence the costliest of all meats. Indian goatskins are considered to be of high quality because of their relatively larger size, minimal blemishes and high quality processing.

There are twenty distinct breeds of goats in India. The native breeds are adapted to local management and feeding and are resistant to certain tropical diseases. Cross breeding with exotic breeds is not recommended, as past experience had shown that the crosses with exotic genotypes could not adapt

to Indian conditions. The genetic improvement of native goat breeds should therefore be limited to selective breeding.

The Malabari goats are widely present in the Northern parts of Kerala. This breed seems to have its antecedents in the goats brought centuries ago to the Kerala coast by Arab merchants (Pattabiraman, 1955). Indiscriminate cross breeding with the local goats and inbreeding among progeny resulted in the breed, as seen today. It is the recognised breed of goats in this state. These goats are noted for their high milk yield and meat production qualities. They represent a unique genetic resource by virtue of their adaptability, resistance to many infectious diseases and prolificacy in the humid tropics of Kerala. They also exhibit considerable variation in individual performance in milk production, growth rate and fecundity.

Creation of high yielding animals has long been the primary objective of animal breeding programmes. Production of superior animals requires identification of superior parents. The advances in biotechnology and molecular genetics offer great opportunities for the identification, replication and transfer of desired genes and manipulation of the reproductive processes. Potentially, it is possible to select superior animals for breeding by direct genotyping, rather than through indirect selection processes based on the phenotypes with all their uncertainties.

Watson and Crick in 1952 postulated the molecular structure of DNA, which opened the door to a new era in modern science. During 1970s, the use of

restriction enzymes capable of restricting DNA at specific sites led way to the recombinant DNA technology. Advances in molecular genetics during 1980s revealed new techniques dealing directly with genes as physical entities. Prime characteristics of DNA are its robustness, capability of replicating itself and the ability of its constituent nucleotides to code for specific protein products.

In 1986, Kary Mullis invented the Polymerase Chain Reaction (PCR) technique, which permits minute amount of DNA to be increased exponentially (amplification) so that they can be used in various types of molecular genetic studies, which revolutionised many bench techniques. PCR is a relatively simple and inexpensive method and has greatly reduced the cost and time involved for many laboratory procedures with DNA. This technique is also sensitive, rapid and specific in detecting, modifying and amplifying DNA sequences.

Substitutions, insertions or deletions at restriction enzyme cleavage sites may produce changes in the resulting length of DNA fragments known as restriction fragment length polymorphism (RFLP). The PCR based RFLP method is relatively easy, fast and more sensitive than the traditional RFLP method based on Southern blotting and hybridisation.

Growth hormone stimulates deposition of proteins and growth in almost all tissues of the body. Its most obvious effect is to increase the growth of the skeletal frame. It enhances deposition of body protein, uses up the fat stores, and conserves carbohydrates. So it has a major impact on growth, lactation and mammary gland development in dairy animals. Therefore, the growth hormone

gene is a potential target for studies of molecular variation because of its possible direct or indirect effects upon growth and milk production.

Though the polymorphism at the growth hormone locus has been investigated extensively in cattle and sheep, there is a paucity of information in goats. Realising the importance of goats in Indian economy particularly in rural economy, the present study of the polymorphism at the growth hormone gene in Malabari goats was undertaken with the following objectives:

1. To study DNA polymorphism at the growth hormone locus using PCR-RFLP technique.
2. To estimate the allele and genotype frequencies at the growth hormone locus.
3. To find out association, if any, between different genotypes and body measurements, milk yield and composition in Malabari goats.

Review of Literature

2 REVIEW OF LITERATURE

2.1 Genome analysis

With the development of recombinant DNA technique came several methods for resolving genetic polymorphisms at the DNA sequence level. Such polymorphisms promise to be exceedingly numerous and well distributed throughout the genome of any species of interest. This opens up the possibility of statistically robust testing of putative genetic linkage relationships between marker and quantitative trait loci (QTL) alleles and provides greater confidence in the reliability of decision making for marker-assisted selection (MAS). The theoretical basis for experimental designs approaching the mapping of QTLs to markers and subsequent MAS has advanced considerably both in terms of general theory (Geldermann, 1975; Soller and Plotkin-Hazan, 1977; Soller and Beckmann, 1983) and applications specific to livestock.

Various authors (Smith, 1967; Soller, 1978; Smith and Simpson, 1986; Hallerman, 1989; Brascamp *et al.*, 1993; Falaki *et al.*, 1996; Israel and Weller, 1998; Mitra *et al.*, 1999; Beuzen *et al.*, 2000) have considered the value of selection based on quantitative trait loci (QTL) and of indirect selection through linked marker loci. Both these approaches would result in a considerable increase in the over all effectiveness of selection and in the rate of genetic progress (Smith and Simpson, 1986; Lande and Thompson, 1990).

Genetic makers can be defined as any stable and inherited variation that can be measured or detected by a suitable method, and can be used subsequently to detect the presence of a specific genotype or phenotype other than itself, which otherwise is nonmeasurable or very difficult to detect. In genetic analysis, various types of genetic markers such as morphological, chromosomal, biochemical and molecular markers are used (Mitra *et al.*, 1999).

The chance of detecting markers depends upon the relative contribution of the trait, the degree of linkage between the marker and the trait, and the frequency of desirable marker alleles in the population (Beuzen *et al.*, 2000).

The exploitation of DNA polymorphisms that are associated with multifactorial traits depends upon whether the polymorphism defines a marker or defines the QTL itself (Beuzen *et al.*, 2000). Utilisation of marker-based information for genetic improvement depends on the choice of an appropriate marker system for a given application.

2.1.1.1 Molecular markers

The location of markers and their linkage with QTL can be determined by within family analysis of performance data and then used in livestock improvement. Molecular variability at certain loci, for instance at genes coding for immunocompetence-related molecules or for protein hormones, might have direct physiological effects upon quantitative traits (Hallerman, 1989).

The markers revealing variations at DNA level are referred to as molecular markers (Mitra *et al.*, 1999). The use of molecular markers to define the genetic makeup (genotype) and predict the performance of an animal is a powerful aid to animal breeding (Beuzen *et al.*, 2000). Three types of DNA polymorphisms are particularly well characterised. They are restriction fragment length polymorphisms (RFLPs), microsatellites, and single nucleotide polymorphisms (SNPs).

2.1.1.1.1 Restriction Fragment Length Polymorphisms (RFLPs)

Nucleotide changes occur in all eukaryotic genome. If the change results in the creation or abolition of a restriction endonuclease recognition site, then the DNA sequence acquires or loses the ability to be cleaved by a particular restriction endonuclease resulting in a RFLP. With the proposition of theoretical models by Soller *et al.* (1976), Soller and Genizi (1978) and Weller *et al.* (1990), the foundations were laid for the application of RFLPs as markers for QTL.

RFLPs have been exploited in human genetics where they comprise the basis of a genetic map that can be used to diagnose and predict a number of genetic diseases including Huntington disease (Gusella *et al.*, 1983) and cystic fibrosis (Beaudet *et al.*, 1986).

The RFLP mapping is a powerful and extensively used technique for the identification of genetic variation, providing valuable markers for use in animal and plant genetics (Theilmann *et al.*, 1989; Rocha *et al.*, 1992).

2.1.1.1.2 Microsatellites

Microsatellites are one to six nucleotide repeats, interspersed throughout the genome. The mutation rate of microsatellites is thought to be high and there are often large numbers of alleles that vary in size at a single locus. A simple repeat family in human genome has been shown to reveal numerous polymorphisms (Ali *et al.*, 1986). Microsatellite-derived markers represent a powerful way of mapping genes controlling economic traits (Beuzen *et al.*, 2000).

2.1.1.1.3 Single Nucleotide Polymorphisms (SNPs)

The SNPs involve the substitution of one nucleotide for another, or the addition or deletion of a nucleotide. In human genomic DNA, there appears to be an SNP in approximately every 1000 bases (Landegren *et al.*, 1998).

2.1.1.2 Properties of molecular markers

Molecular markers are numerous and distributed ubiquitously throughout the genome. The DNA samples can be isolated easily from blood, sperm, hair follicles and even from archival preparations. It can be stored for long time and can be readily exchanged between laboratories. The analysis of DNA can be performed even in early embryonic stage, irrespective of the sex and the results are repeatable (Mitra *et al.*, 1999).

Table 2.1. Properties of different molecular markers: A comparison

Features	Marker system				
	RFLP	**CAPS (PCR-RFLP)**	**DFP**	**MAAP e.g. RAPD**	**Microsatellites**
Method of detection	Hybridisation	PCR	Hybridisation	PCR	PCR
Type of probe /primer used	Genomic DNA/cDNA sequence of structural genes	Sequence-specific primer	Minisatellite/synthetic oligos	Arbitrarily designed primer	Sequence-specific primer
Need for radioactivity	Yes	No/Yes	Yes	No/Yes	No/Yes
Extent of genomic coverage	Limited (coding sequence)	Limited (coding sequence)	Extensive	Extensive	Extensive
Degree of polymorphism	Low (SLDA)	Low (SLDA)	High (MLMA)	Medium to high (MLDA)	High (SLMA)
Phenotypic expression	Co-dominant	Co-dominant	Codominant/ dominant	Dominant	Codominant
Possibility of automation	No	Yes	No	Yes	Yes

Source: Mitra *et al.* (1999)

2.1.1.3 Applications of molecular markers

To maximise genetic response, markers should be combined with performance information (Smith, 1967 and Soller, 1978). The information may work in two ways. One is to allow increases in accuracy of selection. This may be particularly useful for traits which cannot be measured directly on live animal, such as carcass traits, or traits with expression sex limited to female such as milk yield and litter size. The other way is to affect the time of selection.

Another use could be the introgression of a gene, or genes, from one stock into another, by selective backcrossing to crosses with a rare or unique marker haplotype (Soller and Beckmann, 1983), which includes the gene.

The contribution of genetic markers to increase genetic improvement has been studied by Beckmann and Soller (1983); Smith and Simpson (1986); Hallerman (1989); Lande and Thomson (1990); Kashi *et al.* (1990); Meuwissen and Van Arendonk (1992); and Brascamp *et al.* (1993) in different ways.

With an enlarged set of polymorphic markers available, parentage checking can be more comprehensive and accurate. Numerous polymorphic markers could make accurate identification of breeding stocks and their derivatives possible so that patenting of improved stocks could be feasible (Soller and Beckmann, 1983).

Brascamp *et al.* (1993) reported that returns from the utilisation of genetic markers in dairy cattle breeding programs have been computed in two ways. In the first approach, returns accrued from additionally improved milk yield that was due to marker utilisation in selection is calculated. In the second approach, changes in returns from semen sales for a breeding organisation operating in a competitive market are determined.

For MAS to be applied efficiently, individuals with the desired genotypes must first be identified, there must be an incentive for selling these individuals, and the genotypic value must be estimated accurately (Israel and Weller, 1998).

Mitra *et al.*. (1999) reviewed the possible applications of molecular markers in livestock improvement with reference to conventional and transgenic breeding strategies.

2.2 Genomic DNA isolation

The first step in carrying out genome analysis studies is the isolation of pure high molecular weight genomic DNA from the sample population. The common methods for the isolation of DNA from whole blood include the use of phenol:chloroform extraction procedure (Blin and Stafford, 1976; Appa Rao, 1994; Aravindakshan *et al.*, 1997).

2.2.1 Yield and purity of DNA

Beckmann *et al.* (1986) reported 300-500 µg genomic DNA per 10 ml of cattle blood. Montgomery and Sise (1990) extracted genomic DNA from 20 ml of sheep blood and obtained DNA yield of 500 µg and 640 µg for phenol extraction and high salt methods, respectively.

Appa Rao *et al.* (1994) extracted genomic DNA from cattle, buffalo, sheep, goat and swine blood using a modified phenol extraction procedure and obtained a yield of 250-300 µg of DNA from 15 ml of whole blood.

Senthil *et al.* (1996) reported that the mean yields of genomic DNA from 15 ml of cattle blood samples extracted by the phenol and high salt methods were 444.58±21.54 µg and 615.55±20.72 µg, respectively. The ratio of optical density at 260 and 280 nm were more than 1.7 indicating good deproteinisation.

Aravindakshan (1997) observed that the mean yields of DNA extracted from 10 ml of whole blood of cattle and buffaloes by phenol and high salt methods were 394.50±34.26/446.16±26.69 µg and 344.25±16.45/432.83±19.34 µg, respectively. The ratio of optical densities at 260 and 280 nm was consistently between 1.75 and 1.91 indicating good deproteinisation.

2.3 Polymerase Chain Reaction (PCR)

The introduction of polymerase chain reaction (PCR) in conjunction with the constantly increasing DNA sequence data represents a milestone in

genome analysis. PCR has lead to the discovery of several useful and easy-to-screen methods. PCR method was invented and named by Mullis and coworkers of the Cetus Corporation, USA, in the year 1985. Don *et al.* (1991) discussed the touchdown PCR approach to avoid spurious priming during gene amplification.

Depending on the type of primers used for PCR, it can be divided into the sequence-targeted PCR assays and the arbitrary PCR assays. In the sequence-targeted PCR assays, a particular fragment of interest is amplified using a pair of sequence-specific primers. PCR-RFLP, allele specific PCR (AS-PCR), PCR amplification of specific alleles (PASA), simple sequence length polymorphism (SSLP) and sequence-tagged microsatellite site (STMS) markers belong to this category.

In arbitrary PCR assay system, unlike the standard PCR protocol, randomly designed single primer is used to amplify a set of anonymous polymorphic DNA fragments. Randomly amplified polymorphic DNA (RAPD) is a typical example of this category (Mitra *et al.*, 1999).

2.3.1 Components of PCR

2.3.1.1 *Taq* DNA Polymerase

Taq DNA polymerase is a thermo stable DNA polymerase isolated from *Thermus acquaticus*, which lacks 3′ to 5′ exonuclease activity but has 5′ to 3′ exonuclease activity, in addition to 5′ to 3′ polymerase activity. For most

amplification reactions 1 to 2.5 units of enzyme per 100 µl reaction mixture are recommended (Lawyer *et al.*, 1989) and excess of it leads to non-specific amplification.

2.3.1.2 Deoxyribonucleoside triphosphates (dNTPs)

The final concentration of each dNTP in a standard amplification reaction mixture is 200 µM. The optimal concentration of dNTPs depends on several factors including $MgCl_2$ concentration, reaction stringency, primer concentration, length of the amplified product and the number of cycles of PCR (Eckert and Kunnel, 1990).

2.3.1.3 PCR primers

Oligonucleotides used for priming the PCR should be at least 16 nucleotides and preferably 20-24 nucleotides in length. The primers should, if possible be made with approximately equal numbers of each of the four bases, avoiding regions of unusual sequences such as stretches of polypurines, polypyrimidines or repetitive motifs. Sequences possessing significant secondary structures should be avoided. Primer pairs should also be designed so that there is no complementarity of their 3′ ends to reduce the 'primer-dimer' formation, which is an amplification artifact. The presence of high concentrations of oligonucleotides can cause priming at ectopic sites, with consequent amplification of undesirable nontarget sequences. Conversely the PCR is extremely inefficient when the concentration of primers is limiting (Sambrook *et al.*, 1989).

For most applications of PCR, the primers are designed to be exactly complementary to the template DNA. The designing of oligonucleotides (primers) is generally carried out using some simple guidelines (Lowe *et al.*, 1990), although several computer programmes have been devised to aid primer design.

PCR primers developed for one species can sometimes function in related species (Moore *et al.*, 1991; Rubinsztein *et al.*, 1995).

2.3.1.4 PCR buffers

The standard buffer contains 50 mM KCl, 10mM Tris-HCl (pH 8.3 at room temperature) and 1.5 mM $MgCl_2$. Because the optimal concentration of Mg^{++} is quite low (1.5 mM), it is important that the preparation of template DNA does not contain high concentration of chelating agents such as EDTA or of negatively charged ionic groups such as phosphates (Sambrook *et al.*, 1989).

2.3.1.5 Template DNA

DNA containing the target sequences can be added to the PCR mixture in a single-or double-stranded form (Sambrook *et al.*, 1989). DNA can be extracted from a wide variety of sources such as fresh tissues, cytological specimens, fixed paraffin-embedded tissues and dried blood (Jackson *et al.*, 1992; Ivinson and Taylor, 1992).

2.3.2 Conditions of PCR

Aravindakshan *et al.* (1997) carried out the PCR reaction in a final volume of 25 µl with initial denaturation for 2 min at 94°C, followed by 35 cycles of 1 min at 94°C, 1 min at 60°C for primer annealing and 1 min at 72°C for primer extension. After the last cycle, a final primer extension was carried out for 7 min at 72°C for PCR-RFLP of growth hormone gene in cattle.

2.3.3 Inhibitors of PCR

Heparin is a potent inhibitor of PCR and therefore should not be used as an anticoagulant if PCR is to be performed on the extracted DNA. Residual traces of phenol, proteinase K, detergents such as SDS that are used for DNA extraction are inhibitors of PCR and these can be eliminated during DNA isolation (Sambrook *et al.* 1989; Newton and Graham, 1994).

2.4 PCR-RFLPs in growth hormone gene

2.4.1 Growth hormone (GH)

Bovine GH is a single chain polypeptide hormone about 22,000 daltons in size, consisting of 191 amino acids (Dayhoff, 1978) and is secreted by the anterior pituitary gland. Growth hormone has a significant influence on growth, mammary gland development and lactation in dairy animals (Tucker, 1981; Collier *et al.*, 1984; Bauman *et al.*, 1985; Hoj *et al.*, 1993). The proposed mechanisms of action of bovine somatotropin (bST) including those of galactopoiesis have been reviewed by McBride (1988).

The administration of bST has the potential to dramatically increase milk production and improve milk production efficiency in well managed dairy herds (Bauman *et al.*, 1985; Eppard *et al.*, 1985; Soderholm *et al.*, 1988; Annexstad *et al.*, 1990; Burton *et al.*, 1990; Erdman *et al.*, 1990; Lormore *et al.*, 1990; McBride *et al.*, 1990; Eppard *et al.*, 1991; Jordan *et al.*, 1991; McGuffey *et al.*, 1991; Adriaens *et al.*, 1992; Cole *et al.*, 1992; Gibson *et al.*, 1992; Lynch *et al.*, 1992). Burton *et al.* (1990) reported that the increased milk yields were accompanied by increased component yields, except for protein due to bST administration.

Bovine GH is associated with important biological actions such as mammary growth, lactation and regulation of metabolism; and its genomic DNA composition makes it a promising candidate gene for studying gene-specific DNA polymorphism (Zhang *et al.*, 1993).

Hansen *et al.* (1994) reported that the administration of bST in the commercial dairy herd was effective in increasing milk production for two consecutive years.

2.4.2 Growth hormone gene

Miller *et al.* (1980) developed DNA sequence complementary to bovine growth hormone mRNA. Kidd and Saunders (1982) described the linkage arrangements of placental lactogen and GH genes in human.

The bovine GH gene has been sequenced and it is composed of five exons coding for 786 bp long mRNA and four introns spreading over 1.8 kb (Woychick *et al.*, 1982; Gordon *et al.*, 1983). The GH gene has been assigned to chromosome region 19q26-qter in cattle and 11q25-qter in sheep (Hediger *et al.*, 1990).

Zhang *et al.* (1992) identified the nucleotide sequence of bST gene through sequencing of the PCR product.

2.4.3 RFLP in GH gene

Growth hormone has a key role in mammary gland development and milk production (Daughaday *et al.*, 1975; Bauman *et al.*, 1985). Also, GH has a tissue-specific action that is either direct or indirect via insulin like growth factor-I (IGF-I), and the effect of this action depends on the GH receptor (GHR) and several other hormones.

The RFLP may directly affect gene expression by changing the splicing of mRNA, stability of mRNA, rate of gene transcription, or the sequence of the gene product. The RFLP may also serve as genetic markers if linked to QTL (Beckmann and Soller, 1983; Smith and Simpson, 1986). Hence, the determination of the contribution (direct or as a marker) of RFLP to production traits before their subsequent use in selection schemes is of great interest.

Investigations of bovine GH gene have resulted in detection of two RFLPs: an insertion (I) / deletion (D) of approximately 0.9 kb in the 3' region of

the gene and a polymorphic *Msp*I restriction site in the third intron. These RFLPs have been shown to be present in several breeds of cattle (Beckman *et al.*, 1986; Hallerman *et al.*, 1987; Cowan *et al.*, 1989; Hilbert *et al.*, 1989; Theilmann *et al.*, 1989, Hoj *et al.*, 1993; Aravindakshan *et al.*, 1997).

Hoj *et al.* (1993) developed a PCR procedure to detect the polymorphic *Msp*I site in the third intron of bovine GH gene. They obtained two alleles: viz., *Msp*I (+) and *Msp*I (-), representing the presence and absence of *Msp*I site respectively. GH/*Msp*I polymorphism is created by the insertion of a T at position +837 and C-G transition at position +838 in the intron III of the GH gene, which is detected as an *Msp*I PCR-RFLP.

Lagziel *et al.* (2000) reported that the *Msp*I (-) allele originated in the *Bos indicus* breeds of the Indian subcontinent, from which it diffused through the humpless *Bos taurus* breeds of the Eastern Europe, the Mediterranean basin, eventually reaching North-Western Europe and Western Africa in low frequencies.

2.4.4 Relationship of GH/RFLP polymorphism to production traits

Bovine somatotropin offers potential as a candidate for improved milk production via the enhancement of gross biological efficiency (Burton *et al.*, 1990).

A *Taq*I RFLP of growth hormone gene has been associated with the birth weight of beef cattle (Rocha *et al.*, 1992).

In the Red Danish lines the frequency of the allele lacking a functional *Msp*I site (*Msp*I (-) allele) was 0.28 and 0.05 in high and low fat production lines, respectively; this difference was significant ($p<0.01$). The corresponding frequencies in the Norwegian Red lines were 0.09 in the high fat production line and 0.0 in the low fat production line (Hoj *et al.*, 1993).

Lee *et al.* (1993) found a significant association of *Msp*I (-) with higher fat yield in a high milk-yield selection line. They also observed that bovine GH/*Alu*I polymorphisms were not associated with milk yield. The breeding values for meat traits were significantly different for different GH/*Alu*I genotypes but no effects on breeding values for milk yield were apparent even though those of milk composition approached significance (Schlee *et al.*, 1994).

Falaki *et al.* (1996) found the *Msp*I (-) allele to be associated with higher fat and protein yield and percentage in Italian Holstein dairy cattle, although the effect was not statistically significant. In contrast, Yao *et al.* (1996) found the *Msp*I (-) allele to be associated with a statistically significant decrease in milk, fat and protein yield.

In cattle, the frequencies of *Msp*I (+) and *Msp*I (-) alleles were 0.56/0.44 and 0.07/0.93 for crossbred Jersey and Ongole breeds, respectively. No significant variation ($p>0.05$) could be observed in the mean birth and adult body weights between different GH/*Msp*I genotypes. In Indian buffalo, all animals irrespective of the breed were of +/+ genotype indicating that

monomorphism at this locus may be a species-specific characteristic of buffalo (Aravindakshan *et al.*, 1997).

Lagziel *et al.* (1999) reported that the heterozygous (+/-) genotypes, as compared to homozygous (+/+), showed a significantly high milk protein percentage and kg protein per year and a significantly low ($p<0.10$) milk somatic cell counts (MSSC) for the *Msp*I polymorphism at the bovine GH gene. They also observed that in the general Israeli Holstein dairy cattle none of the animals were of the homozygous *Msp*I (-) genotype.

2.5 Milk analysis

2.5.1 Milk yield

Mishra (1979) reported that the mean lactation yield, length of lactation and average daily yield in Malabari goats are 37.5 kg, 101.3 days and 0.37 kg respectively. Milk production is affected by several factors such as breed, season, order of lactation, plane of nutrition, health and management of goats. Age has significant effect on daily milk yield and lactation yield (Anjaneyulu *et al.*, 1985). Parity has also been reported to have significant effect ($p<0.01$) on lactation yield, lactation length and dry period (Singh and Mukherjee, 1998).

2.5.2 Milk composition

Parkash and Jenness (1968) reviewed the composition and characteristics of goats' milk. The variation in composition of milk was reported to be due to age, breed, season and stage of lactation and plane of

nutrition (Agarwal and Bhattacharya, 1978; Mittal, 1979). Fat, protein, and lactose contents of milk of dwarf goats are higher than those of other breeds (Jenness, 1980). Research in the past decade has extended knowledge of composition of goat milk and properties of its constituents (Jenness, 1980). Gross chemical composition of milk of different breeds of goats varied widely (Anjaneyulu *et al.*, 1985).

2.5.2.1 Fat

Butterfat, protein, and lactose values were significantly affected by stages of lactation ($p<0.05$) and tended to rise with advancing lactation (Mba *et al.*, 1975). Verma and Chawla (1984) reported that more than 50 per cent Beetal does produced milk with higher than 4.5 per cent fat. Fat content ranges among different breeds from 3.6 to 5.0 per cent (Anjaneyulu *et al.*, 1985).

2.5.2.2 Protein

There was marked variation in the amount and distribution of nitrogen in milk due to breeds and stage of lactation (Bhagel and Gupta, 1980; Singh and Singh, 1980a; Singh and Singh, 1980b).

Anjaneyulu *et al.* (1985) observed that the protein content of goat milk (3.65±0.15 per cent) was almost similar to that of cow milk (3.34±0.09 per cent).

2.5.2.3 Total solids

Verma and Chawla (1984) reported that more than 50 per cent Beetal does produced milk with higher than 10 per cent SNF and 14.5 per cent total solids. Koushik and Kupta (1989) observed a marked variation in the total solids content, which was affected significantly by breed variation.

2.6 Estimation of body weight from body measurements

Bhattacharya *et al.* (1984) reported that relationships between body weight, heart girth and length or body weight, heart girth and circumference of neck, in the multiple regression models are best prediction equations for body weight estimation in Black Bengal goats. In male goats, heart girth was slightly better predictor of live weight than body length and body length was slightly better predictor of live weight than heart girth in female goats of Black Bengal breeds (Islam *et al.*, 1991). Mohammed and Amin (1996) concluded that the body weight of Sahel goats could be estimated in the field using morphometric measurements taken with tape.

Materials and Methods

3. MATERIALS AND METHODS

3.1 Experimental animals

A random sample of 196 genetically unrelated Malabari does in second lactation formed the material for this study. The experimental animals were selected from the home tract of the breed, namely, Malappuram, Kozhikkode, Wyanad, Kannur and Kassaragode districts of Northern Kerala.

3.2 Collection of samples

3.2.1 Blood

Blood samples (5 ml each) were collected from jugular vein aseptically using sterile disposable syringes and transferred immediately to sterile disposable centrifuge tubes containing EDTA (1 mg/ml of blood) as anticoagulant. The samples were brought in ice to the laboratory and stored at 4°C till processed.

3.2.2 Milk

Milk samples (about 50 ml) were collected from the experimental animals in sterile milk bottles without any preservatives. The samples were brought to the laboratory in frozen condition and stored at -20 °C till processed.

3.2.3 Data collection

Information about each experimental animal and data on production and reproduction were collected and recorded in the data-recording sheet. (Annexure-I).

3.3 Extraction of genomic DNA

Genomic DNA from whole blood was extracted by using the standard phenol: chloroform extraction procedure.

[1]3.3.1 Phenol: chloroform extraction procedure

1) To five ml of blood, two volumes of ice-cold RBC lysis solution was added and mixed and kept in ice with occasional mixing for 10 min to allow the complete lysis of erythrocytes.

2) The tubes were centrifuged at 4000 rpm for 10 min to pellet the contents. The red coloured supernatant containing the lysed erythrocytes and haemoglobin was discarded leaving the pellet of white blood cells.

3) Steps 1 and 2 were repeated till the pellet was clear without any unlysed erythrocytes. The pellet was washed twice with TBS buffer by centrifugation at 3000 rpm for 10 min.

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[1]The composition and methods of preparation of the reagents and buffers used are presented in the Annexure-II

4) Washed white cell pellet was resuspended completely by vortexing in 5 ml of SE buffer so that no cell clumps remained.

5) To this cell suspension 25 µl of proteinase K (20 mg/ml in water) and 0.25 ml of 20 % SDS were added and mixed gently.

6) The samples were then incubated at 50 °C in a water bath with occasional shaking for at least three hours.

7) The digested samples were cooled to room temperature and 300 µl of 5 M NaCl was added to each sample and mixed by vortexing.

8) An equal volume of saturated phenol (pH 7.8) was added and mixed thoroughly by inversion for 10 min. The tubes were centrifuged at 4000 rpm for 15 min and the aqueous phase was collected in fresh tubes with the help of wide bore Pasteur pipettes.

9) An equal volume of saturated phenol: chloroform: isoamyl alcohol (25:24:1) was added to the aqueous phase and mixed thoroughly by inversion for 10 min. The tubes were centrifuged at 4000 rpm for 15 min and the aqueous phase was collected in fresh tubes using wide bore Pasteur pipettes.

10) To the aqueous phase an equal volume of chloroform: isoamyl alcohol (24:1) was added and mixed for 10 min and centrifuged at 4000 rpm for 10 min. The upper aqueous phase was transferred in to a fresh tube with the help of a wide bore Pasteur pipette and the step was repeated.

11) The aqueous phase was transferred to a sterile 50 ml beaker and one-tenth volume of 3 M sodium acetate (pH 5.5) was added and mixed well.

12) An equal volume of isopropyl alcohol was added and the precipitated DNA was spooled out on a clean micropipette tip, washed in 70 % ethanol, air-dried and resuspended in 500 µl TE buffer (pH 8.0) and stored at –20 °C.

3.3.2 Determination of the yield and purity of DNA samples

From the DNA stock solution 20 µl of solution was dissolved in 980 µl of sterile distilled water, giving a dilution of 50 times. Optical densities (OD) were measured at 260 nm and 280 nm wavelengths using disposable cuvettes in an UV spectrophotometer (Jenway, UK) using sterile distilled water as blank. The yield and purity of DNA samples were estimated as follows.

3.3.2.1 Yield

An OD of 1 at 260 nm wavelength corresponds to approximately 50 µg/ml for double stranded DNA.

Concentration of DNA stock solution (µg/ml) = OD_{260} X Dilution factor X 50

From the concentration of DNA stock solution, the total yield of DNA was calculated by multiplying the concentration and the volume of DNA stock solution.

3.3.2.2 Purity

Purity of DNA stock solution was estimated by finding the ratio between the readings at 260 nm and 280 nm wavelengths (OD_{260}/OD_{280}). Pure DNA samples have OD_{260}/OD_{280} ratios 1.8 and above.

3.3.3 Agarose gel electrophoresis for checking quality of genomic DNA

To determine the quality and molecular weight of DNA samples, approximately 0.5 to 1 µg DNA were checked electrophoretically using 0.7 per cent agarose in 1 X TAE buffer in a horizontal submarine gel electrophoresis unit.

The agarose in 1 X TAE buffer containing 0.5 µg/ml of ethidium bromide was heated until it was a clear solution and was cooled to 50 °C. The comb was kept in proper position in the gel tray on a level surface and the molten agarose was poured carefully in to the gel tray avoiding air bubbles. After gelling, the comb was removed gently and the gel tray was immersed in the buffer tank filled with 1 X TAE buffer. From DNA stock solution 0.5 to 1 µg DNA was mixed with one-sixth volume of 6 X gel loading buffer and the samples were loaded into the wells carefully. Electrophoresis was carried out at 2 V/cm at room temperature until the bromophenol blue dye migrated more than two-third of the length of the gel. The gel was visualised under a UV transilluminator (Hoefer, USA).

3.4 PCR-RFLP typing

PCR-RFLP analysis was carried out at the growth hormone (GH) locus. The PCR amplified product was digested with *Msp*I restriction enzyme to analyse the polymorphic patterns.

3.4.1 Template DNA preparation for PCR

Template DNA for PCR was prepared by diluting the DNA stock solution in sterile triple distilled water to a concentration of 50 ng/µl and was stored at –20 °C.

3.4.2 Selection, reconstitution and dilution of primers

The primers for the present study were selected from published reports (Hoj *et al.*, 1993) and were custom synthesised (Invitrogen (India) Pvt. Ltd.). The primers obtained in lyophilised form were reconstituted in sterile distilled water to a concentration of 200 pM/µl. The tubes were kept at room temperature with occasional shaking for one hour. The tubes were spun briefly to pellet down the insoluble particles, if any and the stock solution was distributed into 10 µl aliquots and stored at –20 °C. At the time of use, the aliquots were thawed and further diluted 10 folds before using for PCR.

3.4.3 Setting up of PCR

A master mix was prepared just before setting up the PCR assay combining 10X PCR buffer, 50 mM $MgCl_2$, dNTP mix, primer pairs, *Taq* DNA

polymerase and sterile water in such a way to get a final concentration of 1X PCR buffer, 1.5 mM $MgCl_2$, 200 µM dNTPs, 20 pM of each primer and one unit *Taq* DNA polymerase in a total volume of 25 µl in 200 µl reaction tubes.

To each reaction tube 24 µl of master mix and 1 µl (50 ng) of template DNA were added. The tubes were spun briefly and placed in the thermal cycler. One negative control without template DNA was also included to monitor contamination, if any.

The standardised thermal cycling profile consists of an initial denaturation of 3 min at 94 °C and 35 cycles of 1 min at 94 °C, 1 min at 60 °C and 1 min at 72 °C. This was followed by a final extension of 7 min at 72 °C. The PCR amplified products were stored at –20 °C till analysed.

3.4.4 Checking of target DNA amplification

The PCR products were checked by agarose gel electrophoresis to confirm the amplification before analysing for polymorphism.

Two microlitre aliquots of the PCR products were checked eletrophoretically using 1 per cent agarose gel in 1X TAE buffer. Either ϕX174 RF DNA/*Hae*III digest or 100 bp DNA ladder was used as DNA size marker.

3.4.5 Analysis of PCR amplified products

For restriction enzyme analysis, 10 µl of the amplified product was digested with 15 units of *Msp*I enzyme at 37 °C for 2 hr in a final volume of 15

µl. The amplified products were taken separately in to the tubes and 5 µl of the master mix containing the enzyme, 10X assay buffer and sterile distilled water was added to each tube. Following the digestion the enzyme was inactivated by incubating the tubes at 65 °C for 30 min and the digested products were stored at 4 °C till analysed.

3.4.6 Separation of restriction fragments

The DNA fragments were separated by electrophoresis either in 3 per cent agarose gels in 1X TAE buffer containing ethidium bromide at 2V/cm for 3 hr or in 8 per cent polyacrylamide gels (PAGE) in 1X TBE buffer (pH 8.3) at 2V/cm for 3 hr. As DNA size markers, ϕX174 RF DNA/*Hae*III digest and 100 bp DNA ladder were used. The PAGE gels were stained with ethidium bromide (0.5 µg/ml in 1X TBE) solution for 30 to 45 min. The gels were visualised and the images were documented in a gel documentation system (Bio rad Laboratories, USA).

3.5 Milk samples

At the time of analysis milk sample bottles were transferred in to a water bath maintained at 40 °C and kept for 15 min. Then samples were mixed thoroughly by pouring repeatedly from one container to another.

The milk samples were analysed for the following parameters.

3.5.1 Fat

The fat percentage in milk was estimated by Gerber's method as per the procedure described in IS: 1224-Part I (1977) as follows.

In a clean dry butyrometer 10 ml of Gerber's sulphuric acid was taken and 10.75 ml of well-mixed sample of milk and 1 ml of amyl alcohol were added. The contents of the butyrometer were mixed well and maintained at a temperature of 65±2.0 °C. Butyrometers were centrifuged for 4 min at maximum speed in a Gerber's centrifuge. The fat per cent was read after adjusting the fat column to a main graduation in the butyrometer.

3.5.2 Total Solids

The percentage of total solids in milk was estimated by the Gravimetric method as described in IS: 1479-Part II, 1961.

Empty stainless steel dishes, cleaned and dried, were weighed with their lids. About five millilitres of milk was pipetted into each dish. The dish was again weighed with the lid. The open dish was placed in a boiling water bath for 30 min in order to dry the milk. It was then transferred to a well-ventilated oven maintained at 99 to 100 °C. After three hours the dish was covered with lid and transferred immediately to the desiccator. The dish was allowed to cool for about 30 min and the weight was noted. The dish was once again transferred to the oven and heated further for one hour. It was again transferred to the desiccator for cooling and weighed as before. This procedure was repeated until

the loss of weight between successive weighings did not exceed 0.5 mg. The lowest weight was recorded. The percentage of total solids was calculated by using the following formula.

$$\text{Total solids (\%) by weight} = \frac{w}{W} \times 100$$

Where,

w- weight in grams of residue after drying

W- weight in grams of milk sample taken for the test.

3.5.3 Solids-not-fat

Solids-not-fat (SNF) content of milk was determined by finding the difference between solids content and fat content of milk.

3.5.4 Protein

Total protein content in milk was estimated by the dye-binding method described by AOAC (1980) and Dolby (1961).

3.5.4.1 Preparation of dye

The dye solution was prepared by dissolving 0.7 g of Amido black 10B, 10.56 g of citric acid, 1.32 g of di-sodium hydrogen orthophosphate and 2 ml of 10 % thymol solution in methanol in one litre of distilled water. The pH of the dye was below three.

3.5.4.2 Procedure

Five millilitres of the milk sample was diluted to 100 ml with distilled water. Five ml of the diluted milk was mixed with 10 ml of the dye solution in 15 ml centrifuge tubes and centrifuged for 5 min at 2500 rpm. Three ml of the supernatant was diluted to 100 ml with distilled water and optical density was measured at 620 nm in spectrophotometer against blank solution prepared by mixing 5 ml of water and 10 ml of the dye solution and diluted in the same manner. The difference in optical densities between blank and sample was recorded. The protein content was arrived at from a standard curve.

3.5.4.3 Preparation of the standard curve

The standard curve for the estimation of protein by the dye-binding method was prepared by the method described by Sathian (2001), using condensed and diluted milk as follows.

Ten litres of pooled goat milk was divided into two portions of one litre and nine litres. The nine litres portion of milk was condensed at 55 to 60 °C and at 64 cm of Hg using a vacuum condenser (Anhydro, UK) and samples were drawn at 5, 10, 15 and 20 min intervals.

The other one litre portion of milk was diluted in the following proportions: 50 ml of milk + 0 ml water, 50 ml of milk + 10 ml water, 50 ml of milk + 20 ml water, 50 ml of milk + 40 ml water, 50 ml of milk + 50 ml water, 50 ml of milk + 70 ml water and 50 ml of milk + 80 ml water. All these

condensed and diluted milk samples were subjected to the dye-binding method as described above as well as to Kjeldahl estimation of total nitrogen (AOAC, 1980).

A standard curve was plotted between total protein percentage (Kjeldahl) on the Y-axis and the difference between the optical densities of the blank and the sample (Do-Dx) on the X-axis.

3.6 Prediction of body weight

Linear regression equations were derived for the does from the known body weightswith corresponding body measurements like body length, height and heart girth from 52 does (Table 3.1). The prediction equation based on heart girth ($Y= -13.4480+0.5663\ X_3$) was found to be the most simple and effective with a coefficient of correlation of 0.842 and this equation was used for predicting the body weights of the experimental animals.

3.7 Statistical analysis

The experimental animals were grouped into three populations based on the geographical locations, viz. Population I (Kozhikkode and Malappuram districts), Population II (Kasaragode district) and Population III (Kannur and Wyanad districts).

The gene and genotype frequencies at the GH/*MspI* locus were calculated by direct counting method for the three populations separately as well as for the whole population. The variation of the gene frequencies among

Table 3.1. Different prediction equations with partial regression coefficient of respective variables, coefficient of determination (R^2) and multiple correlation coefficient (R)

Prediction equation	a	b_1	b_2	b_3	Residual mean square	R^2	R
1. $Y = a + b_1X_1$	-8.4997	0.5508**	-	-	13.109	0.540	0.735
2. $Y = a + b_2X_2$	-5.3597	-	0.4849**	-	15.341	0.462	0.680
3. $Y = a + b_3X_3$	-13.4480	-	-	0.5566**	8.291	0.709	0.842
4. $Y = a + b_1X_1 + b_2X_2$	-10.4266	0.3999**	0.1786^{NS}	-	12.733	0.563	0.750
5. $Y = a + b_1X_1 + b_3X_3$	-14.8596	0.1234^{NS}	-	0.4773**	8.181	0.719	0.848
6. $Y = a + b_2X_2 + b_3X_3$	-14.8292	-	0.0931^{NS}	0.5017**	8.234	0.717	0.847
7. $Y = a + b_1X_1 + b_2X_2 + b_3X_3$	-15.2656	0.0897^{NS}	0.0533^{NS}	0.4646**	8.297	0.721	0.849

Y= Body weight in kg; X_1= Length in cm; X_2= Height at withers in cm; X_3= Heart girth in cm; b_1, b_2 and b_3 are the respective partial regression; 'a' is the respective intercept.

NS – Not significant

** Significant at 1 per cent level

the three population groups was analysed by the χ^2-test of significance as described by Snedecor and Cochran (1994).

Mean and standard errorswere estimated for body weight, body length, height at withers, heart girth, peak milk yield, milk fat, protein, and SNF percentage with respect to different GH/*Msp*I genotypes. The effect of GH/*Msp*I genotypes and different population groups were worked out using least squares analysis of variance as described by Harvey (1986) for non-orthogonal data. Model used was

$$Yijk = \mu + Gi + Pj + eijk$$

Where

Yijk = k^{th} observation of j^{th} population of the i^{th} genotype

μ = General mean

Gi = Effect of i^{th} genotype (g = 1-2)

Pj = Effect of j^{th} population (p = 1-3)

eijk = Random error.

The effect of GH/*Msp*I genotypes on different growth and production traits within each population group as well as for the whole population was analysed using Students 't' test. The litter size for different GH/*Msp*I genotypes were analysed using the χ^2-test.

Results

4. RESULT

4.1 Isolation of Genomic DNA

The DNA used in this study was isolated from venous blood samples of 196 lactating female Malabari goats using the phenol-chloroform extraction method.

4.1.1 Yield and Quality of DNA

The average (mean ± SE) yield of DNA obtained from 5 ml of blood was 231.097±11.65 µg. The ratios of optical density at 260 and 280 nm were more than 1.7 indicating good deproteinisation. On agarose gel electrophoresis the DNA samples were found to be of high molecular weight and appeared as single bands without sheared fragments.

4.2 PCR-RFLPs

PCR reactions were set up for GH locus using primers based on bovine sequences for the detection of RFLPs.

4.2.1 Optimisation of PCR Parameters

For the efficient amplification of the target sequences individual reaction components, time and temperature parameters were optimised. The optimum PCR conditions standardised for the GH locus are presented in Table 4.1.

4.2.2 Amplification of GH gene by PCR

The DNA samples isolated from blood samples of goats could be amplified by PCR at the GH locus without any major non-specific amplified products (Fig. 4.1). In all the animals, the size of the amplified product was 768 bp indicating conservation of DNA sequences at the GH locus in bovine and caprine species.

4.2.3 PCR-RFLP analysis in GH gene using *Msp*I

The amplified DNA was digested with restriction enzyme *Msp*I and the restriction fragments were resolved by agarose or acrylamide gel electrophoresis. Upon electrophoresis two restriction digestion patterns could be observed (Fig. 4.2). These allowed the identification of two alleles viz., *Msp*I (+) (612, 93 and 63 bp fragments) and *Msp*I (-) (705 and 63 bp fragments). The gene and genotype frequencies of GH/*Msp*I polymorphism in the three population groups as well as in the pooled population are presented in Table 4.2.

The frequencies for (+/+) and (+/-) genotypes in the pooled population were 0.40 and 0.60 respectively. Of the 196 animals typed, none of the animals were of the (-/-) genotype. The gene frequencies for *Msp*I (+) and *Msp*I (-) alleles in the pooled population were 0.70 and 0.30 respectively. The population was found to be not under Hardy-Weinberg equilibrium (Table 4.3).

Table 4.1 Optimum PCR conditions standardised for GH locus

Primers Sequence	$MgCl_2$	Denaturation	Primer annealing	Primer extension	Number of cycles
5'-AGA ATG AGG CCC AGC AGA AAT C-3' 5'- GTC GTC ACT GCG CAT GTT TG-3'	1.5 mM	94°C for 1 min	60°C for 1 min	72°C for 1 min	35

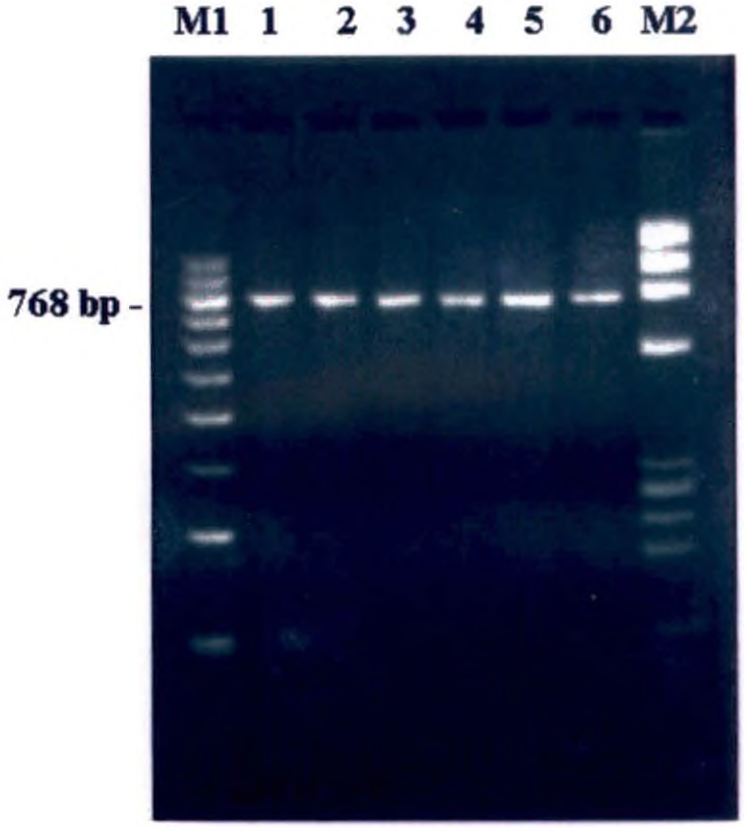

Fig. 1 **The 768 bp fragment of the growth hormone gene amplified from genomic DNA by PCR in Malabari goats on 2 % agarose gel. M1: 100 bp ladder and M2: ϕX174 RF DNA/*Hae*III digest as DNA size markers.**

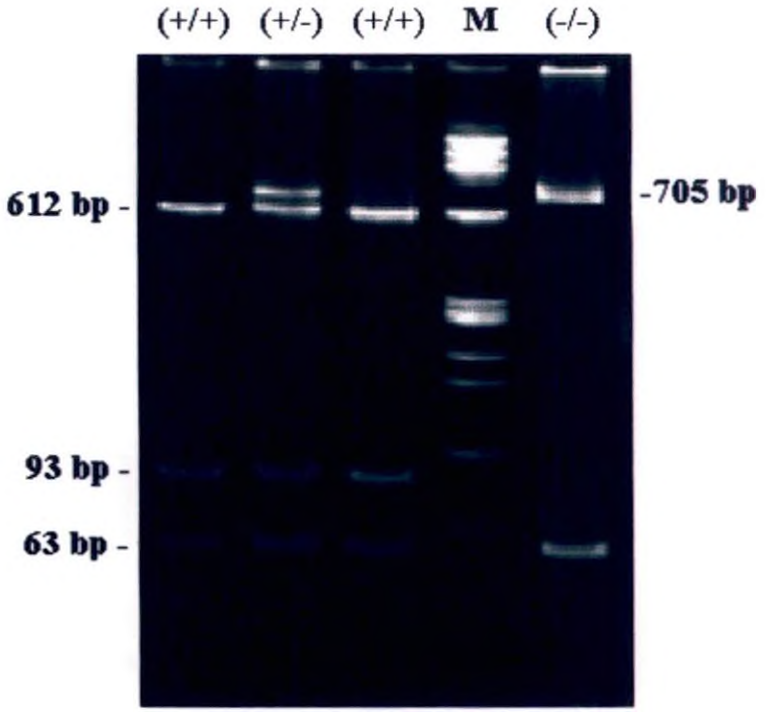

Fig. 2 The GH/*Msp*I genotypes resolved on 8 % non-denaturing polyacrylamide gel and stained by ethidium bromide. '+/+' (612, 93 and 63 bp fragments), '+/-' (705, 612, 93 and 63 bp fragments) and '-/-' (705 and 63 bp fragments) genotypes. The '-/-' genotype shown is from cattle DNA sample. M: ϕX174 RF DNA/*Hae*III digest as DNA size markers.

Table 4.2 Genotype and allele frequencies of GH/*Msp*I polymorphisms in Malabari goats

Population	Genotype frequency			Allele frequency	
	(+/+)	(+/-)	(-/-)	(+)	(-)
Population –I (n=67)	0.40 (27)	0.60 (40)	0.0 (0)	0.70	0.30
Population –II (n=66)	0.35 (23)	0.65 (43)	0.0 (0)	0.67	0.33
Population –III (n-63)	0.44 (28)	0.56 (35)	0.0 (0)	0.72	0.28
Pooled population (n=196)	0.40 (78)	0.60 (118)	0.0 (0)	0.70	0.30

Figures in parenthesis are actual numbers

Table 4.3 Testing of Genotypes of GH/*Msp*I polymorphisms for Hardy-Weinberg Equilibrium in Malabari goats

Source	GH/*Msp*I genotype			χ^2 value
	(+/+)	(+/-)	(-/-)	
Observed	78	118	0	34.594**
Expected	96.04	82.32	17.64	

Figures in parenthesis are genotype frequencies

** Significant at 1 per cent level

A comparison of the GH/*Msp*I allele frequencies among different population groups was carried out employing the χ^2-test. It showed that the frequencies of (+) and (-) alleles observed in different population groups did not differ significantly (Table 4.4).

4.3.1 GH/*Msp*I polymorphism and body weight

The average predicted body weight of Malabari does in the pooled population and in the three population groups with respect to different GH/*Msp*I genotypes is given in Table 4.5. The statistical analysis by Student's t-test revealed no significant variation in the body weights of different genotypes in the pooled population as well as in the different population groups.

Least square analysis of variance showed that differences in body weight for different population groups were highly significant. The body weights of different GH/*Msp*I genotypes did not differ significantly (Table 4.5).

4.3.2 GH/*Msp*I polymorphism and body length

The average body length of Malabari does in the pooled population and in the three population groups with respect to different GH/*Msp*I genotypes is given in Table 4.6. There was no significant variation in the body lengths of different genotypes in the pooled population as well as in the different population groups.

Table 4.4 Comparison of the frequencies of GH/*Msp*I alleles in the three population groups in Malabari goats

GH/*Msp*I Alleles	Population Groups			χ^2 (df=2)
	Population I	Population II	Population III	
(+)	0.70	0.67	0.72	0.712 NS
(-)	0.30	0.33	0.28	

NS – Not significant

Table 4.5 The average predicted body weights with respect to different GH/*Msp*I genotypes in Malabari goats

Population	GH/*Msp*I Genotype		't' value	Total
	(+/+)	(+/-)		
	Mean±SE (kg)	Mean±SE (kg)		Mean±SE (kg)
Population I	26.02±0.59 (27)	26.73±0.52 (39)	0.895 NS	26.44±0.39 a (66)
Population II	24.88±0.40 (23)	25.28±0.50 (43)	0.624 NS	25.14±0.35 b (66)
Population III	26.60±0.79 (28)	27.07±0.61 (35)	0.481 NS	26.8±0.48 a (63)
Pooled population	25.89±0.37 (78)	26.00±0.32 (117)	0.824 NS	26.14±0.24 (195)

Figures in parenthesis are actual numbers
NS – Not significant
Values bearing different superscripts are least square means differ between population groups significantly (P≤0.05)

Least square analysis of variance showed that differences in body lengths among different population groups were highly significant. The GH/*Msp*I genotypes did not exhibit significant effect on the character (Table 4.6)

4.3.3 GH/*Msp*I polymorphism and height at withers

The mean height at withers for different GH/*Msp*I genotypes are given in Table 4.7. No significant variation in height at withers could be observed among different genotypes in different population groups and in the pooled population.

Least square analysis of variance showed highly significant variation in height at withers among different population groups. The variation in height among different GH/*Msp*I genotypes was not significant (Table 4.7).

4.3.4 GH/*Msp*I polymorphism and heart girth

The mean values of heart girth for different GH/*Msp*I genotypes are given in Table 4.8. The statistical analysis revealed no significant variation in heart girth for different GH/*Msp*I genotypes in the different population groups and in the pooled population.

Least square analysis of variance showed that the change in heart girth for different population groups was highly significant. The different GH/*Msp*I genotypes did not exhibit significant effect on the heart girth. (Table 4.8)

Table 4.6 The average body length with respect to different GH/*Msp*I genotypes in Malabari goats

Population	GH/*Msp*I Genotype		't' value	Total
	(+/+)	(+/-)		
	Mean±SE (cm)	Mean±SE (cm)		Mean±SE (cm)
Population I	63.50±1.01 (26)	63.30±0.86 (38)	0.206 NS	63.38±0.65 a (64)
Population II	62.00±0.96 (23)	62.60±0.94 (43)	0.382 NS	62.36±0.69 a (66)
Population III	59.70±1.10 (28)	59.40±0.95 (35)	0.212 NS	59.51±0.71 b (63)
Pooled population	61.68±0.62 (77)	61.83±0.55 (116)	0.181 NS	61.77±0.41 (193)

Figures in parenthesis are actual numbers
NS – Not significant
Values bearing different superscripts are least square means differ between population groups significantly (P≤0.05)

Table 4.7 The average height at withers with respect to different GH/*Msp*I genotypes in Malabari goats

Population	GH/*Msp*I Genotype		't' value	Total
	(+/+)	(+/-)		
	Mean±SE (cm)	Mean±SE (cm)		Mean±SE (cm)
Population I	65.80±0.96 (26)	65.30±0.71 (38)	0.412 NS	65.48±0.57 a (64)
Population II	63.50±0.54 (23)	64.20±0.69 (43)	0.764 NS	63.96±0.48 b (66)
Population III	62.30±0.68 (28)	63.80±0.85 (35)	1.288 NS	63.13±0.56 b (63)
Pooled population	63.84±0.46 (77)	64.42±0.43 (116)	0.894 NS	64.19±0.32 (193)

Figures in parenthesis are actual numbers
NS – Not significant
Values bearing different superscripts are least square means differ between population groups significantly (P≤0.05)

4.3.5 GH/*Msp*I polymorphism and peak milk yield

The mean values of peak yield for different GH/*Msp*I genotypes in different populations are given in Table 4.9. The analysis showed that the change in peak milk yield did not differ significantly with GH/*Msp*I genotypes.

Least square analysis of variance showed that the difference in peak yield for different population groups were highly significant. The GH/*Msp*I genotypes did not exhibit significant variation in the peak milk yield (Table 4.9).

4.3.6 GH/*Msp*I polymorphism and milk fat percentage

Fat percentage in the goat milk samples was estimated by Gerber's method. The mean values of milk fat percentage for different GH/*Msp*I genotypes in different population groups are presented in Table 4.10. There was no significant variation in the milk fat percentage of GH/*Msp*I genotypes.

Least square analysis of variance showed that GH/*Msp*I genotypes as well as different population groups did not exhibit significant effect on the milk fat percentage (Table 4.10).

4.3.7 GH/*Msp*I polymorphism and milk protein percentage

The total protein percentage in milk was estimated using the dye-binding method. The mean values of milk protein percentage for GH/*Msp*I genotypes in different population groups are presented in Table 4.11. The

Table 4.8 The average heart girth with respect to different GH/*MspI* genotypes in Malabari goats

Population	*GH/MspI* Genotype		't' value	Total
	(+/+)	(+/-)		
	Mean±SE (cm)	Mean±SE (cm)		Mean±SE (cm)
Population I	70.00±1.09 (26)	70.42±0.77 (38)	0.324 NS	70.25±0.63 a (64)
Population II	67.70±0.70 (23)	68.37±0.88 (43)	0.600 NS	68.14±0.62 b (66)
Population III	70.71±1.40 (28)	71.54±1.07 (35)	0.480 NS	71018±0.85 a (63)
Pooled population	69.57±0.67 (77)	70.00±0.53 (116)	0.503 NS	69.83±0.42 (193)

Figures in parenthesis are actual numbers
NS – Not significant
Values bearing different superscripts are least square means differ between population groups significantly (P≤0.05)

Table 4.9 The average peak milk yield with respect to different GH/*MspI* genotypes in Malabari goats

Population	GH/*MspI* Genotype		't' value	Total
	(+/+)	(+/-)		
	Mean±SE (ml)	Mean±SE (ml)		Mean±SE (ml)
Population I	552.50±108.79 (20)	668.20±100.89 (33)	0.747 NS	624.53±74.78 a (53)
Population II	341.70±39.45 (18)	263.40±24.35 (28)	1.788 NS	294.02±21.89 b (46)
Population III	1092.90±182.3 (21)	893.5±133.64 (27)	0.903 NS	980.73±109.33 c (48)
Pooled population	680.51±85.30 (59)	608.52±61.95 (88)	0.669 NS	637.42±50.38 (147)

Figures in parenthesis are actual numbers

NS – Not significant
Values bearing different superscripts are least square means differ between population groups significantly (P≤0.05)

analysis by t-test showed that the variation in milk protein percentage between GH/*Msp*I was highly significant in population-I where as no significant variation could be observed in population II, III and in the pooled population.

Least square analysis of variance showed that GH/*Msp*I genotypes as well as population groups did not exhibit significant effect on the milk protein percentage (Table 4.11).

4.3.8 GH/*Msp*I polymorphism and milk SNF

The average SNF percentages for GH/MspI genotypes in different population groups are presented in Table 4.12. The analysis showed that the variation in the milk SNF percentage differed significantly with GH/*Msp*I genotypes in population II and pooled population. The difference in milk SNF percentage for GH/*Msp*I genotypes in population-I and III was not significant.

Least square analysis of variance showed that the differences in milk SNF percentage for GH/*Msp*I genotypes were significant. The different population groups did not exhibit significant effect on the milk SNF percentage (Table 4.12).

4.3.9 GH/*Msp*I polymorphism and litter size

The litter sizes such as single, twin, and triplet and more observed in Malabari goats in the present study are presented in Table 4.14. A comparison of the litter sizes with respect to GH/*Msp*I genotypes among different

Table 4.10 The average milk fat percentage with respect to different GH/*MspI* genotypes in Malabari goats

Population	GH/*MspI* Genotype		't' value	Total
	(+/+)	(+/-)		
	Mean±SE	Mean±SE		Mean±SE
Population I	5.66±0.33 (14)	5.52±0.40 (28)	0.263 NS	5.57±0.29 a (42)
Population II	5.18±0.64 (13)	4.53±0.32 (20)	1.018 NS	4.79±0.31 a (33)
Population III	6.07±0.40 (27)	5.22±0.37 (32)	1.566 NS	5.61±0.27 a (59)
Pooled population	5.75±0.27 (54)	5.15±0.22 (80)	1.731 NS	5.39±0.17 (134)

Figures in parenthesis are actual numbers
NS – Not significant
Values bearing different superscripts are least square means differ between population groups significantly (P≤0.05)

Table 4.11 The average milk protein percentage with respect to different GH/*MspI* genotypes in Malabari goats

Population	GH/*MspI* Genotype		't' value	Total
	(+/+)	(+/-)		
	Mean±SE	Mean±SE		Mean±SE
Population I	3.32±0.08 (16)	3.99±0.21 (25)	2.936**	3.72±0.14 a (41)
Population II	4.20±0.19 (12)	3.69±0.27 (19)	1.368 NS	3.89±0.18 a (31)
Population III	3.75±0.17 (25)	3.64±0.15 (30)	0.488 NS	3.69±0.11 a (55)
Pooled population	3.72±0.10 (53)	3.77±0.12 (74)	0.326 NS	3.75±0.08 (127)

Figures in parenthesis are actual numbers
NS – Not significant
** Significant at 1 per cent level
Values bearing different superscripts are least square means differ between population groups significantly (P≤0.05)

population groups is given in Table 4.13. The analysis by χ^2-test of significance showed that the litter size did not differ significantly with genotypes in Malabari goats. The litter sizes in three population groups are given in Table 4.15. The statistical analysis revealed highly significant difference in litter sizes for different populations.

Table 4.12 The average SNF percentage in milk with respect to different GH/*Msp*I genotypes in Malabari goats

Population	GH/*Msp*I Genotype		't' value	Total
	(+/+)	(+/-)		
	Mean±SE	Mean±SE		Mean±SE
Population I	9.34±0.50 (14)	10.35±0.35 (28)	1.639	10.01±0.30 [a] (42)
Population II	8.64±0.57 (13)	10.39±0.40 (20)	2.592*	9.70±0.36 [a] (33)
Population III	9.26±0.33 (27)	9.46±0.43 (32)	0.361	9.37±0.28 [a] (59)
Pooled population	9.13±0.25 (54)	10.01±0.24 (80)	2.471*	9.65±0.18 (134)

Figures in parenthesis are actual numbers
NS – Not significant
* Significant at 5 per cent level
Values bearing different superscripts are least square means differ between population groups significantly (P≤0.05)

Table 4.13 Comparison of the Litter size in percentage for GH/*Msp*I genotypes in Malabari goats

Population groups	GH/*MspI* genotype	Litter sine			χ^2 value (d.f=2)
		Single	Twin	Triplet & more	
Population- I	(+/+) (n=21)	42.86 (9)	47.62 (10)	9.52 (2)	0.276 NS
	(+/-) (n=32)	40.62 (13)	53.13 (17)	6.25 (2)	
Population- II	(+/+) (n=11)	54.55 (6)	45.45 (5)	0 (0)	0.257 NS
	(+/-) (n=22)	63.64 (14)	36.36 (8)	0 (0)	
Population- III	(+/+) (n=27)	22.22 (6)	62.96 (17)	14.82 (4)	0.587 NS
	(+/-) (n=34)	26.47 (9)	64.71 (22)	8.82 (3)	
Pooled population	(+/+) (n=59)	35.59 (21)	54.24 (32)	10.17 (6)	1.22 NS
	(+/-) (n=88)	40.91 (36)	53.41 (47)	5.68 (5)	

Figures in parenthesis are actual numbers

NS – Not significant

Table 4.14 The Litter size in Malabari goats

Litter size	Number	Percentage
Single	57	38.78
Twin	79	53.74
Triplet & more	11	7.48
Total	147	100.00

Table 4.15 Comparison of the Litter size in percentage among different populations of Malabari goats

Population groups	Litter size			χ^2 value (d.f=4)
	Single	Twin	Triplet & more	
Population - I	41.51 (22)	50.94 (27)	7.55 (4)	13.611**
Population -II	60.61 (20)	39.39 (13)	0 (0)	
Population- III	24.59 (15)	63.93 (39)	11.48 (7)	

Figures in parenthesis are actual numbers

** Significant at 1 per cent level

Discussion

5. DISCUSSION

5.1 Isolation of genomic DNA

The phenol-chloroform extraction method is routinely used for the isolation of high molecular weight DNA from blood samples. In the present study, the mean yield of DNA from 5 ml of goat blood samples extracted by the phenol-chloroform method was found to be 231.097±11.65 µg. Beckmann *et al.* (1986), Senthil *et al.* (1997) and Arvindakshan *et al.* (1997) reported 300-500 µg, 444.58±21.54 µg and 394.50±34.26 µg respectively per 10 ml of whole blood of cattle using the phenol-chloroform method. The yield of DNA per unit volume of blood was high in goat as compared to that of cattle. This might be due to the high leukocyte count in goat (Swenson, 1996) and better suitability of this procedure for goat blood. The ratios of optical density at 260 and 280 nm were more than 1.7 indicating good deproteinisation. Senthil *et al.* (1997) and Aravindakshan (1997) also reported similar ratios in cattle.

5.2 PCR-RFLPs

The literature availability regarding goat for this study was scanty. In this study, PCR-RFLP analysis at growth hormone gene was attempted to characterise goats using primers specific to cattle.

5.2.1 Amplification of GH gene by PCR

The polymorphism of GH gene has been investigated in goats by PCR amplification using bovine specific primers (Hoj *et al.*, 1993) and subsequent digestion of the amplified product with *Msp*I enzyme. In all the animals the size of the amplified product was 768 bp indicating conservation of DNA sequences in related species. The finding of this study is in close agreement with the reports made earlier by Moore *et al* (1991) and Rubinsztein *et al.* (1995).

5.2.2 Optimisation of PCR parameters

PCR parameters used in this study are given in Table 4.2. Aravindakshan (1997) recommended the same parameters for cattle and buffalo. Though the primers used were specific for cattle, the amplification was satisfactory suggesting that a strong conservation of DNA sequences occurs at this locus among related species.

5.2.3 PCR-RFLPs analysis in GH gene using *Msp*I

The amplified DNA was digested with *Msp*I restriction enzyme and fragments were separated by electrophoresis. The amplified 768 bp fragment has a non-polymorphic *Msp*I site suitable for control of *Msp*I digestion giving rise to two fragments of 705 and 63 bp. The 705 bp fragment was restricted into a 612 bp fragment and a 93 bp fragment when the polymorphic *Msp*I site (+) was present. This allowed the identification of two alleles viz., *Msp*I (+) (612, 93 and 63 bp fragments) and *Msp*I (-) (705 and 63 bp fragments). Hoj *et al.*

(1993) reported that in cattle, the *Msp*I (-) allele is created by the insertion of a T at position +837 and C→G transition at position +838. Therefore, the findings in the present study are in close agreement with reports in cattle made earlier by Hoj *et al.* (1993) and Aravindakshan *et al.* (1997). The results are suggestive of strong conservation of DNA sequences between cattle and goat at this locus.

Among the 196 tested animals, 78 (+/+) and 118 (+/-) genotypes were found giving rise to genotype frequencies of 0.40 and 0.60 respectively. The gene frequencies were 0.70 and 0.30 for (+) and (-) alleles, respectively. Out of the three possible genotypes viz., (+/+), (+/-) and (-/-), the (-/-) genotype was not observed in the present study. This finding is in close agreement with the findings made earlier by Lagziel *et al.* (1999) in Israel Holstein and Hoj *et al.* (1993) in Danish low line and Norwegian cattle. Aravindakshan *et al.* (1997) reported all the three genotypes viz., (+/+), (+/-) and (-/-) in Indian cattle. It is suspected that absence of (-/-) genotype in goat might be due to the lethal effect of the *Msp*I (-) homozygote (-/-) or due to the linkage of a lethal gene to the *Msp*I (-) allele in Malabari goats. Usha (1990) reported a similar finding in the biochemical polymorphism of the transferrin allele (Tf^C) in Rabbits.

The population of the Malabari goats investigated in the present study was not under Hardy-Weinberg equilibrium though it was a large random mating one with no known mutation, or migration. Hence, it is suspected that the natural selection process acting against the (-/-) genotype through reduced

viability or early embryonic death may be a possible reason for the disagreement of the Hardy-Weinberg equilibrium for this locus in Malabari goats. However, further detailed investigations are required to confirm this finding.

The gene frequencies of the GH/*Msp*I polymorphism in different population groups investigated were not significantly different. This indicates the genetic homogeneity of Malabari goats as a breed though the different population groups differ significantly with respect to morphological features such as colour patterns, body shape etc.

5.3.1 GH/*Msp*I polymorphism and Body Weight

The mean body weight did not differ significantly in different population groups and GH/*Msp*I genotypes. It was inferred that body weight increased or decreased more or less in a similar pattern in different population groups as well as in the different GH/*Msp*I genotypes. These findings are in agreement with the reports of Aravindakshan *et al.* (1997) in crossbred Jersey.

5.3.2 GH/*Msp*I polymorphism and Body Length

The average body length was not exhibiting significant difference between two GH/*Msp*I genotypes within different population groups as well as in the pooled population. Least square analysis of variance revealed highly significant differences in body lengths among different population groups that

could be possibly attributed to the variation in the management and other environment factors influencing the growth of animals.

5.3.3 GH/*Msp*I polymorphism and height at withers

GH/*Msp*I genotypes did not exhibit any significant changes in the average height at withers in different population groups and in the pooled population. A significant difference in height at withers was noticed among the population groups. The significant difference in height at withers in different population groups might be due to the influence of management and other environmental factors.

5.3.4 GH/*Msp*I polymorphism and heart girth

The average heart girth was not significantly different in different population groups and pooled population with respect to GH/*Msp*I genotypes. A significant difference was noticed between population groups possibly due to the effect of management and other environmental factors as in the case of body length and height though the effect of genetic factors cannot be ruled out.

5.3.5 GH/*Msp*I polymorphism and peak milk yield

The mean peak milk yield with respect to GH/*Msp*I genotypes in different population groups and in the pooled population did not differ significantly. Population groups exhibited significant effect on the character. The difference could be largely attributed to the variation in feeding and

management practices and other environmental factors influencing production in different population groups.

5.3.6 GH/*Msp*I polymorphism and milk fat percentage

Statistical analysis revealed that GH/*Msp*I genotypes in pooled population as well as population groups did not exhibit significant effect on milk fat percentage. It may be inferred that the GH/*Msp*I genotypes and the geographical regions are not having significant influence on milk fat percentage in Malabari goats. Falaki *et al.* (1996) found the *Msp*I (-) allele to be associated with higher fat yield and percentage in Italian Holstein dairy cattle but the effect was not statistically significant. Hoj *et al.* (1993) found a significantly higher frequency of *Msp*I (-) in Red Danish and Norwegian Red dairy cattle selected for high as compared to low fat production. Lee *et al.* (1993) observed a significant association of *Msp*I (-) with higher fat yield in a milk-yield selection line. In contrast, Yao *et al.* (1996) found that the *Msp*I (-) allele was associated with a statistically significant decrease in milk fat yield.

5.3.7 GH/*Msp*I polymorphism and milk protein percentage

A significant variation in milk protein percentage has been observed between the GH/*Msp*I genotypes in population-I. It is possible that the (+/-) genotype is associated with high milk protein percentage in Malabari goats though significant variation was not observed in the other populations investigated. These findings are in contrast with earlier reports by Hoj *et al.*

(1993) and Yao *et al.* (1996). Falaki *et al.* (1996) found the *Msp*I (-) allele to be associated with higher protein percentage and yield in Italian Holstein cattle, although the effect was not statistically significant.

5.3.8 GH/*Msp*I polymorphism and milk SNF

The analysis showed that the GH/*Msp*I genotypes exhibited significant effect on the milk SNF in pooled population and population II. The results indicated that the (+/-) genotype is possibly associated with high milk SNF percentage in Malabari goat. The GH/*Msp*I genotypes did not exhibit any significant variation in the character in populations III and I, which could be possibly attributed to different non-genetic factors.

5.3.9 GH/*Msp*I polymorphism and litter size

The litter size in different population groups with respect to GH/*Msp*I genotypes did not differ significantly. But the different population groups exhibited significant effect on the litter size which could be on account of variations in the feeding, management and other environmental factors influencing litter size and less likely to be of genetic factors.

Summary

6. SUMMARY

Molecular markers are gaining more and more importance as a potential tool to geneticists and breeders to evaluate the existing germplasm and to manipulate it to create animals desired and needed by the society. In goats, limited information is available on the molecular genetics. The present study was undertaken with the objective of analysing the polymorphism at the growth hormone gene locus in Malabari goats using the PCR-RFLP technique.

Genomic DNA was isolated from 196 female Malabari goats belong to three different population groups based on geographical locations, viz., populationsI, II and III. The genomic DNA was extracted from whole blood using the phenol-chloroform method. The mean yield of DNA was 231.097±11.65 µg from 5 ml of whole blood. The ratio of optical densities at 260 and 280 nm was above 1.7 indicating good deproteinisation. Agarose gel electrophoresis of the DNA indicated that the samples obtained were of good quality and high molecular weight. All the DNA samples obtained were suitable for PCR-RFLP studies.

Cattle primers were used for amplification of GH gene in goats. The successful amplification of the expected 768 bp fragment indicated the suitability of cattle primers in goats and conservation of DNA sequence in related species. The digestion of the amplified product with *Msp*I restriction enzyme revealed two alleles viz., *Msp*I (+) and *Msp*I (-) with gene frequencies

length, heart girth and height at withers, peak milk yield and litter size. These results revealed the influence of management and other environmental effects on growth traits in Malabari goats. It is concluded that the typing of the *MspI* polymorphism using PCR-based procedure is a very efficient way to identify the GH genotypes in Malabari goats.

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Annexures

ANNEXURE – I

MOLECULAR GENETIC CHARACTERISATION AND GENETIC IMPROVEMENT OF MALABARI GOATS

DATA COLLECTION SHEET

Date of blood sample collection :

Date of blood processing :

1. Sample No. :
2. Animal I.D. No. :
3. Source/ Address of owner :

4. Colour pattern :
5. Date of birth/Age :
6. Sex :
7. Sire :
8. Dam :
9. Milk sample taken : Yes/No
10. Stage of lactation/ date of kidding :
11. Details of production: :
    (a) Milk yield (peak) :
    (b) Parity :
12. Body weight/Measurements:
    (a). Birth weight :
    (b). Body weight :
    (c). Height at withers (cm) :
    (d). Length (cm) :
    (e). Girth (cm) :
13. Incidence of diseases :
14. Type of kidding :
15. Tassels : Present/Absent
16. Beard : Present/Absent
17. Horned/ Polled :
18. Remarks :

ANNEXURE – II

COMPOSITION OF REAGENTS AND BUFFERS USED IN THE STUDY

30% Acrylamide

Acrylamide	29 g
N, N-methylenebisacrylamide	1 g
Water	to 100 ml

10% Ammonium persulphate

APS 1 g

Water to 10 ml

Prepared fresh at the time of use.

EDTA (0.5M, pH 8.0)

Dissolve 18.61 g of EDTA (disodium, dihydrate) in 80 ml of distilled water by bringing the pH to 6.0 with NaOH and make up the volume to 100 ml. Filter, autoclave and store at room temperature.

Phenol (saturated, pH7.8)

Melt the commercially available crystalline phenol at 68°C in a water bath. Add hydroxyquinolone to a final concentration of 0.1%. To the molten phenol, add equal volume of 0.5 M Tris.HCl (pH 8.0). Stir the mixture for 30 min on a magnetic stirrer. Transfer the content into a separating funnel and when the two phases have separated, collect the lower phenolic phase. Add an equal volume of 0.1 M Tris.HCl (pH8.0) to the phenol. Stir the mixture on a magnetic stirrer for 30 min. Collect the phenolic phase as above.

Repeat the extraction with 0.1 M Tris. HCl (pH 8.0), until the pH of the phenolic phase is more than 7.8. Finally add 0.1 volume of 0.01M Tris. HCl (pH 8.0) and store in a dark bottle at 4°C.

8% Polyacrylamide gel

30% Acrylamide	26.6 ml
Triple distilled water	52.7 ml
5X TBE	20.0 ml
10% APS	0.7 ml
Total	100.0 ml

To the above mixture add 35µl of TEMED and mix by swirling. Draw the solution into the syringe, invert and expel the air and pour into the sandwitch of glass plates.

RBC Lysis Buffer

Ammoniun Chloride	150 mM	8.0235 g
Potassium Chloride	10 mM	0.7455 g
EDTA	0.1 mM	0.0372 g

Add distilled water up to 1000 ml, stir, filter and autoclave. Store at 4°C

Sodium acetate (3M, pH5.5)

For 100 ml, dissolve 40.824 g of sodium acetate in 70 ml of distilled water. Adjust the pH to 5.5 with glacial acetic acid. Make up the volume to 100 ml, autoclave and store at 4°C.

Sodium Chloride (5M)

For 100 ml, dissolve 29.22 g of sodium chloride in 80 ml of distilled water, make up the volume to 100 ml, filter and store at room temperature.

SE Buffer (pH8.0)

Sodium Chloride	75 mM	4.383 g
EDTA	35 mM	9.306 g

Dissolve in 900 ml distilled water. Adjust the pH to 8.0. Make up the volume to 1000 ml. Filter, autoclave and store at 4°C.

Sodium dodecyl Sulphate (SDS) 20%

SDS	20 g
Distilled water to make up to 100ml.	

Stir on a magnetic stirrer, filter and store at room temperature.

TAE Buffer (50X)

Tris base	48.4g
Glacial acetic acid	11.42 ml
0.5M EDTA (pH 8.0)	20 ml
Distilled water	upto 1000ml.

Autoclave and store at room temperature.

Tris-Borate- EDTA (TBE) buffer (pH 8.3) 10X

Tris base	108.0 g
Boric acid	55.0g
EDTA	9.3g

Dissolve in 700ml of distilled water and adjust the pH to 8.3. Make up the volume to 1000 ml, autoclave and store at room temperature.

Tris Buffered Saline (pH 7.4)

Sodium Chloride	140 mM	8.18 g
Potassium Chloride	0.5 mM	0.0373 g
Tris base	0.25 mM	0.0303 g

Dissolve in 900 ml distilled water and adjust the pH to 7.4. Make up the volume to 1000 ml, filter, autoclave and store at room temperature.

Tris-EDTA (TE) Buffer (pH 8.0)

Tris base	10 mM	1.2114 g
EDTA	0.1 mM	0.3722 g

Dissolve in 900 ml distilled water and adjust the pH to 8.0 Adjust the pH to 8.0. Make up the volume to 1000 ml. Filter and autoclave in batches of 100 ml and store at 4°C.

ANNEXURE-III

SOURCES OF IMPORTANT CHEMICALS, ENZYMES AND EQUIPMENTS USED IN THIS STUDY

A. CHEMICALS

Acrylamide	SRL, Bombay
Agarose (Low EEO)	Bangalore Genei Pvt Ltd
Amido Black10B	SRL, Bombay
Ammonium chloride	SRL, Bombay
Ammonium persulphate (APS)	SRL, Bombay
N,N-MethyleneBis-acrylamide	SRL, Bombay
Boric acid	SRL, Bombay
Citric acid	Merk
Crystalline Phenol	Merk
Di-sodium hydrogen orthophosphate	SRL, Bombay
dNTPs	Finn Enzymes, OY
EDTA	SRL, Bombay
Ethanol	Merk
Ethidium bromide	BDH Lab, England
6 X Gel loading buffer	Bangalore Genei Pvt Ltd
Glacial acetic acid	BDH-E-Merk (India) Ltd
Hydroxyquinolone	Qualigens Fine Chemicals, Mumbai
Methanol	SRL, Bombay
Potassium chloride	SRL, Bombay
Sodium acetate	SRL, Bombay
Sodium chloride	SRL, Bombay
Sodium dodecyl sulphate (SDS)	SRL, Bombay
TEMED	SRL, Bombay
Tris base	SRL, Bombay

B. ENZYMES

Taq DNA polymerase	Finn Enzymes, OY
Proteinase K	Bangalore Genei Pvt Ltd

Restriction endonuclease	
*Msp*I	Bangalore Genei Pvt Ltd
C. MOLECULAR MARKERS	
ϕ174X RF DNA/*Hae*III digest	Bangalore Genei Pvt Ltd
100 bp ladder DNA	Bangalore Genei Pvt Ltd
D. PRIMERS	Invitrogen (India) Pvt. Ltd
E. EQUIPMENTS	
Autoclave	Labo Autoclave, Sanyo, Japan
Centrifuge	REMI Motors Ltd, Mumbai
Deep freezer	Kripscold
Disposable plastic centrifuge tubes	Tarsons
Gel documentation system	Biorad Laboratories
Hot Plate & Stirrer	Jenway 1000, UK
Laminar Floor	Labline Instruments, Cochin
Microcentrifuge	Mikro 22R-Hettich Zentrifugen, Germany
Microwave oven	BPL, India
Micropipettes	Labsystems, Finland
PAGE Unit	Amersham Pharmacia Biotech
pH meter	Systronics, India
Spectrophotometer	Jenway, Genova, UK
Submarine Electrophoresis Unit	Bangalore Genei Pvt Ltd
Thermal cycler	PTC-200, MJ Research Inc. USA
PCR tubes (0.2ml)	Bangalore Genei Pvt Ltd
Vortex	Bangalore Genei Pvt Ltd
UV Transilluminator	MacroVue-20, Pharmacia Biotech
Water bath	KEMI Lab Equipments
Weighing balance	Sartorius, Germany

POLYMORPHISM OF GROWTH HORMONE GENE IN MALABARI GOATS (*Capra hircus*)

By

CHITRA, R.

ABSTRACT OF A THESIS

Submitted in partial fulfilment of the requirement for the degree of

Master of Veterinary Science

Faculty of Veterinary and Animal Sciences
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ABSTRACT

Malabari goats are noted for their high milk yield and meat production qualities. They represent a unique genetic resource by virtue of their adaptability, resistance to many infectious diseases and prolificacy in the humid tropics of Kerala. They also exhibit considerable variation in individual performance in milk production, growth rate and fecundity.

The growth hormone (GH) gene is a potential target for studies of molecular variation because of its possible direct or indirect effects upon growth, lactation and mammary gland development in dairy animals. The polymorphism in the third intron of GH gene was investigated by polymerase chain reaction (PCR) and restriction endonuclease digestion.

Genomic DNA was isolated from a random sample of 196 genetically unrelated Malabari does in second lactation belonging to three different population groups based on geographical locations. The mean yields of DNA from 5 ml of whole blood extracted by phenol-chloroform was 231.097±11.65 µg. The ratio of optical densities at 260 and 280 nm was above 1.7 indicating good deproteinisation.

Oligonucleotide primers based on bovine sequences were used for amplification of GH gene in goats. The successful amplification of the expected 768 bp fragment indicated the suitability of bovine primers for goats and conservation of DNA sequence in related species. The digestion of the

amplified product with *Msp*I restriction enzyme revealed two alleles viz., *Msp*I (+) and *Msp*I (-) with gene frequencies of 0.70 and 0.30, respectively. In the Malabari goats tested in this study only (+/+) and (+/-) GH/*Msp*I genotypes were encountered where as none of the animal was of the (-/-) genotype. It is suspected that the *Msp*I (-) allele in the homozygous condition might be lethal or linked to a lethal gene. The population of the Malabari goats investigated in the present study was not under Hardy-Weinberg equilibrium though it was a large random mating one with no known mutation, or migration. Hence, it is suspected that the selection process acting against the (-/-) genotype through reduced viability or early embryonic death may be a possible reason for the disagreement of the Hardy-Weinberg equilibrium in Malabari goats for this locus. It was observed that the goat population was in genetic equilibrium with respect to growth hormone locus in all population groups. The genetic equilibrium might be indicative of the homogeneity of different population groups though they differed significantly with respect to morphological and other phenotypic characters.

Studies on the association of GH/*Msp*I genotypes with growth and milk traits in Malabari goats revealed the following results. The (+/-) genotype was superior to (+/+) genotype with respect to milk protein percentage in population-I. The (+/-) genotype was found to be associated with significantly high milk SNF percentage in population II and pooled population. The GH/*Msp*I genotypes did not exhibit significant effect on body weight, body measurements, peak milk yield, milk fat and the litter size. The different

population groups differed significantly with respect to body weight, body measurements like length, heart girth and height at withers, peak milk yield and litter size. These results revealed the influence of management and other environmental effects on growth traits in Malabari goats. It is concluded that the typing of the *Msp*I polymorphism using PCR-based procedure is a very efficient way to identify the GH genotypes in Malabari goats.