STUDY OF GROWTH HORMONE GENE POLYMORPHISM IN BERARI GOAT

T H E S I S

Submitted

In partial fulfillment of the requirements for the Degree of

MASTER OF VETERINARY SCIENCE

IN

ANIMAL GENETICS AND BREEDING

BY

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(INDIA)

2022

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I hereby declare that the experimental research work and interpretation of the thesis entitled **"STUDY OF GROWTH HORMONE GENE POLYMORPHISM IN BERARI GOAT"** or part there of has not been submitted for any other degree or diploma of any University, nor the data have been derived from any thesis/publication of any University or scientific organization. The sources of materials used and all assistance received during the course of investigation have been duly acknowledged.

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This is to certify that the thesis entitled, **" STUDY OF GROWTH HORMONE GENE POLYMORPHISM IN BERARI GOAT "** submitted by **KACHAVE MUKUND RAMESH** to the Maharashtra Animal and Fishery Sciences University, Nagpur in partial fulfillment of the requirement for the degree of **MASTER OF VETERINARY SCIENCE** in **ANIMAL GENETICS AND BREEDING** has been approved by the Student's Advisory Committee after examination in collaboration with the External Examiner.

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ACKNOWLEDGEMENTS

"Absorb what is useful, Discard what is not, and what is uniquely your own."
-Anonymous.

First of all, I bow my head in front of my parents. I do not have proper words to express gratitude and respect to my beloved parents, mother (Aai), father (Baba), grandmother (Aajji), late grandfather (Aajoba), my sisters Rutuja and smt Rajnee, who took pains to bring me up to this stage. Their love, sacrifice, extra-ordinary patience, inspiration and blessings encouraged and inspired me and because of them I am at such a great stage of life.

It gives me a great pleasure to express my deepest sense of profound respect & whole hearted gratitude towards my honorable research guide ***Dr. S. V. Kuralkar****, M.V.Sc., Ph.D. (IVRI), Professor and University Head, Department of Animal Genetics & Breeding, PGIVAS, Akola, for his invaluable guidance, execution and pursuance of the present research project. I shall forever remain an admirer of his interest in work, his deep knowledge and his approach to the subject, his confidence, his love for his students and department and his constant invaluable guidance, constructive counsel, parental kindness and unreserved help throughout my entire research work and post-graduation studies, all of which make him unforgettable personality. I will forever remain proud of him for his continuous encouragement and appreciation.*

I render my absolute and sincere gratitude from the bottom of my heart to ***Dr. P. S. Bankar****, Assistant Professor, Dept. of AGB, PGIVAS, Akola for his unreserved and continuous support, critical evaluation and constructive suggestion as member of advisory committee, throughout the research, which helped me in successful execution of this scientific endeavor.*

I thankful to ***Dr. G. J. Panchbhai****, (LPM) In charge-DDR, and* ***Dr. H. S. Kanadkhedkar****, SMS (AGB) of Berari Goat and Deccani sheep Research Training and Demonstration Centre, Borgaon Manju, Akola for their keen interest, continuous encouragement, suggestion, and guidance during my post-graduate studies and research.*

I am also grateful towards the members of my advisory council ***Dr. Prajakta S. Kuralkar****, Assistant Professor and Head, Department of Veterinary*

Physiology, and ***Dr. S. G. Deshmukh****, Assistant Professor, Dept. of ARGO, PGIVAS, Akola for their kind and encouraging and professional guidance during my entire course of time in PGIVAS.*

I have debt of gratitude and respectfully extend my regards to ***Dr. V. D. Aher****,* ***Dr. A. U. Bikhane, Dr. C. S. Pawshe,*** *Ex-Associate Dean PGIVAS, Akola and* ***Dr. D. G. Dighe****, Associate Dean, PGIVAS, Akola for his cooperation in providing the facilities required, which helped me a lot to complete my research work.*

I am especially grateful to ***Dr. R. S. Ingole****, Assistant Professor, Department of Veterinary Pathology, PGIVAS, Akola for providing the requisite facilities of UV transilluminator for successful completion of the research.*

I am also thankful to Dr. Sanjay Chintamani and Dr. Suhas Kathe. I feel sincere thanks to Sanjay Solunke, Sunil Giri, Dinesh Chavhan, Ramesh Ingale, from Berari Goat and Deccani sheep Research Training and Demonstration Centre, Borgaon Manju, Akola, for his expedite and kind help during the visits for data and blood collection.

I extend my sincere thanks to ***Dr. D. R. Rathod*** *(Assistant Professor)****,*** *Ms. Vividha Shirsath, Mr. Vishal Patil, Dept. of plant Biotechnology, Dr. PDKV, Akola for their help during the research.*

I will be failing in my duties if I do not mention my sincere thanks to ***Smt. Tayade Mavashi*** *Department of Animal Genetics and Breeding for extending co-operation from time to time & indispensable help during my P.G. studies.*

Throughout my life I will cherish the beautiful memories of the days in the department with my colleagues Dr. A. P, Bhavekar, Dr. Mrunal More, Dr. Roja chhetri and my juniors Dr. Rahul, Dr. Shubhangi, Dr. Ragini and Dr. Ajay, for helping me in completion of my research work and supporting me in daily endeavors in the college.

I am very thankful to all authors and researchers, whose articles help me to perceive knowledge and make it possible to mould my literature to shape.

Place: Akola **(Kachave Mukund Ramesh)**

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LIST OF ABBREVIATIONS

Abbreviation	Full form
%	Per cent
°C	Degree Celsius
µg	Micro gram
µl	Micro litre
bp	Base pair (s)
DNA	Deoxyribonucleic Acid
EDTA	Ethylene Diamine Tetra acetic Acid
GH	Growth hormone
GHR	Growth hormone receptor
GHBP	Growth hormone binding protein
IGF	Insuline like growth factor
mg	milli gram
min	Minutes
ml	milli litre
mMol	milli Molar
NCBI	National Center for Biotechnology Information
NDRI	National Dairy Research Institute
ng	nano-gram
PCR	Polymerase Chain Reaction
pmole	pico-mole
QTL	Quantitative trait loci
RE	Restriction Enzyme
RFLP	Restriction Fragment Length Polymorphism
rpm	Rotations per minute
s	Seconds
SNP	Single Nucleotide Polymorphism

CHAPTER I

INTRODUCTION

Goat is a valuable livestock species that are mainly raised for meat, milk, fiber, and skin/hides. Goats are known for their ability to grow on limited feed resources. The goat has distinct social, economic, managmental, and biological advantages over other livestock species. Since ancient times, goats are valuable due to their flexibility to adapt a variety of agro-climatic situations and low input cost for production and maintenance than that of output benefits. A village community can rely on the goat rearing for a steady source of income.

Presently, among the total livestock population in India, Goat contribute 148.88 million (20th livestock census, 2019), which is increased by 10.14% over previous 19th livestock census (2012). This shows goats are preferred for rearing by the farmers. Goat contributes 27.8 % of the total number of livestock reared in India (Anonymous, 2019). Goat contributes about 2.95 % of total milk production and 13.72 % of total meat production in India (Anonymous, 2020). The population of Goats in Maharashtra is 10.60 million and ranks 6th in statewise goat population after Rajasthan, West Bengal, Uttar Pradesh, Bihar, Madhya Pradesh. In India, 34 breeds of goat have been registered by the National Bureau of Animal Genetic Resources, NDRI, Karnal (NBAGR, Karnal). Maharashtra harbored four goat breeds namely Osmanabadi, Sangamneri, Berari & Konkan Kanyal (ICAR-NBAGR, 2021).

The Berari goat is from the Vidarbha region of Maharashtra which is mostly utilised for meat purpose in this region. Berari is a breed that thrives in both rainy and dry tropical environments. Average age for first successful conception is 10 to 11 months in Berari goats, an average daily milk yield is 533.28 g/day, and a total 130 days lactation yield is 78 kg. Berari goat is a medium-sized meat breed and coat color ranges from light tan to dark tan. The head is convex, with a slightly Roman nose. The ears are flat, leafy, and pendulous, wattles and beard rarely present in Berari goats, and horns are flat, upward, and backward. Distinctive characteristics include light to dark stripes on

the lateral sides from the base of the horn extending up to the nostrils in adults and in adults males black ring around the neck and along vertebral column black hairline lengthens up to the tail. (Kuralkar *et al*., 2013).

Rapid growth is a result of rapid body-weight gain, this influences net profit from sale of meat which is vital aspect in goats farmed for meat. Various physiological processes in the regulation of body growth in animals are monitored by various genes like Growth Hormone (GH), Growth Hormone Receptor (GHR), and Insulin-like Growth Factor-1 (IGF-1) results in overall body growth.

Growth hormone plays an important role in the immunological response, wound healing, and haematopoiesis of the body. IGF-1 production is stimulated by GH, and IGF-1 is also known as somatomedin C. This hormone has anabolic properties and promotes growth. As a result, selection based on high levels of endogenous growth hormone aids in the exploration and utilisation of superior development potential.

The gene for goat GH is physically located on chromosome no.19q22 (Schibler *et al*., 1998; Pinton *et al*., 2000). The approximate size of growth hormone gene is 2544 base pairs (Alakilli *et al*., 2012) and this gene is encoded by five exons and four intervening introns (Kioka *et al*., 1989). Growth hormone (GH) is a polypeptide anabolic hormone i.e. polymerised and secreted by somatotroph cells of the anterior pituitary gland in a regular and pulsatile fashion, and it is used as a promising and effective gene marker for improving milk and meat production in goats and other farm animals. This hormone is responsible for influence in development of body tissues and various organs like bone, muscle, and visceral organs (Ikonen *et al.,* 2001). GH has an impact on postnatal development, a variety of physiological processes viz. lactation, reproduction, metabolism (protein, lipid, and carbohydrate metabolism) in vertebrates (Ayuk and Sheppard, 2006). GH is associated with animal growth (Hua *et al*., 2009), development, and milk characteristics across several livestock animals (Malveiro *et al*., 2001; Marques *et al*., 2003). Animal's morphometric characteristics such as birth-weight, body-weight, chest-girth, body-length, etc. are good indicators of growth and production performance. In a diversity of livestock species, Growth

hormone (GH) is a well-known candidate gene that liable to alter either growth measures or lactation yield attributes (Supakorn, 2009).

The human activity for Goat's selection influences the conformation and endocrinology of upcoming generations. Selection for milk traits resulted in a slender, wedge-shaped doe and rather selected for meat resulted in square, stocky-shaped body goats. Typically growth genes controls growth and development, and interaction of solitary or multi, external and internal environmental stimulus has influenced the extent of expression of these genes. The quantitative aspects of growth are best defined by variation in size, mass, height, or length, but the qualitative aspects of growth are best described by changes in conformation. In terms of quantitative growth features, goats have rapid weight gain immediately after birth, followed by an accelerating growth up to weaning. This is the interval in which goats gain the most weight (Webb, EC. and N.H. Casey, 2005).

During postnatal period the muscle development is characterized by the proliferation of satellite cells located between the sarcolemma and the basal lamina of myofibrils. The most notable impacts of age on growth and development are related to increased live mass, which results in noticeable changes in body composition and conformation. Growth and development in goats are influenced by the sex of the animal. Buck shows faster growth rates and yields lean meat compared to does or castrates (Webb, EC. and N.H. Casey, 2005).

The present investigation was undertaken to study the genetic polymorphism in Berari Goats. The studies of genetic screening of growth hormone gene in Berari goat and its association with growth performance are not available in literature. Therefore, the present study is proposed to genetically characterize and explore the polymorphism in growth-related gene (GH1) in Berari.

Objectives:

1) To study the growth performance in Berari goats.

2) To investigate genetic polymorphism in growth hormone gene in Berari goats using PCR RFLP.

3) To explore the association of various genotypes of GH gene with growth performance.

CHAPTER II

REVIEW OF LITERATURE

Animals with high genetic merit for growth (body weight, body measures, weight gain, and carcass qualities) are prioritized in breeding programs for meat-type animals. The economy and livelihood of asset-poor farmers in Asian developing countries significantly depends on small animals like goat and Sheep (Dixit *et al*., 2010).

A complex process of growth is regulated by the coordination of a variety of neuroendocrine pathways, and also by coordinated activity of many hormones (Falaki *et al*. 1997). Significant contribution in the biological function shown by Several genes viz. Growth Hormone (GH), Growth Hormone Receptor (GHR), Insulin-like Growth Factor-1 (IGF-1), and Myostatin (MSTN), have been identified (Hossner *et al*. 1997; Supakorn 2009).

The Genetic diversity that is genetic variance between and within breeds is considered as a starting point by animal breeders for improving the genetics of various livestock species. Detection of polymorphism at the DNA level by using markers has been used to investigate the population structure and genetic variation among breeds. Molecular genetic techniques are one of the recent technologies that are being successfully applied to the conservation of animals of a particular breed around the world. By minimization of genetic relatedness maximum genetic diversity can be achieved in upcoming generations (Joshi *et al*. 2012). As the advancement of molecular genetic technology, several techniques like DNA-based markers greatly aided gene mapping, allowing the identification of the part of genes that govern variability of phenotypic features.

Genetic markers are the DNA sequence or gene that has a defined location on a chromosome and can be used to distinguish between different individuals or species. Due to a mutation or modification in the genomic locus, variation in genome can be observed (Muhammad *et al*. 2018). For population and evolutionary investigations, an ideal genetic marker should be extensively

distributed across the genome (Sunnucks 2000). Variation at the genetic level in native breeds is a key thrust area because preservation of unique and valuable diversity is crucial for meeting emerging demands. Genetic characterization of animals is necessary to preserve the possible greatest amount of genetic variety and to define conservation priorities.

Considering the objectives of the present study review of literature has been discussed under the following subheads.

2.1 GENETIC POLYMORPHISM

A difference in DNA sequence between individuals, populations, or groupings is known as genetic polymorphism. Polymorphisms can occur at the genomic level in both the coding and non-coding sections of a gene, with the majority of them being silent, indicating that they occur in the non-coding areas and so do not affect the expression or function of a gene, whereas those occurring in the coding regions can affect the gene's expression and produce variation (Hrdlickova *et al*., 2014).

Genetic polymorphisms may be induced by external agents or they may be due to the consequence of random processes. Single nucleotide polymorphism (SNP), sequence repetitions, insertions, deletions, and recombination are all sources of polymorphism. A variety of genetic tools, like allele-specific polymerase chain reaction (PCR), microarray technique, restriction fragment length polymorphism (RFLP), single-strand conformation polymorphism (SSCP), whole-genome sequencing, allozymes, and tandem repeats (mini and microsatellite), have been used to detect these polymorphisms and have proven to be useful in various research areas such as population genetics, evolutionary genetics, molecular phylogeny and forensics (Singh, 2001; Pool *et al*., 2010; Yin *et al*., 2017).

The two experimental strategies viz. candidate gene approach and linkage studies are being employed for the study of variability (Zhu and Zhao 2007). The candidate gene approach is concerned with the analysis of genetic variability of candidate genes based on biological and physiological information of the traits to

be involved for a portion of the trait variations, whereas linkage findings are concerned with the pursuit of quantitative trait loci (QTL) based on an understanding of genetic maps in comparison with the segregation pattern of the genetic marker and the traits of curiosity.

During the twenty-first century molecular genetic technology had been modified greatly, This led to countless breakthroughs, including the discovery of DNA-based markers, which has greatly aided the development of gene mapping. This would make it easier to find genes that regulate some of the phenotypic trait variability. Genetic markers for demographic and evolutionary studies should be plentiful, and data from different genotype scoring systems should be comparable across laboratories (Sunnucks, 2000).

Two experimental strategies that are linkage studies and the candidate gene approach are presently used in the study of variability. The linkage studies are involved in the search of quantitative traits loci (QTL) based on the knowledge of genetic maps in comparison with the segregation pattern of the genetic marker and the interested traits. The second method entails analyzing genetic polymorphism of candidate genes based on biological and physiological information about the attributes that will be implicated for the component. When it comes to trait variation as a result, association studies are carried out to see if a particular trait is securely linked to a particular genotype.

RFLP is associated with nucleotide changes in a DNA molecule such as deletions, insertions, or rearrangements that delete, generate, or translocate the cleavage sites of restriction enzymes (RE) (Bostein *et al*., 1980). An insertion, deletion, or point mutation might affect the size of future restriction fragments by creating or removing the recognition site for a certain RE at a given locus. Inversion, on the other hand, alters the size of restriction fragments by varying the distance between two RE sites.

Changes in a DNA sequence associated with an allelic alteration at a certain locus will be observed on gel electrophoresis by the varying mobility of restriction fragments, and distinct band patterns will be shown by individuals carrying different allelic variations of the gene. RFLPs are defined as variances in

the number and location of bands caused by changes in fragment size. Because gene expression is not needed for RFLP analysis, variation in the flanking regions or introns of genes may also be discovered. PCR-RFLP is a simple, fast, and precise approach for SNP genotyping that has proven to be particularly beneficial.

2.2 GROWTH HORMONE

Growth hormone (GH) also known as somatotropin, is a polypeptide hormone produced and secreted by somatotroph cells in the anterior pituitary gland. It has a molecular weight of 22000 Daltons. It accounts for 4%–10% of the pituitary's total weight when wet. It's a peptide hormone, which includes 191 amino acids, and it is a member of the somatolactogenic hormone family. Different mammalian species have varying degrees of lactogenic, somatotropic, and metabolic effects. (Miller and Eberhardt, 1983; Mc Cutcheon and Bauman, 1986; Nicoll *et al*., 1986; Berczi and Nagy, 1987).

The roles of GH in the body are as follows:

2.2.1 Effect on growth

Postnatal growth is aided by the pituitary growth hormone. The major role of GH in stimulating body growth is to stimulate the liver and other tissues to secrete IGF-1. This hormone proliferate chondrocytes resulting in bone (Neathery *et al*., 1991).

2.2.2 Metabolic effects

GH had effect on protein, fat, and glucose metabolism. Protein synthesis is stimulated and protein oxidation is reduced by GH. Increase in fat utilization by the breakdown and oxidation of adipocytes are aided by GH. It also aids in the maintenance of blood vessels. It maintains sugar level within typical limits. GH inhibits insulin to uptake glucose absorption in peripheral tissues and improve glucose synthesis in the liver.

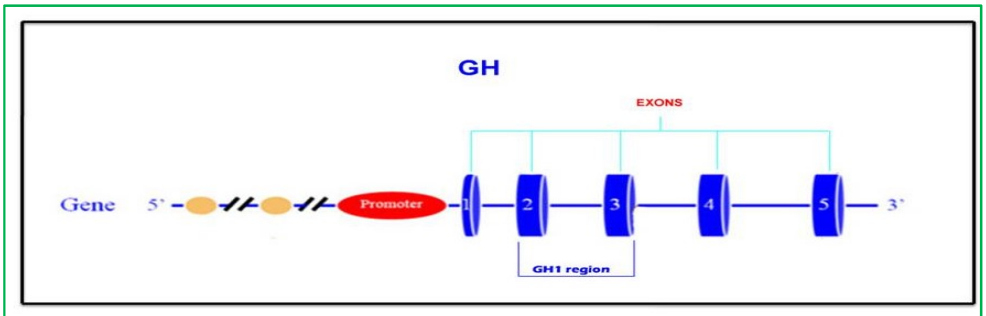

Fig. 2.1 Structural organization of growth hormone gene

AGGGCCCAGCAGAGACCAATTCCAGGATCCCAGGACCCAGTTCACCAGACGACTCAGGGT
CCTGCTGACAGCTCACCAACTATGATGGCTGCAGGTAAGCTCACAAAAATCCCCTCCATT
AGCGTGTCCTAAGGGGGTGATGCGGGAGAACTGCCGATGGATGTGTCCACAGCTTTGGGT
TTTAGGGCTTCTGAATGCGAACATAGGTATCTGCACCCAGACATTTGGCCAAGTTTGAAA
TGTTCTCAGTCCCTGGAGGGAAGGGCAGGCGGGGGCTGGCAGGAGATCAGGCATCCAGCT
CTCTGGGCCCCTCCGTCGCGGCCCTCCTGGTCTCTCCCTAGGCCCCCGGACCTCCCTGCT
CCTGGCTTTCACCCTG*CTCTGCCTGCCCTGGACT*CAGGTGGTGGGCGCCTTCCCAGCCAT
GTCCTTGTCCGGCCTGTTTGCCAACGCTGTGCTCCGGGCTCAGCACCTGCATCAACTGGC
TGCTGACACCTTCAAAGAGTTTGTAAGCTCCCCAGAGATGTGTCCTAGAGGTGGGGAGGC
AGGAAGGGGTGAATCCGCACCCCCTCCACACAATGGGAGGGAACTGAGGACCTCAGTGGT
ATTTTATCCAAGTAAGGATGTGGTCAGGGGAGTAGAAATGGGGGTGTGTGGGGTGGGGAG
GGTTCCGAATAAGGCAGTGAGGGGAACCACACACCAGCTTAGACCCGGGTGGGTGTGTTC
TCCCCCCAGGAGCGCACCTACATCCCGGAGGGACAGAGATACTCCATCCAGAACACCCAG
*GTTGCCTTCTGCTTC*TCCGAAACCATCCCGGCCCCCACGGGCAAGAATGAGGCCCAGCAG
AAATCAGTGAGTGGCCACCTAGGACCGAGGAGCAGGGGACCTCCTTCATCTTAAGTAGGC
TGCCCCAGCTCTCTGCACCGGGCCTGGGGTGGCCTTCTCCCTGAGGTGGCAGAGGGTGTT
GGATGGCAGTGGAGGATGATGGTTGGTGGTGGTGGCAGGAGGTCCTCGGGCAGAGGCCGA
CCTTGCAGGGCTGCCCCGAGCCCGCGGCACCCACCAACCACCCATCTGCCAGCAGGACTT
GGAGCTGCTTCGCATCTCACTGCTCCTTATCCAGTCGTGGCTTGGGCCCCTGCAGTTCCT
CAGCAGAGTCTTCACCAACAGCCTGGTGTTTGGCACCTCGGACCGTGTCTATGAGAAGCT
GAAGGACCTGGAGGAAGGCATCCTGGCCCTGATGCGGGTGAGGATGGCGTTGTTGGGTCC
CTTCCATGCTGGGGGCCATGCCCACCCTCTCCTGGCTTAGCCAGGAGAACACACGTGGGC
TGGGGGAGAGAGATCCCTGCTCTCTCTCTCTTTCTAGCAGCCCAGTCTTGACCCAGGA
GAAACCTCTTCCCCTTTTGAAACCTCCTTCCTCGCCCTTCTCCAAGCCTATAGGGGAGGG
TGGAAAATGGAGCGGGCAGGAGGGAGCCGCTCCTGAGGGCCCTTCGGCCTCTCTGTCTCT
CCCTCCCTTGGCAGGAGCTGGAAGATGTTACCCCCCGGGCTGGGCAGATCCTCAAGCAGA
CCTATGACAAATTTGACACAAACATGCGCAGTGACGACGCGCTGCTCAAGAACTACGGTC
TGCTCTCCTGCTTCCGGAAGGACCTGCACAAGACGGAGACGTACCTGAGGGTCATGAAGT
GTCGCCGCTTCGGGGAGGCCAGCTGCGCCTTCTAGTTGCCAGCCATCTGTTGTTACCCCT
CCCCGTGCCTTCCTAGACCCTGGAAGGTGCCACTCCAGTGCCCACTGTCCTTTCCTAATA
AAGCGAGGAAATTGCATCACATTGTCTGAGTAGGTGTCATTCTATTCTAGGGGGTGGGGT
CAGGCAGGATAGCGAGAGGGAGGATTGGGAAGACAATAGCAGGGATGCTGTGGGCTCTAT
GGGTACCCAGGTGCTGAATAATTGACCCGGTTCTTCCTGGGCCAGAAGGAAGCAGGCACA
TCCCCTTCTCTGTGACACACCCGGTCCTCGCCCCTGGTCCTTAGTTCCAGCCCCACTCAT
AGGACACTCATAGCTCAGGAGGGCTCTGCCTTCAGTCCCACCCGCTAAAGTGCTTGGAGC
GGTTTCTCCTTCCCTCATCAGCCCACCAAACCAAACCTAGCCTCCAAGAGTGGGAAGAAA
+

CT..... - Exonic nucleotides
CT.....- Intonic nucleotides

CT.....- Primers
CT....- Amplified segment

Fig. 2.2 Nucleotides sequence of Growth hormone gene and amplified GH1 region (422 bp)

2.3 GROWTH HORMONE GENE

The growth hormone gene is part of the growth hormone gene axis, which includes GHRH, Somatotropin, GHBP1, GHBP2, and GHBP3, as well as IGF-1, IGF-2, and GHR (Laron, 2001).

The growth hormone gene is found on the 19th chromosome (Kioka *et al.*, 1989). The caprine growth hormone gene is made up of five exons separated by four introns (Figure 2.1). The overall sequence length of base pairs is approximately 2.5 kbp (Figure 2.2), with the prime transcript spanning 374-2169 bp. Exon 1 is short, spanning 432-444 bp and encoding amino acids 1-4(04), whereas exon 2 extends 692-852 bp and encodes for amino acids 5-58(54). Exon 3, which spans 1080 to 1196bp, encodes for amino acids 59-93(35). Exon 4 runs from 1426 to 1587bp and encodes for amino acids 94-146(53). Exon 5, spans from 1864 to 2064bp and encodes for amino acids 147-217(71). The GH gene encodes 217 amino acids in total. Intron 1 runs from 445 to 691, separating exons 1 and 2. Intron 2 separating the Exons 2 and 3 which runs from 853 to 1079. Exons 3 and 4 are separated by intron 3, which runs from 1197 to 1425. Exons 4 and 5 are separated by intron 4, which runs from 1864 to 2064.

2.4 GENETIC SCREENING OF GOAT GH GENE USING RFLP

Chitra *et al*. (2004) investigated the 768 bp size amplified segment of the GH gene in 196 genetically unrelated Malabari goats DNA samples and discovered two alleles by digesting the amplified product with *MspI (+)* and *MspI (-)* with frequencies of 0.70 and 0.30, respectively.

Lan *et al*. (2007) discovered variation in the GH gene in Chinese goat populations. Amplified the GH gene using five pairs of primers: GH1 and GH2 (for the 5' UTR area), GH3 and GH4 (for exon-2 and surrounding sections), and GH5 (for exon-5 and flanking 3' UTR region), and polymorphic SSCP patterns were observed in all amplified regions. Only a significant relationship was identified between SSCP in exon-5 and milk production in the third and fourth lactations. The 1651T-to-G mutation in exon-5 and the adjacent 3' UTR region

was used for PCR-RFLP genotyping of several goat breeds with *KpnI*, which detected two alleles, GH-T and GH-G, as well as two genotypes (TT/TG). For Xinong Sannen, Laoshan, Guanzhong, Shaannan White, Guizhou White, Angora, and Boer goat populations breed in China, GH-G allele frequencies were 0.142, 0.092, 0.032, 0.131, 0.059, 0.050, and 0.000 respectively. The study found that the relationship between the TT and TG genotypes and litter size, body weight, and milk performance could be relevant for assessing breed characteristics.

Hua *et al.* (2009) analyzed polymorphism of growth hormone (GH) gene as a genetic marker candidate for growth traits in Boer goat bucks. A genomic DNA isolated from the blood and primers (Forward '5'-CTCTGCCTGCCCTGGACT-3' and Reverse 5'-GGAGAAGCAGAAGGCAACC-3') was used for polymerase chain reaction (PCR) amplification and products obtained (422bp) on restriction digestion of amplicon with enzyme *HaeIII*. Two single nucleotide polymorphisms (SNPs) – A781G (Ser/Gly35) and A1575G (Leu147) were identified.

Alakilli *et al*. (2012) discovered variation in two separate growth hormone gene regions in Egyptian and Saudi goat breeds. They discovered that exon 2 amplification resulted in 422 bp fragments, whereas exon 4 amplification resulted in 116 bp fragments. When 422bp fragments were digested with restriction enzymes, three bands were formed: 422 (non-cut), 366, and 56 bp representing alleles A and B, respectively, whereas 116 bp fragments produced three bands: 116 (non-cut), 88, and 28 bp representing alleles C and D, respectively. Allele A and B frequencies in GH1 were 0.41 and 0.59 in the Barki breed, 0.62 and 0.38 in the Zaribi breed, and 0.43 and 0.57 in the Ardi breed, respectively, and 0.49 and 0.51 for the Masri breed.

Marini *et al*. (2012) analysed two regions in growth hormone (GH1 and GH5) in Savanna and Kalahari goats using *HaeIII*-RFLP. The study of GH1 shows a 422 bp fragment revealed polymorphism with two genotypes AA (366 and 56bp) and AB (422, 366, and 56bp). In Savanna and Kalahari goats, the frequency of AA genotype was 0.07 and 0.36, whereas the frequency of AB genotype was 0.88 and 0.64, respectively. In both goats, the AB genotype was

more common than the AA genotype. The study of a 404-bp GH5 fragment revealed polymorphism with three genotypes: GG (228, 78, and 53bp), GH (228, 150, 78, and 53bp), and HH (150, 78, and 53bp). In Savanna and Kalahari goats, the frequency of the GG genotype was 0.57 and 0.14, the GH genotype was 0.37 and 0.5, and the HH genotype was 0.05 and 0.36.

Singh *et al*. (2015) studied polymorphism of exon 2 and exon 3 region of growth hormone (GH) gene of size 422 bp (Forward '5'-CTCTGCCTGCCCTGGACT-3' and Reverse 5'-GGAGAAGCAGAAGGCAACC-3') in Sirohi and Barbari breeds of goat using *HaeIII* PCR-RFLP. Digestion revealed AB and BB genotypic variants with genotypic frequencies of AB and BB were found to be 0.82 and 0.18 in Sirohi and 0.90 and 0.10 in Barbari goats, respectively. The respective allelic frequencies of A and B were 0.41 and 0.59 in Sirohi and 0.45 and 0.55 in Barbari.

Othman *et al*. (2015) used PCR-RFLP to investigate genetic polymorphisms in exons 2 and 3 of the GH gene in Egyptian goat and sheep breeds. *HaeIII* endonuclease digestion of PCR-generated fragments indicates two genotypes, GG and AG. In 101 tested sheep, the frequencies for GG and AG genotypes were 43.56 and 56.44 percent, respectively, while in 48 tested goats, the frequencies for GG and AG genotypes were 12.5 and 87.5 percent, respectively. The existence of an SNP (G6A) at locus 55 in the amplified segment was responsible for the degradation of the restriction site GGCC and, as a result, the presence of two distinct alleles G and A, according to the sequencing analysis. The association showed that heterozygous genotypes had a substantial effect on some growth parameters. Because of the association between the A/G genotype and growth traits, the selection was recommended to increase the frequency of the A/G genotype to improve production efficiency. It was also concluded that the detected SNP could be of potential application in carrying out marker assisted selection and bringing genetic improvement to the studied breeds.

Marini *et al*. (2015) investigated the GH gene's inheritance and the link between GH gene polymorphism and pre-weaning growth in Savanna goats in Malaysia. GH1 and GH2 regions were combinations chosen for breeding based on

body condition score of 2.5 or higher, history of kidding, and reproductive soundness. F1 progeny were examined for genotypic patterns and growth performance, based on GH1 and GH5 regions. The GH1 gene revealed polymorphisms with two genotypes, AA and AB, and the GH5 gene revealed polymorphisms with three genotypes, GH, GG, and HH. The four parental GH1 and GH5 gene combinations resulted in F1 genotypes that followed Mendelian inheritance. The GH1 gene revealed polymorphisms with two genotypes: AA (366, 56 bp) and AB (422, 366, 56 bp) and GH5 gene with three genotypes GH (228, 150, 78, and 53 bp) and GG (228, 78, 53 bp) and HH (150, 78 and 53 bp). The combination of GH1 and GH5 genotypes of P1 Savanna parents revealed four genotypic variants of ABGG, ABGH, AAGH, and AAHH with frequencies of 0.47, 0.39, 0.06, and 0.08, respectively. The birth weight of the ABGG-F1 Savanna kid was greater ($p<0.05$) than that of the ABGH genotype. According to the findings of the study, the genetic polymorphism of the GH gene can be employed as a possible marker in genomic selection and breed development of goats under good management and environmental conditions.

Radhika *et al*. (2016) investigated the polymorphism of exon 2 and 3 of the GH gene, local goat breeds in Kerala. A polymorphism in the GH gene at 781A > G was found during study on 343 goats of native breeds of Kerala, Malabari, and Attappady Black, as well as Malabari crossbreds. Restriction digestion with the enzyme *HaeIII* produced three genotypes: AA, AB, and BB. The frequency of genotype AB was higher in all populations, indicating a selective advantage, but no link between genotypes and growth traits was found. The BB genotype was with a low frequency of 0.02. This genotype was seen mostly in the Attappady Black goat population which comes under the 'insecure' category of conservation and hence cannot afford to undergo strict selection measures, which might be the reason for the presence of this rare genotype.

Ilham *et al*. (2016) used the PCR-RFLP method to study genetic polymorphisms of the GH gene in 168 Kacang goats in Indonesia, revealing two genotypes; AA and AB with frequencies of 0.095 and 0.904. The respective frequencies of alleles A (0.547) and B (0.452) suggested a polymorphism in the

GH A781G gene. The observed (Ho) and excepted (He) heterozygosities were 0.0904 and 0.496, respectively. The distribution of GH alleles in Kacang goat populations was not in Hardy and Weinberg equilibrium. Statistical analysis showed no difference in body size between genotypes AA and AB.

Susilorini *et al*. (2017) used PCR-RFLP to investigate the genetic polymorphism of the GH2 region of gene in 94 Etawah crossbred goats. *HaeIII* endonuclease was used to digest the PCR-generated fragments. The findings revealed the presence of two genotypes, CC and CD. The genotype frequencies were 0.47 (CC) and 0.43 (CD) respectively. Statistical analysis showed that CD genotype had a higher birth weight and weaning weight than the CC genotype. The study suggests that the GH gene might be employed in MAS for goat selection.

Maharous *et al*. (2018) analysed GH gene for polymorphism of 422 bp segment. The amplified fragment digested with the restriction enzyme *HaeIII* resulted in two different alleles, A (uncut 422 bp fragment) and B (366 bp and 56 bp fragments). Only two genotypes, the homozygous BB (366 bp and 56 bp) and the heterozygous AB (422 bp with 366 bp or 56 bp) were found, and absence of the genotype AA. Genotype AB had the highest frequency in Egyptian goat breeds under study. The AA genotype (366 and 56 bp) resulted in a significant decrease in birth chest girth (P=0.03) and weaning weight (P=0.014), compared to the AB genotype (422, 366, and 56 bp).

Bayan *et al*. (2018a) investigated GH gene polymorphism using *HaeIII* PCR-RFLP in Surti and Mehsana goats. The PCR amplified region of 422 bp (Exons 2 and 3) indicated the polymorphism, AA and AB genotypes with genotypic frequencies of 0.24 and 0.76 in Surti goats and 0.20 and 0.80 in Mehsana goats respectively. Both the population of Surti and Mehsana goats were not found to be in genetic equilibrium for GH locus exon 2-3 indicating selection pressure for growth.

Similarly, Bayan *et al*. (2018b) using Primers GH 2 (F-5'TCA GCA GAG TCT TCA CCA-3'and R 5'-CAA CAA CGC CAT CCT CAC-3'), screened the exon-4 region of GH gene of 116 bp size, wherein the researchers reported only

one genotype CC in both Surti and Mehsana goat breeds. The allelic frequency of C was 1.00 in both the breeds of goats with the absence of the D allele.

Gooki *et al*. (2018) detected GH gene polymorphism in Raini Cashmere goat using *HaeIII* -PCR RFLP. A 422 bp fragment encoding exon 2 and 3 of GH gene was amplified resulted in the occurrence of AA and AB genotype with genotype frequencies as 0.15 and 0.85, and the allele frequencies of A as 0.575 and B as 0.425, respectively. Fleece weight and birth type traits of AB genotype showed higher estimated breeding value.

Moneva *et al*. (2020) studied crossbred Anglo-Nubian goats of the Philippines, for the target A781G locus in GH1 region. The target region extends from exon 2 (126), intron 2 (227 bp bp), to part of exon 3 (69 bp). Two genotypes (AA and AB) were obtained from the RFLP analysis, and BB genotype was absent, the frequency of AB genotype (0.76) was higher than AA (0.24).

Genetic polymorphism for growth hormone (GH) gene in Assam Hill Goat was studied by Sarmah *et al*. (2020) using a synthetic primer (F: 5'-TCCCTGCTCCTGGCTTTCAC-3'and R: 5'-GGAGAAGCAGAAGGCAACC-3'). PCR-RFLP product digested with *HaeIII* restriction enzyme revealed two genotypes, viz. AA (25.39%) and AB (74.61%) with the gene frequency 0.63 (A) and 0.37 (B). The genotype frequency for AA and AB were found to be 0.40 and 0.60 respectively. The evaluated mean body weight of AA and AB genotypes at 0 day, 3, 6, 9, and 12 months of age were 1.18± 0.03, 5.37±0.11, 7.50±0.12, 9.69±0.14, and 12.74±0.17 for AA genotype and 1.16±0.02, 4.78±0.06, 7.14±0.08, 10.03±0.11 and 13.32±0.11 kg, for AB genotype respectively.

Aradhana *et al*. (2021) explored the genetic variation of the GH gene using *HaeIII* PCR-RFLP and its association with morphometric traits of Ganjam and Baigani goats of Odisha. Genomic DNA was isolated and the target segment 422 bp amplified and digested, which yielded only two variants in both the populations and genotypes, viz. AA (25.39%) and AB (74.61%) while the gene frequency 0.63 (A) and 0.37 (B). The genotype frequency for AA and AB were found to be 0.40 and 0.60 respectively. Variant A had only one restriction recognition site on the target gene segment yielding two bands with the size of

366 bp and 56 bp whereas variant B did not have any restriction site with a single band of 422bp.

Pandya *et al*. (2021) screened GH gene polymorphism in Surti goat using PCR-RFLP technique, Amplified the exon 2 and 3 regions of GH gene and digestion with *HaeIII* enzyme of GH PCR products (422bp) revealed two genotypes (AB and BB). The genotypic frequencies of AB (422, 366, and 56 bps) and BB (366 and 56 bps) were 0.8 and 0.2, respectively.

Saputra *et al*. (2021) analysed the growth hormone gene role in regulating body growth and in developing mammary gland in Etawah grade, Saanen, Saanpe, and Sapera breeds of Indonesia. PCR-RFLP of respective regions was carried using *HaeIII* enzyme (exon 2) or *Pst*I (exon 4). The amplified product of the GH exon 2 was digested fragments of 186 and 12 bp were genotyped as AA; fragments with 186, 97, 89, and 12 bp was AG genotype; and fragments with 97, 89 and 12 bp bands was GG genotype The frequency of AG genotype was very high (0.8377) in Boer goat, whilst the frequency of GG genotype was very low (0.1623). RE digested GH exon 4 region fragments of 200 bp was genotyped as AA genotype, fragments of 200, 131, and 69 bp was AC genotype, fragments of 131, 69 bp was CC genotype. The exon 4 region region were monomorphic pattern with CC genotype. And concluded that GH exon 2 could be employed as a useful marker for helping the selection of Sapera goat.

Table 2.1 Reports of polymorphism in Growth hormone gene

Sr. no.	Species/ Breed	Region	Amplification Segment length(bp)	RE	Genotypes observed	Reference
1	Goat	3rd Intron	768	*Msp*I	*Msp*I *(+/+)* and *Msp*I *(+/-)*	Chitra *et al*. (2004)
2	Goat	GH1	422	*HaeIII*	AA (422), AB(422, 366 & 56) and BB(366 & 56)	Alakilli *et al*. (2012)
		Exon 4	116		CD (116 & 88)	
3	Goat	GH1	422	*HaeIII*	AA (366 & 56bp) and AB (422, 366 & 56).	Marini *et al*. (2012)

Sr. no.	Species/ Breed	Region	Amplification Segment length(bp)	RE	Genotypes observed	Reference
4	Goat	GH1	422	*HaeIII*	AA (366 & 56) and AB (422, 366 & 56).	Singh *et al*. (2015)
5	Goat	GH1	422	*HaeIII*	GG (366 & 56) and AG (422, 366 & 56).	Othman *et al*. (2015)
6	Goat	GH1	422	*HaeIII*	AA (366 & 56bp) and AB (422, 366 & 56).	Marini *et al*. (2015)
7	Goat	GH1	422	*HaeIII*	AA (366 & 56bp) and AB (422, 366 & 56), BB (422).	Radhika *et al*. (2016)
8	Goat	GH1	422	*HaeIII*	AA (366 & 56) and AB (422, 366 & 56).	Ilham *et al*. (2016)
9	Goat	Exon 4	116	*HaeIII*	CC (116 & 88) and CD (116, 88 28)	Susilorini *et al*. (2017)
10	Goat	GH1	422	*HaeIII*	BB (366 & 56) and AB (422, 366 & 56).	Mahrous *et al*. (2018)
11	Goat	GH1	422	*HaeIII*	AA (366 & 56bp) and AB (422, 366 & 56).	Bayan *et al*. (2018[a])
12	Goat	Exon 4	116	*HaeIII*	CC (116 & 88).	Bayan *et al*. (2018[b])
13	Goat	GH1	422	*HaeIII*	AA (366 & 56) and AB (422, 366 & 56).	Gooki *et al*. (2018)
14	Goat	GH1	422	*HaeIII*	AA (366 & 56) and AB (422, 366 & 56).	Moneva *et al*. (2020)
15	Goat	GH	466	*HaeIII*	AA (366 & 80) and AB (446, 366 & 80).	Sarmah *et al*. (2020)
16	Goat	GH1	422	*HaeIII*	AA (366 & 56), AB (422, 366 & 56) & BB (422).	Aradhana *et al*. (2021)
17	Goat	GH1	422	*HaeIII*	BB (366 & 56) and AB (422, 366 & 56)	Pandya *et al*. (2021)
18	Goat	Exon 2	198	*HaeIII*	AA (186 & 12), AG (186, 97, 89 & 12) and GG (97, 89 & 12)	Saputra *et al*. (2021)

2.5 GENETIC SCREENING OF SHEEP GH GENE USING RFLP

Malewa *et al*. (2014) studied seventy fat-tailed sheep (Donggala and East Java sheep) for the GH gene portion, including an intron 2, a portion of exon 3, a portion of exon 4, and a portion of intron 4, was amplified. The 934-bp-long amplified product was digested with *HaeIII* restriction enzymes and revealed the existence of GH *HaeIII* polymorphism with three genotypes (AA, BB, and AB). Genotype AA was identified as bands with ten restricted pieces of 277, 202, 110, 100, 94, 68, 49,22, 8, and 4 bp. Bands with 256, 202, 110, 100, 94, 68, 49, 22, 21, 8, and 4 bp restricted fragments were classified as genotype BB. AB was used to genotype bands with 12 limited fragments of 277, 256, 202, 110, 100, 94, 68, 49, 22, 21, 8, and 4 bp. The difference between allele A and allele B was caused by an AG (AG-CC) to (GGCC) transition in exon 3 of the gene at 727 bases or at 255 bases of the PCR product, which leads to the site being identified by the *HaeIII* enzyme. The AA and AB genotypes had the same frequency of 0.357 in Donggala sheep, while the BB genotype had a frequency of 0.286. The AA genotype is the most common in East Java sheep (0.464), with a frequency of 0.286 and 0.250 for BB and AB, respectively. Growth rate and weaning weight were modified by the GH/*HaeIII* polymorphism in both Donggala and East Java sheep. AA was shown to have a considerably faster growth rate than BB in Donggala sheep. When compared to both homozygotes (AA and BB), AB as a heterozygote, showed no significant changes in growth rate. In both the Donggala (11.6 kg vs. 9.68 kg) and East Java (10.83 kg vs. 9.37 kg) sheep breeds, genotype AA had a considerably greater weaning weight than genotype BB. In both Donggala and East Java sheep, genotype AB showed non-significant variations in weaning weight when compared to genotypes AA and BB.

Kumari *et al*. (2014) investigated the GH gene at A1575G and A781G locus. The 324 random blood samples from Nine sheep breeds from distinct agro-ecological zones of India were collected, PCR-RFLP was used to analyse the samples. Restriction digestion investigation at the A781G gene revealed two allelic variants A and B. The gene frequency of A=0.6016 and B=0.3983 and genotype frequency were AA=0.2032 and AB=0.7968. At a A1575G locus all individuals were homozygous (CC).

Seevagan *et al*. (2015) screened GH1 and GH5 region by of GH gene by Polymerase Chain Reaction - Restriction Fragment Length Polymorphism in Vembur sheep. Results revealed the existence of two genotypes viz., AA (366 and 56 bp) and AB (422, 366 and 56 bp), and two alleles viz., A and B. Genotypic frequencies for AA and AB were 0.43 and 0.57, and allelic frequencies were 0.71 and 0.29 for A and B respectively, BB genotype was absent.

Gorlov *et al*. (2017) used PCR-RFLP and the *HaeIII* enzyme to investigate the connection of the GH gene polymorphism with growth traits in Salsk sheep. The genotypes AA, AB, and BB were discovered with frequencies of 57, 36, and 7%, respectively. The values of the weight at weaning, and at the age of 9 months with the AB genotype exceeded the values of the parameters of the ram lamb with the AA genotype by 0.92 kg and 10.67 kg. The presence of a heterozygous AB genotype in the Salsk sheep breed improved growth traits. The AB genotype outperformed the AA genotype and was determined to have the highest meat productivity.

Mahdi *et al*. (2018) amplified GH1 and GH2 with PCR technology and enzymatic digestion (PCR-RFLP) was performed using the *HaeIII* enzyme in Iraqi sheep breeds (Awassi, Hamdani, and Karadi). The results showed that there are two locus of PCR gene in the sheep genome. The first region was located in the second and third exons with 422 bp (GH1), while the second region was in the fourth exon with 116 bp (GH2). The genotypes BB and AB of GH1 were found in both Karadi (0.70, 0.30) and Awassi (0.60, 0.40) respectively . The genotypes AA, BB and AB were found in Hamdani (0.10,0.50 and 0.40) breed. The frequency of alleles A, B and C in GH1 and GH2 was 0.45, 0.55 and 1.00 respectively in the Hamdani , while in the Awassi frequency for alleles A, B, and D was 0.15, 0.85, and 1.00 respectively, and in the Karadi the alleles frequency for A, B, C and D was 0.20,0.80, 0.90 and 0.10, respectively.

Senkal *et al*. (2021) studied Iraqi awassi sheep for polymorphism of growth hormone gene (exon2/A781G) region (422bp). The amplification was conducted by using PCR-RFLP with *HeaIII* restriction enzyme. There were three genotypes (AA, Aa, aa) with genetic distribution 0.44, 0.49 and 0.07 respectively..

The AA genotype was superior to other genotypes in breeding value and dominant deviation also the dominant variance superior to additive variance in weight traits. The average allele substitution and the average allele effect A allele was more prevalent than a allele in Weaning weight. The study showed the importance of the A allele in the weaning weight selection programme for the characteristic of weaning weight.

Alsaedi *et al*. (2021) examined sheep breed for GH gene polymorpism by PCR-RFLP method, three genotypes were spotted AA, GG and AG with frequency of 0.153, 0.247 and 0.6, respectively.

2.6 MORPHOMETRIC TRAITS IN BERARI GOATS.

Berari is the 23rd recognized goat breed of India with accession number INDIA_GOAT_1100_BERARI_0623. The Berari goat breed is from central region of India that is Vidarbha region of maharshatra, this goat breed is mainly reared for meat purpose by the farmers of this region.

In animals reared for meat purpose body weight had prime importance, and it is based and correlated with body characteristics viz. body height, chest girth and body length.

2.6.1 Bodyweight

It is an important feature in meat animal production since it determines carcass yield and dressing percentages (Mandakmale *et al.,* 2009c).

Pujari *et al.* (2004) studied the body conformation traits and their inheritance in Berari goats and reported birth weight for male and female kids were 2.20±0.13 kg and 1.84±0.10 kg, and body weight at 12 months were 18.05±0.64 and 15.18±0.56 kg for adult male and female, respectively.

Kharkar *et al*. (2014) reported the birth and final body weights (90 days) were 2.41 and 8.92 kg respectively, in Berari goat kids. The birth weight of male kids (2.46 kg) was higher than female kids (2.36 kg). The overall body weights at birth, 3, 6, 9, and 12 months of age were 2.46, 9.22, 15.41, 18.33 and 22.64 kg in males and 2.36, 8.70, 14.65, 18.04 and 20.41 kg in females. In all the age groups,

higher body weights and body conformations (height, length, chest girth and punch girth) was recorded in males than females.

Suranagi *et al*. (2005) measured 53 Bidri goats for live body weight, body length, chest girth, and height. The correlation coefficients between many measures were highly significant.

Singh *et al*. (2006) studied growth traits of Beetal goat, the overall means for body weights at birth, 3, 6 and 12 months were 2.54 ±0.04, 8.56 ±0.18, 12.87 ±0.23 and 18.98 ±0.34, respectively.

Kumar *et al*. (2006) discovered the body weight of Kutchi goat at 3 and 6 months of age were 12.82 and 18.98 kg, respectively.

Malik *et al*. (2006) examined the Beetal and Jhakhrana kids for birth, 3, 6 and 12 months body weights, the respective means were 2.28 ±0.08, 9.94 ±0.80, 12.40 ±0.40 and 23.15 ±1.20 kg for Beetal goats and 2.31±0.11, 9.30 ±0.46, 12.33 ±1.57 and 22.00 ±1.02 kg, respectively for Jhakharana goats.

Rao *et al*. (2009) measured the average body weight of Ganjam goats adult male 41.5 kg and female 37.6 kg. The body weight at birth, 3, 6, 9 and 12 month of age were 2.55, 6.15, 8.41, 11.76 and 16.64 kg in females and 2.70, 7.16, 9.33, 13.7, 18.35 kg for males, respectively.

Mandakmale *et al.* (2009a) studied the growth performance of Sangamneri kids under field condition, the overall means for body weights in Sangamneri kids at 1, 3, 6, 9, 12 months of age were 4.95 ± 0.05, 8.72± 0.08, 13.69 ±0.18, 17.79± 0.41 and 22.56 ±0.62 kg respectively. The sex and sire had significant influence ($P < 0.01$) on body weight at different ages. And the effect of season had significant effect up to 6 months age. Type of birth affected the birth and 3 months weight significantly.

Mandakmale *et al.* (2009b) summarized growth performance of Sangamneri goats under farm condition, the overall means for body weights in Sangamneri goats at birth, 3, 6, 9, 12 months of age were 2.06 ± 0.41, 6.77± 0.99, 11.63 ±1.14, 17.05± 1.78 and 24.31±1.68 kg respectively.

Gaikwad *et al.* (2009) computed the growth performance of Osmanabadi goats under field condition, the overall means for body weights at birth, 3, 6 and 9 months were 2.10 ± 0.25, 8.24± 0.02, 12.40 ±0.02 and 17.20± 0.58, respectively for males and 1.98 ± 0.87, 7.85± 0.21, 11.50 ±0.56 and 16.50± 0.12, respectively for females.

Hassan *et al*. (2010) obtained overall leasts squares mean of body weight at birth, 3, 6, 9 and 12 months was 1.6 ± 0.6, 7.9 ± 2.3 , 12.2 ± 3.5, 16.8 ± 3.9, 21.4 ± 3.8 and 21.4 ± 3.8kg, respectively in Jamunapari goat.

Kumar *et al*. (2010) elaborated that the least-squares means in Sirohi kids were 2.35±0.04, 12.80±0.19, 16.31±0.21, 19.34±0.25 and 23.27±0.36 at birth, 3, 6, 9, and 12 months of age, respectively. The genetic correlation between birth weight and body weight at 12 months of age was discovered low and positive, but it was low and negative after three, six, and nine months body weight. The genetic correlation between 3 months, 6 months, 9 months, and 12 months was discovered positive and high.

Birari *et al*. (2012) recorded the growth performance of Osmanabadi goats, the overall birth weight of males (1.92±0.03 kg) was higher than the females (1.69±0.04 kg). The overall body weights of males and females recorded at 3 months of age was 7.29±0.01 and 6.92±0.04 kg, respectively. The overall body weight at 6 months of age was higher in males (14.46±0.02 kg) than females (14.02±0.03 kg). The body weights at 12 months of age in females (20.61±0.03 kg) were significantly ($P<0.01$) higher than males (19.80±0.03 kg).

Sundaram *et al*. (2012) examined Tellicherry goats in organized farm, results showed that the birth weight was 2.62±5.04 kg for male and 2.34±0.72 kg for female. The average body weight at 3, 6, 9, and 12 months for males were 2.3±0.68, 9.2 ±1.40, 20.0 ±3.36, and 27.4 ±2.52, and for female animals of age 3, 6, 9, and 12 month were 2.6±0.49, 8.5±1.49, 16.8±1.85, 24.1±2.22, respectively.

Tyagi *et al*. (2013) studied goats from 22 villages from 6 clusters of Gujarat. The body weight varies between16.50 to 30.50 kg at 12 months of age.

Murali *et al*. (2014) elaborated Tellicherry goats, The least-squares means for birth, 3, 6, 9 and 12 months body weights were 2.26±0.02, 8.58±0.11,

12.37±0.15, 15.81±0.19 and 19.69±0.29 kg, respectively. Males were significantly heavier and had a higher weight gain than females during all stages of growth and their differences increases with age. The birth weight of kids born between March and May was significantly lower than those born in other months.

Dudhe *et al*. (2015) monitered Sirohi kids born during 2007-2013 in farmers' flocks and data on body weights was recorded. The overall least-squares means for body weight at 12 months of age was 25.80±0.49 kg.

Karna *et al*. (2016) quantified body weights of Ganjam goats at different intervals up to 12 months. The overall least squares means of body weight at birth, 3, 6, 9 and 12 months age were 2.38±0.01, 7.08±0.01, 9.64±0.02, 14.03±0.02 and 18.02±0.03, kgs respectively. The bodyweight of the kids ranged from 0.8 to 3.8 kg at birth, 4.6 to 11.8 kg at 3 months, 6.4 to 13.6 kg at 6 months, 10.8 to 17.5 kg at 9 months and 14.6 to 22.3 kg at 12 months of age.

Bansode *et al*. (2017) compared Osmanabadi kids under conventional and loose housing system, The average weekly body weight (kg) of Osmanabadi goat kids under conventional housing system for 0-16 weeks were 5.75±0.35, 5.75±0.35, 5.91±0.32, 6.10±0.34, 6.31±0.33, 6.53±0.34, 6.70±0.35, 6.90±0..34, 7.05±0.35, 7.24±0.35, 7.40±0.34, 7.60±0.33, 7.76±0.35, 8.00±0.36, 8.21±0.37 and 8.40±0.35, respectively. The overall average body weight of Osmanabadi goat kids under the loose housing system was 6.97±0.14 kg. The average weekly body weight (kg) of Osmanabadi goat kids under loose housing system for 0-16 weeks were 6.00±0.29, 6.00±0.29, 6.28±0.28, 6.56±0.29, 6.83±0.29, 7.13±0.29, 7.41±0.27, 7.85±0.20, 8.06±0.20, 8.26±0.19, 8.50±0.20, 8.71±0.21, 8.91±0.19, 9.18±0.15, 9.46±0.14 and 9.78±0.19 respectively. The overall average body weight of Osmanabadi goat kids under the loose housing system was 7.81 kg.

Khadda *et al*. (2017) tabulated overall least-squares means for body weight at birth, 3, 6, 9 and 12 months of age were found to be 1.89±0.02, 9.49±0.20, 13.09±0.18, 16.38±0.19 and 18.84±0.22 kg, respectively, in Pantja goats under field conditions. The random effect of sire was very significant ($P<0.01$) on body weight at birth, 3, 6, and 9 months of age, although it was not significant ($P<0.05$) on body weight at 12 months of age. The cluster exhibited a

highly significant (P<0.01) influence on body weight at birth, 3, 6, and 12 months body- weights. The kind of birth and gender of the kid were discovered to be extremely influential on birth, 3, 6, 9, and 12 month body weights. Bodyweight genetic and phenotypic relationships to body weight at successive ages were found to be both high and positive.

Gond *et al*. (2019) inspected Black Bengal and Crossbreed goats for body weights, The mean birth weights (Kg) of Black Bengal and crossbred kids were estimated as 1.42±0.17and 2.36±0.35 respectively, whereas it was 5.75 ±0.29 and 9.54 ±0.35 at age of 3 months and 6 months, respectively, for Black Bengal goats and while it was 10.85 ±0.40 and 16.00 ±0.40, respectively, for Crossbred goats.

Sahu *et al*. (2020) measured body weights in Black Bengal goats, the least-squares means for body weights at birth and 3 months of age were 1.48 and 6.06 kg, respectively. The study showed that sex and season had significant effect (P<0.05) that influencd the average birth-weight. The average birth weight of male kids (1.56) was recorded to be higher than female kids (1.41) of Black Bengal goats. The highest birth weight was recorded in the summer season than rainy and winter and the highest body weight was recorded in single birth (1.61) than twin (1.40kg) birth kids.

Jat *et al*. (2020) recorded growth performance of Sirohi kids under organized farm. The average birth weight recorded was 2.67±0.051 kg for male kids and 2.46±0.41 kg for female kids. The average body weight recorded at 30, 60 and 90 days was 6.24±0.132 and 5.72±0.119, 8.91±0.229 and 7.73±0.193 and 10.41±0.245, 8.91±0.192 kg in male and female kids, respectively.

Gupta *et al*. (2021) summarized the performance of growth pattern of Sojat goat, The body weight at 3, 6, 9 and 12 months were 22.40±0.15, 29.28±0.07, 42.78±0.21 and 50.37±0.64 kg in male and the corresponding values in female were 19.50±0.14, 25.65±0.10, 30.93±0.23 and 37.45±0.15 kg, respectively.

2.6.2 Growth Performance

Growth is an early expressed trait and has a direct bearing on the age of maturity, which in turn is stated to be highly genetically and non-genetically

correlated with lifetime production and reproduction. Based on their growth, meat animals can be evaluated at an early age, which can significantly enhance/ promote/ push-up the economics of the goat industry. More overgrowth is an indication of the health and adaptability of the animal. It can be used as a useful check of the systems of feeding and management. Genetic studies on growth performance in Berari goat and other Indian goats are depicted briefly as below.

Kuralkar *et al.* (2013) studied morphometric characteristics in Berari goats and reported mean for body weight, heart girth, body length, height at wither, paunch girth, horn length, ear length and tail length as 25.7±0.5 kg, 69.5±0.6 cm, 57.0±0.8 cm, 69.6±0.7 cm, 71.0±0.9 cm, 9.3±0.4 cm, 15.3±0.4 cm and 12.3±0.3 cm, respectively in Berari goats belonging to 1–2 years of age. However, the mean values for the same traits in Berari goats above 2 years of age were observed as 31.4±0.6 kg, 73.3±0.6 cm, 63.1±0.7 cm, 71.3±0.6 cm, 76.2±0.8 cm, 9.6±0.4 cm, 15.7±0.3 cm and 13.7±0.3 cm, respectively.

Kharkar *et al*. (2014) investigated the effect of non-genetic factors on body weight and measurements (body length, height, heart girth, and punch girth) in Berari goats at birth, 3, 6, and 12 months of age. At birth, 3, 6, and 12 months of age, the least-squares means were 2.43 ±0.04, 10.60 ±0.67, 15.08 ±0.30, and 21.14 ±0.39 kg, respectively. The overall mean for height, length, heart girth, and paunch girth were 32.24, 30.09, 31.40, and 29.41 cm at birth, 46.39, 44.48, 45.59, and 48.58 cm at 3 months, 58.08, 52.23, 55.86, and 59.08 cm at 6 months, and 60.06, 57.07, 64.35, and 65.86 cm at 12 months. For bodyweight at birth and 12 months of age, the effects of birth type and sex were substantial sources of variation. Initially, the three groups' kids had identical body weights and body conformations. The birth and final body weight of G3 kids (3.06 and 8.84 kg, respectively) were considerably higher ($P<0.05$) than those of G1 and G2 kids. Results showed that the birth and final body weight (90 days) of Berari goat kids were 2.43 and 10.60 kg, respectively.

Patil *et al*. (2013) computed the overall least squares mean for bodyweight at 1,3, 6, 9, and 12 months of age were 5.09±0.03, 9.18 ±0.05, 14.05 ±0.12, 19.10± 0.26 and 22.38± 0.36 kg, respectively. The mean for body measurements

viz, chest girth, body length, and wither height for 1st month (39.18± 0.12, 35.24±0.10 and 40.27± 0.12 cm, respectively), 3rd month (48.18 ±0.13, 42.55±0.10, 48.59±0.12 cm, respectively) and at 6th month (55.51±0.22, 48.99±0.21, 55.71 ±0.26, respectively). Body measurements like chest girth and height at wither are affected by year of birth of kid. The season of birth had significant effect on chest girth. The effect of sex is dominant in male animals.

Panda *et al*. (2014) recorded 446 medium-sized indigenous sheep with an average adult body weight of 26.02±0.11 kg in males and 23.02±0.08 kg in females. Males had an average body length, height, heart girth, and punch girth of 55.52±0.18, 60.22±0.29, 68.19±0.27 and 74.43±0.45 cm, respectively, while females had 51.86±0.16, 56.60±0.24, 63.67±0.30 and 69.64±0.38 cm, respectively. Body conformation traits were found to be medium to high in the majority of these cases, with a medium to high correlation between body length and other morphometric characteristics.

Mondal *et al*. (2015) found that male Changathangi X Non-descript goats had higher body weight, height, length, and heart girth ($P<0.05$) than females. The overall mean body weights at three, six, nine, and twelve months of age were 8.35, 12.32, 16.38, and 28.51 kg, respectively. At 3 months of age, the overall mean for height, length, and heart girth was 34.31, 20.55, and 32.21cm, respectively; at 6 months of age were 46.25, 45.77, and 40.52 cm, respectively; at 9 months of age, were 55.19, 52.11, and 58.11 cm, respectively; and at 12 months of age were 57.86, 62.17, and 61.54 cm, respectively.

Karna *et al*. (2016) elaborated body morphometry of Baigani goats, The mean body weight, body length, wither height and chest girth was 26.42±2.05 kg, 58.31±2.57 cm, 65.44±1.96 cm and 66.80±2.63 cm respectively for males whereas the similar measurements for females were 22.94 ±0.67 kg, 56.01±0.97 cm, 63.44±0.75 cm and 64.59±1.05, respectively.

Waiz *et al*. (2018) analysed body weights in Sirohi, least-squares means of body weights at birth, three, six, nine and twelve months of age were 2.50 ± 0.39, 11.21 ± 0.36, 15.29 ± 0.41, 18.00 ± 0.57 and 21.86 ± 0.77 kg, respectively. The

results revealed that the effect of sex and type of kidding significantly ($p<0.01$) affected body weight and body measurements viz, heart girth, height at withers and body length at birth, three, six, nine and twelve months of age. The influence of season was highly significant ($p<0.01$) on body weight and body measurements at 6 months of age.

Sarma *et al*. (2020) examined effect of genetic and non genetic factors on growth performance in Assam hill goats, The least-squares means for body weight, height at withers, heart girth and body length were 7.557 ± 0.049 kg, 41.231 ± 0.121 cm, 44.621 ± 0.115 cm and 50.778 ± 0.172 cm at 6 months; 9.934 ± 0.044 kg, 43.902 ± 0.120 cm, 48.890 ± 0.099 cm and 55.552 ± 0.170 cm at 9 months and 12.549 ± 0.046 kg, 46.791 ± 0.112 cm, 52.765 ± 0.090 cm and 58.392 ± 0.207 cm at 12 months of age, respectively. Season of birth exerted a significant effect on body weight at 9 and 12 months; on a height at withers at 12 months and on body length at 6, 9 and 12 months of age. Significant effect of sex was observed on body weight at 9 and 12 months, on a height at withers at 6, 9 and 12 months and on heart girth and body length at 9 and 12 months of age.

CHAPTER III

MATERIALS AND METHODS

The present study was designed to investigate the association between the polymorphic genotypes of the growth hormone gene and body weight trends in Berari goats.

3.1 MATERIALS

3.1.1 Experimental Animals

The present study was carried on Berari Goat. For the study, 42 animals of different age groups were constituted as material. Animals were selected from Berari Goat and Deccani Sheep Research, Demonstration and Training Centre, Borgaon Manju, PGIVAS Akola.

3.1.2 Chemicals and Reagents

The chemicals and reagents used in the present study were of molecular biology grade and are presented in Appendix I and II

3.1.3 Plastic-wares and glasswares

Autoclaved 10 ml sterile EDTA vacutainer tubes (BD), Eppendorf tubes (0.5 ml, 1.5 ml, and 2.0 ml), tips (make Axygen Scientific) and pipettes (Eppendorf), collection tubes and Graduated Elution tubes (FavorGenPrep), PCR tubes (Eppendorf) and glasswares (Make Scott Duran) were used for the experiment.

3.1.4 Equipment

The equipment available in Molecular Genetics Laboratory of Department of Animal Genetics and Breeding, PGIVAS, Akola were used for the present study, viz. Electronic balance (Anamed), Digital pH meter (MAC), Distillation Assembly (Quartz), Micro-centrifuge (Tarson-Spinwin), Autoclave (Osworld), Vortex shaker (Tarsons), Microwave Oven (LG), Magnetic Stirrer with hot plate, Vertical Deep Freezer (Siemens), Master Cycler Pro S-PCR (Eppendorf), Refrigerated Centrifuge with the standard rotor (Eppendorf), Horizontal Gel Electrophoresis Unit (Axygen and Genco), UV Transilluminator (Bioera), etc.

The nano-photometer of Biotechnology Laboratory, Department of Plant Biotechnology, Dr. PDKV, Akola was used.

3.2. BLOOD COLLECTION AND DNA ISOLATION

3.2.1 Collection of Blood Sample From Goat

A 5 to 10 ml of venous blood was collected aseptically from the jugular vein of 32 unrelated Berari goats of different sex and age groups in sterile vacutainers coated with ethylene-diamine tetra acetic acid (EDTA) as an anticoagulant. After collection, the tubes were delicately shaken to facilitate thorough mixing of blood with the anti-coagulant. The tubes containing blood samples were transported to the laboratory in an icebox containing ice packs and kept in a deep freezer of Molecular Genetics Laboratory of Department of Animal Genetics and Breeding, PGIVAS, Akola, at a temperature of –20°C until further processing for DNA isolation.

3.2.2 Genomic DNA extraction

Genomic DNA is necessary to test the sample for molecular genetic characterization. The quality of extracted DNA has a large influence on the results of such tests. The term "quality" denotes genomic DNA having a high molecular weight (>100 kb) and being free of contaminants such as protein, RNA, organic solvents, or salt. Any biological substance, such as blood, hair follicles, tissue, or sperm, can be used to extract genomic DNA. The most preferred source for quality DNA extraction was peripheral blood of live animal. DNA was extracted from the whole blood using 'FavorGen Prep^{TM} Blood Genomic DNA Extraction Mini Kit.'

Procedure:

1. The blood samples stored at -20^{o}C were thawed to room temperature.
2. A 200 µl of whole blood was taken in an autoclaved microcentrifuge tube.
3. In each microcentrifuge tube 20 µl of proteinase K was added and vortexed for a few sec.
4. A 200 µl of FABG Buffer was added to the sample and content was mixed thoroughly by pulse-vortexing.

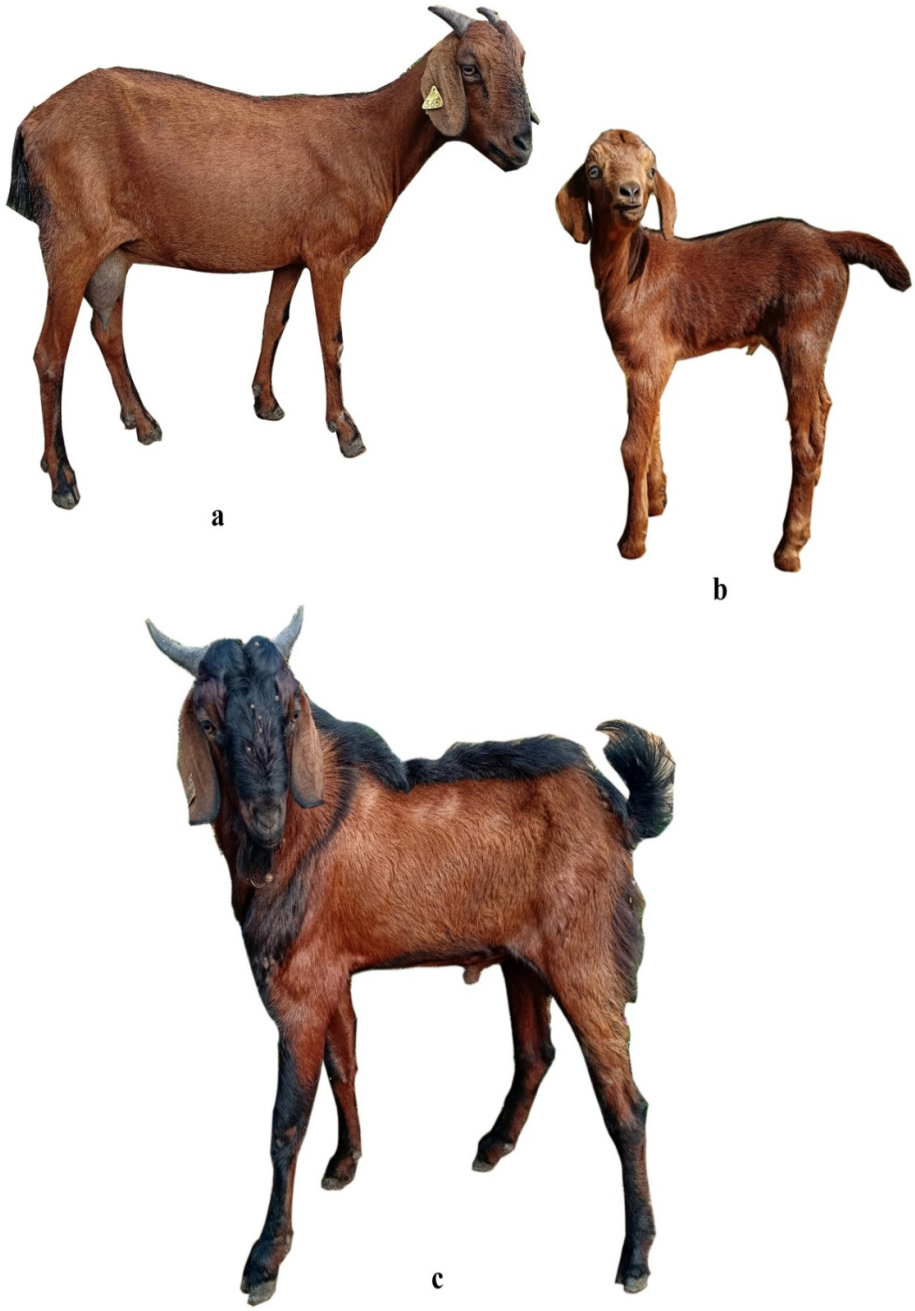

Plate 3.1 Berari goat
a) Berari female b) Berari kid c) Berari male

5. The microcentrifuge tube was incubated at 60ºC for 15 minutes in a preheated water bath to Iyse the sample. During incubation, microcentrifuge tubes were vortexed after every 5 minutes.
6. The tubes were briefly spun to remove drops from the inside of the lid.
7. A volume of 200 µl of ethanol (99 %) was added to the microcentrifuge tube, followed by mixing thoroughly by pulse-vortexing for 10 sec.
8. The tubes were briefly spun to remove drops from the inside of the lid.
9. A well labeled FABG Mini Columns were Placed on a Collection Tube. Then the mixture was transferred carefully to the respective FABG Mini Column. Tubes were centrifuged at 6,000 x g for 1 min then FABG Mini Column was placed to a new Collection Tube.
10. A 400 µl of W1 Buffer was added to the FABG Mini Column and centrifuged at full speed for 30 sec then flow-through in collection tubes was discarded.
11. Then 750 µl Wash Buffer was added to the FABG Mini Column and centrifuged at full speed for 30 sec then flow-through in collection tubes was discarded.
12. The tubes were Centrifuged at full speed for an additional 3 minutes to dry the column.
13. The FABG mini-column was then placed in the previously labeled elution tubes.
14. A volume of 50 µl preheated (at 65°C) elution Buffer was added to the membrane center of the FABG mini-column. And FABG mini-columns were allowed to stand for 3 minutes.
15. Tubes were centrifuged at full speed for 1 minute to elute total DNA.
16. Elution tubes containing extracted DNA were Stored at 4 ℃ or -20 ℃.

3.2.3 Qualitative assessment of DNA samples

After the isolation of DNA, a quality evaluation of DNA samples was carried using agarose gel electrophoresis, steps for quality evaluation are as follows:

1. The gel casting tray was cleaned with spirit and properly placed in the gel casting assembly.

2. A Puragene^{TM} Agarose powder 1% for 40 ml TAE buffer i.e 0.4 gm of agarose powder measured by electric digital balance was taken.
3. The 40 ml of 1% TAE buffer (PH 8.5) was taken in a conical flask, then weighted agarose powder was poured in a conical flask.
4. The conical flask containing buffer and agarose gently shaken and then solution is melted in microwave oven to obtain a colorless homogenous solution.
5. The Conical flask was cooled to a warmer state, then ethidium bromide (1%) @ 5 µl /100 ml of TAE buffer i.e. 2 µl was added to the solution and mixed gently.
6. The solution was slowly poured into the gel casting tray and let to solidify for 40/45 min.
7. After the solidification of gel, comb was removed carefully and casting tray is submerged in an electrophoresis unit containing 1X TAE buffer, placed in such a way that the well was nearer to the cathode.
8. The buffer should be 5-10 mm above the gel.
9. Then on the paraffin sheet, 7 µl DNA sample and 1 µl of 6X DNA loading dye was mixed with help of 10 µl micropipette, the mixture was loaded in a individual well.
10. Gel glectrophoresis was carried at a voltage of 120 for 10 min and then at 100 volts for 30 min.
11. After completion of the gel run, the gel was observed under a UV transilluminator and analyzed.

3.2.4 Estimation of DNA concentration and purity using nanophotometer

1. The Quantitative analysis of DNA (concentration) was done by the NanoDrop method, nanophotometrically. The ratio between OD-260 and OD-280 was used to evaluate DNA samples by using the formula:

DNA concentration (µg/ml) = OD-260 x Dilution factor x 50.

2. A unit of measurement of DNA concentration is a nanogram/ microlitre. The blank was set using elution buffer.

3. The DNA samples were vortexed for few seconds, then 1 µl of DNA sample at a time placed on measuring slot of nanophotometer, the obtained results pertaining to OD ratio and DNA concentrations were noted carefully.

4. Samples OD ratio ranging between 1.7-2.0 and concentrations above 100 are used for PCR amplification.

3.3 PRIMERS

3.3.1 Primer sequences used for PCR

The primers were synthesized from Eurofins Genomics India Pvt. Ltd., Bangalore. A forward primer having 18 bp and reverse primer 19 bp in length (Hua *et al*., 2009), used in PCR amplification to amplify exon 2 and exon 3 region of the growth hormone gene.

Table 3.1 Forward and reverse primer sequences

Primer name	Primer sequence (5'-3')		Product size	Reference
GH1	Forward	5'-CTCTGCCTGCCCTGGACT-3'	422	Hua *et al.* (2009)
	Reverse	5'-GGAGAAGCAGAAGGCAACC-3'		

3.3.2 Primer dilution

Primers supplied in freeze-dried powder form were reconstituted in nuclease-free water in a prescribed format to create 100 pmoles/µl of stock solution and further diluted to give a final concentration of (20 pmoles/µl) that were used as working solution.

Table 3.2 Dilution of Primers for Stock and Working Solution

Gene	Sequence	Stock Solution		Working Solution	
		Primer in moles	Nuclease Free Water	Primer (Stock solution)	Nuclease free water
GH1	Forward	48.80 nmol	487.50 µl	10µl	50µl
	Reverse	35.30 nmol	353 µl	10µl	50µl

3.4 PCR AMPLIFICATION OF REGION OF EXON 2 AND 3 OF GROWTH HORMONE GENE LOCUS

The growth hormone genes in the goat code for Growth hormone and it is composed of 5 exons and 4 introns. The target locus was a 422 bp DNA segment which is in between the exon 2 and exon 3 region of GH gene.

3.4.1 PCR composition for amplification

Table 3.3 PCR composition

Pcr component	Final concentration	1X	32X
Dream taq Green Master mix(2X)	10 µl	10 µl	320 µl
Forward primer (20pmole/ µl)	20pmol/ µl	0.8 µl	25.6 µl
Reverse primer (20pmol/ µl)	20pmol/ µl	0.8 µl	25.6µl
Template DNA	30 nanogram/ µl	3.0 µl	96 µl
Nuclease Free Water		5.4 µl	172.8 µl
Total		**20 µl**	**640 µl**

Table 3.4 PCR protocol for GH1 region of Growth Hormone gene

Steps	Temperature	Duration	Cycle	Reference
Initial denaturation	94^0C	5 minutes	1	Bayan *et al.* (2018a)
Denaturation	95^0C	30 seconds	11	
Annealing temperature	65^0C (-1^0C per cycle)	30 seconds		
Extension	72^0C	30 seconds		
Denaturation	95^0C	30 seconds	24	
Constant Annealing	54^0C	30 seconds		
Extension	72^0C	30 seconds		
Final Extension	72^0C	10 minutes	1	

For the PCR amplification a final reaction volume of 20 µl consisting of 10 µl of 2X PCR dreamtaq green master mix, 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 were taken in autoclaved PCR tubes. Proper cooling was maintained by placing

ice packs in the tray. After adding all components in PCR tubes, tubes were gently tapped and spun (Tarson) @1000 rpm for 40 sec. The tubes were then placed in a thermal cycler (Eppendorf) for amplification.

The PCR was carried out using the protocol mentioned in Table no. 3.4 to amplify a 422bp fragment of the exon 2 and exon 3 region of GH gene.

3.4.2 Conformation and quality check of PCR Product by agarose gel electrophoresis

The quality check of PCR amplicons was confirmed by using the agarose gel electrophoresis technique. An amplified segment is confirmed by running mixture from PCR tubes on an agarose gel. A 1.5%, agarose gel was prepared by adding 0.6 gm of agarose powder in 40 ml 1X TAE buffer. The 5 µl ethidium bromide was incorporated to 40 ml agarose gel solution. The solution is melted in microwave oven then allowed to solidify.

The 7 wells in agarose gel were filled with PCR amplified product against molecular marker of 100 bp size (DNA ladder) in one well. The gel was run in 1% TAE buffer at a voltage of 80 for 75 minutes. Then the bands were visualized under UV light in a UV transilluminator.

3.5 RESTRICTION FRAGMENT LENGTH POLYMORPHISM

The PCR amplified products were subjected to restriction endonuclease digestion. The digestion was carried out using fast-digesting *HaeIII (Haemophilus aegipticus)* at 37^{o}C for 15 min and the reaction composition was as follows. The restriction sequence for *HaeIII* was 5'GGCC and 3'CCGG. The cut site was 5'---GG/ CC---3'and 3'---CC/GG---5'.

3.5.1 RFLP reaction composition

Table 3.5 Restriction endonuclease digestion composition

Reagent	Quantity
PCR amplified product	8µl
Nuclease free water	5µl
10 X Buffer	1.5µl
HaeIII Restriction endonuclease	0.5µl
Total	**15µl**

3.5.2 Agarose gel electrophoresis of RFLP product

The restriction digested products were analyzed by agarose gel electrophoresis. A 9 µl of digested products along with 1 µl of DNA loading dye was loaded in wells of a 2 % agarose gel in electrophoresis tank. The samples were ran against a standard DNA ladder (100 bp size) and the electrophoresis was carried out at 80 V for 75 min. The gel was then visualized in UV-light using UV-Transilluminator and genotyping was carried out.

3.5.3 Recording of the PCR-RFLP fragments and Scoring of alleles

The genotyping was performed after agarose gel electrophorosis of the restriction-digested PCR fragments with *HaeIII*. The standard 100 bp DNA ladder was used as a marker against which the alleles of each sample were assigned scores and recorded for further comparison. After the scoring of bands, gene and genotypic frequencies were evaluated.

3.6 RECORDING OF MORPHOMETRIC TRAITS

3.6.1 Source of Data on Measurement of Body Weight for association studies.

Data of birth weights of Berari goats recorded from Berari Goat and Deccani Sheep Research, Demonstration and Training Centre, Borgaon Manju, PGIVAS Akola during September 2020 to November 2021.

Data for association studies include:

A) Birth weights (weight of an animal at birth)
B) Body weights (weight of animals during 1, 3, 6, 9, and 12 months)

Were measured using an electronic weighing scale (BPL Engineers) and recorded in kilograms.

3.6.2 Classification of data

The whole data were classified according to sex, type of birth, and season. Codes for data were given in table 3.6.

a) Sex

Sex was divided into two subgroups as male and females. Sex of kid is one of the important factors which may have certain influence on body weights of Berari goats.

b) Type of birth

The type of kidding was classified as single born and twin born kids.

c) Season

A year is classified into 3 season's as summer, rainy, winter based on climatic condition. Season of birth of kid is one of important environmental factor that may have influence on body weights of Berari goats.

Table 3.6 Collection of data

Sr. no.	Effect	Particular	Code	Number of animals
1	Sex	Male	M1	22
		Female	M2	20
2	Type of birth	Single	T1	16
		Twin	T2	26
3	Season	Summer (February-may)	S1	20
		Rainy (June-September)	S2	10
		Winter (October-January)	S3	12

3.6.3 Data analysis of morphometric traits

The data on birth weights and body weights during the different intervals were analyzed for fixed effects.

The data about body weights during different intervals were subjected to an analysis software package of LSML (Harvey, 1990).

The general linear model used was indicated as follows:

$$Y_{ijk} = \mu + P_i + S_j + A_k + e_{ijk}$$

Where,

Y_{ijk} = Body weight of animal of i^{th} Sex, j^{th} Type of birth and k^{th} Season

μ = overall mean

P_i = fixed effect of i^{th} sex (i= 1, 2)

S_j = fixed effect of j^{th} type of birth (j=1, 2)

A_k = fixed effect of k^{th} season (k=1, 2, 3)

e_{ijk} = random error associated with Y_{ijk} weight character corresponding to and is assumed to be independently and normally distributed about mean zero and with variance "e".

CHAPTER IV

RESULTS AND DISCUSSION

Under the present study, the birth weight and body weights of 42 goats were recorded at regular different intervals from birth to 12 months of age during the period from September 2020 to November 2021. The data on body weight were analyzed using Least-squares analysis of variance technique (Harvey, 1990).

For the molecular examination of Growth Hormone gene, the blood samples were collected from 32 unrelated Berari goats from Berari Goat and Deccani sheep Research Training and Demonstration Centre, Borgaon Manju, Akola. DNA were extracted from the whole blood sample using 'FavorGen Prep^{TM} Blood Genomic DNA Extraction Mini Kit, Then amplified using reported primers for 422 bp size of the Growth hormone (GH) gene and the amplified fragments were enzymatically digested with *HaeIII* endonucleases. The resultant fragments were devised for examining the genotypes based on restriction fragments size in the studied samples.

4.1 GENETIC POLYMORPHISM OF GH GENE IN BERARI GOATS

4.1.1 Extraction of genomic DNA

Whole blood samples of unrelated 32 Berari goats were collected aseptically and were stored at -20^{o}C in the Molecular Genetics Laboratory of Department of Animal Genetics and Breeding, PGIVAS, Akola.

Extraction of genomic DNA using 'Favor Prep TM Blood Genomic DNA Extraction Mini Kit' was carried out as per the working protocol manual provided by the manufacturers.

4.1.2 Qualitative and quantitative evaluation of extracted DNA

The qualitative assessment and purity of isolated DNA were checked by running it on 1 percent agarose gel at 100 V for 30 minutes. (Plate no. 4.1).

Table 4.1 DNA concentration and OD (260/280 nm) ratio of DNA in Berari Goat

Sr. No.	Sample ID	DNA concentration	OD (260/280) ratio
1	130	250	1.81
2	132	364	1.75
3	134	275	1.76
4	135	220	1.79
5	137	260	1.81
6	138	210	1.83
7	139	310	1.81
8	140	231	1.77
9	142	196	1.78
10	143	250	1.79
11	144	266	1.81
12	145	195	1.81
13	146	234	1.80
14	252	222	1.80
15	253	162	1.77
16	254	134	1.72
17	256	267	1.96
18	258	158	1.90
19	259	150	1.85
20	261	163	1.79
21	262	153	1.89
22	263	274	1.81
23	264	111	1.80
24	265	178	1.93
25	266	241	1.86
26	267	171	1.79
27	268	259	1.80
28	269	267	1.85
29	270	102	1.78
30	271	108	1.87
31	272	117	1.89
32	273	183	1.78

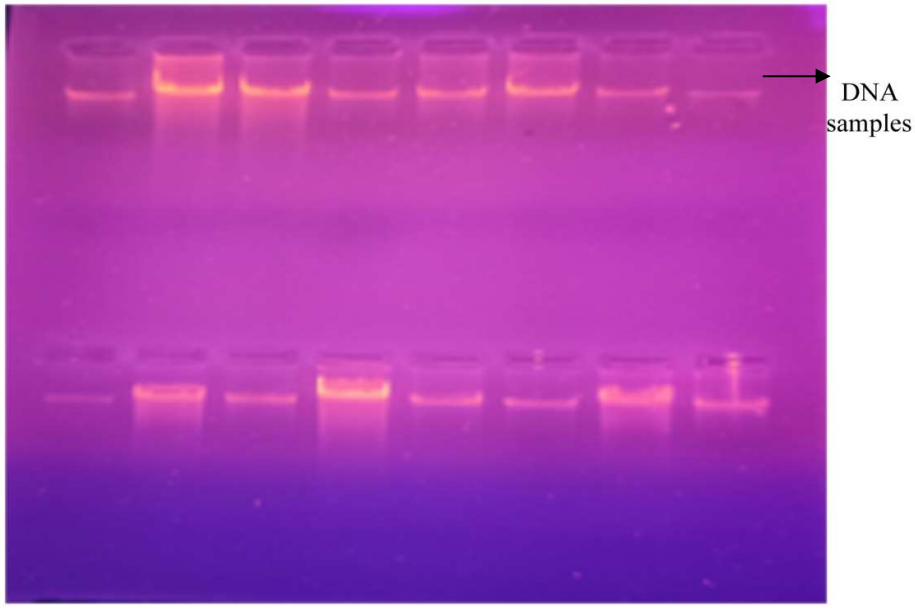

Plate 4.1 Quality assessment DNA samples of Berari goat on Agarose gel

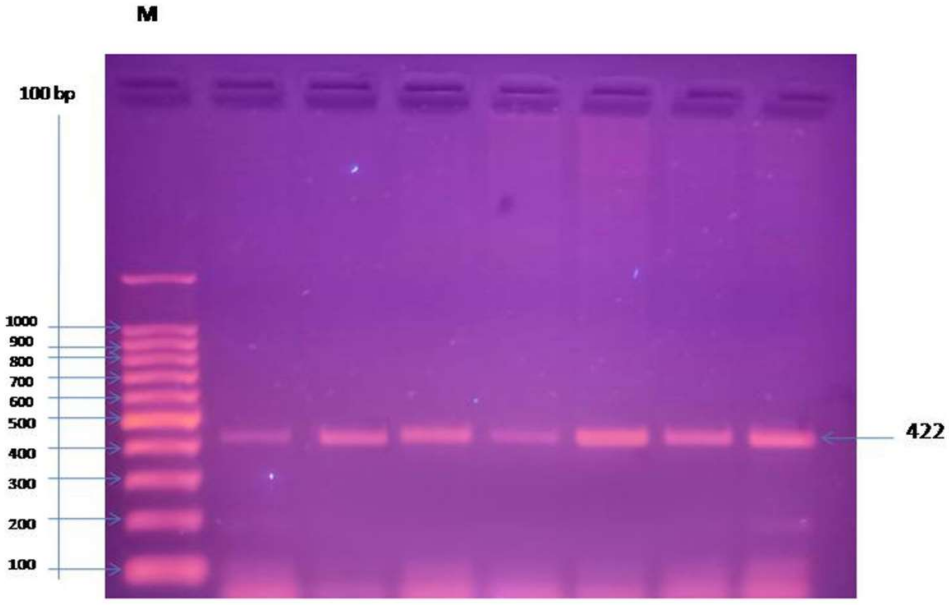

Plate 4.2 Quality check of PCR amplified products on Agarose gel

The isolated DNA samples were quantified and evaluated under Nanophotometer. Those samples yielded DNA from 102 to 364 $^{\mu g}/_{ml}$ with the OD (260/280) ratio ranging from 1.72 to 1.93 indicating purity of DNA samples were selected for PCR amplification. The concentration and OD ratios of samples 32 DNA samples from Berari goat were depicted in Table 4.1.

4.1.3 PCR amplification of genomic DNA

To amplify the 422 bp region of GH gene, Forward primer- 5' CTCTGCCTGCCCTGGACT 3' and Reverse primer- 5' GGAGAAGCAGAAGGCAACC 3' as per Hua *et al*. (2009) and Bayan *et al.* (2018a) were selected for the present research.

Many studies on the GH gene (422 bp size) have been reported in various goats viz. in Boer bucks (Hua *et al*., 2009), Egyptian goats (Alakilli *et al*., 2012), Savanna and Kalahari goats (Marini *et al*., 2012), Sirohi and Barbari goats (Singh *et al*., 2015), Egyptian goats (Othman *et al*., 2015), Kacang goats (Ilham *et al*., 2016), Malabari and Atapaddy Black goats (Radhika *et al*., 2016), Raini cashmere goat (Gooki *et al*., 2018), Barki, Zaraibi and Damascus goats (Maharous *et al*., 2018), Anglo-Nubian Goats (Moneva *et al*., 2020), Gaddi goats (Gitanjali *et al*., 2020), Surati goats (Pandya *et al*., 2021), Ganjam goats (Aradhana *et al*., 2021).

Simlilarly, Kumari *et al*. (2014), Seevagan *et al.* (2015), Othman *et al*. (2015), Alsaedi *et al*. (2021) reported amplification of growth hormone gene region in various sheep breeds.

4.1.4 Optimization of PCR protocols

Several PCR protocols were practiced initially to obtain satisfactory yield PCR product of size 422 base pairs. The protocol mentioned in table 4.2 worked out successfully to amplify the target gene segment of the desired length of 422 base pairs without any unspecific products (Bayan *et al.,* 2018a).

A PCR amplified products were checked by running on 1.5 % agarose gel against a standard 100bp DNA ladder and visualized (Plate 4.2) under UV Trans-illuminator. For all the amplified samples prominent bands of 422 bp were observed.

Table 4.2 PCR protocol for GH1 gene

Steps	Temperature	Duration	Cycle
Initial denaturation	94^0C	5 minutes	1
Denaturation	95^0C	30 seconds	11
Annealing temperature	65^0C (-1^0C per cycle)	30 seconds	
Extension	72 ^{0}C	30 seconds	
Denaturation	95^0C	30 seconds	24
Constant Annealing	54^0C	30 seconds	
Extension	72^0C	30 seconds	
Final Extension	72^0C	10 minutes	1

4.1.5 RFLP of PCR amplicons

PCR amplicons of GH1 gene (422 bp) were subjected to restriction endonuclease i.e. fast digest *HaeIII* and digested at 37^{o}C for 15 minutes.

To examine the polymorphism, the enzymatically digested products were electrophoresed in 2% agarose gel against a standard 100 bp DNA ladder, then digested products visualized and photographed for genotyping. The results of PCR-RFLP analysis are depicted in Plates 4.3 to 4.7 for Berari goat.

4.1.6 Genotyping of Berari goat for the GH1 locus

The RFLP of PCR product with *HaeIII* leads to formation of fragments of GH1 gene. The cut in target segment of 422 bp led to the production of fragments of 366 and 56 bp size showing one restriction site was scored A type. The sample variant with no restriction site was scored as B type.

In the present study all 32 samples of Berari goat on PCR-RFLP digestion resulted in fragmentation of the 422 bp PCR product into the 422, 366 & 56 bp bands which were noted as heterozygous AB genotype, showing the presence of restriction site on one allele and absence of restriction site on other allele. The homozygous genotype AA (366 and 56 bp) & BB (422bp) were not found in the present study. Sample-wise allotment of genotypes is tabulated in Table 4.5.

The use of growth hormone gene as a marker for selection had been widely used in several livestock, in cattle (Beauchemin *et al*., 2006; Thomas *et al*.,

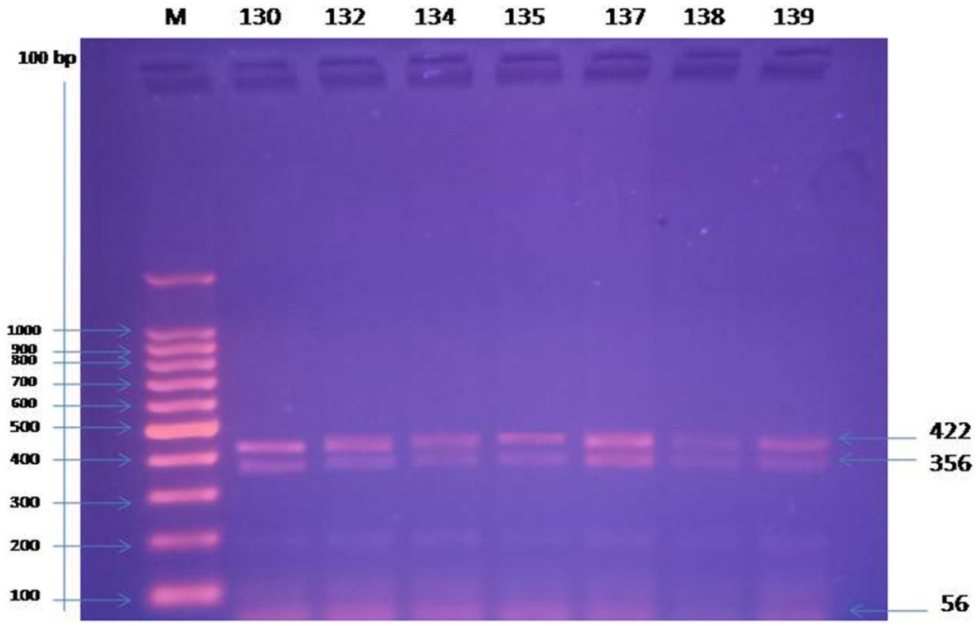

Plate 4.3 PCR-RFLP products of GH1 gene digested with *HaeIII*
(Samples 130, 132, 134, 135, 137, 138, 139)

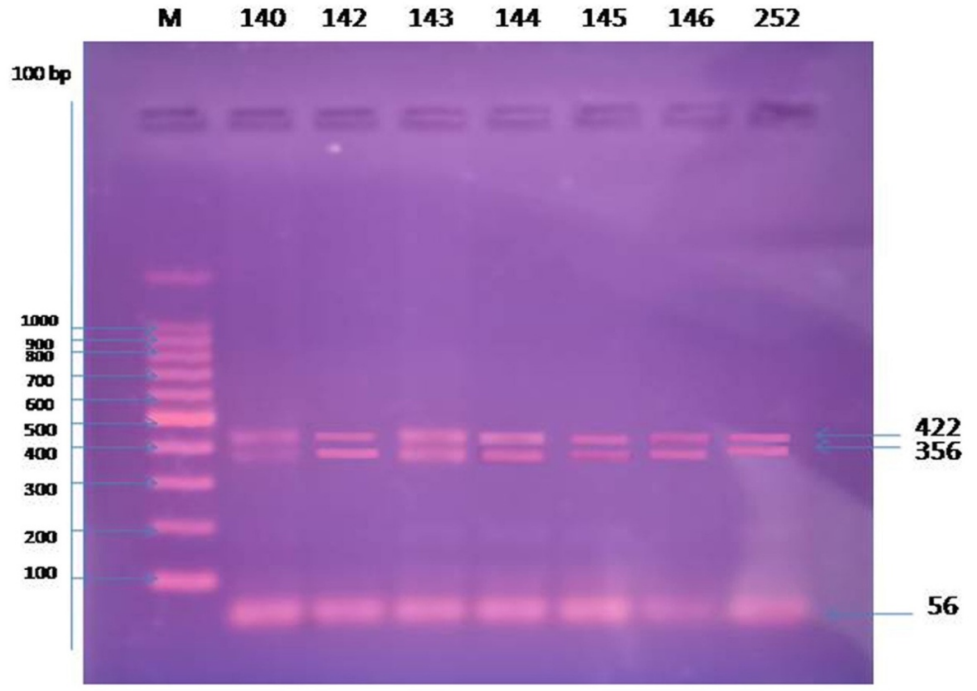

Plate 4.4 PCR-RFLP products of GH1 Gene digested with *HaeIII*
(Samples 140, 142, 143, 144, 145, 146, 252)

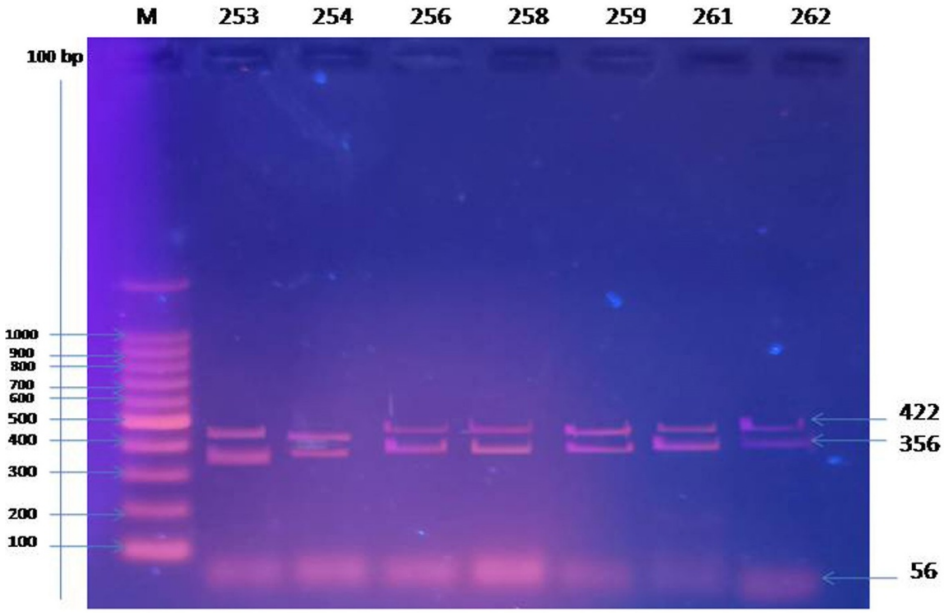

Plate 4.5 PCR-RFLP of GH1 gene digested with *HaeIII*
(Samples 253, 254, 256, 258, 259, 261, 262)

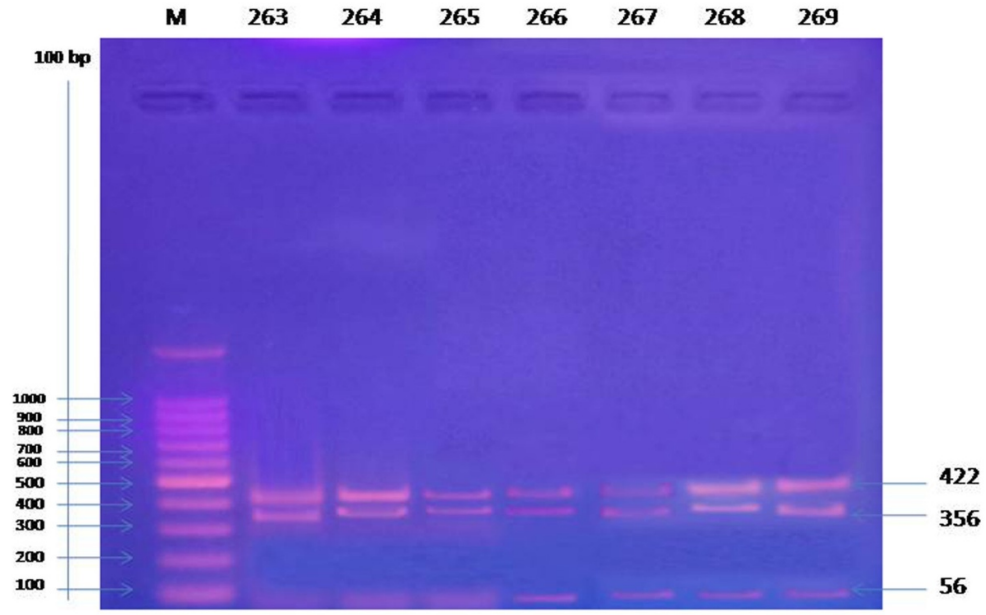

Plate 4.6 PCR-RFLP of GH1 gene digested with *HaeIII*
(Samples 263, 264, 265, 266, 267, 268, 269)

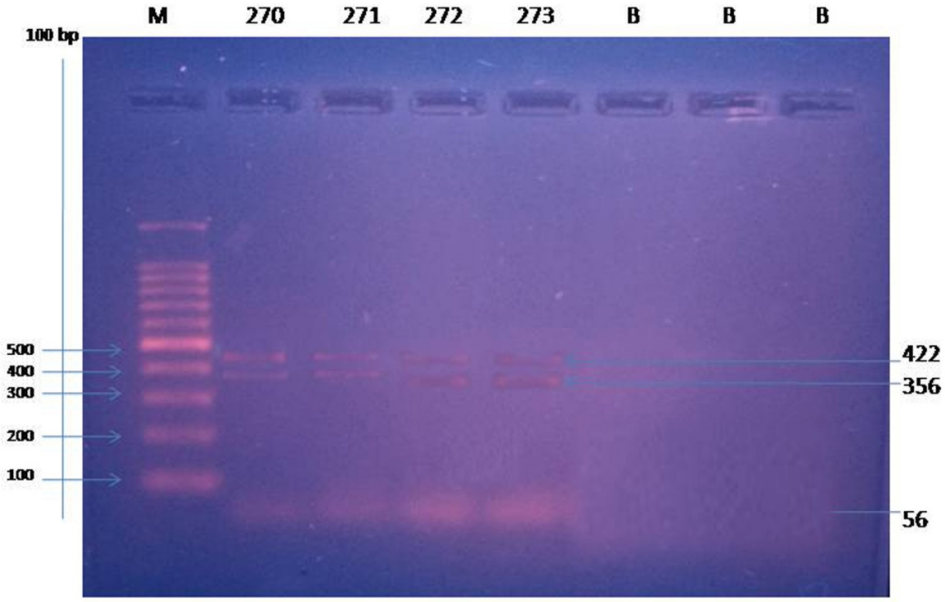

Plate 4.7 PCR-RFLP of GH1 gene digested with *HaeIII*
(Samples 270, 271, 272, 273, Blank, Blank, Blank)

2007; Katoh *et al*., 2008), sheep (Marques *et al*., 2003) and goat (Malveiro *et al*., 2001; Boutinaud *et al*., 2003; Chitra and Aravindaksan, 2004; Hua *et al*., 2009;).

Table 4.3 Gene and Genotype Frequencies

Berari Goat N (32)	Gene frequency		Genotypic frequency		
	A	B	AA	AB	BB
	0.50	0.50	00	1.00	0

This is maiden attempt to see the effect of growth hormone gene polymorphism on body weight in Berari goat. In the present study, the observed genotypes were of AA, AB and BB are 0, 100% and 0 respectively. So samples studied were found to be monomorphic. Similar finding of monomorphic pattern was reported by Othman *et al*. (2015) in Baladi goat were bands of (422, 366 and 56 bp) and genotype was considered as AG in contrast to AB genotype of present study on Berari goats.

The present results are not in agreement with the results of polymorphism reported by Hua *et al.* (2009) in Boer bucks, Zhang *et al*. (2011) in Matou and Boer breeds of goat, Marini *et al*. (2012) in Savanna and Kalahari goats, Singh *et al*. (2015) in Sirohi and Barbari goats, Marini *et al*. (2015) in Savanna and Kalahari goats, Bayan *et al*. (2018a) in Surati and Mehsani goats, Gooki *et al*. (2018) in Raini Cashmere goat, Moneva *et al*. (2020) in Anglo-Nubian goats, Maharous *et al*. (2018) in Egyptian goat breeds, and Pandya *et al*. (2021) in Surati goats, and Othman *et al*. (2015) in Egyptian goats as they reported polymorphic pattern with two genotypes.

The polymorphism with three possible genotypes was reported by Alakilli *et al*. (2012) in four Egyptian and Saudi goat breeds (Barki, Zaribi, Ardi and Masri), Radhika *et al*. (2016) in Malabari and Atapaddy Black goats and by Aradhana *et al*. (2021) in Ganjam goats was in disagreement with monomorphic pattern revealed in present investigation for GH1 gene in Berari goats.

Table 4.4 Genotyping of Berari Goat for GH1 gene (422bp)

Sr. No.	Sample ID	RFLP fragment (s)	Genotype
1	130	422, 366 & 56	AB
2	132	422, 366 & 56	AB
3	134	422, 366 & 56	AB
4	135	422, 366 & 56	AB
5	137	422, 366 & 56	AB
6	138	422, 366 & 56	AB
7	139	422, 366 & 56	AB
8	140	422, 366 & 56	AB
9	142	422, 366 & 56	AB
10	143	422, 366 & 56	AB
11	144	422, 366 & 56	AB
12	145	422, 366 & 56	AB
13	146	422, 366 & 56	AB
14	252	422, 366 & 56	AB
15	253	422, 366 & 56	AB
16	254	422, 366 & 56	AB
17	256	422, 366 & 56	AB
18	258	422, 366 & 56	AB
19	259	422, 366 & 56	AB
20	261	422, 366 & 56	AB
21	262	422, 366 & 56	AB
22	263	422, 366 & 56	AB
23	264	422, 366 & 56	AB
24	265	422, 366 & 56	AB
25	266	422, 366 & 56	AB
26	267	422, 366 & 56	AB
27	268	422, 366 & 56	AB
28	269	422, 366 & 56	AB
29	270	422, 366 & 56	AB
30	271	422, 366 & 56	AB
31	272	422, 366 & 56	AB
32	273	422, 366 & 56	AB

4.2 GROWTH PERFORMANCE IN BERARI GOATS

The growth performance trend of body weight (kg) in Berari goats were recorded at birth, 1, 2, 3, 6, 9, 12 month of age in male and female animals using electric weighing balance for 42 goats under study. The overall least-squares means (LSM) and standard errors (SE) for birth weight and body weights at various age stages are presented in Table 4.5. The observed growth performance of Berari goats at different stage of age are depicted in fig 4.5.

The least-squares mean for overall body weight (kg) at birth, 1 month, 2 month, 3 month, 6 month, 9 month and 12 month of age in Berari goat were recorded as 2.24±0.05, 5.75±0.23, 8.94±0.28, 11.65±0.29, 18.58±0.43, 24.18±0.85 and 26.82±0.88 kg, respectively. The corresponding body weights in males were 2.32±0.07, 5.93±0.29, 9.46±0.36, 12.19±0.37, 20.35±0.56, 25.21±0.87 and 28.32±0.77 kg, respectively and in females 2.12±0.08, 5.57±0.34, 8.42±0.42, 11.10±0.43, 16.82 ±0.65, 21.35±1.25 and 24.03±1.01 kg, respectively (Table 4.5). The male kids were heavier than female kids at the corresponding stage of age.

The present findings of body weight at birth in Berari goat were in close agreement with the earlier reports of birth weight of 2.20 kg reported by Pujari *et al*. (2004), however, the higher birth weight of 2.41 kg was observed by Kharkar *et al*. (2014).

The higher birth weight estimates than present study were reported by Patel *et al.* (2005) in Jamunapuri and Marwari goats, Singh *et al.* (2006) in Beetal goat, Rao *et al*. (2009) in Ganjam goats, Thiruvenkadan *et al*., (2009) in Tellichery goat, Kumar *et al*. (2010) in Sirohi goats, Sundaram *et al*. (2012) and Murali *et al*. (2014) in Tellicherry goats, Gupta *et al*. (2016) in Mehsana goats, Dudhe *et al*. (2015) and Jat *et al.* (2020) in Sirohi kids.

The lower births weights than present study were reported by Mandakmale *et al.* (2009b) in Sangamneri goat, Gaikwad *et al.* (2009) and Birari *et al*. (2012) in Osmanabadi goats, Hassan *et al*. (2010) in Jamunapari goat, Khadda *et al.* (2017) in Pantja goats, Sahu *et al*. (2020) in Black Bengal goats.

The results of present study revealed higher estimates of least-squares mean for overall body weight at 3 months (11.65±0.29), 6 months (18.58± 0.43), 9 months (24.18±0.85) and 12 months (26.82±0.88) kg, than that reported by Kharkar *et al*. (2014), viz. 8.92 kg, 15.02 kg, 18.14 kg and 21.32 kg, respectively. Higher estimates of body weights were reported in present study then the findings of Pujari *et al*. (2004) and Kuralkar *et al*. (2013) for body weight at 12 month of age in Berari goat.

The difference in body weight of Berari goats might be due to improvement of Berari goat over a period of time and also as data for the present study was collected from organized farm.

The lower body weight estimates were reported by Patil *et al*. (2013), Mandakmale *et al.* (2009b) in Sangamneri goats at 1, 3, 6, 9 and 12 months age than the present study. However, lower body weight estimate at 3, 6, 9, 12 month of age were reported by Kumar *et al*. (2010) in Sirohi goats, Murali *et al.* (2014) in Tellicherry goats, Khadda *et al*. (2017) in Pantja goats, Selvam *et al*. (2021) in Native goats of Tamilnadu.

The higher body weight estimates than present results for birth, 3, 6, 9, 12 months body weight was reported by Gupta *et al*. (2021) in Sojat goats. The body weights at different stages in Berari goats confirm that Berari is medium sized goat breed.

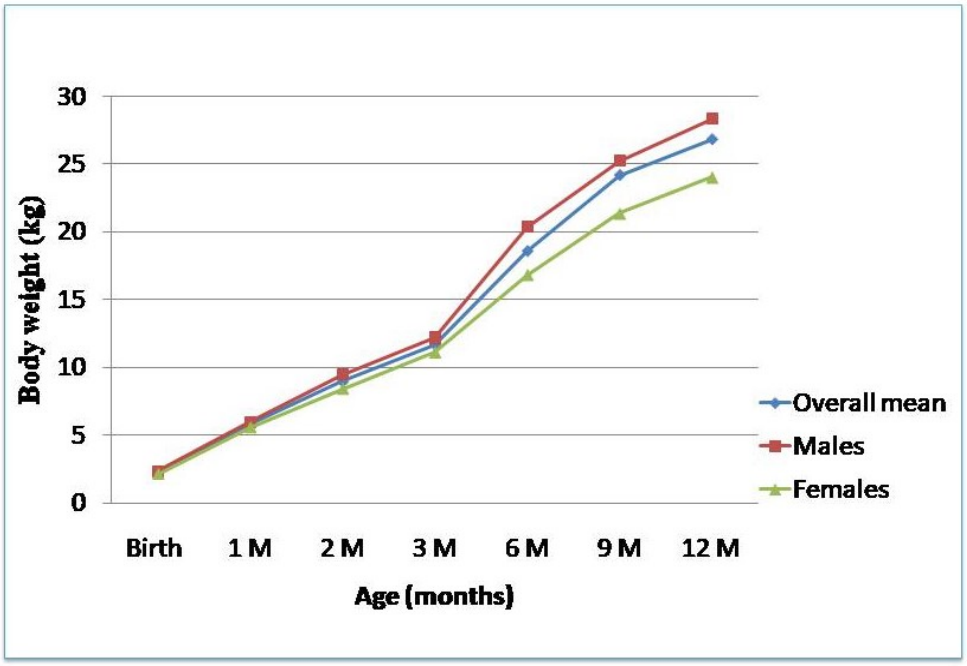

Figure 4.1 Growth curve indicating effect of sex on body weights.

Table 4.5 Least squares mean (± SE) for body weight (kg) at different stages of age in Berari goats

Source of Variation		N	Birth	15 Days	1month (30 days)	45 Days	2 month (60 Days)	75 Days	3 months (90 Days)	6 Months	N	9 Months	N	12 Months
	Overall Mean	42	2.24±0.05	4.33±0.15	5.75±0.23	7.51±0.25	8.94±0.28	10.37±0.30	11.65±0.29	18.58± 0.43	19	24.18±0.85	11	26.82±0.88
Sex	**Male**	22	2.32±0.07	4.54±0.19	5.93±0.29	7.79±0.32	9.46±0.36	10.91±0.38	12.19±0.37	20.35±0.56	13	25.21±0.87	7	28.32±0.77
	Female	20	2.12±0.08	4.12±0.23	5.57±0.34	7.23±0.37	8.42±0.42	9.82± 0.45	11.10±0.43	16.82 ±0.65	6	21.35±1.25	4	24.03±1.01
			NS	NS	NS	NS	NS	NS	NS	**		*		*
Type of Birth	**Single**	16	2.21±0.83	4.51±0.25	6.17±0.37	8.02±0.40	9.29±0.45	10.60±0.48	11.97± 0.46	18.59±0.70	8	22.56±1.16	5	25.16±1.03
	Twin	26	2.28±0.64	4.15±0.19	5.33±0.28	7.00±0.31	8.60±0.35	10.13±0.37	11.32± 0.36	18.58±0.54	11	24.00±1.00	6	27.18±1.00
			NS	NS	NS	NS	NS	NS	NS	NS		NS		NS
Season	**Summer**	20	2.34±0.07	4.76±0.21	6.05±0.31	7.41±0.33	8.37±0.38	9.64 ±0.41[a]	10.83 ±0.39[a]	16.62[a] ±0.59		-		-
	Rainy	10	2.19±0.10	4.26±0.30	5.69±0.44	7.72±0.48	9.27±0.54	9.90 ±0.58[a]	10.99 ±0.55[a]	19.41[b] ±0.84	10	24.77±1.03	5	26.51±1.03
	Winter	12	2.20± 0.10	3.98± 0.29	5.51± 0.43	7.40± 0.46	9.18± 0.52	11.57 ±0.56[b]	13.12 ±0.53[b]	19.72[b] ±0.81	9	21.79±1.12	6	25.83±1.00
			NS	NS	NS	NS	NS	*	**	**		NS		NS

*N- Number, * - Significant (P<0.05), ** - Significant (P<0.01), NS-Non –significant, Means with different superscript within the columns differ significantly with each other.*

Table 4.6 Least squares analysis of variance for body weight at different stages of age in Berari goats

Source of variation	df	Mean sum of sqaures										
		Birth	15 days	30 days	45 days	60 days	75 days	90 days	6 months	df	9 months	df	12 months
Sex	1	0.21^{NS}	1.74^{NS}	1.17^{NS}	2.89^{NS}	10.34^{NS}	11.26^{NS}	11.27^{NS}	118.31**	1	60.05*	1	46.67*
Type of birth	1	0.46^{NS}	1.06^{NS}	6.03^{NS}	8.66^{NS}	3.97^{NS}	1.82^{NS}	3.54^{NS}	0.0028^{NS}	1	8.09^{NS}	1	6.62^{NS}
Season	2	0.11^{NS}	2.36^{NS}	1.14^{NS}	0.34^{NS}	3.71^{NS}	27.69*	19.81**	45.04**	1	35.74^{NS}	1	0.76^{NS}
Error	37	0.94^{NS}	0.86^{NS}	1.87^{NS}	2.18^{NS}	2.79^{NS}	118.84^{NS}	2.93^{NS}	6.73^{NS}	15	9.33^{NS}	7	4.11^{NS}

** - Significant (P<0.05), ** - Significant (P<0.01), NS-Non –significant, df- Degrees of freedom*

4.2.1 EFFECT OF NON GENETIC FACTORS ON BODY WEIGHTS IN BERARI GOAT

Various non-genetic factors act as a source of variation for the body weights at different age intervals in Berari goats. In the present study sex, type of birth and season were considered as a source of variation. The results associated with non genetic factors affecting body weights were depicted in Table 4.6.

a) Effect of sex

The results of present study revealed that sex of Berari kids as non-significant source of variation for pre-weaning body weights (at birth, 1, 2 and 3 months of age). However, sex as significant source of variation in post weaning body weight i.e. at 6, 9, 12 month of age.

The body weight estimates of Berari male kids were significantly higher at 6, 9 and 12 months of age than that of females. The results of present investigation revealed that highly significant ($P<0.01$) difference in body weight due to sex was observed at 6 month of age (males: 20.35±0.56, females: 16.82±0.65), and significant ($P<0.05$) difference at 9 month (males: 25.21±0.87, females: 16.82±0.65) & 12 months (males: 28.32±0.77, females: 24.03±1.01) of age.

Findings of Selvam *et al.* (2021) was in close agreement with present results, as they observed sex as non-significant source of variation at for birth, 1 and 3 months of age, however, reported sex as significant ($P<0.01$) source of variation at 6, 9 and 12 month of age in Native goat breeds of Tamilnadu.

The significant effect of sex on body weight at 6 month of age was in agreement with findings of Thiruvenkandan *et al*. (2009) and Murali *et al*. (2014) in Tellichery goat, Patil *et al*. (2013) in Sangamneri goats, Tyagi *et al.* (2015) in Surati goats, Gupta *et al* (2016) in Mehasana, Waiz *et al.* (2018) in Sirohi, Khadda *et al*. (2017) in Pantja goats, similarly, significant effect of sex on body weight at 9 month of age was reported by Patil *et al*. (2013) in Sangamneri goats.

The accelerated growth of male goats might be attributed due to the early activation of male gonads than the female gonads. Influence in growth due to sex

hormone (androgens) i.e. testosterone which causes anabolic effect and higher metabolism in post-weaning period than in pre-weaning period.

b) Effect of type of birth (single & twin)

The results of present investigation revealed that effect of type of birth (single & twin) was non-significant source of variation for body weight at different stages of age.

The present findings for effect of type of birth were not in agreement with findings of Thiruvenkadan *et al*. (2009) in Tellicherry goats, Tyagi *et al*. (2015) in Surati goats, Khadda *et al*. (2017) in Pantja goats, Waiz *et al*. (2018) in Sirohi goats, who noted effect of type of birth was significant source of variation on body weights at birth, 1, 2, 3, 6, 9 and 12 month of age. Banerjee and Jana (2010), Meel *et al*. (2010) and Jat *et al*. (2020) in Sirohi goat kids reported significant difference in body weights of single born and twin kids from birth to 3 months body weight.

Type of birth as non-significant source of variation was reported by Rai *et al*. (2004) at 9 and 12 months body weight in Marwari goat, Kumar *et al*. (2005) at birth and 3 months of body weights in Tellicherry goat, Yadav *et al*. (2008) in Kuchi goat and Patil *et al.*(2013) in Sangamneri goat at 6, 9 and 12 months body weight, Alex *et al*. (2010) at birth, 3, 6, 9 and 12 months body weight, Bhusan (2012) at 3, 9 and 12 months body weights in Jakhrana goats, Tyagi *et al.* (2015) in Surati goats and Talekar *et al.* (2015) in Sangamneri goats at 12 month of age.

c) Effect of Season of birth

The results of present study revealed season as non significant source of variation for body weights at birth, 1, 2, 9 and 12 months of age, however significant source of variation at 3 and 6 months of age of Berari goat.

The non significant effect of season for body weight are in corroborated with the findings of Thiruvenkandan *et al*. (2009) in Tellichery goats, Gupta *et al.* (2016) in Mehasana, Waiz *et al.* (2018) in Sirohi goats for birth weight, and with Patil *et al*. (2013) in Sangamneri goats at 1 and 12 months of age respectively.

Murali *et al*. (2014) in Tellichery goat, Patil *et al*. (2013) in Sangamneri goats, Tyagi *et al.* (2015) in Surati goats and Waiz *et al.* (2018) in Sirohi, on body weights at 3 and 6 month of age. Gupta *et al*. (2016) in Mehasana at 3 month of age, Paul *et al*. (2014) in Black Bengal goats at 6 month of age respectively, observed highly significant ($P<0.01$) effect of season of birth. These findings are agreement with present study for effect of season on body weights at 3 and 6 months age.

The season as non significant source of variation on body weights reported by Khadda *et al*. (2017) in Pantja goats at 3 and 6 months, Dudhe *et al*. (2015) in Sirohi goat and Sarma *et al*. (2020) in Assam hill goat at 6 month body weights.

The significantly higher body weight at 3 and 6 months of age for the goat born during the winter season might be due to exposure to better environmental conditions, and availability of green fodder and healthy management practices.

4.2.2 Study of association of various genotypes of GH gene with body weights.

In the present study monomorphic pattern of AB genotype for GH1 gene was observed. Hence association of genotype of GH1 gene with body weight was not possible.

CHAPTER V

SUMMARY AND CONCLUSIONS

The present research was conducted on Berari goats to visualize the growth hormone gene polymorphism in exon 2 and exon 3 regions and growth performance in goats. The Berari is medium-sized breed from the Vidharbha region of Maharashtra. Berari goats are mostly reared for meat purpose, the birth and body weights of goats were an important factor for the selection and improvement of these goats for meat production.

The growth hormone regulated by the growth hormone gene is necessary for various physiological processes like postnatal growth, metabolism, reproduction, and lactation. The research intended to analyze the association of growth hormone gene with growth performance for genetic improvement of breed.

The blood samples were collected aseptically in sterile vacutainers from the jugular vein of unrelated goats irrespective of sex. The DNA isolation was carried out from blood samples of 32 goats using 'Favor Gen Prep^{TM} Blood Genomic DNA Extraction Mini Kit. The quality evaluation of DNA samples was carried by electrophoresis using 1% agarose gel and the quantitative evaluation of DNA concentration and optical density ratios using a nanophotometer.

The PCR amplification of isolated DNA for the target region of 422 bp size segment was carried out using primers described by Hua *et al.,* (2009) were brought from Eurofins Genomics India Pvt. Ltd. The amplification protocol was given by Bayan *et al.,* (2018a).

The restriction digestion of amplified PCR segment of 422 bp was carried using *HaeIII* restriction enzyme at 37^{o}C for 15 min. The PCR-RFLP digestion resulted in fragments, segment that had one cut and lead to production of two fragments the allele noted as A and segment which is uncut noted as allele B. The AB genotype was noted in present study for all 32 samples. The resulting

genotypic frequency for AB genotype is 1 and allelic frequency for A and B were 0.5 and 0.5, respectively.

The growth performance in terms of body weights of Berari goats was recorded from birth to 12 months of age at different stages of age and analyzed for the influence of fixed factors (sex, type of birth, season) on birth weight and body weights were undertaken during the study. The data on body weights for the present study was recorded from September 2020 to November 2021 from Berari Goat and Deccani Sheep Research, Demonstration and Training Centre, Borgaon Manju, PGIVAS Akola. The data on body weights during different intervals were analyzed using least-squares analysis of variance technique (Harvey, 1990).

The least-squares means for overall body weight (kg) was 2.24±0.05, 5.75±0.23, 8.94±0.28, 11.65±0.29, 18.58±0.43, 24.18±0.85, and 26.82±0.88 kg, for birth, 1, 2, 3, 6, 9, and 12 months of age, respectively. The analysis of fixed factors as a source of variation was as under.

In the results of the present study, the body weights of males were higher than females. However, sex of kids as a non-significant source of variation for pre-weaning body weights (at birth, 1, 2, and 3 months of age) and sex as a source of variation on body weights had significant ($P<0.01$) effect at 6 month and significant ($P<0.05$) effect at 9 and 12 months of age in post-weaning body weights.

Type of birth (single & twin) of kids was non-significant source of variation for body weight at different stages of age.

In the present research, season was a non-significant source of variation for body weights at birth, 1, 2, 9, and 12 months of age. However, highly significant effect ($P<0.01$) on body weights at 3 and 6 months of age. Winter body weights of winter-born kids were significantly high at 3 and 6 months of age than that of summer and rainy seasons.

Conclusions:

The conclusions for the present experiment are as follows:

1. The growth hormone 1 gene (422 bp locus) was found monomorphic in the studied population of Berari goats.
2. The results of the present study revealed that sex was a significant source of variation in post-weaning body weight. However, the male and female body weights differ highly significantly ($P<0.01$) at 6, and significantly ($P<0.05$) at 9 and 12 months of age in Berari goats.

THESIS ABSTRACT

a) Title of the thesis	**:**	**STUDY OF GROWTHHORMONE GENE POLYMORPHISM IN BERARI GOAT**
b) Full name of student	**:**	**Kachave Mukund Ramesh**
c) Name and address of major advisor	**:**	**Dr. S. V. Kuralkar** Professor and University Head, Department of Animal Genetics & Breeding, PGIVAS, Akola.
d) Degree to be awarded	:	M.V.Sc. (Animal Genetics and Breeding)
e) Year of award of degree	**:**	**2022**
f) Major subjects	**:**	Animal Genetics and Breeding
g) Total number of pages in thesis	**:**	53
h) Number of words in abstract	**:**	288
i) Signature of student	**:**	
i) Signature, name and address of forwarding authority	**:**	

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Department of Animal Genetics &
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ABSTRACT

The present research was undertaken to investigate the growth hormone gene polymorphismin Berari goats. The data of 42 unrelated Berari goats on body weights was collected from BSDSRTDC, Borgaon Manju, PGIVAS, Akola. The blood samples of 32Berari goat was collected for molecular screening of locus exon 2 and exon 3 of GH gene (422 bp).DNA samples were amplified for PCR

using forward (5'-CTCTGCCTGCCCTGGACT-3') and reverse (5'-GGAGAAGCAGAAGGCAACC-3') primers. In PCR-RFLP,amplified product GH1 (422 bp) was digested with RE*(HaeIII)* whichrevealedAB genotype (422, 366 & 56) in GH gene locus in all 32 studied Berari goats, therefore monomorphic pattern for GH gene (422 bp) was assigned to all studied samples.

Simultaneously, the body weights from birthto 12 months of age in 42 Berari kids were recorded duringSeptember 2020 to November 2021.The data analyzed for effect of factors (sex, type of birth, and season). The results of least-squares mean for overall body weight (kg) at birth, 1, 2, 3, 6, 9 and 12 month of age were 2.24±0.05, 5.75±0.23, 8.94±0.28, 11.65±0.29, 18.58±0.43, 24.18±0.85 and 26.82±0.88 kg, respectively.

In the present study effect of sex was significant source of variation at 6, 9and 12 months of body weights. The males had higher body weights than that of females at all stages of age.The effect of type of birth as non significant source of variation at all stages of age. The season had significant ($P<0.01$) influence on body weight at 3 and 6 months of age.

The present study revealed that, the polymorphism screening of GH gene (422 bp) was observed as monomorphic for AB genotype & the sex of kid was significant source of variation for body weight in Berari goat.

प्रबंध सांराश

१) प्रबंधाचे शीर्षक	:	**बेरीरी शेळ्यांमध्ये वाढ संप्रेरक जनुकाच्या बहुरूपतेचा अभ्यास**
२) विद्यार्थ्याचे पूर्ण नाव	:	**कच्छवे मुकुंद रमेश**
३) नाव आणि पत्ता प्रमुख सल्लागार	:	**डॉ. एस. व्ही. कुरळकर** प्राध्यापक आणि विदयापीठ विभाग प्रमुख, पशुअनुवंशिकी आणि प्रजननशास्त्र विभाग, स्नातकोत्तर पशुवैदयक व पशुविज्ञान संस्था, अकोला.
४) पदवी प्रदान केली जाणार आहे	:	एम.व्ही.एस.सी.
५) पदवी प्रदान करायचे वर्ष	:	२०२२
६) प्रमुख विषय	:	पशुअनुवंशिकी आणि प्रजननशास्त्र.
७) प्रबंधामध्ये एकूण पृष्ठांची संख्या	:	५३
८) प्रबंध सांराशमध्ये एकूण शब्दसंख्या	:	२३९
९) विद्यार्थ्यांची स्वाक्षरी	:	
१०) प्रबंधक कार्यवाहीस्तव पाठवणारया अधिकारयाची स्वाक्षरी, नाव आणि पत्ता	:	

(डॉ. एस. व्ही. कुरळकर)
प्राध्यापक आणि विदयापीठ विभाग प्रमुख,
पशुअनुवंशिकी आणि प्रजननशास्त्र विभाग,
स्नातकोत्तर पशुवैदयक व पशुविज्ञान संस्था,
अकोला.

सांराश

सध्याचे संशोधन हे बेरारी जातीच्या शेळ्यांमधील वजनाच्या जनुकाच्या बहुरूपतेच्या शोधासाठी तपासणीसाठी करण्यात आले होत, बेरारी शेळी आणि दख्खनी मेंढी संशोधन, प्रात्यक्षिक आणि प्रशिक्षण केंद्र, बोरगाव मंजू, अकोला, येथील ४२ असंबंधित बेरारी शेळ्यांची माहिती गोळा करण्यात आली. वजन वाढीच्या जनुकाच्या (४२२ बीपी) आण्विक परिक्षणासाठी एकुण ३२ बेरारी शेळयांमधुन रक्ताचे संकलण करून त्यातील डीएनए काढले. डीएनएचे पुढील (५'-CTCTGCCTGCCCTGGACT-३') आणी उलट (५'-GGAGAAGCAGAAGGCAACC-३') प्राइमर्ससह पीसीआर प्रवर्धन केले गेले. प्रवधीत जनुकाचे *HaeIII* प्रतिबंध विकराची प्रक्रिया केली असता AB जीनोटाइप (४२२, ३६६ आणि ५६) आढळला . अभ्यासलेल्या सर्व ३२ बेरारी शेळ्यांमध्ये वजन वाढीच्या जनुकात एकरूपता आढळून आली.

त्याचप्रमाने, ४२ बेरारी शेळ्यांचे, सप्टेबंर २०२० ते नोव्हेंबर २०२१ या कालावधीत, त्यांच्या शरीराचे जन्माचे ते वयाच्या १२ महीनेपर्यत वजनाच्या नोंद घेण्यात आली. व शेळ्यांच्या वजनावर करडयांचे लिंग, जन्मप्रकार आणि ऋतू या घटकामुळे होणारया परिणामासाठी त्यांचे विश्लेषण केले गेले. लिस्ट स्क्वेअर मीन पद्धतीच्या विश्लेषणानुसार करडांच्या शरीराचे प्रामुख्याने जन्माचे, १, २, ३, ६, ९ आणि १२ महिन्याचे वजन अनुक्रमे २.२४±०.०५, ५.७५±०.२३, ८.९४±०.२८, ११.६५±०.२९, १८.५८±०.४३, २४.१८±०.८५ आणि २६.८२±०.८८ किलो आढळुन आले.

सदर संशोधनात शेळयांचे ६, ९ आणि १२ महिन्यांच्या वजनात करडयांचा लिंगाचा लक्षणीय परीणाम आढळुन आला. सर्व वयोगटात मादीपेक्षा नर करडयांत शरीराचे वजन जास्त आढळुन आले. वयाच्या सर्व टप्प्यावर जन्माचा प्रकार भिन्नतेचा महत्त्वपूर्ण स्रोत नसल्याचे निद्रक्षणास आले. तथापि ऋतूचा ३ आणि ६ महिने वयाच्या शरीराच्या वजनावर लक्षणीय परीणाम आढळुन आला.

सध्याच्या अभ्यासातून असे दिसून आले आहे की, वजन वाढीच्या जनुकाची (४२२ बीपी) तपासणी केली असता सर्व ३२ बेरारी शेळ्यांमध्ये एकरूपता आढळून आली तसेच करडयांचे लिंग हा बेरारी शेळीच्या शरीराच्या वजनात फरकाचा लक्षणीय घटक होता.

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VITA

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APPENDIX - I

Chemicals used in the Agarose Gel Electrophoresis

A) Gel loading dye (6X)

0.25% bromophenol blue

0.25% Xylene cyanol FF

15% Ficoll.

Stored at room temperature

B) Ethidium bromide (1%)

10 mg Ethidium bromide

1.0 ml distilled water

C) 10X TAE Buffer

Nuclease free water : 800 ml

Tris Base : 48.4gm

Glacial Acetic acid : 11.42 ml

0.5 M EDTA 0.2 ml : 20 ml

Make up to 1000 ml with autoclaved double distilled water.

D) 1X TAE Buffer

10X TAE Buffer 100 mlDouble distilled water (up to) 1000 ml

APPENDIX - II

Equipments used in the study

No.	Equipments
1	Refrigerated centrifuge (Eppendorf)
2	Weighing balance (Anamed)
3	Micropipettes (Eppendorf, Germany)
4	Spectrophotometer (NanoDrop P-330,IMPLEN)
5	Gel electrophoresis apparatus (GenAxy, India)
6	Cold Cabinet (SIEMENS)
7	Thermocycler (Eppendorf, Germany)
8	U.V.Transilluminator (Bio Era)
9	Microcentrifuge (Minispin, TARSON)
10	P.H.Meter (MAC)