T.R.
VAN YUZUNCU YIL UNIVERSITY
INSTITUTE OF NATURAL AND APPLIED SCIENCES
ANIMAL SCIENCES

GROWTH HORMONE (GH) GENE POLYMORPHISMS IN NORDUZ SHEEP

M.Sc. THESIS

PREPARED BY: Rasul Mahmood MOHAMMED
SUPERVISOR: Assist. Prof. Dr. Hasan KOYUN

VAN-2018

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ACCEPTANCE and APPROVAL PAGE

This thesis entitled "**GROWTH HORMONE (GH) GENE POLYMORPHISMS IN NORDUZ SHEEP**"presented by Rasul Mahmood MOHAMMED under supervision of Assist. Prof. Dr. Hasan KOYUN in the department of Animal Sciences has been accepted as a M. Sc. thesis according to Legislations of Graduate Higher Education on 24/04/2018 with unanimity / majority of votes members of jury.

Chair: Prof. Dr.Hayrettin OKUT Signature:

Member: Doç. Dr. Ayhan YILMAZ Signature:

Member: Yrd. Doç. Dr. Hasan KOYUN Signature:

This thesis has been approved by the committee of The Institute of Natural and Applied Science on 06.../04.../2018 with decision number ...2018...17

Signature

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THESIS STATEMENT

All information presented in the thesis obtained in the frame of ethical behavior and academic rules. In addition all kinds of information that does not belong to me have been cited appropriately in the thesis prepared by the thesis writing rules.

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Rasul Mahmood MOHAMMED

ABSTRACT

GROWTH HORMONE (GH) GENE POLYMORPHISMS IN NORDUZ SHEEP

MAHMOOD, Rasul Mahommed
M.Sc.Thesis, Department Animal Sciences
Supervisor: Assist. Prof. Dr. Hasan KOUYN
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Genetic markers and their applications to animal molacular genetics have commonly been used to determine polymorphisms. Restriction Fragment Length Polymorphism (RFLP) is a molecular method of genetic analysis exploiting variations in homologous DNA sequences, consequently being considered as one of the informative genetic markers.The growth hormone (GH) gene plays major roles in growth regulation and development of higher organisms and the growth traits are complex traits involving multiple genes, loci and interactions. The aim of the research was to detect GH polymorphisms in Norduz sheep. Genomic DNAs were isolated from blood samples of Norduz sheep and GH1 primers based on the 5 'flanking sequence of the sheep GH1 gene (Gene Bank Accession Number: D00476) designed by (Hua et al., 2009) and GH gene designed by (Kuulasma, 2002) were amplified. The amplified PCR product with the length of 422 bp for GH1 locus and 934 bp for GH locus. Both loci were digested with *HaeIII* restriction enzyme and the results displayed the presence of *GH1/HaeIII* homozygous AA genotype with fragments 366 bp and 56 bp and heterozyogous AB genotype with fragments 422 bp and 366 bp. However, homozygous BB genotype did not appear. Considering *GH/HaeIII* digestion, all expected genotypes AA, AB, BB were detected. Additionally, the population under investigation did not exhibite to be in Hardy-Weinberg equilibrium (HWE) for both loci. The present study concluded that *GH1and GH/HaeIII* could be used as a genetic marker in sheep for growth characters.

Keywords: Growth hormone, (GH1 and GH) genes, *HaeIII* digestion, Norduz sheep, PCR-RFLP.

ÖZET

NORDUZ KOYUNUNDA BÜYÜME HORMONU (GH) GENİ POLİMORFİZMLERİ

MAHMOOD, Rasul Mohammed
Yüksek Lisans Tezi, Zootekni Anabilim Dalı
Tez Danışmanı : Dr. Öğr. Üyesi Hasan KOUYN
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Genetik belirteçler ve bunların hayvan moleküler genetiğine uygulamaları yaygın olarak polimorfizmleri belirlemek için kullanılmaktadır. Sınırlı Parça Uzunluğu Polimorfizmi (RFLP), homolog DNA dizilerindeki varyasyonları kullanan bir genetik analiz molekülü yöntemidir ve bilgilendirici genetik belirteçlerden biri olarak kabul edilmektedir. Büyüme hormonu (GH) geni canlının büyüme ve gelişiminde önemli rol oynar Araştırmanın amacı Norduz koyunlarında GH polimorfizmini tespit etmektir. İzole edilen koyun genomik DNA'sında Büyüme hormaonuna etkiyen 422 bp uzunluklu GH1 (Hua ve ark., 2009) geni ve 934 bp uzunluklu GH (Kuulasma, 2002) genleri PCR ile çoğaltılmış ve HaeIII kesim enzimi ile kesilmişlerdir. Buna göre GH1 lokusunda fragmanları 366 bp ve 56 bp olan AA genotipi ve 422 bp ve 366 bp'lik fragmanlara sahip heterozigot AB genotipi Ancak homozigot BB genotipi görülmemiştir. Ayrıca GH lokusu için her üç genotipte AA, AB, BB saptanmıştır. Araştırılan sürü popülasyonun her iki lokus için Hardy-Weinberg dengesinde (HWE) olmadıkları tespit edilmiştir. Bu çalışma da *GH1ve GH / HaeIII'* nin büyüme karakterleri için koyunlarda genetik bir marker olarak kullanılabileceği sonucuna varmıştır.

Anahtar kelimeler: Büyüme hormonu, (GH1 ve GH) geni, *HaeIII* sindirim, Norduz koyunu, PCR-RFLP,

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Rasul Mahmood MOHAMMED

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SYMBOLS AND ABBREVIATIONS

Some symbols and abbreviations used in this study are presented below, along with descriptions.

Symbols	Description
Mg	Microgram
µL	Microliter
µM	Micromole
Da	Dalton
M	Minute
g	Gram
kDa	Kilo Dalton
Kg	Kilogram
M	Molar
Kb	kilobase pair
mg/ml	Milligram/milliliter
mL	Milliliter
mM	Millimolar
MW	Molecular weight
Nm	Nanometer
Mol	Moles
U	Unit
V	Volt
v/v	Volume/volume
w/v	Weight/volume

Abbrevations	Description
1GFBO-3	Insulin as Growth Factor binding protein-3
BG	Boar Goats
BH	Body height
BL	Body length
BSGD	Between the shovels chest depth
BW	Body wight
CC	Chest circumference
CSN3	κ-casein
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic Acid
DNTPs	Deoxy Nucleoside Triphosphate
EAR	East Anatolian Red
EDTA	Ethylene Diamine Tetra Acetic Acid
EtBr	Ethidium Bromide
FAO	Food and Agricultural Organization
G	Genotype
GD	Chest depth
GH	Growth Hormone
GHRL	Growth Hormone Receptor Ghrelin
H	Heterozygosis
HCL	Hydrogen Chloride
Hr	Hour
K	Potassium
LALBA	Alpha-Lactoalbumin
LEP	Leptin
LGB	Beta-Lactoglobulin

Abbrevations	Description
Mg	Magnesium
$MgCl_2$	Magnesium Chloride
NaCl	Sodium Chloride
PBS	Phosphate buffered saline
PCR	Polymerase Chain Reaction
PIC	Polymorphism Information Content
PIC	Polymorphic Informative Content
PRL	prolactin
RFLP	Restriction Fragment Length Polymorphism
RPM	Round Per Minute
SAS	Statistical analyses
SDS	Sodium Dodecyl Sulfate
SG	Saanen Goats
SSCR	Single Strand Confirmation Polymorphism
TGF	Transforming Growth Factor
TGF- β3	Transforming_growth_factor,_beta_3
UV	Ultraviolet

1. INTRODUCTION

Sheep keeping is one of the important activities in livestock farming not only in Turkey but also all around the world in term of economically producing red meat, milk and fleece. There are 629 sheep breeds (Ovis aries) currently maintained in the 52 European countries and 233 maintained in the Asian and Pacific countries (FAO, 2008). In many developing countries, there are a number of indigenous sheep genotypis that represent unique. They were classified on the bases of morphological characteristics and were given local names (Galal et al., 2005). Sheep and goat play an important economic role and make a significant contribution to both domestic and export markets through provide of food (meat and milk) and non-food (manure, skin and wool) products (Alvarez et al., 2009;Duguma et al., 2011). Although sheep and goat plays a significant role in national economy of the country to date the benefit obtained from these livestock are hampered by different challenge (Jilo et al., 2016; Abdela ., 2016). The Eastern Anatolian region has low vegetation period, drought and harsh climate. Therefore, these conditions enforce farmers to livestock production, such as small ruminant production (Karaca et al., 1990). Norduz sheep is a type within Akkaraman breed. Norduz sheep is raised in region called “Norduz” in Gürpınar, Van Norduz sheep are generally white, but grey, grey-white, brown white Norduz sheep can also be found. Different parts of body, especially head, chest and legs can have black spots. Norduz sheep generally have long legs, and its neck is covered with fleece. Tail consists of three parts, and middle part is longer (Bingöl, 1998).

Genetic polymorphism arises from mutation. It refers to the different in DNA sequence among individuals. groups or population and can be caused by mutations extending from a single nucleotide base change to variations in several hundred bases (Yahyaoui, 2004). In a formal sense, There are only two kinds of polymorphisms; those due to additional of DNA bases and those due to insertion or deletion of pairs. The humblest kind of genetic polymorphism is the single nucleotide polymorphism (SNP). A position is referred to as an SNP when it exist in interest two variants, being the seldom allele more abundant 1% in the general population. Other kind of genetic polymorphism result from the insertion or deletion of a section of DNA, which include

repeat sequence (mini and microsatellites)and gross genetic losses and rearrangement (Yahyaoui, 2004). In addition, other molecular techniques such as polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) are used in animal breeding domain as a powerful tool with the aim of providing breeders with the opportunity to carefully identify better animals immediately (Zhang et al., 2013). RFLP as a technique analyzes variable lengths of DNA fragments result from digesting a DNA sample with specific enzymes. This enzyme, so called a restriction endonuclease, breaks DNA at a specific sequence pattern known as a restriction endonuclease recognition site (Chages et al., 2007).

GH is a polypeptide hormone synthesized and secreted by acidophilic or somatotropic cells of the anterior pituitary gland. It was first discovered by Evans and Long in 1921 in the extracts of the anterior pituitary which can promote growth and maturation (Evans and Long, 1921). Therefore, the GH gene, with its functional and positional potential, has been widely used as a marker in several livestock species. Several polymorphisms of the caprine GH gene have been identified. Moreover, exon-4 and exon-5 accounted for the higher diversity and can be correlated with growth performance of animal (Malveiro et al., 2001; Marques et al., 2003; Gupta et al., 2007; Gupta et al., 2009; Hua et al., 2009; Mousavizadeh et al., 2009; An et al., 2010; Wickramaratne et al., 2010). Growth hormone (GH) has been used as a functional and positional candidate gene in genotype to phenotype association studies in several species, including bovines (i.e., Bos Taurus and Bos indicus) and Homo sapiens (Ge et al., 2003; Beauchemin et al., 2006; Rudd et al., 2006). There are several genes that influence the growth and body size of the animal. Growth hormone gene (GH), growth hormone receptor (GHR) and Insulin-like growth factor-1 (IGF-1) genes have been identified as the major genes in this biological process (Ge et al., 2000; Ge et al., 2003). It is a polypeptide hormone of about 22kDa molecular weight, composed of 190 or 191 amino acids (Ayuk et al., 2006). Growth hormone contains many metabolic and physiological actions (Rahbar et al., 2013). Moreover, the Growth hormone (GH) is single polypeptide chain protein hormone that is synthesized and secreted by the anterior pituitary eosinophils cells in vertebrates (Chen et al., 2002). The GH can accelerate metabolism and promote growth of many organs and tissues, especially the

bone, muscle, and visceral organs (Ikonen et al., 2001). The GH gene has a direct effect on the synthesis and secretion (Gadelha et al., 2012).

Molecular genetics has lead to the discovery of individual genes or candidate genes with substantial effects on the traits of economic importance. Candidate gene strategy has been proposed by direct search for quantitative trait loci (QTL) (Mehmannavaz et al., 2012). Molecular markers usually do not have any biological effect. Instead, they can be thought of as constant landmarks in the genome. They are identifiable DNA sequences, found at specific locations of the genome, and transmitted by the standard laws of inheritance from one generation to the next. They rely on a DNA assay, in contrast to morphological markers that are based on visible traits, and biochemical markers that are based on proteins produced by genes (Ruane et al., 2007). The molecular marker systems allow high-density DNA marker maps through many markers of known location which are interspersed at relatively short intervals throughout the genome, to be constructed for a range of economically important agricultural species, thus providing the framework needed for eventual applications of MAS (Dekkers, 2004). stated that the benefits of MAS to sheep breeding programmes depend on a number of conditions that are relevant for most breeding programmes across species. These conditions include the existence of a genotype test predicting phenotypic differences, the economic value of these differences and the value of the genotypic information within the breeding programme. The value of genetic information will depend heavily on the socio-economic context of the breeding programme and the production system. In a technical sense, the value of this information is basically driven by the increase in selection accuracy resulting from knowledge of genotypes, which in turn will differ between animals from different age classes. In particular, the relative increase in selection accuracy of the youngest selection candidates will be critical to the value of MAS. However, technical arguments about increased selection accuracy are of little value if these selection criteria are poorly developed or accepted within the production system (Van der Werf, 2007).

The growth hormone (GH) gene plays major roles in growth regulation and development of higher organisms and the growth traits are complex traits involving multiple genes, loci and interactions. The aim of this research is to detect

polymorphisms of GH related genes (GH1 and GH) in Norduz sheep born in the same season.

2. LITERATURE REVIEW

In this study amplified product have been used with the length of 934 bp was digested with *HaeIII* restriction enzymes in Awassi sheep breed (Iraqi and Turkish) (Luma et al., 2015). showed the appearance of GH *HaeIII* homozygotes AA genotype, with fragments 277, 202, 110, 100, 94, 68, 49 and 22, 8, 4 did not appeared on agarose gel under UV light, Homo and heterozygous genotype with fragments 277, 256, 202, 110, 100, 94, 68 and 49bp showed in Iraqi Awassi sheep.

The aim of this to investigate the correlation between polymorphisms in the 5' regulatory region, exon 4, and 3' untranslated region (UTR) of the sheep *GH* gene and sheep growth traits. The DNA from 510 adult sheep was analyzed by DNA sequencing and polymerase chain reaction single-strand conformation polymorphism. Two alleles (A and B) and 3 genotypes (AA, AB, and BB), 2 alleles (A and B) and 3 genotypes (AA, AB, and BB), and 3 alleles (A, B, and C) and 4 genotypes (AA, AB, BB, and AC) were found within the 5' regulatory region, exon 4, and 3' UTR, respectively (Jia et al., 2014).

According to Indian sheep breeds research, that a total of 324 random blood samples were analyzed by PCR-RFLP (Polymerase chain reaction-Restriction fragment length polymorphism) technique. Restriction digestion analysis at A781G locus revealed two allelic variants (A= 0.6016 and B= 0.3983) and two genotypes (AA= 0.2032, AB= 0.7968). At A1575G locus, all individuals were homozygous (CC) in all 9 breeds. The allelic frequency differences for both alleles across the Indian breeds (Kuma et al., 2014).

Another study have been used fragment of GH gene, consist in a part of intron 2, complete exon 3, complete intron 3, complete exon 4, and a part of intron 4, was amplified. The amplified product with the length of 934 bp was digested with *HaeIII* restriction enzymes and showed the presence of GH *HaeIII* polymorphism with three *HaeIII* genotypes (AA, BB and AB). Bands with ten restricted fragments of 277, 202, 110, 100, 94, 68, 49, 22, 8 and 4 bp bands were identified as genotype AA. Bands with eleven restricted fragments of 256, 202, 110, 100, 94, 68, 49, 22, 21, 8 and 4 bp bands were identified as genotype BB. Bands with twelve restricted fragments of 277, 256, 202, 110, 100, 94, 68, 49, 22, 21, 8 and 4 bp were genotyped with AB. In Donggala

sheep, AA and AB genotypes had the same genotype frequency of 0.357, however BB genotype was 0.286. In East Java sheep AA genotype is the most frequent genotype (0.464), however BB and AB were 0.286 and 0.250, individually. Up to this data showed the allele frequencies of A and B in Donggala sheep were 0.536 and 0.464, respectively, whereas those for East Java sheep were 0.589 and 0.411. The degree of heterozygosity of the GH|HaeIII polymorphismsin Donggala and East Java sheep are 0.503 and 0.493, respectively (Malewa et al., 2014).

Furthermore, Genomic DNA was isolated from a total of 20 animals from four Egyptian breeds (five each) of sheep namely Rahmani, Ossimi, Awassi, and Barki. A segment of insulin-as growth factor binding protein-3 IGFBP-3 gene, consisting of a part of exon 2, complete intron 2, exon 3, and a part of intron 3, was amplified (Amr et al., 2009). The amplified segment was found to be 654 bp in sheep. On digestion of 654 bp with HaeIII restriction enzyme yielded single restriction pattern of five segments of sizes 201, 201, 87, 67, 57 bp in all the animals belonging to the four Egyptian breeds studied revealing absence of polymorphism in those four Egyptian sheep breeds.

Evaluation of the genetic diversity for GH gene in 100 animals of Iranian indigenous sheep breed (Makooei sheep) was done by Hajihosseinlo and Negadhary (2013). using exon 4 of the GH gene through PCR-SSCP. The fragment exon 4 showed five conformational patterns. These data provided evidence that Iranian indigenous Makooei sheep had genetic variability, which opened interesting prospects for future selection programme and also preservation strategies.

Hao et al. (2010) detected SNPs of exon 1 of GH gene in Gansu modern meat sheep by PCR-SSCP. They observed polymorphism in GH gene, which had two genotypes (AA, AB). Gansu sheep, had a T/A mutation in 301 bp and G/A mutation in 305 bp of exon 1 of GH gene. The genotypes did not influence body weight at birth, one month, two months and three months. It was concluded that GH gene possibly was a major gene or linked to a major gene that affect the body weight traits of sheep.

Single stranded conformation polymorphism (SSCP) patterns of PCR products were studied 5 banding patterns AA (p1), AB (p2), BB (p3), CC (p4), and CD (p5) were obtained with frequencies of 31.3, 64.6, 1, 1, and 2.1, respectively. Associations of the observed polymorphisms with withers height, rump height, body length, chest girth,

testis girth and leg girth were analyzed using a general linear model procedure for Makooei sheep (Abbas et al., 2013).

Determine whether the used primers a 422 bp fragment from exons 2 and 3 of GH gene in sheep and goat. The *HaeIII* endonuclease cutting enzyme used in PCR amplified fragments showed the presence of two genotypes; GG and AG with the absence of AA genotype in 149 tested animals for this gene. The total frequencies were 43.56 and 56.44% for GG and AG genotypes, respectively in 101 tested sheep animals whereas in goat animals, the total frequencies were 12.5 and 87.5% for GG and AG genotypes, respectively in 48 tested goat animals. The sequence analysis of purified PCR products declared the presence of a SNP (GA) at position 55 in the amplified fragment which was responsible for the destruction of the restriction site GG^CC and consequently the presence of two different alleles G and A which were named in this work according to the detected SNP (Othman et al., 2015).

According to the research on goat that the DNA isolated from kids of Sirohi and Barbari breeds of goat on digestion with the restriction enzyme *HaeIII.* It was revealed two genotypic variants AB and BB. None of the two breeds was in Hardy–Weinberg equilibrium for these variants (Singh at el., 2015).

In an experiment the Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) were used to detect polymorphisms of five candidate genes in four Egyptian and Saudi goat breeds (Barki, Zaribi, Ardi and Masri), to detect the genotype of GH, IGF1, POUIF1, MSTN and BMP15 genes in the goat breeds and their allele frequencies by (Alakilli et al., 2012). Results of GH gene which encloses a Haelll endonuclease restriction site show four unique PCR-RFLP banding patterns (genotypes AA, AB, CC and CD). The frequencies of the A allele in the samples from the goat breeds varied from 0.410 to 0.620. While IGF-1gene revealed three fragments after digestion with HaeIII with genotype AA, AB and BB and the frequencies of allele A varied from 0.432 to 0.731.

An et al. (2011) analyzed that, the polymorphisms of growth hormone (GH) gene 5′ promoter region and intron 8, exons 4 and 10 of growth hormone receptor (GHR) gene were analyzed in Xinong Saanen goats (SG) and Boer goats (BG). Two alleles (A and B) and three genotypes (AA, AB and BB) were detected at P1 locus of GH gene, and two alleles (G and T) and two genotypes (GG and GT) were detected

at P4 locus of GHR gene by PCR-RFLP analysis. In addition, two single nucleotide polymorphisms (SNPs)-A73C (P1 locus) and G114T (P4 locus), were identified by DNA sequencing. The frequencies of alleles A and B in the two goat breeds were 0.61–0.62, and 0.39–0.38, respectively, and the frequencies of alleles G and T in the two goat breeds were 0.82–0.86, and 0.18–0.14, respectively. The SNP loci were in Hardy–Weinberg disequilibrium in both goat breeds Polymorphisms of GH and GHR genes were shown to be associated with growth traits in BG breed. AA and GG genotypes were associated with superior growth traits in 1-,2- and 3-month old individuals.

Revealed point mutations of G200T, A815G, A1753, C1763T and A1789G in GH gene sequence of both goat breeds. In contrast, A497G, A499G C500G C501-2 C730T C781T and C2055T were observed specific to Osmanabadi and Sangamneri breeds respectively indicating the possibility of using them as breed specific markers. G200T resulted GG, GT and TT genotypes and of them, GT was associated with heavy body weight and GG with low body weight in both the breeds. Sangamneri GT genotypes were 6.5% taller than GG. Similarly, AG of A815G revealed low body weight. AA of A1753 insertion revealed heavy body weight in both the breeds and 7% longer body in Sangamneri breed. Heterozygote counterparts for C1763T and A1780G SNPs exhibited heavy body weights. It was concluded that SNPs and their association with body weight may be useful in selecting goats for higher growth traits (Wickramaratne et al., 2011).

In other study result viewed that the LL genotype was the only genotype found in Aceh cattle population, and the allele frequency of allele L is 1 for two group of Aceh cattle population, i.e., Banda Aceh (12), Aceh Besar (30). The techinc that was used for amplify 404 bp of GH gene for total 42 DNA genes samples. This finding sign to there was not evidence of polymorphism of GH/*Alu*I in Aceh cattle, and there was not correlation of GH/ *Alu*I gene with carcass quality of Aceh cattle (Sumantri et al., 2012).

The Pit-1 gene has been identified as the pituitary specific transcription factor that regulates the expression of the growth hormone (GH) and prolactin (PRL) genes in the anterior pituitary. A total of 252, 181 Holstein cows and 71 native East Anatolian Red (EAR) cattle, were genotyped for polymorphism of Pit-1/*Hinf1* gene by the polymerase chain reaction and restriction fragment length polymorphism (PCR-RFLP) methods. In the Pit-1 gene, the frequency of AA, AB, and BB genotypes was 14, 54,

and 32% for EAR, and 4, 31, and 65% for Holstein, respectively. The frequency of A allele was 41% for EAR, and 20% for Holstein. Both populations were in Hardy-Weinberg equilibrium (Ozdemir et al., 2012).

As stated by another research was conducted to identify *Alu*-I locus of growth hormone (GH) gene in Bali cattle by using 232 blood samples collected from Bali and Lombok islands. PCR-RFLP and sequencing methods were used to detect the polymorphism and nucleotide sequence at *Alu*-I locus of GH gene. The result showed that Bali cattle from Bali island has one genotype (LL genotype), whereas Bali cattle originating from Lombok island has two genotypes, namely LL and VV genotypes, respectively. The L and V allele frequencies from Bali and Lombok islands were 1.00 and 0.00; 0.99 and 0.01, respectively (Jakaria et al., 2011).

PCR-RFLP and sequencing methods used to detect the polymorphism and mutation at MspI and AluI loci of GH gene. The results showed that at MspI locus, the Bali, Limousine and Simmental cattle had one genotype (-/-), three genotypes(+/+, +/- , -/-) and two genotypes (+/+, +/-), respectively whereas for AluI locus, the Bali, Limousine and Simmental cattles had one genotype (LL), two genotypes (LL, LV) and three genotypes (LL, LV, VV), respectively. The allele frequencies of + and – alleles in Bali, Limousine and Simmental cattle were 0.000 and 1.000; 0.636 and 0.364; 0.889 and 0.111 respectively (Jakaria et al., 2009).

This study was conducted to determine DNA-polymorphism of a *Mu*\ RFLP at bovine growth hormone (bGH) gene in (Zavot. East Anatolian Red, Simmental and Brown Swiss) cattle breeds. A total of 246 cattle were genotyped for the bGH-*Alu*ı. Polymorphism by polymerase chain reaction and restriction fragment length polymorphism (PCR-RFLP). In the study, two alleles (L and V) and three genotypes (LL, W and LV) were revealed after than digestion of amplification product with *Mu*\ restriction enzyme. Allelic Frequencies for EAR, SIM, BS and Zavot breeds were determined as 0.775, 0.734, 0.781 and 0.760 respectively for L allele; 0.225, 0.266, 0.219 and 0.240 respectively for V allele. Otherwise, genotypic frequencies were 0.65,0. 57, 0.63 and 0.63 for LL, 0.10, 0.11, 0.06 and 0.10 for W, and 0.25, 0.32, 0.31 and 0.27 for LV respectively (Ağaoğlu et al,. 2013).

It has been taken 93 blood samples from 93 hybrid cows randomly, DNA extraction from blood samples and used PCR for amplification of 249-bp fragment of

Exon 1 of the gene were performed. The digestion by cutting *SnaBI* enzyme. The data showed that the genotype frequencies of AA, AB and BB, respectively are as 0.06, 0.53 and 0.41. AB genotype and AA genotype had the highest and the lowest frequencies in the herd, respectively. The frequencies of allele A and allele B in the population were estimated respectively as 0.33 and 0.67 (Ahmad et al., 2013).

3. MATERIALS AND METHODS

3.1. Animal Supply and Ethics Permission

The animal material of the experiment was obtained from VYYU-Faculty of Agriculture, Livestock Research and Application Farm. Norduz lambs of the same ages (6-9 months), born in 2017. were picked up.

In addition, this study was initiated by taking the project work permit with the official certificate of ethics committee numbered 27552122-604.01.02-E.59510 dated 26/08/2017 of VYYU.

3.2. Method

3.2.1. Animals, trait and measurements

There are 43 females, 6 males, Norduz lambs of having different monthly ages born in the same year. Norduz growth characteristics of lambs were gender, body weight (BW), body height (BH), body length (BL),chest depth (GD), between the shovels the chest depth (BSGD) and chest around (CA). Taking into account quantitative measurement values, it was started measuring the animals in mid July, 2017 and with an average period of 24 days. Five growth parameters were measured and final measurement was recorded in November, 2017. To obtain the quantitative data there were used a scale, a measuring stick, and a hamstring.

3.2.2. DNA ısolation

The 5ml peripheral strand from the sheep genomic DNA individuals is shown below was obtained by the standard phenol-chloroform method (Sambrook et al., 1982).

- $2x10^6$ cells are centrifuged at 1500 rpm for 5 minutes and the supernatant is removed, then the pellet is washed 2 times with PBS (Phosphate Buffered Saline).

- Resuspend in 300 µl lysis solution (5 mM EDTA, 10 mM Tris-HCl, pH = 8.0, 100 mM NaCl, 0.5% SDS) (sodium dodecyl sulfate), 0.5 mg / ml Proteinase-K).
- The following day, an equal volume of saturated phenol is added to the solution and the mixture is then centrifuged at 1500 rpm for 20 minutes.
- Remove the supernatant clean lure and add an equal volume of chloroform: isogamy alcohol mixture (24: 1) and mix.
- Centrifuge at 1500 rpm for 20 minutes and transfer the supernatant to a clean scarf.
- Add an equal volume of pure ethanol to the solution and allow for the DNA to precipitate by gentle mixing. The precipitated DNA is transferred to a 1.5 ml microfuge tube with micropipette.
- Once pure ethanol is centrifuged once with 70% ethanol for 10 minutes at 13000 rpm, the supernatant is discarded and the DNA is allowed to dry.
- The dried DNA is resuspended by adding 10 mM Tris pH = 7.5, 1 mM EDTA and incubated at -20 ℃ for future use.

3.2.3. Oligo determination and primer desing

GH1 and GH genes were first determined and used by entering the gene bank (access: http://www.ncbi.nlm.nih.gov/genome/guide/sheep/index.html), in sheep genome The loci were on sheep chromose 3 (OAR3) Taking into account the exon-intron boundaries, PCR from the coding gene regions Primer3Plus for the replication of gene regions (access:http://www.bioinformatics.nl/cgibin/primer3plus/primer3plus.cgi/) by utilizing the software program, replication for each gene (amplification) primers were designed and are shown in (Table 3.1).

Table 3.1. Growth Hormone Genes (GH) and PCR Conditions

Locus Name	Chromosome	Forward Primer	Reverse Primer	TM F/R	PCR product
GH1	OAR 3	5'-ctctgcctgccctggact-3'	3'-ggagaagcagaaggcaacc-5'	57.5	422bp
GH	OAR 3	5'-ggaggcaggaagggatgaa-3'	3'-ccaagggagggagagacaga-5'	58	934bp

3.2.4. PCR and gel electropheresis

The PCR conditions for each locus were in particular the amount of MgCl2 and hybridization heat and the following PCR mixture solutions and concentrations were used (Table 3.2). Gel Electropheresis were performed at different densities (%0.8, %1, %1.5, %2) with Ethidium Bromide (EtBr) and visualized under ultraviolet (UV) light.

Table 3.2. PCR Master Mixtures and Concentrations

PCR master karışımı	µl
dH_2O	15.1
10X buffer	2.5
$MgCl_2$	1.5
DMSO	0.35
dNTP	1
Primer F	1
Primer R	1
Taq Polimeraz	0.2
DNA	2.5
	Total= 25.15

3.2.5. PCR programmes:

Initial Denaturation	95 ºC 10 m
Denaturation	96 ºC 40 Sc
Annealing	58 ºC 35 Sc
Extension	72 ºC 45 Sc
Final Extension	72 ºC 10 m
Storage	4 ºC ∞(İnfinite)
Total 30 Cycles	

3.2.6. PCR-RFLP

The PCR amplicons on GH and GH1 DNA regions were digested with DNA cutting enzyme *HaeIII*, (DNA endonuclease) shows the results (Table 3.3).

Table 3.3. Virtual Cutting and Expected Lengths of Locus with DNA Cutting Enzymes

Locus	DNA Cutting Enzyme	Cutting Outcome Expected DNA Fragment Lengths (bp)
GH1	*HaeIII*	366+56= 422bp
GH	*HaeIII*	277+256+202+110+100+94+68+49= 934bp

3.3. Hardy-Weinberg Equilbrium (HWE) Analysis

Allele varieties determined on agarose gel were counted baded on related references and online calculated via (http://www.oege.org/software/hwe-mr-calc.shtml).

3.4. Statistical Analysis

SAS 9.4 Statistical software package was used to obtain basic statistics and Pearson correlation analyses between and among the growth measures.

4. RESULTS

A basic statistical analysis of the growth characteristics of Norduz sheep grown and free-grazing in farmland is shown in (Table 4.1.) Phenotypic features on the chart; sex, body weight (BW), body height (BH), body length (BL) the chest depth (GD), between the shovels the chest depth (BSGD) and the chest around (CA). At the beginning, a total of 49 sheep (43 females, 6 males) were observed, records on phenotypic yields July, August, In September, October and November 5 times in average 24 days period measurements were taken, but the blood required for 7 sheep genomic DNA the number of sheep surveyed was reduced to 42 for genetic analyzes because of not taken blood samples from lambs which were grazing in meadow on the day of their sapmles intake.

4.1. Statistical Analysis

Table 4.1. Correlation Coefficients among Body Weight (BW) during five different measurement periods (Pearson Correlation Test)

Phenotype	BW1	BW2	BW3	BW4	BW5
BW1	1	.127	.213	.231	.284*
BW2		1	.946**	.952**	.919**
BW3			1	.970**	.962**
BW4				1	.969**
BW5					1

*($p<0.05$), ** ($p<0.01$)

It has been found that when the measurement time progressed, correlations between the other measurements were partially increased. Only a statistically significant relationship was found between BW1 and BW5 ($p <0.05$). Correlations between BW2 and BW5 increased and were found statistically significant ($p <0.01$).

Table 4.2. Correlation coefficients among body height (BH) during five different measurement periods (pearson correlation test)

Phenotype	BH1	BH2	BH3	BH4	BH5
BH1	1	.678**	.464**	.624**	.481**
BH2		1	.731**	.697**	.612**
BH3			1	.789**	.519**
BH4				1	.619**
BH5					1

*(p<0.05), ** (p<0.01)

Table 4.3. Correlation coefficients among body length (BL) during five different measurement periods (pearson correlation test)

Phenotype	BL1	BL2	BL3	BL4	BL5
BL1	1	.324*	.483**	.648**	.561**
BL2		1	.320*	.438**	.397**
BL3			1	.632**	.563**
BL4				1	.768**
BL5					1

*(p<0.05), **(p<0.01)

Table 4.4. Correlation coefficients among chest depth (GD) during five different measurement periods (pearson correlation Test)

Phenotype	GD1	GD2	GD3	GD4	GD5
GD1	1	.432**	.489**	.729**	.603**
GD2		1	.483**	.625**	.496**
GD3			1	.769**	.763**
GD4				1	.685**
GD5					1

*(p<0.05), **(p<0.01)

Table 4.5. Correlation coefficients among between the shovels chest depth (BSGD) during five different measurement periods (pearson correlation test)

Phenotepe	BSGD 1	BSGD 2	BSGD 3	BSGD 4	BSGD 5
BSGD 1	1	.360*	.280	.429**	.284
BSGD 2		1	.478**	.582**	.607**
BSGD 3			1	.650**	.672**
BSGD 4				1	.697**
BSGD 5					1

*(p<0.05), **(p<0.01)

Table 4.6. Correlation coefficient samong chest around (CA) during five different measurement periods (pearson correlation test)

Phenotype	CA1	CA2	CA3	CA4	CA5
CA1	1	.450**	.728**	.632**	.639**
CA2		1	.684**	.761**	.611**
CA3			1	.856**	.805**
CA4				1	.781**
CA5					1

*(p<0.05), **(p<0.05)

Table 4.7. T-Test Values; Introductory Statistics by Sex

	Gender	N	Mean	Std. Deviation	Std. Error Mean
BW2	1	43	28.9767	3.70745	.56538
	2	7	29.6714	8.26251	3.12293
BW3	1	41	29.7878	3.51527	.54899
	2	8	30.9750	5.75593	2.03503
BW4	1	41	29.9878	3.68430	.57539
	2	16	29.6125	5.84669	1.46167
BW5	1	42	29.0643	3.69646	.57038
	2	14	29.1286	5.71939	1.52857
BH1	1	44	58.4773	3.43199	.51739
	2	5	61.8000	.44721	.20000
BH2	1	43	59.0465	2.88637	.44017
	2	7	58.7143	7.93125	2.99773
BH3	1	41	60.2439	2.50779	.39165
	2	8	65.6250	7.24938	2.56304
BH4	1	41	60.6585	2.88626	.45076
	2	16	60.8750	5.94278	1.48570
BH5	1	42	60.9286	2.90830	.44876
	2	14	61.0714	3.17355	.84817
BL1	1	44	53.5568	3.06749	.46244
	2	5	53.6000	3.78153	1.69115
BL2	1	43	53.6512	3.84759	.58675
	2	8	51.3750	5.60453	1.98150
BL3	1	41	54.6707	2.64483	.41305
	2	8	55.0000	2.72554	.96362
BL4	1	41	55.3171	3.13998	.49038
	2	16	54.8750	5.25198	1.31300
BL5	1	42	54.8452	3.41176	.52645
	2	14	53.6786	3.29106	.87957
GD1	1	44	23.7159	2.11692	.31914
	2	5	26.6000	3.28634	1.46969

Table 4.7. T-Test Values; Introductory Statistics by Sex (continue)

	Gender	N	Mean	Std. Deviation	Std. Error Mean
GD2	1	43	23.0581	1.50479	.22948
	2	7	23.7143	2.28869	.86504
GD3	1	41	23.8902	1.42088	.22190
	2	8	24.8750	2.16712	.76619
GD4	1	41	23.7561	1.29481	.20221
	2	16	24.1875	2.10456	.52614
GD5	1	42	23.5190	1.46024	.22532
	2	14	23.5000	2.18386	.58366
BSGD1	1	44	15.1136	1.66658	.25125
	2	5	15.6000	1.51658	.67823
BSGD2	1	43	14.0000	1.34519	.20514
	2	7	14.1429	3.23669	1.22336
BSGD3	1	41	13.7073	1.05461	.16470
	2	8	14.1250	1.45774	.51539
BSGD4	1	41	13.4146	1.01798	.15898
	2	16	13.2188	1.12500	.28125
BSGD5	1	42	12.9048	.98920	.15264
	2	14	12.8571	1.46009	.39023
CC1	1	44	71.1136	5.15894	.77774
	2	5	76.4000	8.61974	3.85487
CC2	1	43	70.6279	3.51204	.53558
	2	7	70.0000	7.72442	2.91956
CC3	1	41	69.2195	3.60910	.56365
	2	8	70.0000	4.78091	1.69031
CC4	1	41	68.8293	3.03235	.47357
	2	16	68.6250	5.28993	1.32248
CC5	1	42	67.4286	3.74259	.57749
	2	14	68.0000	5.21831	1.39465

Table 4.8. Correlations Among Growth Parameters.

		BW	BH	BL	GD	BSGD	CA
BW	Pearson Correlation	1	.120	.208**	.260**	.193**	.234**
	Sig. (2-tailed)		.082	.002	.000	.005	.001
	N	210	210	210	210	210	210
BH	Pearson Correlation	120	1	.551**	.596**	.256**	.479**
	Sig. (2-tailed)	.082		.000	.000	.000	.000
	N	210	210	210	210	210	210
BL	Pearson Correlation	.208**	.551**	1	.428**	.287**	.432**
	Sig. (2-tailed)	002	.000		.000	.000	.000
	N	210	210	210	210	210	210
GD	Pearson Correlation	.260**	.596**	.428**	1	.536**	.678**
	Sig. (2-tailed)	000	000	.000		.000	.000
	N	210	210	210	210	210	210
BSGD	Pearson Correlation	.193**	.256**	.287**	.536**	1	.710**
	Sig. (2-tailed)	.005	.000	.000	.000		.000
	N	210	210	210	210	210	210
CA	Pearson Correlation	.234**	.479**	.432**	.678**	.710**	1
	Sig. (2-tailed)	.001	.000	.000	.000	.000	
	N	210	210	210	210	210	210

**. Correlation is significant at the 0.01 level (2-tailed).

The correlation between BW and BH is statistically significant

The correlation between BH and GC is statistically significant

The correlation between BL and BH is statistically significant

The correlation between BSGD and GC was found statistically significant.

The correlations between BW and BH, BH and GC, BL and BH and BSGD and GC was found statistically significant.

4.2. Molecular Genetics Analyzes

4.2.1. Genomic DNA isolation

VYYÜ-Faculty of Agriculture, Livestock Research and Application Farm February, DNA was isolated as a result of blood samples from Norduz lambs of the same age (6-9 months) following births in 2017.An agarose gel image of the isolated genomic DNA was then given in. (figure 4.1).

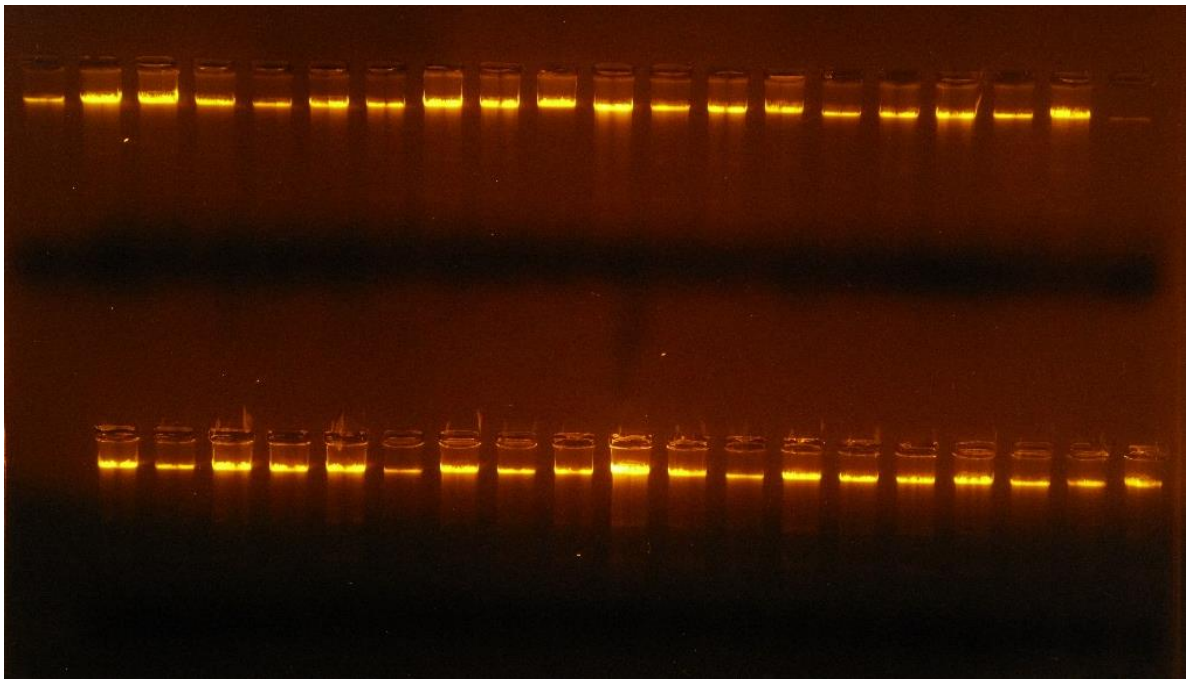

Figure 4.1. Sheep Genomic DNA Isolation. % 1 Agarose Gel, 75 Volt, 30min.

4.2.2. PCR Results

Among the isolated blood samples, Growth Hormone Genes; reference genomic sequence determined from two different loci (GH1 and GH) and genetic screening of all individuals under investigation were defined for optimal PCR conditions.GH1 and GH loci, the optimum Tm temperatures were determined using gradient PCR and touch-down PCR (Table .3.1).

The GH1 locus was amplified by PCR at an optimal extension temperature of 57.5 ºC .422-bp PCR product was obtained and 34 animals were genotyped for this locus (figure 4.2).

The GH locus was amplified by PCR at an optimal extension temperature of 58 ºC, 934 bp PCR product was obtained and but 26 animals were genotyped for this locus (figure4.3).

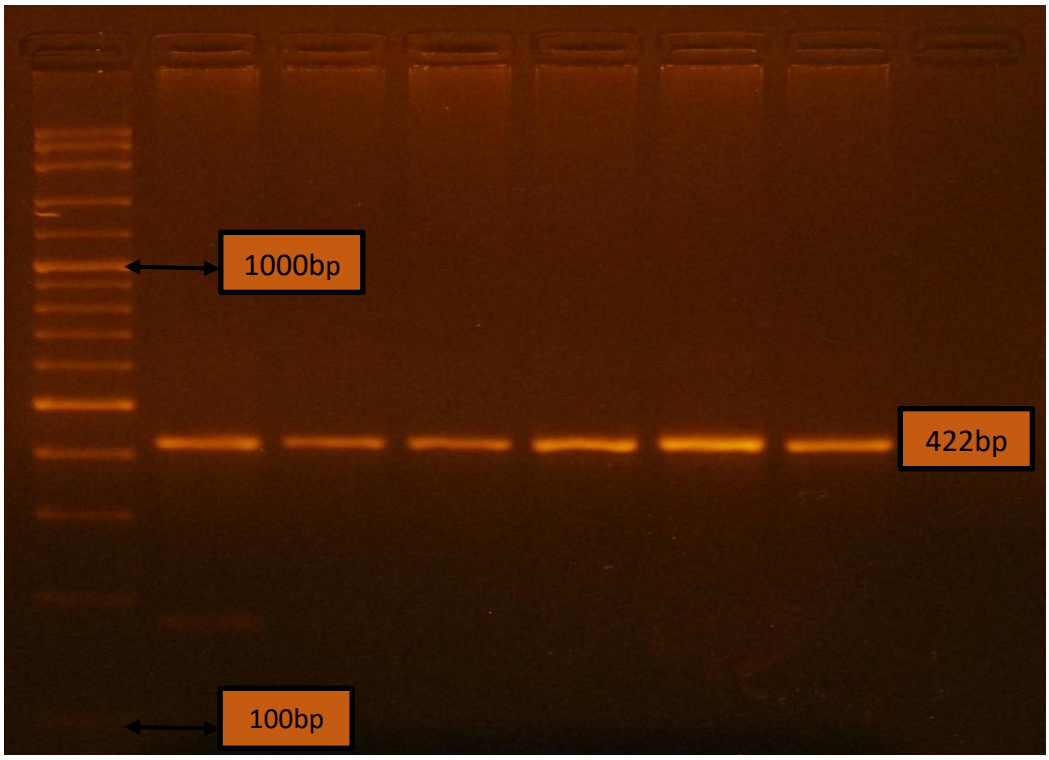

Figure 4.2. PCR results of GH1 (422 bp) locus; Extension Temperature: 57.5 ºC.

Table 4.9. PCR Master Mix for GH1

PCR master mix	µl	x6
dH_2O	15.1	90.6
10X buffer	2.5	15
$MgCl_2$	1.5	9
DMSO	0.35	2.1
Dntp	1	6
Primer Reverse	1	6
Primer Forward	1	6
Taq Polimeraz	0.2	1.2
DNA	2.5	
	Total= 25	

4.2.3. PCR Programmes

The PCR conditions applied for the GH1 gene are as follows.

Initial Denaturation	95 ºC 10 m
Denaturation	96 ºC 40 sc
Annealing	57.5ºC 35 sc
Extension	72 ºC 45 sc
Final Extension	72ºC 10 m
Storage	4 ºC ∞(infinite)
Total 30 cycles	

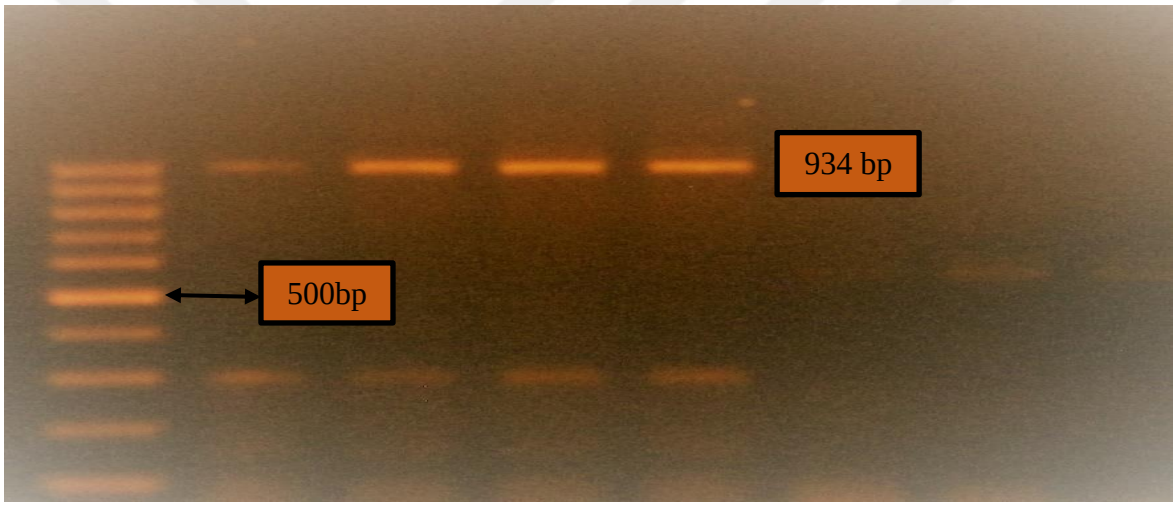

Figure 4.3. PCR results of GH (934 bp) locus; Extension Temperature: 58 ºC. %1.5 Agarose Gel, 80V, 2h.

Table 4.10. PCR Master Mix for GH(934bp)

PCR Master Mix	µl	x4
dH_2O	17.64	70.56
10 X Buffer	5	20
$MgCl_2$	2	8
DMSO	0.5	2
dNTB	1	4
Reverse Primer GH	1	4
Forward Primer GH	1	4
Taq DNA Polymerase	1	4
1 µl DNA sample	1	
Total Volume	30	116.56

4.2.4. PCR-RFLP Results and genotyping

The PCR products obtained from the two locusts were analyzed in accordance with (Table 4.11) PCR-RFLP treatment was performed by treatment with HaeIII DNA cutting enzyme at 37 ºC for 10 min. And polymorphic in both loci.

Table 4.11. DNA Digestion Reaction with HaeIII Enzyme for GH1 (422bp)

Master Mix	µl	6× µl
dH_2O	17	102
10×buffer	2	12
Enzyme	1	6
DNA-PCR	10	126
Total	30	180

The 422 bp PCR result was obtained that. Two parts of 422 bp and 366 bp was observed as DNA bands, but the band of 56 bp was not seen (figuer 4-4.5.6.7).

Thirty-four sheep were screened, based on (Seevagan et al., 2015). 5 of the 34 animals of the AA, the 29 animals had the AB genotypes, the BB genotype could not be found.

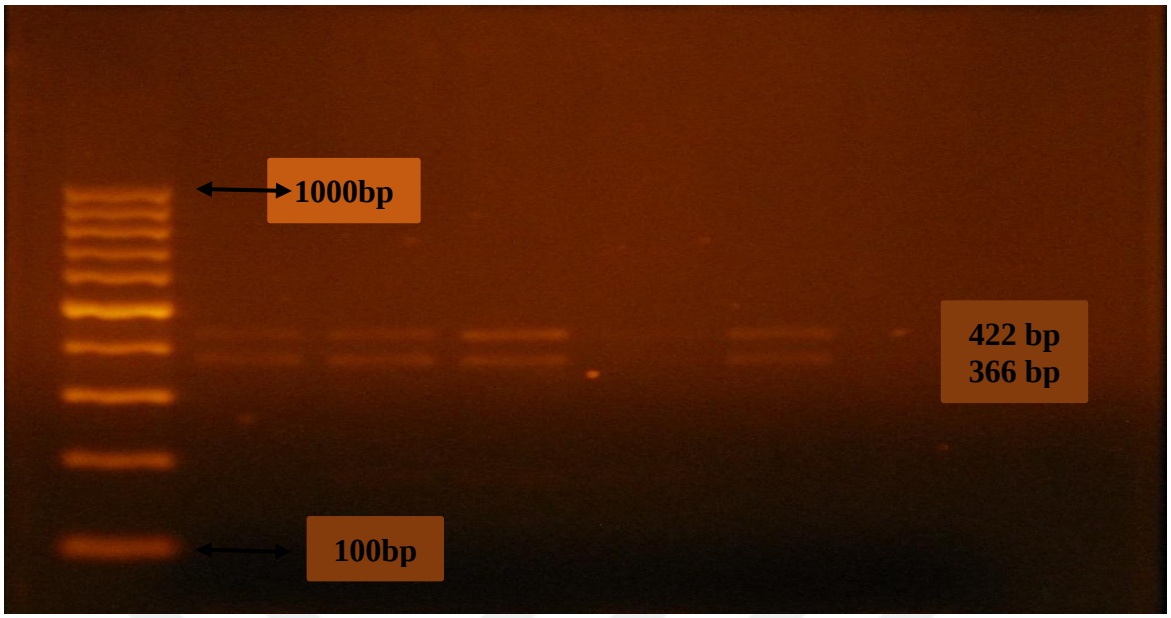

Figure 4.4. PCR-RFLP Results and Genotyping with HaeIII Enzyme of GH1 (422 bp) Locus. %1.5 Agarose Gel, 80V, 2h .Genotyping; 1-AB, 2-AB 3-AB 4-AB 5-AB 6-AB.

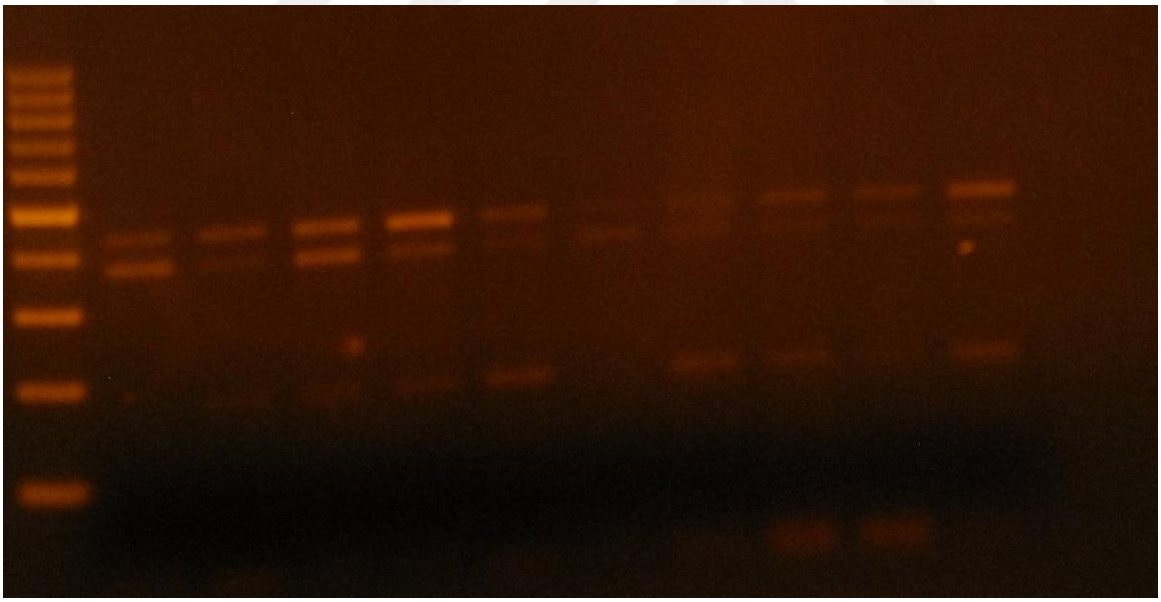

Figure 4.5. PCR-RFLP Results and Genotyping with HaeIII Enzyme of GH1 (422 bp) Locus %2 Agarose Gel, 80V, 2h Genotyping; 7-AB 8-AB 9-AB 10-AB 11-AB 12-AA 13-AB 14-AB 15-AB 16-AB.

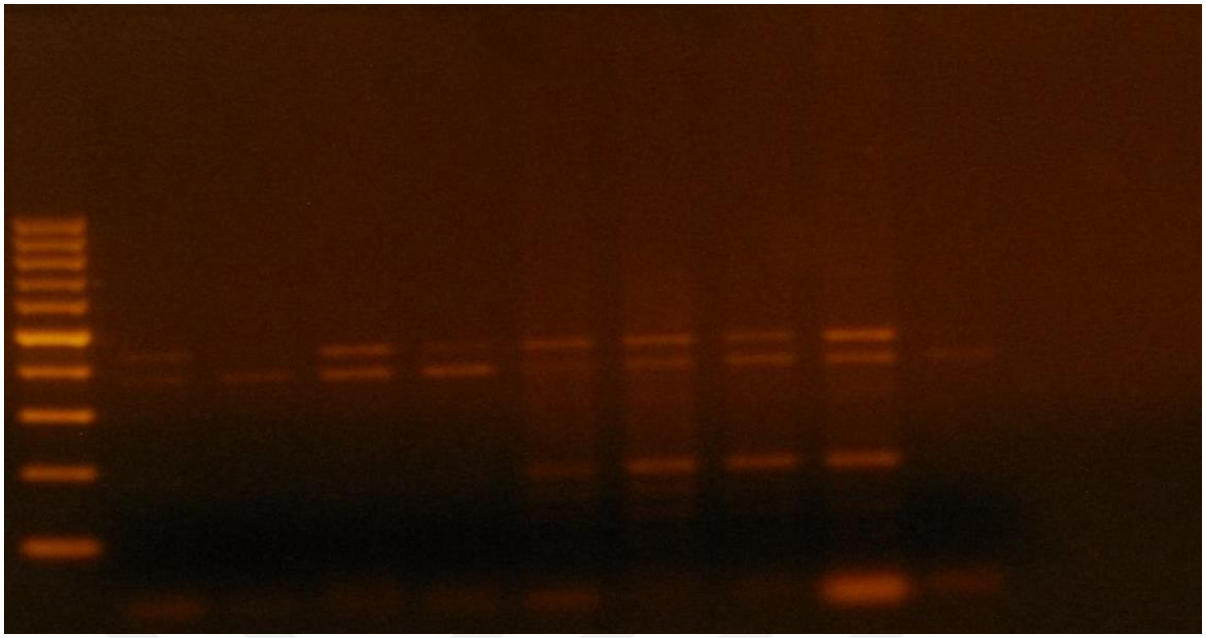

Figure 4.6. PCR-RFLP Results and Genotyping with HaeIII Enzyme of GH1 (422 bp) Locus Genotyping; 17-AB 18-AA 20-AB 21.AB 22-AA 23-AB 24-AB 25-AB 26-AA. %1.5 Agarose Gel, 80V, 2h.

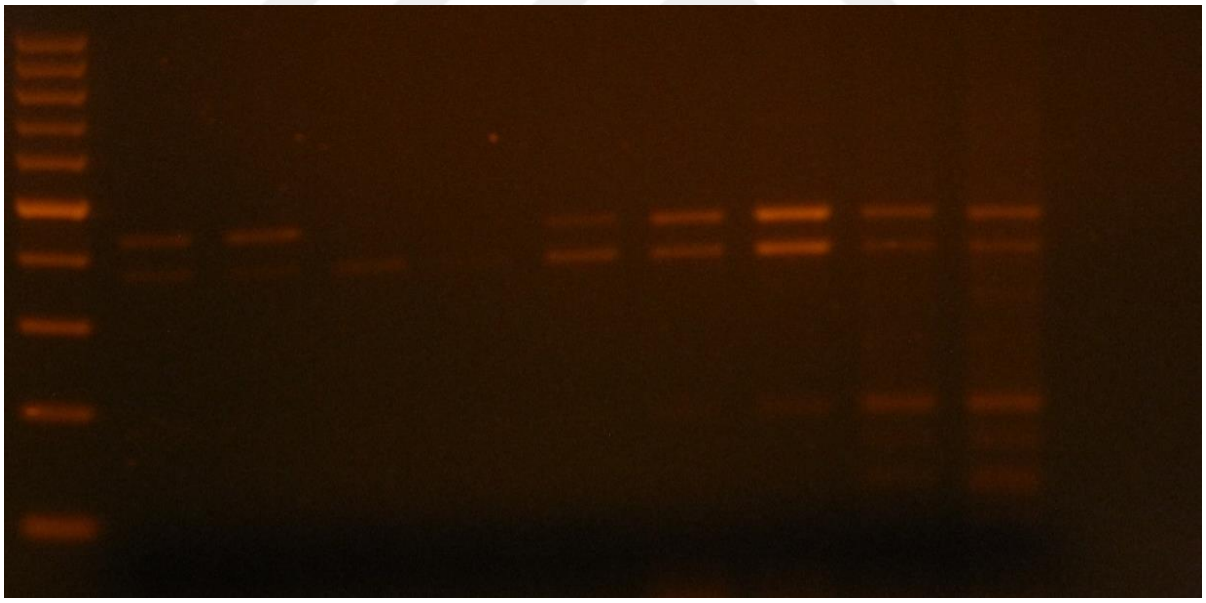

Figure 4.7. PCR-RFLP Results and Genotyping with HaeIII Enzyme of GH1 (422 bp) Locus Genotyping; 30. AB 33. AB 35. AA 36. AA 40. AB 43. AB 44AB 45. AB 46. AB.

Total 5/34 AA, 29/34 AB, 0 BB animals scanned for GH1 (422bp)

The 934 bp PCR result showed that the DNA band 277 bp, 256 bps, and 202 bps, but no bands with DNA fragments smaller than 100 bps were observed (Figure 4.8. and 4.9.). 26 sheep were collected from the genotype screen and Based on (Gorlov et al., 2017).

5 of 26 animals are AA, 17 of the AB and 4 of them have BB genotypes have been identified.

Table 4.12. DNA Digestion Reaction with HaeIII Enzyme for GH (934).

Master Mix	µl	6× µl
dH_2O	17	102
10×buffer	2	12
Enzyme	1	6
PCR-product	10	126
Total	30	180

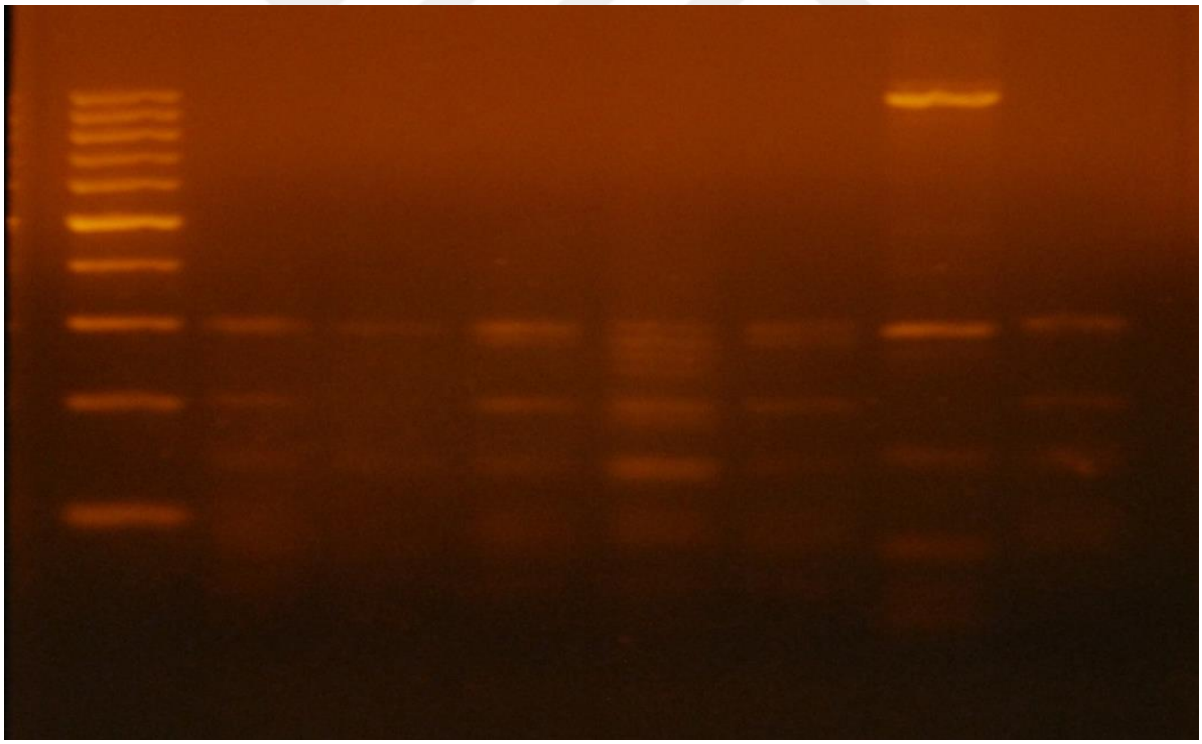

Figure 4.8. PCR-RFLP Results and Genotyping with GH (934 bp) Locus HaeIII Enzyme Genotyping; 1.AA 2.BB 3.AB 4.AB 5.AB 6.BB 7.AA. %1.5 Agarose Gel, 80V, 2h.

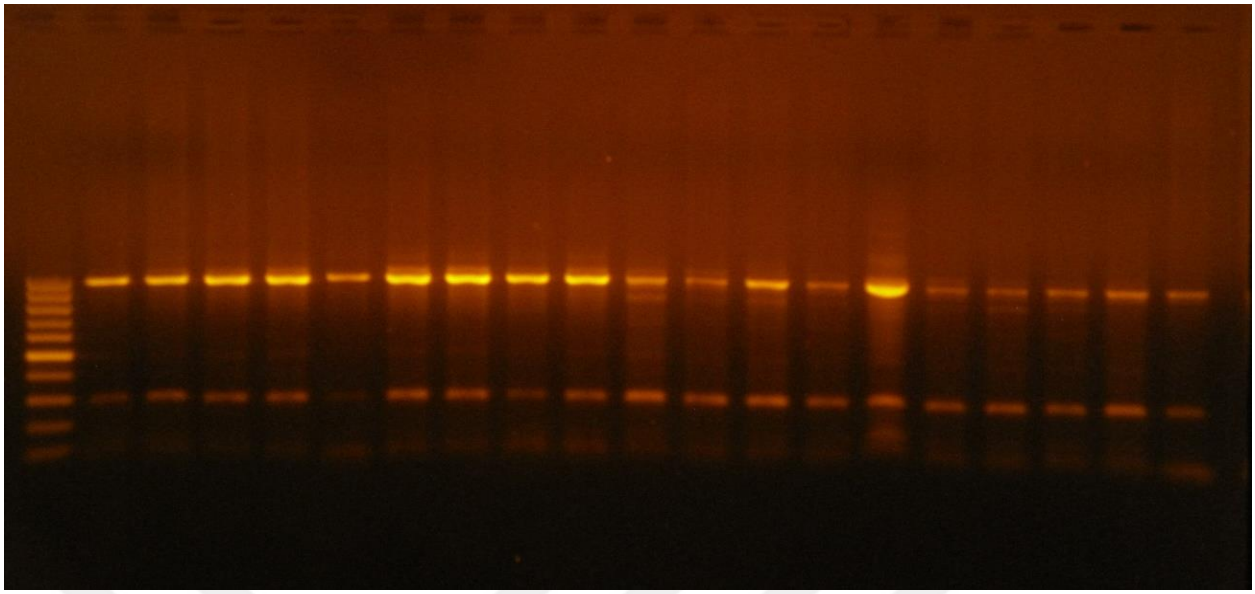

Figure 4.9. PCR-RFLP results and genotyping with HaeIII enzyme of GH (934 bp) locus Genotyping; 1.BB 2. AA 3.AB 4. AB 5. BB 6.AB 7 .AB 8 .AA 9.AB 10. AB 11. AB 12. AB 13. AB 14 .AA 15. AB 16. AB 17. AB 18. AB 19. AB.

Table 4.13 Genotype and allele frequencies for (GH and GH1)

	Genotypes	Frequency		Allele	Frequency	
Locus	AA	BB	AB	A	B	Total
GH1	0.26(4)	0	0.74(29)	0.57	0.43	34
CH	0.27(5)	0.23(4)	0.50(17)	0.52	0.48	26

4.3. Hardy-Weinberg Equilibrium (HWE) and Statistical Tests

4.3.1. Result for GH1 (422bp)

$X^2 = 18.8$

(34 samples counted)

Table 4.14. For likelihoods of calculated X2 value see below.

Genotype	Expected	Observed
Common homozygotes	11.18	5
Heterozygotes	16.63	29
Rare homozygotes	6.18	0

p allele freq = 0.57; q allele freq = 0.43

4.3.2. Result for GH(934)

X^2 =5.59

(26samples counted)

Table 4.15. For likelihoods of calculated X2 value see below.

Genotype	Expected	Observed
Common homozygotes	7.01	5
Heterozygotes	12.98	17
Rare homozygotes	6.01	4

p allele freq = 0.52; q allele freq = 0.48

5. DISCUSSION AND CONCLUSIONS

Norduz sheep are well-adapted in the Van Lake region, believed to originate in Eastern Anatolia, Van-Gürpınar, but are endemic and indigenous gene source in danger due to unconscious mating and crossbreeding. Therefore, it is quite important for not only keeping their unique genotype in the region but also detection the genotypes with desirable alleles for gene maintenance and genomic selection programmes in future.

In this study, polymorphic structures of 422 bp GH1 and 934 bp GH loci from growth hormone gene family affecting Growth Parameters in Norduz Sheep were examined. This is the first research performed in Norduz sheep from the farm animals raised in our country based on Growth Hormone genes.

Taking into account the growth parameters almost all the growth parameters showed gradually increased correlations during five different measurament time periods (Table 4.1-4.6). Moreover, looking at the (Table .4.7) BH at 1st and 3rd measurement and GD at 1st measurement males were found statistically significant. For the rest of the growth parameters, there were no differences between genders. Also, the correlations between BW and BH, BH and GC, BL and BH and BSGD and GC was found statistically significant (Table 4.8).

Unfortunately, due to the unfavorable conditions of VYYU Research Farm where the Norduz sheep trying to be extensively cultivated in pasture conditions. 34 animals in the GH1 locus and 26 animals in the GH locus were able to be investigated . If PCR-RFLP were used with equal number of animals (42 sheep) in for both loci, the HWE values previously observed were expected to show different results.

As for GH1 gene polymorhism in 34 Norduz sheep was due to transition at A781G locus. *HaeIII*-RFLP of the GH1 gene (422 bp) revealed the existance of two genotypes; AA (366 and 56bp) and AB (366 and 56bp). Genotypic frequencies for AA and AB were 0.26 and 0.74, and allelic frequencies were 0.57 and 0.43 for A and B respectively. Higly significant ($p<0.001$) Chi-square value18.8 has displayed that the population is not under Hardy-Weinburg equilibrium (HWE) at the GH1 locus.

BB genotype was not observed in this study. Similarly a lack of the homozygous mutant genotype was reported in Vembur sheep (Seevagan et al., 2015). Moreover, have also indicated that complete absence of homozygous BB genotype is due to the natural

selection. Same resarch group also explained that heterozygous animals were well grown and free from all physical deformities. Their results suggests that the mutation A781G in GH1 gene is a recessive lethal. It would be quite helpful effort to add the lethal effects of GH1 locus for the further studies.

Considering *HaeIII*-RFLP of the GH gene (934 bp) polymorhism in 26 Norduz sheep showed the existance of all three genotypes; AA (277, 202 and 110 bp), AB (277, 256, 202 and 110 bp) and BB (256, 202, and 110 bp). Genotypic frequencies for AA and AB and BB were 0.27 and 0.50 and 0.23, and allelic frequencies were 0.52 and 0.48 for A and B respectively. Moderately significant ($p<0.001$) Chi-square value 5.59 has displayed that the population is not also under Hardy-Weinburg equilibrium (HWE). Unlike the research performed by (Othman et al., 2015) HWE analysis displayed narrow ranges in terms of allelic and geneotypic frequencies. One of the possible reasons for GH locus it is just because of screened animal size.

This research has been perfomed on Norduz sheep in Turkiye in terms of GH related genes and the growth trait parameters. Moreover, it is very important to make further statistical analysis to detect the relationships between the growth trait data and genotypic data in order for the ones to make better genetic decisions. The results in this research indicates that molecular markers associated with growth hormone related to growth quantitative characterisitics in Norduz sheep would be explored and exploited for their use in Marker-assisted selection (MAS) in future.

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EXTENDED TURKISH SUMMARY
(GENİŞLETİLMİŞ TÜRKÇE ÖZET)

GİRİŞ

Genetik polymorfizm mutasyondan kaynaklanır. Bireyler arasındaki DNA dizimi farklılığını ifade eder ve gruplar ya da popülasyon tek bir nükleotid baz değişikliğinden yüzlerce bazın değişikliğine kadar uzanan bir mutasyondan dolayı oluşur (Yahyaoui, 2004). Bu yapı, DNA bazlarına eklenme sonucu olanlar ve çiftlerin eklenmesi ya da silinmesi sonucu oluşur. Tek nükleotid polimorfizmi (SNP), purin-purin, primidin-primidin ve purin-primidin pozisyon değişikliklerinden kaynaklanır Genetik polimorfizmin bir diğer türü de DNA tekrar dizileridir (mini ve mikrosatelitler) (Yahyaoui, 2004). Buna ek olarak, PCR ve DNA kesim enzimleri kullanılarak yapılan restriksiyon fragment uzunluk polimorfizmi (PCR-RFLP) gibi moleküler yöntemler , hayvan ıslahı alanında verim yönüne bağlı olarak (et, süt yumurta vb.) daha iyi genotipe sahip hayvanların belirlemesini sağlamak ve gelecekte damızlık olarak kulanmak amacıyla kullanılmaktadır (Zhang, ve ark., 2013). PCR, polimeraz zincir reaksiyonu için kullanılan bir kısaltmadır ve bu yöntemle belli bir DNA bölgesinden milyonlarca eş kopya DNA elde edilir. (Verma, S. 2016). Yani, bir teknik olarak restriksiyon fragment uzunluk polimorfizmi (RFLP) DNA parçalarının farklı uzunluklarını analiz eder ki bu da bir DNA örneğinin spesifik enzimlerle kesilmesi sonucu olur.

Büyüme hormonu (GH), sirkadiyen ve pulsatil bir tarzda hipofiz bezinin ön lobundan somatotrof hücreleri tarafından sentezlenen ve salgılanan bir anabolik hormondur. Yaklaşık 22 kDa moleküler ağırlığa sahip, 190 ya da 191 amino asitten oluşan bir polipetit hormondur. (Ayu ve ark., 2006). Büyüme hormonu birçok metabolik ve fizyolojik eylemleri içerir (Rahbar ve ark., 2013).

Büyüme hormonu, metabolizmayı hızlandırır, ve bilhassa kemik, kas ve iç organlar gibi, çok sayıda organ ve dokunun büyümesini teşvik eder (Ikonen ve ark., 2001). Büyüme hormonu geni, büyüme hormonu salgılanmasında doğrudan bir etkiye sahiptir ve hayvanın büyümesinde önemli rol oynar (Gadelha ve ark., 2012). Bu nedenle, Büyüme hormonu geninin yapısı ve işlevini araştıran çalışmalara, son yıllarda

ilgi oldukça artmıştır. Özel varyasyonu etkileyen majör genlerin belirlenmesi, genetik verinin seçim şemasında kullanılmasını sağlayacak durumla oluşturur.

Öne çıkan genlerden, en çok talep edilen genlere sahip hayvanların seçilmesi, hayvan kazanç miktarını önemli şekilde arttırabilir (Godesky, 2006). Arzu edilen verim özelliklerine etkiyen genlerin polimorfizmi hakkındaki bilgi, ve onun bu varyasyonlar üzerindeki biyolojik etkilerini anlamak, damızlık hayvan seleksiyon programlarında genomik verinin en etkili şekilde ullanılmasını sağlayacaktır. Ekonomik olarak önemli verimlere sahip çiftlik hayvanların seleksiyon programlarının genotipik verilere göre belirlenmesine ve Genetik belirteç yardımlı seleksiyon (MAS) denir (Mehmannavaz ve ark., 2012) .

AMAÇ

Büyüme hormonu (GH) geni organizmanın büyüme gelişiminde önemli rol oynar Bu araştırmanın amacı aynı sezonda doğan Norduz koyunlarında GH ile ilintili genlerin (GH1 ve GH) polimorfizmlerini tespit etmektir.

3. MATERYAL VE YÖNTEM

3.1. MATERYAL

3.1.1. Hayvan Temini

Denemenin hayvan materyali VYYÜ, Araştırma ve Uygulama Çiftlik Müdürlüğüne bağlı koyunculuk biriminde Şubat, 2017 tarihinde doğmaya başlamış yaklaşık 6- 9 aylık yaşta 43 baş dişi ve 7 baş erkek olmak üzere toplamda 50 baş Norduz ırkı kuzudan oluşmuştur.

Ayrıca bu çalışma, VYYÜ Rektörlüğü'nün 26/08/ 2017 tarih ve 27552122-604.01.02-E.59510 sayılı etik kurulu resmi belgesiyle proje çalışma izni alınarak başlatılmıştır.

3.2. Yöntem

3.2.1. Sürüden Hayvan Seçimi, Verim Kayıtları ve Ölçümü

Temmuz, 2017 ayının son haftasından itibaren işletmede doğal merada otlatılan Norduz kuzuları büyüme özellikleri (cinsiyet, canlı ağırlık (CA), cidago yüksekliği (CY), vücut uzunluğu (VU), göğüs derinliği (GD), kürekler arası göğüs derinliği (KAGD) ve göğüs çevresi (GÇ)) gibi kantitatif değerler ölçülmüştür. Ölçümler ortalama 20 günlük periyodik dilimlerde, toplamda 5 kez yapılmış ve son ölçüm 2017 Kasım ayında gerçekleştirilmiştir. Büyümeye ilişkin Kantitatif veriler, terazi, ölçü bastonu ve mezro kullanılarak elde edilmiştir.

3.2.2. DNA İzolasyonu, Gen ve Oligo Belirleme

Kuzuların genomik DNA'sı her bireyden alınan 5ml periferik kandan aşağıda belirttilen standart fenol-kloroform yöntemiyle elde edilmiştir (Sambrook ve ark.,1982).

- $2x10^6$ hücre 1500 rpm'de 5 dakika santrifüj edilip süpernatan uzaklaştırıldıktan sonra pelet 2 kez PBS (Phospahate Buffered Saline) ile yıkanır.
- 300µl lizis solüsyonu (5mM EDTA, 10mM Tris-HCl , pH=8,0, 100mM NaCl, %0,5 SDS (Sodium dodecyl sulfate), 0,5 mg/ml Proteinaz-K) ile yeniden süspanse edilir.
- Vortekslenen karışım bir gece 37^0C'de etüvde inkübe edilir.
- Ertesi gün solüsyon üzerine eşit hacimde doymuş fenol eklenip karıştırıldıktan sonra 1500 rpm'de 20 dakika santrifüj edilir.
- Süpernatan temiz bir tüpe alınıp üzerine eşit hacimde kloroform:izoamil alkol karışımı (24:1) eklenir ve karıştırılır.
- 1500 rpm'de 20 dakika santrifüj edilir ve süpernatan temiz bir tüpe aktarılır.
- Solüsyon üzerine eşit hacimde saf etanol ilave edilir ve yavaşça karıştırılarak DNA'nın presipite olması beklenir. Presipite olan DNA mikropipet ile 1.5 ml'lik mikrofüj tüpüne aktarılır.
- Bir kez saf etanol, bir kez de %70 etanol ile 10 dakika 13000 rpm'de santrifüj edilir, süpernatan atılır ve DNA kurumaya bırakılır.

- Kurutulan DNA, 10mM Tris pH=7,5, 1 mM EDTA eklenerek yeniden süspanse edilir ve ileride kullanılmak üzere -20^{0}C'de bekletilir.

Tablo 3.1. Büyüme Hormonu Genleri (GH) ve PCR Koşullarına İlişkin Bilgileri

Lokus Adı	Kromozom	Forward Primer	Reverse Primer	TM F/R	PCR ürün bç
GH1	OAR 3	5'-ctctgcctgccctggact-3'	3'-ggagaagcagaaggcaacc-5'	57.5	422 bç
GH	OAR 3	5'-ggaggcaggaagggatgaa-3'	3'-ggagaagcagaaggcaacc-5'	58	934 bç

3.2.4. PCR ile Gen Çoğaltma ve Jel Yürütme

Her bir lokus için PCR koşulları özellikle $MgCl_2$ miktarı ve hibridizasyon ısısı açısından optimize edilmiş ve aşağıdaki PCR karışım çözeltileri ve derişimleri kullanılmıştır (Tablo 3.2). Değişik yoğunluklardaki (% 0.8, %1.0, %1.5, %2) Etidium Bromür (EtBr) ile boyanmış agaroz jelde yürütülerek, morötesi (UV) ışık altında görüntülenmiştir.

Tablo 3.2. PCR Master Karışım ve Derişimleri

PCR master karışımı	µl
dH_2O	15.18
10X buffer	2.5
$MgCl_2$	1.5
DMSO	0.32
dNTP	0.5
Primer F	1.5
Primer R	1.5
Taq Polimeraz	0.14
DNA	2.0
	Toplam= 25

3.2.5. PCR programları

95 ºC 10m

96 ºC 40Sc

58 ºC 35Sc

72 ºC 42Sc

72 ºC 10m

3.2.6. PCR-RFLP

PCR ile çoğaltılan GH DNA bölgeleri, DNA kesim enzimi *HaeIII*, (DNA endonukleazı) ile kesilecek şekilde düzenlenmiştir.(Kısaca Tablo 3.3) te gösterilmiştir.

Çizelge 3.3. Lokusların DNA Kesim Enzimleri ile Sanal Olarak Kesilmesi ve Beklenen Parça Uzunlukları

Lokus	DNA Kesim Enzimi	Kesim Sonucu Beklenen DNA Parça Uzunlukları (bç)
GH1	*HaeIII*	366+56= 422
GH	*HaeIII*	277+256+202+110+100+94+68+49= 934

3.3. Allel Çeşitleri ve Frekasının Bulunması

Allel çeşitleri değişik derişimlerindeki (konsatrasyonlarındaki) agaroz jel üzerinde belirlenip, sayılarak aşağıdaki formül kullanılarak sürü içerisindeki heterozigotluk (H) ve Polimorfizm Bilgi Katsayısı (PIC) hesap edilmiştir (Ott, 1992).

Heterozigotluk (H) için;

$H= 1- (\Sigma p_i^2)$

p_i: genin bir allelilin frekansı

Polimorfizm Bilgi Katsayısı (PIC) için;

$PIC= 1- (\Sigma p_i^2)- \Sigma p_i^2 p_j^2$

p_i: genin bir allelilin frekansı

p_j: genin diğer allel frekansları

3.4. İstatiksel Analiz

SAS (Version 9.4) istatistik yazılım paketi kullanılarak büyüme ölçütlerine ilişkin tanıtıcı istatistikler ve bu ölçütler arasındaki korelasyon değerleri hesaplanmıştır.

4. SONUÇ

Kuzuların beslenmesi işletmenin mevcut yemleme prosedürü bağlı olarak yapılmıştır. Otlatma dönemi boyunca VYYÜ Araştırma ve Uygulama Çiftliği Müdürlüğü'ne ait merada anneleri ile birlikte serbest olarak otlatılmış olan Norduz kuzularının büyüme özelliklerine ilişkin temel istatistiki veriler Çizelge 1' de gösterilmiştir. Çizelgede ki fenotipik özellikler; cinsiyet, canlı ağırlık (CA), cidago yüksekliği (CY), vücut uzunluğu (VU), göğüs derinliği (GD), kürekler arası göğüs derinliği (KAGD) ve göğüs çevresi (GÇ) olarak belirlenmiştir.

Başlangıçta toplam 49 koyun (43 dişi, 6 erkek) gözlem altına alınıp, fenotipik verimlerine ilişkin kayıtlar Temmuz, Ağustos, Eylül, Ekim ve Kasım aylarında ortalama 24 günlük periyotlarda 5 defa ölçümler alınarak tutulmuş ancak bunlardan 7 adet koyun genomik DNA için gerekli kan örneklerinin alım gününde merada otlamakta oldukları için araştırma yapılan koyun sayısı genetik analizler için 42 'ye indirgenmiştir.

4.1. İstatistik Analizler

Çizelge 4.1. Canlı Ağırlık (CA) 5 farklı peryodu arasındaki korelasyon katsayıları (pearson korelasyon testi)

Fenotip	CA1	CA2	CA3	CA4	CA5
CA1	1	.127	.213	.231	.284*
CA2		1	.946**	.952**	.919**
CA3			1	.970**	.962**
CA4				1	.969**
CA5					1

*($p<0.05$), **($p<0.01$)

Denetim zamanları ilerledikçe CA diğerleri arasında korelasyonlarında arttığı saptanmıştır. Yalnız CA1 ile CA5 arasında istatistiksel olarak önemli bir ilişki bulunmuştur ($p<0.05$).

Denetim 2 denetim 5'e doğru gidildiğinde denetim 2 ile ilgili korelasyonlar azalmakla birlikte tamanen istatiksel olarak oldukça önemli olduğu anlaşılmıştır ($p<0.01$).

Çizelge 4.2. Cidago Yüksekliği (CY) 5 farklı peryodu arasındaki korelasyon katsayıları (pearson korelasyon testi)

Fenotip	CY1	CY2	CY3	CY4	CY5
CY1	1	.678**	.464**	.624**	.481**
CY2		1	.731**	.697**	.612**
CY3			1	.789**	.519**
CY4				1	.619**
CY5					1

*(p<0.05), **(p<0.01)

Çizelge 4.3. ücut Uzunluğu (VU) 5 farklı peryodu arasındaki korelasyon katsayıları (pearson korelasyon testi)

Fenotip	VU1	VU2	VU3	VU4	VU5
VU1	1	.324*	.483**	.648**	.561**
VU2		1	.320*	.438**	.397**
VU3			1	.632**	.563**
VU4				1	.768**
VU5					1

*(p<0.05),**(p<0.01)

Çizelge 4.4. Göğüs Derinliği (GD) 5 farklı peryodu arasındaki korelasyon katsayıları (pearson korelasyon testi)

Fenotip	GD1	GD2	GD3	GD4	GD5
GD1	1	.432**	.489**	.729**	.603**
GD2		1	.483**	.625**	.496**
GD3			1	.769**	.763**
GD4				1	.685**
GD5					1

*(p<0.05),**(p<0.01)

Çizelge4.5. Kürekler Arası Göğüs Derinliği (KAGD) 5 farklı peryodu arasındaki korelasyon katsayıları (pearson korelasyon testi)

Fenotip	KAGD 1	KAGD 2	KAGD 3	KAGD 4	KAGD 5
KAGD 1	1	.360*	.280	.429**	.284
KAGD 2		1	.478**	.582**	.607**
KAGD 3			1	.650**	.672**
KAGD 4				1	.697**
KAGD 5					1

*(p<0.05),**(p<0.01)

Çizelge 4.6. Göğüs Çevresi (GÇ) 5 farklı peryodu arasındaki korelasyon katsayıları (pearson korelasyon testi)

Fenotip	GÇ1	GÇ2	GÇ3	GÇ4	GÇ5
GÇ1	1	.450**	.728**	.632**	.639**
GÇ2		1	.684**	.761**	.611**
GÇ3			1	.856**	.805**
GÇ4				1	.781**
GÇ5					1

*(p<0.05),**(p<0.01)

Çizelge 4.7. T-Testi Değerleri; Cinsiyete Göre Tanıtıcı İstatistikler

	Cinsiyet	N	Mean	Std. Deviation	Std. Error Mean
CA2	1	43	28.9767	3.70745	.56538
	2	7	29.6714	8.26251	3.12293
CA3	1	41	29.7878	3.51527	.54899
	2	8	30.9750	5.75593	2.03503
CA4	1	41	29.9878	3.68430	.57539
	2	16	29.6125	5.84669	1.46167
CA5	1	42	29.0643	3.69646	.57038
	2	14	29.1286	5.71939	1.52857
CY1	1	44	58.4773	3.43199	.51739
	2	5	61.8000	.44721	.20000
CY2	1	43	59.0465	2.88637	.44017
	2	7	58.7143	7.93125	2.99773
CY3	1	41	60.2439	2.50779	.39165
	2	8	65.6250	7.24938	2.56304
CY4	1	41	60.6585	2.88626	.45076
	2	16	60.8750	5.94278	1.48570
CY5	1	42	60.9286	2.90830	.44876
	2	14	61.0714	3.17355	.84817
VU1	1	44	53.5568	3.06749	.46244
	2	5	53.6000	3.78153	1.69115
VU2	1	43	53.6512	3.84759	.58675
	2	8	51.3750	5.60453	1.98150
VU3	1	41	54.6707	2.64483	.41305
	2	8	55.0000	2.72554	.96362
VU4	1	41	55.3171	3.13998	.49038
	2	16	54.8750	5.25198	1.31300
VU5	1	42	54.8452	3.41176	.52645
	2	14	53.6786	3.29106	.87957
GD1	1	44	23.7159	2.11692	.31914

Çizelge 4.7. T-Testi Değerleri; Cinsiyete Göre Tanıtıcı İstatistikler(devam et)

	Cinsiyet	N	Mean	Std. Deviation	Std. Error Mean
	2	5	26.6000	3.28634	1.46969
GD2	1	43	23.0581	1.50479	.22948
	2	7	23.7143	2.28869	.86504
GD3	1	41	23.8902	1.42088	.22190
	2	8	24.8750	2.16712	.76619
GD4	1	41	23.7561	1.29481	.20221
	2	16	24.1875	2.10456	.52614
GD5	1	42	23.5190	1.46024	.22532
	2	14	23.5000	2.18386	.58366
KAGD1	1	44	15.1136	1.66658	.25125
	2	5	15.6000	1.51658	.67823
KAGD2	1	43	14.0000	1.34519	.20514
	2	7	14.1429	3.23669	1.22336
KAGD3	1	41	13.7073	1.05461	.16470
	2	8	14.1250	1.45774	.51539
KAGD4	1	41	13.4146	1.01798	.15898
	2	16	13.2188	1.12500	.28125
KAGD5	1	42	12.9048	.98920	.15264
	2	14	12.8571	1.46009	.39023
GÇ1	1	44	71.1136	5.15894	.77774
	2	5	76.4000	8.61974	3.85487
GÇ2	1	43	70.6279	3.51204	.53558
	2	7	70.0000	7.72442	2.91956
GÇ3	1	41	69.2195	3.60910	.56365
	2	8	70.0000	4.78091	1.69031
GÇ4	1	41	68.8293	3.03235	.47357
	2	16	68.6250	5.28993	1.32248
GÇ5	1	42	67.4286	3.74259	.57749
	2	14	68.0000	5.21831	1.39465

Çizelge 4.8. Correlations Among Growth Parameters

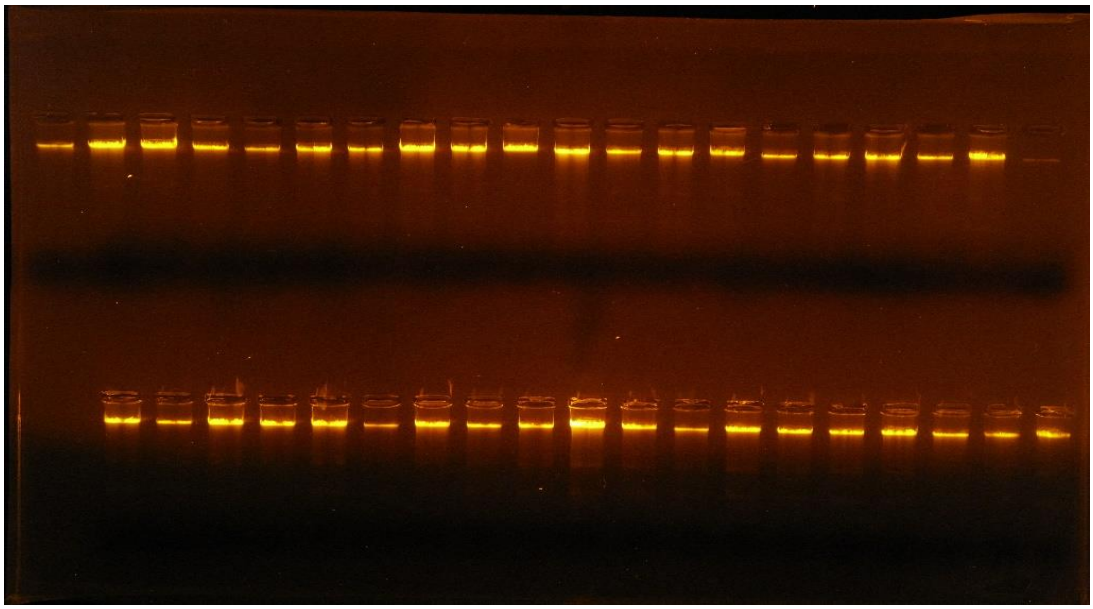
		Canlı ağırlığı	Cidago yüksekliği	Vücut uzunluğu	Göğüs derinliği	Kürekler arası göğüs genişliği	Göğüs çevresi
CanlıAğırlığı	Pearson Correlation	1	.120	.208**	.260**	.193**	.234**
	Sig. (2-tailed)		.082	.002	.000	.005	.001
	N	210	210	210	210	210	210
CidagoYüksekliği	Pearson Correlation	.120	1	.551**	.596**	.256**	.479**
	Sig. (2-tailed)	.082		.000	.000	.000	.000
	N	210	210	210	210	210	210
VücutUzunluğu	Pearson Correlation	.208**	.551**	1	.428**	.287**	.432**
	Sig. (2-tailed)	.002	.000		.000	.000	.000
	N	210	210	210	210	210	210
GöğüsDerinliği	Pearson Correlation	.260**	.596**	.428**	1	.536**	.678**
	Sig. (2-tailed)	.000	.000	.000		.000	.000
	N	210	210	210	210	210	210
KüreklerArasıGöğüsGenişliği	Pearson Correlation	.193**	.256**	.287**	.536**	1	.710**
	Sig. (2-tailed)	.005	.000	.000	.000		.000
	N	210	210	210	210	210	210
GöğüsÇevresi	Pearson Correlation	.234**	.479**	.432**	.678**	.710**	1
	Sig. (2-tailed)	.001	.000	.000	.000	.000	
	N	210	210	210	210	210	210

**. Correlation is significant at the 0.01 level (2-tailed).

4.2. Moleküler Genetik Analizler

4.2.1. Genomik DNA İzolasyonu

VYYÜ, Araştırma ve Uygulama Çiftlik Müdürlüğüne bağlı koyunculuk biriminde yetiştirilen Norduz kuzularından alınan kan örneklerinden DNA izolasyonu yapılmıştır. Daha sonra izole edilen DNA'ların agaroz jel görüntüleri elde edilmiştir. (Şekil 4.1)



Şekil 4.1. Koyun Genomik DNA İzolasyonu % 1 Agaroz Jel, 75 Volt, 30 dk.

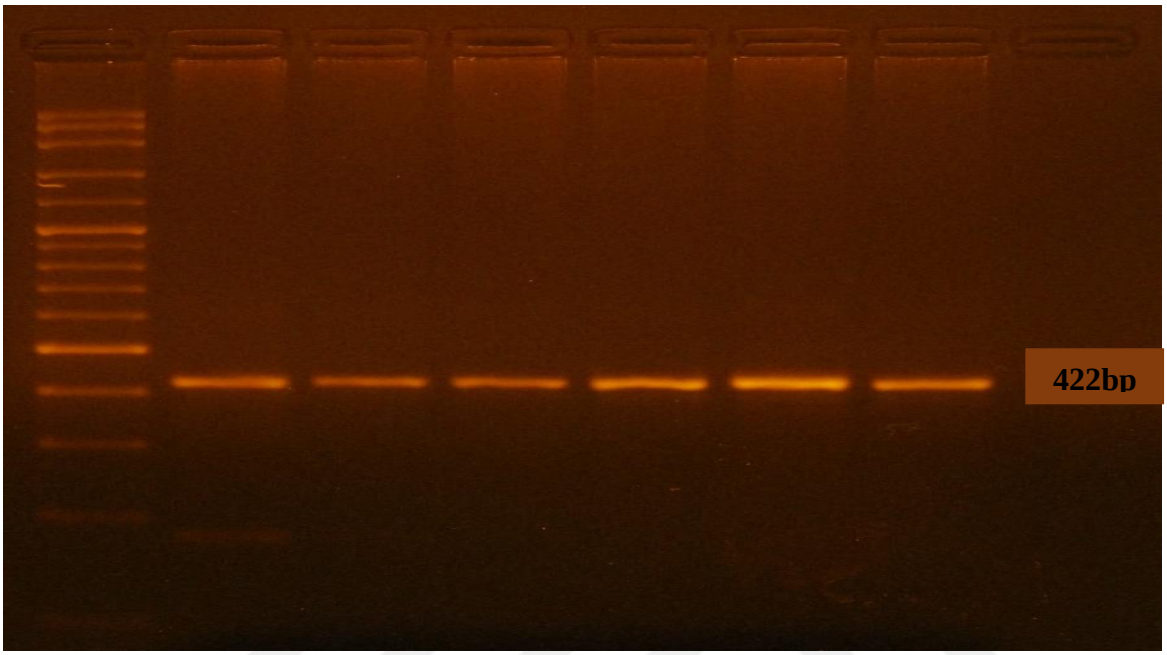
4.2.2. PCR Sonuçları

İzole edilmiş kan örneklerinden Büyüme Hormonu Genleri; referans genomik diziden 2 farklı lokus (GH1 ve GH) belirlenmiş ve belirlenen optimal PCR koşullarında inceleme altına anılana tüm bireylerin genetik taraması yapılmıştır.

GH1 ve GH lokusları, gradient PCR ve touch-down PCR yöntemleri ile değişik uzama sıcakları ile optimum Tm ısıları belirlenmiş ve koyun genomunda PCR ile çoğaltılamamıştır (Çizelge 3.l).

GH1 lokusu optimal uzama sıcaklığı olan 57.5 ºC'de PCR ile çoğaltılmış, 422 bç'lik PCR ürünü elde edilmiş ve 34 baş kuzu bu lokus bakımından genotiplenmiştir. (Şekil 4.2).

GH lokusu optimal uzama sıcaklığı olan 57.5 ºC'de PCR ile çoğaltılmış, 934 bç'lik PCR ürünü elde edilmiş ve 26 baş kuzu bu lokus bakımından genotiplenmiştir.(Şekil 4.3).



Şekil 2.2. GH1 (422 bp) lokusuna ait PCR Sonuçları; Uzama Sıcaklığı: 57.5 ºC
% 2 Agaroz Jel, 75 Volt, 2 sa.

Çizelge 4.9. PCR Master Mix for GH1

PCR master mix	µl	x6
dH_2O	15.1	90.6
10X buffer	2.5	15
$MgCl_2$	1.5	9
DMSO	0.35	2.1
Dntp	1	6
Primer Reverse	1	6
Primer Forward	1	6
Taq Polimeraz	0.2	1.2
DNA	2.5	
	Total= 25	

4.2.3. PCR Programları

GH1 geni için uygulanan PCR koşulları aşağıdaki gibidir.

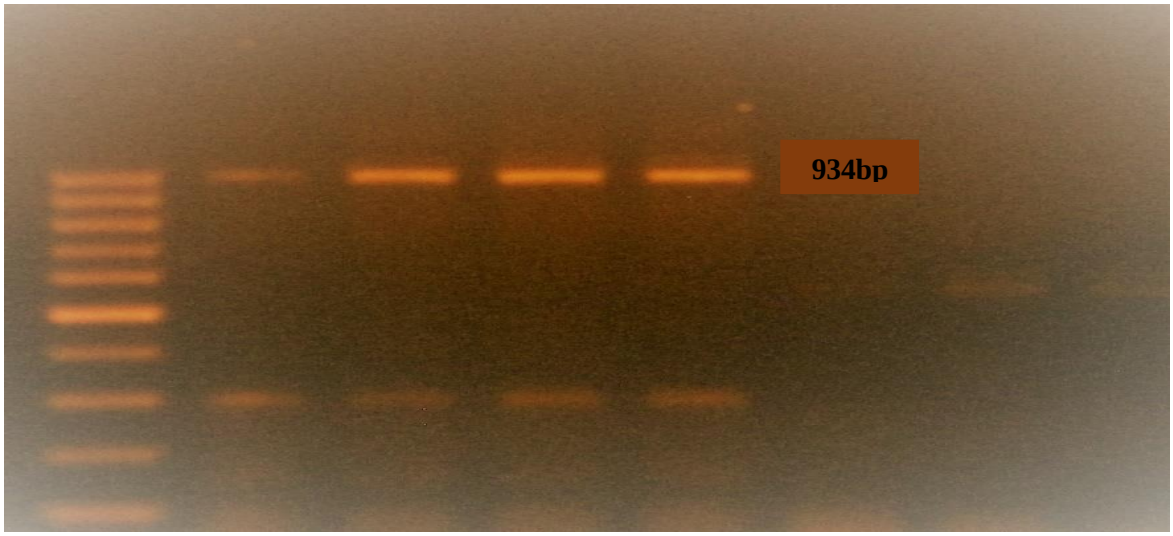
95 ºC 10m

96 ºC 40Sc

58 ºC 35Sc

72 ºC 42Sc

72 ºC 10m



Şekil 4.3. GH (934bp) lokusuna ait PCR Sonuçları; Uzama Sıcaklığı: 57.5 ºC %1.5 agarose, 80V, 2

Çizelge 4.11. PCR Master Mix for GH

PCR Master Mix	µl	x4
dH_2O	17.64	70.56
10 X Buffer	5	20
$MgCl_2$	2	8
DMSO	0.5	2
dNTB	1	4
Reverse Primer GH	1	4
Forward Primer GH	1	4
Taq DNA Polymerase	1	4
1 µl DNA sample	1	
Total Volume	30	116.56

CURRICULUM VITAE

He was born in Sulaymaniyah - Iraq, in 1987. he was completed the primary education in Balfat school. And secondary Asos. During the years of 2008-2012. he had studied in Sulaymaniyah University, the college of Agriculture and Department of animal science in 2012. he had graduated from there. At the February of 2015. he started master study in VAN.

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