

**Investigation of single nucleotide polymorphisms of the *growth hormone* gene  
and its association with the growth traits of Dorper sheep**

by

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## DECLARATION

I declare that the Investigation of single nucleotide polymorphisms of the *Growth hormone* gene and its association with the growth traits of Dorper sheep (mini-dissertation) hereby submitted to the University of Limpopo, for the degree of Master of Science in Agriculture (Animal Production) has not previously been submitted by me for a degree at this or any other university; that it is my work in design and in execution, and that all material contained herein has been duly acknowledged.

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## **DEDICATION**

This mini dissertation is dedicated to my family (Molabe Granny, Molabe Dineo, Molabe Rodney and Molabe Bontle).

## ABSTRACT

Determining body weight from growth attributes is the best, cheapest, and simplest approach, especially in rural areas where farmers lack the resources to purchase weighing instruments to assess an animal's body weight. Gene polymorphisms are regarded as elements to evaluate the animal's economic value and thus serve as a viable technique in improving traits of critical economic importance. The study sought to uncover genetic markers of the growth hormone gene that could be utilized for selection during breeding to improve Dorper sheep growth traits. A total of fifty Dorper sheep (forty-three ewes and seven rams) between the ages of one and two years old were used in the study. Pearson's correlation, simple linear regression analysis, PCR-FRLP, DNA sequences and marker-traits association were used for analysis to achieve the objectives. Coefficient of determination ( $R^2$ ) and mean square error (MSE) were used as the goodness of fit criteria to select the best regression model. Growth traits were measured from all randomly selected Dorper sheep. Growth traits: Heart girth (HG), Body length (BL), Withers height (WH), Sternum height (SH) and Rump height (RH) were measured with a centimetre-calibrated measuring tape (cm). At the same time, Body weight (BW) of each sheep was individually weighed in kilograms (kg) using a balanced weighing scale. Blood samples (2-3ml) were collected from each animal via the external jugular vein in the morning and DNA was isolated and purified using the Noegen's Genomic DNA Isolation kit process. Correlation coefficients ( $r$ ) in ewes indicated that BW had a positive significant relationship with HG ( $r = 0.51$ ), WH ( $r = 0.49$ ) and BL ( $r = 0.41$ ). Whereas in rams, BW showed to be positively and statistically correlated to WH ( $r = 0.78$ ) and SH ( $r = 0.78$ ). Simple linear regression results in ewes demonstrated the highest  $R^2$  value with the lowest MSE on HG while in rams SH and WH had the highest  $R^2$  with the lowest MSE. PCR-RFLP and DNA sequence findings showed a synonymous SNP (T/A) on position 735 of the coding region of the *growth hormone* gene in exon 4 and were named T735A. Moreover, Marker-trait association results showed that there was no statistical relationship between genotypes (AA and AB) and growth traits except for withers height whereby genotype AA had the highest impact on withers height. Correlation results suggest that increasing HG, WH and BL in ewes might cause BW to increase and an increase

in WH and SH might result in an increase in BW in rams. Ram's regression equation with SH shows that an increase in one centimetre of SH will increase BW by 0.84 kg and a model consisting of wither height reveals that an increase in one cm of WH will lead to a body weight increase of 0.60 kg. In ewes, the model with HG implies that an increase in one cm of HG will result in 0.62 kg of body weight. PCR-RFLP and DNA sequence results suggest that animals with genotype AA of *growth hormone* gene might be used when improving withers height. In conclusion, the findings of the current study will assist breeders in advising rural farmers who lack weighing equipment on how to predict the body weight of their animals using growth traits for a variety of reasons, including feeding, medication and breeding purposes. Furthermore, the findings will assist breeders in selecting animals based on molecular genetic markers to optimize withers height. Farmers should be educated on the association between body weight and growth traits, single nucleotide polymorphisms, and the significance of body weight in making good management decisions when feeding, medicating, marketing, and selecting replacement animals. However, more research on growth hormone gene polymorphisms and their association with growth traits need to be done with bigger sample size and more growth traits.

**Keywords:** Correlation, Regression, Heart girth, Withers height, Sternum height

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## LIST OF ABBREVIATIONS

BL	Body length
BW	Body weight
WH	Withers height
RH	Rump height
SH	Sternum height
HG	Heart girth
Cm	Centimetre
CV	Coefficient of variance
DNA	Deoxyribonucleic acid
EDTA	Ethylene diamine tetraacetic acid
GH	Growth hormone
<i>GH1</i>	<i>Growth hormone</i> gene
GLM	General linear model
Kg	Kilogram
G	gram
MAS	Marker assisted selection
ml	Millilitre
MSE	Mean square error
Ns	Non-significant
NCBI	National Center for Biotechnology Information
PCR	Polymerase chain reaction

r	Correlation coefficient
R <sup>2</sup>	Coefficient of determination
RFLP	Restriction fragment length polymorphism
SD	Standard deviation
SNPs	Single nucleotide polymorphisms
SNP	Single nucleotide polymorphism
SPSS	Statistical Package for Social Sciences
μl	Microliter
°C	Degree Celsius
SAS	Statistical analysis software

**CHAPTER ONE**  
**INTRODUCTION**

## 1.1 Background

Sheep farming is amongst the most important sector in the agricultural industries in South Africa, supplying a significant amount of food to the human population (Gorlov *et al.*, 2017). It is extensively practised because it serves as a source of nutrients of significant biological relevance, particularly protein, in the human diet (Saldanha *et al.*, 2022). Valencia *et al.* (2022) documented that sheep contribute to the economy through the production of wool, milk, meat, and skin, and their faecal matter is used as manure. Furthermore, Wahyono *et al.* (2022) stated that sheep are important small-stock that play a role in most rural areas as a source of income, source of food, and they are also used at ceremonial occasions. Dorper sheep are a South African breed that resulted from the crossbreeding of Dorset Horn and Blackhead Persian sheep (Joy *et al.*, 2020). Dorper sheep, according to Issakowicz *et al.* (2018), are distinguished by excellent productivity, good body shape, and good carcass quality.

According to Jahan *et al.* (2013), it is a mutton sheep breed that can survive and adapt to harsh climatic circumstances better than other animals such as Merino breed. Based to Oyieng *et al.* (2022), the breed is resilient and produces a high-quality carcass at a younger age. Body weight has been identified as the most significant attribute in the animal industry when it comes to marketing, additionally, knowing an animal's body weight is crucial for optimal feeding, medicine administration and breeding purposes (Abbas *et al.*, 2021). Goal of the livestock sector is to maintain food security, to have a technique for assessing economically relevant features, and to decide on carcass worth and value (Agamy *et al.*, 2015). In the animal industry, growth traits are of key interest during breeding for economic traits, particularly for meat purposes (Cobra *et al.*, 2013).

Traditional genetic selection methods are selection strategies which only focuses, or selection is based on the animal phenotypic information, they improve important traits such as body weight significantly, however, phenotypic and genetic correlations are estimation of breeding values, and regressions without improving the animal genetically, moreover they are not accurate (Zhao *et al.*, 2015). Marker assisted selection approach is the powerful tool in which researchers recognize, map, and analyse single nucleotide polymorphism (SNPs) of genes associated with growth traits (Bayraktar and Shoshin, 2022; Valencia *et al.*, 2022). Growth hormone (GH) has effect

on animals before birth because it influences cell division, muscle and visceral tissue, cartilage production, growth, and bone formation (Valencia *et al.*, 2022). According to Malewa *et al.* (2014), the *growth hormone* gene is a somatotropin hormone that is released from the pituitary gland's front flap and stimulates bone and skeletal muscle growth and plays a significant part in biological processes such as lactation, metabolism, and animal growth. Hence as documented by Al-Sharif *et al.* (2022), the *growth hormone* gene (*GH1*) has been the candidate gene employed in the majority of research studies since it is connected with nutrient partitioning, which contributes to rapid growth and high performance of the animal. Based on animal selection from gene's perspective, it is critical to maximize the animal's growth and development (Sharma *et al.*, 2013). Hence the aim was to determine the presence of single nucleotide polymorphism of growth hormone gene and its relationship with growth traits of Dorper sheep.

## 1.2. Problem statement

Growth is a biological process that involves the delicate interplay of various components, including genes (Ekegbu *et al.*, 2008). According to Depison *et al.* (2017), it is difficult to make genetic improvements using standard methods (correlation and regression) because they are inaccurate in measuring an animal's genetic value. Customers, on the other hand, have a high desire for heavier sheep, thus why the growth performance of sheep requires greater attention order to match the customers' demand and requirements (Rajni *et al.*, 2014).

The majority of animal genetic improvement for traits has typically been based on animal predicted breeding values, with no knowledge of the gene responsible for the trait (Ibrahim, 2014). Due to a shortage of measuring scales, such as weighing scales, rural farmers rely solely on animal physical appearance and body weight estimates, resulting in improper decisions on drug dosages, marketing and feeding management, as well as poor selection criteria (Lambe *et al.*, 2008; Abdel-Mageed and Ghanem, 2013).

According to Koopaee and Koshkoiyeh (2014), a new class of markers known as DNA molecular markers was developed to improve body weight due to significant advancement in the field of molecular biology, which would assist breeders to make

productive decisions on animal selection and breeding goals. Currently, single-nucleotide polymorphism (SNP), or variation of nucleotide at a single place in a DNA sequence across individuals, is emerging as the standard marker for genetic improvement of animal body weight and growth traits (Othman *et al.*, 2015). Developments in molecular genetics introduced the use of molecular markers to improve animal important traits such as body weight (Ibrahim, 2014), and the usefulness of SNPs has been demonstrated in many studies (Depison *et al.*, 2017; Abd Al-Muhsen *et al.*, 2018; Altwaty *et al.*, 2020). However, few studies have been done on Dorper sheep, hence the current study will contribute to animal selection and breeding.

### 1.3. Rationale

According to Lambe *et al.* (2008), estimating body weight using growth traits is the cheapest and easiest way that communal farmers can adopt. According to Abbas *et al.* (2015), there is improvement in employing the *growth hormone* gene as a marker when increasing economically important traits. Many promising candidate genes for farm animal features are found within the growth hormone axis. In sheep and goats, genetic technology for improved selection of heritable traits through marker-assisted selection enhances genetic production, and research on ruminants verifies the crucial role of *GH1* in growth regulation (Othman *et al.*, 2015).

Genetic gene-associated markers (candidate genes) are widely used in national breeding programs to boost meat yield, and the *growth hormone* gene is the most promising candidate since it has a wide range of biological activity and affects all body cells (Gorlova *et al.*, 2017). Bahrami *et al.* (2013) confirmed the importance of *GH1* as a gene for marker-assisted selection in sheep carcass-trait improvement.

According to Rajni *et al.* (2014), as molecular biology and biotechnology advance, the utilization of marker-assisted selection (MAS) will help sheep breeders in achieving more precise selection aims, as well as in enhancing animal key qualities such as body weight (Ibrahim, 2014) because of its efficacy and promise. Furthermore, *GH1* has been utilized as a marker in various livestock species around the world. According to Cobra *et al.* (2013), there have been documented investigations on genetic variants of the *growth hormone* gene in domestic animals, primarily cattle. According to Koopaee and Koshkoiyeh (2014), molecular markers play an essential role in livestock

structure, genetic variety, and the adoption of marker-assisted selection have a potential to assist breeders in their herds with breeding selection.

It is crucial to note that, single nucleotide polymorphisms of the *growth hormone* gene and their association with growth traits in a Dorper sheep are unknown, therefore, current study on the association of polymorphic gene with economically important traits will help breeders carry out selection programs for economic traits based on genetic markers that predict body weight and growth traits.

#### 1.3.1. Aim

The current study sought to find genetic markers of the *growth hormone* gene that could be used for selection during breeding to improve Dorper sheep growth features.

#### 1.3.2. Objectives

The objectives of the study were to:

- I. Determine the relationship between body weight and growth traits (body length, heart girth, sternum height, withers height and rump height) of Dorper sheep.
- II. Establish the model for estimation of body weight using growth traits (body length, heart girth, sternum height, withers height and rump height) of Dorper sheep.
- III. Identify single nucleotide polymorphisms of the *growth hormone* gene and their relationship with growth traits (body length, heart girth, sternum height, withers height and rump height) in Dorper sheep.

#### 1.3.3. Hypotheses

The null hypothesis tested were:

- I. There is no relationship between body weight and growth traits (body length, heart girth, sternum height, withers height and rump height) of Dorper sheep.
- II. Growth traits (body length, heart girth, sternum height, withers height and rump height) cannot be used to estimate body weight of Dorper sheep.



- III. There is no association between single nucleotide polymorphisms of the *growth hormone* gene and growth traits (body length, heart girth, sternum height, withers height and rump height) of Dorper sheep.

**CHAPTER TWO**  
**LITERATURE REVIEW**

## 2.1. Introduction

Dorper sheep have been identified as the second most popular sire breed for meat characteristics in South Africa by national and international producers because they produce meat with greater tenderness and milder flavor than wool sheep breeds (Villatoro, 2019) and are typically raised under extensive management Cloete *et al.* (2013). Dorper sheep were categorized as an adaptive fast-growing animal providing heavy meat resulting in appealing cuts for retailers and consumers by Villatoro *et al.* (2021). Shackelford *et al.* (2012) stated Dorper sheep have an ability to resist dehydration and drought. According to Sandeep *et al.* (2017), Dorper sheep have a long unrestricted reproductive season, meaning they may breed at any time of year and produce lambs that grow faster, has a greater weaning weight to enable food security. Cloete *et al.* (2021) stated that Dorper sheep mature early, while König *et al.* (2017) agreed that Dorper sheep are valued for their fast growth rate potential and higher mature size.

The motivation for this chapter is to provide a detailed review of literature from various sources on the investigation of single nucleotide polymorphism of the *growth hormone* gene and its association with Dorper sheep growth traits. This chapter will reveal some understanding into various concepts of this investigation and to facilitate the easy exchange of information among different concerned studies in order to improve comprehension of the current study. The origin, characteristics, and significance of body weight of the Dorper sheep are explained. The importance of growth features (heart girth, withers height, body length, sternum height, rump height) as parameters for predicting body weight is also discussed. The effect of the *growth hormone* gene in sheep, as well as Molecular Marker Assistance Selection (MAS) in breeding as the study's focal point, are also discussed.

## 2.2. Origin and features of Dorper sheep

Dorper sheep are a superior South African meat breed developed in 1930 by crossbreeding Black Head Persian sheep and Dorset Horn sheep (Gavojdian *et al.*, 2013) in response to a demand for sheep breed development in terms of adaptability and growth performance (Ayichew, 2019). Dorper sheep, according to Souza *et al.* (2016), can live and survive in harsh environments and climatic conditions. Christensen *et al.* (2011) stated that Dorper sheep are the most adaptable sheep

breed because they are hardy in nature, and their thick skin and coat of a mixture of wool and hair protects them from harsh temperatures and ticks. Dorper sheep are characterised by a fat tail which acts as a fat reserve to be used when there is a lack of water and feed and they possess outstanding carcass traits (Burger *et al.*, 2013).

### 2.3. Significance of body weight

Growth is defined as result of the cooperation of numerous neuro-endocrine processes (Rasouli *et al.*, 2016). An *et al.* (2015) defined gene interaction as the coordinated activity of hormones such as growth, thyroxine, insulin, and prolactin released from important endocrine glands and controlled by the action of their corresponding genes. Growth is defined by Akpa *et al.* (2011) as the entire sum of structural body components measured from growth traits and live weight, and it is mostly used in the livestock business. According to Eydurán *et al.* (2013), live weight is important for proper management, which includes the proper supply of medication and feeds. Body weight is an important factor in animal industry when it comes to selecting animals for various objectives such as breeding and marketing purposes (Lakew *et al.*, 2017).

Animal breeding practices attempt to improve economically significant features. According to Idorenyin *et al.* (2016), body weight is a quantitatively relevant economic feature in the selection of animals for breeding and maximizing production (Kumar *et al.*, 2018). Shirzeyli *et al.* (2013) stated that body weight also helps to monitor animal growth, allowing good decision making in selecting male and female replacements, establish the value of animals, and the efficiency of rearing. Furthermore, knowing the body weight of an animal assist breeders to understand breeding in terms of carcass production per animal, know pedigree to select replacement looking at the weight to avoid complications such as dystocia (Iqbal *et al.*, 2013; Yilmaz *et al.*, 2013).

According to Kumar *et al.* (2018), body weight is the most economically significant attribute, although some other farmers in with limited resources are disadvantaged when selling their animals due to a shortage of weighing scales. Aside from using body weight to decide on breeding, Tsegaye *et al.* (2013) highlighted that knowing the weights of animals helps in calculating market pricing to avoid selling your animal or their product for a price they do not deserve. Chitra *et al.* (2012) agreed and added that animal body weight helps in determining correct drug dosage, whereas Asefa *et al.* (2017) added that knowledge about the body weights of small ruminants such as

sheep plays a vital role in ensuring proper management in terms of feeding the correct amount of feeds to avoid obesity, high body condition score, and anorexia.

According to Prasad. (2010), an animal's body weight is vital for calculating a balanced feed, evaluating growth, and inspecting the animal's health. Birteebi and Ozoje (2012) demonstrated that knowing the exact body weight ensures that farmers or producers are fairly compensated for their efforts in raising the animals. The primary goals of animal breeding are to improve economically important features such as body weight for improved selection and production improvement (Shirzeyli *et al.*, 2013).

#### 2.4. Significance of growth traits as parameters to predict body weight

Determining body weight from developmental features is the best, cheapest, and simplest way, mostly in communal areas where there is a lack of capital for precise weighing equipment (Sandeep *et al.*, 2017). The purpose of the livestock and meat industry, according to Younas *et al.* (2013), is to establish a reliable and objective assessment method for analyzing economic features of animals and predicting weight, prices, and merit of the carcass of a live animal. In the animal industry, growth features are of key interest during breeding for economic traits, particularly for meat production purposes (Cobra *et al.*, 2013). Recent literature revealed that growth traits can be used to supplement body weight, as Babale *et al.* (2018) suggested and wither heights, body length, heart girth and rump height have been found to be reliable in estimating live weight on both traditional and institutional levels worldwide, and they can also be used as indicators of breed origin and linkage within species.

Growth traits evaluate the quantitative characteristics of a carcass or meat and aid in the development of selection criteria depending on the farmer's aims (Kumar *et al.*, 2018). Asefa *et al.* (2017) further revealed that because they are easy and quick to measure, they can be used indirectly to determine body weight. The ability to properly measure and assess features of economic importance, determine animal merit, and value of the corpse while the animal is still alive is a critical goal of the animal industry (Agamy *et al.*, 2015).

Correlation is a prominent measure for determining the link between two qualities (Shirzeyli *et al.*, 2013). Evaluating the association between live body weight and growth traits simplifies the selection of breeding animals that perform best in body

weight (Jahan *et al.*, 2013). Growth traits vary depending on parameters such as sex, breed, and age, but they are an important source of information for determining breed standards (Shirzeyli *et al.*, 2013). Many studies have been conducted in which growth traits serve as predictors of body weight in various livestock species (Babale *et al.*, 2018). Kumar *et al.* (2018) concluded that growth traits serve as animal growth predictors and are important in estimating body weight and carcass characteristics, particularly in rural areas where measuring scales are scarce, and they have been used by other authors to predict body weight in exotic sheep breeds (Birteeb *et al.*, 2012).

According to Kumar *et al.* (2018), growth traits in livestock can be used by farmers to recognize an animal's morphological genetic strength and weakness, as well as to track inheritance. It was also concluded that growth traits can indirectly provide an accurate estimate of body weight, especially when measured in the morning before animals are released to graze or given feeds (Shirzeyli *et al.*, 2013). According to Lakew *et al.* (2017), growth traits are important data sources in terms of reflecting breed standards and can also be used as qualitative growth indicators that reflect animal body changes that occur when the animal grows. Furthermore, knowledge on body weight with several growth traits is an important estimator of genetic correlations between body weight and growth traits for better improvement and management in terms of adequate medical administration for health care (Mohammad *et al.*, 2012). When it comes to selecting superior animals with the goal of making more genetic advancement on reproductive yield, body traits are significant (Tariq *et al.*, 2012).

Growth traits, according to Verma *et al.* (2016), provide information on an animal's skeletal structure, growth, and development capabilities. Several authors have used growth traits to predict body weight in many breeds of sheep (Younas *et al.*, 2013) because growth traits provide important details about the morphological structure and potential of development of the animals (Shirzeyli *et al.*, 2013). Furthermore, a study was conducted in which growth traits such as rump height, withers height, body length, and heart girth were used to predict body weight (Olawumi and Farinnako, 2017).

The ability and capability of knowing and understanding growth traits is critical to producers and purchasers (Babale *et al.*, 2018). As documented by Kumar *et al.* (2018), growth parameters in sheep are utilized to evaluate quantitative meat

attributes. Furthermore, Eghahi *et al.* (2011) revealed the application of growth traits in forecasting body weight and assisting in the establishment of acceptable meat prices. Berhe (2017) stated that growth features, particularly in small-holder farms aid in the long-term improvement of indigenous sheep, and that frequent qualities employed for breed improvement include body length, heart girth, and withers height. Weldeyesus and Yayneshet (2016) showed that the aforementioned growth traits are the most important traits utilized for animal improvement selection, and most researchers conducted studies on this trait as predictors of bodyweight in small animals such as sheep and goats (Eyduran *et al.*, 2017).

#### 2.4.1. Heart girth

The heart girth is the circumference of the body around the chest right below the front legs and withers (Kumar *et al.*, 2018). According to majority of the research, heart girth can be used as a metric to predict body weight in various animals and provide quite accurate findings. Lakew *et al.* (2017) conducted a study in which body weight was predicted from growth traits in Awassi Crossbred sheep and a highly significant, consistent link between body weight and heart girth was discovered, explaining more variation than other variables in all animal age groups. Kumar *et al.* (2018) additionally demonstrated that body weight can be predicted from heart girth. As a result, it was recommended and concluded that heart girth can be used as an independent trait to estimate body weight in rural areas lacking weighing scales, as well as a developing agent for animal genetic improvement, feeding, retail, and medicating services.

In west African Dwarf goats, the association between body weight and growth traits was investigated, and heart girth was found to have a substantial relationship to body weight regardless of gender or location of rearing, when compared to other traits Olawumi and Farinnako, (2017). Furthermore, Temoso *et al.* (2017) found that heart girth was the feature with the highest significant connection with body weight regardless of the animal's sex. According to a study conducted on Hissardale sheep by Younas *et al.* (2013), main growth features such as body length, height at withers, and heart girth revealed to have a significant influence in the early phases of the animal's life cycle, however there is a strong association between heart girth and body weight.

Body weight was estimated using growth traits in three Egyptian Fat-Tailed sheep, and it was reported that a significant relationship between heart girth and body weight was found in Barki sheep Agamy *et al.* (2015); additionally, literature mostly states heart girth as the reliable trait to predict body weight (Abd-Alla, 2014).

According to a study conducted by Sun *et al.* (2020) on Jamuna basin sheep in Bangladesh of various ages, height girth was found significantly associated to body weight in the 1-9 months' group and 1.9-2 years' group. Positive and statistical association shows that body weight can be improved by improving heart girth; additionally, a positive relationship between body weight and heart girth suggests that body weight can be evaluated and improved using heart girth dimension. On other literature, however, showed similar results on animal aged 2 to 6 years (Yilmaz *et al.*, 2013). A study on sheep aged 13 to 24 months indicated that height girth is the best feature to use to determine body weight. This link suggests that animals with comparatively high heart girth are more likely to have high body weight (Mahmud *et al.*, 2014).

Reports on exotic sheep breeds used growth features to measure body weight (Birteeb *et al.*, 2012). According to Kumar *et al.* (2018), the most vulnerable feature is heart girth, which can be used to estimate body weight, particularly in rural locations where weighing instruments are unavailable. Jafari and Hashemi (2014); Petrovic *et al.* (2012), made similar ideas using other sheep breeds. Kumar *et al.* (2018) proposed that farmers who lack the resources to weigh their animals utilize heart girth to measure animal weight.

#### 2.4.2. Body length

Body length is measured from the occipital protuberance to the base of the tail, according to Babale *et al.* (2018). Growth traits, such as body length, are valuable in judging quantitative meat features and suitable selection criteria, and because they are relatively easy to measure, they can be utilized as an indirect predictor of live weight and carcass traits (Agamy *et al.*, 2015). Prasad. (2010) conducted a study and concluded that growth traits can predict body weight. Furthermore, growth traits usually employed in assessing body weight in small ruminants such as goats, sheep, and pigs are body length, chest girth, and height at withers.



Shirzeyli *et al.* (2013) found that various growth features can predict body weight in four sheep breeds, and body length was one of the traits connected to body weight in the Shaal breed. Animal growth features vary by breed, sex, and age, and their association with body weight provides information about an animal's ability to grow and develop. Tariq *et al.* (2012), in a sheep study, body length showed a good association with body weight (Shirzeyli *et al.*, 2013). In addition, Kumar *et al.* (2018) revealed a positive statistical relationship between body weight and body length from a Harnali sheep study. Berhe (2017) stated showed a positive high association between body weight and growth traits in a study on indigenous highland sheep in Tigray were body length had a positive relationship with body weight.

#### 2.4.3. Withers height

According to Agamy *et al.* (2015), an animal's withers height is the distance from the bottom of the front foot (phalanges) to the highest point of withers between the shoulders when the animal is standing. Growth features may function as growth and breed standard indicators, particularly in rural regions. In a study of indigenous sheep breeds, withers height was discovered to be the largest independent variable influencing body weight (Mohammad *et al.*, 2012).

Jahan *et al.* (2013), used multiple statistical analyses to estimate body weight from body features on Balochi male sheep (2013). It was documented that body weight can be predicted from various growth traits, and according to their findings, withers height was one of the traits found to be statistically significant to body weight, implying that an increase in withers height may cause an increase in body weight. Shirzeyli *et al.* (2013) conducted a study on four different breeds namely Mehrabani, Macoei, Zandi, and Shaal breed, withers height was observed to be the trait having positive relationship with body weight in Shaal breed. Agamy *et al.* (2015) reported in Ossimi and Rahmani ram-lambs that withers height was discovered to be associated to body weight, the results agree with reports of Shehata (2013) who indicated that body weight and withers height had a high significant association.

Rather *et al.* (2021) using Kashmir Merino's sheep to assess body weight, discovered a positive phenotypic connection between body weight and all the traits measured, withers height being the greatest predictor of body weight. Petrovic *et al.* (2012) reported genetic and phenotypic correlations between various growth parameters in

Merino and schaf sheep, Jafari and Hashemi (2014) in Makuie sheep, and Kumar *et al.* (2018) in Harnali sheep. Furthermore, withers height was found to be associated with body weight in a study conducted by Mahmud *et al.* (2014) in sheep aged 37 months and up, and it was determined that body weight may be improved indirectly through withers height.

#### 2.4.4. Sternum height

Sternum height was defined by Tyasi *et al.* (2020) as the vertical distance from the lower tip of the sternum to the ground when the animal is upright. Sun *et al.* (2020) predicted body weight using growth features; the results revealed a positive correlation coefficient between body weight and sternum height in a group of sheep aged 1-9 months; sheep of this age are more likely to have relatively high body weight than sheep of other ages. Furthermore, Temoso *et al.* (2017) did a study in Botswana on both sheep and goats, disregarding sex, and found that shoulder height had the highest association with body weight, which is consistent with the findings of Norris *et al.* (2015) on indigenous goats.

According to Tyasi *et al.* (2020), sternum height is the best feature to predict body weight from growth traits of Nguni cattle breed. The results suggested that enhancing sternum height might also improve body weight in Nguni cattle. Yilmaz *et al.* (2011) defined shoulder height as the distance between the height point of the wither and the ground; their findings from a study on the determination of live weights and growth traits of Kangal Sheep support shoulder height as a candidate trait for estimating body weight. Patbandha *et al.* (2018), who reported that sternum height can be used to predict body weight and enhance genetics, reports are consistent with results of the above study.

According to the Pearson correlation results between growth features and body weight on female Ettawa Grade Goats, body weight was shown to be related to shoulder height (Dakhlan *et al.*, 2020). Similarly, Baleseng *et al.* (2016) found that shoulder height has the potential to act as a predictor of weight in both sheep and goats. Previously, Musa *et al.* (2012) found that heart girth was a better proxy measure of weight than shoulder height, but both can be used to estimate the weight of sheep and goats under communal grazing.

#### 2.4.5. Rump height

Rump height is defined by Olawumi and Farinnako, (2017) as the distance between the surface of a platform to the rump when the animal is standing. According to Sun *et al.* (2020), rump height exhibited a high statistical association with body weight in the 1.9-2 years' age group of Jamuna sheep, indicating that sheep in the 1.9-2 years' age group were more likely to have a high body weight. The stronger the association between rump height and BW, the more likely it can be employed as an indirect selection criterion in the field. Lavvaf *et al.* (2012) and Melesse *et al.* (2013) findings agree with results obtained from Jamuna sheep. Body weight was predicted from growth traits in highland sheep, and it was discovered that body weight had a strong positive link with growth traits, and rump height was one of the connected traits (Berhe, 2017).

Deribe *et al.* (2018) on Begait, Gumz, and Rutana sheep to predicted body weight from growth traits, a positive relationship between body weight and some growth traits was found with rump height as one of the traits. Asefa's (2017), reports on indigenous sheep in the Bale zone are comparable to the findings above.

Mahmud *et al.* (2014) documented a study on the estimation of body weight using cannon bone length and other body measurements in Nigerian sheep breeds breeds sheep, and body weight was found to be correlated to growth traits, with rump height being one of them in sheep less than 12 months old. Lavvaf *et al.* (2012) reported similar results in a sheep study. Furthermore, rump height was reported to be the best trait to predict body weight in Awassi sheep where growth traits were used to predict body weight. Furthermore, more studies in the animal industry need to be investigated on prediction of body weight using growth traits to help disadvantaged farmers to know the body weight of their livestock for management purposes, and rump height appeared to be the best candidate to be used to estimate body weight. Ambacolu *et al.* (2017).

#### 2.5. Effects of *growth hormone* gene in sheep

Growth hormone (GH) is encoded by the *growth hormone* gene, which in sheep can be a single copy or duplication (Dettori *et al.*, 2015). Growth hormone (GH), also known as somatotrophic hormone, is a protein containing 191 amino acids (Malewa *et al.*,

2014) and five exons separated by four intervening intron sequences located on the chromosome (Hajihosseini *et al.*, 2013). It is a single polypeptide hormone that improves milk and meat production in farm animals (Moradian *et al.*, 2013) and is involved in a variety of biological processes in farm animal species, including growth, metabolism, lactation, and reproduction (Seevagan *et al.*, 2015).

GH is an anabolic hormone that is released in a circadian and pulsatile way by the somatotroph cells of the anterior lobe of the pituitary gland, influencing all body cells (Ibrahim *et al.*, 2016; Gorlova *et al.*, 2017). According to Kumari *et al.* (2014), growth hormone has a pattern that has a significant impact in postnatal longitudinal growth and development as well as metabolism of protein, carbohydrates, and fats, and its effect is usually spotted on all three body tissues; similarly, An *et al.* (2011) discovered that its effects indirectly relate to bone and skeletal development via the insulin-like growth factor gene. The *growth hormone* gene regulates appetite, aging, reproduction, and immunological responsiveness (Cobra *et al.*, 2013).

The *growth hormone* gene has been utilized as a marker in numerous species, including goats and cattle (Kumari *et al.*, 2014), goats (Mohammadabadi, 2012), and sheep (Ghazi *et al.*, 2014), and its polymorphism has been linked with carcass weight as well as production qualities (Depison *et al.*, 2017) Furthermore, it is the first gene to be used as a candidate in genotype-phenotype studies related to growth and carcass characteristics because it is responsible for aspects of prenatal growth, postnatal growth, lactation, and carbohydrates metabolism (Sarmah *et al.*, 2020). Furthermore, it accelerates metabolism to aid in organ and tissue growth, particularly bones, muscles, and visceral organs.

Yousefi and Azari (2012) concluded that the *growth hormone* gene has an effect on wool quality; additionally, Mahrous *et al.* (2018) reported that higher growth hormone levels resulted in high-yielding animals. GH1 promotes the synthesis of proteins, DNA, RNA, and glycogen, as well as the mobilization of depot fat, fatty acids, and glucose in tissues (Gorlov *et al.*, 2017), and it has a direct effect on the formation and secretion of GH (Jia *et al.*, 2014).

## 2.6. Molecular marker assisted selection in sheep breeding

According to Altwaty *et al.* (2020), it is critical to investigate gene variation in sheep in order to define sheep breeds from a genetic standpoint, and the study allows for the

use of breeding improvement tactics. Animals can now be identified by looking at candidate genes of economic importance or on the DNA level using developments in molecular genetics techniques such as polymorphism because of its potential to aid in genetic selection for future production, and the use of single polymorphic sites (SNPs) has been widely accepted in different livestock namely cattle, sheep, and goats (Abdelmoneim *et al.*, 2017).

According to Kumar *et al.* (2018), accurate and cost-effective procedures that are more exact than traditional breeding strategies or programs are being introduced on a daily basis, with the usage of single nucleotide polymorphism being one of the most recent in molecular breeding. Single nucleotide polymorphism (SNPs), defined as variation at a single location in a DNA sequence between individuals, is currently the most common type of approach used to detect genetic diversity in individual genomes (Deng *et al.*, 2017). Furthermore, Single-Nucleotide Polymorphism (SNP) is emerging as the gold standard for genetic enhancement of animal body weight and physical features (Othman *et al.*, 2015). The use of molecular markers to improve animal key qualities such as body weight was introduced by advances in molecular genetics (Ibrahim, 2014), and the efficacy of SNPs has been established in various research. Single nucleotide polymorphisms can be found in a variety of locations on genes, including exons and introns (He *et al.*, 2016).

Tohidi *et al.* (2013) added that gene-associated markers are now approved in most countries for use in animal husbandry, and there is a significant increase in technology in the molecular industry on DNA-marker-based technology, and they play a role in improving and increasing meat production in terms of composition and quality to meet food security as human population grows, as well as affecting production traits (Abd Al-Muhsen *et al.*, 2018) Furthermore, Kumar *et al.* (2014) emphasized that this breeding technique that takes phenotype and genotype into account in order to acquire precise variation of genes between animals is used not only in animal production but also in plant development around the world.

Furthermore, Depison *et al.* (2017) observed that the use of molecular genetics aids in the genotyping of animals for specific genetic loci. The productivity of genes into the herd using marker assisted selection (MAS) could boost the accuracy of animal traits selection, resulting in substantial genetic gain (Sharma *et al.*, 2013). Implementing

molecular genetics to identify polymorphism of candidate genes associated with growth via marker assisted selection (MAS) provides important and functional information for animal genetic improvement (Cauveri *et al.*, 2016), and the *growth hormone* gene is widely regarded as the best candidate gene for marker-assisted selection in various livestock (Gorlov *et al.*, 2017). Furthermore, Jahan *et al.* (2013) noted that the selection of animals with better genetic features is a priority in sheep breeding. According to Mahrous *et al.* (2018), studying the molecular structure of genes and function in the breeding industry helps to know genetic information carried by an individual and decide on passing the trait to the herd. There are also several applications that have been implemented to determine genetic variation within populations, and single nucleotide polymorphism is one of them.

According to Ibrahim *et al.* (2016), the use of nucleotide patterns of candidate genes plays a role in the growth, development, lactation, and reproduction of farm animals. Furthermore, genetic markers are used in the wool industry as an aid for animal assessment for potential wool production and also assist in accurate selection. GH1, with its functional and positional potential, has been utilized as a marker in numerous livestock species, including cattle and goats, and marker assisted selection (MAS) can achieve an accurate and efficient selection target (Kumari *et al.*, 2014).

## 2.7. Conclusions

There is high demand of food by consumers as human population increases. Agricultural industries especially sheep industry requires greater attention to ensure food security. Breed improvement is vital, hence the goal of this study was to look into single nucleotide polymorphisms in the *growth hormone* gene and its relationship to Dorper sheep growth qualities. Growth qualities are the best and cheapest way to forecast body weight, and they are largely utilized by disadvantaged farmers who lack weighing equipment and need to know the weights of their animals for breeding, nutrition, marketing, and health management. Body length, heart girth, and withers height are desirable sheep breed features. Because of amazing progress in the field of molecular biology, a new class of markers known as DNA molecular markers has been identified to enhance body weight, allowing breeders to make more informed decisions about animal selection and breeding. The advancement of molecular biology and biotechnology, as well as the use of marker-assisted selection (MAS), will assist

sheep breeders in achieving more accurate selection goals, as well as in improving animal important traits such as body weight (Ibrahim, 2014), and *GH1* has been widely used as a marker in several livestock species due to its functionality and potential. However, studies on single nucleotide polymorphism of the *growth hormone* gene and its association with growth traits of a Dorper sheep are not yet known. Current study on the relationship of polymorphic gene with economically important traits will assist breeders in carrying out selection programs for economic traits based on genetic markers that predict body weight and growth traits.

**CHAPTER THREE**  
**METHODOLOGY AND ANALYTICAL PROCEDURES**



### 3.1. Ethical approval

Ethical approval (AREC) number AREC/08/2021: PG was granted by the University of Limpopo Animal Research Ethics Committee (ULAREC) prior to the commencement of the study.

### 3.2. Study area

The current study was carried out at the University of Limpopo's Experimental farm, which is located 10 kilometers west of the University. The farm has semi-arid climatic conditions, with winter temperatures ranging from 5°C to 28°C and summer temperatures ranging from 10°C to 36°C, and an annual rainfall of less than 400mm (Kutu and Asiwe, 2010). The laboratory work was carried out in the Department of Biochemistry, Microbiology, and Biotechnology at the University of Limpopo, South Africa.

### 3.3. Experimental animals

The experiment was conducted using fifty (forty-three ewes and seven rams) Dorper sheep between the age of one and two years.

### 3.4. Animal management

The animals were raised under extensive management system which allows animals to graze outside during the day and recalled back to their camps made of 20m length 8m width 3m height steel with a steel gate built in the afternoons. *Ad libitum* access to clean water was provided. To prevent all possible diseases, vaccination and dipping programs were carried out on a regular basis prior to lambing, weaning, and breeding. Animals received a dose of Multivax-P Plus vaccine at an interval of 4 to 6 weeks, and the dipping (Organophosphate and Pryrethroids) program was carried out at an interval of 14 days per season. Animal's life routine was not changed, they were allowed to live their normal lives, including the ability to remain in their natural habitat during and after the study. Animals were visually monitored and evaluated for well-being twice a day, once in the morning before they left to graze and once in the afternoon after they returned from grazing. To restrain the animal and reduce movement during data collection, a well-built functional handling facility with a crowding pen, working crush, and head gate was used.

### 3.5. Research design

The current study used a cross-sectional experimental design where data will be collected once per animal. Data was collected from Dorper sheep once in the morning. The data collection period lasted one day.

### 3.6. Data collection

#### 3.6.1. Measurement of traits

Body length (BL), heart girth (HG), sternum height (SH), withers height (WH), and rump height (RH) of Dorper sheep were measured using a measuring tape calibrated in centimetres (cm). At the same time, the body weight of each sheep was measured in kilograms (kg) using a ST-0606 sheep, goat and pig professional scale (crate scale 300kgx100g) from Scale Tronic services (46 Platinum Street Polokwane). Growth trait measurements were taken in accordance with the recommendations of (Birteeb *et al.*, 2012). Shortly, withers height was measured as the vertical distance from the highest point of the shoulder (withers) to the ground surface in relation to the level of the forelegs, body length was measured from the anterior shoulder point to the posterior extremity of the pin bone, sternum height as the vertical distance was measured from the lower tip of the sternum to the ground as the animal stands, heart girth was measured as the body circumference just behind the scapula and rump height was measured from the top of the pelvic girdle to the ground surface (Figure 3.1). To avoid bias, measurements were all taken by the same person.

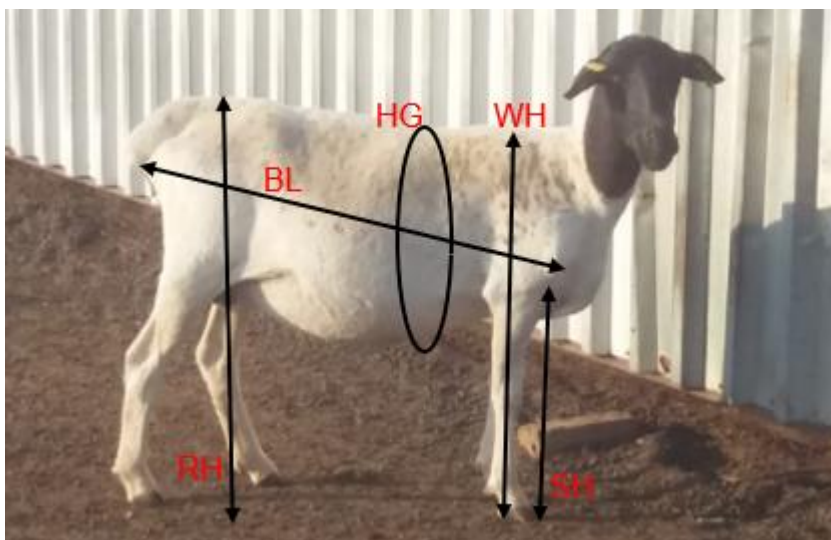


Figure 3.1: Growth traits that were measured in Dorper sheep

### 3.6.2. Blood sample collection and DNA purification

Blood samples (2-3 ml) were collected via the external jugular vein of each Dorper sheep (n = 50) at once by a veterinarian using a 21-gauge needle and a 5 (ml) syringe. Blood samples were collected into 10 mL EDTA-coated tubes and stored at 4 °C until use. Following the manufacturer's protocol, DNA was isolated and purified from blood samples using the Norgen's Genomic DNA Isolation kit (Norgen Bioteck Corp, Canada). A UV spectrophotometer (Thermo Fisher Scientific, India) was used to determine the purity and concentration of DNA samples, while agarose gel electrophoresis was used to determine their integrity.

### 3.6.3. DNA amplification by polymerase chain reaction (PCR)

The *growth hormone* gene was amplified using PCR. Primers to amplify the *growth hormone* gene were designed using Primer Premier 5.0 software based on the sequence in the National Centre for Biotechnology Information (NCBI) database sequences (GenBank accession No.GQ452268) (PREMIER Biosoft, Palo Alto, CA, USA). Table 3.1 lists the primers used to amplify the *growth hormone* gene.

Table 3.1: Identity of primers used for *Growth hormone* gene amplification

Primer	Sequence	Annealing temperature (°C)
Forward	GGAGGCAGGAAGGGATGAA	60
Reverse	CCAAGGGAGGGAGAGACAGA	

PCR reaction was carried out in a 50 µl reaction mixture containing 7 µl of genomic DNA, 25 µl of master mix, 1 µl of each forward and reverse primer and 16 µl of de-ionised double-distilled water. PCR amplification was carried out following the thermal cycling conditions as described by Gorlov *et al.* (2017): Pre-denaturation at 95 °C for 5 min and then 33 cycles of 95°C for 45s for denaturing, 60°C for 45s for annealing, 72°C for 45s for extension and final synthesis at 72°C for 10 min. The resulting products were separated by electrophoresis on 1.2 % agarose gel stained with ethidium bromide, visualised and captured under a UV trans-illuminator (Spectroline).

#### 3.6.4. Genotyping

Restriction fragment length polymorphism (RFLP) was used for genotyping PCR products. PCR products were digested with *HaeIII* restriction endonuclease and electrophoretically separated. For RFLP analysis, 20 µl of PCR products, 5 µl of PCR buffer, 23 µl of distilled water and 2 µl of *HaeIII* enzyme together to make a total of 50 µl were incubated in the Thermo scientific incubator for 20 hours at 36.9°C. The restriction endonuclease digestion solutions were mixed with 4 µl of loading dye, then loaded on 1.2% agarose gel stained with ethidium bromide, visualised, and photographed by U.V. trans-illuminator (Spectroline).

#### 3.6.5. DNA sequencing

PCR amplicons of the *growth hormone* gene were purified and sent to Inqaba Biotechnology Company (525 Justice Mahommed St Muckleneuk, 200, Pretoria, South Africa) for DNA sequencing. Following sequencing, the NCBI/BLAST/blastin site was used to perform sequence alignment.

#### 3.7. Statistical analysis

Statistical Package for Social Sciences (IBM SPSS, 2020) software version 27 was used for statistical analysis. To achieve objective 1, Pearson's correlation coefficient was used to estimate the relationships between body weight and growth traits. objective 2 which is to estimate the best model to predict live body weight using various growth traits was achieved by simple linear regression analysis. Body weight was used as a dependent variable while growth traits were used as independent variables; only correlated variables were used, beginning with the most highly correlated.

The following model was used for regression analysis:

$$Y = a + b_1X_1$$

Where Y = dependent variable (body weight)

a = regression intercept

b<sub>1</sub> = coefficient of regression

$X_1$ = independent variable (s) (withers height, body length, sternum height, heart girth and rump height).

Coefficient of determination ( $R^2$ ) and mean square error (MSE) were used to select the best-fitted regression model.

PCR-FRLP and DNA sequencing were used to determine single nucleotide polymorphisms, and the general linear model (GLM) was performed for Marker-Trait Association analysis to achieve objective 3. The following GLM model of SAS 9.4 software (IBM SAS, 2019) was used for marker-trait association analysis:

$$Y_{ij} = \mu + G_i + e_{ij}$$

Where:

$Y_{ij}$  = Phenotypic values of  $i^{\text{th}}$  trait on  $j^{\text{th}}$  genotype

$\mu$  = Population mean

$G_i$  = Fixed effect of  $i^{\text{th}}$  genotype

$e_{ij}$  = Random residual error

Genetic equilibrium of the population under Hardy- Weinberg theorem was measured using chi-square test and for Population Genetic Analysis, POPGENE version 1.32 software was used to calculate allelic and genotypic frequencies.

**CHAPTER FOUR**  
**RESULTS**

#### 4.1. Descriptive statistics

Data of all the measured traits was summarised by descriptive statistics. Table 4.1 displays descriptive statistics for Dorper sheep growth traits for both ewes and rams. The summary revealed that ewes had a higher numerical mean value of 33.35 kg than rams (30.53 kg). When compared to rams, ewes had higher WH, SH, HG and lower RH and BL. In ewes, the coefficient of variance ranged from 5.98 percent to 20.42 percent, while in rams, the coefficient of variance ranged from 3.81 percent to 84.81 percent.

Table 4.1: Descriptive statistics of growth traits of Dorper sheep

Traits	Minimum	Maximum	Mean	Std. Deviation	CV (%)
----- Ewes -----					
BW (kg)	20.40	44.50	33.35	6.81	20.42
WH (cm)	55.00	69.00	60.74	3.63	5.98
RH (cm)	55.00	72.00	62.28	4.78	7.68
BL (cm)	60.00	88.00	68.47	6.33	9.27
SH (cm)	37.00	49.00	41.21	3.07	7.45
HG (cm)	74.00	97.00	83.95	5.69	6.78
----- Rams -----					
BW (Kg)	25.20	36.00	30.53	4.13	84.81
WH (cm)	56.00	69.00	60.71	5.41	8.91
RH (cm)	61.00	78.00	66.86	6.23	9.32
BL (cm)	69.00	88.00	74.86	6.57	8.78
SH (cm)	38.00	49.00	40.86	3.85	9.42
HG (cm)	78.00	87.00	82.71	3.15	3.81

BW: Body weight, WH: Withers height, RH: Rump height, BL: Body length, SH: Sternum height, HG: Heart girth, CV: Coefficient of variance, Std. Deviation: Standard deviation.

#### 4.2. Phenotypic correlation between growth traits of Dorper sheep

Pearson's correlation was used to establish the relationship between measured variables. Table 4.2 demonstrates the relationship between growth traits (BW and HG, WH, BL, SH, RH) of Dorper sheep with ewes results below diagonal and rams' results above diagonal. Pearson's correlation in ewes demonstrated that the relationship between growth traits ranged from 0.10 to 0.51, BW showed a highly positive and showed to be statistically correlated to HG at  $p < 0.01$ , positive statistical correlation to WH and BL at  $p < 0.05$ , however showed no significant relationship with RH and SH ( $p > 0.05$ ). Amongst traits, WH had a positive relationship with RH and HG ( $p < 0.05$ ) whereas showed no significance to BL and SH.

Correlation results above diagonal (rams) revealed that the relationship between growth traits ranged from - 0.28 to 0.78. Correlation reports showed BW to be highly positively and statistically correlated to WH and SH at  $p < 0.01$ . However, had a negative statistical correlation with RH and positive statistical correlation with HG at  $p < 0.05$ . No significant relationship was observed between BL and BW ( $p > 0.05$ ). Relationship within traits, WH was negatively and highly correlated to RH, highly and positively correlated to SH at  $p < 0.01$ , positively correlated to BL ( $p < 0.05$ ) however, no significance correlation with HG ( $p > 0.05$ ). However, RH was not significantly correlated to HG ( $p > 0.05$ ), however, negatively correlated to BL and SH ( $p < 0.05$ ). BL had no statistical relationship with SH ( $p > 0.05$ ) however, it was found be negatively correlated to HG ( $p < 0.05$ ) lastly SH showed a highly statistical correlation with HG ( $p < 0.01$ ).



Table 4.2: Phenotypic correlation between growth traits of Dorper sheep with ewes below diagonal and rams above diagonal

Traits	BW	WH	RH	BL	SH	HG
BW (Kg)		0.78**	-0.28*	0.06 <sup>ns</sup>	0.78**	0.34*
WH (cm)	0.49*		-0.76**	0.39*	0.79**	0.19 <sup>ns</sup>
RH (cm)	0.10 <sup>ns</sup>	0.35*		-0.41*	-0.45*	0.14 <sup>ns</sup>
BL (cm)	0.41*	0.14 <sup>ns</sup>	0.11 <sup>ns</sup>		0.05 <sup>ns</sup>	-0.36*
SH (cm)	0.10 <sup>ns</sup>	0.25 <sup>ns</sup>		-0.15 <sup>ns</sup>		0.70**
HG (cm)	0.51**	0.34*	0.04 <sup>ns</sup>	0.17 <sup>ns</sup>	0.19 <sup>ns</sup>	

BW: Body weight, WH: Withers height, RH: Rump height, BL: Body length, SH: Sternum height, HG: Heart girth, \*\*: Correlation is significant at the 0.01 level (2-tailed), \*: Correlation is significant at the 0.05 level (2-tailed), <sup>ns</sup>: non-significant.

#### 4.3. Simple linear regression for estimation of body weight

Models for prediction of BW using WH, BL, SH and RH as independent variables generated from simple linear regression analysis of ewes and rams are shown in Table 4.3. In ewes, highest  $R^2$  (0.27) was obtained from HG indicating that the model ( $BW = -18.47 + 0.62HG$ ) may be used to estimate BW of Dorper sheep. In rams, SH and WH may be used to predict and improve body weight. Their model ( $BW = -3.70 + 0.84SH$ ) and ( $BW = -5.55 + 0.60WH$ ) showed equal  $R^2$  value of 0.61.

Table 4.3: Simple linear regression of ewes and rams of the Dorper breed

Regression parameters	Model	MSE	R <sup>2</sup>	P value
Ewes				
HG (cm)	-18.47 + 0.62HG	34.88	0.27	0.00
WH (cm)	-22.61 + 0.92WH	36.07	0.24	0.00
BL (cm)	3.29 + 0.44BL	39.07	0.17	0.00
SH (cm)	24.11 + 0.22SH	47.02	0.10	0.52
RH (cm)	24.35 + 0.14RH	47.01	0.10	0.52
Rams				
SH (cm)	-3.70 + 0.84SH	7.95	0.61	0.04
WH (cm)	-5.55 + 0.60WH	8.03	0.61	0.04
RH (cm)	42.72 - 0.18RH	18.87	0.08	0.55
BL (cm)	27.66 + 0.04BL	20.34	0.00	0.90
HG (cm)	5.54 + 0.30HG	19.33	0.05	0.62

WH: Withers height, RH: Rump height, BL: Body length, SH: Sternum height, HG: Heart girth, MES: Mean square error, R<sup>2</sup>: Coefficient of determination.

#### 4.4. Amplification and Restriction fragment length polymorphism (RFLP) analysis of the *growth hormone* gene

A segment of exon 4 of the *GH1* of Dorper sheep was amplified using PCR to determine the size of the gene. A 934 bp PCR amplicon size was obtained as shown in Figure 4.1A. A total of 50 Dorper sheep *GH1* amplified products were genotyped with PCR-RFLP analysis to detect the polymorphism on exon 4. The PCR-RFLP electrophoretic results as presented in Figure 4.1B revealed different band patterns, some with one band and the others with two bands. In all samples of Dorper sheep assessed in this study, PCR-RFLP recognised two genotypes (AA and AB) in the coding region of the *GH1*.

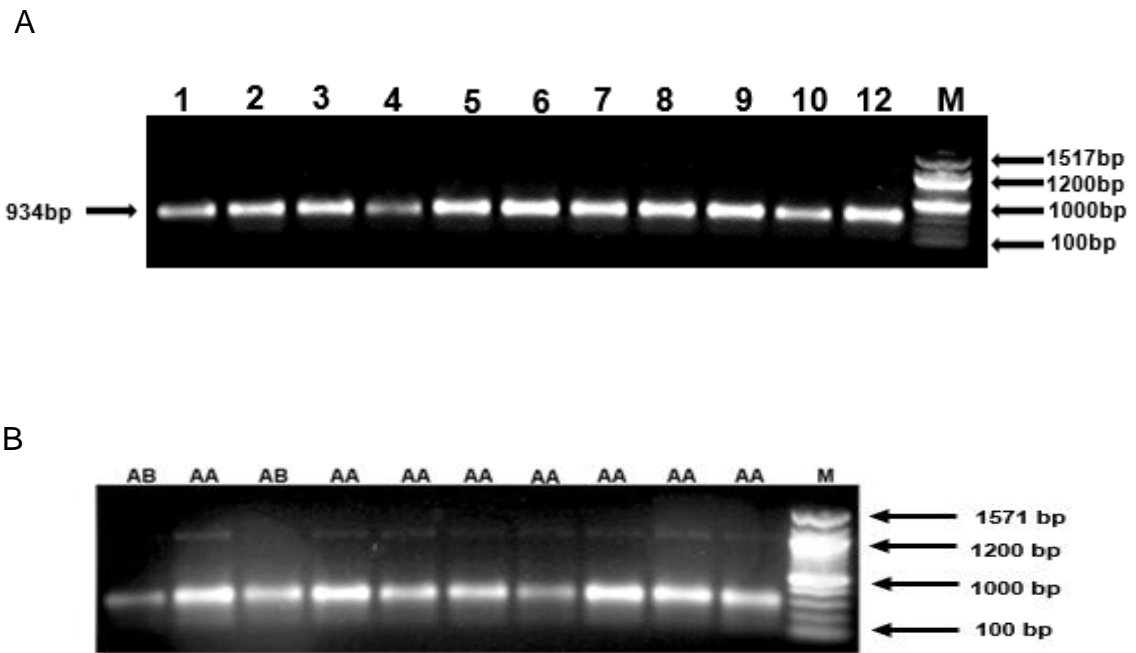


Figure 4.1: PCR products from the amplification of exon 4 region of *GH1* gene in Dorper sheep(A). PCR-RFLP band patterns of exon 4 PCR product of the *growth hormone* gene in Dorper sheep (B).

#### 4.5. Amplified sequence analysis of *growth hormone* gene

DNAMAN and chromas was used to analyse the sequence. In the *GH1* fragment of exon 4, T/A transition at base position 735 of the coding region was detected as the single nucleotide polymorphism following DNA sequencing (Figure 4.2) and was named as single nucleotide polymorphism (SNP) T735A.

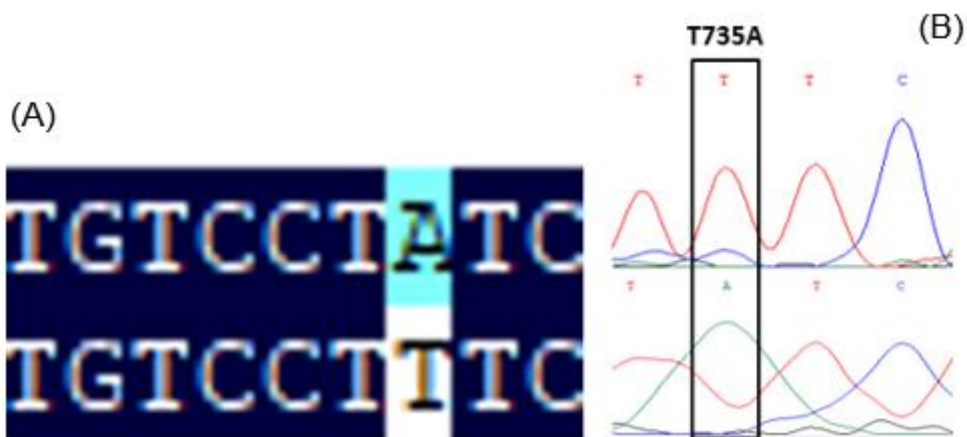


Figure 4.2: Nucleotide sequence analysis showing the SNP T735A site on exon 4 of the Dorper sheep *growth hormone* gene(A). Sequence analysis using DNAMAN; (B): Sequence analysis using chromatograms.

#### 4.6. Gene sequence alignment

Pairwise DNA sequence alignment and blast software from the NCBI database was carried out to identify the single nucleotide polymorphism position. As shown in Figure 4.3, a SNP was noted on position 735 of the exon 4 PCR product.

SUBJECT	601	TACCTGAGGGTCATGAAGTGTGCGCCGCTTCGGGGAGGCCAGCTGCGCCTT	650	
Query	601	TACCTGAGGGTCATGAAGTGTGCGCCGCTTCGGGGAGGCCAGCTGCGCCTT	650	
SUBJECT	651	CTAGTTGCCAGCCATCTGTTGTTACTACCCCTCCCCTGTGCCTTCCTAGA	700	
Query	651	CTAGTTGCCAGCCATCTGTTGTTACTACCCCTCCCCTGTGCCTTCCTAGA	700	
SUBJECT	701	CCCTGGAAGGTGCCACTCCAGTGCCCACTGTCCTTTCCTAATAAAGCGAG	750	
Query	701	CCCTGGAAGGTGCCACTCCAGTGCCCACTGTCCTATCCTAATAAAGCGAG	750	
SUBJECT	751	GAAATTGCATCAC	763	
Query	751	GAAATTGCATCAC	763	

↓  
SNP of *Growth hormone* gene  
in Dorper sheep

Figure 4.3: Sheep *GH1* nucleotide sequence retrieved from NCBI with accession number: NM\_001009315.3. The query is the sheep *GH1* nucleotide sequence of an SNP nucleotide sequence.

#### 4.7. Protein sequence alignments

Pairwise *GH1* protein sequence alignment and blast software from the NCBI database was carried out to identify the type of single nucleotide polymorphism. A pairwise *GH1* protein sequence alignment (Figure 4.4) demonstrated that transition T/A referred to as T735A leads to a synonymous amino acid exchange. Change in nucleotide (T/A) on position 210 does not change in amino acid F(Phenylalanine).

Score	Expect	Method	Identities	Positives	Gaps
449 bits(1156)	2e-168	Compositional matrix adjust.	217/217(100%)	217/217(100%)	0/217(0%)
Query 1		MMAAGPRTSLLLAFTLLCLPWTQVVGAFPAMSLSGLFANAVLRAQHLHQLAADTFKEFER			60
Sbjct 1		MMAAGPRTSLLLAFTLLCLPWTQVVGAFPAMSLSGLFANAVLRAQHLHQLAADTFKEFER			60
Query 61		TYIEGQRYSIQNTQVAFQCFSETIPAPTGNKAEQKSDLELLRISLLLIQSWLGPLQFLS			120
Sbjct 61		TYIEGQRYSIQNTQVAFQCFSETIPAPTGNKAEQKSDLELLRISLLLIQSWLGPLQFLS			120
Query 121		RVFTNSLVFGTSDRVYEKLDLEEGILALMRELEDVTPRAGQILKQTYDKFDRNMRSDDA			180
Sbjct 121		RVFTNSLVFGTSDRVYEKLDLEEGILALMRELEDVTPRAGQILKQTYDKFDRNMRSDDA			180
Query 181		LLKNYGLLSCFRKDLHKTETYLKRMKCRFFGEASCAF	217		
Sbjct 181		LLKNYGLLSCFRKDLHKTETYLKRMKCRFFGEASCAF	217		

↓  
Synonymous SNP

Figure 4.4: Pairwise *GH1* protein sequence alignment. Subject is a sheep *GH1* protein sequence retrieved from NCBI with accession number: >NP\_001009315.2. Query is the sheep *GH1* protein sequence of a SNP nucleotide sequence.

#### 4.8. Genotypic and allelic frequencies

Population Genetic Analysis was used to calculate allelic and genotypic frequencies of the studies population and genetic equilibrium of the population under Hardy-Weinberg theorem was measured using chi-square test. Allelic and genotypic frequencies for the *GH1* single nucleotide polymorphism are presented in Table 4.4. Two allele (A and B) and two genotypes (AA and AB) were noted where frequency of allele A was found to be higher than of allele B, moreover genotypic frequency of genotype AA was also found to be higher than of genotype AT. Chi-square test showed that the population's genotypic and gene frequencies were similar to the expectations of Hardy-Weinberg ( $X^2 = 1.56$ ). Chi-square test results demonstrated that the allelic and genotypic frequencies *GH1* SNP are under Hardy-Weinberg equilibrium, suggesting that the population's gene and genotypic frequencies remain constant from generation to generation.

Table 4.4: Genotypic and allelic frequencies at the single nucleotide polymorphism locus of *GH1* in Dorper sheep

Genotype	Number of animals	Genotypic frequency	Allele	Allele frequency	$\chi^2$
AA	35	0.7	A	0.85	1.56 <sup>ns</sup>
AB	15	0.3	B	0.15	

\*  $p < 0.05$ : Statistically significant when the data were analysed using a Pearson's goodness-of-fit chi-square test (degree of freedom = 1), <sup>ns</sup>: non-significant ( $p < 0.05$ ).

#### 4.9. Polymorphism information analysis

Polymorphism information analysis and genetic diversity of the population was achieved by Population Genetic Analysis. *Growth hormone* gene genetic diversity parameters ( $H_o$ ,  $H_e$ ,  $N_e$  and  $PIC$ ) for the SNP are shown in Table 4.5. Outcomes revealed that gene homozygosity was lower than gene heterozygosity with the effective allele number of 1.34 and lower polymorphism information content.

Table 4.5: Polymorphism information analysis of *growth hormone* gene of Dorper sheep

Gene homozygosity ( $H_o$ )	Gene heterozygosity ( $H_e$ )	Effective allele number ( $N_e$ )	Polymorphism information content ( $PIC$ )
0.74	1.56	1.34	0.22

#### 4.10. SNP genotypes of *growth hormone* gene associated with growth traits of Dorper sheep

General linear model (GLM) was performed for marker-trait association. Association between marker-traits are displayed in Table 4.6. Reports showed that no significant difference ( $pp > 0.05$ ) was observed between AA and AB genotype regarding BW, RH, BL, SH, and HG. T735A (*GH1*) showed to have a statistical relationship with WH ( $p < 0.05$ ), genotype AA was remarkably related to higher WH than genotype AB.

Table 4.6: Association of the polymorphism of *GH1* with growth traits of Dorper sheep

Traits	AA (n = 35) (Mean ± SE)	AB (n = 15) (Mean ± SE)
Body weight (Kg)	33.17 ± 1.19	33.47 ± 1.41
Rump height (cm)	62.46 ± 0.83	62.27 ± 0.88
Body length (cm)	68.97 ± 1.04	67.33 ± 1.66
Sternum height (cm)	40.97 ± 0.51	41.87 ± 0.73
Withers height (cm)	61.06 ± 0.61 <sup>a</sup>	57.53 ± 1.55 <sup>b</sup>
Heart girth (cm)	83.83 ± 1.63	84.00 ± 1.47

SE: Standard error, n: Number of animals. Different superscript on the same row shows significant difference ( $p < 0.05$ ).

**CHAPTER FIVE**  
**DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS**



## 5.1. DISCUSSION

Body weight estimation using growth traits is the simplest and most cost-effective method available to communal farmers (Sun *et al.*, 2020). Shirzeyli *et al.* (2013), indicated that there is an increase in studies on the prediction of body weight using different growth traits on different animal breeds. The current study's first objective was to use Pearson's correlation to determine the relationship between body weight and body length, heart girth, sternum height, withers height, and rump height in Dorper sheep. Except for rump height and sternum height in ewes, body had a positive statistical correlation with all measured traits. Body length was the only trait in rams that did not have a statistical relationship with body weight.

Temoso *et al.* (2017) study on communal goats and sheep in Botswana rangelands, is in agreement with the current study report on the positive correlation between BW and HG in ewes, BW and SH in rams. However, results from the same study disagrees with the current correlation reports whereby body weight had no statistical relationship with sternum height in ewes and heart girth in rams, the contrary may be due to use of different breeds and environmental conditions during data collection.

Boujenane and Halhaly (2015) estimated body weight from height girth in Sardi and Timahdite sheep using different models. Their results are in line with current reports that there is a strong relationship between body weight and heart girth. Current correlation results are in agreement with the reports of Yakubu's (2010) on Yankasa lambs, which show a positive statistical relationship between body weight and heart girth in both ewes and rams. Furthermore, Shirzeyli *et al.* (2013) reported that growth traits can be used as a predictor of body weight, and it was concluded that body length can be used as a selection criterion in females because it had a high correlation with body weight.

Rather *et al.* (2021) reported that wither height is the best predictor of body weight for farmers who lack a weighing scale. Furthermore, Kumar *et al.* (2018) observed a positive and statistical phenotypic relationship between body weight and body length, heart girth on the prediction of body weight from growth traits in sheep, which agrees with the current study results. Reports of Musa *et al.* (2012) in Sudanese Shogur, Ravimurugan *et al.* (2013) in Kilakarsal sheep, and Kumar *et al.* (2018) in Harnali

sheep are in line with the current study results that heart girth can be used to estimate live weight.

Current phenotypic correlation results in ewes suggest that an increase in heart girth may result in an increase in body weight. According to current ram correlation findings, increasing withers height or sternum height may increase body weight. The relationship between growth traits suggests that these traits are influenced or controlled by the same gene (s) (pleiotropy) (Mathapo *et al.*, 2022).

Correlation experiments only measure the association between two variables and neglect how they affect each other (cause-effect relationship) (Yakubu *et al.*, 2015). As a result, the second study objective was to use simple linear regression to develop models for estimating body weight from body length, heart girth, sternum height, withers height, and rump height in Dorper sheep.

According to the results of the ewe regression analysis, heart girth contributed 27 percent of the variation in body weight, as shown by the coefficient of determination. In rams, sternum height and withers height had the same coefficient of determination, accounting for 61 percent of the variation in body weight. The results of the regression analysis agree with the findings of Kumar *et al.* (2018), who established a model to predict body weight of sheep from growth traits, and the highest coefficient of determination was found on heart girth, indicating that it has the greatest variation in body weight, making it a suitable trait to be used as a predictor of body weight.

Recent study regression analysis reports are consistent with the findings of Rather *et al.* (2021) on Kashmir merino sheep, which concluded that a model consisting of withers height is the best equation to use when predicting body weight. The findings of the study suggest that heart girth, as an independent trait in ewes, and sternum height and withers height, as independent traits in rams, could be used as predictors of body weight in resource limited farms. In rams, equation with SH shows that an increase in one cm of sternum height will increase body weight by 0.84 kg and for the prediction of body weight from withers height reveals that an increase in one cm of withers height will lead body weight to increase by 0.60 kg. In ewes, the model with HG implies that an increase in one cm of heart girth will result in 0.62 kg of body weight. Ashwini *et al.* (2019) explained that regression equation with heart girth for crossbred

cattle indicated that an increase in one cm of heart girth gave an increase of 2.048 kg of body weight.

Correlation and regression techniques are traditional breeding strategies for herd improvement in the breeding industry, however, they neither improve the animal genetically nor improve the animal DNA-wise (Depison *et al.*, 2017). Furthermore, Sarmah *et al.* (2020) demonstrated that molecular genetics plays a role in identifying candidate genes with a significant effect on economically important traits. To achieve the third goal of the current study, single nucleotide polymorphisms of the *growth hormone* gene and their relationship with growth traits in Dorper sheep, DNA sequencing and measured Marker-traits association using the General Linear Model (GLM) were examined.

Current study results on single nucleotide polymorphism (SNP) revealed a synonymous SNP (T/A) on position 735 of exon 4 of the *growth hormone* gene, which was named T735A. In their study of the relationship between the *growth hormone* gene polymorphism and the estimated body weight of 100 Harri sheep, Abdelmoneim *et al.* (2017) discovered a novel single nucleotide polymorphism in exon 4. However, they discovered another SNP (G871A) on intron 2 and another SNP on intron 4 at position 1383. (A1509G). Study on *growth hormone* gene polymorphisms and growth traits in Chinese Tibetan sheep documented that two SNPs (G616A and G624G) were mapped on intron 2 and SNP (G498C) which was noted to be a synonymous meaning the mutation does not change the structure of the encoded protein was present on exon 2 (Han *et al.*, 2016).

The 365 bp amplified fragments of *GH1* was analysed using PCR-SSCP and two patterns were noted on study in Egyptian sheep and the sequence analysis revealed a single nucleotide polymorphism (C/T) of *GH1* on exon 5 (Farag *et al.*, 2016). Youji and Guohong (2010) reported that in Gansu modern meat sheep, single nucleotide polymorphism was noted on exon 1 of *growth hormone* gene. Contrary, Cauveri *et al.* (2016) study on the single nucleotide polymorphisms in *GH1* associated with growth traits in Nilagiri sheep of Tamil Nadu found no SNP in all exons. However, two SNP(s) were noted on transition G/A on position 480 and transition G/A on position 871 in intron 1 and intron 2 respectively on the same research study. Disagreement could be due to differential expression of genes which influences animal's physiology. SNP

findings of the current study suggest that the change in the DNA sequence does not change nor have an effect on the protein structure and function.

Chi-square results of the current study demonstrated that the population used was under Hardy-Weinberg equilibrium (HWE). Chi-square reports on the study conducted by Han *et al.* (2016) demonstrated two SNPs (G616A and G624G) to be under HWE in OT sheep while other individual gene and genotypic frequency deviated from HWE. The current study suggest that the studied population is under HWE implying that the genotypic and allelic frequency of *GH1* of Dorper population does not change from generation to generation.

Marker-trait association results showed that there was no statistical relationship between genotype AA and AB regarding the growth traits except withers height. Genotype AA had the highest impact on withers height. Gorlov *et al.* (2017) results on the association of the *growth hormone* gene polymorphism with growth traits in Salsk sheep breed are not in parallel with the current study, they discovered 3 genotypes (AA, AB and BB), and AB genotype was associated with positive effect on carcass weight. Furthermore, Malewa *et al.* (2014) on sheep of Donggala and East Java breeds and Hajihosseini *et al.* (2013) on Makoei sheep breed revealed the presence of genotype AB result with high impact on carcass weight.

Contrary to current study was reported by Malewa *et al.* (2014) on Indonesia fat tailed sheep were *GH1* polymorphism showed to affect growth traits such as weaning weight in both sheep breeds as it was noted that in Donggala sheep, genotype AA had a significant on growth traits than genotype BB, moreover same study reported higher significance of genotype AB than genotype AA and BB on growth traits of East Java sheep. Contrary might be due to use of different sheep breeds. Report by Moradian *et al.* (2013) in Makoei sheep is in accordance with the findings of the current study that there was no association between SNP genotypes and body weight.

Concerning the results found on our association, genotype AA in the *GH1* might be used as the potential genetic marker when improving withers height. Presence of association implies that the genotype of polymorphism has a chance of occurring more often than expected by chance in an organism having position of the trait (Abousoliman *et al.*, 2021).

Gene polymorphisms serve as the potential approach in improving major economically essential traits such as growth traits as they are regarded as aspects to evaluate an animal's economic value (Han *et al.*, 2016) hence more breeders improve molecular genetic associated with growth traits through marker-assisted selection in most livestock species such as in goats (Sarmah *et al.*, 2020), chickens (Tyasi *et al.*, 2018) and in cattle (Agung *et al.*, 2018).

Bayraktar and Shoshin (2022); Valencia *et al.* (2022) inspects the association of growth traits with the genotypes of growth candidate genes in various breeds world widely utilizing numerous molecular techniques such as marker assistance selection of animals for faster genetic improvement especially for economically important traits. However, more studies need to be conducted on single nucleotide polymorphisms of the *growth hormone* gene and its association with the growth traits of Dorper sheep in a larger sample size and more growth traits to be included.

## 5.2. CONCLUSIONS

Pearson's correlation technique was employed to determine the relationship between body weight and growth traits, the current study revealed that body weight had a relationship with heart girth, withers height, and body length in ewes while having relationship with withers height, sternum height, and heart girth in rams of Dorper sheep.

Simple linear regression was employed to establish models for prediction of body weight from growth traits, the ewe results showed the highest  $R^2$  (0.27) from HG indicating that the model ( $BW = -18.47 + 0.62HG$ ) may be used to estimate BW of Dorper sheep whereas in rams, SH and WH showed highest  $R^2$  indicating that their model ( $BW = -3.70 + 0.84SH$ ) and ( $BW = -5.55 + 0.60WH$ ) may be used to estimate body weight.

PCR-RFLP was used to identify the single nucleotide polymorphism, results showed two genotypes (AA and AB). DNA analysis revealed a synonymous SNP on exon 4 of *growth hormone* gene of Dorper sheep.

General Linear Model was used for marker-traits association, and it was noted that withers height was the only growth trait found to have association with the genotype (AA and AB) with genotype AA contributing more on withers height.

## 5.3. RECOMMENDATIONS

The recommendations of the current study are:

- For herd improvement, it is critical that resource limited farmers should be taught more about the relationship between body weight and growth traits such as heart girth, wither height, body length, sternum height, and rump height since they affect body weight and are easy to measure.
- Importance of body weight should be emphasized to small holder farmers especially at rural farmers for management purposes such as feeding, medication, marketing, and breeding.
- Dorper sheep with genotype AA may be selected for improvement of withers height which revealed to have positive correlation with body weight.

- More studies need to be conducted on *growth hormone* gene polymorphisms and their association with growth traits using larger sample sizes and more growth traits.

## **CHAPTER SIX**

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