

INVESTIGATION OF SINGLE NUCLEOTIDE POLYMORPHISMS OF *INSULIN-LIKE GROWTH FACTOR 1 (IGF-1)* GENE AND THEIR ASSOCIATION WITH GROWTH TRAITS IN KALAHARI RED GOAT

BY

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DECLARATION

I declare that this dissertation hereby submitted to the University of Limpopo for the degree of Master of Science in Agriculture has not previously been submitted by me for a degree at this or any other university, that it is my own work in design and execution, and that all materials contained herein has been duly acknowledged.

Signature

A handwritten signature in black ink, appearing to be 'Mokoena K', with a long horizontal stroke extending to the right.

Date 06/10/2022

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DEDICATION

This dissertation is dedicated to my late uncle (Mokoena Moraba David Scara) may your soul continue to rest in peace. I will always cherish the moments we had.

ABSTRACT

Weighing scales are typically out of reach for small-scale farmers due to expensive cost and a lack of operational expertise. However, understanding body weight and its relationship to linear body measures are critical for farmers making management decisions. Single nucleotide polymorphisms (SNPs) are significant because they influence the coding area of the DNA, leading to changes to the amino acid sequences, which might affect the animal's phenotype. The current study sought to find genetic indicators of the *insulin-like growth factor 1* gene that may be exploited for breeding selection in order to improve the growth traits of Kalahari Red goats. The research was carried out at the Zuurfontein farm in Polokwane. As experimental animals, fifty ($n = 50$) Kalahari Red goats (8 males and 42 females) aged 2 to 3 years were used. A balance weighing scale was used to record body weight, and a measuring tape was used to capture linear body measures. Blood samples were obtained from the jugular vein once per animal using vacutainer blood collecting tubes. The deoxyribonucleic acid (DNA) was extracted and purified according to the methodology provided by Noegen's Genomic DNA isolation kit. Pearson's correlation was used to achieve the correlation between the growth traits, Simple linear regression was performed to predict body weight from linear body measurements, Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) was utilized to discover the single nucleotide polymorphism, Chi-square test (χ^2) was performed to assess the allele frequencies for Hardy-Weinberg equilibrium and General Linear Model (GLM) was performed for marker-trait association analysis. The mean square error (MSE) and coefficient of determination (R^2) were exercised to choose the best regression model. Correlation results indicated a high positive significant correlation ($P < 0.01$) among BW and RH ($r = 0.69$), BL ($r = 0.92$), HG ($r = 0.91$), WH ($r = 0.85$) in bucks. While it does, all the linear body measurements indicated a high positive significant correlation ($P < 0.01$) except SH ($r = 0.41$) which had a positive significant correlation ($P < 0.05$). Simple linear regression findings highlighted that BL had minimum MSE and highest R^2 in bucks while in does HG had minimum MSE and highest R^2 . PCR-RFLP results indicated that two fragment patterns (two fragments and one fragment) were identified. Two genotypes were identified, KK with one fragment and KM with two fragments. The

genotype frequency of KK was higher than that of KM and K allele had a higher allelic frequency than the M allele. The χ^2 results showed that the Kalahari Red goats population used was not in Hardy Weinberg equilibrium (HWE) ($\chi^2 = 0.39^*$). Marker-trait association findings by GLM indicated that the genotypes (KK and KM) had no association with the growth traits measured.

In conclusion, correlation findings suggest that BW had a higher relationship with BL and HG in Kalahari Red goats. The regression results suggest that in bucks, an increase of 1 cm of BL might increase body weight by 1.24 kg, whereas it does, a 1 cm increase of HG might increase the body weight by 0.73 kg. The χ^2 results suggest that the studied population gene and genotypic frequencies keep on changing from generation to generation and the marker-traits association results suggest that the genotypes identified had no relationship with growth traits in Kalahari Red goats. Further studies need to be conducted on single nucleotide polymorphism of *IGF-1* and their relationship with growth traits using a larger sample, more growth traits and targeting more exons.

Keywords: Correlation, Body weight, Coefficient of determination, Regression, Linear body measurements.

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LIST OF ABBREVIATION

<i>IGF-1</i>	<i>Insulin-like growth factors 1</i>
HG	Heart girth
RH	Rump height
WH	Withers weight
SH	Sternum height
BL	Body length
BW	Body weight
EDTA	Ethylene diamine tetraacetic acid
Cm	centimetre
Kg	kilogram
DNA	Deoxyribonucleic acid
PCR	Polymerase chain reaction
µl	Microliter
°C	Degree Celsius
bp	Base pairs
RFLP	Restriction fragment length polymorphism
NCBI	National Centre for Biotechnology Information
SPSS	Statistical Package for Social Sciences
R ²	Coefficient of determination

MSE	Mean square error
GLM	General linear model
SD	Standard deviation
CV	Coefficient of variance
SE	Standard error
Df	Degree of freedom
r	correlation coefficient
SNPs	single nucleotide polymorphism
HWE	Hardy Weinberg equilibrium
χ^2	chi-square test
H_0	Gene homozygosity
H_e	Gene heterozygosity
N_e	Effective Allele number
PIC	Polymorphism information
ANOVA	Analysis of variance

CHAPTER ONE
INTRODUCTION

1.1 Background

The Kalahari Red goat is a South African indigenous goat that was selected around 1990. There are now two lines that arose from red head Boer goats and unchanged indigenous goats (Campbell, 2003). The Kalahari Red goat is a meat-type goat with a medium to big framed red body, round horns that lean backward and loose skin with folds (Snyman, 2014a). This breed is more resilient, naturally adaptive, and resistant to illness and parasite infestation than other breeds (Sanni *et al.*, 2018). It is also noted for its quick growth, ability to give birth to twins or triplets, and ability to produce enough milk to support its offspring (Amie-Marini *et al.*, 2012).

Growth features such as body weight have always provoked the curiosity of those involved in the production of animal meat because they are controlled by a complicated system in which the somatotrophic axis plays a crucial role (Liu *et al.*, 2012). *The insulin-like growth factor 1 gene (IGF-1)* is a critical part of somatotrophic axis, which regulates development and metabolism in mammals, including farm animals (Lestari *et al.*, 2020).

1.2 Problem statement

The indigenous goats of South Africa are known to have low growth performance, necessitating genetic enhancement of growth traits (Ssewanyana *et al.*, 2004; Bhattarai *et al.*, 2019). Single nucleotide polymorphisms (SNPs) as genetic markers and their association with growth traits may aid goat farmers in improving economically significant traits (Zhang *et al.*, 2008). According to Naicy *et al.* (2017), genetic growth traits may be improved by choosing genotypes related with growth traits based on the discovery of *IGF-1* single nucleotide polymorphisms in the Malabari and Attappady Black goat breeds. However, we are unaware of any accepted genetic markers of *IGF-1* related with growth traits in South African Kalahari Red goats.

1.3 Rationale

According to Othman *et al.* (2016), productivity can be improved by employing modern genetic technology for trait selection via markers-assisted selection. It has been

observed that *IGF-1* genetic polymorphisms are associated with growth features in chickens (Seo *et al.*, 2001) and cattle (Li *et al.*, 2004). Naicy *et al.* (2017) analysed polymorphisms in *IGF-1* and found a strong correlation between cytosine substituted with thymine in position 80 and growth features in a population of Malabari and Attappady Black goat breeds. Pehlivan (2019) discovered a link between *IGF-1* and the body measurement trait in white Angora goat babies.

This study will help in the identification of SNPs of *IGF-1* that might be used as potential genetic markers to improve growth traits in Kalahari Red goats breeding.

1.4 Aim

The aim of the current study was to identify genetic markers of the *insulin-like growth factor 1* gene that might be used for selection during breeding to improve the growth traits of Kalahari Red goats.

1.5 Objectives

The objectives were to:

- I. Estimate the relationship between body weight and heart girth, rump height, withers height, sternum height and body length traits of Kalahari Red goats.
- II. Establish a model for the prediction of body weight using heart girth, rump height, withers height, sternum height and body length.
- III. Identify single nucleotide polymorphisms of the *insulin-like growth factor 1* gene of Kalahari Red goats.
- IV. Determine the association of the single nucleotide polymorphisms of *insulin-like growth factor 1* gene with body weight, heart girth, rump height, withers height, sternum height and body length of Kalahari Red goats.

1.6 Hypotheses

- I. Body weight has no relationship with heart girth, rump height, withers height, sternum height and body length of Kalahari Red goats.

- II. Heart girth, rump height, withers height, sternum height and body length cannot be used to establish a model for the prediction of body weight.
- III. Kalahari Red goat's *insulin-like growth factor 1* gene has no single nucleotide polymorphisms.
- IV. Single nucleotide polymorphisms of *insulin-like growth factor 1* gene have no association with body weight, body weight, heart girth, rump height, withers height, sternum height and body length of Kalahari Red goats.

CHAPTER TWO
LITERATURE REVIEW

2.1 Introduction

The goals of this chapter were to (1) offer a complete analysis of the reported origin and characteristics of South African indigenous goat breeds, (2) reflect on the use of body measurements to estimate the body weight in goats and (3) emphasize genetic variations of *IGF-1* by SNPs and their relationship with growth traits in other animals. This review was structured as follows to fulfil the study objectives: (a) South African goat breeds such as Angora goat, Boer goat, Savanna goat, and Kalahari Red goat, (b) Kalahari Red goat characteristics, (c) Growth traits as a predictor of body weight in goat breeds, (d) The insulin-like growth factor 1 gene (*IGF-1*), (e) Single nucleotide polymorphisms (SNPs), (f) Genetic diversity of *IGF-1* in different animals such as sheep, goats

2.2 South African goat breeds

South African goat breeds include indigenous goats as well as commercial varieties like the Savanna goat, Kalahari Red goat, Boer goat, and Angora goat (Visser, 2019). Indigenous goats are typically termed for the geographical places in which they are kept because they lack unique breed identification (Pedi, Nguni, and Xhosa indigenous goats) and are distinguished by their horn shape, color variation, and ear length (Monau *et al.*, 2020). They are well recognized for having a petite body frame, a poor carcass yield, and being highly resistant to illnesses and hard environmental circumstances such as inadequate feed and excessive cold (Campbell 2003; Ramukhithi *et al.*, 2019; Mataveie *et al.*, 2021). The following are some of the South African goat breeds.

2.2.1 Angora goat

The Angora goat is a breed that was introduced to South Africa in 1838 from Asia's Angora district; they adapted and thrived, and are distinguished by big hanging ears, white hair (mohair), and a compact body frame (Figure 2.1) (Snyman, 2014b). This breed is occasionally used as a meat (chevon) goat, but only to a limited extent because their meat is lean and favoured when they are young (Visser and Marle-Köster, 2014). Mohair is produced by this goat breed (Snyman, 2014b).



Figure 2.1: Angora goat

Source: Snyman (2014b).

2.2.2 Boer goat

The Boer goat is a regionally created chevon-type breed that originated in South Africa's Eastern Cape Province through selection with indigenous goats (Pieters *et al.*, 2009). The Boer goat is well-known for its adaptability to both broad and intense pastures (Snyman, 2014c). It has a huge body structure (Figure 2.2), redhead with a white body, lob ears, circular horns that bend toward the back, and fold skin (Visser and Marle-Köster, 2017). Females are known to give births to twins, triplets and quadruplets at once (Kamarudin, 2011).



Figure 2.2: Boer goat

Source: Snyman (2014c).

2.2.3 Savanna goat

The Savanna goat was created in 1955 near the Vaal River in a closed environment where they were expected to adapt and survive (Visser and MarleKöster, 2017). This breed (Figure 2.3) consists of medium to large-sized goats with a smooth coat and dark black oval-shaped horns that grow backward (Snyman, 2014d). The savannah goat has an excellent development rate and carcass confirmation, as well as well-muscled forequarters and a long neck for convenient browsing (Pieters *et al.*, 2009).



Figure 2.3: Savanna goat

Source: Snyman (2014d).

2.2.4 Kalahari Red goat

The indigenous goats were brought to Namibia and South Africa by migrating tribes who kept goats traditionally, and records show that the Western, Eastern, and Northern Cape provinces had lob eared goats, and some Northern Cape farmers and Namibia part of Kalahari Desert began selecting goats that are slightly smaller than the improved Boer goats (Snyman, 2014a). According to the literature, the red goats had few

breeders, and in the year 1990, Mr Albie Horn began choosing this breed, which he termed the Kalahari Red goat breed (Figure 2.4). The goats were shown with the Savanna goat at Bloemfontein in 1998 as Brown Savanna, and a National Championship for the Kalahari Red goats breed was held in Bloemfontein in 2000 (Campbell, 2003).



Figure 2.4: Kalahari Red goat

Sources: Snyman (2014a).

2.3 Characteristics of Kalahari Red goat

The Kalahari Red goat breed is a crucial chevon-producing breed in South Africa, with traits such as tolerance to semi-arid and arid savannah due to highly pigmented coats and long ears that provide exceptional heat resistance (Pieters, 2007). They also have good foraging abilities, rapid growth, and excellent mothering abilities, as well as the ability to produce more milk to assist the growth of their children (Sanni *et al.*, 2018). The breed can walk well and gives birth three times every two years (Kotze *et al.*, 2004). They are considered a "minimal care / maximum profit" breed since they are less prone to diseases and require less frequent vaccination than other goat breeds (Adewumi *et al.*, 2017).

2.4 The growth traits as a predictor of body weight in goat breeds

On the farm, a goat's body weight is used to anticipate the dose of medicine to be supplied, alter nutrition, and make a breeding decision (Iqbal, 2013). Body weight varies according on breed, gender, age, health, and the overall environment in which the goats are housed (Babale *et al.*, 2018).

The body weight of goats can be determined using tape measures making it simple, inexpensive, faster, and useful in rural areas where resources such as weighing scales are few (Panda and Ghorpade, 2016). The live body weight of goats increases at an increasing rate until the first set of incisors appear at the age of 1 year 4 months, after which it gradually decreases. Body measurements as quantitative growth indicators represent the changes that occur during the goats' life span (Hagos, 2016; Semakula *et al.*, 2010).

It is not often easy to measure the live body weight due to the lack of a weighing scale (Singh *et al.*, 2020). However, in order to maintain proper goat husbandry, measures of body weight are required for breeding and herd management (Sam *et al.*, 2016). Goats' live body weight can be approximated using body parameters such as body length, heart girth, and wither height as essential attributes for determining body weight (Berhe, 2017).

2.5 The *insulin-like growth factor 1* gene

IGF-1 is a metabolic factor in regulating cellular development and metabolism (Al Qasimi *et al.*, 2019). Sharma *et al.* (2014) identified *IGF-1* as one of 271 potential genes found in goats that regulate growth and meat production and proposed its usage as a molecular marker to increase growth and meat output. *IGF-1* is coded by a single gene on chromosome 5, which consists of three leader exons (1W, 1 and 2) and three exons (3, 4 and 6), with exon 3 and exon 4 encoding the mature IGF-1 peptide (Sarmah *et al.*, 2019).

The *IGF-1* hormone develop the growth of foetal organs, skeletal maturation, and endocrine glands in a variety of livestock species by increasing foetal amino acid and glucose intake in sheep (Naicy *et al.*, 2017). It promotes longitudinal bone growth, cartilage synthesis, and muscle expansion, *IGF-1* is an important element in animal

linear growth (Othman *et al.*, 2019). *IGF-1* plays several roles in prenatal and postnatal growth, it's been suggested that this gene could be linked to growth and carcass characteristics in livestock species (Zhang *et al.*, 2008).

2.6 Single nucleotide polymorphisms (SNPs)

Single nucleotide polymorphisms (SNPs) (pronounced "snips") are deoxyribonucleic acid variations that arise when a single nucleotide: thymine (T), adenine (A), guanine (G), or cytosine (C) in the genome sequence is altered. When Snips occur inside a gene, they form distinct variants or alleles of that gene (Koopae and Koshkoiyeh, 2014). According to Seidel (2010), the usage of SNIPs is powerful for population genetics because they are the most prevalent sequence variations seen in a genome. According to Li *et al.* (2010), the usage of SNPs is crucial because they modify the coding area of the DNA, resulting in changes to the amino acid sequences, which affect the phenotypic of the animal. Wenne (2018) showed that SNPs can be utilised to create genetic linkage maps, find quantitative trait loci (QTL) for relevant attributes such as growth, body weight, resistance to stress and illnesses, map sex determination loci, and identify progeny in aquaculture selection.

Koopae and Koshkoiyeh (2014) listed the following as some of the advantages of SNP markers: 1. the direct effect of SNP markers on protein function because they are located in the DNA coding region. 2. SNPs are more stable than other DNA markers in terms of inheritance, making them excellent for long-term selection markers. 3. SNPs are more suitable for high throughput genetic research than microsatellites, and 4. SNPs are more prevalent than other types of polymorphisms, and therefore provide more potential markers near the locus of interest. Figure 2.5 show the SNP of *insulin-like growth factor 1* gene in Kejobong goat breed.

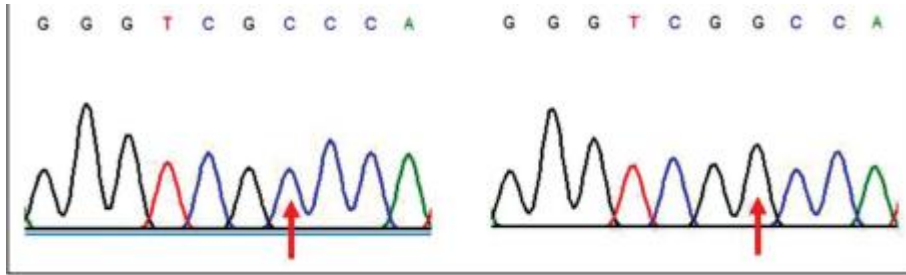


Figure 2.5: Identified snip of *insulin-like growth factor 1* gene in Kejobong goat breed.

Source: Lestari *et al.* (2020)

2.7 Genetic diversity of *IGF-1* in different animals

Genetic diversity of *IGF-1* has been observed using SNPs in different livestock and their associations with economically important traits such as growth traits.

2.7.1 Sheep *IGF-1*

Grochowska *et al.* (2017) discovered that variation in the 5'-flanking regions of *IGF-1* influences not only body size and growth but also meat and carcass quality indices in Merino sheep. According to Meira *et al.* (2019) internal carcass length, rump girth, rib yield, and neck weight have all been linked to SNPs in *IGF-1* intron 1 in Santa Ines sheep.

2.7.2 Goat *IGF-1*

Zhang *et al.* (2008) reported a new SNP (G to C transversion) in *IGF-1* intron 4 that was related to birth weight, six-and twelve-month body weight, chest girth at two months, six-month body length, six-and twelve-month wither height, and twelve-month heart girth. Naicy *et al.* (2017) revealed that at exon 2, two genotypes (CC and CT) were found, and association analysis of the loci revealed that CT genotypes have longer body length, chest circumference, and body length than CC genotypes.

2.7.3 Chicken *IGF-1*

It has been observed that there is an association between *IGF-1* genetic polymorphisms with growth features and slaughter characteristics in chicken, with the polymorphism influencing leg muscle, breast muscle, and liver weight (Kadlec *et al.*, 2011). According to Amills *et al.* (2003), the chicken *IGF-1* gene is found on chromosome 1 and spans 50 kb. *IGF-1* has been linked with eggshell weight and egg weight in the White Leghorn chicken population.

2.7.4 Cattle *IGF-1*

According to Mullen *et al.* (2011), *IGF-1* has been shown to be related with growth, fertility, and development in cattle and further demonstrates the multifaceted influences of *IGF-1* growth-related traits and on milk production in cattle.

2.8 Conclusions

The origin of South African goat breeds has been proven in literature, and it has been underlined that the indigenous goats are characterised by distinct body shapes, although they have different colour patterns, the horns are common in the diverse goat breeds. The presence of *IGF-1* genetic variety within different species was discovered in the literature, and this genetic diversity was linked to growth features in animals such as sheep, goats, chickens, and cattle. The research, however, indicates that SNPs of the *insulin-like growth factor 1* gene in Kalahari Red goats and their connection with growth features are unknown.

CHAPTER THREE
METHODOLOGY AND ANALYTICAL PROCEDURES

3.1 Study area

The study was performed at Zuurfontein farm, which is situated under Polokwane Local municipality, Limpopo province, South Africa. The farm is located 1154 meters above sea level at latitude -23.57660° S and longitude 29.52090° E. The temperature fluctuates from 7°C to 21°C in the winter and 16°C to 28.10°C in the summer, with an annual rainfall of more than 600 mm (Shabalala *et al.*, 2019). The laboratory work was conducted at the Department of Biochemistry, Microbiology and Biotechnology, University of Limpopo, Limpopo Province, South Africa.

3.2 Ethical approval

All procedures were performed following the standards and protocols set by the University of Limpopo Animal Research Ethics Committee (AREC) project number AREC/14/2021: PG.

3.3 Experimental animal and management

The study included fifty Kalahari Red goats of both sexes (8 males and 42 females), all of whom were between the ages of two and three years. The Kalahari Red goats were raised in an intensive manner. The goats were housed in the kraals at night and released into the veld during the day to eat on the various foods available on the property. The males, on the other hand, were kept in pans and fed a ration devised by the farmer. The goats were dewormed weekly, and dipping occurred every Wednesday of the week. Males and females were housed in separate enclosures. The kraals had water troughs and free access to clean water. Identification was accomplished through the use of ear tags.

3.4 Research design

One replicate per goat was employed in the cross-sectional design. The cross-sectional design is a sort of observational study in which data is collected once for each goat from

a population at a certain point in time (Kohlmann, 2008; Zangirolami-Raimundo *et al.*, 2018). However, sick and pregnant does were excluded from data gathering process.

3.5 Measurements of growth traits

Body weight and five linear body measurements were collected following the procedure described by Cam *et al.* (2010). Briefly, the body weight of the goats was measured using a hanging scale calibrated in kilograms (kg). The linear body measurements were measured using a measuring tape (cm) and a ruler (cm). The linear measurements that were measured are as follows: heart girth (HG): was measured of body circumference just behind the scapula, rump height (RH): was recorded as vertical distance from the top of the pelvic of the scapula to the ground, withers height (WH): as the distance from the highest point of the shoulder (withers) and the ground surface in relation to the level of the forelegs, sternum height (SH): as the vertical distance from the lower tip of the sternum to the ground as the animal standing and body length (BL): was measured diagonally from the lateral tuberosity on the scapula to the pin-bone. One individual was allowed to take measurements to avoid errors in data collection.

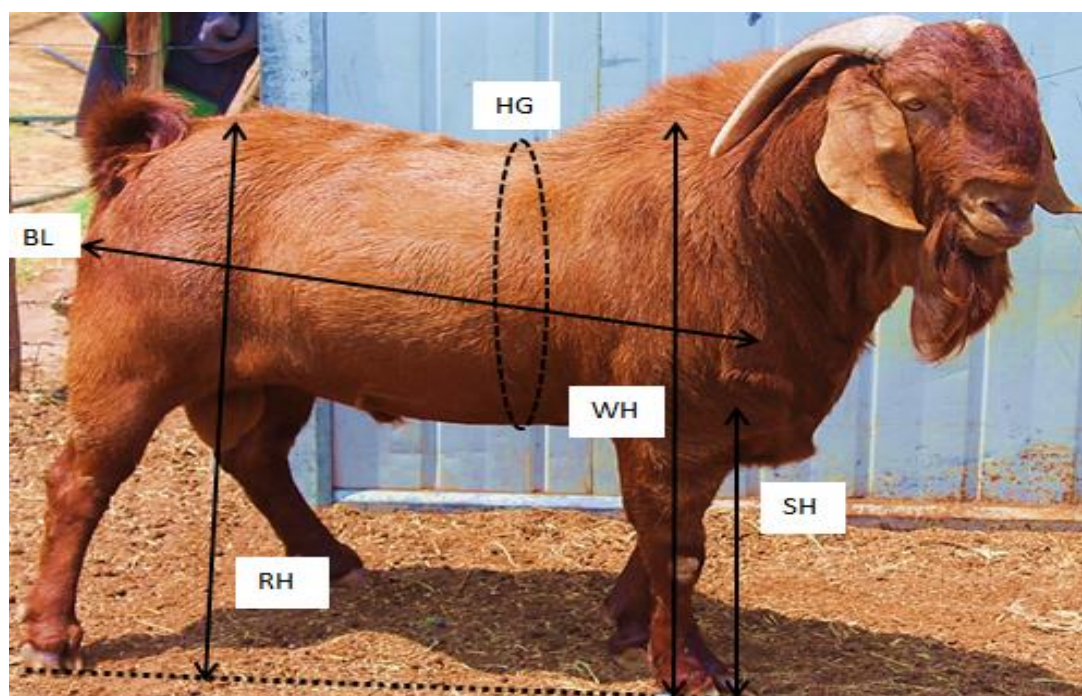


Figure 3.1: A Kalahari Red goat showing the points at which measurements were taken.

3.6 Blood sampling and DNA extraction

At the Zuurfontein farm, 50 blood samples were collected from Kalahari Red goats. Blood samples for DNA analysis were taken in Vacutainer blood collection tubes with an anticoagulant by the university veterinarian using a 3 ml syringe from the jugular vein (EDTA). The samples were maintained at 4 degrees Celsius until they were used. The DNA was extracted and purified according to the methodology provided by Noegen's Genomic DNA isolation kit.

3.7 DNA Amplification

IGF-1 was amplified using Polymerase chain reaction (PCR). Primers to amplify the *insulin-like growth factor 1* gene were designed based on the sequence in the National Centre for Biotechnology Information (NCBI) database sequences (GenBank accession No. D26118.1) using Primer Premier 5.0 software (PREMIER Biosoft, Palo Alto, CA, USA). Table 3.1 shows the primers that were used to amplify the *IGF-1*.

Table 3.1: Primer sequence, amplified region, and fragment size for PCR amplification of *IGF-1*

Amplified region	Primer sequence (5'-3')	Genbank accession No.	Fragment size and location (bp)	Annealing temperature
Exon 4	gctgggtgtagcagtgaca gttgcttcagccgataact	D26118.1	320 (308 – 627)	60°C

PCR mixture of 50 µl, containing 25 µl of Master Mix, 1µl of each primer forward and reverse, 5 µl DNA template and 18 µl deionised double-distilled water. The PCR program was left at 95°C for 5 min to denature, followed by 34 cycles of 94°C for 30s, 60°C for 30s, 72°C for 30s and a final extension at 72°C for 10 min. The resulting PCR products were separated by electrophoresis on a 1.2% agarose gel. The gel was stained with ethidium bromide visualised and photographed under a U.V. trans-illuminator (Spectroline).

3.8 Genotyping

The PCR products were genotyped using the Restriction Fragment Length Polymorphism (RFLP) using *HaeIII* enzyme. A total of 50 µl reaction mixtures consisting of 30 µl of the PCR product, 5 µl of 10 X buffer, 13 µl water and 2 µl of *HaeIII* enzyme were incubated at 37°C for 24 hours. The restriction digest reaction products were electrophoresed on a 1.2% agarose gel, visualised and photographed using a U.V. trans-illuminator (Spectroline).

3.9 Statistical analysis

Statistical Package for Social Sciences (IBM SPSS, 2019) version 26.0 software was used to analyse data. Standard deviation, standard error, coefficient of variance and mean were compute as descriptive statistics. To fulfil the first objective, Pearson's correlation was used to find a link between growth traits. A probability of 5% was chosen for significant and 1% for highly significant differences between characteristics. To estimate the model for predicting body weight from body measuring traits, simple regression analyses were utilized. The following regression model was used:

$$BW = a + b_1X_1$$

Where:

BW = dependent (body weight), a = regression intercept, b's = regression coefficient of linear body measurements and X's = independent (WH, RH, HG, SH, BL).

The selection of the best-fitted regression model was chosen using the coefficient of determination (R^2) and mean square error (MSE).

To determine single nucleotide polymorphism, PCR-Restriction Fragment Length Polymorphism (RFLP) was employed. The POPGENE software (version 1.32, University of Alberta, Canada) for population genetic analysis was utalized to calculate allele and genotype frequencies. Chi-square (χ^2) test was performed to assess the allele frequencies for Hardy-Weinberg equilibrium. The general linear model (GLM) was performed for marker-trait association analysis. The following model was used:

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

Where:

Y_{ij} = Phenotypic values of traits, μ = Population mean, G_i = Fixed effect of genotype and e_{ij} = Random residual error.

CHAPTER FOUR

RESULTS

4.1 Descriptive statistics of measured traits in Kalahari Red goats

Descriptive statistics were used to analyze a summary of the acquired data. Table 4.1 shows descriptive statistics for Kalahari Red goat bucks' and does' body weight and linear body measurements. The average body weight of bucks was found to be greater than that of Kalahari Red does. When compared to other features in bucks, withers height showed the lowest coefficient of variance. Despite doe having a lower average body weight than bucks, the does had the lowest coefficient of variance in withers height, heart girth, and rump height.

Table 4.1: Descriptive statistics of body weight and growth traits of Kalahari Red bucks and does

BUCKS				DOES		
Traits	Mean ± SE	SD	CV (%)	Mean ± SE	SD	CV (%)
BW	60.75 ± 4.85	13.72	22.58	48.92 ± 1.04	6.73	13.76
RH	76.75 ± 2.24	6.34	8.26	68.71 ± 0.78	5.05	7.35
BL	88.50 ± 3.63	10.27	11.60	79.14 ± 0.98	6.37	8.05
SH	44.25 ± 1.13	3.20	7.23	41.48 ± 0.73	4.76	11.48
HG	99.00 ± 3.73	10.54	10.65	84.69 ± 1.12	7.26	8.57
WH	74.38 ± 2.94	8.31	11.17	66.55 ± 0.75	4.83	7.26

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

4.2 Phenotypic correlation of bucks and does in Kalahari Red goats

Association between growth traits was performed using Pearson's correlation. Table 4.2 shows phenotypic correlation between body weight and growth traits in Kalahari Red bucks. In bucks, the results indicated that there was a highly positive significant correlation ($P < 0.01$) among BW and BL, BW and HG, BW and WH, BW and RH respectively. All the traits measured had a highly negative correlation to SH ($P < 0.01$). A high coefficient of correlation was recorded between BL and WH, HG and WH, HG and BL ($P < 0.01$), respectively.

In does, the results revealed that all growth traits taken had a positive correlation with body weight. Briefly, BW and BL, BW and HG, BW and RH, BW and WH were recorded to be highly positively correlated at significance ($P < 0.01$). BW and SH had a significant correlation with the lowest coefficient of correlation among all the traits ($P < 0.05$).

Table 4.2: Phenotypic correlation between body weight and linear body measurements in Kalahari Red does above diagonal and bucks below diagonal.

Traits	BW	RH	BL	SH	HG	WH
BW		0.58**	0.76**	0.41*	0.79**	0.56**
RH	0.69**		0.64**	0.68**	0.71**	0.56**
BL	0.92**	0.66**		0.59**	0.73**	0.45*
SH	-0.83**	-0.70**	-0.80**		0.67**	0.50**
HG	0.91**	0.66**	0.83**	-0.84**		0.51**
WH	0.85**	0.72**	0.91**	-0.73**	0.85**	

** . Correlation is significant at the 0.01 level: * Correlation is significant at the 0.05 level: ns non-significance. BW: Body weight, BL: Body length, SH: Sternum height, RH: Rump height, HG: Heart girth, WH: Withers height.

4.3 Simple linear regression analysis for different growth traits in Kalahari Red goats

4.3.1 Effect of rump height on body weight

Regression analysis was used to establish the simple linear models. The results of simple regression analysis between body weight and rump height of bucks indicated a coefficient of determination (R^2) of 0.48 and mean square error (MSE) of 114.86 (Table 4.3). The results further revealed that rump height explained about 48% of variation in body weight of bucks. The linear regression model was established as follows:

$$BW = -53.90 + 1.49RH$$

Where; BW = body weight, RH = rump height, -53.90 = constant, 1.49 = regression coefficient of rump height. The regression model of rump height highlighted that for every increase in one centimetre (1 cm) of rump height, body weight will increase by 1.49 kilograms (kg). In does, the simple regression analysis between body weight and rump height showed a R^2 of 0.34 and MSE of 30.73. Rump height explained about 34% of variation in body weight of does and the linear regression below was established.

$$BW = -4.37 + 0.78RH$$

Where; BW = body weight, RH = rump height, -4.37 = constant, 0.78 = regression coefficient of rump height. Linear regression model revealed that an increase of 1cm in rump height of does will increase body weight by 0.78 kg.

Table 4.3: Regression between body weight and rump height

Source	Sum of squares	DF	Mean square	R	R ²	Adjusted R ²
Bucks						
Regression	628.14	1	628.14	0.69**	0.48	0.39
Residual	689.36	6	114.89			
Total	1317.50	7				
Does						
Regression	628.26	1	628.26	0.58**	0.34	0.32
Residual	1229.38	40	30.73			
Total	1857.64	41				

df: Degree of freedom; r: correlation coefficient; R² : Coefficient of determination; Adjusted R² : Adjusted coefficient of determination; ** Significant at P < 0.01; * Significant at P < 0.05

4.3.2 Effect of body length on body weight

Simple linear regression between body weight and body length is shown in Table 4.4. The results indicated a R² = 0.85 and MSE = 33.80. The outcome of the results indicated that body length explained about 85% of variation in body weight. The following model was established:

$$BW = -48.02 + 1.23BL$$

Where; BW = body weight, BL = body length, -48.02 = constant, 1.23 = regression coefficient of body length. The regression model for body length showed that an increase in 1cm of body weight will increase body weight by 1.23kg. In does, the results

revealed a $R^2 = 0.58$ and $MSE = 19.47$. There was 58% of variation in body weight of Kalahari Red does from the results.

$$BW = -14.78 + 0.81BL$$

Where; BW = body weight, BL = body length, -14.78 = constant, 0.81 = regression coefficient of body length. The regression model for body length revealed that there will be 0.81kg increase in body weight for every increase in 1cm of body length in does.

Table 4.4: Regression between body weight and body length

Source	Sum of squares	DF	Mean square	R	R^2	Adjusted R^2
Bucks						
Regression	1114.70	1	1114.70	0.92**	0.85	0.82
Residual	202.80	6	33.80			
Total	1317.50	7				
Does						
Regression	1078.67	1	1078.67	0.76**	0.58	0.51
Residual	778.97	40	19.47			
Total	1857.64	41				

df: Degree of freedom; r: correlation coefficient; R^2 : Coefficient of determination; Adjusted R^2 : Adjusted coefficient of determination; ** Significant at $P < 0.01$; * Significant at $P < 0.05$

4.3.3 Effect of sternum height on body weight

Table 4.5 presents the simple regression between body weight and sternum height in Kalahari Red bucks. The results indicated that $R^2 = 0.68$ and $MSE = 69.78$. The results

suggested that 68% of variation in body weight was explained by sternum height. Simple linear regression model for sternum height was developed as follows:

$$BW = 217.64 - 3.55SH$$

Where; BW = body weight, SH = sternum height, 217.64 = constant, -3.55 = regression coefficient of sternum height. The results indicated that for every increase in 1cm of sternum height there was a decrease in 3.55 kg of body weight. Whereas in does, the results highlighted that a $R^2 = 0.16$ and $MSE = 58.80$. The result revealed that sternum height explained only about 16% of variation in body weight, the simple linear regression model is shown below:

$$BW = 25.12 + 0.57SH$$

Where; BW = body weight, SH = sternum height, 25.12 = constant, 0.57 = regression coefficient of sternum height. The results highlighted that there was an increment in body weight (0.57kg) for every increase in 1cm of sternum height.

Table 4.5: Regression between body weight and sternum height

Source	Sum of squares	DF	Mean square	R	R ²	Adjusted R ²
Bucks						
Regression	898.77	1	898.77	0.83**	0.68	0.63
Residual	418.73	6	69.79			
Total	1317.50	7				
Does						
Regression	305.59	1	305.59	0.41*	0.16	0.14
Residual	1552.05	40	38.80			
Total	1857.64	41				

df: Degree of freedom; r: correlation coefficient; R² : Coefficient of determination; Adjusted R² : Adjusted coefficient of determination; ** Significant at P < 0.01; * Significant at P < 0.05

4.3.4 Effect of heart girth on body weight

Simple regression between body weight and heart girth is presented in Table 4.6. The result showed that heart girth explains 82% of variation in body weight in bucks. Simple linear regression is presented below:

$$BW = -56.45 + 1.18HG$$

Where; BW = body weight, HG = heart girth, -56.45 = constant, 1.18 = regression coefficient of heart girth. For every increase in 1cm of hearth girth, there was an increase of 1.18kg of body weight. The results in does revealed that about 63% of variation in body weight was explained by heart girth.

$$BW = -13.25 + 0.73HG$$

Where; BW = body weight, HG = heart girth, -13.25 = constant, 0.73 = regression coefficient of heart girth. The simple linear regression of heart girth explained that for every increase of 1cm in heart girth there was 0.73kg increases in body weight.

Table 4.6: Regression between body weight and heart girth

Source	Sum of squares	DF	Mean square	R	R ²	Adjusted R ²
Bucks						
Regression	1090.28	1	1090.28	0.91**	0.82	0.80
Residual	227.22	6	37.87			
Total	1317.50	7				
Does						
Regression	1164.18	1	1164.18	0.79**	0.63	0.62
Residual	693.46	40	17.34			
Total	1857.64	41				

df: Degree of freedom; r: correlation coefficient; R² : Coefficient of determination; Adjusted R² : Adjusted coefficient of determination; ** Significant at P < 0.01; * Significant at P < 0.05

4.3.5 Effect of withers height on body weight

Simple linear regression between withers height and body weight MSE = 60.9 and R² = 0.72 as depicted in Table 4.7. The results means that about 72% of body weight variation in bucks was explain by withers height. Linear regression model was established as follows:

$$BW = -43.58 + 1.40WH$$

Where; BW = body weight, WH = withers height, -43.58 = constant, 1.40 = regression coefficient of withers height. The model indicated that for an increase of 1cm in withers height, the body weight is expected to increase by 1.4kg. The simple linear regression between withers height and body weight indicated that withers height explained only about 31% of variation in body weight of does. The following model was developed from the does:

$$BW = -2.99 + 0.78WH$$

Where; BW = body weight, WH = withers height, -2.99 = constant, 0.78 = regression coefficient of withers height. The results indicated that the body weight of does will increase by 0.78kg when withers height is increased by 1 cm.

Table 4.7: Regression between body weight and withers height

Source	Sum of squares	DF	Mean square	R	R ²	Adjusted R ²
Bucks						
Regression	952.11	1	952.11	0.85**	0.72	0.68
Residual	365.39	6	60.9			
Total	1317.50	7				
Does						
Regression	581.91	1	581.91	0.56**	0.31	0.30
Residual	1275.73	40	31.89			
Total	1857.64	41				

df: Degree of freedom; r: correlation coefficient; R² : Coefficient of determination; Adjusted R² : Adjusted coefficient of determination; ** Significant at P < 0.01; * Significant at P < 0.05

4.4 Analysis of *IGF-1* amplification

Amplification of DNA was conducted using Polymerase Chain Reaction (PCR). Following the electrophoresis of the amplified region of *IGF-1*, a 320 bp DNA was observed in Figure 4.1).

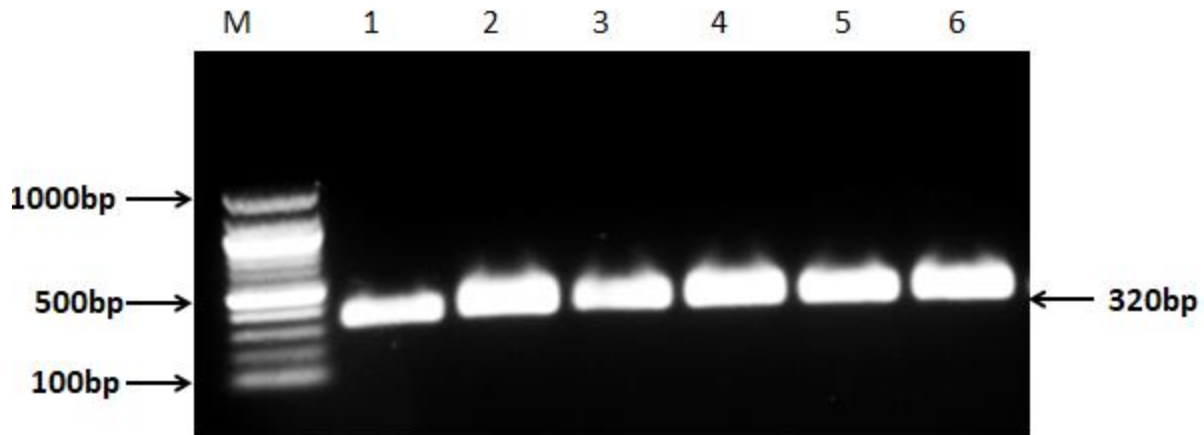


Figure 4.1: PCR products of *IGF-1* on 1.2% agarose gel.

4.5 Polymerase Chain Reaction - Restriction Fragment Length Polymorphism (PCR-RFLP) analysis

PCR-RFLP was employed to determine the single nucleotide polymorphism (SNPs). The resulting gel patterns from PCR-RFLP are presented in Figure 4.2. PCR-RFLP of amplicons revealed 2 fragment patterns. The first pattern showed one fragment of 210bp and the second pattern showed two fragments (110bp and 210bp). It was further revealed that there were two genotypes, KK with one fragment and KM with two fragments.

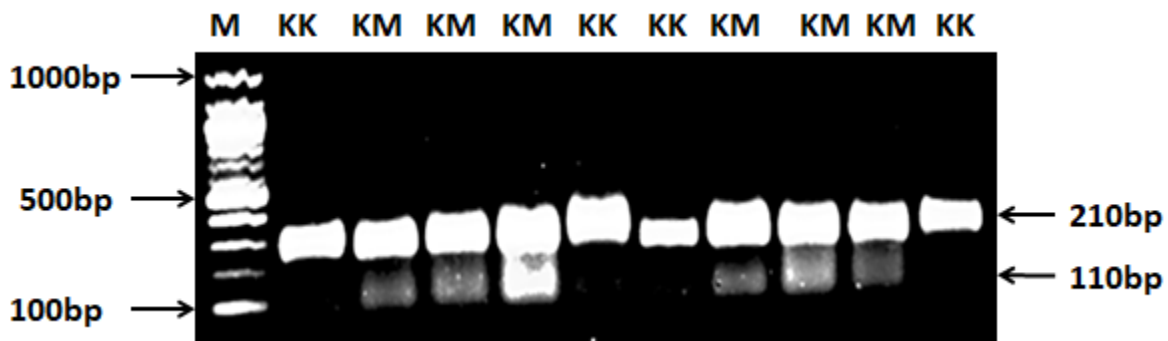


Figure 4.2: Polymerase chain reaction-restriction fragment length polymorphism of *IGF-1*

4.6 Allelic and genotypic frequencies

The genotypic and allelic frequencies were determined by POPGENE software. The estimated allelic and genotypic frequencies of *IGF-1* in Kalahari Red goats were determined in the current study (Table 4.8). Allele K and M were found and K had the highest frequency than the M allele. There were two genotypes KK and KM which were determined, and the frequency of KK genotype was higher as compared to KM genotype. The genotype distribution was statistically different ($P < 0.05$) from Hardy Weinberg equilibrium (HWE).

Table 4.8 Allelic and genotypic frequencies at the single nucleotide polymorphism locus of *IGF-1* in Kalahari Red goats

Genotype	Number of goats	Genotypic frequency	Allele	Allele frequency	χ^2
KK	28	0.56	K	0.78	3.98*
KM	22	0.44	M	0.22	

χ^2 : chi-square test, degree of freedom = 1, $P < 0.05$

4.7 Polymorphism parameters

POPGENE software was used to determine the polymorphism parameters as presented in Table 4.9. The results indicated that there was high homozygosity of *IGF-1* in Kalahari Red goats as compared to heterozygosity. Based on the results of PIC, it was revealed that Kalahari Red goats possessed medium polymorphism ($0.25 < \text{PIC} < 0.5$) at *IGF-1*.

Table 4.9 Polymorphism parameters

Gene homozygosity (H_0)	Gene heterozygosity (H_e)	Effective number (N_e)	Allele	Polymorphism information (PIC)
0.66	0.34	1.52		0.28

4.8 Associated of genetic variants with growth traits

GLM was employed to determine the association between genotypes and growth traits. To investigate the effect of *IGF-1*, the association between genotype and their effect on differences in body weight, rump height, sternum height, body length, withers height and heart girth was analysed. The results of the association were highlighted in Table 4.10. There was no significant association between the genotypes and the growth traits measured in Kalahari Red goats.

Table 4.10 Association between the polymorphism in *IGF-1* and body measurement traits of Boer goats

Traits	<i>IGF-1</i> Genotypes		F value	Significance
	KK (n = 28) (Mean ± SE)	KM (n = 22) (Mean ± SE)		
Body weight	47.82 ± 1.835	53.45 ± 1.79	0.61	0.44
Rump height	66.59 ± 1.20	72.68 ± 0.92	0.57	0.45
Body length	76.41 ± 1.47	84.12 ± 1.41	0.91	0.35
Sternum height	38.82 ± 1.01	44.36 ± 0.51	0.01	0.91
Heart girth	81.91 ± 1.67	90.96 ± 1.63	0.64	0.43
Withers height	66.64 ± 1.05	65.93 ± 2.43	1.66	0.20

SE: standard error

CHAPTER FIVE

DISCUSSION, CONCLUSION AND RECOMMENDATIONS

5.1 Discussion

Body weight is an economically essential feature that necessitates precise estimate in order to improve cattle breeding and output through selection (Sam *et al.*, 2016). The study first used Pearson's correlation to establish the association between Kalahari Red goat growth parameters such as body weight, withers height, heart girth, rump height, sternum height, and body length. The findings demonstrated a positive relationship between all growth traits in does. Except for SH, which was adversely connected to all other growth parameters in bucks, other traits exhibited a positive connection. The current study supports the findings of Tsegaye *et al.* (2013), who discovered that heart girth was highly associated with body weight in Hararghe Highland goat males and females. Temoso *et al.* (2017) conducted a study on small stock of communal rangelands in Botswana, and found that heart girth was correlated to body weight in both sexes, with females having a higher coefficient of correlation than the current study; however, the same study disagreed with the finding that sternum height is negatively correlated to body weight in bucks. Abd-Allah *et al.* (2019) discovered a positive association between body weight and heart girth in male Shami goats and a positive relationship among body weight and body length in female Shami goats, which is consistent with the current findings. Tyasi *et al.* (2020) investigated the relationship between growth traits in South African non-descript goats, and the results agree with the findings that body length and withers height are correlated to body weight in both bucks and does, but heart girth showed no relationship to body weight in bucks, which contradicts the current study's findings. Furthermore, the findings are consistent with those of Maylinda and Busono (2019) on body weight estimation in Fat Tailed sheep, Rashijane *et al.* (2021) in Boer goats raised at Farm Tivolie, South Africa, Ouchene-Khelifi and Ouchene (2021) on statistical models on morphological traits based on live weight of goats in twenty-one Algerian regions, and Lan *et al.* (2021) in goats of PNGUNRE farm. Dakhlan *et al.* (2021) investigated the link between body weight, body length, shoulder height, and heart girth in Saburai female goats in Lampung province, Indonesia, and identified a strong positive relationship between body weight, body

length, shoulder height, and heart girth, which is consistent with the current study's findings.

The findings show that increasing measured linear body measurements in does improves body weight, whereas increasing measured linear body measurements in bucks' decreases body weight except for an increase in sternum height, which causes a drop in body weight. According to Mathapo *et al.* (2022), the link between the growth traits suggests that the attributes are governed by the same genes.

While correlation results demonstrate a relationship between variables, they do not reveal the varying effect sizes of predictor and response (Loftus, 2022). As a result, the second objective was to predict a model to estimate body weight in Kalahari Red goats utilizing heart girth, rump height, withers height, sternum height, and body length using basic linear regression analysis.

Simple linear regression results in bucks revealed that all linear body measurements measured can be applied to predict body weight, however, body length and heart girth revealed the highest variation in body weight with coefficients of determination of 85 percent and 82 percent, respectively. Heart girth was the leading predictor of body weight in does, with a 63 percent coefficient of determination, followed by body length, with a 58 percent coefficient of determination. In a study on Saburai goats conducted by Dakhlan *et al.* (2021), it was discovered that heart girth was the strongest predictor of body weight in female goats compared to body length, which is consistent with the current study's findings. Furthermore, Ibrahim *et al.* (2021) discovered that heart girth in Batur sheep from the Banjarnegara district of Indonesia is the ideal predictor of body weight in ewes. The findings of the research are in agreement with Dakhlan *et al.* (2020) indicated that heart girth was used to predict body weight in female Ettawa Grade goats. Chitra *et al.* (2012) highlighted that heart girth had the highest coefficient of determination ($R^2 = 0.709$) as compared to withers height and body length in female Malabari goats, these results are in agreements with the current results. The current study's regression results are in line with the findings of Sabbioni *et al.* (2020), who discovered that in male and female Cornigliese sheep, heart girth and body length are the most important indicators of body weight. The current study's findings imply that

heart girth and body length can be utilized as independents to estimate the body weight of both Kalahari Red bucks and does among communal farming without making use of a weighing scale. The results also showed that an increase in 1cm of body length causes an increment in body weight of 1.2kg in bucks and an increase in 1cm of heart girth causes an increment in body weight of 1.1kg in does, whereas an increase in 1cm of heart girth causes a rise of 0.79kg in body weight of does.

Mendelian genetics has been used to improve phenotypic traits in domesticated animals for a long time; however, these breeding programs do not allow for optimal control over specific phenotypic traits; thus, using genetic markers for animal selection for breeding purposes via marker-assisted selection is the best method (Jin *et al.*, 2010). Furthermore, Lestari *et al.* (2020) noted that a lack of animal pedigrees and production records over a short period of time has rendered the traditional breeding program less appealing to marker-assisted selection. As a result, the third objective was to look at single nucleotide polymorphisms in the *insulin-like growth factor 1* gene and its association with growth traits in Kalahari Red goats. In Kalahari Red goats, the researchers discovered a single nucleotide variation in exon 4 of the *insulin-like growth factor 1* gene. The findings are consistent with those of Sarmah *et al.* (2019), who discovered an SNP at 5752bp with a nucleotide transversion from G to C in Assam hill goats. Lestari *et al.* (2020) did research on Kejobong goats and found an SNP at intron 4 as a transversion, which contradicts the existing findings. The evaluation of polymorphism of *insulin-like growth factor 1* gene in Egyptian small ruminant breeds (sheep breeds; Barki, Ossimi and Rahmani, goat breeds; Baladi, Barki and Zaraibi) revealed a variation (C > G) at position 282 (Othman *et al.*, 2016). The sequencing analysis of Mongolia cashmere goats by Liu *et al.* (2012) revealed a transition from C > G at nucleotide 69, causing a missense mutation in exon 2 which lead to the observed polymorphism. The results of this study suggest that there was a variation of *insulin-like growth factor 1* gene in Kalahari Red goats.

The results obtained from chi-square indicated that the population used in the current study is not under Hardy-Weinberg equilibrium (HWE). The results of the current study were in line with the study of Lestari *et al.* (2020) which detected that the genotype

distribution of Kejobong goats was statistically different from HWE. Furthermore, the findings of the study are in harmony with the result of Rasouli *et al.* (2017) in Markhoz goats revealed that the allelic and genotypic distributions for *IGF-1* and insulin-like growth factor binding protein 3 genes (*IGFBP-3*) were not in Hardy Weinberg equilibrium. The current study suggests that the allelic and genotypic frequencies of *insulin-like growth factor 1* gene changed over time in the Kalahari Red goats. The results of Zhang *et al.* (2008) reported that chi-square test on two genotypes within the Nanjiang Huang goat population were not in Hardy-Weinberg equilibrium.

The results of the genetic variation association with growth traits revealed that the genotypes KK and KM had no significant link with any of the growth traits in Kalahari Red goats. The current findings are in line with the findings of Sarmah *et al.* (2019), who found that despite the presence of SNP on *IGF-1* in Assan hill goats, there was no association between genotypes and body weight. In contrast to the current study's findings, Zhang *et al.* (2008) found that *IGF-1* polymorphism was associated with body weight in Nanjiang Huang goats, with goats with genotype CC being heavier than those with genotype GG and GC. Lestari *et al.* (2020) discovered that animals with genotype GG were substantially larger and heavier than animals with genotype CC. Othman *et al.* (2016) discovered a relationship between *IGF-1* polymorphism and other growth parameters in Egyptian small ruminant breeds (sheep breeds; Barki, Ossimi and Rahmani, goat breeds; Baladi, Barki and Zaraibi). The study's findings indicate that genotypes KK and KM cannot be employed as prospective genetic markers in Kalahari Red goats for improving growth traits since evaluated traits may be impacted by other genes and the environment. According to Sarmah *et al.* (2019), the lack or absence of correlation could be related to the presence of mutations that disrupt protein regulation.

In the past, breeding for growth traits relied much on the phenotypic measurements and performance records, this led to less improvement which took a long time to achieve due to some key traits having low heritability (Ekegbu *et al.*, 2019). Hence, the studies conducted by EL-Magd *et al.* (2017) in buffalo, Lazar *et al.* (2018) in Carpatina breed, Abdalhag *et al.* (2015) in Jinghai yellow chickens were aimed at improving animal breeding using phenotype and genetic markers. However, more research needs to be

conducted on the association of single nucleotide polymorphisms of *insulin-like growth factor 1* gene and growth traits in Kalahari Red goats using a larger sample size and including the DNA sequencing to confirm the single nucleotide polymorphisms identified, the growth traits included should be increased.

5.2. Conclusions

Pearson's correlation was utilized to examine the association between Kalahari Red goat growth traits. Correlation results demonstrated that all tested linear body measurements in does show a link with body weight, whereas all measured attributes in bucks exhibited a relationship with body weight. Sternum height, on the other hand, indicated a negative association. The findings imply that by taking linear body measures, body weight can be improved. The findings in bucks suggest that other traits can enhance body weight while sternum height can lower body weight. The coefficient of determination was utilized to assess the association among body weight and assessed traits. The results showed that heart girth had the highest coefficient of determination in does. Body length and heart girth had a significant coefficient of determination in bucks. The heart girth of Kalahari Red goats can be used to predict body weight in does, but both body length and heart girth can be used to predict body weight in bucks. The single nucleotide polymorphism was identified using Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP). According to PCR-RFLP results, two genotypes (KK and KM) were discovered, indicating that there was a difference in the *insulin-like growth factor 1* gene in Kalahari Red goats. The link between growth traits and identified genotypes was investigated using marker-trait association analysis. The data revealed that there was no correlation. As a result, changes in Kalahari Red goat growth traits may be attributed to other genes or the environment; thus, detected genotypes cannot be utilized as genetic markers in enhancing Kalahari Red goat growth traits.

5.3. Recommendations

- The association results revealed a relationship between body weight and heart girth, sternum height, body length, rump height and withers height in does. In

buck, body weight had a relationship with rump height, body length, heart girth and withers height. Hence, it is recommended that these traits can be used to improve body weight in Kalahari Red goats. Although there were positive results yielded on the correlation of growth traits, it is recommended that farmers be taught on the association and what it means.

- Regression analysis showed the traits that farmers can use to predict body weight in Kalahari Red goats. Body length and heart girth can both be used to forecast body weight in bucks, but only heart girth can be used to predict body weight in does. It is recommended that the researcher educate the farmers how to use the easy method of predicting body weight using linear body measurements.
- It is recommended that there should be more studies conducted on the single nucleotide polymorphisms in Kalahari Red goats, with the increased population size and more genes of interest taken into account, and their association with growth traits.
- Furthermore, sequencing methods such as Sanger sequencing are required to confirm the PCR-RFLP finding on single nucleotide polymorphisms.

CHAPTER SIX
REFERENCES

6.1 References

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