Gene Expression Profiling of South African Indigenous Goat Breeds using RNA-Seq Technologies In Search of Genes Associated With Growth and Carcass Quality Traits

by

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PREFACE

The research contained in this thesis was completed by the candidate while based in the Discipline of Genetics, School of Life Sciences of the College of Agriculture, Engineering and Science, University of KwaZulu-Natal, Pietermaritzburg, South Africa and the Agricultural Research Council-Biotechnology Platform. The research was financially supported by University of KwaZulu-Natal, Department of Science and Innovation-National Research Foundation (DSI-NRF Freestanding, Innovation and Scarce Skills Masters and Doctoral Scholarships), United States Department of Agriculture, Agricultural Research Services and the Agricultural Research Council-Biotechnology Platform.

The contents of this work have not been submitted in any form to another university and, except where the work of others is acknowledged in the text, the results reported are due to investigations by the candidate.

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Date: 01 December 2020

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My role in each paper and presentation is indicated. The * indicates corresponding author.

Chapter 3

1. Ncube, K.T., Hadebe, K., Dzomba, E.F., Soma P., Frylink L., and *Muchadeyi, F.C. 2017. Growth profiles and genomic population structure of South African indigenous goat populations. 5th African Goat Improvement Network Workshop held on 30th October to 03rd November 2017. Work was presented at the Goat Farmers Workshop on 30th October 2017. Presented by Ncube K.T.
2. Ncube K.T., Hadebe, K., Dzomba, E.F., Soma P., Frylink L., and *Muchadeyi, F.C. 2018. Growth profiles and genomic population structure of South African indigenous goat populations. Work was presented at the Indigenous Veld Goats Information and Club Establishment day held on 10th November 2018. Presented by Ncube K.T.
3. Ncube K.T., Hadebe, K., Dzomba, E.F., Soma P., Frylink L., and *Muchadeyi, F.C. 2019. Relationship between population genomic structure and growth profiles of South African goats under different production systems. *Tropical Animal Health and Production*. DOI 10.1007/s11250-019-02128-1.

Chapter 4

4. Ncube, K.T., Mdladla, K., Dzomba, E.F., Soma P., Frylink L., and *Muchadeyi, F.C. 2019. Genomic analysis and identification of candidate genes for carcass quality traits in South African goat populations. Work was presented at the University of Kwa-Zulu Natal Postgraduate Research day held on 22nd May 2019.

Chapter 3 and 4

5. Ncube, K.T., Mdladla, K., Dzomba, E.F., Soma P., Frylink L., and *Muchadeyi, F.C. 2018. Growth profiles and genome wide association of meat quality traits of South African Indigenous goat populations. A poster presented for the 5th Professional Development Program Post Graduate Conference, held on 27 to 29 August 2019, Agricultural Research Council, Vegetable and Ornamental Plants Institute, Roodeplaat, South Africa. Presented by Ncube K.T.

Chapter 5

6. Ncube, K.T., Rosen B.D., Dzomba, E.F., Schroeder S., Van Tassell C.P., and *Muchadeyi, F.C. 2020. Differential gene expression and identification of growth and carcass quality associated genes in the pituitary gland of South African goats. Submitted to BMC Genomics. Work was Presented at PAG XXVIII held on 11-15 January 2020 San Diego, CA, United States of America.

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ABSTRACT

Growth performance and carcass quality are economically essential traits in the chevon industry. There is a high demand for goat meat on the African continent which is unmatched by the existing goat populations due to untapped genetic potential and challenging environmental factors leading to low growth potential, poor carcass quality and yield. In a country where over 60 % of the goats are kept in communal farms with poor infrastructure and resources, challenges faced by these goats affect growth performance leading to low mature weights. Due to the importance of these traits, this study investigated growth profiles, carcass quality, genetic diversity and gene expression profiles of South African goat populations using the Illumina goat SNP50K and RNA sequencing technologies. Boer goats were used as a benchmark with which to compare the indigenous veld goats namely; i) Northern Cape Speckled ii) Xhosa lob ear iii) Mbuzi/Nguni as well as iv) Uncharacterized village goats. Two experiments were set up, one at the Agricultural Research Council, Animal Production to raise goats intensively and the second one was set in Pella village under the extensive production system. The SA Boer had a higher mature live weight (28.96±0.30 kg) as compared to other populations. The village goats raised in Pella village were relatively smaller (17.55±0.37 kg) than those raised on the research farm (19.55±0.36 kg). SA Boer goats had heavier warm and cold carcass weights (17.2±2.3 kg and 16.3±2.3 kg). Pella village goats raised under intensive system had heavier warm and cold carcass weights (9.9±1.1 kg and 9.2 ±1.2 kg) than those raised extensively (9.1± 2.0 kg and 8.4± 1.9). Candidate genes (*GADD45G, IGF2R, GAS1, VAV3, CAPN8, CAPN7, CAPN2, GHSR, COLQ, MRAS* and *POU1F1*) were associated with carcass characteristics. Transcriptomic analysis of the pituitary gland revealed differentially expressed growth-related genes such as the *POU3F4* and *CHKB*, were highly expressed in the extensively raised populations for the within-breed and across production system experiment, while differentially expressed *FGFR2* and *SMPX* genes were highly expressed in the Boer goat for between breeds raised under similar production systems experiment. Ballgown analysis revealed a high expression of *GH1* and *IGF1* in the intensively raised village goats compared to extensively raised village goats. Gene expression profiling of the *longissimus* muscle, on the other hand, unravelled genes such as the *MIR214, PDK4* and *KLF15* that were highly expressed in intensively raised versus extensively raised goats within populations raised under different production systems. Muscle-development related genes such as *COX2, ND3, ANAPC4, IGFBP3, MSTN, SUCO, LHCGR* and *KLF15* genes were highly expressed in Boer goats versus village goats raised under a similar production system. In addition, gene expression analysis in the pituitary gland revealed stress-related genes such as the *MAPK* and immune response genes (*CXCL9, CXCL10, CXCL11* and *BATF2*) from the gene expression analysis of the *longissimus* muscle were highly expressed in the extensively raised village populations further indicating that village goat populations are under a greater amount of stress. Overall, the study concludes that the production systems affects the attainment of genetic potential in village goat populations. Genes identified have the potential to be used in selection programs.

Keywords: *Carcass quality, gene expression, growth profiles, Illumina goat SNP50K, village goats*

EXTENDED ABSTRACT

Goats play a significant role in impoverished, marginalized communities of South Africa for food security and socio-economic purposes. The majority of goats are raised in villages with poor infrastructure and resources, therefore, facing challenges that affect growth performance leading to low mature weights. South Africa consists of two production systems where goats are raised under different farming conditions. The intensive production system is where goats are raised in large-scale commercial systems with suitable housing, adequate feed and veterinary networks, where goats such as the well-established, high-performing South African Boer are raised. The village goat populations, on the other hand, are raised in communal farms under the extensive system with unstructured management systems, feed scarcity and no veterinary assistance. These village goats are well adapted to their local production systems and are suitable for low input production systems. Apart from these, South Africa has another group of goats characterized as the indigenous veld goats that are raised under improved management systems by the Indigenous Veld Goat Society (IVG) farmers. Though there have been significant developments in South African goat genomic resources and reporting on the adaptation of local breeds, studies on growth, carcass and gene expression profiles of South African indigenous goat populations have not been reported. The advancement of technologies such as the Illumina Bead Chip which can be used in the understanding of goat populations, genetic composition and association studies, and RNA Sequencing as well as bioinformatics tools which have been used to measure relative gene expressions as well as in gene discovery studies, will assist in the research of growth, carcass and gene expression profiles in the investigation and profiling of growth genomic resources in order to reveal the genetic potential of South African goats. This study utilized five goat breeds including the Boer as a representative of the intensive production system which was compared with the indigenous veld goats namely; i) Northern Cape Speckled ii) Xhosa lob ear iii) Mbuzi/Nguni as well as iv) Uncharacterized village goats, where two experiments were set up at the Agricultural Research Council, Animal Production to raise goats intensively under controlled conditions and the second one was set in Pella village under the extensive production system. The study was conducted in two phases which included a genome-wide association study on carcass quality characteristics. The second phase was the investigation of gene expression profiles in search of growth, carcass quality and muscle development genes and an understanding of the mechanisms underlying growth and carcass quality in SA goat populations.

For the first experiment of this study, growth profiles, carcass quality and genomic population structure of SA indigenous breeds raised in different production systems were investigated in order to unravel the genetic potential of indigenous goat populations. In this experiment, live weights and morphological body measurements were collected from a total of 83 kids representing the commercial meat-producing SA Boer ($n=14$), the indigenous veld goats (IVG) of NC Speckled ($n=14$), Mbuzi/Nguni ($n=13$), and Xhosa lob ear ($n=14$) raised under intensive systems, and non-descript village goat populations ($n=14$) raised in the intensive, and others ($n=14$) raised in the extensive production systems. Of these 83 phenotyped goats, 72 were genotyped using the Illumina goat SNP50K BeadChip. The SA Boer had a higher mature weight (28.96±0.30 kg) compared to other populations. The Mbuzi/Nguni population had the smallest (14.83±0.33 kg) mature weight, while the village goats raised in Pella village were relatively smaller (17.55±0.37 kg) than those raised on the research farm (19.55±0.36 kg). Principal component analysis showed the Boer to be a different breed from the rest of the populations, while suggesting that the NC Speckled is a unique population. The rest of the IVG populations clustered together with the village populations. This is consistent with the ADMIXTURE results. The study concluded that genetic improvement and improved management and nutrition can lead to improved growth performance. The outputs of this study can be used to identify suitable breeds and potential genotypes for optimal growth and establish optimal goat management systems ideal for communal farmers for improved productivity.

As a build up from the first study, the second experiment further investigated carcass quality traits as essential and economic traits in goat meat production. Meat samples were collected from the same animals that were profiled for growth and genotyped on the Illumina Caprine SNP50K in the first experiment. SA Boer goats had heavier warm and cold carcass weights ($17.2^{a}\pm2.3$ kg and $16.3^{a}\pm2.3$ kg) compared to the rest of the populations. Pella village goats raised under intensive system ha d heavier warm and cold carcass weights ($9.9^{d}\pm1.1$ kg and 9.2^{c} ±1.2 kg) than those raised extensively ($9.1^{d}\pm$ 2.0 kg and $8.4^{c}\pm$ 1.9). A total of 2412 SNPs on chromosomes 6, 10, 12-13, 19 and 21 were reported significant at a -log10 of $P<0.05$. Candidate genes associated with carcass characteristics (*GADD45G, IGF2R, GAS1, VAV3, CAPN8, CAPN7, CAPN2, GHSR, COLQ, MRAS* and *POU1F1*) were observed. Results did shed light on the genetics of carcass traits in improved and non-improved goat populations and can find use in breed improvement programs.

The second phase of the study employed RNA-seq technologies on specific tissues hypothesised to play an important role in growth and carcass quality. The third experiment, therefore, investigated differentially expressed and associated growth-related genes from the caprine pituitary gland transcriptome of South African indigenous goat breeds of varying growth performance. Tissue samples were collected from three village ecotype goats and three SA Boer goats all raised in similar conditions simulating intensive commercial production systems to investigate between breed differences and from three village goats raised under extensive village conditions (on-farm in Pella village farming community) and three village goats raised under intensive commercial production system in order to investigate within-breed and between production system differences. Total RNA isolated from the pituitary gland of 36 weeks old animals (*n=9*) was sequenced individually in triplicates, and an average of 28 298 512 trimmed in total, and quality-controlled reads were mapped to the goat genome (*Capra_hircus.ARS1.94*) using HiSat2 software. Transcript assembly and quantification from RNA sequence yielded 104 differentially expressed genes for extensive system and 62 for the intensive system at the false discovery rate (FRD) of 0.05 % and a fold change of ≥2 which were retained for downstream analysis. Growth-related genes such as the *POU3F4* and *CHKB,* which were highly expressed in the extensively raised populations for the within-breed and between production system experiment. Conversely, growth-related genes such as *FGFR2* and *SMPX* genes were highly expressed in the Boer goats in the between breeds raised under similar production systems experiment. Ballgown analysis revealed a high expression of *GH1* and *IGF1* in the intensively raised goats compared to those raised under the extensive production system while this gene was also highly expressed in the village goats compared to the Boer. The *POU1F1* gene was moderately expressed between and within breeds in both experiments. The differential gene expression data provided insights into genes and molecular mechanisms associated with growth and growth development in goats.

The fourth and last experiment of the study analysed the transcriptome of the *longissimus* muscle to identify genes associated with muscle growth and development. Total RNA isolated from the *longissimus* muscle tissue of 36 weeks old animals (*n=9*) from the SA Boer and village goat populations was sequenced individually in triplicates and an average of 62 991 716 trimmed in total, and quality-controlled reads were mapped to the goat genome (*Capra_hircus.ARS1.94*) using HiSat2 software. Transcript assembly and quantification from RNA sequence yielded 12 differentially expressed genes for the extensive system and 9 for the

intensive production system at the false discovery rate (FRD) of ≤0.05 and a fold change of ≥2 which were retained for downstream analysis. Genes such as the *MIR214, BMP4, PDK4 and KLF15* were highly expressed in intensively raised versus extensively raised goats within populations raised under different production systems. For between breeds raised under similar production systems muscle-development related genes such as *COX2, ND3, ANAPC4, IGFBP3, MSTN, SUCO, LHCGR* and *KLF15* genes were highly expressed in Boer goats. Data from this study revealed genes and molecular mechanisms involved in muscle development and these have the potential to be used in breeding and development programs.

Overall, the study sheds light into the growth profiles and carcass quality traits of South African goat populations and their genetic potential, which gives more scope for the selection of desirable traits. Candidate genes identified in this study demonstrate a significant genetic potential in terms of their use in genomics-assisted breeding. Furthermore, the differential gene expression analysis provided insights into genes and molecular mechanisms associated with growth and muscle development in goats.

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CHAPTER 1: INTRODUCTION

1.1 Rationale for the research (nature and scope)

Goats, well known as the “poor man’s cow” are by far the most essential and useful livestock to humans (Sharma *et al.*, 2012). Goat farming in South Africa is predominantly done by communal farmers (63%) who are distributed in all the provinces of the country, with the Eastern Cape, KwaZulu-Natal and Limpopo provinces being the major goat producing provinces (Lahiff & Cousins, 2005; Morokolo, 2011; Dzomba *et al.*, 2018). The communal farming system, under which most goats are raised in South Africa, is characterized by poor management and suboptimal goat performance as compared to commercial goat farming systems (Masika *et al.*, 2007). Goats raised by communal farmers are used to improve food security and alleviate poverty through sales (Simela & Merkel, 2008; Gwaze *et al.*, 2009). In communal areas, goats are also used in ceremonial and traditional functions (Masika *et al.*, 2007), and most commonly for burials and rituals by traditional healers (Morokolo, 2011).

Goat meat is of great importance as it provides an easily accessible, high-quality protein to low-income families (Gwaze *et al.*, 2009). Goat meat contains less fat and has high levels of proteins, cholesterol and iron (NAMC, 2005). Even though goat farming is well practised in South Africa, goat meat production, consumption and offtake are still below optimum (Morokolo, 2011). Communal goat production is characterized by poor management practices often associated with suboptimal nutrition and housing, inadequate veterinary services and lack of breed/animal improvement programs (Gwaze *et al.*, 2009). Masika *et al.*, (2007) reported that poor management, shortage of feed, environmental stress and diseases and parasites are the main production constraints in communal agriculture.

It has been indicated that the goats kept by communal farmers are of low genetic merit particularly in terms of growth and reproductive potential (Visser *et al.*, 2004). The primary goat genotypes in communal farming systems (also referred to as “village goats”) are non-descript populations named by location or geographical regions such as Northern Cape Speckled in the Northern Cape Province and Eastern Cape Xhosa lob ear in the Eastern Cape Province (Morrison, 2007). Village goats are slow growing and therefore smaller in size and of low meat quality in comparison to commercial breeds (Masika *et al.*, 2007). This poses a limitation when raising village goats for meat production.

In South Africa, the most dominant meat-type goats are the SA Boer, Savannah and Kalahari Red, which are recognized as commercial breeds and are associated with high growth rates and meat yields (Visser *et al*., 2004; NAMC, 2005). They contribute good quality meat and income in the chevon industry. However, despite their desirable traits, commercial breeds cannot be sustainably grown or kept in communal farming systems. NAMC (2005) reported that fast-growing breeds, such as the SA Boer goat require more feed and nutrients for maintenance as well as health support, hence raising Boer goats becomes expensive and unaffordable particularly for smallholder farmers. The indigenous goats kept by most communal farmers travel long distances in search of feed and water and can use low-quality forage, in comparison to commercial goats which lack this ability (Gwaze *et al*., 2009). The majority of smallholder farmers do well with these indigenous goats that are well adapted to the local environments and production conditions (Pieters, 2007). Therefore, improving the production performance of the adapted indigenous goats is a more feasible option for these communal farmers.

Although considered highly adapted and suitable for communal farming systems, there is still a gap in knowledge about the actual genetic potential of indigenous goats. Indigenous goats are non-descript breeds that have not been selected for any production trait and are rather linked to their geographical locations (Visser *et al*., 2004). The indigenous goats are well known for their significant advantages of disease resistance, poor quality feed conversion to energy and adapt to a wide variety of climatic conditions (Webb & Mamabolo, 2004). Previous studies on landscape genomics (Mdladla *et al*., 2017) and the growth hormone gene (Ncube *et al*., 2016) of the South African goat populations have proven that there is room for within-population selection for improvement in traits of economic importance and adaptation. With the use of the Illumina goat SNP50K chip, Mdladla *et al*. (2017) was able to unravel valuable information on the demographic history of South African goat populations and further shed light into the understanding of the goat populations and their genomic composition. Ncube *et al*., (2016) revealed high polymorphisms in the *growth hormone 1* gene showing more potential for within-population selection and thus the high potential for this gene to be used in selection and breeding programs.

Growth performance and carcass characteristics are some of the most important economic traits in goat production. Therefore, it is of high importance to have high performing breeds with high carcass yields. Pophiwa *et al*. (2017) described live weights and carcass characteristics of the

Boer and indigenous goat populations where the Boer was proven to be a much better performing breed than indigenous breeds in terms of weight and carcass yields. Other studies like that of Mdladla *et al*. (2017) have described the use of body measurements as a useful tool to measure growth. Growth is one of the primary factors influencing meat and carcass yield and it is affected by genes such as the *growth hormone1*, *caprine myostatin* and *caprine pituitary specific transcription factor-1* genes (Supakorn, 2009). Polymorphisms in these genes, such as the *growth hormone1* gene, are associated with pre- and post-natal growth in animals (Alakilli *et al.*, 2012). The polymorphisms in the *growth hormone1* gene can be used as markers for growth association studies and as a tool for high growth performance selection (Wickramaratne *et al*., 2010). High-throughput targeted gene sequencing of the *growth hormone 1* gene has shown the locus to be polymorphic and capable of differentiating goat populations from different breeds and production systems (Ncube *et al*., 2016).

Variations in the ability to use poor quality feed, disease resistance and adaptation to a wide variety of environmental conditions have also been reported in indigenous goat populations (Visser *et al*., 2004; Webb & Mamabolo, 2004). It is, therefore hypothesized that indigenous goats have good genetic potential and can be selected for optimum growth under the compromised production systems.

Studying the growth potential, muscle development and carcass quality traits as well as the associated genes and genetic mechanisms in crucial particularly where efforts are being made to improve productivity of local goat populations. Some of the methods that can be used to study the genes and genetic mechanisms associated with growth and carcass quality are RNA sequencing and genome-wide SNP genotyping technologies. These methods will generate information on SNPs, genes and gene expression profiles associated with growth and carcass quality performance, which can be used in genomic assisted selection and breeding.

1.2 Justification

A large percentage of goats in South Africa are generally non-descript village goats that are kept by communal farmers in small communities and rural areas (Morokolo, 2011). Communal farming is generally characterized as resource-poor management (low input) and lacking in modern methods of goat farming (Masika *et al.*, 2007). Keeping fast growing commercial breeds in smallholder farms is challenging as they are too costly to keep because they require more feed and feed supplementation (NAMC, 2005) which communal farmers cannot afford (Morokolo, 2011). The non-descript village goats kept by communal farmers are unimproved, uncharacterized and have not been subjected to any form of artificial selection (Morokolo, 2011). Non-descript village goats show a great genetic potential due to the phenotypic diversity in growth rates and mature body weights that they exhibit (Visser *et al.*, 2004). Variation in ability to use poor quality feed, disease resistance and adaptation to a wide variety of environmental conditions have also been reported in indigenous goat populations (Visser *et al.*, 2004; Webb & Mamabolo, 2004).

Although there have been several studies that have unravelled the genetic potential of South African goat populations, limited information exists in the genetic factors that influence traits such as growth and carcass quality. Across species, genomics have helped unravel the genetic potential of species for traits of economic and socio-economic importance. The Illumina goat SNP50K Bead chip is a medium-density SNP chip consisting of markers evenly spaced across the goat genome and is a useful tool for population genetic studies in goats (Tosser-Klopp *et al.*, 2014; Lashmar *et al.*, 2015; Mdladla *et al.*, 2017). The Illumina goat SNP50K chip was used to reveal valuable information on the demographic history of South African goat populations (Mdladla *et al*. 2017). This study therefore, opted to use the Illumina goat SNP50K to investigate the population genetic structure and associated growth and carcass quality traits of South African indigenous goats under different production systems.

There are several factors that influence growth and carcass yield, some of which are environmental, breed and genetic factors (Webb, 2014). RNA sequencing is a high-throughput sequencing method for characterizing gene expression in specific tissues. This technology provides accurate counts of transcripts to measure relative expression and to discover new exons or genes (Wang *et al*., 2009). This approach is widely used for mapping, and quantifying transcriptomes developed to analyze gene expressions on various tissues (Cánovas *et al*., 2010). Gene expression differs between breeds and is triggered by different environmental conditions and as such been used to investigate genes and molecular mechanisms influencing traits of interest in domestic livestock species (Song *et al*., 2019). Studying the transcriptome and differential gene expression of SA goat populations will shed light on the genetics of growth, muscle development and carcass quality in these unique genotypes. The study therefore aimed to use transcriptomic analysis to investigate the effect of production system and breed differences on gene expression profiles.

It is hypothesized that:

- Village goats raised under the intensive production system have better growth performance compared to those raised extensively, therefore, suggesting that improved goat management systems can lead to expression of the genetic potential of different goat breeds through improved growth.
- The South African Boer is a well-characterized breed and genetically superior for growth and carcass quality traits when compared to indigenous and non-descript village populations.
- Gene expression is affected by the environment and production system that animals are raised in and as such different genes were expected to be expressed between village goats raised under different production systems.
- Gene expression is affected by the breed of the animal and as such different genes were expected to be expressed between different breeds raised under the same production.

1.3 Aims

The study aims to use genomics and transcriptomics to build an understanding of the growth potential, carcass quality and associated genetics of South African goat populations.

1.4 Objectives

Specific research objectives are:

- To investigate the population genetic structure and associated growth potential of South African indigenous goat breeds and ecotypes.
- To describe the carcass characteristics of South African indigenous goat breeds and populations and investigate the associated SNPs and genetic mechanisms using the Illumina goat SNP50K chip.

- To use RNA-Seq based gene expression profiling to investigate genes and molecular mechanisms associated with growth and carcass quality of South African goat breeds and populations.
- To investigate differential gene expression and genetic mechanisms associated with muscle development and carcass quality i) within breeds, across production systems and ii) between breeds within the same production systems.

In so doing, the study also sought to identify genes that are associated with growth and muscle development that could potentially act as selection targets in goat breeding and improvement programs.

1.5 Outline of the thesis structure

Each chapter has an abstract, introduction which incorporates a literature review, materials and methods, results and discussion and conclusions.

Chapter 2 is a review of the available knowledge on the South African goat populations genetic resources as well as production systems and their challenges. The chapter further reviews three groups of South African goats describing their characteristics and a brief overview of their growth performance, and explores the potential of village goats as well as indigenous veld goats for their selection for desirable growth and carcass quality traits. The review also discusses transcriptomics as one of the genomics approaches in studying genes that are associated in growth and muscle development.

Chapter 3 draws more attention to the growth profiles of South African indigenous goats. This chapter also looked at the populations genomic structure of these populations, in addition the chapter utilized body conformation indices as an additional tool to analyze diversity as well as differentiate between breeds or populations.

Chapter 4 involved a genome wide association analysis of carcass quality traits in SA goat populations. This experimental chapter described the carcass quality and yield of different goat populations and identified candidate genes that are associated with carcass quality traits using the Illumina goat SNP50K genotypes.

Chapter 5 investigated gene expression profiling within breeds under different production systems as well as between breeds under a similar production system in search of growth- and carcass-related genes and associated genetic mechanisms using transcriptome data of the pituitary gland.

Chapter 6, analysed the transcriptome of the longissimus muscle in search of differentially expressed genes within breeds raised under different production systems as well as between breeds raised under similar production system. The chapter also looked at biological pathways that help in the understanding of biological mechanisms involved in growth and development of muscles.

The last chapter, Chapter 7, provided an overall discussion of the study findings, conclusions and recommendations for future analysis.

1.6 Ethical consideration

The Animal Ethics Committee of the Agricultural Research Council, Animal Production, South Africa (Ethics approval number “APIEC16/010”) has approved all the work and animal management undertaken in this study.

CHAPTER 2: GENE EXPRESSION PROFILING FOR GROWTH AND CARCASS QUALITY TRAITS IN SOUTH AFRICAN INDIGENOUS GOATS: A REVIEW

2.1 Abstract

Growth performance and carcass quality are economically essential traits in chevon production. South African goat populations are found in either the commercial (commercialized meat breeds) or the extensive production system (non-descript populations). The commercial production system consists of proper management and housing that is believed to contribute to optimum growth performance. Understanding current production systems will aid in developing better management practices for improved growth and chevon production. Though genomics studies have unravelled the genetic potential of South African non-descript goats, more still needs to be done to understand the genetics of traits of economic importance in these goat populations. One of the challenges of conducting genomic studies in non-descript populations is the absence of farm records on growth performance under these production systems.

Gene expression profiling provides insights into genes and molecular mechanisms associated with traits of economic importance such as growth, muscle and skeletal muscle development. Profiling these genes will shed light into the complex genetic mechanisms used by goat populations to thrive under diverse and often challenging production systems. This review explores the current state of goat genomics in South Africa and how gene expression profiling can help in the future understanding of the genetic potential of South African goats under different production systems. Understanding the molecular mechanisms that contribute to growth and growth performance will have a significant impact on breeding and management strategies.

Keywords: *Gene expression, goats, growth, growth performance, production systems*

2.2 Introduction

Goats are produced worldwide, with a total count of about 1 billion (Mazhangara *et al*., 2019). Sub-Saharan countries such as South Africa, Nigeria, Namibia and Botswana are the major goat producing countries in Africa. The South African goat industry alone is estimated at 7.8 million live goats with over 63 % raised by communal farmers in rural areas (Ncube *et al*., 2016; Dzomba *et al*., 2018). Goats play an important socio-economic and food security role in rural communities (Ngambi *et al*., 2013; Visser & van Marle-Köster, 2018; Wodajo *et al*., 2020). Despite their important roles and the high number of goats in South Africa and other African countries, there is still scarcity and low consumption of goat meat (Webb, 2014).

South Africa is one of the few countries across the world with well-defined goat breeds such as the Boer, Savannah, and Kalahari Red that are predominant in the commercial farming systems for meat production (Mdladla *et al*., 2017; Dzomba *et al*., 2018). There also exists phenotypically characterized indigenous veld goats (Morrison, 2007) named according to their geographical location and phenotype (Northern Cape Speckled, Xhosa lob ear and Mbuzi), which are kept by organized farmers within the Indigenous Veld Goat Society (IVG). The non-descript village goats in communal farming systems are often uncharacterized and commonly named according to their geographical locations (Mohlatlole *et al*., 2015) and include Venda goats from Vhembe District in Venda, Limpopo Province of South Africa (Gwaze *et al*., 2009; Morrison, 2007), Namibian Ovambo from the Ovambo region in Namibia (Els *et al*., 2004), West African Dwarf from West Africa (Fasae *et al*., 2012) and Tswana goats from Botswana (Gwaze *et al*., 2009). These uncharacterized goat populations constitute an important genetic resource necessary to meet food security and improve livelihoods of marginalized communities.

Non-descript village goats are known for their ability to use poor quality feed, resistance to prevalent diseases and parasites and their adaptation to harsh environmental and climatic conditions that are characteristic of their habitats (Bester *et al*., 2009; Mataveia *et al*., 2018; Ncube, 2016). Regardless of these desirable characteristics, growth performance and carcass quality characteristics of non-descript village goat populations have not been comprehensively characterised and reported. Village goats have been observed to have various body sizes both within and between populations, with an average mature live body weight of 38 kg (Lusweti, 2000). Mature live body weights vary according to populations, the Mbuzi/Nguni has an average adult weight of 40 kg in bucks and 34 kg in does, while the Xhosa lob ear is 32 kg for bucks and 29 kg for does (Snyman, 2014), body weights of other ecotypes such as the Northern Cape Speckled have not been documented. Such variation can be exploited through selection and breeding to improve productivity.

Breeds such as the SA Boer that have been specially developed for meat production are fast-growing (NAMC, 2005), whereas the non-descript goats have a wide variation in growth rates (Kosgey *et al*., 2008; Gaddour *et al*., 2012; Hassen, 2012). High variations in body weights and a below-average mature body weight of about 38 kg have been observed in the village goats (Visser *et al*., 2004). Since commercial goat farmers select for fast-growing animals and culling against poor growth performance, commercial breeds demonstrate a much faster growth rate when compared to the non-descript communal goats (Webb & Mamabolo, 2004).

It's hypothesized, however, that as uncharacterized and not artificially selected for economically important traits, non-descript village goat populations should possess an untapped genetic potential for growth and body weight traits (Webb & Mamabolo, 2004; Peacock & Sherman, 2010). Improvement of growth in non-descript village goat populations would therefore result in increased chevon (goat meat) yields, which would contribute to improved food security amongst marginalized communities (Visser & van Marle-Köster, 2018).

Growth is the primary factor influencing the amount and expense of meat produced at mature weight (Supakorn, 2009). Several genes affect growth and these include the growth hormone (*GH*), growth hormone receptor (*GHR*), insulin-like growth factor I (*IGF-I*), leptin (*LEP*), caprine pituitary specific transcription factor-1 (*POU1F1*), caprine myostatin (*MSTN*) and bone morphogenetic protein (*BMP*) genes. These genes can be used as genomic markers for the improvement of muscle and other growth traits (Supakorn, 2009). Variations in these genes are associated with variability of growth traits in goats (Alakilli *et al*., 2012). In addition to growth, carcass quality is one of the most important traits in livestock production, influencing meat quality (Agbeniga & Webb, 2018).

Growth and carcass quality are quantitative traits that are influenced by several genes (Alakilli *et al*., 2012). Genomic studies on growth and carcass quality traits in South African goat populations will, therefore, assist in evaluating the potential of candidate genes for use in selection programs and growth performance improvement. In Africa and other developing countries, non-descript goats harbours great genetic potential with their variation in size and ability to use poor quality feed. Genetic profiling of this genetic diversity could provide fundamental information for future selection programs.

A number of studies profiling growth and carcass traits and the genomic associations emerged in the past years (Robledo *et al*., 2017; Wu *et al*., 2018). Expression of growth-related genes such as the *MSTN* and *PRKAG3* in slow-growing chickens have been associated with meat quality traits (Wu *et al*., 2018). Ncube *et al*. (2016) analysed the *growth hormone 1* gene and identified mutations that resulted in amino acid changes that could result in phenotypic alterations. The study by Ncube *et al*. (2016) also suggested that the South African goat populations, especially the non-descript ecotypes, have a genetic potential for within-population selection for good growth. More genomic association studies are, however, still required particularly in conjunction with performance records on growth and carcass quality. To successfully perform a growth performance study and analyse growth-related genes, one needs to have in place records such as weights and body measurements, which are scarce under communal farming systems.

Next-generation sequencing (NGS) as a high-throughput technology brings about an array of sequencing tools and approaches to study genes and their differential expression for specific traits. NGS is a rapid technology that reduces turn-around time of sample processing. Next-generation sequencing is a technology that allows one to do routine sequencing of either complete genomes or targeted sections in a faster and more effective manner (Myllykangas *et al*., 2011). One of the tools used within the NGS platform is RNA sequencing (RNA-Seq) that is used to generate gene expression data on specific genes of interest, providing information to make inferences on genetic associations and candidate genes. RNA sequencing (RNA-Seq) provides more accurate levels of analysed transcripts and is capable of discovering genes and associated pathways for affected phenotypes (Wang *et al*., 2009).

The present review explores the current genetic resources of South African goat populations and explores literature on the growth profiles and carcass characteristics of indigenous goat breeds and populations. The review looks at different meat breeds in the South African goat industry as well as their growth and carcass characteristics. In addition, more light is shed on the South African characterized indigenous veld goats (IVG) and non-descript village goat populations. Furthermore, the review explores the use of genomics in the South African goat sector and how gene expression profiling can aid in the future understanding of the genetic potential of South African goats under different production systems. The utility of differential gene expression profiling as a tool to understand molecular mechanisms that contribute to growth performance and improved carcass quality are explored.

2.3 South African goat populations

The primary role of goats is chevon. South African goat populations can be grouped into three categories of i) Commercial meat type ii) Indigenous Veld Goats and iii) Extensively raised village goat populations. The South African goat meat industry utilizes indigenous goat breeds raised commercially under large scale production systems (Dzomba *et al*., 2018).

Commercial meat breeds such as the indigenous Boer, Savannah and Kalahari Red are bred for high meat yield and raised in large scale systems characterized by well-designed housing systems, adequate feed, feed supplementation and regular health care systems (Mdladla *et al*., 2016). Morrison (2007) phenotypically characterized a group of indigenous goats as indigenous veld goats namely, i) Northern Cape Speckled, ii) Xhosa lob ear and iii) Mbuzi/Nguni and these are kept by farmers of the Indigenous Veld Goat Society (IVG). The extensive production system is one of the important sectors where communal farmers use non-descript village goat ecotypes that are adapted to low-input communal farming (Mdladla *et al*., 2017; Dzomba *et al*., 2018). These goats fend for their food under the extensive system.

2.3.1 Commercial meat goat breeds

2.3.1.1 The South African Boer

The South African Boer is a commercial meat breed that was developed and established in South Africa in the early 1900s and was bred for meat production (Erasmus, 2000; Campbell, 2003; Ncube, 2016; Dzomba *et al*., 2018; Visser & van Marle-Köster, 2018). Breeders of the Eastern Cape developed it using Xhosa lob ear goats, including a specific big, robust dapple coloured male goat, that formed the basis of the well-known Buffelsfontein Boer Goat Stud (Morrison, 2007). There are about six types of Boer goats, namely, the ordinary Boer, long-hair, polled white red-headed, bridle/briekwa, the mouse-ear and short-ear Boer goats (João & King, 2009). The common commercial Boer goat is characterized by horns, short white-haired body with red-brown colour on the head, a solid white stripe on the forehead and long droopy ears (Snyman, 2014; Dzomba *et al*., 2018). This stud influenced the development of the Boer goat in South Africa to a marked extent (Morrison, 2007). It is one of the best goat breeds developed for meat production (Dzomba *et al*., 2018) with excellent body conformation, fast growth rate and performance with an average weight of 120 kg for bucks and 80 kg for does, good carcass quality and carcass yield of 25 kg carcass weight for bucks and 22 kg for does 100 days post-natal (Sapkota *et al*., 2016). It is a highly fertile breed where triplets and quadruplets are common (Sapkota *et al*., 2016). It's yearlings have been reported to have a high pre-slaughter weight which leads to high carcass yield as compared to village goat populations (Pophiwa *et al*., 2017). Using targeted high-throughput sequencing, Ncube *et al*. (2016) observed differences between the Boer goats and non-descript village and feral goat populations in the *growth hormone 1* gene, which was expected since the Boer was specifically selected for growth and could have diverged from other populations in such growth-related genes.

2.3.1.2 The Kalahari Red

The Kalahari Red was selected from lob ear animals for its uniform red coat colour by the South African and Namibian breeders and is hence characterized by long ears, a solid red coat colour together with an ability to thrive in sweltering conditions (Pieters, 2007). Initially identified as a landrace goat in 1998, the Kalahari Red is reported to comprise a mixture of the Savanna and Boer goats and was named after the red dunes of the Kalahari (Farmer's weekly 2010). The Kalahari Red was initially restricted to the Eastern, Northern and Western Cape provinces and Namibia but has since spread all over South Africa and into other countries such as the United States of America (Farmer's weekly 2010). Management of this breed is as costly as the Boer as it also requires a high level of feed supplementation and minerals with accessible copper (Little, 2010). It has tender meat with an average mature weight of 75 kg and weaning weight of 25 kg for bucks and 21 kg for does (Snyman, 2014). The carcass and meat quality characteristics have not been reported.

2.3.1.3 The Savannah

Messers Cilliers and sons developed the Savannah goat from a mixture of indigenous white ewes and a white ram in 1957 (Visser *et al*., 2004). Though developed in 1957, it was only recognized as a unique breed in 1993 (Campbell, 2003). It is characterized by its solid white colour, long, slightly curved face and long-oval shaped ears said to be of Southern African origin (Pieters, 2007). It is well known for high fertility and fast growth rate (http://www.extension.org/pages/19384/goat-breeds-savanna, accessed 15 August 2019). At a young age, the Savanna has a lean, balanced carcass with low-fat tender meat (Snyman, 2014). It has a good growth rate with an average mature weight of 60 kg and weaning weight of 30 kg for bucks and 25 kg for does (Snyman, 2014).

2.3.2 Indigenous Veld Goats

2.3.2.1 Xhosa lob ear

Xhosa lob ear is a medium to large frame goat, well-muscled, with long lob ears. Dapple and marble patterns of different colour combinations are generally observed among this ecotype, although single colours and combinations are found. The Xhosa lob ear originates in the Eastern Cape regions with medium to lower rainfall and played a role in the development of the SA Boer goat (Morrison, 2007). High inbreeding levels have been reported within this population which led to low production performance (Dube *et al*., 2016). The average mature weight of the Xhosa lob ear is 32 kg for bucks and 29 kg for does (Snyman, 2014).

2.3.2.2 Nguni/Mbuzi

Widely distributed in Southern Africa, specifically in the higher rainfall areas stretching from the Eastern Cape, Kwa-Zulu Natal, Swaziland, Mpumalanga, Northern Province, Botswana, the Caprivi, and the extreme northern, high rainfall area of Namibia (Morrison, 2007). With regards to areas mentioned above, it is observed that these goats have similar phenotypes, with minute geographic variations. It has medium to small and semi-pendulous ears, but not dwarf- and milk goat ears. They are medium to small framed, well-proportioned goats. Sexual dimorphism is a characteristic of the breed, with small females and much larger males (Morrison, 2007). The Nguni yearlings weigh about 18.6 kg for bucks and 14.6 kg for does, furthermore, the adult doe weight at 8 teeth is 34 kg while the buck adult weight has not been reported (Snyman, 2014).

2.3.2.3 Northern Cape Speckled

Speckled goats are normally medium to large framed, rather well-muscled, with large drooping lob ears. The body is covered with red, red-brown or black spots with the lower part of the legs having an almost solid dark pigmented colour (Morrison, 2007). The head is protected by a concentration of colour around the muzzle, eyes and on the ears, with a white blaze on the forehead (Snyman, 2014).They are very heat and sunlight tolerant (Morrison, 2007). Their productivity is equal to or higher than any of the other goat types in South Africa, with an average of twins every eight months (Snyman, 2014). The Speckled goat originates in the dry Northern Cape, Karoo area, stretching from Sutherland to Upington (Morrison, 2007).

2.3.3 Extensively raised village goat populations

Communal farmers keep non-descript goats in rural areas (Ncube *et al*., 2016). The primary purpose of non-descript goats is for meat production (Morokolo, 2011) although they are also used for traditional ceremonies (Morokolo, 2011) while their manure is used for fertilization of soil in communal areas (Gwaze *et al*., 2009). Non-descript village goats are characterized by a demonstration of a variety of coat colours, hair and ear lengths and body sizes (Morrison, 2007), none of which are not characterized (Visser *et al*., 2004). In addition, the SA non-descript village goats are reported to demonstrate disease tolerance and an ability to utilize poor quality feed (Gwaze *et al*., 2009). They are believed to have come to South Africa as the black communities were migrating to different parts of Southern Africa (Campbell, 2003). Their actual centre of origin and evolution thereafter is not well known. Non-descript village goats are mostly owned by smallholder farmers in poor communities and are named based on the area in which they are found, for instance, Nguni goats in Kwa-Zulu Natal and Venda goats in the Venda village of the Limpopo Provinces of South Africa (NAMC, 2005). The genetic diversity of non-descript village populations was studied by Mdladla et al. (2016), where these populations were differentiated according to their origins. A further study analyzed genomic regions under selection and associated with different environmental and climatic conditions (Mdladla et al., 2018).

Regardless of the progress made in developing commercial breeds, the majority of SA's non-descript village goat populations remain uncharacterized with very little information available on their origin, production characteristics and genetic attributes. Goat performance in communal farms is said to be lower than that of commercial farming systems (Masika *et al*., 2007). This has been mainly due to the sub-optimal management practices of village farming systems (Masika *et al*., 2007). Since size and body weight are essential traits in goat meat production, the improved commercial breeds are preferred over village breeds as they grow fast and yield higher mature weights (Kosgey *et al*., 2008). The average yearling weight of non-descript village populations in the Gauteng region is about 33.7 kg (Pophiwa *et al*., 2017). Non-descript village goats usually fend for their food in crop fields, bushes or around the household, and no supplement feed is given (Webb & Mamabolo, 2004). High level of diseases and parasites combined with poor nutrition lead to poor performance of village goats (Gwaze *et al*., 2009). Non-descript village goats that can survive and thrive under such production challenges are then preferred for farming.

The SA non-descript village goats have great genetic potential due to high phenotypic variation within populations (Simela & Merkel, 2008; Mdladla *et al*., 2016; Ncube *et al*., 2016). Recent studies on these goats populations have revealed their genetic potential (Ncube *et al*., 2016; Mdladla *et al*., 2018) however, more studies are required to further reveal this genetic potential such that it can be utilized to benefit South African goat farmers.

Masika et al. (2007) reported poor performance and high within-population variation in South African village goat populations. More studies are still required to profile the growth performance, carcass characteristics and future growth potential of these goats. This will provide insight into what the optimal selection programs for these goats are and how to improve them for better growth performance.

2.4 Growth performance and carcass traits

Growth performance is one of the important traits in goat production. Goats in communal farms fend for their food while those in large scale commercial farms have adequate feed and healthcare, and these are some of the factors that affect growth performance. Apart from genetics, other factors that affect growth performance include nutrition and management practices. It is, therefore, of great importance to study growth performance and further investigate how it can be improved, especially in communal farms. Investigating growth profiles and carcass quality traits of South African goats is key to understand the potential of local goat populations. Some studies have been performed in an endeavour to investigate growth performance in South African goats mainly focusing on live weight. Snyman (2014) studied the growth performance of South African indigenous goats using live weights where it was reported that the Boer was superior in growth performance with a 100-day weaning weight of 27 kg for buck kids, and 24 kg for doe kids. A study by Pophiwa et al. (2017) investigated carcass quality between indigenous goats and the Boer and observed that the Boer goats had expectedly high carcass yields compared to indigenous goats; further indicating high growth performance in this breed. With the existing research on growth performance, there still exists a gap in the growth performance of South African goats, particularly growth at different growth stages.

Growth profiling is one of the tools that can be used to understand growth performance of South African goat populations. Some of the methods that can be used to measure growth have been described by Chacón *et al*. (2011) and Mdladla *et al*. (2017) where body measurements data can be collected using a flexible measuring tape. Furthermore, Chacón *et al*. (2011) have described the use of body conformation indices as an additional measure to profile and characterize growth. Weight measurements have also been used as a method to measure growth performance (Snyman, 2014). In the extensive system goats roam freely to fend for food and there are no formal managements practices in place, therefore, no record keeping by farmers in order to track growth performance of goats. With the advancement of goat studies in Africa and the establishment of community based breeding programs, studies such as those by Mdladla *et al*. (2017) describe other methods that can be used for body measurements and these can also be incorporated in communal farms especially through community based breeding programs. In other African countries such as Ethiopia, rural communities use chest girth measurements to estimate the live weight of their small ruminants due to limitations of having a scale (Asefa *et al*., 2017).

2.5 Goat genomic tools in the Next-generation sequencing era

Advances in the genomic era have shown that more can be explored in terms of using genomics to reveal and aid in the understanding of genetic mechanisms. Approximately 271 genes have been found to be associated with the economic traits such as milk, fibre and meat production, disease resistance, reproduction and growth in goats (Supakorn, 2009). Seven genes were specifically associated with growth and are presented in Table 2.1 (Supakorn, 2009).

Some of these genes show potential for use in optimizing animal growth for production purposes. For example, the *leptin* and *caprine myostatin* genes can be used in meat production and improvement (Alakilli *et al*., 2012). The *leptin* gene plays a role in carcass traits with studies suggesting the use of this gene as a marker for weight improvement (Javanmard *et al*., 2010). The caprine *myostatin,* on the other hand, can be used in meat improvement for desirable traits such as meat tenderness (Jain *et al*., 2012).

The next-generation era offers a range of tools that can generate high-quality data in a short period of time (www.illumina.com). This data can then be used to study informative genes that can assist in bringing about useful information and hence allow for a better understanding of the South African goats and more specifically, the SA non-descript village goats. Their potential can hence be ascertained and developed for their improvement. Several tools can be used in goat genomics studies. One such tool is Single Nucleotide Polymorphisms (SNPs) that can explain most of the genetic discrepancies evident between animals (Salem *et al*., 2012).

Single Nucleotide Polymorphisms (SNPs) are single base-pair modifications within the genome (Dough, 2010) comprising of non-synonymous and synonymous (Sharma *et al*., 2012) as well as coding and non-coding variations. Coding SNPs are found near the coding regions of various genes such as the mitochondrial DNA (Salem *et al*., 2012). Several techniques have been developed to study SNPs, which include SNP arrays, whole-genome sequencing (WGS) and targeted gene sequencing (Sharma *et al*., 2012).

Table 2.1 Growth hormone genes and their role in growth performance and other growth traits

Gene	Populations	Key findings	References
Growth hormone	Chinese Boer	Association with growth traits	(Hua *et al*., 2009)
Growth hormone receptor	Alpine, Polish Fawn Improved, Saanen, Polish White Improved and Boer	Associated with some metabolic processes in the liver and may have an effect on the growth of goats.	(Strzelec & Nikowski, 2011)
Insulin-like growth factor I	Egyptian and Saudi	Major role in protein synthesis, growth of mammals, skeleton and hair growth. Plays a role in reproduction, ageing and growth development	(Alakilli *et al*., 2012)
Leptin	Boer	Positive regulator of the carcass, growth and production traits	(Javanmard *et al*., 2010)
Caprine pituitary	Chinese indigenous	Polymorphisms of the coding gene of POU1F1 have a positive influence on	(Lan *et al*., 2007)

specific transcription factor-1		animal weight, milk performance and reproduction	
Caprine Myostatin	*Capra hircus*	The use of gene knock out of the caprine myostatin gene to improve goat meat and develop desirable traits such as high protein content, soft and tenderness and less fat	(Jain *et al*., 2012)
Bone morphogenetic protein	Egyptian and Saudi	i) Influences goat growth ii) Influence on chest circumference; body height and trunk index whereas the sub-division	(Supakorn, 2009; Alakilli *et al*., 2012)

2.5.1 The Illumina Goat SNP50K

The Illumina goat SNP50K is a high-density SNP chip consisting of markers evenly spaced across the goat genome and is a useful tool for population genetics, genetic diversity and genomic association studies (Tosser-Klopp *et al*., 2014). It was developed using six goat breeds and has been validated to be suitable for use in a variety of goat breeds (Tosser-Klopp *et al*., 2014; Lashmar *et al*., 2015; Mdladla *et al*., 2016). This technology has been used in various studies in South African goats. One such study was performed on Angora goats, where the suitability of the Illumina goat SNP50K Bead Chip for specific breeds was explored (Lashmar *et al*., 2015). Lashmar . (2015) also suggested that the chip could be used as a tool for genome-wide association, genetic variation, signatures of selection and genomic selection studies as well as for parentage verification. The Illumina goat SNP50K chip was used in the analysis of population structure of South African goat populations (Mdladla *et al*., 2016). In this study, Mdladla *et al*. (2016) reported the feral Tankwa population to be a genetically distinct population with South African goat populations clustering according to their historical origins. This study by Mdladla and co-workers (2016) further described the usefulness of the Illumina goat SNP50K in the investigation of population structure, genetic diversity and relationships between the SA non-descript goats and the feral Tankwa populations. The Illumina goat SNP50K chip also revealed valuable information on the demographic history of South African goat populations (Mdladla *et al*., 2017). The Illumina goat SNP50K Bead chip was used for body morphological traits in Sudanese goats (Rahmatalla *et al*., 2018). Using this chip, SNPs and genes associated with growth, body metabolism and other adaptive traits were reported to be segregating and differentiating goat populations from different geographic and production environments of South Africa (Mdladla et al., 2018). The use of very few breeds (six) to develop the goat SNP50K (Tosser-Klopp et al., 2014), poses limitations in that, breeds that were used are not of African origin and there may be some genetic variations due to ascertainment bias. Though studies like that of Lashmar *et al*. (2015) and Mdladla *et al*. (2017) have proven its usefulness in South African goat populations, it still hasn't been tested in other breeds such as the Northern Cape Speckled for its usefulness.

2.6 Next Generation Sequencing (NGS)

Next generation sequencing (NGS) is a high throughput technology based on massively parallel sequencing, where millions of sequences are deciphered concurrently (Franklin *et al*., 2013). The technology originated in the early 2000s and it was proven a success through the complete sequencing of the human genome (Franklin *et al*., 2013). NGS consists of platforms that have proven to be highly quantitative and adaptive with the promise to eliminate micro-array limitations (Malatji, 2017). It has the ability to identify transcriptomes without knowledge of a particular gene, therefore, providing insights on alternative splicing and alternative variations in identified genes, and it also gives complete coverage in a relatively short period of time (Malatji, 2017; Wang *et al*., 2009). Platforms such as the Illumina HiSeq 2500 can produce about 500Gb and NovaSeq 6000 can produce about 3 TB of sequence data in one run in less than 10 days.

2.6.1 Whole Genome Sequencing

Whole-genome sequencing (WGS) is a method whereby the whole genetic makeup of an organism or animal is sequenced (www.illumina.com). Whole-genome sequencing in practice does, however, not necessarily cover all components of the genome (www.acmg.net). For sufficiency in data generated, a large number of sequence reads is needed (approx. 6.3 million) and, multiplexing is also a challenge (Boon & Faas, 2013). These alone make sequence cost per sample very high, thereby placing limitations on sample numbers (Boon & Faas, 2013).

WGS studies done on the South African feral Tankwa goat revealed variants associated with cellular pathways, defence and immunity (Mohlatlole *et al*., 2015). SNPs that show a potential role in goat growth performance, diseases and adaptation processes have also been identified (Mohlatlole *et al*., 2015). This technology has also been used in the identification of selection of signatures in important traits such as coat colour, growth, reproduction and high-altitude adaptation in goats (Guo *et al*., 2018; Wang *et al*., 2016). The number of thoracic vertebrae is one of the important economic traits that influences carcass length and meat production in livestock and WGS revealed candidate genes that were associated with thoracic vertebrate number in sheep (Li *et al*., 2019). Through WGS in Pakistan cattle breeds, it was revealed that most of the altered genes are significantly enriched in economically important biological processes such as heat tolerance, immune response, development and sensory perceptions (Iqbal *et al*., 2019). However, as mentioned above, the main limiting factor of this technology is the high cost per sample making it too expensive; especially in the case of multiple samples.

Whole genome sequencing has been very useful in the development of goat genomic studies especially in the sequencing of goat reference genomes. The first draft genome achieved through WGS was from the female domestic goats Yunnan black goat with genomic size of approximately 2.66 Gb (Mohlatlole *et al*., 2015). This was followed by other goat genome sequences that have been successfully used in the identification of CNVs, SNPs and other genomic variants (Mohlatlole *et al*., 2015).

2.6.2 RNA-Sequencing

RNA-Seq is a high throughput sequencing method for gene expression profiling widely used for mapping and quantifying transcriptomes and to analyse gene expressions in various tissues (Cánovas *et al*., 2010); providing more accurate levels of transcripts used to measure transcriptome composition and to discover new exons or genes (Wang *et al*., 2009). RNA-seq is one powerful approach that is widely The *myostatin* gene is one of the economically important genes due to its negative regulation of skeletal muscle mass in mammals (Supakorn, 2009). Mutations in this gene can result in muscle-doubling (Alakilli *et al*., 2012). RNA-Seq studies have shown that the knockout of this gene has a positive impact in muscle growth and development in goats (Wang *et al*., 2017). RNA-Seq technology has also proven its usefulness in gene expression profiling of the intramuscular muscle in Nellore cattle where several genes that were associated to lipid metabolism and fatty acid composition were identified (Berton *et al*., 2016). Transcriptome analysis studies were able to associate expressed genes to pathways that play a role in cell stimulations and neutral growth in chickens, as well as phenotypes in slow and fast-growing chickens (Wu *et al*., 2018). These among many other studies demonstrate the potential for RNA-Seq as a tool to be used for gene expression profiling as there are associations that can be linked to growth traits.

The transcriptome of any tissue is affected by factors like breed, physiological and environmental conditions, which makes RNA-seq experimental designs challenging as these conditions need to be controlled in order to reduce noise and get accurate associations. Because of these requirements, RNA-seq studies works very well for commercial breeds or experimental populations that have uniform genetics, constant production systems, good and uniform management systems etc. The design of RNA-seq experiments for extensively raised population is challenged by genetic variations and inconsistent production and management systems. In an uncontrolled environment a high number of differentially expressed genes may be observed. Another such example is from a study by Malatji *et al.* (2019) who profiled the transcriptome of small intestines of extensively raised chickens in an *Ascaridia galli* infested environment. In this study, multiple genes were reported during differential gene expression analysis and attributed to the experiment being conducted in an uncontrolled extensive production environment. Under a controlled environment Berton *et al*. (2016) conducted gene expression profiling of intramuscular muscle in Nellore cattle and reported only fatty acid specific differentially expressed genes. The requirement for high computational power and resources, as well as high data storage is another limitation for RNA-seq based gene expression profiling studies (Linde, 2018).

Though there has been a significant advancement in genomic studies of South African goat populations, transcriptomics is one method that has not been explored to address some of the research questions for this sector. The majority of the goats in South Africa are raised in communal farms with no structured management and breeding systems (Ncube, 2016) and coupled to the high genetic variation in village goats, may present challenges in setting up gene expression profiling studies. In other livestock species such as cattle, transcriptomic studies have been conducted using well defined breeds such as the Bonsmara that have uniform genetics and are raised under controlled conditions (Linde, 2018).

2.8 Conclusion and recommendations

South Africa consists of well-developed meat goat breeds as well as the characterized indigenous populations dubbed the Indigenous Veld Goats (IVG) that are kept in organized farms by the indigenous veld goats society farmers. There also exists the non-descript village populations; that have not yet been characterized and have been naturally selected for adaptation to harsh conditions.

There is limited knowledge on the growth performance and carcass characteristics of South African goat populations particularly the non-descript village goats that form the majority of the goats kept in the country. Profiling growth at different growth stages is important in understanding the growth potential of South African goat populations. In addition, carcass characteristics are highly valuable to the producer as it gives an idea of yield and quality of meat offtake. There is limited information of goat carcass characteristics in South African populations. This information is important is selection programs in village populations as it can aid in management improvement for better yields. Transcriptomics is one of the most useful and interesting fields of study involving both genetics and other factors such as breed and production system which affect gene expression with significant effects in growth and development. Setting up gene expression profiling experiments in village goats is extremely challenging and further studies need to be performed in order to make these possible. One recommendation would be to genetically characterize the animals in order to find uniform genetics within the populations, then use those for gene expression profiling.

CHAPTER 3: RELATIONSHIP BETWEEN POPULATION GENOMIC STRUCTURE AND GROWTH PROFILES OF SOUTH AFRICAN GOATS UNDER DIFFERENT PRODUCTION SYSTEMS

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3.1 Abstract

Goats play a major role in poor marginalized communities of South Africa for food security and socio-economic purposes. Majority of the goats are raised in villages with poor infrastructure and resources, therefore, facing challenges that affect growth performance which leads to low mature weights. Investigating growth profiles will shed light on growth performances and will aid in goat improvement and selection. This study investigated the growth profiles and genomic structure of SA indigenous breeds raised in different production systems to unravel the genetic potential of indigenous goat populations. Live weights and morphological body measurements were collected from a total of 83 kids representing the commercial meat-producing SA Boer (*n=14*), the indigenous veld goats (IVG) of NC Speckled (*n=14*), Mbuzi/Nguni (*n=13*), and Xhosa Lob ear (*n=14*) raised under the intensive system, and non-descript village goat populations (*n=14*) raised in the intensive and another (*n=14*) raised in the extensive production systems. The remaining 72 of 83 phenotyped goats were genotyped using the Illumina goat SNP50K BeadChip. The SA Boer had a higher weight (28.96±0.30 kg) gain as compared to other populations. The Mbuzi population was the smallest (14.83±0.33 kg), while the village goats raised in Pella village were relatively smaller (17.55±0.37 kg) than those raised on the research farm (19.55±0.36 kg). The study concluded that both genetics and management systems can lead to improved growth performance in goat production. The outputs of this study can be used to identify suitable breeds and potential genotypes for optimal growth and establish optimal goat management systems suitable for communal farmers for improved productivity.

Keywords: *Growth profiles, population structure, morphological measurements, production systems, genomics*

3.2 Introduction

Goats play a key role in poor low-income communities for food security, socio-economic roles as well as a source of financial security (Visser & Marle-Köster, 2017). There are two goat production systems in South Africa (Mdladla *et al.*, 2017). The commercial/intensive production system is characterized by well-designed housing systems, adequate feed, feed supplementation and regular health care systems (Mdladla *et al.*, 2016). Breeds such as the Boer, Savannah and Kalahari Red goats that are raised in the commercial production system (Campbell 2003; Mdladla *et al.*, 2016; Ncube *et al.*, 2016), have high growth performance and high meat yield (NAMC, 2005). The Xhosa Lob ear, Mbuzi/Nguni and the Northern Cape Speckled goats were characterized by Morrison (2007) as South African indigenous veld goats (IVG) and are kept by an organized farmer association under semi-intensive production systems.

The extensive production system is characterized by low input management practices often associated with suboptimal nutrition and housing, inadequate veterinary services and lack of breed/animal improvement programs (Gwaze *et al.*, 2009; Masika & Mafu, 2004). Diseases such as heartwater (Mdladla *et al.*, 2017), coccidiosis and helminth parasite infections are major constraints to goat productivity in communal farming systems (Gwaze *et al.*, 2009). The uncharacterized village ecotype populations are kept and grown under extensive systems by communal farmers in rural areas (Simela & Merkel 2008; Bester *et al.*, 2009; Moloko 2011; Mdladla *et al.*, 2016; Ncube *et al.*, 2016).

The village goat populations that are kept and adapted to most of these harsh conditions are small breeds with lower growth rates compared to commercial goats (Masika & Mafu 2004). The village goat populations have shown high diversity in growth rates and mature weights (Visser *et al.*, 2004) indicating that some goats are better performers thereby giving room for within-population selection. There is, however, no written records on production performance in most communal farming systems (Masika & Mafu, 2004).

Several tools that can be used in goat genomics studies to help unravel the genetic potential of communal goats include the Illumina goat SNP50K BeadChip. The Illumina goat SNP50K is a high-density SNP chip consisting of markers evenly spaced across the goat genome and is a useful tool for population genetic studies in goats (Tosser-Klopp *et al.*, 2014; Lashmar *et al.*, 2015; Mdladla *et al*., 2017). It was developed using six goat breeds and has been validated to be suitable for use in a variety of goat breeds (Tosser-Klopp *et al*., 2014). This technology has been used in various studies in South African goats. The SNP50K chip can reveal valuable information on the demographic history of South African goat populations (Mdladla *et al*., 2017). As described by Mdladla *et al*. (2017), the Illumina goat SNP50K can bring about the understanding of the goat populations and their genetic composition. The indigenous veld goats have only been phenotypically characterized by Morrison, (2007) and have not been genetically characterized. The BeadChip technology will, therefore, unravel the genetic make-up of these populations.

This study sought to investigate the population genetic structure and associated growth potential of South African indigenous ecotypes under different production systems. The growth profiles of the Boer goats were investigated and set as a benchmark with which to compare the indigenous veld goats namely; i) Northern Cape Speckled ii) Xhosa Lobear iii) Mbuzi/Nguni as well as iv) Uncharacterized village goats.

3.3 Materials and methods

3.3.1 Animals and management

The study was performed under two management systems i) extensive and ii) intensive from October 2016 to March 2017 (Table 3.1). A total of 83 weaned goat kids born in July 2016 were purchased from the Indigenous Veld Goat Society (IVG) (*n=55*), and from Pella village (*n=28*), North West Province (Figure 3.1). Goat kids were weighed on arrival and they all had varying weaning weights ranging from 5 kg to 15 kg.

In the intensive production system 69 goats from the South African Boer (SAB) (*n=14*), Northern Cape Speckled (NCS) (*n=14*), Xhosa lob ear (XL) (*n=14*), Nguni/Mbuzi (MBZ) (*n=13*) and the village (Tswana) goats (VTI) (*n=14*) were kept in a browsing camp at the Small Stocks Unit of the Agricultural Research Council, Animal Production (ARC-AP). They were kept on a management diet of game pellets (110g/kg, 25-70 g/kg crude fat, 110-200 g/kg crude fibre, 6-10 g/kg calcium, 2.5 g/kg phosphorus and 3.68% non-protein nitrogen) provided at 3% of live weight/animal/day. Lucerne hay and clean water were available *ad libitum*.

Table 3.1 Number of animals phenotyped and genotyped per breed/ecotype and production system

Production system	Ecotype	Acronym	N	No. Phenotyped	No. Genotyped	Experimental location
Intensive						**ARC-AP**
	Boer	SAB	14	14	13	
	Northern Cape Speckled	NCS	14	14	14	
	Xhosa Lob ear	XL	14	14	13	
	Mbuzi	MBZ	13	13	12	
	Village (Pella)	VTI	14	14	9	
TOTAL			69	69	61	
Extensive						**Pella Village**
	Village (Pella)	VTE	14	14	11	
TOTAL			14	14	11	
ALL			**83**	**83**	**72**	

They were evaluated for signs of diseases and treatment was administered or the animal was taken to a vet/hospital in cases of diseases. Dipping was performed twice a month to prevent external parasites.

In the extensive system, weaned village (Tswana) goats (VTE) (*n=28*) were purchased from 14 farms (2 kids per farm) at Pella Village, North West Province where one kid per farm was taken to ARC-AP (*n=14*) to be raised under the intensive system as described above, while the remaining (*n=14*) were raised under typical communal farming systems. In an extensive system, the animals are penned at night and left to forage during the day, and there was no feed supplementation given to the animals. Pella village is in the arid North West province characterized by low rainfall leading to very little pasture resulting in poor quality feed (Holmgren *et al*. 2006; Mdladla *et al*. 2017).

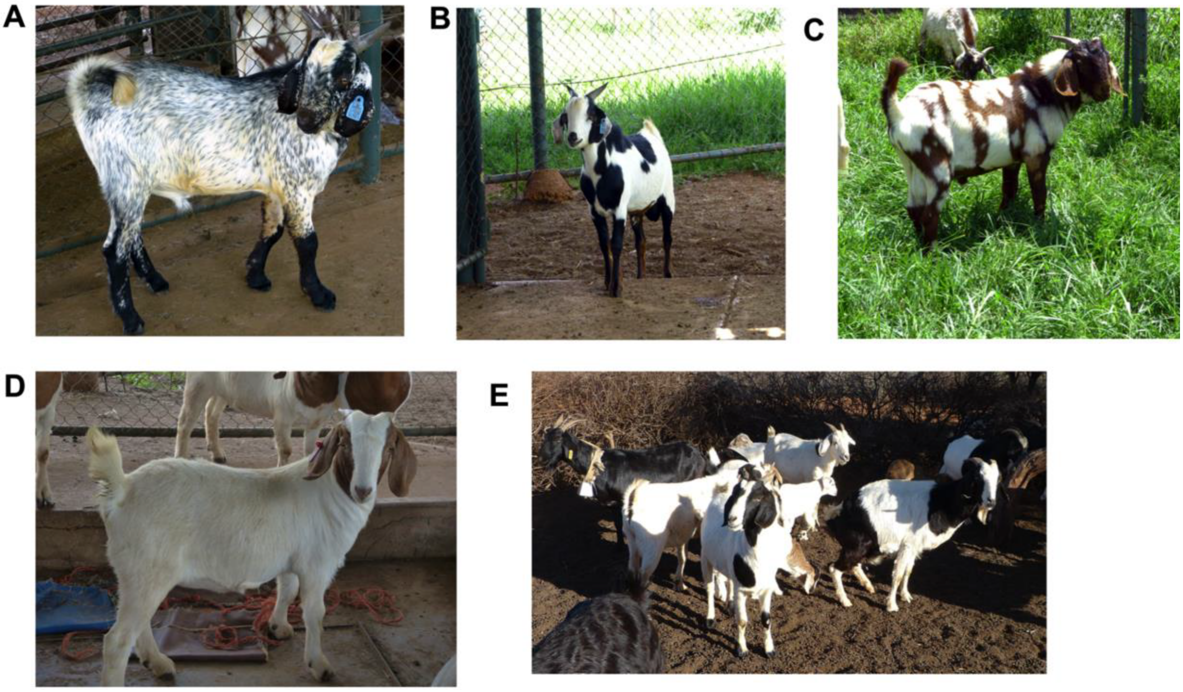

Figure 3.1 South African indigenous goat ecotypes used in this study A) NC Speckled (NCS), B) Xhosa Lobear (XL), C) SA Boer (SAB), D) Mbuzi (MBZ), and E) Village populations from Pella Village (VTI and VTE).

3.3.2 Blood collection and DNA Isolation

Seventy-two (72) out of 83 kids belonging to South African Boer (n = 13), Northern Cape Skilder/Speckled (n = 14), Xhosa lob ear (n = 13), Nguni/Mbuzi (n = 12) and the village (Tswana) goats (n = 9) and extensively raised village Tswana (n = 11) remained after some were lost due to heartwater and pulpy kidney disease. Venous blood (2 ml) was collected from the jugular vein of 72 kids and transported in an icebox to the Agricultural Research Council, Biotechnology Platform laboratories, after sampling and stored at− 20 °C in a freezer till further use. DNA was isolated using the optimized Qiagen DNeasy blood and tissue kit (www.qiagen.com) according to manufacturer's instruction with modifications such as increasing sample volume to 200 µl blood and an increased incubation period of 120 min. DNA quantification was performed on the Qubit® 2.0 Fluorometer using the Invitrogen's QubitTM dsDNA BR Assay Kit (Invitrogen, Life Technologies). The quality of the DNA was investigated electrophoretically on 1% agarose gel and with 4 µl of ethidium bromide at 80 V for 30 min.

3.3.3 Genotyping and quality control

After isolation and quantification, 50 ng/µl genomic DNA of 72 goats were genotyped on the Illumina goat SNP50K BeadChip using the Infinium assay. The SNP chip was then scanned on the Illumina HiScan SQ genotyping platform at the Agricultural Research Council, Biotechnology Platform Core Facility, South Africa. SNP genotypes were called using the genotyping module in GenomeStudio™ V2010.1 (Illumina Inc.).

The SNP marker map file was updated using Golden Helix SNP Variation Suite version 8.7.2 (Golden Helix, Inc., Bozeman, MT, USA). PLINK v1.07 (Purcell et al., 2007) was used to filter individuals genotypes of 95% and samples with a low call rate were excluded. The data was pruned for low-quality markers with call rate ≤ 0.95%, minor allele frequency (MAF≤ 0.05) and markers that significantly deviated from Hardy-Weinberg equilibrium (HWE; $P<0.001$). For population structure and ADMIXTURE analysis, LD pruning was performed at (LD >0.2) as well as for related individuals (IBD > 0.5).

3.3.3 Population structure analysis

Population structure was performed using Principal component analysis (PCA) in Golden Helix SNP Variation Suite (SVS) Version 8.7.2 (Golden Helix Inc., Bozeman, MT, 2012). ADMIXTURE v1.3.0 was used for the most probable number of ancestral populations based on the SNP genotype data. ADMIXTURE was performed from K=2 to K=10 and the optimal number of clusters (K=3) was determined as that which had the lowest cross-validation error (CV-error).

3.3.4 Phenotypic measurements and calculations

Weaning and post-weaning weights (kg) were measured using an LS4H Electronic Sheep Scale (TAL-TEC) at 12 weeks for weaning weight and monitored fortnightly between 14-36 weeks for post-weaning weight. The livestock scale was calibrated on the day of use and tarred before each goat was weighed. Animals were weighed on the same day of the week to ensure accuracy (Thursdays at the research farm, and Fridays at village farms).

In addition, sixteen body measurements were collected at each weighing. Head Length (HL), Head width (HW), Ear Length (EL), Muzzle Diameter (MD), Cannon Bone (CB), Withers Height (WH), Sternum Height (SH), Body Depth (BD), Bicoastal Diameter (BISC), Chest Girth (CG), Body Length (BL), Rump Height (RH), Rump Width (RW), Rump Length (RL) Chest width (CW) and Pin Bone (PB) were collected according to parameters described by Chacón et al., (2011) and Mdladla et al., (2017) using a flexible measuring tape.

Fortnightly recording of live body weight (kg) was used to measure average daily gain (ADG) using the following formula:

$$ADG = Weight\ gained\ (kg) \div number\ of\ days\ fed\ (14\ days)$$

3.3.5 Body conformation indices

Body measurements were used to calculate conformation indices according to Chacón et al. (2011) and Khargharia et al. (2015) and defined as:

1) Cephalic index (IC) = Head width x 100 / Head length,
2) Proportionality (Ipr) = Withers height x 100 / Body length,
3) Pelvic index (IP) = Rump width x 100 / Rump length,
4) Weight: Singh and Mishra (2004) = (0.63 x HG) – 19.5,
5) Width slope (WS) = rump width / chest width,
6**)** Balance (BAL) = (rump length x rump width) / (body depth x chest width),

7) Transverse pelvic (IPT) = rump width x 100 / rump height,

8) Longitudinal pelvic (IPL) = rump length x 100 / rump height,

9) Relative depth of thorax (IPRT) = chest depth x 100 / wither height,

10) Relative body index (RBI) = BL x 100/ wither height,

11) Dactyl thorax index (DTI) = (Cannon bone circumference / Chest girth) * 100,

12) Body ratio (BR) = Sternum height / Rump height,

13) Baron and Crevat (BC) or Conformation index = (Chest girth)^2 / Height at withers,

14) Pectoral index (PI) = ((Sternum height + Rump height) / 2) / Sternum height,

15) Thoracic development (TD) = Chest Girth / Sternum height,

16) Height slope (HS) = Rump height – height at withers,

17) Depth index (DI) = Body depth / height at withers,

18) Body index (BI) = (Body length/ Chest girth) * 100,

19) Area index (AI) = Height at withers x Body length,

20) Relative cannon thickness index (RCI) = (Cannon circumference / Height at withers) x 100.

3.3.6 Statistical analysis

Least square means and standard errors of the body measurements and body conformation indices were estimated using the General Linear Model procedure (PROC GLM) within the Statistical Analysis System (SAS Institute Inc. 2013). The model factored in ecotype, sex and age as well as their interaction (Ecotype X Age and Ecotype X Age X Sex interactions).

3.3.7 Morphological traits

Stepwise regression with backwards elimination procedure was performed using PROC STEP DISC from SAS Institute Inc. 2013 to rank the growth traits according to their discriminatory power to separate populations.

3.4 Results

3.4.1 Population structure analysis using genomic data

3.4.1.1 Principal component analysis based clustering

The PCA (PC1 and PC2) produced 3 main clusters of the (i) NC Speckled, (ii) SA Boer goat and (iii) a cluster that had the Mbuzi, Xhosa lob ear and village goats from Pella (Figure 3.2).

PC1 shows a cluster of the NC Speckled which separates this population from the rest of the populations. PC2 shows a variation between the SA Boer and the village ecotypes by separating the SA Boer population from them. Some of the indigenous veld goats formed one cluster together with the Tswana village populations. It was observed that the Tswana village populations formed a cluster in between the Xhosa Lobear and the Mbuzi populations. Some of the SA Boer was observed within the Mbuzi cluster. The Xhosa lobear cluster also consisted of some of the SA Boer and the Mbuzi populations.

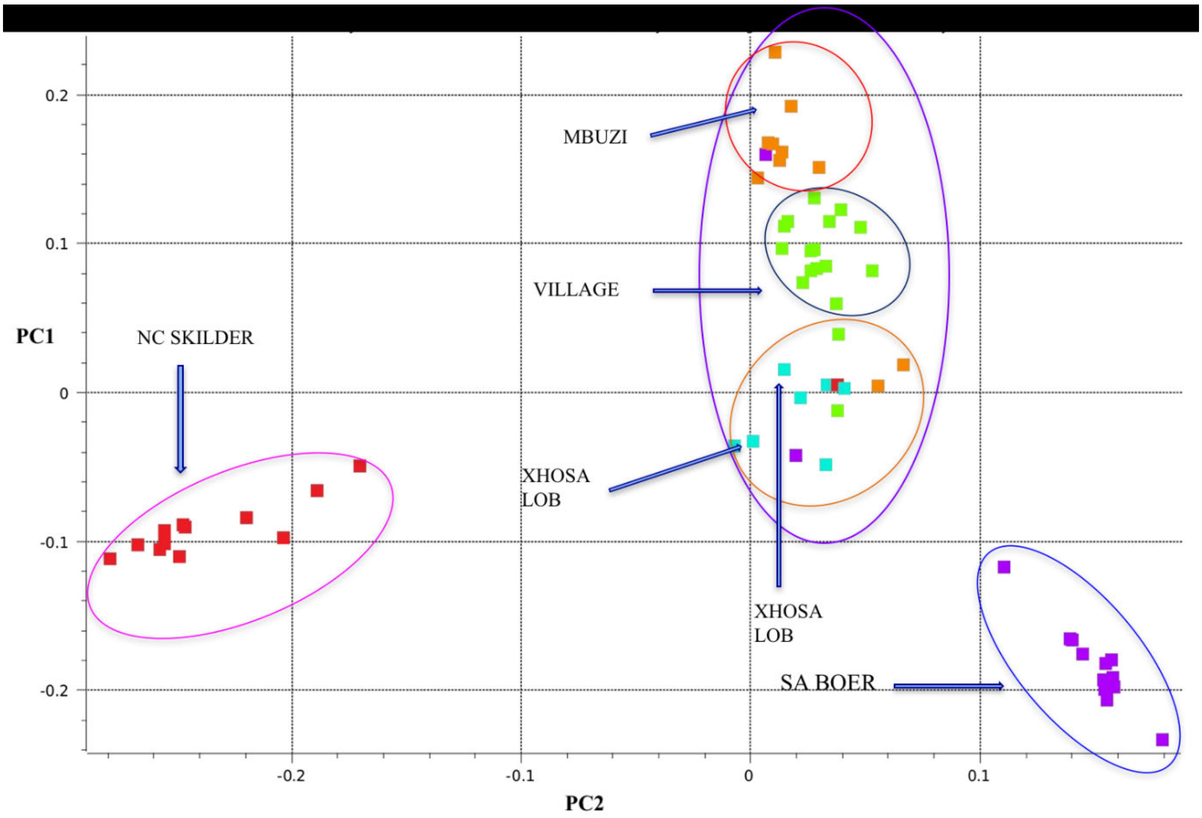

Figure 3.2 Principal component analysis of the indigenous goat populations of South Africa.

3.4.1.2 ADMIXTURE based population clustering (Optimal K value of K=3)

The admixture-based clustering revealed NC Speckled breed separating into an independent population at K=2 (Figure 3.3), followed by Boer at K=3, village goats at K=4. and Mbuzi and Xhosa Lobear at K=5. The NC Speckled split into two subpopulations at K=7 to K=10. Clustering of all the populations under study was observed at K=5 to K=10. The Xhosa cluster was admixed with some of the Mbuzi goat populations.

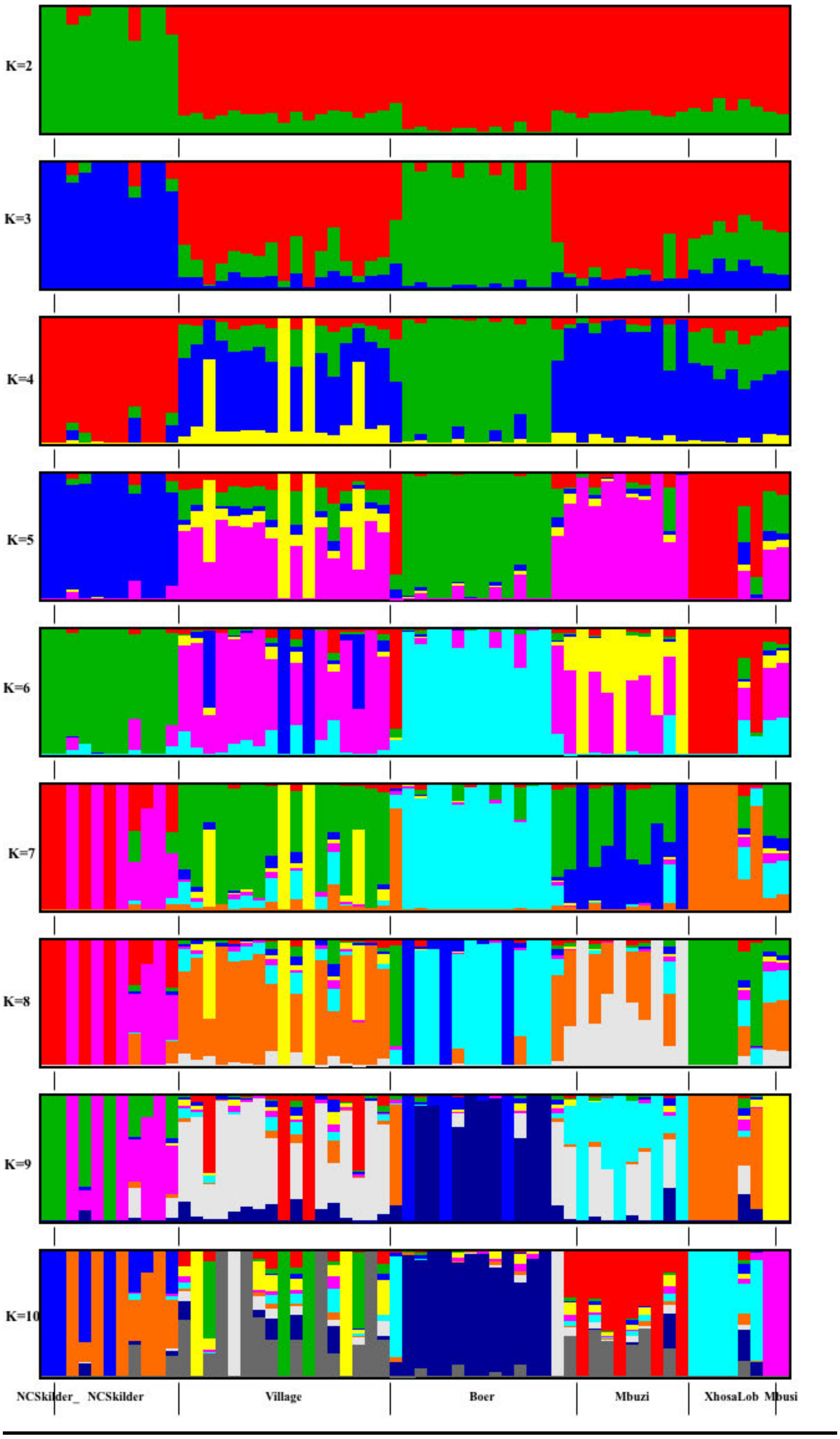

Figure 3.3 ADMIXTURE based clustering of the South African indigenous goat populations with optimal K value of K=3 (CV=0.65).

3.4.2 Phenotypic calculations and measurements

Least square means (LSM) for live weight and body measurements of indigenous goats are described in Appendix A-C. All kids had varying weaning weights with the overall average weaning weight of 13.15±0.47 kg (Appendix C). The highest live weight (LW) was observed in the SAB population (28.96±0.30 kg) followed by the NC Speckled (24.21±0.31 kg) and the Xhosa Lobear (20.00±0.29 kg) populations. The Mbuzi population had the lowest LW mean (14.83±0.33 kg). The Pella population raised at the research farm had a slightly higher LW mean (19.55±0.36 kg) as compared to the population that was raised extensively at Pella village (17.55±0.37 kg). The LW comparison between the sexes showed the males to be heavier (21.49±0.21 kg) than the females (20.21±0.16 kg).

The mean body length (BL) ranged from 47.01±0.36 cm to 58.11±0.3 cm, with the SAB having the longest body 58.11±0.33 cm and the MBZ with the shortest 47.01±0.36 cm. All the populations had similar proportions of withers height ranging from 51.14±0.37 cm to 58.48±0.35 cm. The SAB and the NCS had larger chest girths (CG) 67.81±0.43 cm and 66.35±0.45 cm respectively, the XL, VTI and VTE had slightly similar proportions (61.55±0.41 cm, 61.04±0.51 cm and 61.42±0.52 cm) respectively while the MBZ was the smallest 56.48±0.48 cm. The males had longer (57.90±0.27 cm) bodies than the females (55.29±0.22 cm) as well as their height (57.07±0.23 cm and 54.31±0.18 cm) respectively. The males also had larger CG than the females (63.63±0.29 cm and 61.25±0.23 cm) respectively.

3.4.3 Average daily gain

Table 3.2 shows a representation of the average daily weight gain of the indigenous goat populations. The SAB, NCS and XL had the highest daily weight gain of 0.13±0.01 kg, 0.12±0.01 kg and 0.10±0.01 kg respectively. The females and males had similar daily weight gain 0.10±0.002 kg and 0.10±0.004 kg, respectively. Daily gain fluctuated at different ages with the highest observed at 14 weeks 0.14±0.01 kg and the lowest 0.07±0.01 kg at 22 weeks; at 34 weeks the ADG was 0.11±0.01 kg.

Table 3.2 Least square means and standard error of average daily weight gain for South African goat ecotype populations

Category	ADG LSMEAN	Significance					
Ecotype		Ecotype	Sex	Age	Ecotype*Age	Ecotype*Age*Sex	Ecotype*Sex*Age
MBZ	0.09±0.01	***			NS	NS	NS
NCS	0.12±0.01	***			NS	NS	NS
SAB	0.13±0.01	***			NS	NS	NS
VTE	0.07±0.01	***			NS	NS	NS
VTI	0.09±0.01	***			NS	NS	NS
XL	0.10±0.01	***			NS	NS	NS
Sex							
F	0.10±0.002		NS		NS	NS	NS
M	0.10±0.004		NS		NS	NS	NS
Age							
14	0.14±0.01			***	NS	NS	NS

16	0.09±0.01	***	NS	NS	NS
18	0.08±0.01	***	NS	NS	NS
20	0.11±0.01	***	NS	NS	NS
22	0.07±0.01	***	NS	NS	NS
24	0.08±0.01	***	NS	NS	NS
26	0.09±0.01	***	NS	NS	NS
28	0.09±0.01	***	NS	NS	NS
30	0.10±0.01	***	NS	NS	NS
32	0.10±0.01	***	NS	NS	NS
34	0.11±0.01	***	NS	NS	NS

*****Ecotype: SAB=South African Boer, NCS=Northern Cape Speckled, XLE=Xhosa lob ear, MBZ=Mbuzi, VTI=Village Tswana raised at API research farm, and VTE= Village Tswana raised in extensive conditions at village farms. Highly significant values of ($P \leq 0.05$) is indicated by ***. NS= Non-Significant.

3.4.4 Body conformation indices

Body conformation indices derived from morphological measurements of South African indigenous goats are represented in Appendix D-F. The cephalic index (IC) showed similarities between the populations ranging from 87.37±0.69 to 87.98±0.82 except the MBZ population with the IC of 90.65±0.75.

Sex did not influence any difference in IC with having similar indexes between the males 88.14±0.37 and the females 89.67±0.48.

Dactyl Thorax Index (DTI) was relatively high (16.62±0.12 to 18.31±0.11) across all the populations indicating that they were all heavy animals. The MBZ was slightly lower 16.62±0.12. Both the males (17.39±0.06) and the females (17.35±0.08) were heavy. All the populations were balanced and had lower withers than the rump with the body ratio (BR) ranging from 0.68±0.0 to 0.71±0.004. The males and the females had the same body ration (0.70±0.004). It was also observed that the ratio was slightly higher from 12-24 weeks (0.70±0.006 to 0.72±0.006), and then from 26 weeks, the ratios dropped to (0.69±0.006) right through to 34 weeks with no change.

The body index (BI) indicated that all populations are medigline with the BI ranging from 83.20±0.47 to 85.55±0.43. Both sexes were also medigline with 83.76±0.24 and 84.09±0.34 for males and females, respectively. Age also showed no variation in body indexes, the measurements indicate that the animals were medigline from 12 to 34 weeks of age. The sternum height of all populations both sexes and for all ages was nearly half of the rear height with the pectoral index (PI) ranging from 1.20±0.01 to 1.24±0.004.

Proportionality (Ipr) and pelvic index (IP) indicate how well proportional the animal is. All ecotypes were proportional with Ipr 101.20±0.63 to 109.53±0.81, and IP of 113.43±0.87 to 124.89±0.92. The males were more proportional than the females with Ipr (107.39±0.45 and 105.86±0.31) and IP (120.22±0.65 and 116.91±0.50), respectively. The transverse pelvic (IPT) and longitudinal pelvic (IPL) indices are indications of hip-width. The NCS, XL, MBZ and VTE had narrow hips with 29.35±0.26 to 29.85±0.29 IPT and 25.04±0.21 to 26.34±0.19 IPT and IPL, respectively while the SAB and the VTI had slightly wider hips with 33.26±0.24 and 30.50±0.31 IPT and 26.77±0.21 and 25.36±0.26 IPL, respectively. Both the males and females had relatively wider hips 30.35±0.13 and 30.43±0.17 IPT and 26.77±0.21 and 25.04±0.21 respectively.

3.4.5 Discriminating power of morphological traits

Morphological measurements were subjected to a stepwise selection to select the highly significant measurements. Only 10 measurements were highly significant ($p < 0.05$), while EL was the highest at ($p < 0.01$). This procedure revealed the EL, RH, RW, MD, LW, SH, CB, BD, CG and HW as quantitative traits had significant discriminatory power (Table 3.3).

Table 3.3 Significant traits using stepwise selection

Step	Trait	Partial R^2	F Value	Pr > F	Wilks' Lambda	ASCC
1	**EL**	0.6258	19.40	<.0001	0.37424292	<.0001
2	**RH**	0.2657	4.13	0.0029	0.27480051	<.0001
3	**RW**	0.3459	5.92	0.0002	0.17974044	<.0001
4	**MD**	0.2876	4.44	0.0018	0.12803915	<.0001
5	**LW**	0.3313	5.35	0.0005	0.08562095	<.0001
6	**SH**	0.2476	3.49	0.0085	0.06441760	<.0001
7	**CB**	0.2118	2.79	0.0261	0.05077585	<.0001
8	**BD**	0.2302	3.05	0.0175	0.03908617	<.0001
9	**CG**	0.2216	2.85	0.0244	0.03042349	<.0001
10	**HW**	0.1795	2.14	0.0757	0.02496114	<.0001

*EL=Ear Length, RH=Rump Height, RW=Rump Width, MD=Muzzle Diameter, LW=Live Weight, SH=Sternum Height, CB=Canon Bone, BD=Body Depth, CG=Chest Girth and HW=Head Width.

3.5 Discussion

South Africa consists of two main production systems which are the commercial/intensive and the extensive/communal production systems. The commercial system is mainly the large-scale goat farming system while the communal system is where village goats are raised in backyards under extensive conditions (Mdladla *et al.*, 2017). This study investigated the population genomic structure and the growth profiles of the South African goat populations using the Boer goat as a benchmark. The discriminating power of body conformation indices in categorizing animals according to their breed/population affiliation was investigated and provided more understanding of the diversity in the ecotype populations.

The Principal component analysis (PCA) and ADMIXTURE were used to cluster populations and determine breed or population differences and confirm the diversity of the populations under study. Both the PCA and the ADMIXTURE were consistent with the observation that the SAB population differed from the rest of the populations. Historically, it has been reported that the Boer was developed from village goats (Campbell, 2003; Mdladla *et al*., 2016), however, the Boer did not cluster with the village goats but separated from them instead and this is consistent with Mdladla's findings in 2016. This separation may be due to the Boer being selected for specific traits over the years, therefore, the genetic make-up may have changed with farming practices playing a role. The Boer goats are raised in large scale commercial farms while the village populations are kept in communal farms. The PCA showed the Northern Cape Speckled (NCS) to be a unique genetic resource which separated from the rest of the populations. PCA showed one cluster of the Mbuzi (MBZ), Xhosa lob (XL) and uncharacterized village populations where the village populations were clustered in between the MBZ and the XL suggesting that even though they may be related they do not have the same genetic make-up possibly due to geographic locations. Admixed individuals were observed in village and Mbuzi populations indicating high genetic variations. This study observed within-population diversity and the presence of population genomic structure between SA goat populations. The ADMIXTURE results showed that some of the ecotypes are admixed. These findings demonstrate the importance of genetic conservation of local breeds as a source of within and between population diversity.

Overall, these results indicated that goat breeds/populations sampled for the study were genetically different from each other. Based on both PCA and admixture analysis, the study went on to analyse the mature body weights and growth profiles of populations under study on the assumption of genetically structured populations. On this basis, differences in growth traits were attributed to the genetic variation.

Mature live body weights varied in line with the population genetic clusters reported from the PCA analysis. The Nguni/Mbuzi had an average adult weight of 40 kg in bucks and 34 kg in does, while the Xhosa lob ear was 32 kg for bucks and 29 kg for does (Snyman, 2014).

The body weights of other ecotypes such as the Speckled have not been previously documented. The SAB goat was reported to be a high performing breed with early maturity and high meat yields. Body weight of an animal is one of the most important traits to farmers who raise these goats for meat production (Moela, 2014). The South African Boer was developed for meat production and was therefore used as a reference population to compare to other indigenous ecotypes that have not been selected for meat. As expected, the SAB population had the highest average weight gain at 28.96±0.30 kg. The NCS is a population that is mainly developed and used for its hide. However, this study suggests its potential as a high growth performing breed with its performance close to that of the SAB. The XL population was used in the development of the SA Boer and the Kalahari Red meat-type goats found in the commercial sector (Dube *et al*., 2016). Based on this, it was expected that it would perform well in terms of growth, which was in contrast to observations in this study where it was observed that the Speckled performed better. Village ecotypes that were raised at the research farm performed better than those raised in village farms. Village farms are characterized by poor management practices (Ncube *et al*., 2016) and face challenges such as diseases, drought and they also walk long distances to browse which puts a strain on them; these may be some of the causes of poor growth performance. In a controlled management system, the animals are fed a management diet, they receive adequate medical attention when there is a need and always have clean water and good shelter, which then reduces the challenges the animal goes through, thus improving growth performance. This study, therefore, suggests that improved management systems can bring about improved growth performance.

There was only about 1 kg difference between the weight of the males (21.49±0.21 kg) and the females (20.21±0.16 kg) observed in this study. Moela (2014) observed less than 1 kg (0.23 kg) in weight variations between the males and females of the indigenous goats in the Limpopo province of South Africa. Most studies have reported that males are heavier than the females in various populations (Pophiwa *et al*., 2016). There was a large variation between breeds in body weight and other body measurements which could be important for genetic improvement of these traits.

The growth profiles analysed for the genetically distinct populations demonstrated the potential for growth and within-population selection in the study populations. Most communal farmers believe that cross-breeding is the solution for improved genetics, however, this study showed that the goat populations are genetically diverse from each other and differ in growth and morphological conformation, a scenario which presents other opportunities of improving performance without crossbreeding and diluting the local genetics. The current study together with previous studies on the growth hormone gene (Ncube *et al*., 2016) and landscape genomics of South African goats (Mdladla *et al*., 2017) implies possibility for within-population selection for improvement in traits of economic importance and adaptation.

The village goats have been reported to have poor growth performance irrespective of their good genetic characteristics and ability to adapt and thrive in harsh environmental conditions. The present study raised uncharacterized village goats under controlled and improved production system, therefore demonstrating that improved management systems lead to improved growth genetic. This then suggests that farmers in communal areas should work on improving animal management systems that will enable goats to reach their highest genetic potential.

Over the years, body measurements have been greatly used as a means to calculate or measure the weight of an animal especially in cases where scales are unavailable. Such measurements can be used as indicators or proxy measures of weight and included withers height, chest/heart girth, body length, rump height and rump width (Chacón *et al*., 2011). Understanding the relationship between these measurements and weight will, therefore, be useful in livestock management and growth performance.

The mean body length (BL) ranged from 47.01±0.36 cm to 58.11±0.3 cm, with the SAB having the longest body 58.11±0.33 cm and the MBZ with the shortest 47.01±0.36 cm. Mdladla *et al*. (2016) reported that the adult commercial Boer had longer bodies than other populations while the Tswana populations had longer bodies than the rest of the village populations. In this study, the village goats were only longer than the MBZ populations. All the populations had around the same withers height ranging from 51.14±0.37 cm to

58.48±0.35 cm. The SAB and the NCS had larger chest girths (CG) 67.81±0.43 and 66.35±0.45 cm, respectively, the XL, VTI and VTE had slightly similar proportions (61.55±0.41 cm, 61.04±0.51 cm and 61.42±0.52 cm), respectively while the MBZ was the smallest 56.48±0.48 cm. The males had longer (57.90±0.27 cm) bodies than the females (55.29±0.22 cm) as well as their height (57.07±0.23 cm and 54.31±0.18 cm), respectively. The males also had larger CG than the females (63.63±0.29 cm and 61.25±0.23 cm), respectively.

To know the type and origin and relationship of breeds, the cephalic index (IC), which is derived from the facial measurements is used (Chacón *et al*., 2011). The IC of all the populations were similar except for the MBZ population indicating that there is a relationship between the SAB and the other ecotypes. Campbell (2003) and Mdladla *et al*. (2017) had reported that the Boer goats were developed from the indigenous populations, therefore, it is not surprising that there is a relationship between the breed and other ecotypes. The Boer is also a breed that most farmers including communal farmers use for cross-breeding for livestock improvement purposes, therefore, similarity in IC between the Boer and the village goat ecotypes was expected. The MBZ, on the other hand, had been reported to be a smaller breed and slightly different from other populations according to Snyman (2012). This is consistent with the observation in this study that the IC of the MBZ is different from the rest of the populations. The dactyl thorax index (DTI) is used as an indication of whether an animal is a light or heavy meat animal. A reading of 11.5 or higher means that the animal is a heavy meat type. The SA populations were more than 16.62±0.12 indicating that they are heavy meat types and heavier as compared to the Assam Hill goats in India (Khargharia *et al*., 2015) and Creole goats (Chacón *et al*., 2011) which were 9.82±0.38 and 9.58±0.57, respectively.

In communal farming systems, the animals go around browsing and searching for food, at times they walk long distances and climb hills. Therefore, it is key for these animals to be well balanced for them to be able to climb up and down the hills effectively (Khargharia *et al*., 2015). This study observed that all populations were balanced irrespective of their production system. This was also consistent with Chacón *et al*. (2011) and Khargharia *et al*. (2015), respectively.

This study reported morphological measurements that had significant discriminating power to separate populations. Some studies have suggested using chest girth as a proxy for live weight estimation especially in situations where a livestock scale cannot be accessed (Pesmen & Yardimci, 2002). Proxy traits such as Ear Length, Chest Girth, Head Width etc. are usually easy to measure and thus more suitable for smallholder farming systems when compared to bodyweight measurements.

3.6 Conclusion

Overall, the Boer goat has shown high growth performance as expected due to its development as a high performing meat breed. Potential for growth was observed in breeds such as the Northern Cape Speckled. Though the NC Speckled is mainly used for its hide, this study shows its potential as a high-performing breed and should be considered in selection programs. A higher weight gain was observed in the intensively raised village goat populations suggesting that improved management can lead to improved genetics and potential genotypes for growth performance. Bodyweight and morphological measurements of goats are observed as useful tools for improved management practices. Body conformation indices corresponded with linear body measurements and could be used in differentiating breeds or populations.

CHAPTER 4: CARCASS QUALITY PROFILES OF SOUTH AFRICAN GOAT POPULATIONS AND THE ASSOCIATED GENOMIC REGIONS INVESTIGATED USING GOAT SNP50K GENOTYPES

4.1 Abstract

Carcass quality traits are essential economic traits which play a significant role in influencing farmer breed preferences. Samples of South African Boer (*n=14*), Northern Cape Speckled (*n=14*), Eastern Cape Xhosa Lob ear (*n=12*), Nguni/Mbuzi (*n=13*), Village (*n=20)* were genotyped on the Illumina goat SNP50K and phenotyped for carcass quality traits. SA Boer goats had heavier warm and cold carcass weights (17.2±2.3 kg and 16.3±2.3 kg). Pella village goats raised under intensive system had heavier warm and cold carcass weights (9.9±1.1 kg and 9.2 ±1.2 kg) than those raised extensively (9.1± 2.0 kg and 8.4± 1.9). A total of 40 SNPs located on chromosomes 6, 10, 12,13, 19 and 21 were significant for association at (-log10 [P <0.05]). Candidate genes associated with carcass characteristics (*GADD45G, IGF2R, GAS1, VAV3, CAPN8*, *CAPN7*, *CAPN2*, *GHSR*, *COLQ*, *MRAS* and *POU1F1*) were observed. Results can find use in breed improvement programs as well as give insight on the suitable production system for improved carcass yields.

Keywords: *Candidate genes, carcass quality, goats, goat ecotypes, GWAS, SNP50K*

4.2 Introduction

World goat population is approximately 1 billion (Mazhangara *et al*., 2019) with the South African industry producing about 7.8 million goats (Lehohla, 2016; Dzomba *et al*., 2018). South African goat production contributes about 3 % of goats in Africa as a whole (Morokolo, 2011). About 63% of goats in South Africa are non-descript and unselected genotypes raised in communal farms in the rural areas (Dzomba *et al*., 2018). There is a high demand of goat meat on the African continent, however this demand is unmatched by the existing goat populations. Reasons for this disparity include low growth potential, and carcass quality due to genetic and environmental factors (Webb, 2014). South Africa is one of the few countries that has developed meat-type breeds, from their own indigenous genetic resource, that have been recognized as commercial breeds. These include the South African Boer, Savanna and Kalahari Red goats (Casey & Webb, 2010; Moloko, 2011; Mdladla *et al*., 2016). Breeds such as the Boer dominate the commercial industry with good carcass quality and high carcass yields of 25 kg for bucks and 22 kg for does 100 days post-natal (Sapkota *et al*., 2016). Morrison (2007) described the Northern Cape Speckled, Mbuzi/Nguni and the Eastern Cape Xhosa Lob ear as the indigenous veld goat populations which are kept by the Indigenous Veld Goats (IVG) club farmers in organized farming and breeding societies usually under semi-intensive production systems. Additionally, there are uncharacterized village ecotypes that are raised by communal farmers, that have not been developed for any traits and have multipurpose functions, i.e. meat, milk and skin (Mdladla *et al*., 2016; Ncube *et al*., 2016). Carcass quality characteristics of indigenous veld goats and village goat have not been reported. Despite being a major goat producer, the country contributes only <1% of chevon production in the South African red meat market (DAFF, 2015), this low production may be because chevon is regarded as inferior meat through market preferences/perceptions and shortage of product supply (Mazhangara *et al*., 2019).

The primary role of goats in African countries is meat (chevon) which serves as a source of easily accessible protein to poor communities (Ncube *et al*., 2016). Although chevon has excellent meat quality characteristics and health benefits, it is the least preferred among consumers, mainly due to unfavourable perceptions relating to toughness and strong odour (Pophiwa *et al*., 2016). With majority of goats being kept by communal farmers in rural areas, poor growth performance which subsequently leads to low carcass yields remains a challenge in the chevon industry (Ncube *et al*., 2019). Low chevon production is a growing concern in

South Africa. Some of the causes of this low productivity are because the majority of the goats are farmed in the rural areas characterized by inefficient feeding and management, disease constrains, lack of characterization and inadequate exploitation of genetic resources (Webb, 2014). Limited information exists on the genetic factors/potential that influence increased productivity in indigenous goats raised in communal areas. The differences in growth performance and carcass characteristics of different genotypes under similar or varying production systems is poorly understood. Village goat ecotypes have not been characterised adequately in terms of their growth, and carcass quality attributes. There have been limited efforts made for the commercial promotion of chevon as well as the development of uncharacterized goat populations for chevon production. There are no reported/written records that describe the carcass characteristics and genetics of carcass quality traits of the indigenous goats including South African populations.

Several goat genomics tools are used to unravel the genetic potential of breeds and populations. High-throughput targeted gene sequencing of the *growth hormone 1* gene has shown the locus to be polymorphic and capable of differentiating goat populations from different breeds and production systems (Ncube *et al*., 2016). The Illumina goat SNP50K is a high-density SNP chip consisting of markers evenly spaced across the goat genome and is a useful tool for population genetics and association studies (Tosser-Klopp et al., 2014; Lashmar et al., 2015; Mdladla et al., 2016). The Illumina goat SNP50K Bead chip was used for body morphological traits in Sudanese goats (Rahmatalla *et al*., 2018). Using this chip, SNPs and genes associated with growth, body metabolism and other adaptive traits were reported to be segregating and differentiating goat populations from different geographic and production environments of South Africa (Mdladla *et al*., 2018). The current study describes the carcass characteristics of five South African indigenous goat populations inclusive of the uncharacterized village ecotypes from Pella village. This was followed by a genomic association analysis to investigate genes and molecular mechanisms associated with carcass quality in different breeds and populations using the goat SNP50K genotypes.

4.3 Materials and methods

4.3.1 Animal description and management

The study involved four well-established goat breeds of the South African Boer (SAB) (*n=14*); Northern Cape Speckled (NCS) (*n=14*), Eastern Cape Xhosa lob ear (XL) (*n=14*), Nguni/Mbuzi (MBZ) (*n=13*) that were purchased and sampled from farmer members of the Indigenous Veld Goat Society (IVG) at 12 weeks of age and brought to the experimental farm at the Agricultural Research Council, Animal Production (ARC-AP), South Africa. Goat kids were weighed on arrival, and they all had varying weaning weights ranging from 5 kg to 15 kg. These goats were kept in a browsing camp at the Small Stocks Unit of the ARC-AP. They were kept on management diet of game pellets (110 g/kg, 25-70 g/kg crude fat, 110-200 g/kg crude fiber, 6-10 g/kg calcium, 2.5 g/kg phosphorus and 3.68 % non-protein nitrogen) provided at 3% of live weight/animal/day. Lucerne hay and clean water were available *ad libitum*. They were evaluated for signs of diseases, and in cases of diseases, either treatment was administered, or the animal was taken to a vet or hospital. Dipping was performed twice a month to prevent external parasites.

In addition, village goat ecotypes (*n=28*) were purchased and sampled from Pella village at weaning age of 12 to 12.5 weeks in the North West province of South Africa. The animals were sampled from 14 farms, and two kids of similar age were purchased per farm. A proportion (*n=14*) of these village goats (VTI) were moved to Small Stocks Unit of the Agricultural Research Council, Animal Production (ARC-AP) where they were weighed on arrival and raised similarly to the other breeds of IVG as described above. The second set of these goats (VTE; *n=14*) were raised under typical communal farming systems at their respective village farms. They were penned at night and left to forage during the day. There was no feed supplementation given to this set of animals.

4.3.2 Blood collection and DNA isolation

Venous blood (2 ml) was collected from the jugular vein of 72 goat kids at 24 weeks of age and transported in an icebox to the Agricultural Research Council, Biotechnology Platform laboratories and stored at -20°C in a freezer till further use.

DNA was isolated using the optimized Qiagen DNeasy blood and tissue kit (www.qiagen.com) according to manufacturer's instruction with modifications such as increasing sample volume to 200 µl blood and an increased incubation period of 120 minutes. DNA quantification was performed on the Qubit® 2.0 Fluorometer using the Invitrogen's Qubit^{TM} dsDNA BR Assay Kit (Invitrogen, Life Technologies). The quality of the DNA was investigated electrophoretically on 1% agarose gel and with 4 µl ethidium bromide at 80V for 30 minutes.

4.3.3 SNP Genotyping

After isolation and quantification, 50 ng/µl genomic DNA of 72 goats were genotyped on the Illumina Goat SNP50K BeadChip using the Infinium assay. The SNP chip was scanned on the Illumina IScan genotyping platform at the Agricultural Research Council-Biotechnology Platform Core Facility, South Africa. SNP genotypes were called using the genotyping module in GenomeStudio™ V2010.1 (Illumina Inc.).

The SNP marker map file was updated using Golden Helix SNP Variation Suite version 8.7.2 (Golden Helix, Inc., Bozeman, MT, USA). PLINK v1.09 (Purcell *et al*., 2007) was used to filter individuals with genotypes of 95% call rate. SNPs were pruned for call rate ≤ 0.95%, minor allele frequency (MAF ≤ 0.05) and deviation from Hardy-Weinberg equilibrium (HWE; P <0.001). For population structure analysis, linkage disequilibrium (LD) pruning was performed at (LD >0.2) as well as for related individuals (IBD > 0.5).

4.3.4 Slaughter procedure and carcass quality measurements

All kids were raised until they were 36 weeks old then slaughtered using approved procedures at the ARC-AP abattoir. Kids raised at the ARC-AP were transported from the Small Stocks unit to the holding pens at the ARC-AP Meat Science Building abattoir where they were kept overnight before slaughter. Feed and water were available at all times. Extensively raised kids were transported from Pella village, North West to the holding pens at the ARC-AP Meat Science Building abattoir where they were kept overnight before slaughter as described above. Pre-slaughter weight (WS) was measured 24 hours before the animals were transported to the abattoir to be slaughtered.

4.3.1.1 Slaughter procedure

Goats were electrically stunned for 5 seconds at 200 volts rendering them unconscious after which they were slaughtered, skinned and allowed to bleed for 5 minutes by suspending them by both Achilles heels (Cloete et al., 2004). After bleeding, the head was cut at neck point from the spinal column at the occipital-atlantal joint followed by the removal of trotters at the joint from the metacarpus and the ulna of the forelimbs, and the joint between the metatarsus and the fibula in the hind limbs. The offals were removed from the abdominal cavity during evisceration and were not included in this study. Seven to eight (7-8) goats were slaughtered per day.

4.3.1.2 Carcass quality measurements

Following evisceration, head, trotters and offal removal carcass measurements were collected. Warm carcass weight (WCW) was measured one-hour post mortem before the carcass was chilled by hanging it from both hind legs, and the carcass was immediately chilled at 4°C. Cold carcass weight (CCW) was measured as the weight of chilled carcass 24 hours post mortem. The kidneys, kidney fat and tail, were removed after the carcass was chilled and were not used in this study. The fat code classification and distribution of subcutaneous fat were performed by a visual appraisal of the carcass by a trained official who then assigned the carcasses a fat code (FC) between 0 to 6 according to the fat classification criteria set out in the Agricultural Product Standards no 119 of 1990 (Kwa-Zulu Natal Department of Agriculture and Rural Development, the Beef Carcass Classification System) where 0 is no fat and 6 is excessively fat.

Dressing percentage (DP) and chilling loss percentage (CL) were calculated as:

a) $\text{DP } (\%) = \frac{\text{CCW}}{\text{WS}} \times 100$

b) $\text{CL } (\%) = \frac{\text{WCW - CCW}}{\text{WCW}} \times 100$

Where CCW is cold carcass weight, WS is weight at slaughter and WCW is warm carcass weight.

4.3.5 Statistical analysis

General Linear Model procedure (PROC GLM) within the Statistical Analysis System (SAS Institute Inc. 2013) was used to estimate the effect of breed and sex and the following model was factored.

$$y_{ijkl} = \mu+\alpha_i +\beta_j+ (\alpha x\beta)_{ij} + \varepsilon_{ijk}$$

Where y_{ijkl} = observed value; μ = overall mean for each trait; α_i = breed effect (SAB, NCS, XL, MBZ, VTE and VTI), β_j = sex effect (Bucks and Does), $(\alpha x\beta)_{ij}$ = breed x sex interaction and ε_{ijk} = random error.

4.3.6 Association analysis

For those traits that were significantly affected by breed, a mixed linear model ((MLM) in Golden Helix SNP Variation Suite version 8.7.2 (Golden Helix, Inc., Bozeman, MT, USA) was used to investigate SNPs that were significantly associated with various carcass traits using the model:

$$y = X\alpha + K\mu + e$$

Where y was the carcass trait, *X* the genotype (49000 SNPs), α was regarded as vector of fixed effect (breed), while K was the relative kinship matrix and *e* the random error. Dressing percentage was excluded in the association analysis because there were no significant differences between breeds/populations.

4.3.7 Gene annotation and association analysis

The Ensemble BioMart tool (https://www.ensembl.org) was used to identify genes within a 1Mb region of the significant SNP (-log10 [P <0.05]).

4.4 Results

4.4.1 SNP Genotyping Quality control

After quality control, sixty-nine individuals with 49000 SNPs were retained for downstream analysis. For population structure, 47 individuals were retained after LD pruning and IBD for removal of related individuals and outliers. For association analysis, 15711 SNPs and 53 animals were used.

4.4.2 Carcass quality traits

Means and standard deviations for carcass quality traits per population are described in Table 4.1. Breed differences had an effect on all traits except for drip loss percentage (DP) where there were no significant differences between the breeds. The highest pre-slaughter weight (WS) mean was observed in the SAB (40.9±3.6 kg) followed by the NCS (33.1±4.1 kg) goats. The VTE goats had the lowest pre-slaughter weight of 22.0±4.6 kg when compared to the VTI, with an average of 24.2±2.8 kg. Warm carcass weight (WCW) was measured post-slaughter after the animal had been skinned and the head, feet and 5th quarter had been removed. The SAB and the NCS had the heaviest WCW of 17.2±2.3 kg and 14.1± 1.9 kg, respectively. The MBZ, VTE and the VTI had similar WCW of 9.4 ± 1.5 kg, 9.1± 2.0 kg and 9.9±1.1 kg, respectively. The SAB cold carcass weight (CCW) was the highest, followed by the NCS at 16.3±2.3 kg and 9.9±1.1 kg. The intensively raised village population had a higher CCW than the extensively raised ones (9.2 ±1.2 kg and 8.4± 1.9 kg, respectively).

The SAB, NCS and XL were judged to have a higher fat code (FC) (1.3±0.5, 1.2±0.5 and 1.2±0.4, respectively). The VTI had a higher FC (0.4±0.3) than the VTE (0.3±0.0). The NCS produced the highest dressing percentage (DP) (42.5±2.5 %), followed by SAB (41.9±2.4 %). The extensively raised village goats were higher (41.2±2.6 %) than the intensively raised populations (40.8±1.7 %). The chilling loss (CL) was higher in the NCS (8.4±3.5 %) population, the MBZ and the VTE had similar proportions (7.4±1.9 % and 7.8±2.7 %, respectively). The intensively raised VTE had higher CL (7.8±2.7 %) than the VTI population (6.6±2.1 %).

The interaction effects of goat genotype and sex on pre-slaughter weight and carcass characteristics are presented in Table 4.2. Interactions of goat genotype and sex only had pre-slaughter weight effects on XL carcasses where bucks had heavier carcasses than does (34.4 kg and 25.8 kg). Other genotypes (MBZ, NCS, SAB, VTE and VTI) had similar live weights for both bucks and does. The warm carcass weight of the XL bucks was higher than that of does (14.3 kg and 10.5 kg), while bucks and does of MBZ, NCS, SAB, VTV and VT had similar warm carcass weight. The XL bucks had higher cold carcass weight (13.3 kg and 9.9 kg) than that recorded for does. On average, there were no significant differences between the bucks and does of MBZ, NCS, SAB, VTV and VT in terms of cold carcass weight. On the other hand, XL bucks had similar weights to NCS (does and bucks combined) and does of XL had similar weights as MBZ, VT and VTV (does and bucks combined). There were no significant (p>0.05) interaction effects between genotypes and sex on the dressing percentage and chilling loss.

Table 4.1 Mean values and standard deviation for carcass traits of South African indigenous goat populations

	ϒEcotype					
	SAB	**NCS**	**XL**	**MBZ**	**VT**	**VTV**
***Trait**						
WS (kg)	40.9^{a}±3.6	33.1^{b}± 4.1	29.8^{c}±5.9	23.0^{d}±3.4	24.2^{d}±2.8	22.0^{d}±4.6
WCW (kg)	17.2^{a}±2.3	14.1^{b}± 1.9	12.2^{c}±2.6	9.4^{d} ± 1.5	9.9^{d}±1.1	9.1^{d}± 2.0
CCW (kg)	16.3^{a}±2.3	12.9^{b}± 1.7	11.4^{b}±2.4	8.7^{c} ± 1.4	9.2^{c} ±1.2	8.4^{c}± 1.9
FC	1.3±0.5	1.1±0.5	1.2±0.4	0.4^{bc}±0.2	0.4^{bc}±0.3	0.3±0.00
DP (%)	41.9±2.4	42.5± 2.5	41.4±1.7	40.9±1.5	40.8±1.7	41.2± 2.6
CL (%)	5.5^{b}± 1.7	8.4^{a}± 3.5	6.7^{ab}±1.7	7.4^{ab}±1.9	6.6^{ab}±2.1	7.8^{a}±2.7

*Carcass quality traits: WS=Weight at slaughter, WCW=Warm Carcass Weight, CCW=Cold Carcass Weight, FC=Fat Code, CL=Chilling loss, and DP (%)=Dressing percentage. ϒEcotype: SAB=South African Boer, NCS=Northern Cape Speckled, XLE=Xhosa Lob/Ear, MBZ=Mbuzi, VT=Village Tswana raised at API research farm, and VTV= Village Tswana raised in extensive conditions at village farms. Means in the same row with different superscripts are significantly different (*P*<0.05).

4.4.2 Carcass quality traits

Genome-wide association analysis was undertaken for WS, WCW, CCW, CL, and FC, carcass quality traits and a threshold of ((-log10 [*P* <0.05]) was to classify SNPs as significantly associated as shown in Figure 4.1. A total of 40 SNPs were found to be significantly associated

(*P*<0.05) with the above-mentioned carcass traits and genes were reported within the 1 Mb region of the significant SNPs across different traits (Table 4.2a to e). The analysis was performed for the general population as well as individually for the different breeds/population under study. Forty genomic regions were associated with carcass quality traits and were distributed across 11 chromosomes as shown in Table 4.3a to e. Eight SNPs were significantly associated with pre-slaughter weight, seventeen were associated with warm carcass weight, nine with cold carcass weight, five with fat code and one with chilling loss.

Of the eight genes that were significantly associated with WS, the *GADD45G* (growth arrest and DNA damage inducible gamma) was absent in the SAB population as shown in Table 4.3a to 4.3d. Among SNPs associated with WCW, 18 candidate genes were identified in all populations. The calpain gene family (*CAPN8* and *CAPN2*), as well as the *VAV3* (Vav guanine nucleotide exchange factor 3) were some of the genes that were identified in the WCW. Seven candidate genes were identified in the FC with the *CAPN7* being one of them. *POU1F1* was the only gene associated with CL.

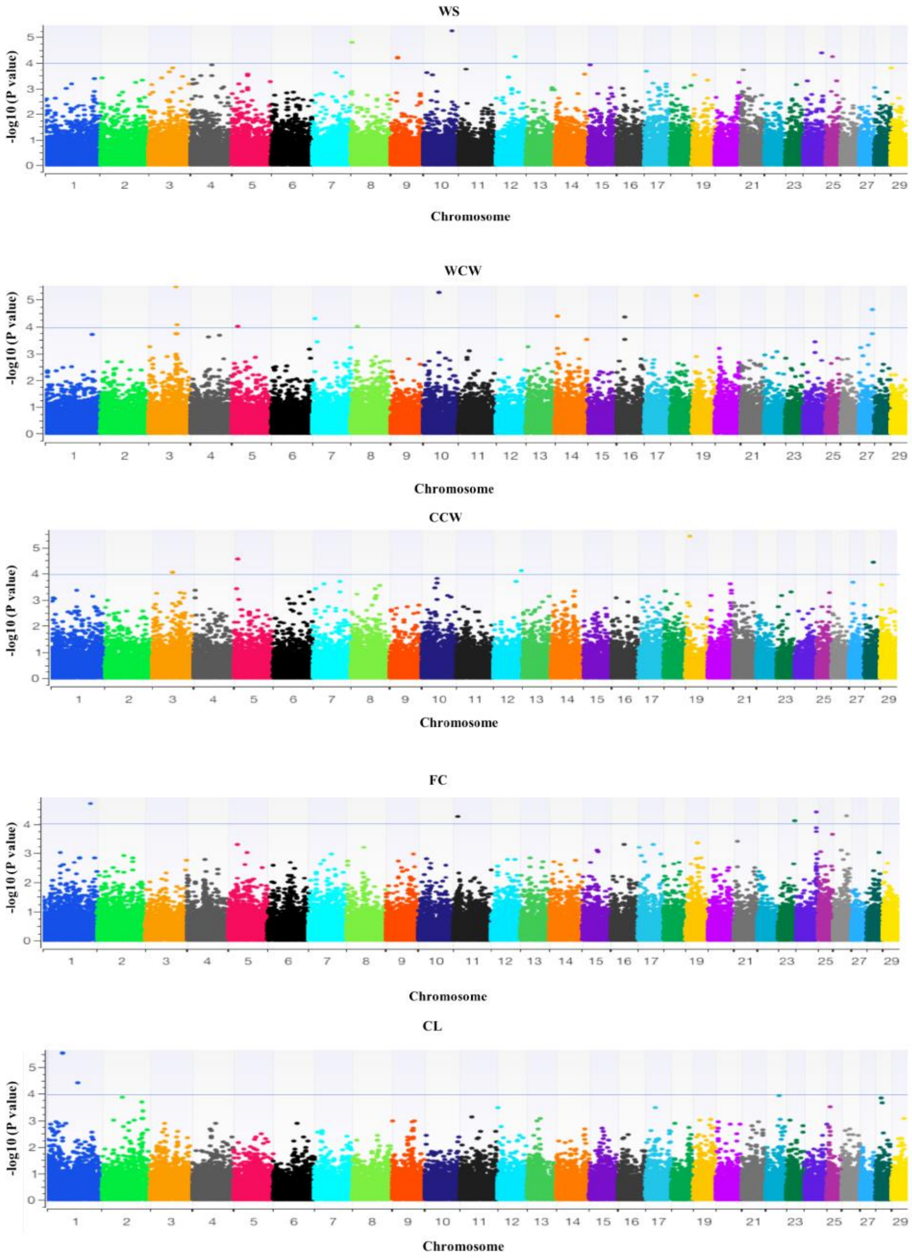

Figure 4.1 Manhattan plots of the GWAS for carcass quality traits in the South African indigenous goat populations. In the Manhattan plots, negative log10 P values of the quantified SNPs were plotted against their genomic positions; different colours indicate SNPs on different chromosomes from chromosome 1 to 29. WS=Slaughter weight, WCW=Warm Carcass Weight, CCW=Cold Carcass Weight, FC=Fat code, and CL=Chilling Loss.

Table 4.2a Marker association with weight at slaughter (WS) of South African goat populations

Trait	SNP	Chr	Position	p value	MAF	Gene	Gene description
WS	snp10578-scaffold1376-2185653	8	72415408	0.005	0.384	*CDCA2*	Cell division cycle associated 2
	snp31819-scaffold356-851991	8	99190229	0.019	0.289	*MUSK*	Muscle associated receptor tyrosine kinase
	snp51334-scaffold750-1385368	8	79156981	0.031	0.402	*GAS1*	Growth arrest specific 1
	snp42388-scaffold56-1009293	8	87724933	0.039	0.100	*GADD45G*	Growth arrest and DNA damage inducible gamma
	snp53226-scaffold802-11917	9	14759064	0.002	0.471	*FABP7*	Fatty acid binding protein 7
	snp36803-scaffold447-444443	9	82960991	0.003	0.478	*IGF2R*	Insulin like growth factor 2 receptor
	snp39508-scaffold501-3016606	10	17332120	0.015	0.435	*VRTN*	Vertebrae development associated
	snp16896-scaffold1766-13800	25	1378654	0.027	0.492	*GFER*	Growth factor, augmenter of liver regeneration

Table 4.3b Marker association with warm carcass weight (WCW) of South African goat populations

Trait	SNP	Chr	Position	p value	MAF	Gene	Gene description
WCW	snp56476-scaffold89-151841	3	11055188	0.001	0.42	*THRAP3*	Thyroid hormone receptor associated protein 3
	snp44023-scaffold595-5580840	3	89714538	0.002	0.196	*CAPZA1*	Capping actin protein of muscle Z-line subunit alpha 1
	snp5156-scaffold118-634690	3	84590504	0.014	0.413	*VAV3*	Vav guanine nucleotide exchange factor 3
	snp15511-scaffold164-2927845	3	41839503	0.039	0.486	*INSL5*	Insulin like 5
	snp11534-scaffold1421-222892	10	16932843	0.001	0.268	*LTBP2*	Latent transforming growth factor beta binding protein 2
	snp16574-scaffold1748-66549	10	15612288	0.005	0.199	*TGFB3*	Transforming growth factor beta 3
	snp39508-scaffold501-3016606	10	17332120	0.009	0.434	*VRTN*	Vertebrae development associated
	snp11543-scaffold1421-651926	10	16503809	0.002	0.427	*PGF*	Placental growth factor

snp23543-scaffold237-2500414	14	46657313	0.008	0.364	*MSC*	Musculin
snp17242-scaffold18-837754	16	24869668	0.012	0.435	*CAPN8*	Calpain 8
					CAPN2	Calpain 2
snp30146-scaffold331-1127999	16	49917608	0.049	0.289	*PERM1*	PPARGC1 and ESRR induced regulator, muscle 1
snp9015-scaffold1328-778798	19	28743439	0.012	0.383	*GAS7*	Growth arrest specific 7
snp43335-scaffold5775-55878	19	55399362	0.032	0.478	*GRB2*	Growth factor receptor bound protein 2
snp55723-scaffold864-1874947	27	25697142	0.001	0.407	*PDGFRL*	Platelet derived growth factor receptor like
snp30755-scaffold34-2405052	27	4053757	0.001	0.478	*THRB*	Thyroid hormone receptor beta
snp44450-scaffold604-977126	27	11448725	0.024	0.301	*FGFR1*	Fibroblast growth factor receptor 1
snp55724-scaffold864-1906315	27	25665774	0.039	0.493	*PDGFRL*	Platelet derived growth factor receptor like

Table 4.4c Marker association with cold carcass weight at slaughter (CCW) of South African goat populations

Trait	SNP	Chr	Position	p value	MAF	Gene	Gene description
CCW	snp11922-scaffold144-3610093	3	68043979	0.004	0.435	*TGFBR3*	Transforming growth factor beta receptor 3
	snp15489-scaffold164-1962025	3	42805323	0.005	0.472	*GADD45A*	Growth arrest and DNA damage inducible alpha
	snp11896-scaffold144-2409377	3	69244695	0.009	0.311	*GFI1*	Growth factor independent 1 transcriptional repressor
	snp43970-scaffold595-3028771	3	92266607	0.020	0.486	*TSHB*	Thyroid stimulating hormone subunit beta
						NGF	Nerve growth factor
	snp12164-scaffold1450-542494	5	99721587	0.0020	0.478	*DPPA3*	Developmental pluripotency-associated protein 3

					GDF3	Growth differentiation factor 3
snp48908-scaffold698-1623875	5	102631469	0.023	0.400	*ING4*	Inhibitor of growth family member 4
snp12642-scaffold1482-13523	5	56734400	0.029	0.130	*GDF11*	Growth differentiation factor 11
snp43888-scaffold593-832260	5	92679764	0.038	0.136	*EPS8*	Epidermal growth factor receptor pathway substrate 8
					RERG	RAS like estrogen regulated growth inhibitor
snp10950-scaffold1393-138987	5	30292487	0.042	0.261	*LMBR1L*	Limb development membrane protein 1 like

Table 4.5d Marker association with fat classification (FC) of South African goat populations

Trait	SNP	Chr	Position	p value	MAF	Gene	Gene description
FC	snp26446-scaffold276-6029619	1	94587913	0.011	0.420	*GHSR*	Growth hormone secretagogue receptor
	snp37534-scaffold46-1530591	1	151789185	0.028	0.272	*COL6A5*	collagen type VI alpha 5 chain
						COLQ	Collagen like tail subunit of asymmetric acetylcholinesterase
						CAPN7	Calpain 7
	snp36302-scaffold435-1967666	1	74307126	0.029	0.351	*FGF12*	Fibroblast growth factor 12
	snp23054-scaffold230-2979377	1	130211629	0.032	0.100	*MRAS*	Muscle RAS oncogene homolog
	snp20787-scaffold204-4829802	11	77889065	0.042	0.447	*LDAH*	Lipid droplet associated hydrolase
						GDF7	Growth differentiation factor 7

Table 4.6e Marker association with chilling loss (CL) of South African goat populations

Trait	SNP	Chr	Position	p value	MAF	Gene	Gene description
CL	snp33063-scaffold385-3390994	1	33867309	0.014	0.321	*POU1F1*	POU class 1 homeobox 1

4.5 Discussion

Goats are primarily kept and used for chevon production. Local goat populations from South Africa and other developing countries are often characterized as of low carcass quality and acceptability to consumers (Webb, 2014). To fill the gap on limited information, this study sought to use five of the South African indigenous populations, including the non-descript populations to investigate carcass characteristics and the associated genetics of South African indigenous goats. The genome-wide association analysis using Illumina goat SNP50K genotypes was used to investigate genes associated with carcass quality traits including their molecular functions.

The SAB population had a high pre-slaughter weight as compared to the rest of the populations. This was expected as the Boer has been developed for high meat yield and growth performance. The SAB is a high performing breed with early maturity of 4-6 months and can weigh 110-135 kg for a buck and a doe about 90-100 kg with an average daily gain of 0.4 kg per day (Mckenzie-Jakes, 2017). The NCS, commonly bred for its hide also showed good growth performance with a slightly high slaughter weight than the rest of the indigenous veld goats (33.1±4.1 kg). There was a 1 kg WS difference between the VTI and the MBZ, which shows that, though the VTI population had lower weight compared to other populations it still had more growth performance potential than the MBZ population. By comparing VTI and VTE goats, the study investigated the role of management, particularly nutrition on local genotypes. In the extensive system, goats typically browse and fend for their food. Harsh climatic conditions and low rainfall lead to scarcity of feed and poor quality feed. Nutritional value of local forage often deteriorates in the dry season (May to August). Regardless of a similar genetic background, differences in WS was observed between the VTI and VTE demonstrating that improved management and nutrition leads to improved performance.

A marketable mature live body weight for goats in their first year is between 30-40 kg (Pophiwa *et al.*, 2016). The present study observed that only the SAB and NCS fall within that range, and the rest of the populations did not meet the threshold. Within population variation in the populations that did not meet the market weight shows that there is still room for selection for optimal growth. This suggests that more still needs to be done on other indigenous veld goats (IVG) as well as non-descript village populations to improve growth performance and attain marketable mature live body weight.

The slaughter weight corresponded to the carcass yield. The breeds with the highest slaughter weight (SAB and NCS) produced high carcass yield post mortem compared to the rest of the populations. The present study is consistent with that of Pophiwa and co-workers (2017) who reported that the Boer had higher cold carcass weight (6.1 kg) compared to the indigenous populations (2.9 kg). Fat class is essential to producers as one of the traits that determine carcass yield. Carcasses with low-fat content are more likely to lose more moisture and cold shortening during chilling (Webb, 2014). The SAB, NCS and XL were classified as very lean carcasses with the fat class that was greater than 1 while the MBZ and the Pella village populations were classified as no fat with a fat classification of zero. According to Webb(2014), the fat class of carcasses is affected by factors such as sex, body weight and age, and the genotype of the animal. The present study observed that large framed genotypes such as the SAB, NCS and XL had a high-fat content while the smaller genotypes (MBZ, VTI and VTV) had low-fat content.

Dressing percentage (DP) is the percent of live weight that is turned into a carcass (Assan, 2012). Dressing percentage can be measured with or without the head (Webb, 2014), in this study the head was removed before taking measurements. DP is a crucial trait in meat production as it aids the farmers to have a target live weight before slaughtering the animals (Pophiwa *et al*., 2016). The highest DP was observed in the NCS population while the rest of the populations had similar proportions. DP observed in this study were generally lower than those reported by Tshabalala *et al*. (2003) where they observed a dressing percentage of 55.72±1.58 % and 55.68±1.29 % in Boer and indigenous goats, respectively. Pophiwa and co-workers (2017) observed 47.5 % in both the Boer and the indigenous populations which was still higher than the present study. Between breed difference was very small and in line with what Tshabalala and co-workers reported in 2003 that there were no significant differences in dressing percentage between breeds. Goats are generally lean and therefore produce lean muscle across breeds (Tshabalala *et al*., 2003).

As described by Webb (2014), thin fat cover of a carcass affects chilling loss. The present study observed high variations of chill loss. Chilling loss has also been observed to be correlated with the live body weight of the animal (Kadim et al., 2007; Pophiwa *et al*., 2017). However, in this study, the SAB had higher body weight and lower chill loss (5.5± 1.7 %), whilst the NCS had a chill loss that was higher (8.4± 3.5 %) than the rest of the populations. Such a contrasting observation may be due to the effect of genotype as described by Webb (2014) that breed is one of the factors that consequently affect chill loss.

The study also investigated SNPs associated with carcass quality traits in the SA goat populations using the Illumina goat SNP50K genotype data. Genome-wide association revealed candidate genes that are associated with carcass quality traits in South African goat populations. For WS, eight candidate genes were identified across breeds. The *GADD45G* gene is a developmental protein that has been associated with immune-related functions and plays a role in the regulation of growth and apoptosis indicating that the extensively raised populations undergo high stress levels (Higgins *et al*., 2019). The p53 signalling pathway is involved in the coordination of response to stress (McCabe *et al*., 2012) and the *GADD45G* gene is one of the genes that play a role in this pathway where its enrichment was associated with feed efficiency in beef cattle (Higgins *et al*., 2019). In the present study, this gene was not observed in the SAB population and the absence thereof is unclear. The village goat production system under harsh climatic conditions, contribute to a stressful environment for the non-descript village populations and the presence of the *GADD45G* gene and the associated P53 signalling pathway attest to that. The *IGF2R* gene, a gene that encodes a transmembrane receptor was associated with WS in the present study. Polymorphisms in this gene were associated with carcass quality traits such as carcass weight as well as growth traits such as animal body size in Irish Holstein-Friesian cattle (Berkowicz *et al*., 2012).

The vertebrate development associated gene (*VRTN*), an important gene in vertebrates was significantly associated with weight at slaughter and warm carcass weight traits. The *VRTN* gene plays a vital role in vertebral development and has shown potential to be used in sheep breeding where it was observed to be associated with carcass length and meat production (Shengwei *et al*., 2019). The ability to convert poor quality feed is one of the traits that characterize village goat populations, and this study showed that the WCW within the Village goat populations were at similar proportions with the VTI having a slightly higher WCW. The *VAV3* gene is a protein-coding gene that has been associated with food efficiency conversion in goats (Brito *et al*., 2017) was also associated with carcass weight in Hanwoo cattle (Lee *et* *al*., 2012). This study demonstrated the association of this gene with warm carcass weight. Some of the calpain gene family members (*CAPN8* and *CAPN2*) were associated with WCW. The calpain gene family produces enzymes that are observed in the proteolysis of muscular proteins during meat ageing and is associated with meat tenderness (Williams, 2008). In South African goat populations, however, this gene family were associated with WCW. The *CAPN2* gene that was associated with warm carcass weight, on the other hand, is one of the gene isoforms identified in skeletal muscle (Lian *et al*., 2013). These are also involved in other processes such as cell differentiation and regulation of the cytoskeleton (Lian *et al*., 2013).

Cold carcass weight (CCW) is that weight achieved 24 hrs post-cooling of the carcass, which is about 1.5% less compared to warm carcass weight. The present study is consistent with that of Pophiwa et al. (2017) who reported that the Boer goat had higher cold carcass weight (6.1 kg) compared to the indigenous populations (2.9 kg). Eleven candidate genes were significantly associated with this trait and included the growth differentiation factor 11 (*GDF11*) that was reported to have skeletal muscle and bone rejuvenation in mice (Idkowiak-Baldys et al., 2019). The transforming growth factor beta receptor 3 (*TGFBR3*) on the other hand, plays an essential role in muscle tissue development in pigs was associated with pork quality (Jeong *et al*., 2016) and was associated with CCW in the present study.

Fat code classification is an important trait as it affects carcass yield and moisture. According to Webb (2014), the fat code of carcasses is affected by factors such as sex, body weight, age, and animal ecotype/breed. In this study, large framed breeds such as the SAB, NCS and XL had a high-fat code while the smaller ecotypes (MBZ, VTI and VTV) had low-fat content. Seven candidate genes that included the calpain family member (*CAPN7*) were identified. As described above, this gene family is associated with meat tenderness and most specifically *CAPN1*. Studies has associated the level of subcutaneous fat with meat tenderness. The tenderness and toughness of meat is therefore dependent on the calpain activity, where high calpain activity post-mortem leads to greater and tougher muscles, and inhibition leads to tender meat thereof (Frylinck et al., 2009). The combination of fat code and calpain activity and the calpain gene family as candidate genes may be used as a selection method for carcass yield and tender meat. Mutations in the Growth hormone secretagogue receptor (*GHSR*) was associated with the impairment of constructive activity receptor in human families with short stature, while the administration of this gene to pregnant and lactating mice resulted in foetal and postnatal weight gain (Chanoine et al., 2009). The identification of this gene as a candidate gene for FC implies that this gene plays a role in the growth and development of goats, therefore, affecting carcass fatness. This can then be one of the most economically important genes in chevon production.

Only one candidate gene, POU class 1 homeobox 1 (*POU1F1*) was associated with chill loss (CL). *POU1F1* plays a role in the expression of growth hormone therefore, leading to variations in growth rates and has been associated with weight gain in chickens at various growth stages (Manjula et al., 2018).

4.6 Conclusions

The intensively raised goat populations performed better in weight and carcass characteristics, indicating that improved management can lead to improved genetics. The study showed that the Northern Cape Speckled usually bred for its hide has potential as a meat breed and should be considered in selection programs. The present study concludes that genetics is one of the factors that affect growth and carcass quality traits and that there is potential for chevon improvement in the South African goat genetic resource pool. Candidate genes identified and associated with carcass quality traits in South African goat populations are potential markers in selection programs. Results from this study can be used for improvement programs for meat and carcass quality traits. The identification of candidate genes associated with carcass quality traits and weight in all the populations, including extensively raised village populations presents potential for integrating genomics for improvement of carcass quality South African goat populations including village populations.

CHAPTER 5: DIFFERENTIAL GENE EXPRESSION AND IDENTIFICATION OF GROWTH-RELATED GENES IN THE PITUITARY GLAND OF SOUTH AFRICAN GOATS

5.1 Abstract

Growth is an economically important trait in goat production. This study investigated differentially expressed and associated growth-related genes from the caprine pituitary gland transcriptome of South African indigenous goat breeds of varying growth performances. Tissues were harvested from the pituitary gland of three village ecotype goats and three SA Boer goats all raised in similar conditions simulating intensive commercial production systems. Within-breed differences were investigated by comparing differential gene expression between three village goats raised under extensive conditions (on-farm in Pella village farming community) and three village goats raised under intensive commercial production system. Total RNA isolated from the pituitary gland of 36 weeks old animals (*n=9*) was sequenced individually in triplicates and an average of 28,298,512 trimmed, and quality-controlled reads were mapped to the goat genome (*Capra_hircus.ARS1.94*) using HiSat2 software. Transcript assembly and quantification from RNA sequence yielded 104 differentially expressed genes for the extensive system and 62 for the intensive production system at the false discovery rate (FRD) of ≤0.05 and a fold change of ≥2 which were retained for downstream analysis. Growth-related genes such as the *POU3F4* and *TSHZ1* were highly expressed within populations raised under different production systems. Conversely, growth-related genes such as *FGFR2* and *SMPX* genes were highly expressed between breeds raised under similar production systems. Ballgown analysis revealed a high expression of *GH1* and *IGF1* in the intensively raised compared to extensively raised goats. This gene was also highly expressed in the village goats when compared to the Boer. The POU1F1 gene was moderately expressed between and within-breeds in both experiments. The differential gene expression data provided insights into genes and molecular mechanisms associated with growth and growth development in goats.

Keywords: *Boer goats; differentially expressed genes; growth; unimproved goats; RNA-seq ;*

5.2 Introduction

Growth and carcass quality are essential traits in goats and other domestic livestock species raised for the provision of meat. Variation in growth performance has been observed between and within goat breeds (Ncube, 2016). The absence of structured selection in communal farming systems were linked to low performance irrespective of the presence of high performing animals in such populations (Masika & Mafu, 2004). Growth performance in South African goats is constrained as a majority (over 60%) of the goat farming is practiced in impoverished, marginalized communities (Dzomba *et al*., 2018) where farmers face various challenges that affect growth and other traits of economic importance.

South Africa is represented by two goat production systems of commercial/intensive and extensive production systems (Mdladla *et al*., 2017). The commercial/intensive production system is characterized by specialized breeds, well-designed housing systems, adequate feed, feed supplementation and regular health care systems (Mdladla *et al*., 2016). Hence breeds such as the South African Boer (SAB) raised in the commercial production system are reported to have high growth performance and meat yield (NAMC, 2005). Non-descript village goats, on the other hand, are raised under the extensive production system characterized by low input management practices often associated with suboptimal nutrition and housing, inadequate veterinary services and lack of breed/animal improvement programs (Gwaze *et al*., 2009; Masika & Mafu, 2004). Village goats fend for food and sometimes travel long distances to browse in undesirable climatic conditions. The use of non-descript breeds coupled with the harsh conditions affects growth performance leading to low mature weights and lean animals. Village goat populations that are kept and adapted to most of these harsh production conditions are small with low growth rates when compared to the commercial populations (Masika & Mafu, 2004). However, village goat populations have diverse growth rates and mature weights (Visser *et al*., 2004) indicating that some individuals may be better performers than typical animals in these communal farms, thus providing room for within-population selection. Unfortunately, there are no records or information on production performance of goats from most communal farming systems (Masika & Mafu, 2004). With limited information, it is challenging to develop breeding programs that enable selection for growth rates and other traits of economic importance.

For optimal breed management and utilization, it is crucial to investigate and understand the genes and genetic pathways that play a role in growth and growth-related traits of goats and other domestic animals. The pituitary gland dubbed the “master gland”, secretes hormones that control other parts of the endocrine system and is the most studied in livestock because of its importance in processes such as growth, reproduction, stress, and lactation (Chapman, 2019). The pituitary is responsible for controlling growth at different developmental stages that involve interactions of numerous hormones and growth factors playing a role in endocrine and paracrine functions (Pareek *et al*., 2016). Therefore, investigating gene expression in the pituitary gland should aid in unravelling the genetic mechanisms influencing growth and carcass quality in South African indigenous goats raised under different production systems. Genes such as the growth hormone (*GH*), growth hormone receptor (*GHR*), insulin like growth factor I (*IGF-I*), leptin (*LEP*), caprine pituitary specific transcription factor-1 (*POU1F1*), caprine myostatin (*MSTN*), bone morphogenetic protein (*BMP*), and others may play important roles in growth (Alakilli *et al*., 2012). For example, mutations in *POU1F1* were associated with dwarfism in mice and humans, while polymorphisms in this gene are associated with production traits in goats (Lan *et al*., 2007). Some of the genes like the *MSTN* are responsible for “double muscling,” a phenotype with significant muscle hypertrophy in sheep and cattle (Gan *et al*., 2008). Knowledge of such genes and their expression in different populations will shed light on growth- and growth-related traits that will in turn empower actions on breed improvement.

RNA sequencing is a high-throughput sequencing method for characterizing gene expression in specific tissues. This technology provides accurate counts of transcripts to measure relative expression and to discover new exons or genes (Wang *et al*., 2009). This approach is widely used for mapping, and quantifying transcriptomes developed to analyze gene expression in various tissues (Cánovas *et al*., 2010). RNA-Seq has been used to study growth traits in rainbow trout using slow- and fast-growing fish and markers that are related to growth traits were identified (Salem *et al*., 2012). Through transcriptome analysis, studies have been able to associate expressed genes to pathways that play a role in cell stimulations and neutral growth as well as phenotypes in slow and fast-growing chickens (Wu *et al*., 2018). These among other studies show potential for RNA-Seq as a tool to be used for gene expression profiling. This study sought to use RNA-Seq based gene expression profiling to investigate genes associated with growth and carcass quality under differing production systems in South African goat breeds.

5.3 Materials and methods

5.3.1 Animals and management

Two separate experiments were designed to study (i) the impact of production systems within a breed and (ii) breed differences within a production system on differential gene expression in indigenous South African goat breeds. To examine the impact of production systems, goats were sampled from non-descript village populations and raised in either extensive or intensive production systems. To examine the effect of breeds, Boer and non-descript village goats were raised under the same intensive production system. The growth trial to investigate the growth profiles and carcass quality of South African goat populations was performed from October 2016 to March 2017 and described in detail in chapter 3 (Ncube *et al*., 2019).

5.3.1.1 Comparisons within the village goat breeds and across production systems

The South African village goat populations (*n*=28) were sampled from 14 farms in Pella village, North West Province of South Africa. These 28 animals were split into two groups of the extensive system (VTE) (*n* = 14) and the intensive system (VTI) (*n* =14), The VTE village goats were purchased and raised in their respective village farms under extensive communal farming conditions. Goats kept at Pella village farms were penned at night and left to browse during the day with no supplementation and healthcare provided. Pella village is in the arid North West province characterized by low rainfall leading to very little pasture which results in scarce and poor quality feed (Holmgren *et al*., 2006; Mdladla *et al*., 2017).

The second group of goats sampled from Pella (VTI) (*n* =14) was transferred to the Agricultural Research Council, Animal Production (ARC-AP), Small Stocks Unit, where they were raised under controlled feed and management conditions. They were kept in a grazing camp on management diet of game pellets (110g/kg, 25-70 g/kg crude fat, 110-200 g/kg crude fiber, 6-10 g/kg calcium, 2.5 g/kg phosphorus and 3.68 % non-protein nitrogen) provided at 3 % of live weight/animal/day. Lucerne hay and clean water were available *ad libitum*. Animals were dipped every second week, vaccinated at recommended intervals by a vet and taken to hospital in the case of diseases.

During the growth trial, live bodyweights were measured fortnightly at three different growth stages (12 weeks, 24 weeks and 36 weeks) as demonstrated in Table 5.1. Animals at the research farm were weighed on Thursdays while those at village farms were weighed on Fridays. The weaning (12 weeks) weights ranged from 9.00-16.40 kg for intensively raised and 7-13 kg for extensively raised goat populations. The Pella population raised at the research farm had higher live weights at 36 weeks (31.00-33.00 kg) as compared to the population that was raised extensively at Pella village (22.50-28.00 kg) (Ncube et al., 2019).

Table 5.1 Body weights of samples used in this study at three different stages (12 weeks, 24 weeks and 36 weeks)

Sample ID	Breed	Production System	12W (kg)	24W (kg)	36W
16-191	Boer	Intensive	20	28.5	41
16-224	Boer	Intensive	15	25.5	39.5
16-225	Boer	Intensive	15	28	42
PAPI01	Village	Intensive	9	22.5	32
PAPI03	Village	Intensive	10.5	19.5	31
PAPI04	Village	Intensive	16.4	24	33
P01	Village	Extensive	7	16	22.5
P03	Village	Extensive	13	20.5	28
P04	Village	Extensive	13	15	27.5

5.3.1.2 Comparison across breeds and within the intensive production system

Samples were collected from a total of 28 goat kids from two production systems representing the commercial South African Boer (*n*=14) and the South African village goat population (*n*=14). The South African Boer goats were sampled from one of the Indigenous Veld Goat (IVG) Society farms and the non-descript village populations were sampled from Pella village, North West farms at 12 weeks of age.

This experiment consisted of the Boer purchased from IVG and the South African non-descript village goat populations both intensively raised at ARC-AP under controlled feed and management conditions as described above. Variations in bodyweight per growth stage are shown in Table 5.1.

5.3.2 Sample collection

Animals were humanely euthanized at 36 weeks and tissue harvesting was completed within 20 min post euthanasia to preserve RNA quality. Tissue samples were collected from the pituitary gland, which is located at the base of the goat brain. The samples were immediately stored in tubes containing the Qiagen RNA stabilization reagent, RNAlater (www.qiagen.com), to preserve the RNA and prevent degradation. The tissue samples were subsequently kept in a -80°C freezer.

5.3.3 RNA isolation and quantification

RNA samples were selected for sequencing based on the population genomic structure, which were derived from admixture analyses using genotypic data from the OvineSNP50K Beadchip (Illumina Inc., San Diego, CA) (Ncube *et al*., 2019). Using this criterion, three animals that clustered within the defined population cluster as observed at K=10 (Ncube *et al*., 2019) were selected for each of the South African Boer (SAB), extensively raised village populations (VTE) and, intensively raised village populations (VTI). RNA isolation from 150mg of the pituitary gland was performed using the Qiagen RNeasy and Qiagen RNA Universal Midi kit for purification of total RNA following the manufacturer’s instructions with slight modifications. The modifications included using 150 mg of tissue sample and homogenization was performed on the 2010 Geno/Grinder® - Automated Tissue Homogenizer and Cell Lyser. RNA was eluted using 100 µl of RNA-free water. The quality of the RNA template was investigated electrophoretically on 1.8 % agarose gel with ethidium bromide of 0.5 µg/mL at

80V for 30min. The gel was examined under UV light for RNA degradation in a BIORAD Imaging System (BIORAD GelDoc XR) (www.bio-rad.com). The samples were submitted to the Agricultural Research Council, Biotechnology Platform (ARC-BTP) Core Facility for sequencing.

5.3.4 RNA sequencing

The cDNA libraries were prepared using an Illumina TruSeq Stranded Total RNA Ribo-Zero H/M/R Gold library prep kit (Illumina Inc, San Diego, CA) preparation. TruSeq universal adapters were ligated to the cDNA fragments, and PCR was performed to produce the final sequencing libraries. RNA was fragmented and randomly primed for reverse transcription to generate double-stranded cDNA fragments. Gel electrophoresis was used to assess the quality of the starting material. The cDNA was colligated, nebulized and then fragmented after which, adapters were ligated to both ends of the fragmented nucleic acid. The fragments were hybridized to a flow cell, which extended a hybridized template or performed bridge amplification. Sequencing was then performed using Illumina HiSeq 2500. All cDNA samples for each animal were sequenced and examined in triplicate.

5.3.5 Data analysis

5.3.5.1 RNA sequence trimming and quality control

Quality Control (QC) was performed on raw sequencing reads using Trimmomatic v0.36 (Bolger et al., 2014). Sequence trimming included the removal of the sequencing adapters, short reads as well as reads containing over 10 % unknown bases (N), resulting in clean reads longer than 30 bp.

5.3.5.2 Sequence read alignment, assembly and, quantification

Reads that remained after QC were aligned and mapped to the goat genome (*Capra_hircus.ARS1.94)* from ENSEMBL (https://www.ensembl.org/Capra_hircus/) using HISAT2 v2.1.0 (Pertea et al., 2016). Genome indexing was performed with a maximum number of multiple hits set to 20 and non-mismatches at splice sites. Mapped reads were then sorted and converted to BAM files using SAMtools v1.3.3 (Pertea *et al.*, 2016).

Transcript assembly and quantification from RNA sequence reads was performed with StringTie (Pertea *et al.*, 2016). Differential gene expression analysis was performed using Ballgown 3.8 and DESeq2 1.18.0 packages within R 3.5.2 using a linear statistical model.

Filtering was performed to remove low abundance genes as well as to identify statistically significant genes between groups under study. Downstream processing after filtering was performed on 6 samples of the VTE (n=3) and VTI (n=3) village goats to compare between extensive and intensive production systems. Processing was also conducted between 6 samples of SAB (n=3) and VTI (n=3) to compare between breeds under similar intensive production system. P-values were adjusted using Benjamini and Hochberg's approach for false discovery rate (FDR) control. Differentially expressed genes were considered those that had the adjusted P-value of ≤ 0.05.

5.3.6 Differential gene expression

For analysis of differentially expressed genes between experimental groups, the DESeq2 R package (Bioconductor, Buffalo, USA) was used with R v3.5.2. Genes with an FDR $\leq$0.05 and a log2 fold change of ≥ 2 and adjusted *P*-value $\leq$0.05 were considered to be differentially expressed genes, and a volcano plot for highly significant genes was performed. Ballgown analysis (Pertea *et al*., 2016) was used to further investigate gene expression levels of genes of interest that are associated with growth within and between breeds using FPKM (Fragments Per Kilobase of transcript per Million mapped reads) values.

5.3.7 Functional annotation and pathway analysis of differentially expressed genes

To gain more insight into the biological processes and implications of the differentially expressed genes (DEGs), gene ontology (GO) annotation and gene enrichment analysis were performed. GO analysis of DEGs was performed using the DAVID v6.7 online server (http://david.abcc.ncifcrf.gov/, (Huang *et al*., 2009). DEGs were further mapped into the KEGG pathway database using KEGG mapper within the Kyoto Encyclopaedia of Genes and Genomes databases (KEGG, http://www.genome.jp/kegg; to predict the significantly enriched pathways. The DEGs genes of the groups under study were classified into cellular component, biological process and, molecular functions. Bar graphs showing differences and similarities between GO terms were plotted using WEGO v2.0 web-based tool (Ye *et al*., 2018) (Figure 5.6a-5.6b).

5.4 Results

5.4.1 RNA sequencing and read mapping

About 28,298,512 reads were generated, and approximately 88.50% of the trimmed and quality-controlled reads were mapped to the goat reference genome (*Capra_hircus.ARS1.94)*.

5.4.2 Differential gene expression

5.4.2.1 Differential gene expression (DGE) within the village goat breed and across production systems

Differentially expressed (DE) genes were considered as those with an FDR ≤ 0.05 and a log2 fold change of ≥ 2 and adjusted *P*-value ≤ 0.05. For within-breed DE, a total of 51 674 genes were expressed in 6 samples of extensively (VTE) and intensively (VTI) raised village goats, and 40 964 were retained after filtering. The distribution of the differentially expressed genes is shown in a volcano plot (Figure 5.1a) in which the top 10 highly significant genes were labelled.

Of the 40 964 expressed genes, 104 genes were differentially expressed, of which 46 were up-regulated, and 58 were down-regulated (Appendix G-H). In the extensive system (VTE), 48 (30.8%) genes were up-regulated while 15 (13.5%) were down-regulated (Figure 5.2a). The intensive system (VTI) had 15 (14.4%) up-regulated genes and 58 (40.4%) down-regulated genes. Both production systems shared 15 up-regulated genes and 16 down-regulated genes.

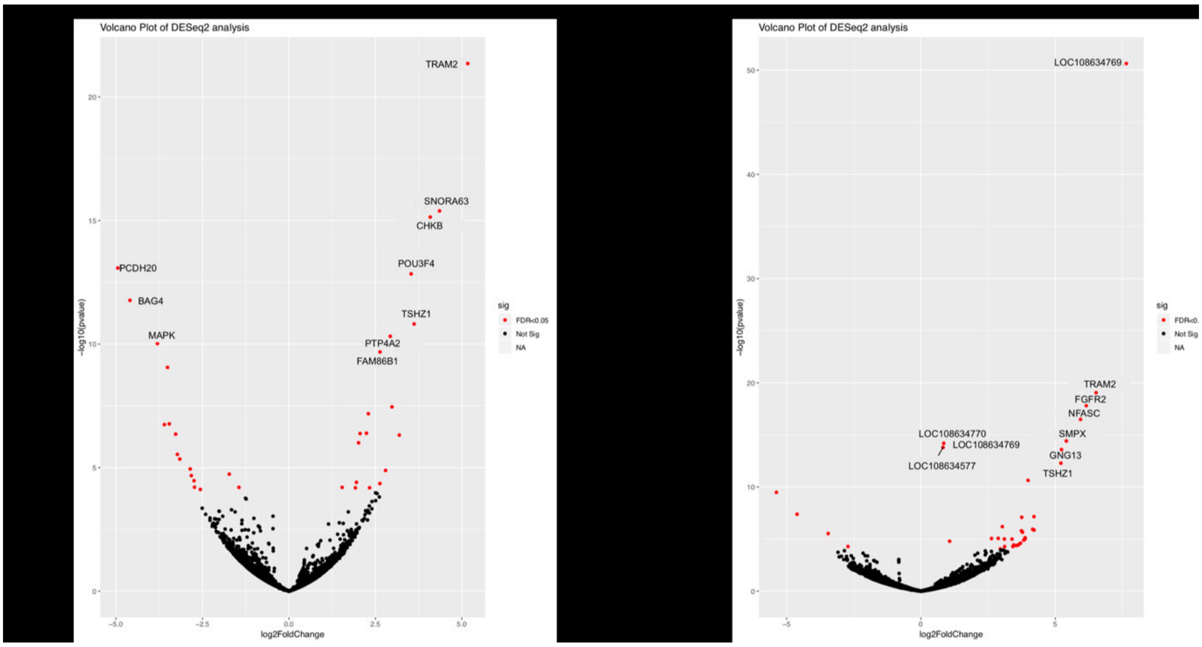

Figure 5.1 Volcano plot of differentially expressed genes a) within populations raised in different production systems b) between populations raised under a similar production system. Highly expressed genes are shown in red.

5.4.2.1 Differential gene expression (DGE) across breeds and within the intensive production system

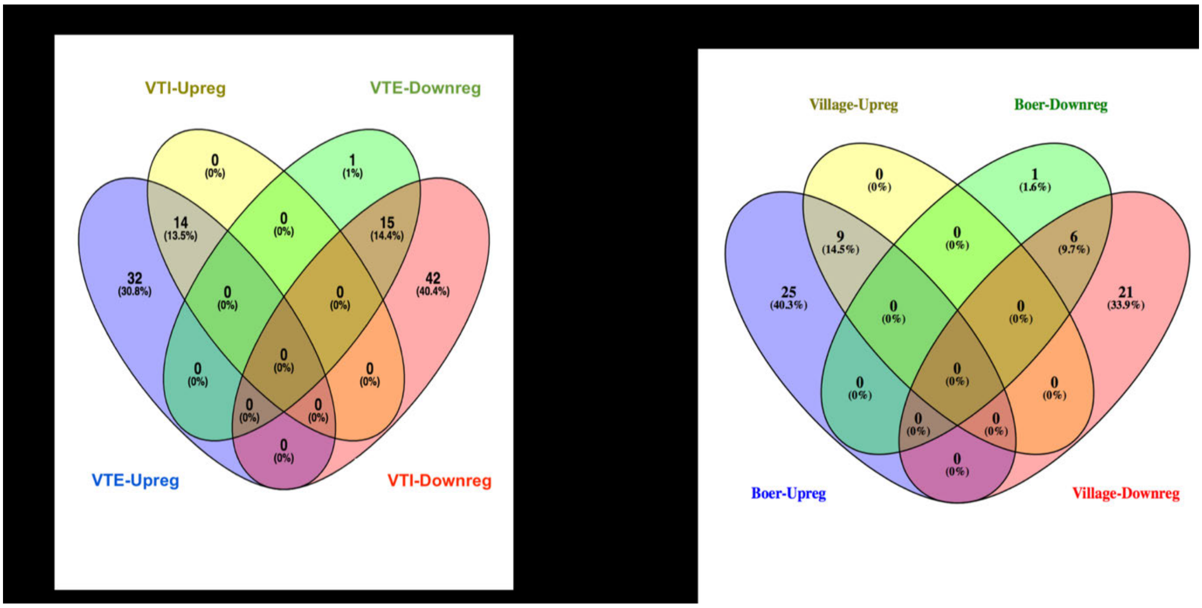

Figure 5.2 Overlapping differentially expressed genes (up and down-regulated) of a) Extensive system (ES) vs Intensive system (IS) and b) Intensively raised Boer vs Village.

The comparison between Boer and village breeds in a similar production system revealed 51 674 expressed genes in 6 samples of Boer and village goats, of which 45 005 were retained after filtering. Differentially expressed gene distribution is shown in a volcano plot (Figure 5.1b) in which the top 10 highly significant genes were labelled.

Sixty-two of the expressed genes were differentially expressed from which 34 (40.3 %) were up-regulated and 28 (6%) down-regulated (Supplementary Table S1.3 and S1.4) as shown in Figure 5.2b. The Boer population demonstrated 34 (40.3 %) up-regulated genes and 7 down-regulated while the village goats had 9 (9.7 %) up-regulated and 27 (33.9%) downregulated genes. Both breeds shared 9 up-regulated genes and 5 down-regulated genes.

5.4.3 Differential expression of genes of interest associated with growth

Certain genes are known to play a significant role in the growth of animals, and this study hypothesized that these genes would be among the most significantly expressed genes. Some of these key genes included *GH1*, *IGF1* and *POU1F1* that work as a network to influence the growth of animals. However, these genes were expressed at lower fold change (≤2) ranging from 0.90 to 0.95 with a *P*-value from 0.61 to 0.83. The *Growth hormone 1* gene, also known as the pituitary growth hormone, is a significant participant in control of several complex physiological processes, including growth and metabolism. Within-breeds, this gene was highly expressed in the intensively raised animals as compared to the extensively raised (Figure 5.3a). For breeds raised under the same production system, the *GH1* was highly expressed in the village populations as compared to the Boer. The *IGF1* gene is a mediator of the effects of growth hormone (GH), stimulates systemic body growth and has growth-promoting effects. Within breeds, these genes were highly expressed in the intensively raised animals as compared to the extensively raised (Figure 5.3a,c). For breeds raised under the same production system, these genes were highly expressed in the village populations as compared to the Boer (Figure 5.3b,d). The pituitary specific transcription factor 1 (*POU1F1)* gene activates growth hormone and its responsible for pituitary development and hormone expression in mammals. For within breed gene expression, this gene was highly expressed in the pituitary tissue for the intensively raised group while moderately expressed in the extensively raised (Figure 5.3e). For between breed gene expression, the *POU1F1* gene was expressed at similar high levels (Figure 5.3f).

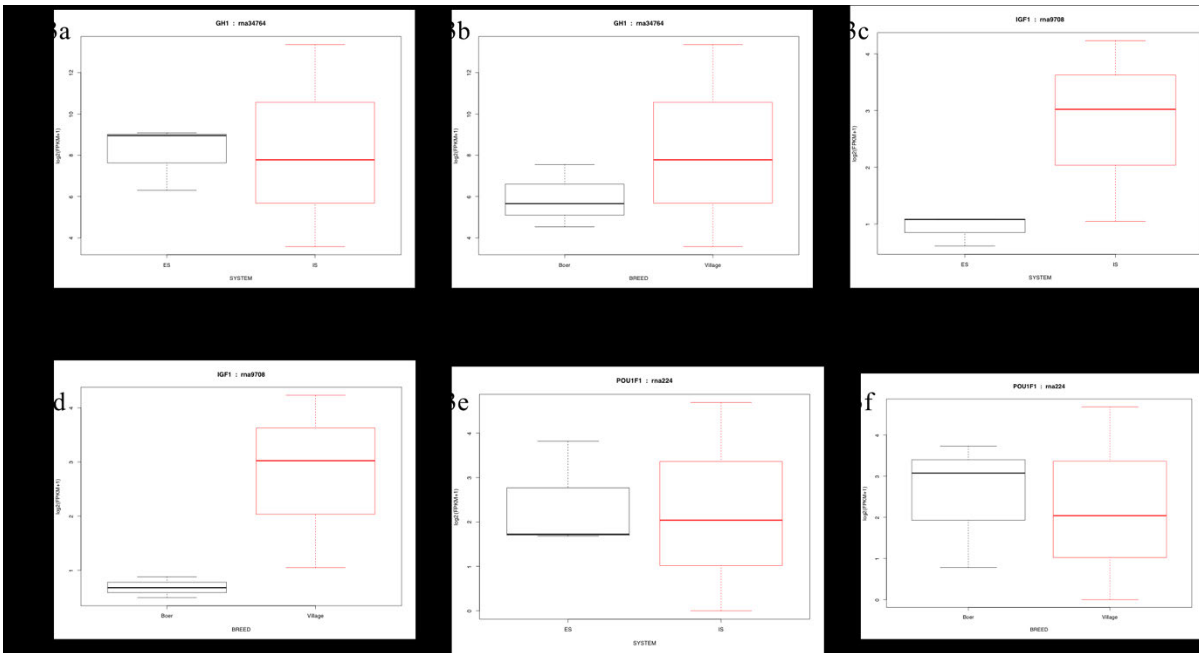

Figure 5.3 FPKM distribution of GH1 gene a) within-breeds raised under different production systems (ES vs IS) b) between-breeds raised under similar production system (Boer vs Village). IGF1 gene c) within-breeds raised under different production systems (ES vs IS) d) between-breeds raised under similar production system (Boer vs Village). POU1F1 gene e) within-breeds raised under different production systems (ES vs IS) f) between-breeds raised under similar production system (Boer vs Village) displayed in box plots.

5.4.4 Functional annotation of differentially expressed genes

To better understand gene networks, gene ontology (GO) annotation was performed using Fisher's exact test and filtering using multiple correlation controls for false discovery rate (FDR) ≤ 0.05. The GO terms were associated with differentially expressed genes in both experiments (within and between population), as shown in Figure 5.4a-5.4b. For within breed analysis that compared extensively raised versus intensively raised village goat ecotypes, significantly annotated gene ontology (GO) terms were within the three major functional groups, cellular component (CC), biological process (BP) and molecular function (MF) (Figure 5.4a). Some of the differentially expressed genes (DEGs) that play a role in the cellular component such as the insulin like growth factor binding protein complex, oxidoreductase complex and growth factor complex were observed only in the extensively raised populations. Lipid binding molecular function was observed only in the extensively raised village populations. GO analysis revealed DEGs that play a role in the biological processes such as muscle adaptation, cell growth, anatomical structure development, anatomical structure morphogenesis involved in morphogenesis, developmental process and growth as well as organ growth in both the VTE and VTI production systems. There were no significantly enriched GOs.

5.4a

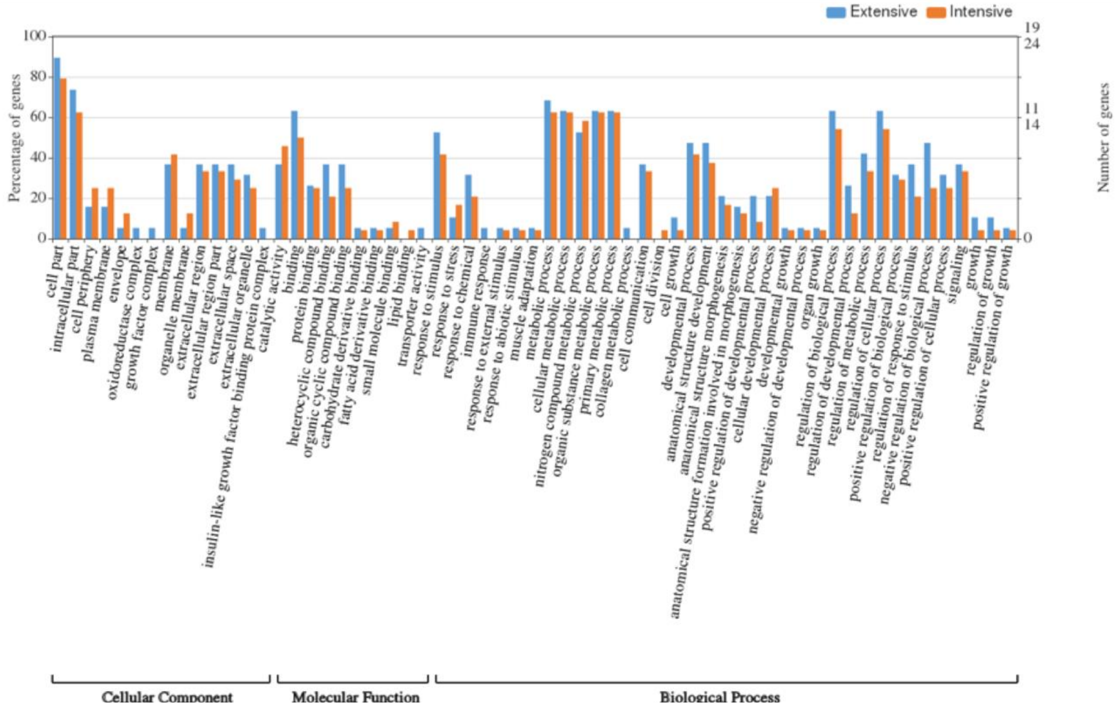

Figure 5.4a Gene ontology (GO) of differentially expressed genes in a village goats raised in the extensive system as well as those raised in the intensive system. The right y-axis indicates the number of genes in a category. The left y-axis indicates the percentage of a specific category of genes in that main category. One gene could be annotated into more than one GO.

5.4b

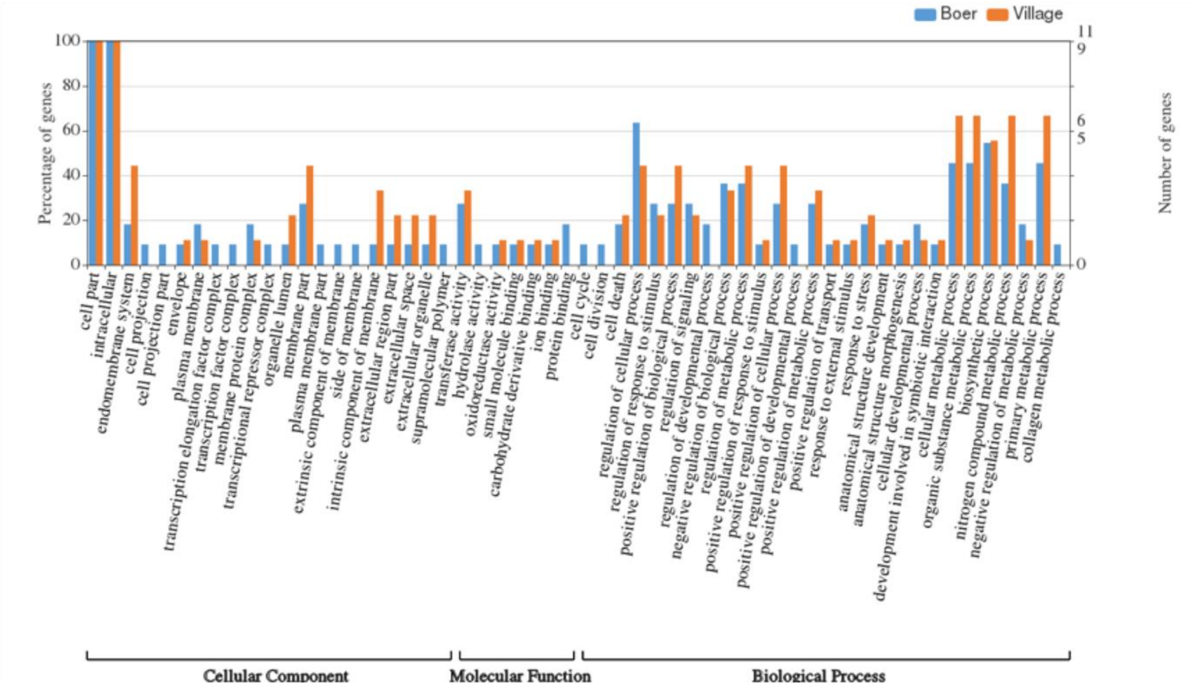

Figure 5.4b Gene ontology (GO) of differentially expressed genes in Boer and village goats raised in the intensive system. The right y-axis indicates the number of genes in a category. The left y-axis indicates the percentage of a specific category of genes in that main category. One gene could be annotated into more than one GO.

Functional annotation analysis between breeds (Boer vs Village) raised under the intensive production system resulted in cellular components, biological processes and molecular functions (Fig 5.4b). There were no significantly enriched GO terms observed. Differentially expressed genes (DEGs) that play a role in the cellular component such as the cell projection, cell projection part, transcription elongation factor complex, transcription factor complex, plasma membrane part, extrinsic component of membrane, side of membrane and supramolecular polymer were observed only in the Boer population. Cell cycle and cell division molecular functions were observed only in the Boer population. The GO analysis revealed DEGs that play a role in the biological processes such as regulation of developmental process, positive regulation of biological process, anatomical structure development, anatomical structure morphogenesis and cellular developmental process involved in symbiotic interactions in both the Boer and village populations except for positive regulation of biological process which was observed only in the Boer population.

5.4.6 Pathway analysis

For an understanding of biological pathways that are involved between and within-breeds raised under different production systems, the KEGG pathway enrichment analysis was performed using the DAVID 6.7 Functional Annotation Tool (http://david.abcc.ncifcrf.gov/) (Table 5.2 and 5.3).

Table 5.2 Pathway analysis of differential gene expression within breeds raised under different production systems (extensively raised vs intensively raised) village populations

Pathway	Gene Symbol	Gene name	Fold enrichment	Benjamini	*P* value
Metabolic	ACADVL; CHKB; DPM3; GNPDA1; RRM2B	Acyl-CoA dehydrogenase, very long chain; choline kinase beta; dolichyl-phosphate mannosyltransferase subunit 3; glucosamine-6-phosphate deaminase 1; ribonucleotide reductase regulatory TP53 inducible subunit M2B	2.18	0.81	0.15
RNA degradation	EXOSC3; PAPD7	Exosome component 3; poly(A) RNA polymerase D7, non-canonical	14.19	0.87	0.12
p53 signalling	IGFBP3; RRM2B	Insulin like growth factor binding protein 3; ribonucleotide reductase regulatory TP53 inducible subunit M2B	15.19	0.98	0.12

Table 5.3 Pathway analysis of differential gene expression between breeds raised under similar production system (Boer raised vs Village) goat populations

Pathway	Gene Symbol	Gene name	Fold enrichment	Benjamini	*P* value
Metabolic	DPM3; MAN1C1; NDUFB7	Dolichyl-phosphate mannosyltransferase subunit 3; mannosidase alpha class 1C member 1; NADH:ubiquinone oxidoreductase subunit B7	2.62	2.93	0.26
N-Glycan biosynthesis	DPM3; MAN1C1	dolichyl-phosphate mannosyltransferase subunit 3; mannosidase alpha class 1C member 1	44.02	0.77	0.04
PI3K-Akt signalling	GNG13; PRKAA2	G protein subunit gamma 13; protein kinase AMP-activated catalytic subunit alpha 2	6.22	0.97	0.25
Non-alcoholic fatty liver	NDUFB7; DPM3; MAN1C1	NADH:ubiquinone oxidoreductase subunit B7; dolichyl-phosphate mannosyltransferase polypeptide 3; mannosidase alpha class 1C member 1	13.48	0.90	0.12

The top three enriched biological pathways involved within-breeds (extensively raised vs intensively raised) were metabolic, p53, and RNA degradation pathways (Figure 5.5a and 5.5b). Five genes (*ACADVL, CHKB, DPM3, GNPDA1,* and *RRM2B*) were enriched in the metabolic pathway while 2 genes (*IGFBP3* and *RRM2B*) and (*EXOSC3* and *PAPD7*) were enriched in the p53 and RNA degradation pathways, respectively.

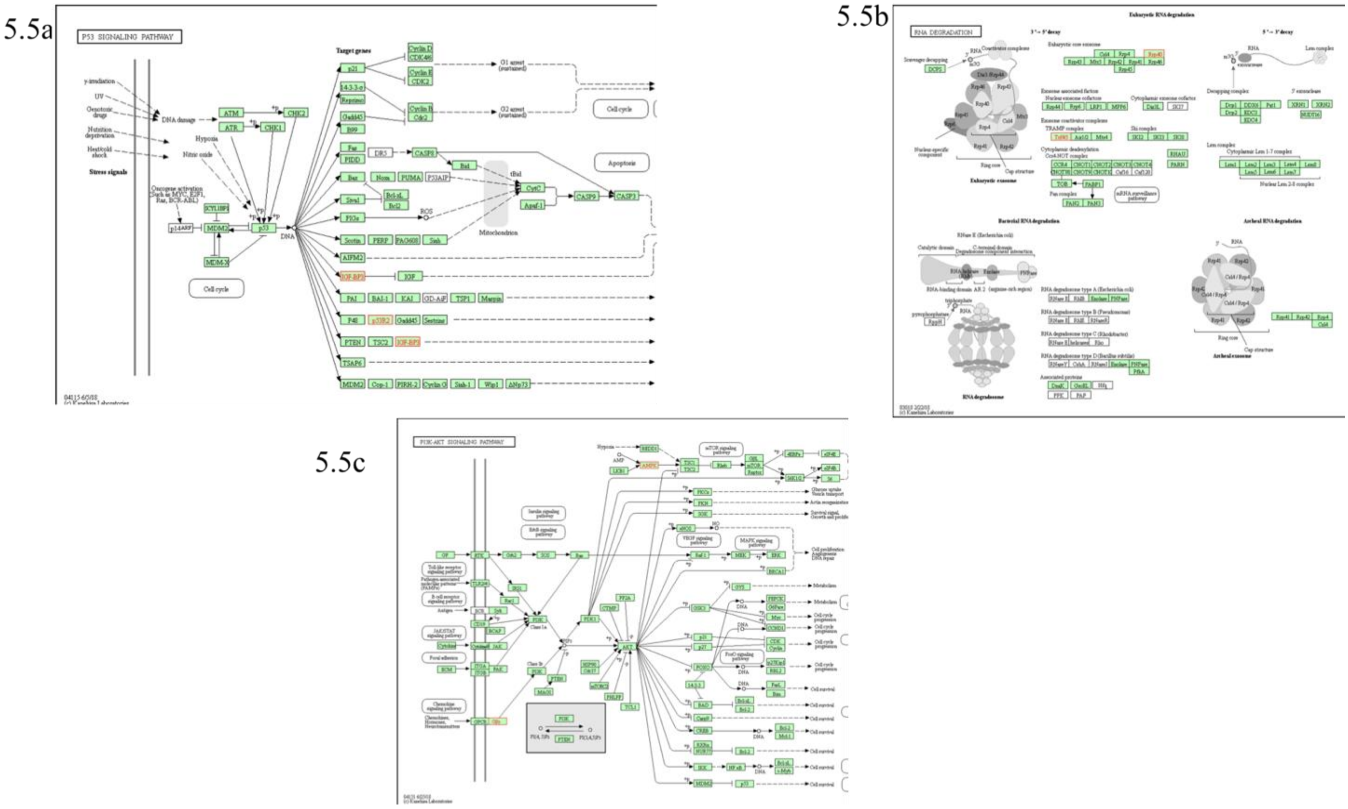

Figure 5.5 a) p53 signalling pathway b) RNA Degradation pathway and c) PI3K Signalling pathway. Genes involved are highlighted in red.

For between breed (Boer vs Village) pathway analysis, the four enriched pathways were metabolic, N-glycan biosynthesis, PI3K-Akt signaling and non-alcoholic fatty liver pathways (Figure 5.5c). Three genes (*DPM3*, *MAN1C1*, and *NDUFB7*) were enriched in both the metabolic and Non-alcoholic fatty liver pathways while 2 genes (*DPM3* and *MAN1C1*) and

(*GNG13* and *PRKAA2*) were enriched in the N-glycan biosynthesis and PI3K-Akt signaling pathways, respectively.

5.5 Discussion

Growth is an essential quantitative trait that influences mature bodyweights and the overall productivity of the goat production system (Supakorn, 2009). In a country where a large number of goats are raised in impoverished, marginalized communal production communities, there exist goat populations that are distinguished for their adaptability to local conditions and low input production systems (Dzomba *et al*., 2018). South Africa has registered and developed meat breeds of which the Boer goat is one. This breed is commonly known for its high meat yield and growth performance and is mainly raised under large scale intensive commercial systems (Pieters, 2007). Village goats, on the other hand, are uncharacterized populations that are raised extensively in communal areas commonly known for poor growth performance. This study hypothesized that the production system and breed differences are some of the critical factors that influence growth and carcass quality.

A high number of genes was differentially expressed within-breeds raised under different production systems. DEGs (*SNORA63*, *CHKB*, *POU3F4*, *MAPK* and *BAG4*) that play a crucial role in growth and development were identified. Small Nucleolar RNA, H/ACA Box 63 (*SNORA63*) is part of the H/ACA class of snoRNAs and is involved in the processing of eukaryotic pre-rRNA where the E3 is encoded in introns in the gene for protein synthesis initiation factor 4AII. The *SNORA63* gene has been identified in various developmental stages, and in different organs in vertebrates (Kehr *et al*., 2014); this suggests that this gene plays a role in the development of goats in the present study. The *chlorine kinase beta* (*CHKB*) on the other hand is a protein-coding gene that belongs to the chlorine/ethanolamine kinase family and encodes for key proteins in phospholipid biosynthesis. The *CHKB* gene that was highly expressed and up-regulated in the extensively raised village populations encodes for chlorine and ethanolamine kinase proteins that play a role in the enzymatic pathway for biosynthesis, and this is the initial step in the biosynthesis of phosphochlorine/phosphoethanolamine in all animals. Goshu *et al*. (2019) indicated a significant association of this gene to growth traits such as chest girth and bodyweight of Datong yaks suggesting that this gene could be a novel marker for growth traits and can be used to design markers for selection in animal breeding.

The *POU domain, class 3, transcription factor 4* (*POU3F4*) is involved in the patterning of the neural tube and both the paraventricular and supraoptic nuclei of the hypothalamus in the developing embryo. The hypothalamus is located near the pituitary gland and plays a role in the endocrine system by releasing hormones such as the growth hormone-releasing hormone (GHRH) that stimulates the pituitary gland to release the growth hormone, or the growth hormone inhibiting hormone (GHIH) which has the opposite effect (Sargis, 2015). The downregulation of this gene will then inhibit the growth hormone from being released; therefore, negatively affecting the growth of animals while up-regulation will aid in the growth of an animal. High expression of this gene in the extensively raised village goats leads to growth hormone inhibition, leading village goats to be slow-growing with low mature bodyweights. *Mitogen activated protein kinase* (*MAPK*) on the other hand directs cellular responses to a variety of stimuli such as proinflammatory cytokinesis, mitogenesis, osmotic stress, etc. and also regulates cell functions such as proliferation, gene expression, differentiation, cell survival and apoptosis. It is highly expressed and up-regulated in the extensively raised animals indicating that village goat populations are under a greater amount of stress. BCL2 associated athanogene 4 (*BAG4*) is one of the growth-related genes that interacts with BAG1, which is an anti-apoptotic protein. It is upregulated in the intensively raised goat populations and has been identified as a candidate gene for residual feed intake in Holstein cows (Hardie et al., 2017). The present study suggests that this gene has potential as a candidate gene for growth in South African populations which is consistent with the above-mentioned study on Holstein cows.

Fewer differentially expressed genes were observed between breeds raised under a similar production system and DEGs that play a role in the growth and development of goats were identified (*FGFR2, NFASC, SMPX* and *LOC108634577*). The *fibroblast growth factor receptor 2 gene* (*FGFR2*) plays a vital role in bone growth during embryonic development. It encodes a protein that is a member of the fibroblast growth factor receptor family which signals some immature cells in the developing embryo to become bone cells and form organs such as the head, hands, feet and other organs (Ornitz *et al*., 2015). This gene was up-regulated in the Boer goats that are reported to be high performing breeds with an average birth weight of 3-4 kg with a 100-day weaning weight of 27 kg (Snyman, 2014). The *small muscular protein* (*SMPX*) gene, on the other hand, is involved in the regulatory network through which muscle cells coordinate their structural and functional states during growth, adaptation and repair. It was isolated in skeletal muscle where it plays a role in protecting sarcolemmal plasma membrane from mechanical stress (Abdelfatah et al., 2013). It can also be found in the myotomal compartment of somites and in developing limb, head and neck muscles in adult mice where it was highly expressed in heart chambers and skeletal muscle (Palmer et al., 2001). The high expression on *SMPX* would then be associated to high muscle development and therefore expected in the Boer which, is associated with high meat yield.

Some of the DEGs though not significantly expressed, are known for their significant impact on growth and development including, *GH1*, *IGF1* and *POU1F1*. As a major participant in control of several physiologic processes including growth and metabolism, *GH1* also known as the pituitary growth hormone, is a member of a family of hormones that are secreted in the anterior pituitary gland including the growth hormone of which the primary function is to stimulate growth in all tissues of the body including bones. Village goats that were raised intensively showed a growth performance improvement under improved management systems which explains why this gene was more expressed in intensively raised as compared to extensively raised populations. The *GH1* gene was reported to be highly polymorphic within village populations and was suggested as a candidate gene for breed selection and improvement programs (Ncube *et al*., 2016).

The effects of growth hormone are primarily mediated by the *IGF1* gene, which stimulates body growth and has growth-promoting effects in almost every cell of the body, such as skeletal muscle, bone, etc (Decourtye *et al*., 2017). The growth hormone is produced in the pituitary gland then released into the bloodstream where it then stimulates the liver to produce *IGF1*. Expression in the extensively raised village populations was extremely low suggesting that its function of mediating growth hormone effects that promote growth in animals is inhibited, therefore leading to poor growth performance. Another reason for low expression may be that, though it is stimulated by the growth hormone from the pituitary gland, it is primarily produced in the liver. On the other hand, relatively high expression in the intensively raised populations further proves that improved management systems lead to improved growth performance.

The *POU1F1* was expressed almost at the same levels both within and between populations. It activates growth hormone and prolactin genes responsible for pituitary development and hormone expression in mammals and is a member of the POU family of transcription factors that regulate mammalian development (Alakilli *et al*., 2012).

Overall, there were more up-regulated genes reported in the extensively raised goats, which might be due to the extreme and diverse environmental conditions experienced by animals in the village farming community (Masika & Mafu, 2004; Webb *et al*., 2005). These goats have adapted to these harsh environmental and climatic conditions, can use poor quality feed as well as resist diseases and parasites (Webb & Mamabolo, 2004). The expression and up-regulation of a wide profile of genes may reflect the genetic coping mechanisms aiding the village goats to adapt to these conditions.

Between breed differences were investigated by comparing the Boer to village goats raised under the same production system. The study observed that there were more genes expressed in the Boer goats than in the village goat population. The Boer is a well-developed breed that was developed from the indigenous goat populations as a meat breed (Mdladla *et al*., 2017). This breed was reported as a fast-growing and high performing breed (NAMC 2005), the high expression of genes in the Boer compared to village populations may be due to the fact that village populations have not been selected for high growth and meat performance.

The gene ontology (GO) analysis in this study revealed DEGs that play a role in cell components such as growth factor and insulin growth factor-binding protein complex in the extensive production system. Biological processes affected included processes such as cell growth, developmental and organ growth, negative regulation of developmental process, growth, regulation of growth, anatomical structure development, developmental process, anatomical structure morphogenesis and anatomical structure formation involved in morphogenesis which were observed in both the extensive and intensive production systems. The p53 signalling pathway observed within-breeds raised under different production systems is induced by several stress signals, including SNA damage and oxidative stress (Feng *et al*., 2007) which are expected in extensively raised populations. Two genes, *insulin like growth factor-binding protein 3* (I*GFBP3*) and *ribonucleic reductase regulatory TP53 inducible subunit M2B* (*RRM2B*) were enriched in the p53 signalling pathway, and these were upregulated in the extensively raised populations. The *IGFBP3* gene plays an essential role in the regulation of postnatal somatic growth and as well as the stimulation of anabolic processes (Ramesha *et al*., 2015). The *IGFBP3* is one of the proteins that bind to insulin growth factors (IGF) which play a vital role in the regulation of cell proliferation and apoptosis (Ramesha *et al*., 2015). Upregulation of *IGFBP3* would lead to cell apoptosis, therefore, causing reduced growth performance in animals that are raised in harsh conditions.

5.6 Conclusions

In summary, this study was able to employ RNA-Seq technology in the identification of growth-related genes in the pituitary gland of South African goat populations raised under different production systems. Differentially expressed genes observed within-breeds raised under different production systems show that management practices and environmental conditions are some of the factors that play a role in growth performance through different genetic and molecular mechanisms. Growth related genes identified in the intensively raised village goat populations suggest that the intensive production system is significant in growth performance. Further suggesting that, improved management systems lead to better growth performance. Growth-related genes such as the *CHKB*, *POU3F4*, *BAG4* and *IGF1* show probable potential as biomarkers for developing selection and breeding programs. Understanding the molecular mechanisms that contribute to growth and growth performance will have a significant impact on breeding and management strategies.

CHAPTER 6: TRANSCRIPTOME ANALYSIS OF MUSCLE DEVELOPMENT FROM THE LONGISSMUS MUSCLE OF THE SOUTH AFRICAN BOER AND VILLAGE GOAT POPULATIONS

6.1 Abstract

Growth performance and meat quality are essential traits in the chevon industry. Goat meat is a good source of high-quality protein and an important nutrient source for poor communities in South Africa. This study aims to use high-throughput RNA sequencing of the *longissimus* muscle tissues to help understand the genomic basis of differences in growth and meat quality traits of uncharacterized village goats compared with SA Boer goats raised under different farming systems. A total of 36 goat kids were sampled at twelve weeks of age from two typical production systems representing the commercial Boer (*n*=14) and the village goat (*n*=28) production systems. Total RNA isolated from the *longissimus* muscle tissue of 36 weeks old animals (*n=9*) was sequenced individually in triplicate and approximately 62 991 716 trimmed reads, and quality-controlled reads were mapped to the goat genome (*Capra_hircus.ARS1.94*) using HiSat2 software. Transcript assembly and quantification from RNA sequence yielded 12 differentially expressed genes for the extensive system and 9 for the intensive production system at the false discovery rate (FRD) of ≤0.05 and a fold change of ≥2 which were retained for downstream analysis. Genes such as *MIR214, BMP4, PDK4* and *KLF15* were highly expressed within populations raised under different production systems. For between breeds raised under similar production systems muscle-development related genes such as *COX2, ND3, ANAPC4, IGFBP3, MSTN, SUCO,* and *LHCGR* genes were highly expressed. Data from this study revealed genes and molecular mechanisms involved in muscle development and these have the potential to be used in breed improvement programs.

Keywords *Boer goats; differentially expressed genes; growth; unimproved goats; RNA-seq.*

6.2 Introduction

The primary role of goats is meat provision, and goat meat is an easily accessible source of protein to rural communities (Ncube *et al*., 2016). However, poor growth performance and low carcass yields are a challenge in the chevon industry particularly in rural farming systems due to inefficient feeding and management, disease constrains, lack of characterization and inadequate exploitation of genetic resources (Webb, 2014). Low chevon production has posed as a significant concern in South Africa amongst other developing countries as the majority (>60%) of the goats are reared in rural areas (Ncube *et al*., 2019). Coupled to low productivity, chevon remains the least preferred among consumers, mainly due to unfavourable perceptions relating to meat tenderness and odour (Pophiwa *et al*., 2016). The production of lean and high quality meat are desirable traits both to the consumer and the producer (Fiems, 2012). There have, therefore, been efforts made to improve carcass quality in livestock focusing on production of lean meat and high meat yield (Fiems, 2012). However, with the majority of goats being kept by communal farmers in rural areas, poor growth performance which subsequently leads to low carcass yields, still remains a challenge in the chevon industry. The African continent experiences a high demand of goat meat, which is unmatched by the existing goat populations. Reasons for this disparity include low growth potential, and carcass quality attributed to both genetic and environmental factors (Webb, 2014). Several studies have demonstrated genetic diversity and population divergence of South African goat breeds and populations (Mdladla *et al*., 2016; Ncube *et al*., 2019).

Ncube *et al*. (2019) in chapter 3 analysed the growth profiles of improved and village goat populations of South Africa and demonstrated that the Boer has superior growth genetics with an average daily weight gain of 0.13±0.01 kg compared to other South African indigenous goats. This study further revealed a higher daily gain in the intensively raised (0.09±0.01) than the extensively raised goats (0.07±0.01). The intensively raised village goats had a better growth performance (19.55±0.36 kg) than those that were raised extensively (17.55±0.37 kg) suggesting that improved management systems lead to improved growth genetics. In a follow up study in chapter 4, the Boer was reported to have better and high carcass characteristics compared to the rest of the populations which was expected based on its development as a meat breed.

Gene expression is affected by breed and the environmental conditions in which it is raised, and has been used to investigate genes and molecular mechanisms influencing traits of interest in domestic livestock species (Song *et al.*, 2019). Muscle transcriptomic studies will therefore shed light on muscle growth and development, as well as the genetics that influence this growth and development. Transcriptomic analysis has been widely used in livestock to study important traits such as milk production where gene expression in dairy cattle revealed key candidate genes for milk production such as *STAT5A* gene that is a key mediator in prolactin signalling which plays a role in lactogenesis (Yang *et al*., 2016). Studies on Jinghai yellow chickens have identified genes (*MSTN* and *PRKAG3*) in slow-growing populations of low meat quality chickens (Wu *et al*., 2018). Bhat *et al*. (2019) observed differential expression of genes involved in melanin production enzymes in black and brown goats. Transcriptomic analysis in Pashmina goats showed a high expression of *TYR* , *TYR1* and *TYR2* genes which code for enzymes involved in melanogenesis to be associated with coat colour phenotypes (Bhat *et al*., 2019). Chapter 5 of the present study observed differential gene expression in the pituitary gland of South African goats associated with differences in growth and meat quality traits. Growth related genes such as *GH1* and *IGF1* were highly expressed in intensively raised goat populations while genes such as *FGFR2* and *SMPX* were highly expressed in the Boer compared to the village populations raised under similar production conditions.

Muscle development and growth are complicated processes encompassing muscle cells as well as the expansion of the extracellular matrix to provide support as well as the blood vessels to provide the oxygen and energy required (Guo *et al*., 2015). The overall livestock body weight consists of 35 to 60 % muscle mass which exhibits a broad range of shapes, sizes, anatomical locations and physiological functions (Listrat *et al*., 2016). The *longissimus* muscle consists of a higher proportion of the carcass value (Kim *et al*., 2019) or presents a good target to identify genes that are associated with muscle growth and development. By analysing the transcriptome of the *longissimus* muscle of Debao and landrace pigs, Song *et al*. (2019) identified genes that played key roles in the metabolic signalling pathways and associated with growth and muscle development. Linde (2018) observed high expression of *insulin-like growth factor 2* (IGF2) in the longissimus muscle of the Bonsmara cattle that were fed high energy diet versus those on a low energy diet. Transcriptome analysis of the *longissimus* muscle of Shaanbei cashmere goats revealed some key pathways such as the skeletal muscle growth and fatty acid metabolism to be enhanced after the knockout of the myostatin (*MSTN*) gene, which is a negative growth modulator. As a follow up to the characterisation of growth and carcass characteristics of South African goat populations, the present study sought to investigate differential gene expression and genetic mechanisms associated with muscle development and carcass quality) within breeds, across production systems and ii) between breeds within the same production systems. In so doing, the study also sought to identify genes that are associated with muscle development and growth that could potentially act as selection targets in goat breeding and improvement programs.

6.3 Materials and methods

6.3.1 Animals and management

Two separate differential gene expression experiments were designed to study the impact of (i) production systems within a breed and (ii) breed differences within a production system in the South African goat populations. In order to investigate the effects of production systems, goats were sampled from non-descript village populations and divided into two subgroups one of which was raised under extensive production system while the second group was reared under intensive methods. The experiment to investigate the effect of breed used Boer goats and non-descript village goats that were both raised under the same intensive production system. The growth trial to investigate the growth profiles and carcass quality of the goats used in these two experiments was performed from October 2016 to March 2017 and reported by Ncube *et al.*, (2019) in chapter 3. Carcass quality traits of goats from these two experiments were evaluated and reported in chapter 4.

6.3.1.1 Experiment 1: Within breed across production system analysis

South African village goats (*n*=28) were sampled from 14 farms in Pella village, North West Province of South Africa. These 28 animals were split into two groups of (i) the extensive system (VTE) (*n* = 14) and (ii) the intensive system (VTI) (*n* =14), The VTE village goats were purchased and raised in their respective village farms under extensive communal farming conditions in Pella. Goats kept at Pella village farms were penned at night and left to browse during the day with no supplementation provided in an arid environment characterised by low rainfall, little and poor quality pasture (Holmgren et al., 2006; Mdladla et al., 2017).

The (VTI) were transferred to the Agricultural Research Council, Animal Production (ARC-AP), Small Stocks Unit, where they were raised under controlled feed and management conditions. They were kept in a grazing camp on management diet of game pellets (110g/kg, 25-70 g/kg crude fat, 110-200 g/kg crude fibre, 6-10 g/kg calcium, 2.5 g/kg phosphorus and 3.68 % non-protein nitrogen) provided at 3 % of live weight /animal /day. Lucerne hay and clean water were available *ad libitum*.

6.3.1.2 Experiment 2: Across breeds and within production system analysis

This experiment consisted of the Boer goats **(***n*=14**)** purchased from IVG and the South African uncharacterized village goats **(***n*=14**)** procured from Pella (VTI) that were both raised intensively at the Agricultural Research Council, Animal Production (ARC-AP) under controlled feed and management conditions as described above and in detail in chapter 3 of this study (Ncube *et al.*, 2019).

6.3.2 Slaughter procedure, sample collection and carcass quality measurements

Growth profiles of the South African goat populations were analysed during a growth trial performed from October 2016 to March 2019 and are described in detail in Ncube et al., (2019). In summary, the highest live weight (LW) was observed in the SAB population (28.96±0.30 kg) as shown in Appendix A-C. The Pella population raised at the research farm had a slightly higher LW mean (19.55±0.36 kg) as compared to the population that was raised extensively at Pella village (17.55±0.37 kg).

The mean body length (BL) ranged from 47.01±0.36 cm to 58.11±0.3 cm, with the SAB having the longest body 58.11±0.33 cm as compared to other populations. All the populations had around the same withers height ranging from 51.14±0.37 cm to 58.48±0.35 cm. The SAB had larger chest girths (CG) 67.81±0.43 cm while the VTI and VTE had slightly similar proportions (61.04±0.51 cm and 61.42±0.52 cm), respectively.

Chapter 4 described carcass characteristics of these South African goat populations under this experimental design. The VTE goats had the lowest pre-slaughter weight of 22.0±4.6 kg when compared to the VTI, with an average of 24.2±2.8 kg. The SAB had the heaviest warm carcass weight (WCW) of 17.2±2.3 kg while the VTE and the VTI had similar WCW proportions of

9.1± 2.0 kg and 9.9±1.1 kg, respectively. The SAB cold carcass weight (CCW) was the highest while the VTI had a higher CCW than the VTE (9.2 ±1.2 kg and 8.4± 1.9 kg) respectively.

The SAB had higher fat code (FC) of 1.3±0.5 compared to the rest of the populations. The VTI had a higher FC (0.4±0.3) than the VTE (0.3±0.0). The SAB produced the highest dressing percentage (DP) 41.9±2.4 %) followed by the VTI (41.2±2.6 %) and the VTE (40.8±1.7 %). The chilling loss (CL) was lower in the SAB (5.5^b± 1.7) population followed by the VTI (6.6±2.1 %) while VTE was the highest (7.8±2.7 %).

6.3.3 Tissue sampling

Animals were humanely slaughtered at an average age of 36 weeks with the average live slaughter weight of 41.17 kg, 28.4 kg and 23.19 kg for the SA Boer, village population raised at ARC-API and village population at the communal farms, respectively. RNA samples were collected immediately after slaughter from the *longissimus dorsi* muscle on the left side region of the 11th and 13th rib of each animal and immediately stored in the Qiagen RNA later tubes containing RNA Stabilization reagent (www.qiagen.com) to stabilize the RNA and prevent degradation and subsequently kept at -80°C. Three animals per group i.e VTI; VTE and SAB were selected as described in Chapter 5.

6.3.4 RNA isolation and quantification

RNA isolation from 150 mg of the *longissimus* tissue was performed using the Qiagen RNeasy and Qiagen RNA Universal Midi kit for purification of total RNA following the manufacturer's instructions with slight modifications. The modifications included using 150 mg of tissue sample and homogenization was performed on the 2010 Geno/Grinder® - Automated Tissue Homogenizer and Cell Lyser. RNA was eluted using 100 µl of RNA-free water. The quality of the RNA template was investigated electrophoretically on 1.8% agarose gel with ethidium bromide of 0.5 µg/mL at 80V for 30min. The gel was examined under UV light for RNA degradation in a BIORAD Imaging System (BIORAD GelDoc XR) (www.bio-rad.com). The samples were submitted to the Agricultural Research Council, Biotechnology Platform (ARC-BTP) Core Facility for sequencing.

6.3.5 RNA sequencing

The cDNA libraries were prepared using an Illumina TruSeq Stranded Total RNA Ribo-Zero H/M/R Gold library prep kit (Illumina Inc, San Diego, CA) preparation. TruSeq universal adapters were ligated to the cDNA fragments, and PCR was performed to produce the final sequencing libraries. RNA was fragmented and randomly primed for reverse transcription to generate double-stranded cDNA fragments. Gel electrophoresis was used to assess the quality of the starting material. The cDNA was colligated and nebulized and then fragmented after which, adapters were ligated to both ends of the fragmented nucleic acid. The fragments were hybridized to a flow cell, which extended a hybridized template or performed bridge amplification. Sequencing was then performed using Illumina HiSeq 2500. All cDNA samples for each animal were sequenced and examined in three technical replicates.

6.3.6 Data analysis

6.3.6.1 RNA sequence trimming and quality control

Quality Control (QC) was performed on raw sequencing reads using Trimmomatic v0.36 (Bolger et al., 2014). Sequence trimming included the removal of the sequencing adapters, short reads as well as reads containing over 10 % unknown bases (N), resulting in clean reads longer than 30 bp.

6.3.6.2 Sequence read alignment, assembly and, quantification

Reads that remained after QC were aligned and mapped to the goat genome (*Capra_hircus.ARS1.94)* from ENSEMBL (https://www.ensembl.org/Capra_hircus/) using HISAT2 v2.1.0 (Pertea *et al*., 2016). Genome indexing was performed with a maximum number of multiple hits set to 20 and non-mismatches at splice sites. Mapped reads were then sorted and converted to BAM files using SAMtools v1.3.3.

Transcript assembly and quantification from RNA sequence reads was performed with StringTie. Differential gene expression analysis was performed using Ballgown 3.8 and DESeq2 1.18.0 packages within R 3.5.2 using a linear statistical model. Filtering was performed to remove low abundance genes as well as to identify genes with statistical significance between groups under study. Downstream processing after filtering was performed on 6 samples of the VTE (n=3) and VTI (n=3) village goats to compare between extensive and intensive production systems. Processing was also conducted between 6 samples of SAB (n=3) and VTI (n=3) to compare breeds under similar intensive production system. *P*-values were adjusted using Benjamini and Hochberg's approach for false discovery rate (FDR) control. Differentially expressed genes are those that had the adjusted *P*-value of ≤ 0.05.

6.3.7 Differential gene expression

For analysis of differentially expressed genes between experimental groups, the DESeq2 R package (Bioconductor, Buffalo, USA) was used with R v3.5.2. Genes with an FDR ≤ 0.05 and a log2 fold change of ≥ 2 and adjusted *P*-value ≤ 0.05 were considered to be differentially expressed genes, and a volcano plot for highly significant genes was generated.

6.3.8 Functional annotations and pathway analysis of differentially expressed genes

To gain more insight into the biological processes and implications of the differentially expressed genes (DEGs), gene ontology (GO) annotation and gene enrichment analysis were performed. GO analysis of DEGs was performed using the DAVID v6.7 online server (http://david.abcc.ncifcrf.gov/; Huang *et al*., 2009). The gene set enrichment of specific GO terms was determined based on Fisher's exact test ($p<0.1$). DEGs were further mapped into the KEGG pathway database using KEGG mapper within the Kyoto Encyclopedia of Genes and Genomes databases (KEGG, http://www.genome.jp/kegg; to predict the significantly enriched pathways. The DEGs of the groups under study were classified into cellular component, biological process and, molecular functions. Bar graphs showing differences and similarities between GO terms were plotted using WEGO v2.0 web-based tool (Ye *et al*., 2018).

6.4 Results

6.4.1 RNA sequencing and read mapping

About 62 991 716 reads were generated from 9 samples of the South African goat populations sequenced in 3 biological replicates, and approximately 87 % of the trimmed and quality-controlled reads were mapped to the goat reference genome (*Capra_hircus.ARS1.94)*.

6.4.2 Differential gene expression

6.4.2.1 Differential gene expression (DGE) for within breeds and across production system experiment

Genes with FDR ≤ 0.05, log2 fold change of ≥ 2 and an adjusted *P*-value ≤ 0.05 were considered as differentially expressed. A total of 32 191 genes were expressed in 6 samples of extensively (VTE and intensively (VTI) raised goats, and 24 849 were retained after filtering. The distribution of the differentially expressed genes is shown in a volcano plot (Fig 6.1a) in which the top 20 highly significant genes were labelled.

Of the 24 849 expressed genes, 12 genes were differentially expressed, 9 of which were upregulated, and 3 were downregulated (Appendix K). There were no overlapping genes between the two production systems.

6.4.2.2 Differential gene expression (DGE) for the across breeds and within production system experiment

The comparison between Boer and village breeds in a similar production system revealed 32 919 expressed genes in 6 samples of Boer and village goats, of which 24 148 were retained post-filtering. Differentially expressed gene distribution is shown in a volcano plot (Fig 6.1b) in which the top 10 highly significant genes were labelled.

Nine genes were differentially expressed from which 8 were upregulated, and 1 was downregulated (Appendix L) as shown in Figure 6.1b. There were no overlapping genes between the two breeds.

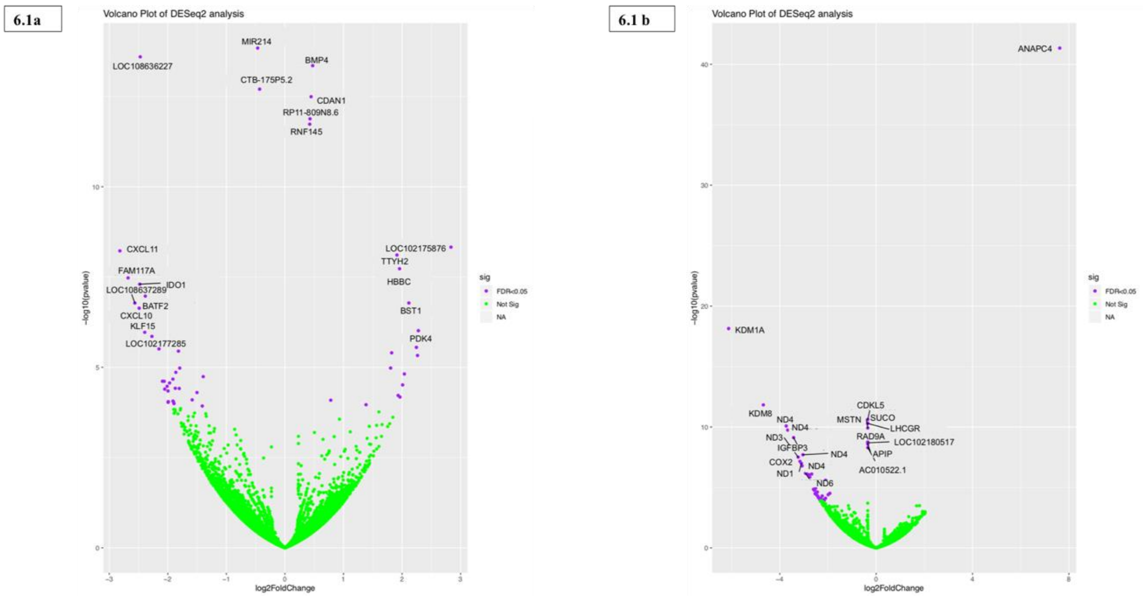

Figure 6.1. Volcano plot of differentially expressed genes a) within populations raised in different production systems and b) between populations raised in similar production system. Highly expressed genes are shown in purple.

6.4.3 Functional annotation of differentially expressed genes

Gene ontology (GO) annotation was performed using Fisher's exact test and filtering using multiple correlation controls for false discovery rate (FDR) ≤ 0.05. The GO terms were associated with differentially expressed genes in both experiments (within and between populations), as shown in Figure 6.2a-b. Genes differentially expressed between village goats extensively raised (VTE) versus intensively raised (VTI), were within the cellular component (CC), biological process (BP) and molecular function (MF) functional groups (Fig 6.2a). Some of the differentially expressed genes (DEGs) that play a role in the cellular component such as the cluster of actin-based cell projections, non-membrane-bounded organelle and contractile fibre were observed only in the extensively raised goats. Tissue remodelling and multicellular organism response to stress biological processes were observed in the intensively raised village populations. GO analysis revealed DEGs that play a role in the biological processes such as regulation of the developmental process, multicellular organism development, developmental process, anatomical structure development and positive regulation of developmental process in both the VTE and VTI goats. There were no significantly enriched GOs.

Functional annotation analysis of differentially expressed genes between Boer versus village goats raised under the intensive production system resulted in cellular components (CC), biological processes (BP) and molecular functions (MF) (Fig 6.2b). The GO analysis revealed DEGs that play a role in the biological processes such as the regulation of growth, cell growth and growth in both the Boer and Village goats. There were no significantly enriched GOs.

6.2a

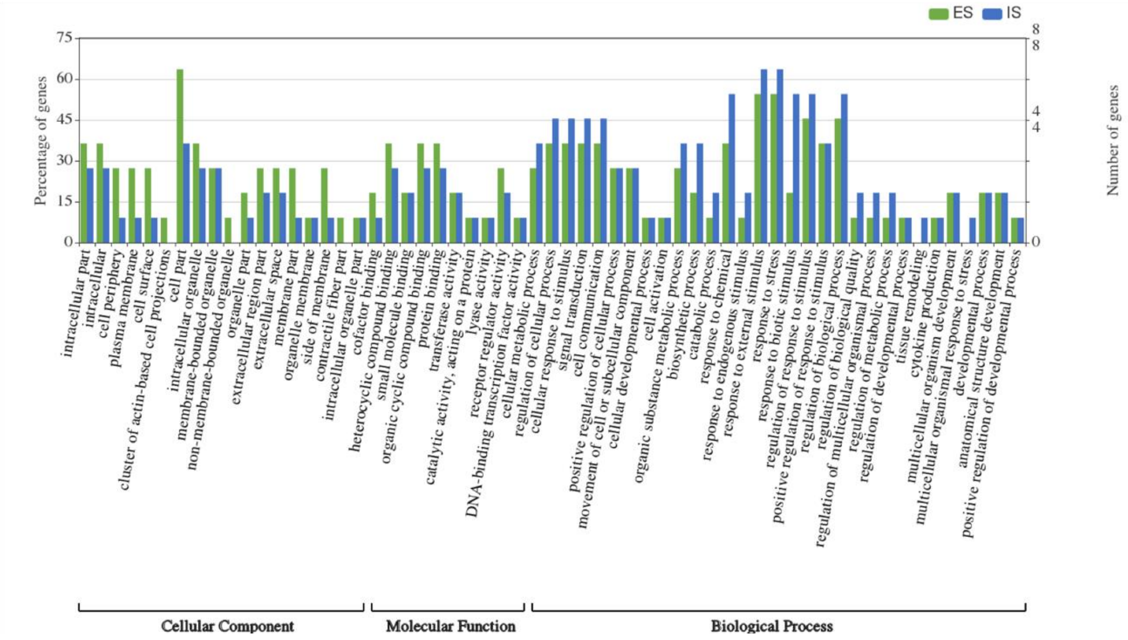

Figure 6.2a Gene ontology (GO) of differentially expressed genes in the village goats raised in the extensive system as well as those raised in the intensive system. The right y-axis indicates the number of genes in a category. The left y-axis indicates the percentage of a specific category of genes in that main category. One gene could be annotated into more than one GO term.

6.2b

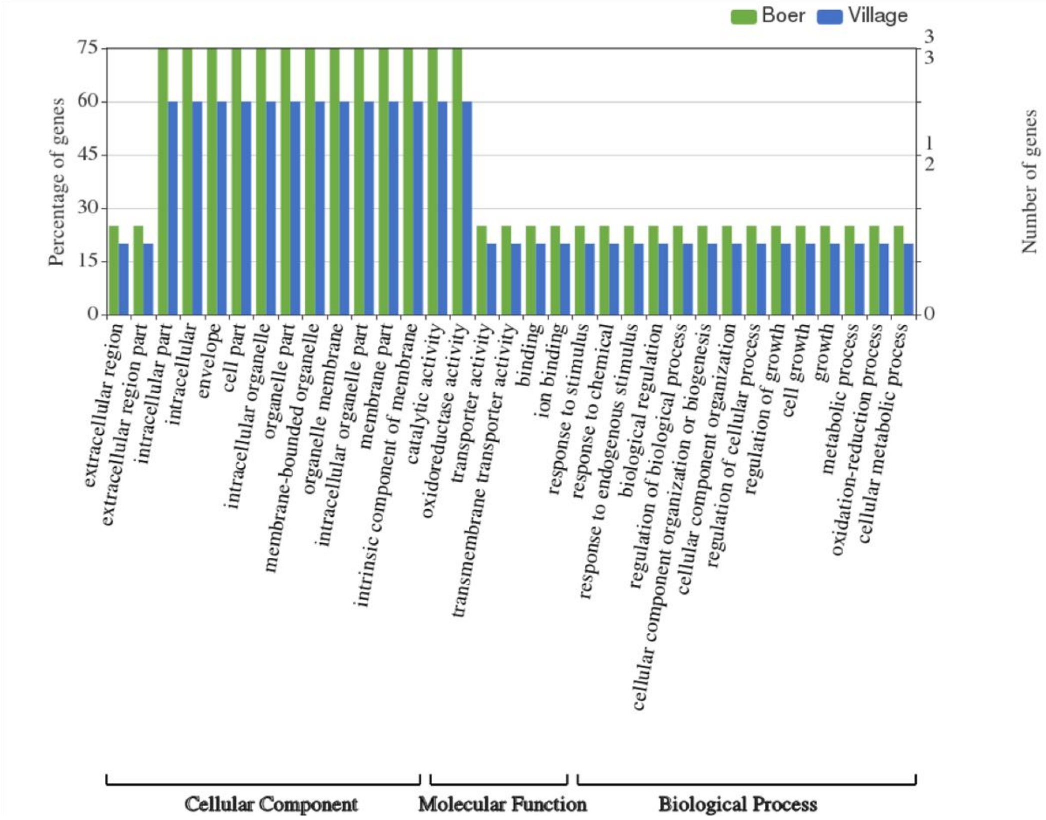

Figure 6.2b Gene ontology (GO) of differentially expressed genes in Boer and village goats raised under the intensive system. The right y-axis indicates the number of genes in a category. The left y-axis indicates the percentage of a specific category of genes in that main category. One gene could be annotated into more than one GO term.

6.4.4 Pathway analysis

To understand the biological pathways that are involved between and within-breeds raised under different production systems, the KEGG pathway enrichment analysis was performed using the DAVID 6.7 Functional Annotation Tool (http://david.abcc.ncifcrf.gov/) (Table 6.1 and 6.2). The top three enriched biological pathways for DEGs within-breeds (VTE vs VTI) were the Toll-like receptor signalling, chemokine signalling, and cytokine-cytokine receptor interaction pathways (Table 6.1).

Table 6.1 Pathway analysis of differential gene expression within breeds raised under different production systems (ES vs IS)

Pathway	**Gene Symbol**	**Gene name**	**Fold enrichment**	**Benjamini**	***P* value**
Toll-like receptor signalling	CXCL9; CXCL10: CXCL11	C-X-C Motif Chemokine Ligand 9 , C-X-C motif chemokine 10, C-X-C motif chemokine 11	35.34	0.03	0.002
Chemokine signalling	CXCL9; CXCL10: CXCL11	C-X-C Motif Chemokine Ligand 9, C-X-C motif chemokine 10, C-X-C motif chemokine 11	20.37	0.04	0.005
Cytokine-cytokine receptor interaction	IGFBP3; RRM2B	Insulin like growth factor binding protein 3; Ribonucleotide-diphosphate reductase subunit M2 B	17.17	0.03	0.007

6.5 Discussion

Growth, muscle mass and development rate are some of the crucial factors in livestock production (Guo *et al*., 2015). Producers have a preference of fast growing animals against slow growing animals associated with low carcass yields. South Africa consists of three registered meat breeds that includes the Boer, Savannah and Kalahari Red (Dzomba *et al*., 2018; Ncube et al., 2016). In addition are the uncharacterized village goats that are raised under poor, marginalized communal production systems as described in chapter 3 (Ncube *et al*., 2019). Previous studies have demonstrated differences in the genetic architecture and associated growth characteristics and carcass quality between the improved breeds of the Boer goat and non-descript village ecotypes (Mdladla *et al*., 2017, 2018; Ncube *et al*., 2019). This study aimed to investigate genes that are associated with growth and carcass quality and their differential expression between breeds and different production systems by comparing the Boer goat with high growth rates (Ncube *et al*., 2019) and high carcass weights (chapter 5) to the village ecotypes that performed lowly in those traits. The study targeted the *longissimus* muscle as it consists of a higher proportion of the carcass value (Kim *et al*., 2019), therefore making it a good target for identifying genes that are associated with growth and muscle development. Based on previous studies and earlier analysis (chapter 3 to chapter 5), the present study hypothesized that the muscle transcriptome is affected by both breed type and the environment in which the animals are raised.

A high number of genes were expressed within populations raised under different production systems with only 12 differentially expressed genes and no overlaps observed. Lawhorn *et al*. (2018) described gene overlap in transcriptomics as a tool to be used to make inferences on similarities between biological processes and that an independent genetic architecture leads to less overlap between the two groups under study. In the present study, the village goats were raised under different production systems and the absence of overlap imply that different production systems triggers different and unique coping mechanisms as described by Lawhorn *et al*. (2018). Genes *MIR214, BMP4, PDK4,* and *KLF15* that play a crucial role in growth and muscle development were differentially expressed between the village goats raised extensively versus those raised intensively. The microRNA 214 (*MIR214*) is part of the microRNA family that are shorth regulatory RNAs that play a role in the post-transcriptional regulation of gene expression in multicellular organisms by affecting the stability and translation of mRNAs as well as the control of physiological processes such as growth and progression (Shivdazani, 2006). This gene has been associated with growth and pre- and postnatal development of skeletal muscles in sheep (Liu *et al*., 2019). Chapter 3 of this study demonstrated that, village goat populations that were raised in the intensive production system had better growth performance compared to those in the extensive system. The high expression of this gene in the experiment and it's up-regulation in the intensive system implies that it is only activated under improved production conditions that allow village goats to attain improved growth rates and carcass characteristics. Village goats raised under poor nutrition in the communal farms (VTE) are underfed due to the harsh conditions and feed scarcity. During skeletal muscle development some miRNAs are specifically enriched in skeletal muscle cells (Ju *et al*., 2015). The *MIR214* which plays a role in physiological processes such as growth was downregulated in the VTE population. This population is raised under extensive conditions with insufficient nutrient supply, therefore, the downregulation suggests that this gene is not enriched in the skeletal muscle therefore leading to poor growth performance and carcass yields. Bone morphogenic protein 4 (*BMP4*) is a polypeptide that belongs to the TGF-β superfamily of proteins which are involved in the development of bones and cartilage and fracture repair (Wang *et al*., 2014). It controls the terminal differentiation into mature white rather than brown fatty cells and has been associated with marketing weight, post-weaning daily gain, height at hips, body mass index, thigh circumference and skeletal muscle index in Bhaki lambs (Ibrahim, 2019). The pyruvate dehydrogenase kinase 4 (*PDK4*) which was upregulated in the intensive system and downregulated in the extensive system plays a role in the decrease of metabolism and conservation of glucose by decreasing its conversion to acetyl-CoA, which enters the citric acid cycle and is converted to ATP. It regulates fatty acid oxidation and de novo fatty acid biosynthesis as well as the protection of detached epithelial cells against anoikis which is a form of programmed cell death. Its downregulation in the extensively raised populations suggests that epithelial cells are not protected against anoikis, and this may lead to poor growth performance and low carcass yields. ATP captures chemical energy from the breakdown of food, in the extensive production system, goats walk long distances scavenging for food so therefore requiring extra energy to move around to fend for food. The upregulation of *PDK4* in the intensive system prevents anoikis therefore leading to muscle growth and improved growth performance. It's role in increase of fat synthesis corresponds with chapter 4 of the present study, where intensively raised village populations had a higher fat code than those raised extensively. This suggest that, in the intensive system energy gets converted to fat. The

Krüppel-like zinc finger factor 15 (*KLF15*) is a direct target of the glucocorticoid receptor in skeletal muscle, which is a gene regulator and controls development, metabolism and immune system (Lyu *et al*., 2014). SNPs in this gene have been associated with chicken growth and carcass traits (Lyu *et al*., 2014). This gene was highly expressed and up-regulated in the intensive system which implies that, under improved management and nutrition conditions this gene is activated therefore leading to improved growth and skeletal muscle development. This further suggests its potential as a candidate gene for growth and carcass traits.

The present study also observed the upregulation of immune response genes (*CXCL9, CXCL10, CXCL11* and *BATF2*) in the extensively raised goat populations. The extensive production system is characterized by poor management and goats are raised under harsh conditions faced with disease prevalence among other challenges (Mdladla *et al*., 2017; Mohlatlole *et al*., 2015; Ncube, 2016). C-X-C Motif Chemokine Ligand 9 (*CXCL9*) induces chemotaxis and promotes differentiation and multiplication of leukocytes while the C-X-C Motif Chemokine Ligand 10 (*CXCL10*) on the other hand mediates immune response by binding to CXCR3 through the activation and recruitment of leukocytes such as T cells, eosinophils, monocytes and NK cells (Tokunaga *et al*., 2019). The C-X-C Motif Chemokine Ligand 11 (*CXCL11*), on the other hand, regulates cell trafficking of various types of leukocytes and plays a role in the development, homeostasis and function of the immune system (Tokunaga *et al*., 2019). Basic leucine zipper ATF-like transcription factor 2 plays a significant role in the control of differentiation of lineage-specific cells in the immune system. The high expression and upregulation of these genes in the extensively raised populations indicate the stress these animals are exposed to as well as their response mechanisms. Disease tolerance and adaptation to harsh conditions has been reported in extensively raised village populations (Mdladla *et al*., 2018). The identification of these genes, as well as those related to growth and muscle development by this study, may facilitate genomic based approaches for improving growth performance and disease tolerance/resistance in village goats.

The between breed experiment had similar proportions of expressed genes as the within breed experiment with only 9 differentially expressed genes and no observed overlap. As with the between production system analysis, the absence of overlap implies unique genetic mechanisms. Differentially expressed genes *COX2, ND3, ANAPC4, IGFBP3, MSTN, SUCO,* and *LHCGR* were observed between Boer and Village goats raised under intensive production system. Cyclooxygenase-2 (*COX2*) produces PGE, which is an inhibitor of apoptosis which is cell death that occurs in multicellular animals, promotes cell proliferation and plays a role in the skeletal muscle pathway (Tokunaga *et al.*, 2019). Its upregulation in the Boer population implies that apoptosis is inhibited therefore cell growth is encouraged leading to increased cell growth and muscle development. This is expected in a breed developed for high growth performance and meat yield. The downregulation in the village population therefore leads to apoptosis linked to poor muscle development and growth, explaining the poor growth profiles and carcass quality of village goats. The (*ND3*) NADK dehydrogenase subunit 3 plays an essential role in protein synthesis and fulfilling the energy requirements of prenatal skeletal muscle development (Yang *et al.*, 2016). This gene was downregulated in the high performing Boer and upregulated in the slow performing village populations. The village goats are normally raised under harsh production systems where they will require high energy to scavenge for feed and water and fight diseases and parasites (Ncube *et al.*, 2019). An upregulation of *ND3* might therefore be a reflection of the inherent preferential channelling of feed to energy versus muscle development in village goats emanating from the production systems under which they are normally raised. Anaphase promoting complex subunit 4 (*ANAPC4*) is a gene that promotes metaphase-anaphase transition by ubiquitinating its specific substrates such as mitotic cyclins and anaphase inhibitor. It plays a role in controlling progression through mitosis and the G1 phase of the cell cycle. This gene was highly expressed and upregulated in the extensively raised populations. The insulin like growth factor binding protein 3 (*IGFBP3*) is a protein that plays a role in the regulation of cell proliferation and apoptosis by binding to insulin growth factors, and it also plays an essential role in the regulation of postnatal somatic growth and the stimulation of anabolic processes (Ramesha *et al.*, 2015). It was upregulated in the Boer in the present study; and SNPs in this gene have been associated with body weight gains and body circumference in Markhoz goats (Rasouli *et al* , 2016) and has been used as a candidate gene for meat production (Priyadi *et al.*, 2017). The myostatin (*MSTN*) gene gives instructions for making the myostatin protein which is a part of the transforming growth factor beta family that helps control the growth and development of tissues throughout the body. It plays a significant role in muscle growth and development and has a potential to be used as a candidate gene for animal muscular growth (Ahad *et al.*, 2017). *MSTN* mutations have been reported to give rise to double muscling in cattle and improvement of average daily gain in swine (Alakilli *et al.*, 2012). Studies have shown that the silencing or gene knockout of *MSTN* leads to improved growth (Wang *et al.*, 2017). It was therefore expected that this gene would be downregulated in the Boer because it produces high meat yield and it has excellent growth performance. The village goats had poor growth performance as compared to the Boer which can be explained by the upregulation of the *MSTN* gene. The SUN domain containing ossification factor (*SUCO*) is a gene that is needed for bone modelling during late embryogenesis and regulates type I collagen synthesis in osteoblasts during their postnatal maturation (Sohaskey *et al*., 2010) while the luteinizing hormone/choriogonadotropin receptor (*LHCGR*) has been associated with body weight at first egg and age at first egg in chickens (Li *et al*., 2013). These were both upregulated in the Boer goats that are associated with good growth performance and muscle development. As expected, the two genes were downregulated in the village populations that are generally low performing populations.

Pathways that were highly enriched within populations raised under different production systems included the toll-like receptor signalling, chemokine signalling and cytokine-cytokine receptor interaction pathways. The toll-like receptor signalling is divided into the dependent and independent pathways and play a critical role in innate immunity. Toll-like receptors are expressed on antigen-presenting cells participating in innate immune responses (Zhenzhen et al., 2014). Three genes that were enriched in this pathway are the *CXCL9*, *CXCL10*, and *CXCL11* which play a significant role in the immune system and were all highly expressed and upregulated in the extensively raised populations reflective of the exposure of village goats to diseases and stressful conditions triggering immune response mechanisms. The cytokine-cytokine pathway on the other hand is where cytokines are released by various cells in the body usually in response to an activating stimulus (Tokunaga *et al*., 2019). The *Insulin like growth factor binding protein 3* (*IGFBP3*) and *Ribonucleotide-diphosphate reductase subunit M2 B* (*RRM2B*) which were upregulated in the extensively raised population were enriched in this pathway. The *IGFBP3* gene plays an essential role in the regulation of postnatal somatic growth and as well as the stimulation of anabolic processes (Ramesha *et al*., 2015). The *IGFBP3* is one of the proteins that bind to insulin growth factors (IGF) which play a vital role in the regulation of cell proliferation and apoptosis (Ramesha *et al*., 2015). Up-regulation of *IGFBP3* would lead to cell apoptosis, therefore, causing reduced growth performance in animals that are raised in harsh conditions.

The oxidative phosphorylation was the only pathway that was enriched between breeds that were raised under the same production system. This is a metabolic pathway where cells use enzymes to oxidize nutrients, thereby releasing the chemical energy of molecular oxygen, and this takes place in the mitochondria. The *NADH dehydrogenase subunit 3* (*ND3*) which was downregulated in the Boer and upregulated in village goats, *NADH dehydrogenase subunit 4* (*ND4*) upregulated in the Boer, and *Cyclooxygenase-2* (*COX2*) upregulated in the Boer are the genes that were enriched in this pathway, and they all play a significant role in the growth and development of the skeletal muscle. The upregulation of this gene in the village goats can be explained by the preferential production and use of energy in the village goats as opposed to growth and muscle production in village goats that are under constant selection for survival in harsh conditions versus Boer goats selected and bred for high meat yield and growth performance.

6.6 Conclusions

The present study demonstrated the usefulness of the *longissimus* muscle in gene expression analysis as a tool to unravel key genes for growth and muscle development in the South African Boer and village goat populations. The non-overlap of DEGs demonstrated unique genetic mechanism between breeds and production systems. Through differential gene expression, the study identified genes such as the *MIR214*, *BMP4* and *PDK4* within breeds and *MSTN* and *IGFBP3* between breeds which show potential as candidate genes for muscle development and can be used as biomarkers for carcass and meat quality. The expression and upregulation of immune related genes such as the *CXCL9*, *CXCL10, CXCL11* and *BATF2* in the extensively raised populations demonstrates the need for immune response mechanisms in village goats raised under harsh and compromised production systems often challenged with diseases and parasites. Overall, results of this study provides an understanding the molecular mechanisms that contribute to growth and growth performance and will have a significant impact on breeding and management strategies.

CHAPTER 7: CONCLUSIONS AND RECOMMENDATIONS FOR FURTHER RESEARCH

7.1 Introduction

Goats are an important livestock species as they are primarily kept and used for chevon production. They play an essential role in rural communities of South Africa for food and socio-economic purposes. Chevon is an important nutrient source to the poor communities of South Africa (SA), where over 60 % of the goats are farmed. South Africa consists of two production systems where goats are either raised extensively or intensively. The intensive system is a large-scale commercial system where high performing breeds such as the Boer are kept while uncharacterized village goats are raised in extensive systems having to fend for their own food and water in harsh production conditions. Extensively raised populations are of low genetic merit in terms of growth, carcass and reproductive potential due to the absence of selection and improvement programs in communal farms. They are slow growing leading to smaller mature weights and poor meat quality as compared to the commercially raised breeds (Masika *et al.*, 2007), which poses a limitation when raising village breeds for meat production. These challenges in the extensive production system lead to low mature body weights and carcass yield thereof. South Africa is one of the major goat producing countries, however, the country contributes only less than 1 % of chevon production in the South African red meat market (DAFF, 2015). Some of the contributing factors to this low production may be because chevon is regarded as an inferior meat through market preferences/perceptions, as well as, supply vs demand challenges where the available supply is unable to match the demand (Mazhangara et *al.*, 2019). While the commercial industry is dominated by superior breeds such as the Boer with high growth performance and meat yields, there are uncharacterized village ecotypes in communal farms which have multipurpose functions such as milk, meat and skin and these have not been selected and developed for any specific traits (Mdladla *et al.*, 2016; Ncube *et al.*, 2016). Low chevon production remains a growing concern in South Africa where the majority of goats are being kept by communal farmers in rural areas with poor growth performance which subsequently leads to low carcass yields posing as a limitation in the industry. Other challenges that lead to low productivity are because majority of the goats are farmed in rural areas characterized by inefficient feeding and management, disease constrains, lack of characterization and inadequate exploitation of the genetic potential (Webb, 2014).

7.2 General discussion

South Africa being one of the countries with well-established goat breeds and production systems, faces several challenges as the majority of the goats are raised in rural areas under the extensive production system. Under this production system, animals experience a high level of stress that contributes to poor growth performance and low carcass yields. With all the advancements in goat genomics and genetics studies, growth and carcass genetics have not been explored, and limited information exists on the genetic potential and factors that affect goat productivity in SA particularly in indigenous goats raised in communal areas. Tools such as the Illumina Goat SNP50K BeadChip and RNA-Seq technology can therefore be employed to study the genetic potential of these goats. The present study sought to use the Illumina Goat SNP50K to investigate the population genetic structure and associated growth and carcass potential of South African indigenous ecotypes under different production systems. In light of the fact that factors such as breed, genetic and environmental factors influence growth and carcass yield this study, therefore, sought to use transcriptomic analysis to investigate the effect of production system and breed differences in differential gene expression of South African goat populations targeting the pituitary gland and the *longissimus* muscles. The pituitary gland is responsible for the control of the endocrine system as well as important processes such as growth at different developmental stages (Pareek *et al*., 2017), therefore, it was targeted as a tissue to study as it can shed light in genetic mechanisms influencing growth, as well as, key genes that play a role in growth and carcass quality. The *longissimus* muscle on the other hand, consists of a higher proportion of the carcass value (Kim *et al*., 2019) and a good target to identify genes that are associated with muscle growth and development. The study therefore, reported on the genetic structure of local goat populations, identified genomic regions associated with growth and carcass quality as well as genes and genetic mechanisms influencing these two traits.

The country consists of three categories/groups of goats; i) Characterized meat breeds (Boer, Kalahari Red and Savannah, ii) Indigenous veld goats (Northern Cape Speckled, Xhosa lob ear and Mbuzi/Nguni) and iii) Non-descript/uncharacterized village goat populations. The present study used the Boer as a benchmark against which the uncharacterised goat populations were profiled. By using the Illumina GoatSNP50K BeadChip, the first experimental study (Chapter 3) genotyped the above-mentioned populations followed by a principal component analysis (PCA) and ADMIXTURE based clustering, to determine population variations as well as confirm their diversity. The commercial South African Boer goat was developed and established in the early 1900s specifically for meat production (Erasmus, 2000; Campbell, 2003; Ncube, 2016; Dzomba *et al*., 2018; Visser & van Marle-Köster, 2018). The PCA and ADMIXTURE clustering separated the Boer from the rest of the populations suggesting its genetic difference from the rest of the populations. Though this breed separated from the rest of the populations it was in the same axis as the village populations which is consistent with the findings of Mdladla *et al*., (2016)'s and reports that the Boer was historically developed from village goats (Campbell, 2003; Mdladla *et al*., 2016). The separate clustering from the village goats may therefore be due to differential selection pressures during breed development of the Boer goats and the harsh and compromising production conditions of village goats. The Northern Cape Speckled population is one of the populations that was characterized by Morrison (2007) as the indigenous veld goats (IVG). The breed is kept by the IVG farmers and commonly used for hides. The PCA revealed the Northern Cape Speckled as a unique genetic resource which separated from the rest of the populations. The Speckled goat originates in the dry Northern Cape, Karoo area, stretching from Sutherland to Upington. Its genomic uniqueness may be due to its origins as well as how the IVG farmers keep them in an organized manner and separated from other breeds. PCA showed one cluster of the Mbuzi (MBZ), Xhosa lob (XL) and uncharacterized village populations where the village populations were in between the MBZ and the XL suggesting that even though they may be related there were some genetic differences possibly due to geographic locations. Admixed individuals were observed in village and Mbuzi populations indicating high genetic diversity and possible interactions between populations. Overall, this analysis observed high within-population diversity and presence of population genomic structure between SA goat populations. The village goat populations were admixed further indicating that there are no formal management practices especially in mating and separating goats from mixing due the extensive production system. Furthermore, farmers in villages share communal land for farming which means they also share pastures and animals meet at the same place to forage and drink water thereby mixing with other animals belonging to other farmers and mating, leading to admixed populations.

One of the most important economic traits of an animal is its mature body weight as high yields indicate more profit for the producers (Moela, 2014). Following the understanding of the population genomic structure, this experimental chapter further analysed the mature body weights and growth profiles of populations under study on the assumption of genetically structured populations. Based on this, variations in growth traits were attributed to the genetic differences, of which mature live body weights varied according to the population genomic cluster as per PCA demonstration. Again, the Boer, which was developed for high performance demonstrated consistency in having higher average weight gain at 28.96±0.30 kg live weights as compared to the rest of the populations. With an average weight gain of 24.21±0.31 kg live weight, the Northern Cape Speckled has potential as a high performing breed. The XL population was used in the development of the SA Boer and the Kalahari Red meat-type goats found in the commercial sector (Dube *et al*., 2016). It was therefore expected that the XL population will perform well in terms of growth, which was in contrast to observations that the Speckled performed better. Village ecotypes that were raised at the research farm performed better than those raised in village farms. Village farms are characterized by poor management practices (Ncube *et al*., 2016) and face challenges such as diseases, drought etc., coupled to walking long distances to browse for feed, which puts a strain on them. Such challenging production systems may be part of the causes of poor growth performance. In a controlled management system, the animals are fed a management diet, they receive adequate medical attention when there is a need and always have clean water and good shelter, which reduces the challenges the animal goes through and presents an opportunity of improved growth rates even of animals of presumed low genetic potential. Overall, there was a significant variation between breeds in body weight and other body measurements which could be important for genetic improvement of such traits.

The growth profiles analysed for the genetically distinct populations demonstrated the potential for growth and within-population selection in the study populations. Most communal farmers believe that using exotic breeds is the solution for improved genetics, however, this study demonstrated that the goat populations are genetically diverse and differ in growth and morphological conformation, a scenario which presents other opportunities of improving performance without introducing exotic breeds into the population and diluting the local genetics. Village goats are reported to have poor growth performance irrespective of their good genetic characteristics and ability to adapt and thrive in harsh environmental conditions. By raising uncharacterized village goats under controlled and improved production systems, the present study demonstrated that improved management systems lead to the optimal performance of animals with similar genetics. This then suggests that farmers in communal areas should in addition to selection and genetic improvement, work on improving animal management systems that will enable goats to reach their highest genetic potential or at least perform optimally.

Breed characterization is one of the key areas that can be used in better understanding South African goat populations as well as characterization of village goats. Body conformation indices were used in this study as one of the tools that can be used to complement genetic characterization. This technique also uses measurements like cephalic indexes for relationships between populations and other measurements that separate animals into different groups, e.g. meat breeds. Breed origin and relationship are important in goat production and cephalic index (IC) which is derived from the facial measurements has proven to be one of the useful tools to use in this area (Chacón *et al*., 2011). The IC of all the populations were similar except for the MBZ population indicating that there is a relationship between the SAB and the other ecotypes, which is consistent with reports by Campbell (2003) and Mdladla *et al*. (2017) that the Boer goats were developed from the indigenous populations, therefore, this relationship was expected. This is consistent with ADMIXTURE analysis in the present study where the Boer was admixed with the rest of the populations except for the Speckled, therefore, this relationship is not surprising even with the village ecotypes. The MBZ, on the other hand, are a smaller breed and slightly different from other populations (Snyman 2012). This is consistent with the observation in this study that the IC of the MBZ were different from the rest of the populations. The dactyl thorax index (DTI) is used as an indication of whether an animal is a light or heavy meat type. All populations under study were more than 16.62±0.12 indicating that they are heavy meat types and heavier when compared to the Assam Hill goats in India (Khargharia *et al*. 2015) and Creole goats (Chacón *et al*., 2011) which were 9.82±0.38 and 9.58±0.57, respectively.

Growth and carcass quality are important traits in the goat production industry and even more important to the producer. Following the growth profiles and the understanding of population structure and growth variations per breed/populations, the study further used genome-wide association studies using Illumina Goat SNP50K BeadChip genotypes for the characterization and association tests in search of candidate genes that play a role in carcass quality of South African goat populations (Chapter 4). Though South Africa has well-established meat breeds, there is still a massive gap in the chevon industry, and there are no reported/written records that describe the carcass characteristics of South African indigenous goat populations as well as genetic associations with carcass quality traits. A marketable mature live body weight for goats in their first year is between 30-40 kg (Pophiwa *et al.*, 2016). The pre-slaughter weight for the Boer population in the present study was higher than the rest of the populations under study as expected, in line with it being bred for high growth performance. The Northern Cape Speckled, commonly bred for its exceptionally beautiful hide, demonstrated unique genetics and slightly higher pre-slaughter weight than the rest of the indigenous veld goats implying potential as a meat breed. The village populations that were raised under the intensive production system had higher pre-slaughter weight than those raised under the extensive production system. Regardless of a similar genetic background, differences in WS were observed between the intensively raised populations and those raised extensively demonstrating that improved management leads to improved performance. Though the intensively raised populations performed better than those raised extensively, they still did not meet the marketable weight as mentioned above, indicating that more work is needed in improving the genetics of these populations. The within population genetic variations in village goats implied that there is still room for selection for optimal growth and carcass traits.

Carcass yield corresponded with pre-slaughter weight in that, breeds with the highest slaughter weight (Boer and Northern Cape Speckled) produced high carcass yields post mortem than the rest of the populations under study. These observations are consistent with that of Pophiwa *et al*. (2017) who reported that the Boer had higher cold carcass weight (6.1 kg) compared to the indigenous populations (2.9 kg). Fat class is essential to producers as one of the traits that determine carcass yield. Carcasses with low-fat content are more likely to lose more moisture and cold shortening during chilling (Webb, 2014). The present study observed that large framed genotypes such as the Boer, Northern Cape Speckled and Xhosa lob ear had a high-fat content while the smaller genotypes (Mbuzi and village) had low-fat content.

Genome-wide association study was also performed for the investigation of SNPs associated with carcass quality traits in the SA goat populations. Genes such as the *GADD45G, IGF2R* and *VRTN* genes were significantly associated with pre-slaughter weight suggesting their importance as candidate genes for pre-slaughter weight. Results were consistent with other studies where genes such as the *IGF2R* gene, a gene that encodes a transmembrane receptor was associated with growth traits such as animal body size in Irish Holstein-Friesian cattle (Berkowicz *et al*., 2012).

With growth and carcass quality being the most important economic traits in goat production and chevon industry, the study went on to further investigate other factors associated with growth and carcass quality. Whilst Chapter 3 and 4 provided information on the population genomic structure, growth profiles and carcass characteristics the following chapters of 5 and 6 further investigated the genes expression profiles and molecular mechanisms that play a role in the growth and carcass quality of local goats. Chapter 5 analysed gene expression profiling through transcriptome sequencing of the pituitary gland of South African indigenous Boer goats and village populations. This study analysed gene expression within breeds across production systems (Village goats kept intensively vs extensively) as well as within the same production system and across breeds (Boer vs Village goats kept intensively). Some of the genes that were differentially expressed across production systems (i.e the *Mitogen activated protein kinase* ;*MAPK*), supported suggestions that the extensively raised goat populations raised under extremely stressful conditions, which probably hinders their growth ultimately leading to low mature body weights. On the other hand, differentially expressed genes such as the *fibroblast growth factor receptor 2 gene* (*FGFR2*) in the Boer breed revealed that the breed has excellent growth performance and high mature body weights attributed to selection for meat production traits. Pathway analysis further confirmed mechanisms that play a role in the growth of goats, that included response to diseases and other production challenges that further hinder the growth of goats in the extensively raised populations. Chapter 6 analyzed differential gene expression associated with growth and carcass quality by using the transcriptome of the *longissimus* muscle. Differentially expressed genes revealed genes that were involved in muscle development, with differences observed within breeds across production systems and between breeds within a production system. Again as with the pituitary, high expression and upregulation of immune related genes (*CXCL9, CXCL10, CXCL11* and *BATF2*) in extensively raised populations further emphasised the challenging conditions under which these populations are raised and the need for immune related genes to cope with the exposure to diseases and disease pathogens.

7.3 Conclusions

High genetic diversity within South African indigenous goats particularly village goat populations was observed. Village goat populations demonstrated variations in growth and carcass quality with relatively poor performance as compared to the Boer and IVG populations. Village goats that were raised intensively at the research farm had better growth performance as compared to those raised in Pella village, suggesting that management does have an effect on growth and carcass quality. The study suggests that these variations in the growth and carcass quality in South African goat populations are brought about by differences in the genomic architecture triggering different gene expressions and molecular mechanisms. Candidate genes identified and associated with growth and carcass quality traits in South African goat populations are potential markers in selection programs. The present study successfully identified and analyzed differentially expressed genes from the pituitary gland and the longissimus muscle of South African Boer and village populations. Gene expression profiling revealed that growth, carcass quality, immune and stress related genetic mechanisms are triggered in order for these goats to produce optimally under different production systems.

7.4 Study limitations and challenges

While poor growth performance of farm animals is a widely discussed problem in communal farming systems where goats are extensively raised, another major challenge is the management system. In communal areas, there are no record-keeping systems which makes data collection and establishing genomic studies an extremely challenging prospect. Chapter 3 of the present study utilized kids at the age of 12 weeks, since recording-keeping in communal farming systems is sparse. Prior to the establishment of the experiments, farmers selected for the study were organized and an agreement was reached for them to record the birth dates of the kids in order to have a record of the birth dates. Because of record keeping challenges, there were no birth weights recorded on village farms. However, the study recorded weaning/starting weight at 12 weeks of age when the growth trial experiment started. The study organized farmers and raised goats under defined extensive production systems in order to have a uniform growth trial and achieve homogeneity of experimental units. The duration of the growth trial was another challenge due to the limitation of funds and the trial could only run for 6 months when the animals were 36 weeks of age. A longer growth trial could have given the study an opportunity to add more growth stages for profiling up to 12 months of age when they reach the adult stage. Therefore, the results of this study can be used as a benchmark for further studies in order to further profile and gain more insight into the growth of South African indigenous goat populations.

The present study utilized five breeds representing i) Commercial/intensive system, ii) Indigenous veld goats and iii) uncharacterized village goat populations at a sample size of *n*=14 per population. Breed representation particularly for the meat breeds and the uncharacterized village goats was a limitation in that, only the Boer goat was used as a representation for the meat breeds and the only one population from one province for village goat populations. More meat breeds such as the Kalahari Red and Savannah could be added in future studies to further have a better understanding and growth profiling of the meat breeds. More village breeds especially from other provinces with different conditions could have been beneficial in the profiling of uncharacterized goats and could also reveal crucial information such as between population growth and genomic variations and whether there can be better growth performance between village breeds depending on geographical regions. This study however, aided with the unravelling of growth and genetic variations in the populations under study and this can be used in future studies.

To have a better understanding of the breed and production system effects on growth performance and carcass quality, the study employed RNA-Seq technology using the pituitary gland and the *longissimus* tissues. Setting-up this experiment was challenging because transcriptomics is affected by factors like breed and environmental conditions. This makes RNA-seq experimental design challenging especially in efforts to reduce extraneous error variation and correctly ascertain effects and associations. RNA-seq studies are suitable for commercial breeds that are genetically uniform, constant production systems, good and uniform management systems etc. In order to resolve some of the challenges that may be caused by genetic variation, the present study first employed a population genomic study (Ncube et al., 2019) to analyze the purity of the breeds particularly village breeds and ADMIXTURE was used to select genetically uniform village goats. It is also important to note that, there may be other factors that could have influenced gene expression in extensively raised population and these can be seen by the expression of immune and stress related genes that were expressed only in the extensively raised populations. This study utilized five breeds of the indigenous

South African goats. However, for the RNA-seq experiment only 2 breeds could be used due to the high research costs of RNA sequencing. The experiment was then set to investigate effect of breed and effect of production system on growth and muscle development. It could have been even more beneficial and enlightening to include the indigenous veld goats in the transcriptomics study as well. Future studies can use these results as a guideline and perhaps a comparative study on gene expression.

7.5 Future possibilities

Some of the future research possibilities that should be given more focus is conducting more experiments on the extensive farming systems in order to further seek ways to improve the management systems for improved genetics. Growth and carcass quality are essential economic traits for goats and it is therefore critical for them to attract research attention. A fast-growing and heavy animal guarantees higher carcass yield which translates to more returns for the farmer. More genetic studies need to be performed in this area to further understand the genes and genetic mechanisms that play a role in the growth performance of goats. Furthermore, studies should bring more focus to the broader spectrum of breed representation, adding more meat breeds as well as studying village goats from other geographical regions that can bring about a better understanding of growth and carcass genomics and profiles of South African goats. As mentioned above, the duration of the growth trial was 6 months. Future studies should conduct longer growth trials from birth up to at least up to 12 months in order to get the fuller growth profile and therefore better understanding of growth.

Transcriptomic analysis in this study revealed that village populations are under a high amount of stress including disease prevalence. Further research should be conducted to understand the interactions of challenging production conditions and the genetic mechanisms at play. Only two tissues (pituitary gland and *longissimus*) were studied during this research project and it is recommended that more tissues such as kidneys, liver, thyroid gland etc. are studied to give a clearer picture of gene expression and molecular mechanisms involved. Tissues such as the thyroid gland play an important role in thermal adaptation and so transcriptomic analysis of this tissue can be beneficial in the identification of differentially expressed genes that play a role in adaptive responses of goats in harsh environmental conditions. Furthermore, its function in the release of hormones that control metabolism can also reveal the usage of energy in village goats since they require high energy levels for scavenging.

Another potential research area should be the role of epigenetics by investigating the impact of this phenomenon on the growth of goats. Causal networks studying the relationship between phenotype-genotype-transcript are another important area of focus that can further bring more understanding to the genetic mechanisms of South African goat populations especially the uncharacterised indigenous veld goat populations predominantly found in rural villages in South Africa.

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APPENDIX A: LEAST SQUARE MEANS AND STANDARD ERROR OF LIVE WEIGHTS AND BODY MEASUREMENTS FOR SOUTH AFRICAN GOAT ECOTYPE POPULATIONS.

Traits	*Ecotype						
	SAB	NCS	XL	MBZ	VTI	VTE	*P*-value
LW (kg)	28.96±0.30	24.21±0.31	20.00±0.29	14.83±0.33	19.55±0.36	17.55±0.37	***
HL (cm)	22.13±0.15	21.25±0.15	19.79±0.14	17.72±0.16	19.18±0.17	18.62±0.18	***
HW (cm)	19.48±0.13	18.58±0.13	17.78±0.12	15.89±0.14	17.23±0.15	16.27±0.16	***
EL (cm)	21.09±0.14	20.45±0.14	18.41±0.13	15.79±0.15	17.79±0.16	17.09±0.16	***
MD (cm)	20.99±0.16	19.65±0.16	19.04±0.15	16.29±0.17	18.04±0.19	18.03±0.19	***
CB (cm)	12.69±0.16	11.28±0.17	10.92±0.16	9.35±0.18	10.52±0.19	10.57±0.20	***
WH (cm)	58.31±0.34	58.48±0.35	54.87±0.32	51.14±0.37	55.62±0.39	55.71±0.41	***
SH (cm)	41.21±0.26	42.02±0.27	39.62±0.25	36.31±0.29	39.97±0.31	39.57±0.32	***
BD (cm)	33.51±0.26	32.58±0.27	30.47±0.25	27.72±0.29	30.80±0.31	31.09±0.32	***
BISC (cm)	60.45±0.33	57.30±0.34	56.07±0.31	51.53±0.36	54.69±0.39	55.24±0.51	***
CG (cm)	67.81±0.43	66.35±0.45	61.55±0.41	56.48±0.48	61.04±0.51	61.42±0.52	***
CW (cm)	19.16±0.25	18.15±0.26	16.42± 0.24	14.41±0.28	16.13±0.29	16.09±0.34	***
BL (cm)	58.11±0.33	55.53±0.34	52.19±0.31	47.01±0.36	51.09±0.39	51.09±0.39	***

RH (cm)	58.22±0.39	61.45±0.41	55.71±0.38	51.63±0.43	56.51±0.46	56.09±0.46	***
RW (cm)	19.48±0.14	18.14±0.14	16.62±0.13	15.17±0.15	17.21±0.16	16.64±0.16	***
RL (cm)	15.62±0.13	15.36±0.13	14.67±0.12	13.22±0.14	14.35±0.15	14.46±15.56	***
PB (cm)	5.63±0.34	5.67±0.04	5.66±0.03	5.37±0.04	5.76±0.04	5.71±0.04	***

$^{\gamma}$Traits: LW=Live Weight, HL=Head Length, HW=Head width, EL=Ear Length, MD=Muzzle Diameter, CB=Cannon Bone, WH=Withers Height, SH=Sternum Height, BD=Body Depth, BISC=Bicoastal Diameter, CG=Chest Girth, CW=Chest Width, BL=Body Length, RH=Rump Height, RW=Rump Width, RL=Rump Length, PB=Pin Bone. *Ecotype: SAB=South African Boer, NCS=Northern Cape Speckled, XLE=Xhosa Lob/Ear, MBZ=Mbuzi, VTI=Village Tswana raised at API research farm, and VTE= Village Tswana raised in extensive conditions at village farms. Highly significant values of ($P \leq 0.0001$) is indicated by ***.

APPENDIX B: LEAST SQUARE MEANS AND STANDARD ERROR OF LIVE WEIGHTS AND BODY MEASUREMENTS FOR SOUTH AFRICAN GOAT ECOTYPE POPULATIONS CATEGORIZED BY SEX.

Traits	Sex		
	F	M	*P*-value
LW (kg)	20.21±0.16	21.49±0.21	***
HL (cm)	19.39±0.08	20.17±0.10	***
HW (cm)	17.05±0.07	18.02±0.09	***
EL (cm)	18.49±0.07	18.37±0.09	***
MD (cm)	18.36±0.09	19.27±0.11	***
CB (cm)	10.73±0.09	11.04±0.12	***
WH (cm)	54.31±0.18	57.07±0.23	***
SH (cm)	38.99±0.14	40.57±0.18	***
BD (cm)	30.52±0.14	31.54±0.18	***
BISC (cm)	55.11±0.18	56.62±0.22	***
CG (cm)	61.25±0.23	63.63±0.29	***
CW (cm)	16.41±0.14	17.04±0.17	***
BL (cm)	51.46±0.18	53.55±0.22	***
RH (cm)	55.29±0.22	57.90±0.27	***

RW (cm)	16.86±0.07	17.56±0.09	***
RL (cm)	14.42±0.07	14.80±0.09	***
PB (cm)	5.69±0.02	5.58±0.02	***

[γ] Traits: LW=Live Weight, HL=Head Length, HW=Head width, EL=Ear Length, MD=Muzzle Diameter, CB=Cannon Bone, WH=Withers Height, SH=Sternum Height, BD=Body Depth, BISC=Bicoastal Diameter, CG=Chest Girth, CW=Chest Width, BL=Body Length, RH=Rump Height, RW=Rump Width, RL=Rump Length, PB=Pin Bone. *Ecotype: SAB=South African Boer, NCS=Northern Cape Speckled, XLE=Xhosa Lob/Ear, MBZ=Mbuzi, VTI=Village Tswana raised at API research farm, and VTE= Village Tswana raised in extensive conditions at village farms. Highly significant values of ($P \leq 0.0001$) is indicated by ***.

APPENDIX C: LEAST SQUARE MEANS AND STANDARD ERROR OF LIVE WEIGHTS AND BODY MEASUREMENTS FOR SOUTH AFRICAN GOAT ECOTYPE POPULATIONS CATEGORIZED BY AGE FROM 12 TO 34 WEEKS.

Trait	Age (Weeks)											
	12	14	16	18	20	22	24	26	28	30	32	34	*P*-value
LW (kg)	13.06±0.47	15.02±0.46	16.41±0.47	17.57±0.46	19.08±0.46	20.12±0.46	21.37±0.46	22.71±0.46	24.01±0.46	25.49±0.46	26.97±0.48	28.41±0.46	***
HL (cm)	16.28±0.22	16.37±0.22	17.65±0.22	18.05±0.22	18.11±0.22	19.52±0.22	19.56±0.22	20.74±0.22	21.77±0.22	22.77±0.22	23.20±0.22	23.37±0.22	***
HW (cm)	14.60±0.19	14.62±0.19	15.15±0.19	15.51±0.19	15.83±0.19	16.74±0.19	16.90±0.19	18.74±0.19	19.49±0.19	20.49±0.19	21.09±0.19	21.33±0.19	***
EL (cm)	15.48±0.21	15.67±0.21	16.51±0.21	16.70±0.21	17.02±0.21	18.39±0.21	18.51±0.21	19.72±0.21	20.33±0.21	20.68±0.21	20.94±0.21	21.28±0.21	NS
MD (cm)	16.42±0.25	16.51±0.25	17.06±0.25	17.30±0.24	17.57±0.25	18.49±0.25	18.69±0.25	19.71±0.25	20.55±0.24	20.82±0.25	21.20±0.25	21.42±0.25	***
CB (cm)	9.67±0.26	9.68±0.25	9.99±0.26	10.11±0.25	10.29±0.25	10.76±0.25	10.77±0.25	10.08±0.25	11.48±0.25	11.74±0.25	12.22±0.25	12.87±0.25	***

WH (cm)	49.15±0.52	49.16±0.51	50.17±0.52	51.23±0.51	51.56±0.51	55.32±0.51	55.69±0.51	58.61±0.51	60.38±0.51	61.47±0.51	62.76±0.51	62.79±0.51	***
SH (cm)	34.75±0.40	34.79±0.39	36.76±0.40	36.93±0.39	37.09±0.39	40.27±0.39	40.32±0.39	41.71±0.39	42.83±0.39	43.45±0.39	44.10±0.39	44.48±0.39	***
BD (cm)	27.01±0.41	27.38±0.40	28.87±0.41	29.33±0.40	29.89±0.40	30.64±0.40	31.00±0.40	32.29±0.40	33.00±0.40	33.46±0.40	34.16±0.40	35.32±0.40	***
BISC (cm)	46.90±0.51	47.32±0.50	50.20±0.51	50.61±0.50	52.16±0.50	55.81±0.50	56.41±0.50	59.37±0.50	61.68±0.50	62.35±0.50	63.36±0.50	64.21±0.50	***
CG (cm)	55.53±0.67	55.30±0.66	57.15±0.67	57.62±0.66	57.93±0.66	61.22±0.66	61.37±0.66	64.28±0.66	67.13±0.66	69.28±0.66	71.22±0.66	71.22±0.66	***
CW (cm)	13.87±0.39	14.31±0.38	14.99±0.39	15.16±0.38	15.31±0.38	16.27±0.38	16.42±0.38	17.37±0.38	18.19±0.38	19.05±0.38	19.32±0.38	20.50±0.38	***
BL (cm)	45.46±0.50	45.63±0.49	47.66±0.50	48.59±0.49	49.29±0.49	51.61±0.49	51.77±0.49	54.35±0.49	56.94±0.49	58.59±0.49	59.74±0.49	60.43±0.49	***
RH (cm)	49.49±0.61	48.83±0.60	50.99±0.61	51.76±0.60	52.13±0.60	56.67±0.60	56.72±0.60	59.70±0.60	61.63±0.60	63.01±0.60	64.34±0.60	63.93±0.60	***
RW (cm)	14.74±0.21	14.72±0.21	15.54±0.21	15.81±0.21	16.04±0.21	16.96±0.21	17.20±0.21	18.02±0.21	18.61±0.21	19.29±0.21	19.66±0.21	19.89±0.21	***
RL (cm)	12.22±0.19	12.08±0.19	12.71±0.19	12.98±0.19	13.18±0.19	14.11±0.19	14.29±0.19	15.59±0.19	16.43±0.19	16.98±0.19	17.41±0.19	17.36±0.19	***

PB (cm)	5.11±0.05	5.13±0.05	5.32±0.05	5.40±0.05	5.45±0.05	5.65±0.05	5.76±0.05	5.88±0.05	5.96±0.05	5.97±0.05	5.99±0.05	6.00±0.05	NS

[γ] Traits: LW=Live Weight, HL=Head Length, HW=Head width, EL=Ear Length, MD=Muzzle Diameter, CB=Cannon Bone, WH=Withers Height, SH=Sternum Height, BD=Body Depth, BISC=Bicoastal Diameter, CG=Chest Girth, CW=Chest Width, BL=Body Length, RH=Rump Height, RW=Rump Width, RL=Rump Length, PB=Pin Bone. Highly significant values of ($P \leq 0.0001$) is indicated by ***. NS= Non-Significant.

APENDIX D: LEAST SQUARE MEANS AND STANDARD ERROR OF BODY INDICES FOR SOUTH AFRICAN GOAT ECOTYPE POPULATIONS.

Body Indices	Ecotype						
	SAB	NCS	XL	MBZ	VTI	VTE	*P*-value
IC	87.96±0.68	87.37±0.69	89.83±0.64	90.65±0.75	89.65±0.87	87.98±0.82	NS
Ipr	101.20±0.63	105.47±0.65	105.17±0.59	109.05±0.69	109.53±0.81	109.32±0.76	***
IP	124.89±0.92	119.09±0.94	113.43±0.87	115.83±1.01	121.52±1.17	116.59±1.11	***
WS	1.04±0.01	1.01±0.01	1.05±0.01	1.06±0.01	1.08±0.01	1.05±0.01	NS
BAL	0.49±0.01	0.48±0.01	0.52±0.01	0.50±0.01	0.51±0.01	0.49±0.01	NS
IPT	33.26±0.24	29.58±0.25	29.79±0.23	29.35±0.26	30.50±0.31	29.85±0.29	***
IPL	26.77±0.21	25.04±0.21	26.34±0.19	25.49±0.23	25.36±0.26	25.75±0.25	***
IPRT	57.17±0.49	55.86±0.51	55.79±0.47	54.41±0.55	55.39±0.64	55.85±0.60	NS
RBI	99.65±0.72	95.11±0.75	96.10±0.69	91.97±0.79	91.81±0.93	91.84±0.88	NS
BR	0.71±0.004	0.68±0.004	0.71±0.004	0.70±0.004	0.71±0.004	0.71±0.004	***
PI	1.21±0.004	1.24±0.004	1.21±0.004	1.21±0.004	1.21±0.004	1.21±0.004	***
TD	1.65±0.01	1.59±0.01	1.57±0.01	1.56±0.01	1.54±0.01	1.56±0.01	***
HS	0.031±0.26	3.13±0.26	1.37±0.24	0.49±0.28	1.06±0.33	0.38±0.31	***
DI	0.57±0.01	0.56±0.01	0.56±0.01	0.54±0.01	0.55±0.01	0.56±0.01	***

BI	85.55±0.43	83.90±0.44	83.98±0.41	83.20±0.47	83.58±0.55	83.34±0.52	NS
DTI	18.31±0.11	17.02±0.12	17.73±0.11	16.62±0.12	17.32±0.14	17.23±0.14	***
BC	79.66±0.69	75.51±0.71	70.88±0.66	62.56±0.76	67.53±0.89	68.02±0.84	***
AI	3415.88±30.19	3272.63±31.09	2913.92±28.59	2451.54±33.19	2911.60±38.67	2879.79±36.54	***
RCI	21.29±0.27	19.29±0.28	20.46±0.25	18.39±0.29	19.05±0.34	18.99±0.32	***
W	23.25±0.26	22.21±0.27	19.37±0.25	16.08±0.28	18.96±0.30	19.20±0.31	***

[γ]Body Indices: IC=Cepalic, Ipr=Proportionality, IP=Pelvic Index, WS=Width Slope, BAL=Balance, IPT=Transverse Pelvic, IPL=Longitudinal Pelvic, IPTR=Relative Depth of Thorax, RBI=Relative Body Index, DTI=Dactyl Thorax Index, BR=Body Ratio, BC=Baron & Crevat (Conformation Index), PI=Pectoral Index, TD=Thoracic Development, HS=Height Slope, DI=Depth Index, BI=Body Index, AI=Area Index, RCI=Relative Cannon thickness Index, W=Weight, and CI=Compact Index. *Ecotype: SAB=South African Boer, NCS=Northern Cape Speckled, XLE=Xhosa Lob/Ear, MBZ=Mbuzi, VTI=Village Tswana raised at API research farm, and VTE= Village Tswana raised in extensive conditions at village farms. Highly significant values of ($P \leq 0.0001$) is indicated by ***. NS= Non-Significant.

APENDIX E: LEAST SQUARE MEANS AND STANDARD ERROR OF BODY INDICES FOR SOUTH AFRICAN GOAT ECOTYPE POPULATIONS CATEGORIZED BY SEX.

Body Indices	Sex		
	F	M	*P*-value
IC	88.14±0.37	89.67±0.48	NS
Ipr	105.86±0.31	107.39±0.45	NS
IP	116.91±0.50	120.22±0.65	NS
WS	1.05±0.01	1.042±0.01	NS
BAL	0.50±0.003	0.49±0.005	NS
IPT	30.35±0.13	30.43±0.17	NS
IPL	26.08±0.11	25.50±0.15	NL
IPRT	56.17±0.27	55.31±0.35	NS
RBI	95.09±0.39	93.73±0.52	NS
BR	0.70±0.002	0.70±0.003	NS
PI	1.21±0.002	1.22±0.003	NS
TD	1.58±0.01	1.57±0.01	NS
HS	1.24±0.14	0.91±0.18	NS
DI	0.56±0.003	0.55±0.004	***

BI	83.76±0.24	84.09±0.34	NS
DTI	17.39±0.06	17.35±0.08	NS
BC	70.03±0.38	71.35±0.49	NS
AI	2840.64±16.56	3107.82±21.49	***
RCI	19.80±0.15	19.36±0.19	NS
W	19.09±0.14	20.59±0.18	***

[γ]Body Indices: IC=Cepalic, Ipr=Proportionality, IP=Pelvic Index, WS=Width Slope, BAL=Balance, IPT=Transverse Pelvic, IPL=Longitudinal Pelvic, IPTR=Relative Depth of Thorax, RBI=Relative Body Index, DTI=Dactyl Thorax Index, BR=Body Ratio, BC=Baron & Crevat (Conformation Index), PI=Pectoral Index, TD=Thoracic Development, HS=Height Slope, DI=Depth Index, BI=Body Index, AI=Area Index, RCI=Relative Cannon thickness Index, W=Weight, and CI=Compact Index. *Ecotype: SAB=South African Boer, NCS=Northern Cape Speckled, XLE=Xhosa Lob/Ear, MBZ=Mbuzi, VTI=Village Tswana raised at API research farm, and VTE= Village Tswana raised in extensive conditions at village farms. Highly significant values of ($P \leq 0.0001$) is indicated by ***. NS= Non-Significant.

APENDIX F: LEAST SQUARE MEANS AND STANDARD ERROR OF BODY INDICES FOR SOUTH AFRICAN GOAT ECOTYPE POPULATIONS CATEGORIZED BY AGE FROM 12 TO 34 WEEKS.

Body Indices	Age (Weeks)												*P*-value
	12	14	16	18	20	22	24	26	28	30	32	34	
IC	91.11±1.06	90.87±1.06	86.42±1.06	86.11±1.06	87.62±1.06	85.83±1.06	86.23±1.06	90.35±1.06	89.69±1.06	90.14±1.06	91.03±1.06	91.46±1.06	***
Ipr	109.01±0.98	108.56±0.99	105.61±0.98	106.12±0.98	105.18±0.98	107.29±0.98	107.68±0.98	108.14±0.98	106.26±0.98	105.18±0.98	105.41±0.98	105.01±0.98	NS
IP	121.96±1.4	121.76±1.42	123.72±1.42	122.71±1.42	122.59±1.42	120.08±1.42	120.24±1.42	116.03±1.42	113.42±1.42	113.97±1.42	113.12±1.42	113.16	***
WS	1.078±0.02	1.07±0.02	1.05±0.02	1.06±0.02	1.06±0.02	1.06±0.02	1.06±0.02	1.05±0.02	1.03±0.02	1.02±0.02	1.03±0.02	1.02±0.02	NS
BAL	0.49±0.01	0.49±0.01	0.47±0.01	0.47±0.01	0.47±0.01	0.49±0.01	0.49±0.01	0.51±0.01	0.51±0.01	0.52±0.01	0.52±0.01	0.52±0.01	***
IPT	29.84±0.37	29.84±0.37	30.60±0.37	30.58±0.37	30.81±0.37	30.03±0.37	30.41±0.37	30.28±0.37	30.24±0.37	30.67±0.37	30.59±0.37	30.78±0.37	NS
IPL	24.6±0.32	24.73±0.32	24.97±0.32	25.09±0.32	25.36±0.32	25.07±0.32	25.38±0.32	26.14±0.32	26.73±0.32	26.97±0.32	27.12±0.32	27.28±0.32	***

IPRT	54.94±0.78	55.04±0.78	58.49±0.78	57.12±0.78	57.83±0.78	55.49±0.78	55.81±0.78	55.25±0.78	54.78±0.78	54.53±0.78	54.59±0.78	55.09±0.78	***
RBI	92.42±1.13	92.75±1.13	96.57±1.13	94.83±1.13	95.68±1.13	93.84±1.13	93.59±1.13	92.84±1.13	94.46±1.13	95.33±1.13	95.15±1.13	95.54±1.13	NS
BR	0.70±0.01	0.70±0.01	0.72±0.01	0.71±0.01	0.71±0.01	0.71±0.01	0.71±0.01	0.69±0.01	0.69±0.01	0.69±0.01	0.69±0.01	0.69±0.01	***
PI	1.22±0.01	1.22±0.01	1.19±0.01	1.20±0.01	1.21±0.01	1.21±0.01	1.21±0.01	1.21±0.01	1.22±0.01	1.23±0.01	1.23±0.01	1.23±0.01	NS
TD	1.60±0.01	1.61±0.01	1.57±0.01	1.57±0.01	1.57±0.01	1.53±0.01	1.53±0.01	1.55±0.01	1.57±0.01	1.59±0.01	1.62±0.01	1.62±0.01	***
HS	0.42±0.39	0.39±0.39	1.04±0.39	0.56±0.39	0.57±0.39	1.36±0.39	1.06±0.39	1.12±0.39	1.29±0.39	1.59±0.39	1.62±0.39	1.84±0.39	NS
DI	0.55±0.01	0.55±0.01	0.59±0.01	0.57±0.01	0.58±0.01	0.55±0.01	0.56±0.01	0.56±0	0.55±0.01	0.55±0.01	0.55±0.01	0.55±0.01	***
BI	81.82±0.67	81.71±0.67	83.25±0.67	84.26±0.67	85.11±0.67	84.62±0.67	84.79±0.67	84.63±0.67	84.91±0.67	84.67±0.67	83.94±0.67	83.41±0.67	NS
DTI	17.42±0.18	17.36±0.18	17.59±0.18	17.51±0.18	17.76±0.18	17.58±0.18	17.59±0.18	17.27±0.18	17.13±0.18	16.97±0.18	17.17±0.18	17.09±0.18	NS
BC	62.934±1.08	63.47±1.08	66.45±1.08	65.12±1.08	65.46±1.08	68.39±1.08	68.17±1.08	70.87±1.08	74.99±1.08	78.29±1.08	81.01±1.08	83.12±1.08	***
AI	2263.07±46.98	2273.14±47.22	2409.92±46.98	2526.89±46.98	2581.32±46.98	2885.63±46.98	2912.21±46.98	3202.58±46.98	3452.02±46.98	3613.71±46.98	3762.88±46.98	3807.38±46.98	***
RCI	19.70±0.42	19.71±0.42	20.73±0.42	19.72±0.42	19.97±0.42	19.49±0.42	19.39±0.42	18.96±0.42	19.07±0.42	19.13±0.42	19.49±0.42	19.61±0.42	NS

W	15.48±0.39	15.27±0.39	16.51±0.39	16.80±0.39	16.99±0.39	19.11±0.39	19.16±0.39	20.99±0.39	22.79±0.39	24.15±0.39	25.37±0.39	25.49±0.39	***

$^{\gamma}$Body Indices: IC=Cepalic, Ipr=Proportionality, IP=Pelvic Index, WS=Width Slope, BAL=Balance, IPT=Transverse Pelvic, IPL=Longitudinal Pelvic, IPTR=Relative Depth of Thorax, RBI=Relative Body Index, DTI=Dactyl Thorax Index, BR=Body Ratio, BC=Baron & Crevat (Conformation Index), PI=Pectoral Index, TD=Thoracic Development, HS=Height Slope, DI=Depth Index, BI=Body Index, AI=Area Index, RCI=Relative Cannon thickness Index, W=Weight, and CI=Compact Index. *****Ecotype: SAB=South African Boer, NCS=Northern Cape Speckled, XLE=Xhosa Lob/Ear, MBZ=Mbuzi, VTI=Village Tswana raised at API research farm, and VTE= Village Tswana raised in extensive conditions at village farms. Highly significant values of ($P \leq 0.0001$) is indicated by ***. NS= Non-Significant.

APPENDIX G: DIFFERENTIAL GENE EXPRESSION OF EXTENSIVELY RAISED VILLAGE GOAT POPULATIONS

Gene Name	Gene Description	Log2 FC	FDR	*P*_value
Extensive System Up-Regulated				
MYDGF	Myeloid derived growth factor	6.04970	0.00858	0.00001
GNPDA1	Glucosamine-6-phosphate deaminase 1	4.55493	0.01862	0.00003
KDM5A	Lysine demethylase 5A	10.16079	0.04019	0.00009
DLG2	Discs large MAGUK scaffold protein 2	11.32892	6.13E-08	8.98E-12
ZFAND5	Zinc finger AN1 type containing 5	11.31772	3.44E-06	1.18E-09
TRAM2	Translocation associated membrane protein 2	14.00741	1.29E-16	2.63E-12
RRM2B	Ribonucleotide reductase regulatory TP53 inducible subunit M2B	7.07406	0.00055	4.36E-07
CELF2	CUGBP Elav-like family member 2	5.73902	0.00322	4.02E-06
LOC102173699	60S ribosomal protein L6 pseudogene	3.94201	0.04164	0.00010
PTP4A2	Protein tyrosine phosphatase 4A2	12.16172	9.91E-08	2.18E-11
EXOSC3	Exome component 3	7.58079	0.03296	0.00007
TPM1	Tropomyosin 1	8.66019	0.00197	2.26E-06

IGFBP3	Insulin like growth factor binding protein 3	9.29734	0.03296	0.00007
FMNL2	Formin like 2	7.98313	0.01311	0.00002
FAM86B1	Family with sequence similarity 86-member B1	12.70133	0.00015	1.05E-07
DST	Dystonin	10.79967	0.00003	1.34E-08
FAM85A	Family with sequence similarity 85-member A	9.95793	0.00315	3.84E-06
WNK1	WNK lysine deficient protein kinase 1	3.28435	0.04391	0.00011
PGRMC2	Progesterone receptor membrane component 2	8.60815	0.00195	2.14E-06
TRPC3	Transient receptor potential cation channel subfamily member 3	8.28544	0.00197	2.26E-06
MESTP2	Mesoderm specific transcript pseudogene 2	4.69934	0.01221	0.00002
ARFIP1	ADP ribosylation factor interacting protein 1	9.29275	0.01544	0.00002
NKX2-1	NK2 homeobox 1	8.86752	0.00055	4.39E-07
SNORA63	small nucleolar RNA, H/ACA box 63	8.95258	6.45E-07	1.73E-10
ENO1P4	enolase 1 pseudogene 4	9.24184	9.71E-07	2.84E-10

RBM22P2	RNA binding motif protein 22 pseudogene 2	9.33545	0.00067	5.53E-07
RABGAP1	RAB GTPase activating protein 1	4.03513	0.00664	8.91E-06
CAPRIN2	Caprin family member 2	7.60697	0.03332	0.00007
CHKB	Chlorine kinase beta	12.78504	4.00E-15	4.09E-11
SLC13A4	Solute carrier family 13 member 4	8.85552	0.03143	0.00006
LOC108637268	Translation machinery-associated protein 7 pseudogene	8.14000	0.00324	4.12E-06
GIGYF1	GRB10 interacting GYF protein 1	3.33623	0.04571	0.00012
TFDP2	Transcription factor Dp-2	8.84442	0.00189	2.03E-06
Extensive System Down-Regulated				
PAPD7	Poly(A) RNA polymerase D7, non-canonical	-3.79685	0.01354	0.00002
RTP4	Receptor transporter protein 4	-3.96664	0.02571	0.00005

GREB1L	GREB1 like retonic acid receptor coactivator	-9.252529	0.00004	2.01E-08
WBSCR16	Williams-Beuren syndrome chromosome region 16	-3.61153	0.03296	0.00007
EDN1	Endothelin 1	-6.91612	0.03929	0.00009
EFNA1	Ephrin A1	-4.45724	0.02799	0.00005
ACADVL	acyl-CoA dehydrogenase very long chain	-5.87293	0.00034	2.41E-07
SKP2	S-phase kinase associated protein 2	-4.84049	0.04799	0.00012
MAPT	Microtubule associated tau	-6.06321	0.00489	6.45E-06
ANKH	ANKH pyrophosphate transport regulator	-4.61531	0.00154	1.43E-06
TP53INP1	Tumour protein p53 inducible protein 1	-4.32553	0.03742	0.00009
AMN1	Antagonist of mitotic exit network 1 homolog	-4.05730	0.01863	0.00003
BAG4	BCL2 associated athanogene 4	-6.63597	8.78E-08	1.50E-11

APPENDIX H: DIFFERENTIAL GENE EXPRESSION OF INTENSIVELY RAISED VILLAGE GOAT POPULATIONS

Gene Name	Gene Description	Log2 FC	FDR	*P*_value
Intensive System Up-Regulated				
MYDGF	Myeloid derived growth factor	6.04970	0.00858	0.00001
GNPDA1	glucosamine-6-phosphate deaminase 1	4.55493	0.01862	0.00003
MBNL1	Muscleblind like splicing regulator	8.36527	0.00419	5.42E-06
ZFAND5	Zinc finger AN1-type containing 5	11.31772	3.44E-06	1.18E-09
LOC102173699	60S ribosomal protein L6 pseudogene	3.94201	0.04164	0.00010
PTP4A2	Protein tyrosine phosphatase 4A2	12.16172	9.91E-08	2.18E-11
WNK1	WNK lysine deficient protein kinase 1	3.28435	0.04391	0.00011
MESTP2	Mesoderm specific transcript pseudogene 2	4.69934	0.01221	0.00002

SNORA63	Small nucleolar RNA, H/ACA box 63	8.95258	6.45E-07	1.73E-10
ENO1P4	Enolase 1 pseudogene 4	9.24184	9.71E-07	2.84E-10
RABGAP1	RAB GTPase activating protein 1	4.03513	0.00664	8.91E-06
LOC108637268	Translation machinery-associated protein 7 pseudogene	8.14000	0.00324	4.12E-06
TMCC1	Transmembrane and coiled-coil domain family 1	3.33623	0.04572	0.00002
Intensive System Down-Regulated				
PAPD7	Poly(A) RNA polymerase D7, non-canonical	-3.79685	0.01354	0.00002
TFB2M	Transcription factor B2, mitochondrial	-9.38026	0.00002	7.07E-09
OSBPL3	Oxysterol binding protein like 3	-7.76187	0.01176	1.69E-05

GREB1L	GREB1 like retonic acid receptor coactivator	-9.25253	-9.25253	2.0E-08
DPY19L1	Dpy-19 like C-mannosyltransferase 1	-7.35928	0.03705	0.00009
IFT43	Intraflagellar transport 43	-9.16435	0.00015	9.63E-08
GAS7	Growth arrest specific 7	-6.62809	0.00143	1.30E-06
CLASP2	Cytoplasmic linker associated protein 2	-5.34690	0.01574	0.00003
WBSCR16	Williams-Beuren syndrome chromosome region 16	3.61153	0.03296	0.00007
MBP	Myelin basic protein	-7.45169	0.03522	0.00008
LOC102175091	Uncharacterized LOC10217509	-7.56588	0.02427	0.00004
SYCP3	Synaptonemal complex protein 3	-10.48507	0.03522	0.00008
QPCT	Glutaminyl-peptide cyclotransferase	-12.03996	3.61E-11	2.64E-15
UBE2V2	Ubiquitin conjugating enzyme E2 V2	-8.87977	0.00854	0.00001
EDN1	Endothelin 1	-6.91612	0.03929	0.00009
EFNA1	Ephrin A1	-4.45724	0.02799	0.00005

DPM3	dolichyl-phosphate mannosyltransferase subunit 3	-9.76981	0.00002	8.21E-09
ACADVL	Acyl-CoA dehydrogenase very long chain	-5.87293	0.00034	2.41E-07
ADAMTSL4	ADAMTS like 4	-12.10545	0.03296	0.00007
ERO1A	Endoplasmic reticulum oxidoreductase 1 alpha	-9.47991	0.00156	1.49E-06
EME2	Essential meotic structure-specific endonuclease subunit 2	-9.52792	0.00008	4.31E-08
SKP2	S-phase kinase associated protein 2	-4.84049	0.04799	0.00012
EXOCL1	Exocyst complex component 3 like 1	-8.58106	0.00055	4.18E-07
LOC108638467	Uncharacterized LOC108638467	-8.80725	0.00310	3.71E-06
MIF4GD	MIF4G domain containing	-12.10544	0.03296	0.00007
MAPT	Microtubule associated tau	-6.06321	0.00489	6.45E-06
MAPK	Mitogen-activated protein kinase	-11.89218	1.54E-06	4.89E-10

RNA5SP209	RNA, 5S ribosomal pseudogene 209	-4.61531	0.00154	1.43E-06
PCDH20	Protocadherin 20	-13.03849	6.19E-13	1.51E-17
TP53INP1	Tumour protein p53 inducible protein 1	-4.32553	0.03742	0.00009
TRDN	Triadin	-8.12883	0.01393	0.00002
TNFRSF19	TNF receptor superfamily member 19	-9.40398	0.00077	6.57E-07
WNK1	WNK lysine deficient protein kinase 1	-10.51757	0.00159	1.55E-06
SNORA22	Small nucleolar RNA, H/ACA box 22	-9.16908	0.00181	1.81E-06
RNA5SP393		-9.72039	0.00130	1.14E-06
AMN1	Antagonist of mitotic exit network 1 homolog	-4.05730	0.01863	0.00003
SPHK2	Sphingosine kinase 2	-8.24149	0.01155	0.00002
RBAG4	BCL2 associated athanogene 4	-6.63597	8.78E-08	1.50E-11
PHBP8	Prohibitin pseudogene 8	-9.72123	0.02849	0.00006

IL20RB-AS1	IL20RB antisense RNA 1	-10.72208	9.91E-08	2.16E-11

APPENDIX I: DIFFERENTIAL GENE EXPRESSION OF VILLAGE GOAT POPULATIONS RAISED UNDER INTENSIVE SYSTEM

Gene Name	Gene Description	Log2 FC	FDR	*P*_value
Village Up-Regulated				
NDUFB7	NADH:ubiquinone oxidoreductase subunit B7	4.28949	0.00303	2.2904E-06
NRCAM	Neuronal cell adhesion molecule	6.65436	0.02848	0.00003
AAMDC	Adipogenesis associated Mth938 domain containing	4.89556	0.00263	1.92971E-06
NEDD4	NEDD4 E3 ubiquitin protein ligase	6.15567	0.01827	0.00002
TMCC1	Transmembrane and coiled-coil domain family 1	3.34148	0.02177	0.00002
Village Down-Regulated				
INO80C	INO80 complex subunit C	-9.47219	0.00052	2.56613E-0

TFB2M	Transcription factor B2, mitochondrial	-9.35579	0.00145	8.71482E-07
CD38	Cluster of differentiation	-4.15993	0.01467	0.00001
LOC108637308	Small nuclear ribonucleoprotein G pseudogene	-9.45119	0.00057	2.88757E-07
SLC30A6	**Solute carrier family 30 member 6**	-10.64268	0.03149	0.00004
DPM3	Dolichyl-phosphate mannosyltransferase subunit 3	-9.74496	0.00087	4.63269
SRRM2	Serine/arginine repetitive matrix 2	-9.49984	0.00360	2.87990
SPHK2	sphingosine kinase 2	-8.92832	0.01827	0.00002
MAN1C1	Mannosidase alpha class 1C member 1	-10.07764	0.04608	0.00006
CAPN13	calpain 13	-11.98484	1.02885E-05	3.20051E-09
GRIN1	Glutamate ionotropic receptor NMDA type subunit 1	-13.21219	0.04813	0.00007
PRKAA2	Protein kinase AMP-activated catalytic subunit alpha 2	-12.62486	0.00254	1.75E-06
SLN	Sarcolipin	-15.34021	0.03149	0.00004
TRDN	Triadin	-5.28741	0.00224	1.49E-06

COMMD2	COMM domain containing 2	-14.51851	0.01829	0.00002

APPENDIX J: DIFFERENTIAL GENE EXPRESSION OF BOER GOAT POPULATIONS RAISED UNDER INTENSIVE SYSTEM

Gene Name	Gene Description	Log2 FC	FDR	*P*_value
Boer Up-Regulated				
MAPRE2	Microtubule associated protein RP/EB family member 2	11.17780	0.00008	3.17E-08
NDUFB7	NADH:ubiquinone oxidoreductase subunit B7	4.28949	0.00303	2.29E-06
TRAM2	Translocation associated membrane protein 2	13.67044	1.01E-11	8.97E-16
ELP2	Elongator acetyltransferase complex subunit 2	18.16198	0.00021	8.85E-08
FMNL2	Formin like 2	9.15837	0.00146	9.08E-07
MLIP	Muscular LMNA interacting protein	10.20452	0.01183	1.10E-05
GNG13	G protein subunit gamma 13	12.56977	1.01E-11	7.45E-16
NRCAM	Neuronal cell adhesion molecule	6.65436	0.02848	0.00003
PPIP5K2	Diphosphoinositol pentakisphosphate kinase 2	10.42396	0.00004	1.49E-08
NCOR2	nuclear receptor corepressor 2	8.50446	0.04831	6.66E-05

NKX2-1	NK2 homeobox 1	9.78257	0.00022	1.01E-07
PPIAP11	Peptidylprolyl isomerase A pseudogene 11	5.31748	0.00094	5.20E-07
NEDD4	NEDD4 E3 ubiquitin protein ligase	6.15567	0.01827	1.91E-05
TMCC1	Transmembrane and coiled-coil domain family 1	3.34148	0.02177	0.00002
TFDP2	Transcription factor Dp-2	8.94258	0.03199	0.00004
USP32	Ubiquitin specific peptidase 32	9.20405	0.007266	6.46E-06
BMPR1B	Bone morphogenetic protein receptor type 1B	9.71264	0.00006	2.16E-08
Boer Down-Regulated				
CD38	Cluster of differentiation	-4.15993	0.01467	1.43E-05
MYZAP	Myocardial zonula adherens protein	-11.20905	0.00003	1.08E-08
MAN1C1	Mannosidase alpha class 1C member 1	-10.07764	0.04608	6.04E-05
PRKAA2	Protein kinase AMP-activated catalytic subunit alpha 2	-12.62486	0.00254	1.75E-06
TRDN	Triadin	-5.28741	0.00223	1.49E-06

APPENDIX K: DIFFERENTIAL GENE EXPRESSION OF EXTENSIVELY VS INTENSIVELY RAISED POPULATIONS UNDER DIFFERENT PRODUCTION SYSTEMS

Gene Name	Gene Description	Log2 FC	FDR	*P* value
Up-Regulated				
FAM117A	Family With Sequence Similarity 117 Member A	9.17687	0.00005	3.91E-08
CXCL11	C-X-C Motif Chemokine Ligand 11	4.39234	0.01055	1.70E-06
BATF2	Basic Leucine Zipper ATF-Like Transcription Factor 2	3.20119	0.01995	6.93E-06
LOC108637289	-	4.19672	0.01995	7.23E-06
LOC108636227	-	2.81510	4.62E-05	1.86E-09
CXCL10	C-X-C Motif Chemokine Ligand 10	3.74676	0.01909	5.38E-06
CXCL9	C-X-C Motif Chemokine Ligand 9	3.16047	0.04654	2.06E-05
IDO1	Indoleamine-pyrrole 2,3-dioxygenase	3.39330	0.00785	9.48E-07
KYAT3	Kynurenine--oxoglutarate transaminase 3	2.92137	0.03699	0.00002
Down-Regulated				
TTYH2	Tweety family member 2	-10.61228	0.01664	4.02E-06
LOC102175876	hemoglobin subunit beta-A-like	-5.06983	0.01362	2.74E-06
PDK4	Pyruvate Dehydrogenase Kinase 4	-3.07692	0.04831	0.00002

APPENDIX L: DIFFERENTIAL GENE EXPRESSION OF BOER GOAT POPULATIONS RAISED UNDER INTENSIVE SYSTEM

Gene Name	Gene Description	Log2 FC	FDR	*P* value
Up-Regulated				
LOC108636730	Putative 60S ribosomal protein L37a	3.80454	0.02883	9.55E-06
COX2	Cyclooxygenase-2	5.24257	0.04873	1.82E-05
ND3	NADH dehydrogenase subunit 3	5.09231	0.01955	5.67E-06
ND4	NADH dehydrogenase subunit 4	6.11651	0.00239	3.97E-07
ND4	NADH dehydrogenase subunit 4	6.10009	0.00376	7.79E-07
KDM1A	lysine demethylase 1A	12.87533	2.81E-10	2.33E-14
KDM8	Lysine demethylase 8	10.66871	0.00036	4.52E-08
IGFBP3	Insulin Like Growth Factor Binding Protein 3	6.92172	0.01375	3.42E-06
Down-Regulated				
ANAPC4	Anaphase Promoting Complex Subunit 4	-19.18249	7.30E-22	3.02E-26