

**A case-control investigation to evaluate resistance to *Haemonchus contortus*
in SA Dohne Merino sheep**

By

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Declaration

I declare that the thesis/dissertation, which I hereby submit for the degree MSc (Agric) Animal Breeding and Genetics at the University of Pretoria, is my own work and has not previously been submitted by me for a degree at this or any other tertiary institution.

Student's Signature

Name of the student

Date

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Abstract

Gastrointestinal parasitism is a major problem to livestock productivity worldwide and small ruminant production is affected the most. Resistance of gastrointestinal nematodes (GIN) to anthelmintics is a widespread problem. Breeding animals resistant to nematode infestation has been proposed as a sustainable alternative. The aim of this study was to use high-throughput genome-wide SNP data to investigate the genetic background of GIN resistance in SA Dohne Merino sheep. The farm Wauldby in the Stutterheim district of South Africa has a history of heavy *H. contortus* challenge and implemented a selection strategy for resistance to *H. contortus* in 2011. Faecal egg count (FEC), body condition scores (BCS) and FAMACHA scores (FAM) were recorded on all lambs from weaning in January until the end of June annually. Lambs were only drenched if they had a FAM of 2.5 or higher. Breeding values (EBV) for FEC were estimated for the Wauldby animals born from 2011 to 2014. The GADI Dohne Merino flock is kept at the Grootfontein Agricultural Development Institute and has never been subjected to selection for resistance to GIN. FEC ranged from 0 to 52 500 among animals, recordings and years. Wauldby lambs that were not dosed had an overall lower FEC and FAM and higher BCS than lambs that were dosed once or more. Wauldby animals that were selected for genotyping based on EBV for FEC. Within years, animals with the highest (n= 48) and lowest (n=48) EBV for FEC were selected among the Dosed (Cases), as well as the Not dosed (Controls) (Low EBV FEC (n=52) and High EBV FEC (n=48). DNA obtained from blood samples were genotyped using the Illumina[®] Ovine SNP50 BeadChip. Principal component analysis (PCA) plot was performed using SNP & Variation Suite (SVS) from Golden Helix. Four distinct genetic clusters were observed, with the GADI Dohne Merino sheep population clustering separately. The Wauldby Dohne Merino population differentiated into 3 distinct clusters. ADMIXTURE version 1.23 was used to investigate population genetic structure. Fixation index (F_{ST}) was estimated between genetic clusters and ranged from low to moderate (0.040563 to 0.091004). The genetic diversity between the populations was assessed and observed heterozygosity (H_o), values of 0.3733 ± 0.1341 and 0.3736 ± 0.1468 were observed for the Wauldby Dohne Merino and GADI Dohne Merino sheep populations, respectively. The Wauldby animals in the different clusters were compared. Cluster 3 had lower FEC, lower FAM and higher BCS ($P < 0.01$) compared to the two other genetic clusters. FEC breeding values of 114 ± 97 , -629 ± 84 and -2 ± 45 were recorded for Cluster 2, 3 and 4, respectively. The distribution of runs of homozygosity was

determined for Clusters 1 to 4. Cluster 4 had the most animals and the highest number of ROH was observed in this genetic cluster. The results from this study indicated that it should be possible to select for resistance to *H. contortus* on the basis of the phenotypic traits.

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Abbreviations

AR	Anthelmintic resistance
ARC-BTP	Agricultural Research Council-Biotechnology Platform
BCS	Body condition score
BLUP	Best linear unbiased prediction
BZs	Benzimidazoles
CV	Coefficient of variation
DADI	Dohne Agricultural Development Institute
DAFF	Department of Agriculture, Forestry and Fisheries
DHCT	Change in hematocrit
DNA	Deoxyribonucleic acid
EBV	Estimated breeding value
EDTA	Ethylenediaminetetraacetic acid
EPW	Eggs per worm
FAM	FAMACHA [®] -score
FEC	Faecal egg count
FECGEN	Fecal egg count
F_{IS}	Inbreeding coefficients
F_{ST}	Fixation index
FWEC	Faecal worm egg count
GADI	Grootfontein Agricultural Development Institute
GDF8	Growth differentiation factor 8
GEBVs	Genomic estimated breeding values

GI	Gastrointestinal
GIN	Gastrointestinal nematodes
GS	Genomic selection
GWAS	Genome-wide association study
<i>H. contortus</i>	<i>Haemonchus contortus</i>
HCT	Hematocrit
H_e	Expected heterozygosity
HFEC	<i>Haemonchus contortus</i> FEC
HFEC_2	<i>Haemonchus contortus</i> FEC
H_o	Observed heterozygosity
HWE	Hardy-Weinberg equilibrium
IFNG	Interferon- γ gene
IRF3	Interferon regulatory factor
IVM	Ivermectin
Kb	Kilo basepairs
LD	Linkage Disequilibrium
LEV	Levamisole
LFEC	Log Faecal egg count
LSmeans	Least-square means
MAF	Minor allele frequency
MAS	Marker assisted selection
Mb	Mega basepairs
MHC	Major histocompatibility complex
MLs	Macrocylic lactones
NFEC	Nematodirus FEC

OAR	<i>Ovis aries</i> chromosome
OLA	Leukocyte Antigen
PCA	Principle component analysis
PCV	Packed cell volume
PPR	Peri-parturient rise
QC	Quality control
QTL	Quantitative trait loci
QTLdb	Quantitative Trait Loci Database
ROH	Runs of homozygosity
SA	South Africa
SAMM	South African Mutton Merino
SD	Standard deviation
SFEC	Strongyle fecal egg count
SNP	Single Nucleotide Polymorphisms
SVS	SNP & Variation Suite
TFEC_1	<i>Trichostrongylus colubriformis</i> FEC
TGF-B1	Transforming growth factor beta-1
TST	Targeted selective treatment
USA	United States of America
WORMCT	Worm count

Chapter 1: Introduction

1.1 Introduction

The domestic sheep (*Ovis aries*) is one of the first wild animals which humans successfully domesticated from the West Asiatic mouflon *Ovis orientalis Gmelin* (Zohary *et al.*, 1998). Sheep breeds arrived in South Africa alongside the Khoi people as they migrated south, to Southwest Africa (Avery, 2004). Sheep farming in South Africa (SA) is practised throughout the country. However, it is concentrated in the more arid areas such as Northern Cape, Eastern Cape, Western Cape, Free State and Mpumalanga provinces (Department of Agriculture, Forestry and Fisheries, 2017). According to Department of Agriculture, Forestry and Fisheries (2017), the total number of sheep in SA is 23,108 million. Of these total sheep numbers, 6,815 million are found in the Eastern Cape Province, 5,716 million sheep in the Northern Cape Province, 4,536 million in the Free State Province and the remaining provinces share 6,041 millions of sheep.

Goats and sheep are vital livestock, especially in Africa and Asia as they provide a range of resources including meat, fibre and milk, as well as cash income, and are a source of social security (Jackson *et al.*, 2012). Sheep in SA are kept mainly for wool and mutton production, with the Eastern Cape and Free State Provinces the biggest and second biggest wool producing provinces in the country respectively (Capewools.co.za, 2015). According to the DAFF Statistics and Economic analysis (2017), SA mutton is mainly exported to Mozambique (46%), Angola (19%) and the Democratic Republic of Congo used only once (18%) in the Southern African Development Community used only once region.

The wool industry is one of the oldest agricultural industries in SA and it plays an important role in the economy of the country (Landman, 2013) and provides stability in the small stock industry. The main sheep breeds used for wool production are the Merino followed by other dual-purpose Merino genotypes such as the Dohne Merino, South African Mutton Merino (SAMM) and the Letelle (Capewools.co.za, 2015). The Dohne Merino breed (named after the Dohne Research Institute where it was developed) was established in the year 1939 in the Eastern Cape Sourveld region of SA, from a cross between the Merino and the German Mutton (presently known as the SA Mutton Merino) and the main aim was to develop a hardy and versatile genotype (Van Wyk *et al.*, 2008). The Dohne Merino's ability to thrive under different conditions has resulted in the breed's expansion to other parts of the

country (Cloete *et al.*, 2007). The Merino and Merino type sheep breeds play an important role in the production of wool and meat in SA and these breeds have been genetically improved to adapt to the local climatic conditions (Soma *et al.*, 2012).

South Africa is the second largest fine wool producer in the world after Australia and about 90% of the wool produced in SA is exported to other countries including the United Kingdom, Germany, Japan, China, France and Italy (Landman, 2013). According to Capewools.co.za (2015), approximately 90% of the wool clip (estimated at 42.7 million kg) in the 2013/14 season (between August and June) was produced in four provinces, namely the Eastern Cape (34%), Free State (23%), Western Cape (20%), and Northern Cape (13%). The remaining 10% was produced in Mpumalanga (6%), KwaZulu-Natal (2%), North West (1%) and Gauteng (1%).

Sheep production in SA is affected by factors such as low fertility, incidences of drought, financial constraints, predators and parasites (Kunene, 2010). Parasitic nematodes are a major constraint for sheep production throughout the world (Vijayasarithi *et al.*, 2016). There is a growing concern about livestock diseases caused by gastrointestinal nematodes (GIN) in both the developing and developed world. Alba-Hurtado & Muñoz-Guzmán, (2013) reported that losses due to gastrointestinal nematodes (GIN) have been estimated to be approximately US\$ 400 million per annum in Australia and up to US\$ 26 million, US\$ 46 million, and US\$ 103 million in Kenya, South Africa, and India respectively.

The control of GINs in sheep is largely based on the use of drugs (Bakunzi, 2003). The presence of anthelmintic resistance (AR) has made the use of anthelmintic drugs to control GINs unsustainable (McManus *et al.*, 2014). The development of new and effective anthelmintics is very expensive and furthermore compounded by a variety of host-parasite related factors (De Souza Chagas *et al.*, 2016). Besides AR, the use of drugs is expensive and many farmers cannot afford it (Mpetile *et al.*, 2015). The use of chemicals have been under scrutiny in the past few years as consumers are concerned about the possible residual effects (Vijayasarithi *et al.*, 2016). These are strong reasons for the development of more sustainable, practical, realistic long-term and cost-effective helminth management strategies (Bath, 2014), such as breeding animals for genetic resistance to parasites (Alba-Hurtado & Muñoz-Guzmán, 2013). Breeding programs with the goal of enhancing host resistance to parasites may help to alleviate problems associated with the use of anthelmintic drugs in the long term (Greer & Hamie, 2016).

Haemonchus contortus is arguably one of the most economically important GIN

infecting hundreds of millions of small ruminants worldwide (Gasser *et al.*, 2016). *H. contortus* resides in the mucosal layer of the abomasum and feeds on blood in the stomach, where it alters abomasal secretion, causing gastritis, hemorrhagic anaemia and haemonchosis (De Souza Chagas *et al.*, 2016). The effects of *H. contortus* infection include reduced growth, compromised reproduction, and elevated mortality and these are due to ubiquitous distribution and severe pathogenicity of *H. contortus* (Guo *et al.*, 2016).

There is a significant variation in resistance to GINs within and between sheep breeds and this variation is due to underlying genetic diversity (McRae *et al.*, 2014a). Within breed genetic variation has been reported in various sheep populations including the Merino (Periasamy *et al.*, 2014). Selection for nematode resistance has mainly been based on the use of indicator traits such as faecal egg count (FEC) (Riggio *et al.*, 2013), FAMACHA[®] scoring (Van Wyk & Bath, 2002), and body condition score (BCS) (Cornelius *et al.*, 2014). The process of collecting and quantifying indicator traits has some challenges, e.g. it is costly, logistically difficult and time consuming, but remains worthwhile (Riggio *et al.*, 2013).

Resistance against parasitoses is based on the immunological capacities of each animal in the flock (Alba-Hurtado & Muñoz-Guzmán, 2013). The resilient sheep are able to perform when exposed to worm challenge and some have high FEC values but are not anaemic thus are not treated (Gray, 1995). Some sheep breeds display low resistance with high resilience, which allow these breeds to be as productive as the naturally resistant breeds (Alba-Hurtado & Muñoz-Guzmán, 2013). Resistance is described as the ability of an animal to suppress development of worm infection whereas resilience is the ability of an animal to maintain good health and productive parameters during the *Haemonchus* season (Riley & Van Wyk, 2009).

Advances made in molecular technology have revolutionized the field of animal breeding and genetics (Gurgul *et al.*, 2014). Next generation sequencing technologies have made the generation of sequence data much easier in comparison to previous years (Bai *et al.*, 2012). Using the whole genome sequencing methods, the genomes of most domesticated livestock including cattle, chickens, pigs, sheep, goats and horses have been sequenced (Eck *et al.*, 2009). The whole genome sequencing of livestock animals has allowed the commercial application of genome-wide Single Nucleotide Polymorphisms (SNP) chips with various densities of markers (Gurgul *et al.*, 2014). These have found application in population genomics, Genome-wide association study (GWAS), diagnostic tests, parentage verification (Moradi *et al.*, 2012) and in breed improvement programs (Cloete *et al.*, 2014). The rapid

advancements in genome sequencing and genomic technologies will help improve our understanding of the ovine host response to *H. contortus* at the molecular level and to identify polymorphisms responsible for nematode resistance (McRae *et al.*, 2014a).

The Ovine SNP50 BeadChip which became commercially available in 2008 provides 54,241 equally spaced SNPs across the sheep genome for association analysis (Mucha *et al.*, 2015). The SNP50 BeadChip also provides a fast way to detect regions under selection and could be used in the identification of genes under selection in sheep resistant or susceptible to GINs, which will help increase our understanding of the biological processes underlying host resistance and susceptibility (McRae *et al.*, 2014a). The availability of a very large number of single nucleotide polymorphisms (SNPs) throughout the genome also provides a way to detect regions where a reduction in heterozygosity has occurred and offers the opportunity to estimate inbreeding and diversity more accurately at the genome level based on runs of homozygosity (ROH) (Mastrangelo *et al.*, 2017). Runs of homozygosity are lengthy, contiguous segments of identical genotypes which are without heterozygosity in the diploid state (Ferenčaković *et al.*, 2013b). Over the past 5 years, the Dohne Merino sheep population at Wauldby has been subjected to selection for GIN resistance therefore the frequency of long ROHs is expected to be high within this population and the parasite resistance traits are expected to be concentrated within these regions.

The farm Wauldby in the Stutterheim district has a well-documented history of heavy *H. contortus* challenge and resistance of the prevalent *H. contortus* to most of the available anthelmintics (Macrocyclic lactones, Imidazoles, Benzimidazoles, Halogenated salicylinalides and Organophosphates) (Snyman, 2016a, 2016b). Over the years, the Dohne Merino sheep on the farm have been subjected to selection for resistance to *H. contortus* using FEC, FAMACHA[®] scoring and BCS methods to identify animals showing clinical signs after natural *Haemonchus* challenge. Since 2012, animals that did not need any anthelmintic treatment were selected and kept as a resistant line. Sires were only selected from animals that did not need any treatment. Data collected on these animals over the past five years were used in this study. Animals from the Grootfontein (GADI) Dohne Merino flock, which have never been subjected to selection for resistance against GINs, were included in the study as a reference population.

This study was designed as a case-control study to determine genetic differences between resistant (Control) and susceptible (Case) sheep. An investigation of the genetic differences in resistant and susceptible lines can provide information that can be used to

control GINs as drenching with anthelmintics has become largely ineffective.

1.2 Aim of the study

1.2.1 Hypothesis

- i. Resilient/Resistant and susceptible Dohne Merino sheep at Wauldby have diverged significantly to constitute distinct genetic clusters.
- ii. Differences in FEC, FAM and BCS are observed amongst the different genetic clusters of the Wauldby and GADI Dohne Merino sheep.

1.2.2 Aim & Objectives

The aim of this study was to use high-throughput genome-wide SNP data to investigate the genetics of GIN resistance in SA Dohne Merino sheep. The first objective of the study was to investigate differences in FEC, BCS and FAM amongst resistant and susceptible Dohne Merino sheep belonging to the Wauldby and GADI flocks. The second objective was to investigate the genetic diversity and flock clustering of the Wauldby and GADI Dohne Merino sheep and its association with resistance / resilience to *H. contortus*. The third objective was to determine the prevalence and distribution of runs of homozygosity in the Wauldby and GADI Dohne Merino sheep populations.

Chapter 2: Literature review

2.1 Introduction

Gastrointestinal (GI) parasitism is a major challenge to livestock productivity worldwide and small ruminant production is affected the most, especially in the tropics where conditions are ideal for the development and transmission of nematodes (Rout *et al.*, 2011). Parasitic roundworms have a significant, long-term effect on animal health and cause animal suffering, reduced animal performance and financial losses in the industry (Preston *et al.*, 2016). The control of nematode infections is of critical importance in all sheep producing regions to prevent production and financial losses. The introduction of broad-spectrum anthelmintic drugs in the early 1960s provided a cheap and supposedly sustainable means to control GINs in livestock (Falzon *et al.*, 2014).

The modern broad-spectrum anthelmintic groups are normally used for the management of nematode parasitism in grazing animals and include Benzimidazoles, imidazothiazoles / tetrahydropyrimidines and macrocyclic lactones (Vijayasarithi *et al.*, 2016). These drugs rapidly became the core for GIN control (Riggio *et al.*, 2013), and reliance on the usage of chemicals as the only treatment strategy for controlling GINs has inevitably led to parasitic nematodes gradually developing resistance against all of the main anthelmintic classes (McManus *et al.*, 2014), threatening the health, welfare and production of small ruminants (Geurden *et al.*, 2014).

Anthelmintic resistance (AR) has been reported in most sheep producing regions such as Australia (Falzon *et al.*, 2014), New Zealand (Hooda *et al.*, 1999; Kenyon *et al.*, 2009), North, Central and South America (De Graef *et al.*, 2013), Africa (Van Wyk & Van Schalkwyk, 1990; Vatta *et al.*, 2002), Asia (Kenyon *et al.*, 2009) and Europe (Jackson & Coop, 2000; Alba-Hurtado & Muñoz-Guzmán, 2013). AR is predominantly prevalent for species such as *Haemonchus contortus*, *Trichostrongylus* and *Teladorsagia circumcincta*, *Fasciola hepatica* and *Nematodirus* (Jackson *et al.*, 2012), *Ostertagia* spp. and *Cooperia* spp. (Marshall *et al.*, 2009; Várady *et al.*, 2011; Greer & Hamie, 2016).

The use of drugs is expensive and many livestock producers can't afford it. Additionally, the extensive use of chemicals in meat products could cause residual effects (Mpetile *et al.*, 2015), thus posing a health hazard to meat consumers. The use of anthelmintic drugs has been under scrutiny in the past few years as consumers are concerned about chemical residues (Vadlejch *et al.*, 2014). There is also an increasing demand by

consumers for inexpensive organic meat and milk products with less drench residues (Guo *et al.*, 2016). The use of anthelmintic drugs to fight against pathogen or parasite infections in livestock is expected to decrease in the future in order to meet consumer demands for chemical-free meat and meat products (Preston *et al.*, 2016).

The challenges associated with the use of anthelmintic drugs calls for alternative nematode control strategies that might be used to reduce anthelmintic usage in sheep without seriously compromising productivity (Bisse *et al.*, 2001). Reduced anthelmintic requirements in lambs could lower animal health cost, extend the useful life of the currently available anthelmintic drugs, meet consumer demands for minimal drug usage in livestock products and reduce the effects of nematodes on production (Dominik, 2005; Morris *et al.*, 2010).

Alternative nematode control strategies that have been proposed in extensive farming systems include increasing the animal's ability to fight infections (natural immune response) through nutritional supplementation, vaccination or through the selection of animals with strong immune systems (Greer, 2008). Targeted selective treatment (TST) and the refugia principle have also been proposed as alternative and viable approaches to limit the selective pressure that leads to AR (Vadlejch *et al.*, 2014). The refugia approach aims to minimize the development of resistance by ensuring the survival of sufficient nematodes of susceptible genotypes in the total population to dilute resistant parasites surviving anthelmintic treatment (Kenyon *et al.*, 2009; Cornelius *et al.*, 2014). TST is a refugia-based method by which only animals suffering significant production loss, showing clinical signs or health effects are subjected to anthelmintic treatment, while the unaffected animals in the flock are not dosed (Van Wyk & Bath, 2002; Kenyon *et al.*, 2009; Chylinski *et al.*, 2015).

Breeding for host genetic resistance is seen as a long-term strategy for controlling GINs in a sustainable way (McRae *et al.*, 2014a). The available options for the management of nematodes are very limited (Nieuwoudt *et al.*, 2002), and breeding for nematode resistance is considered one of the more feasible methods for the management of nematodes (Greer & Hamie, 2016). The aim of this review is to outline the approach to improve genetic resistance to internal parasites in sheep.

2.2 Disadvantages of using anthelmintic drugs

Anthelmintics may not be the most desirable approach of controlling helminth problems due to their cost and the likelihood to slow down or disrupt natural host immunity mechanisms (Thomaz-Soccol *et al.*, 2004; Preston *et al.*, 2016). The cost of the anthelmintics,

together with associated drug resistance and consumer concerns about the increasing use of chemicals in the manufacture of food products are strong reasons for the development of other options such as breeding animals for genetic resistance to parasites (Kloosterman *et al.*, 1992; Alba-Hurtado & Muñoz-Guzmán, 2013; Leathwick & Besier, 2014; McManus *et al.*, 2014).

Anthelmintic drugs are often used indiscriminately and the prolonged use of anthelmintic drugs has led to the emergence of multiple drug-resistant parasites (McManus *et al.*, 2014). According to Shalaby (2013), the use of lower or sub-optimal anthelmintic doses in order to reduce the cost of anthelmintic treatment and to avoid unforeseen outbreaks of parasitism has been reported in developing countries. Helminths are a major problem in humid climates and it is important to understand the levels of parasitism and helminth species present in order to ascertain adequately the frequency and timing of judicious anthelmintic dosing (Miller *et al.*, 2011). In humid climates, regular repeated treatments with anthelmintics may be necessary (Benavides *et al.*, 2016). However, it may not be economically feasible due to the high cost (Kaplan *et al.*, 2004; Fleming *et al.*, 2006). Reducing the frequency of anthelmintic treatments and the correct identification of animals requiring treatment slows the rate of development of *Haemonchus* resistance and also extends the effective life of commercially available anthelmintic drugs (Kearney *et al.*, 2016).

There is a general public anxiety about the increasing use of chemicals in the manufacturing of food products for human consumption. Many consumers are questioning the extensive use of chemicals in animal production because of fears of human food contamination (Greer, 2008; Vadlejch *et al.*, 2014; Mpetile *et al.*, 2015). Alternative approaches for controlling internal parasites are being considered and breeding for animals resistant to parasites will have a direct impact in terms of reducing concerns about food safety, animal welfare and environmental pollution (Morris *et al.*, 2010; Karlsson & Greeff, 2012).

2.3 Development of anthelmintic resistance

Drug resistance occurs when a susceptible population shows a decrease in response to treatment and is complete when the maximum dose of drugs that can be tolerated by the host has no effect (Jackson & Coop, 2000). Resistance is inherited and resistant nematode strains pass their resistance alleles to their progeny (De Graef *et al.*, 2013). According to Vijayasarithi *et al.* (2016), factors such as frequent deworming, treating all animals in the

flock, putting treated animals instantly onto a fresh pasture and giving infected animals an incorrect dosage, contribute to the development of AR. Continuous use of anthelmintics is one of the most important factors leading to the emergence of AR among GIN in sheep raised under commercial farming systems (Van Wyk & Bath, 2002).

The intensive use of the same class of anthelmintic drugs for a long period of time also contributes to the development of AR (Ranjan *et al.*, 2002). This type of resistance is known as side-resistance, whereas resistance against two or more drugs belonging to different anthelmintic drug groups is called multi-drug resistance (De Graef *et al.*, 2013). Identifying the major factors promoting the development of AR is very important, in order to develop appropriate measures to combat AR (Besier, 2012). The factors considered most significant include excessive frequency of treatment, under-dosing (administration of an inadequate dose), the presence of resistance genes in the treated population (Papadopoulos, 2008), the efficacy of anthelmintic drugs to remove worms with resistance genes (Stafford *et al.*, 2009), the inability of susceptible strains to establish resistance genes and number of doses per season (Rout *et al.*, 2011).

Anthelmintic resistance against tetrahydropyrimidines / imidazothiazoles (Gilleard, 2006), benzimidazoles, levamisole, ivermectin and resistance to macrocyclic lactones has been reported in European countries (Geurden *et al.*, 2014), and multiple-drug resistance has been reported in South America (Dolinská *et al.*, 2014). The effects of AR on sheep production and profitability have been reported in several studies (Crawford *et al.*, 2006; Bishop, 2012; Geurden *et al.*, 2014).

2.4 Prevalence of anthelmintic resistance in South Africa

Gastrointestinal nematodes have been studied in sheep raised under commercial farming conditions in the summer rainfall region of South Africa (Nieuwoudt *et al.*, 2002; Van Wyk & Bath, 2002). Van Wyk & Van Schalkwyk (1990) reported that South Africa was one of the countries with a high prevalence of AR in Africa and was already regarded as an AR hotspot almost 30 years ago. Bakunzi (2003) agreed that AR has become a major problem in the South African commercial sheep farming industry, making sheep farming non-sustainable in certain areas. The South African sheep farming industry has been reported as the worst affected in the world with regard to AR (Tsetetsi *et al.*, 2013) and according to Bath (2014), South Africa became an unintended world leader in the development of multiple-anthelmintic resistance. Previous studies in South Africa showed that in

approximately 90% of the sheep-producing regions, parasitic nematodes are resistant to at least 1 or more of the 5 available anthelmintic groups (Nieuwoudt *et al.*, 2002; Bakunzi, 2003). Most AR reports in South Africa from both the commercial sheep and small-scale farming sectors are concerning *H. contortus*, which has been reported as the most important nematode in sheep and goats raised under commercial farming conditions and in sheep and goats raised under small-scale resource-poor farming conditions (Tsoetsi *et al.*, 2013). There is a need for more sustainable, holistic, practical and realistic long-term helminth management strategies in sheep production systems.

2.5 Host resistance to GIN infections

Host resistance is the ability of the host to interact with and control the lifecycle of the parasite, leading to a reduced worm burden (McManus *et al.*, 2014). Genetic improvement made in host resistance against GIN infections affects the transmission of parasite infection (Bishop, 2012). Over the past few years, several efforts have been made to identify genetic variants responsible for resistance (De Souza Chagas *et al.*, 2016). However, the molecular mechanisms and biological pathways underlying host resistance to GIN infections in sheep are still not fully understood (Guo *et al.*, 2016). Variation in resistance to parasites has been found both within and between host populations for a significant number of parasite species and is influenced by both genetic and environmental factors (Hooda *et al.*, 1999).

Some sheep breeds have an ability to protect themselves when exposed to disease-causing parasitic worms. However, there are many factors affecting the individual animal's resistance to GINs, including nutritional status, breed, exposure, host sex, vaccination, age, prior exposure, reproductive status and the genotype of the animal (Bishop, 2012; Colvin *et al.*, 2012; McManus *et al.*, 2014). Knowledge of these factors is quite fundamental for understanding host resistance against nematodes. In addition, physical stress and the total number of potential disease organisms the animal is exposed to (Jovanović *et al.*, 2009), as well as variation in the major histocompatibility complex (MHC) loci (Stear *et al.*, 2009; Yasmeen *et al.*, 2014) contribute to the wide range observed. Animals of the same breed / species respond differently when exposed to pathogens or parasites due to underlying genetic diversity (Stear & Wakelin, 1998; McRae *et al.*, 2014a).

2.5.1 Non Genetic factors influencing host resistance to GIN infections

2.5.1.1 Animal nutrition

Nutrition plays a significant role in the development of immunity to GIN infections and can be used to facilitate the degree of resistance in sheep as recent studies show that sheep on high protein diets are more resistant to GIN infections (Vijayasarithi *et al.*, 2016). Overall animal health can be improved by ensuring that animals have access to quality feed and nutrients (McManus *et al.*, 2014). Introducing supplementation of by-pass protein in small ruminants lead to improved host resistance and resilience to GINs (Torres-Acosta *et al.*, 2012). Recent studies show that feeding most susceptible hosts, namely lactating sheep, pregnant females and young growing animals with dietary protein can improve the animal's resilience and/or resistance to GIN infection (Knox *et al.*, 2006; Kahn & Woodgate, 2012).

According to Louvandini *et al.* (2006), animals infected by nematodes have higher nutritional needs than non-infected animals and diet supplementation with high protein helps to improve resilience and resistance to natural infection by GINs. The increasing challenges regarding the use of anthelmintic drugs (Athanasiadou *et al.*, 2001; Iqbal *et al.*, 2007), led to the investigation of bioactive plants' properties as alternative strategies to control GIN in small ruminants (Hoste *et al.*, 2006). The administration of condensed tannin-rich diet supplementation in small ruminants results in a reduction in nematode numbers, worm fecundity and nematode egg excretion (Hoste *et al.*, 2006) with reductions of 50-60% in FEC reported (Paolini *et al.*, 2003). Some of the benefits of the ingestion of average concentrations of condensed tannins include increased weight gain, wool growth, milk secretion and improved host resistance to GINs in small ruminants (Athanasiadou *et al.*, 2001).

2.5.1.2 Sex

The sex of the host has a significant effect on its susceptibility to nematode infection. Barger (1993) reviewed the influence of host sex on the levels of resistance to nematode parasitism and reported that rams were more susceptible than ewes in natural and experimental infection with *H. contortus* and *T. colubriformis*. He reported that these differences on the levels of resistance between males and females might be due to the immunosuppressive effect of testosterone. Abuargob *et al.* (2014) stated that differences between female and males are due to difference in behavior, morphology or physiological status of sex, with males showing less intense immunity than females.

Barger (1993) reported that the different hormonal status of host sexes could

influence the immunological responses of lambs to *H. contortus*. Differences in the levels of resistance between intact castrated males and females could be due to the effect of female endocrine hormones on the immune system (Abuargob *et al.*, 2014). The consistent and clear sex differences in favor of females regarding response to GIN infections suggest that the male flock should be given more attention in order to maintain lower worm burdens or FEC values (Haile *et al.*, 2007).

2.5.1.3 Age

According to Barger (1993), lambs are less resistant to nematode infection than adult sheep. Older animals show a better response to infection than lambs (Van Wyk & Reynecke, 2011). Resistance to infection with *H. contortus* is directly related to age of lamb at the time of primary infection (Knight & Rodgers, 1974). Young lambs and kids up to approximately 8 months of age are immunologically compromised in terms of the development of immunity to nematode infection (Van Wyk & Reynecke, 2011). Younger lambs are not capable of developing a strong acquired immune response against parasitic nematodes (Napolitano *et al.*, 2008), thus making them less resistant to GIN infections than adult sheep, since acquired resistance appears to improve with age (Leask *et al.*, 2013). Acquired resistance is not manifested until young lambs are 4 to 6 months of age (Qamar *et al.*, 2009).

The significant protective immune capability is developed by 10 to 12 months of age, after lambs are regularly exposed to larval challenge (Gauly *et al.*, 2006). Weaning may also create an important stress for ewes and lambs, and may affect the rate of development of protective immune response to GIN (Barger, 1993). In a study of weaned and unweaned lambs infected with *H. contortus* larvae from 8 weeks of age, Schichowski *et al.* (2010) found that, at the age of 12 weeks, the weaned group had lower packed cell volume (PCV) and twice the mean FEC of the unweaned lambs, which indicates that they had lower resistance to the parasites than the unweaned group.

2.5.1.4 Reproductive status

Response to nematode challenge can be influenced by reproductive status, as adult ewes are relatively resistant to GIN infection except during late pregnancy and early lactation (Zajac *et al.*, 1988). Sebastiano *et al.* (2017) stated that the normal immune mechanisms that regulate GIN infection and nematode egg production in ewes are relaxed during late pregnancy and early lactation, which makes them more susceptible to parasite challenges.

During this period, ewes show a transitory rise in faecal egg count and this is known as the peri-parturient rise (PPR) (Courtney *et al.*, 1985).

Ewes giving birth to more than one lamb, may have decreased immunity to parasites compared to ewes giving birth to only one lamb (Saddiqi *et al.*, 2011; Jones *et al.*, 2012). The PPR may be due to the maturation of arrested larvae (Zajac *et al.*, 1988), a newly acquired GIN infection and increased fecundity of an existing adult worm burden (Courtney *et al.*, 1984). Temporary relaxation in immunity has been reported to begin in late pregnancy, around 3 weeks prior to lambing and continuing through to early lactation up to weaning of the lambs (Baker *et al.*, 1999). The PPR causes increased pasture contamination at the time of lambing, which exposes the very susceptible young lambs to nematode infection (Zajac *et al.*, 1988). It has been reported that lactating ewes are unable to prevent the establishment of newly acquired larvae or incoming larvae (Sebastiano *et al.*, 2017), to suppress fecundity of female worms and to expel adult worms (O'Sullivan & Donald, 1973; Woolaston, 1992).

According to Courtney *et al.* (1985), resistant breeds such as Barbados Blackbelly, St. Croix and Florida Native displayed little or no PPR compared to temperate breeds like the Rambouillet or Dorset x Rambouillet. Courtney *et al.* (1986) reported that Florida Native ewes selected for reduction in FEC showed a reduced PPR when they were grazed on contaminated pastures without drenching. A study by Woolaston (1992) in which lambs were selected for increased or decreased resistance to *H. contortus*, showed that lambs selected for increased or decreased resistance against *H. contortus* displayed similar differences in resistance as PPR ewes. The PPR is affected by factors such as reproductive performance of the ewe; ewes with twins show a higher PPR than ewes with singles (Courtney *et al.*, 1986). Increased supply of metabolizable protein can help to enhance the resistance of lambs and PPR ewes to GIN infection (Sebastiano *et al.*, 2017).

2.5.2 Genetic factors influencing host resistance to GIN infections

2.5.2.1 Between breed variation in host resistance to GIN infections

A number of studies (Wanyangu *et al.*, 1997; Baker *et al.*, 2003; Gruner *et al.*, 2003; Nimbkar *et al.*, 2003) compared locally adapted and commercial sheep breeds with regard to their ability to resist GINs. The results show that the relatively unselected locally adapted breeds are more resistant to and resilient against GIN infections compared to commercial breeds (McManus *et al.*, 2014). Significant variation has been reported between sheep breeds such as the Red Maasai, Garole, Gulf Coast Native, Rhon and Barbados Black Belly on their

ability to resist GIN infections (Periasamy *et al.*, 2014). Marshall *et al.* (2013) reported that the Red Maasai sheep were more resistant to and resilient of GIN infection compared to the Dorper sheep, which were relatively more susceptible under field conditions in sub-humid coastal areas of Kenya. In their study, Dorpers had the highest egg counts and the Red Maasai the least.

Red Maasai lambs had significantly ($P<0.001$) higher packed cell volume than Dorper lambs. This breed difference in performance is probably due to the fact that the Red Maasai are native to specific areas of East Africa, which are prone to high parasite challenges and the Dorper (widely kept in Kenya) originated in South Africa in the 1940s from a cross between the European Dorset Horn and the Black Head Persian breeds (Baker *et al.*, 2004). McManus *et al.* (2014) stated that the Red Maasai flocks were two to three times more productive than the Dorper flocks under these sub-humid conditions favorable to the parasitic nematodes. The increased GIN resistance and resilience of the Red Maasai in comparison with the Dorper has been shown to translate into enhanced performance (Bishop, 2012).

In a study conducted by Mugambi *et al.* (1997), the Red Maasai breed displayed higher resistance to *H. contortus* compared to Blackheaded Somali and Dorper sheep and all three breeds were considerably more resistant than the Romney Marsh breed. A study by Wanyangu *et al.* (1997) confirmed these results as Red Massai sheep displayed higher resistance than the Dorpers, based on FEC values. The Red Massai sheep had lower FEC and higher immunological parameters after infection with *H. contortus*. There was a significant difference in FEC values between Dorper and Red Maasai sheep breeds where the Red Maasai breed had lower FEC after they were exposed to *Haemonchus* challenge (Baker *et al.*, 2003).

Courtney *et al.* (1985) demonstrated that Florida Native sheep were more resistant than the exotic breeds (Suffolk or Rambouillet) when they were exposed to *Haemonchus* challenge. Breeds such as Criollo sheep native to the central Mexican Plateau and Romney sheep display low resistance with high resilience (Alba-Hurtado *et al.*, 2010; Morris *et al.*, 2010), which allow these breeds to be as productive as the naturally resistant breeds (Alba-Hurtado & Muñoz-Guzmán, 2013).

It has been reported that breeds such as Barbados Blackbelly, U.S. St. Croix, Florida Native and Gulf Coast Native breeds, Indonesian Thin tail, Indian Garole, and African Red Maasai appear to have enhanced helminth resistance and are currently being used in genetic studies that aim to identify genes that confer nematode resistance (Bishop, 2012; McRae *et*

al., 2014a; Periasamy *et al.*, 2014). According to Karlsson & Greeff (2012), a high parasite load seems to have induced breed-specific selection pressure resulting in breeds that are more resistant than others. However, according to Amarante & Amarante (2003), resistant breeds are generally poorly productive thus replacing a susceptible, productive breed with a resistant breed is not always a viable option.

2.5.2.2 Within-breed variation in host resistance to GIN infections

Woolaston & Piper (1996) demonstrated that genetic variation within flocks of the same breed exists and can be used to select for internal nematodes resistance. Within-breed genetic variation has been reported in various sheep populations including the Red Maasai (Baker *et al.*, 2003), Merino (Nimbkar *et al.*, 2003), Romney (Morris *et al.*, 2000), Scottish Blackface (McRae *et al.*, 2014b), feral Soay sheep (Smith *et al.*, 1999), Garole (Nimbkar *et al.*, 2003), Gulf Coast Native (Miller *et al.*, 2006) and Barbados Black Belly (Gruner *et al.*, 2003). Parasite resistance in sheep varies among individuals and selective breeding has been successfully carried out with various sheep breeds (Valilou *et al.*, 2015).

Romney sheep and Merino flocks have been successfully selected for resistance against *H. contortus* and *Trichostrongylus colubriformis* (Dominik, 2005). Most within-breed studies of genetic resistance use FEC as the indicator trait for resistance (Morris *et al.*, 2010). Previous studies showed that within-breed selection for the FEC is an effective way of reducing the use of anthelmintic drugs and reducing pasture contamination with the eggs of parasitic nematodes (Bishop & Morris, 2007; Bishop, 2012; McManus *et al.*, 2014). In their reviews, these authors found significant FEC heritabilities ranging between 0.2 and 0.4, together with wide between-animal variation in FEC and concluded that the heritability of the FEC as a measure of resistance varies considerably depending on both the nematode species and breed studied.

2.5.2.3 Variation in immune response genes

There are many physiological pathways involved in the prevention of the establishment of GINs and the pathways that trigger immune responses varies depending on immune state of the sheep (Dominik, 2005). Immune response is undoubtedly involved in the genetic resistance of the host to GIN infections and there is genetic variation in the hosts' ability to respond to infections (Gray, 1995). The major histocompatibility complex (MHC) is believed to be involved in immune response mechanisms which lead to resistance to GINs

(McManus *et al.*, 2014).

The MHC was first discovered in mice (Reese *et al.*, 2007) and since then, the MHC region has been studied extensively in various species due to its involvement in immunological induction and regulation processes and also due to their high polymorphism (Subramaniam *et al.*, 2015). The MHC contains a hallmark of genes that are responsible for the adaptive immune response in vertebrates (Davies *et al.*, 2009), and could be used as markers for breeding to increase animal's resistance to GINs (Valilou *et al.*, 2015). In sheep, the MHC has been mapped to chromosome 20 between the q15 and q23 bands and its polymorphic portion is known as the ovine leukocyte antigen (*OLA*) or sheep lymphocyte antigen (Polat *et al.*, 2015).

Dukkipati *et al.* (2006) stated that alleles of different MHC genes are associated with disease resistance in sheep and also that certain MHC alleles are correlated with parasite resistance in sheep. The *OLA* is poorly described and there's limited information available about this MHC compared to other farm animal species (Yasmeen *et al.*, 2014). Several studies have been done in sheep to investigate the involvement of MHC genes in genetic resistance to diseases caused by gastrointestinal nematodes (Bozkaya & Kurar, 2005; Dukkipati *et al.*, 2006; McManus *et al.*, 2014). It has been shown that *OLA* class I, II and III regions are involved in disease resistance and *Ovar-Mhc* genes have been found to be associated with traits such as marbling and birth weight (Dukkipati *et al.*, 2006). The *OLA* class II plays a significant role in recognizing foreign pathogens and the DRB region of the MHC class II genes is highly polymorphic (Polat *et al.*, 2015). The differences in genetic polymorphism in the MHC genes play a vital role in disease resistance or susceptibility in a population (Miller & Horohov, 2006).

An allele situated in exon two has been associated with a significant reduction in FEC in the Scottish Blackface breed (Sayers *et al.*, 2005). Stear *et al.* (2009) reported that there is a strong association between polymorphism at the DRB1, classII locus and FEC in older Scottish Blackface lambs. This relationship strongly suggests that the polymorphism at the MHC locus could be used as a marker for selective breeding to increase resistance to various diseases (Dukkipati *et al.*, 2006). Two candidate genes (IRF3 = interferon regulatory factor and TGF-B1 = transforming growth factor beta-1) have been identified in chromosome 14 of the ovine genome (Riggio *et al.*, 2014b). IRF3 has been located to a region affecting health traits, with host QTL controlling various pathogens (Jann *et al.*, 2009). TGF-B1 plays a significant role in the regulation of immune responses and is involved, together with IL-10, in

reducing pathology and enhancing tissue repair during GIN infections (Belkaid *et al.*, 2006).

Schallig (2000) demonstrated that young lambs were unable to build immunological responses against *H. contortus* due to weak Th₂ responses. Pernthaler *et al.* (1997) reported that Th₂-type cytokines i.e. elevated IL-4 mRNA expression levels in the gastrointestinal lymphatic tissue, played an important role in immune responses of sheep genetically resistant to *T. colubriformis*. The identification or study of genes in the host immune system makes breeding animals for disease resistance a possibility, impelling researchers to investigate the role played by host genetic variation in disease resistance in livestock (Davies *et al.*, 2009). The correct evaluation of individual allelic diversity at MHC loci is important and can help to understand the functional significance of genetic polymorphism in the MHC genes (Sommer *et al.*, 2013). Understanding how high polymorphic diversity is maintained at the MHC loci and the role played by selection in shaping the MHC diversity and how MHC variation affects disease resistance in farm animals is of major importance (Dukkipati *et al.*, 2006; Yasmeen *et al.*, 2014). This can lead to the development of proper selection techniques to enhance the involvement of the MHC genes for resistance to infectious diseases, which are prevalent in farm animal species (McManus *et al.*, 2014).

2.6 Phenotypic traits associated with nematode resistance

Selection for nematode resistance has mainly been based on the use of indicator traits such as body condition score (BCS) and body weight (Cornelius *et al.*, 2015), faecal egg count (FEC) (Riggio *et al.*, 2013), packed cell volume (PCV) (Guo *et al.*, 2016) and FAMACHA[®] scoring (Van Wyk & Bath, 2002). These traits are obvious goal traits and should be improved (Bishop, 2012). The process of collecting and quantifying indicator traits has some challenges e.g. it's costly and time consuming, logistically difficult and requires the animals to undergo parasitic challenge (Riggio *et al.*, 2013). Susceptible animals succumb to infection and deliver poor performance thus has to be subjected to treatment for parasitism or be culled (Bath, 2014). The best criteria to select animals are chosen based on the epidemiology of GINs and the type of management system used in a particular population (Gallidis *et al.*, 2009). Some of the most commonly used indicator traits will be discussed in more detail.

2.6.1 Faecal egg count

Faecal egg count is a method used to determine the number of internal parasite eggs in a particular dung sample (Marshall *et al.*, 2009). This method gives some valuable information about the presence of potential GINs, however, the interpretation of numbers of eggs per gram (epg) of faeces is subjective and that limits the value of information provided by FEC (Sargison, 2016). The interpretation of FEC depends upon factors such as knowledge of the relative faecal dry matter content, feed intake and the way in which the animals were given food prior to or at the time of sampling (Hooda *et al.*, 1999; Sargison, 2016). Individual FEC values are not regarded as a useful indicator of whether a sheep requires dosing for *Haemonchosis* or not, as resilient sheep normally have high FECs. It is however a useful indicator of parasitic internal nematode resistance development in the flock (Marshall *et al.*, 2009).

FEC has a very wide range of previously reported heritability values, ranging from 0.01 to 0.65 (Zvinorova *et al.*, 2016), with a wide phenotypic variability between individuals (Bishop & Woolliams, 2014). This heritability indicates that FEC can be moderately useful when selecting for resistance or against susceptibility (Alba-Hurtado & Muñoz-Guzmán, 2013). FEC is genetically correlated ($r_g \sim 0.7$) to nematode load in an individual animal (Cloete *et al.*, 2007), although this correlation varies between GIN species and host breed investigated (Hooda *et al.*, 1999).

The FEC level above which treatment with an effective anthelmintic drug should be initiated can differ significantly depending on factors such as the composition of the parasite species, sheep breeds, and the overall health of the animals (Kenyon *et al.*, 2009; Chylinski *et al.*, 2015). Selection for host resistance in small ruminants using FEC and selecting animals with the lowest FEC in the flock would increase host resistance leading to increased genetic gain (Cloete *et al.*, 2007).

2.6.2 FAMACHA[®] scoring & Hematocrit value

FAMACHA[®] is an abbreviation derived from the name of the originator of the idea, Dr. Faffa Malan (FAffa MAlan CHArt) and was developed in South Africa to support parasite control using target selective treatment (Van Wyk & Bath, 2002). This method uses anaemia, determined based on the color of the lower eyelid mucous membrane in goats and sheep, as a disease marker for level of *Haemonchosis* (Di Loria *et al.*, 2009). Only animals with high FAMACHA[®] scores (3-5), with increasing pale color are subjected to anthelmintic

treatment (Riley & Van Wyk, 2009), thus decreasing the number of sheep treated (Stafford *et al.*, 2009). The FAMACHA[®] clinical evaluation system provides a practical, low-cost alternative in areas where *Haemonchus sp.* is prevalent (Riley & Van Wyk, 2009). FAMACHA[®] scoring is a viable method to identify GI resistance/resilience traits in sheep (Di Loria *et al.*, 2009), as FAMACHA[®] scores are heritable (h^2 : 0.24 - 0.55) in sheep and does not require laboratory facilities (Riley and Van Wyk, 2009). This system was developed to help reduce AR in sheep in South Africa by reducing the use of anthelmintic drugs (Vatta *et al.*, 2002).

The FAMACHA[®] scoring has some shortcomings, as the method is not useful if animals are infected with multiple parasites with no predominant species present (Moors & Gauly, 2009), and is not well suited to areas that have a high prevalence of non-haematophagous parasites (Greer *et al.*, 2009). The FAMACHA[®] system is an effective criterion to identify animals that need to be treated or culled (Riley and Van Wyk, 2009), however, it is important to maintain proper FAMACHA[®] records, if this trait is to be used as a tool for the long-term genetic selection of resistant/resilient animals (Van Wyk and Bath, 2002).

Hematocrit count, also called packed cell volume (PCV), represents the proportion of the volume of red blood cells to the total volume expressed as a percentage (Benavides *et al.*, 2016). *H. contortus* is a blood-sucking parasite and results in the depletion of the red blood cells in severely affected animals. PCV indicates the level of infection or worm burden based on the amount of blood available (Moors & Gauly, 2009). Packed cell volumes drop about 10 to 12 days post-infection, and chronic infection with *Haemonchus* results in the reduction in PCVs compared to resistant/resilient animals (Mederos *et al.*, 2014).

Hematocrit values range from 27 to 45, 22 to 38 and 24 to 46 in sheep, goat and cattle respectively (Crawford *et al.*, 2006; Moors & Gauly, 2009). PCV has a heritability ranging from 0.29 to 0.49 (Vanimisetti *et al.*, 2004), and is negatively genetically correlated (-0.09) with FEC and positively correlated (0.35) with live weight (Vanimisetti *et al.*, 2004; Riley & Van Wyk, 2009). Selection for host resistance would thus be to decrease FEC and increase PCV and live weight gain (Bishop, 2012). PCVs are however costly, logistically difficult and time-consuming to collect and therefore impractical for many farmers, especially those in remote areas (Zvinorova *et al.*, 2016). Selection for parasite resistance in sheep using FAMACHA[®] score are feasible, effective, less cost-effective and much more practical alternatives to PCV or FEC (Riley and Van Wyk, 2009).

2.6.3 Body condition score

Body condition score (BCS) is a practical measure used as an indicator of overall body condition of an individual animal and is normally used as an indicator of resilience to parasitic worm infections (Van Burgel *et al.*, 2011; Cornelius *et al.*, 2014). Body condition score ranges from 1 to 5, where a score of 1 indicates emaciated animals and a score of 5 indicates over-fat animals (Cornelius *et al.*, 2015). Animals with BCS ≤ 2 should be treated with anthelmintics (Gallidis *et al.*, 2009). This method is useful in breeding ewes; however it has been reported to be inconsistent in growing lambs (Stafford *et al.*, 2009). Individual BCS is not a good indicator of infection with *H. contortus* (Burke *et al.*, 2007). Recent studies in Western Australia showed that ewes with the lowest BCS demonstrated a greater BCS response to drug treatment compared to ewes with the higher BCS when nutrition was low and they were exposed to a high *Haemonchus* challenge (Cornelius *et al.*, 2014; Cornelius *et al.*, 2015).

This suggest that treatments should be given to ewes in poorest BCS and ewes in better body condition (BCS>3.0) can be used as a source of refugia for worms of lower AR status (Cornelius *et al.*, 2015). Cornelius *et al.* (2014) indicated that selecting sheep for treatment based on low BCS is an appropriate selection indicator for TST. According to Bath & van Wyk (2009), there are several clinical conditions other than GI parasitism that can result in animals losing body condition and these include poor nutrition and paratuberculosis. In a study by Vatta *et al.* (2002) to evaluate the effect of *Haemonchus* infection on FEC, FAMACHA[®] and BCS in sheep raised under resource-poor conditions in South Africa, there was no obvious relationship between FEC and BCS.

2.7 Breeding sheep for resistance to nematode infections

There is a need for control strategies that try to reduce the use of anthelmintics, thus slowing down the process of AR development, or try to replace anthelmintics completely (Vagenas *et al.*, 2002). Two terms are commonly used to describe the conflict between the host animal and parasite, namely resistance and resilience. Resistance is described as the ability of an animal to suppress development of worm infection whereas resilience is the ability of an animal to maintain good health and productive parameters although infested during the challenge season (Riley & Van Wyk, 2009). The highly resistant sheep have a lower parasitic load than susceptible animals, as the host is able to reduce the number of worms that reproduce or survive, with lower impact on production and thus fewer drenches

are required to control the nematode infection (Marshall *et al.*, 2009).

Host resistance to internal nematodes is a physiologically complex and a largely polygenic trait (Dominik, 2005; McManus *et al.*, 2014), while the environment also contributes to the phenotypic variation (Geurden *et al.*, 2014). Resistance against parasitoses is based on the immunological capacities of each animal in the flock (Alba-Hurtado & Muñoz-Guzmán, 2013). The lower number of eggs excreted by the resistant sheep lead to reduced larval contamination of pastures (Bishop, 2012), which benefits all the animals grazing in the same pasture, irrespective of their resistance status (Riley & Van Wyk, 2009). This trait is quantifiable through the performance of individual animals after *Haemonchus* challenges (Benavides *et al.*, 2016).

The process of breeding for enhanced nematode resistance involves correct quantification of a desirable phenotype, and there must be variation in the phenotype that is attributed to genetic variation between animals (Karrow *et al.*, 2013). Quantifying variation in resistance and developing the relationship between indicator traits and performance traits is significant when breeding for nematode resistance (Rout *et al.*, 2011). Breeding of sheep for resistance to nematodes is an alternative method to alleviate the problems associated with the use of anthelmintic drugs as it represents a permanent solution requiring no additional resources for maintenance (McManus *et al.*, 2014).

By using sheep that are inherently highly resistant/resilient to parasites in breeding programs, the costs of managing parasites causing diseases could be managed and consumer and environmental concerns regarding the high rates of use of chemicals could be assuaged (Nieuwoudt *et al.*, 2002). Selection for genetic GIN resistance is aimed at increasing favorable alleles at loci that are related to immune response (Heckendorn *et al.*, 2017).

Breeding for host resistance also adds variety to the available anthelmintic management approaches and reduces dependence on anthelmintic drugs (McRae *et al.*, 2014a). Sheep that are susceptible to nematodes also benefits from the introduction of nematode-resistant sheep in a flock because this lowers pasture parasite load (Besier, 2012). Breeding small ruminants for enhanced resistance and/or resilience to nematode parasites should lead to sustained improvements in animal health and performance (Bishop & Morris, 2007), and rapid genetic progress has been shown in research and commercial breeds (Morris *et al.*, 2000; Kemper *et al.*, 2009; Kemper *et al.*, 2013).

Improving host resistance should lower the rate in which the infection is transmitted between animals, whereas enhancing resilience will reduce the clinical signs of

Haemonchosis but may not necessarily reduce the transmission of GIN infection (McManus *et al.*, 2014). However, there is a possibility that breeding based on a nematode resistance phenotype may have negative effects on other traits (Karlsson & Greeff, 2012). Notwithstanding the potential disadvantages, there are considerable benefits to breeding for enhanced nematode resistance and these include the fact that genetic change is permanent thus resistance will last through the sheep's lifespan (Kloosterman *et al.*, 1992; Morris *et al.*, 2010).

The resilient sheep are able to perform while exposed to worm challenge. Some animals will have high FEC values but will not be anaemic and thus won't be treated (Gray, 1995). The ability of sheep to develop immunity and express resistance to nematode infections varies significantly between and within breeds and is under genetic control (McRae *et al.*, 2014a). Some sheep breeds display high resistance and resilience to *Haemonchus*, simultaneously (Morris *et al.*, 2010).

One of the main benefits of having resilient animals in a flock is to preserve refugia, as the presence of resilient animals' results in fewer animals that are identified as susceptible animals that need to be subjected to anthelmintic treatment (Riley & Van Wyk, 2009). The presence of both resistant and resilient sheep in a flock results in the reduction in selection pressure against AR (Kenyon *et al.*, 2009). Both resistance and resilience are believed to be involved in limiting the detrimental effects of parasitic nematodes on the health and productivity of sheep (Bisset *et al.*, 2001).

The moderate heritability ($h^2=0.01$ to $h^2= 0.65$) (Zvinorova *et al.*, 2016) of resistance suggests that there is a potential to enhance nematode resistance through selective breeding or by introgression (introducing resistance alleles into susceptible breeds) (De Souza Chagas *et al.*, 2016). This heritability suggests that selection for resistance is feasible and selecting sheep that are genetically resistant to parasitic nematodes provides a long-term solution (Vagenas *et al.*, 2002; Crawford *et al.*, 2006). However, mechanisms responsible for resistance are not fully understood (Atlija *et al.*, 2016).

Understanding of mechanisms underlying genetic resistance and accurate identification of genetically resistant individual sheep can help increase the efficiency of worm control. Identification of genetic markers associated with parasitic nematode resistance, resilience or susceptibility is of major importance and could play a significant role in future marker assisted selection of different selection lines without the need to expose animals to parasite challenge (Riggio *et al.*, 2013; McRae *et al.*, 2014a).

It is important that the sheep industry investigate approaches that minimize reliance on chemical control and that non-chemical technologies are developed (Besier, 2012). In the current study the focus was on resistance, not resilience because resilience is less strongly heritable (0.10 to 0.19) and more difficult to measure in commercial farming situations than resistance (Bisset *et al.*, 2001). There is also a poor relationship between FEC and resilience in sheep under *Haemonchus* challenge, as these animals will not necessarily be suffering most from GINs (Morris & Bisset, 1996).

2.8 Genomic tools available to study population genetic structure and genetic diversity

2.8.1 Population genetic structure

Knowledge of the genetic population structure and origin of livestock animal species is fundamental for the successful implementation of genomic selection, targeted marker-assisted breeding, and quantitative trait loci (QTL) detection using genome-wide association studies (GWAS) (Beynon *et al.*, 2015). In genetic case-control studies, different methods are used to compare the frequency distribution of marker alleles or genotypes between case and control groups (Devlin & Roeder, 1999). According to Wu *et al.* (2011), a difference in the frequency of an allele or genotype of a variation between cases and controls suggest the likelihood that the genetic marker may have a contributing effect on the disease or trait of interest.

Population stratification is a major problem in case-control studies if the disease prevalence differs between subpopulations, as differences in ancestry distributions between cases and controls can lead to spurious associations (Marchini *et al.*, 2004). Population stratification is most challenging when some of the SNPs are highly differentiated while the majority of SNPs have comparable allele frequencies across populations and this occurs when a particular SNP is under strong population-specific selection (Luca, 2007).

The advent of high-throughput genotyping assays provided population geneticists with an opportunity to use genome-wide markers to study the histories of many species including sheep (Kijas *et al.*, 2009) and cattle (Gautier *et al.*, 2010). Differentiation between populations is one of the most important subjects of the field of population genetics and the level of variation between subpopulations have been compared with the level of variation in the total population in studies of distributions of genetic variation (Jakobsson *et al.*, 2013).

Population classification approaches such as genomic control, structured association (ADMIXTURE and STRUCTURE) (Patterson *et al.*, 2006), principal component analysis

(PCA) and fixation index (Köhler & Bickeböllner, 2006; Luca, 2007), are used to solve the problem of spurious associations. However, genomic control and structured association approaches have limitations when applied to large samples with huge panels of SNPs, as the effect of stratification increases with sample size (Luca, 2007). The PCA analysis combines principal components with modern statistics to test for population structure and application of this method to genetic data has become a standard tool (Patterson *et al.*, 2006).

Cavalli-Sforza *et al.* (1994) demonstrated that principal components, when displayed in two dimensions, reveal the geographical distribution of populations and also found high correlation between genetic and geographic distances in populations that were geographically close. In GWAS, PCA has been used to investigate ancestry differences between cases and controls along continuous axes of variation (Wu *et al.*, 2011).

Wright's fixation index (F_{ST}), is a measure of genetic differentiation among subpopulations (Wright, 1951). According to Duforet-Frebourg *et al.* (2015), the F_{ST} statistic group individuals into populations. STRUCTURE is a program used to assign the samples to distinct subpopulation clusters and then combines proof of association within each cluster (Price *et al.*, 2004). However, when a large amount of data (thousands of samples and markers) is used, STRUCTURE becomes impractical due to its computational intensive nature (Paschou *et al.*, 2007).

ADMIXTURE and STRUCTURE algorithms estimates the allele frequencies and admixture proportions under the assumption that sampled genotypes are derived from one of "K" ancestral populations (Patterson *et al.*, 2006). These algorithms have been widely used to determine population structure (Gaouar *et al.*, 2015), to measure the ancestral admixture (Ding *et al.*, 2011; Decker *et al.*, 2014) and to understand complex evolutionary theories about population evolution (Köhler & Bickeböllner, 2006; Peter, 2016). In the present study, PCA based clustering, ADMIXTURE and Wright's fixation index (F_{ST}) were used to investigate the population genetic structure of the Wauldby Dohne Merino and Grootfontein Dohne Merino sheep populations

2.8.2 Genetic diversity

Understanding the genetic structure and overall diversity of livestock species is essential to exploit the potential of GWAS, genomic prediction and implementation of successful conservation of genetic resources (Al-Mamun *et al.*, 2015). Maintaining genetic diversity in natural populations is of major importance (Brito *et al.*, 2017). Recent advances

in genomic tools offer opportunities to improve, utilize and conserve farm animal's diversity (Buduram, 2004). The loss of genetic diversity within and among breeds is disadvantageous because lost genes may be of future economic importance (Beynon *et al.*, 2015).

High rates of loss of genetic diversity within breeds, decreases the chances of breed survival owing to decreased fitness as a result of inbreeding depression (Andersson, 2012). The level of genetic diversity in a population is an important indicator of the amount of phenotypic data required to obtain accurate genomic breeding values (GEBVs) and is also crucial when interpreting GWAS data as high levels of genetic diversity reduces the probability that highly significant markers are at a large distance from the QTL responsible for variation in phenotype (Al-Mamun *et al.*, 2015; Brito *et al.*, 2017).

Genetic diversity can be estimated from both pedigree and molecular marker data, the genetic diversity estimates are more accurate when marker data is used (Arranz *et al.*, 2001). The introduction of high-density single nucleotide polymorphism (SNP) arrays have made it possible to estimate genetic diversity parameters in livestock at a much higher level of definition than in previous years when pedigree and molecular marker data were used (Blackburn *et al.*, 2011). There are a number of methods used to estimate genetic diversity using marker data and these include observed heterozygosity (H_o) and expected heterozygosity (H_e) (Zenger *et al.*, 2011), effective population size (N_e) (Flury *et al.*, 2010), runs of homozygosity (ROH) and linkage disequilibrium (Purfield *et al.*, 2012).

Heterozygosity (H_o and H_e) is one of the most extensively used genetic diversity parameters and this method measures the genetic diversity within a population (Al-Mamun *et al.*, 2015). A more genetically diverse population is indicated by a high level of heterozygosity, whereas low genetic diversity and a small N_e is indicated by a low level of heterozygosity (Toro & Caballero, 2005). According to Kijas *et al.* (2012), sheep have high genetic diversity compared to cattle and there is a high level of haplotype sharing between sheep breeds, which suggest a common origin of sheep breeds.

Linkage disequilibrium (LD) between any two markers shows the level of non-random association between the two markers and the degree of LD within a population plays a significant role in MAS, genomic selection (GS) and GWAS (Al-Mamun *et al.*, 2015). Comparison of the level of LD between breeds can provide useful information about the overall level of diversity in a species and can increase our understanding of the patterns of selection that each breed have been subjected to (Wang, 2005). The extent of LD within a population is influenced by factors such as N_e size, selection, migration, mutation and

recombination events (Tenesa *et al.*, 2007). In this study, genetic diversity was studied using methods to estimate inbreeding (ROH), observed and expected heterozygosity, inbreeding coefficients and minor allele frequencies.

2.9 Runs of homozygosity as a measure of inbreeding levels in a population

It has been reported that most of the genetic variation across an individual's genome is composed of sequence and structural changes including single-nucleotide inversions, translocations, insertion or deletions, inversions, rearrangements (copy number variants) (Haraksingh & Snyder, 2013) and single nucleotide polymorphisms (SNPs) or small insertion/deletion polymorphisms (Collins *et al.*, 1998). Within the genomes of different individuals in a population, there are also long continuous sections of homozygous genotypes which are without heterozygosity in the diploid state and these regions are known as runs of homozygosity (ROH) (Purfield *et al.*, 2012).

These stretches allow accurate estimation of levels of inbreeding based on high-throughput, chip-based single nucleotide polymorphism (SNP) genotypes (Mastrangelo *et al.*, 2017). Knowledge of the inbreeding levels in a population can be used in the identification of rare, recessive mutations that are likely to cause inbreeding depression (McQuillan *et al.*, 2008). Recent studies have shown that continued isolation and reduction in population size play a vital role in the formation of ROHs (Nothnagel *et al.*, 2010). However, Ferenčaković *et al.* (2013a) stated that inbreeding is a primary cause of these continuous stretches of homozygous genotypes.

ROH have become a very useful tool in analyzing population history, inbreeding levels and the impact of inbreeding on complex traits and inherited disorders (Howrigan *et al.*, 2011). Long ROH suggest recent inbreeding in a population as there was a limited opportunity for recombination to break up these haplotype segments, whereas shorter ROH suggest loss of genetic diversity either from a population bottleneck or a founder effect (Al-Mamun *et al.*, 2015). The frequency of ROHs may give useful information about the origin of an individual and its population (Ferenčaković *et al.*, 2013a).

The distribution of ROH may be used to identify genomic regions with an unfavorable effect on a phenotype in the homozygous state (Mastrangelo *et al.*, 2017) and also to detect associations between genes in these genomic regions and traits of economic interest (Purfield *et al.*, 2017). The distribution of ROH across the genome may also inform genomic regions under recent or ancient selection pressure, which leads to the fixation of

favorable alleles in a population (Mastrangelo *et al.*, 2017; Purfield *et al.*, 2017). The ability to identify genomic regions that display a reduced level of variation may indicate incidence of recent selection in a population and this information may help to detect QTL and candidate genes in these genomic regions (Szmatola *et al.*, 2016). The availability of high density SNP arrays provides an opportunity to screen the sheep genome for ROHs.

2.10 Genetic tools for investigating resistance and resilience

According to Goddard & Hayes (2009), the majority of the economically important livestock traits are quantitative traits, meaning they are influenced by many different loci spread across the genome. Identifying these loci or regions underlying QTL in the genome is challenging and it requires a sample with a necessary number of marker loci spread across the entire genome (Zhang *et al.*, 2012). Kemper *et al.* (2011) studied nematode resistance in sheep and reported that many different genes located at different loci and different chromosomes, with very few SNPs having large effects or a few genes of large effect, control resistance.

Recent advances in genome sequencing and genomic technologies provide new opportunities and could be applied for breeding if genetic markers associated to nematode resistance or markers closely linked to genes involved in ovine host resistance can be identified (McRae *et al.*, 2014a). These genomic tools will help improve our understanding of the interaction between *H. contortus* and the host (Gasser *et al.*, 2016). According to Dominik (2005), QTLs for difficult to measure, expensive to measure, and low/medium heritability traits such as host resistance against GINs are difficult to incorporate into a breeding program.

2.10.1 Methodology and principles of Marker assisted selection (MAS), quantitative trait loci (QTL) and Genome-wide association studies (GWAS)

2.10.1.1 Marker assisted selection (MAS)

Recent advances in DNA technology have made it possible to identify alleles with large effect on quantitative traits and some genetic markers are associated with improved performance in sheep (McManus *et al.*, 2014). The validation of these markers in particular breeds, can help increase the accuracy of traditional selection methods (Wakchaure *et al.*, 2015). MAS is a method in which marker genes are used to indicate the presence of desirable genes; the trait of interest is selected based on the marker linked to it (Wakchaure *et al.*,

2015). This method depends on identification of association between genetic marker and linked QTLs, and this association depends on distance between marker and target traits (Van der Werf, 2007).

MAS significantly shortens the generation interval through early selection, while increasing the selection accuracy due to the use of marker information (Fulton, 2012). Therefore, a breeding scheme based on MAS can enable rapid genetic gain through selection of markers associated to traits (quantitative traits) of economic importance such as milk and meat production (Wakchaure *et al.*, 2015). MAS is most useful for traits that are difficult or expensive to measure (disease resistance), sex-limited traits (milk yield, egg production), low heritability (litter size, fertility), carcass traits (meat quality), traits that are expressed late in life, and traits that are controlled by a few pairs of alleles (Schwerin *et al.*, 1995; Dekkers, 2004; Wakchaure *et al.*, 2015). Traits related to carcass quality, reproduction and disease resistance would benefit greatly from MAS in meat sheep (Van der Werf, 2007).

MAS using microsatellite markers in sheep has been widely commercially used in the sheep industry and genetic markers are available for major genes associated with fertility and carcass characteristics in sheep. The Booroola gene in Merino-Rambouillet crossbred sheep (Southey *et al.*, 2002), Callipyge gene (Cockett *et al.*, 1994) and the Texel muscling QTL (Macfarlane, 2012) have been successfully mapped. A number of QTLs have been reported for fibre diameter and other wool production and quality characteristics (Henry *et al.*, 1998; Ponz *et al.*, 2001; Dominik *et al.*, 2007).

MAS has been widely used in studies focusing on the selection for disease resistance in sheep (Dominik *et al.*, 2007) and QTLs for diseases such as Spider Lamb Syndrome (Cockett *et al.*, 1999; Kevorkian *et al.*, 2010) and scrapie (Barillet *et al.*, 2002) have been identified. According to Laegreid *et al.* (2008), susceptibility to scrapie is associated with the ovine prion protein amino acid sequence (PrP) as the variation in the sequence of this protein has an impact on scrapie progression. The identification of markers coding for the PrP protein have allowed breeders to select for scrapie resistance in sheep (Houston *et al.*, 2000). The Inverdale marker associated with prolificacy in sheep (Galloway *et al.*, 2000) and a *GDF8* marker haplotype associated with increased muscling (Johnson *et al.*, 2005) have also been successfully mapped.

The advantage of using MAS in a breeding programme is that the effect of genes on production is directly measured on the DNA of the animal and not predicted from the phenotypic information (Moniruzzaman *et al.*, 2015). The limitations of MAS include

increased costs associated with sample collection for genotyping. Complete phenotype and genotype information is a major limitation in MAS breeding programs (Wakchaure *et al.*, 2015).

2.10.1.2 Traditional QTL analysis and nematode resistance

The first QTL studies in livestock were conducted with known sire and dam information (pedigree information) for difficult to measure traits (Benavides *et al.*, 2016). In the classic QTL experiment, the identified region was fine-mapped using additional markers to determine either the casual mutation or a marker in close linkage disequilibrium (LD) that could be used for selection of the trait of interest (Beh *et al.*, 2002), which would result in MAS. Studies using microsatellite-based linkage analysis led to the identification of various regions of the genome containing loci associated with GIN resistance (Beh *et al.*, 2002; Dominik, 2005; Davies *et al.*, 2006; Marshall *et al.*, 2009). In a study by Beh *et al.* (2002), in which Merino selection lines with high and low immune responses to *T. colubriformis* were used, they identified a region with chromosome wide significance for mean FEC after secondary artificial challenge with *T. colubriformis*.

QTL having an effect on resistance to GINs in sheep have been reported (Moreno *et al.*, 2014; Sallé *et al.*, 2014). Most of these studies were based on low-density microsatellite marker maps. Previous QTL mapping results based on microsatellite-based analysis found host resistance for large genomic intervals on several ovine chromosomes (OAR), except for OAR 15, 17, 25, and X (Beh *et al.*, 2002; Moreno *et al.*, 2006; Silva *et al.*, 2012; Marshall *et al.*, 2013). Davies *et al.* (2006) identified regions of particular interest in sheep chromosomes 3, 14 and 20 using the Blackface sheep. Regions on OAR3 and OAR20 has been reported as two of the few QTL for nematode resistance that are common across different QTL studies (Marshall *et al.*, 2009; Bishop, 2012).

QTLs that have been identified indicate that most of the genes associated with parasite resistance have a relatively small effect, thus a large number of QTLs would be required for significant genetic gains to be attained using MAS (Van der Werf, 2007), and to explain the genetic variation in host resistance (McManus *et al.*, 2014). The identification of genes associated with parasite resistance would accelerate the genetic improvement of resistance to GINs and the use of MAS in breeding programs would allow animals to be selected without the need for parasite challenge (Benavides *et al.*, 2002).

The results from the microsatellite-based QTL studies are often difficult and unlikely

to be sustainably included into breeding programs (Bishop, 2012). Crawford *et al.* (2006) reported that the identification of candidate genes through microsatellite-based QTL mapping has, however, proven to be difficult, and as putative fragments often contained millions of base pairs uniformly spaced throughout the genome and contained many potential candidate genes. QTLs were usually detected within families and the linkage phase of markers with causative mutations is family specific (Bishop, 2012), making it difficult to re-establish linkage phase within each family, therefore leading to a constant large-scale requirement for phenotyping (Marshall *et al.*, 2009).

2.10.1.3 SNP-based QTL analysis for internal nematode resistance in sheep

The advent of the cost-effective, time saving sequencing technologies and development of species-specific high density SNP chip tools for livestock have increased our understanding of the genomes of different livestock species (Blasco & Toro, 2014). The introduction of the Illumina[®] OvineSNP50 BeadChip has led to microsatellite-based linkage studies being largely replaced with SNP-based GWAS (Kemper *et al.*, 2011; Sallé *et al.*, 2012; Riggio *et al.*, 2014b). The Illumina[®] Ovine SNP50 BeadChip (50k SNP chip) became commercially available in January 2009 and provides 54,241 equally spaced SNPs distributed across the sheep genome (Mucha *et al.*, 2015), thus providing genome-wide coverage with an estimated one marker per 46 kb on average (Riggio *et al.*, 2013).

This beadchip provides a fast way to detect genomic regions differentiating populations and those under selection and associated with specific traits (Kemper *et al.*, 2011; Atlija *et al.*, 2016). According to Periasamy *et al.* (2014), a number of SNP-based QTL studies on parasite resistance characteristics have been reported in sheep. Animal QTL database revealed a total of 753 QTLs related to economic traits in sheep, of which 81 were associated with parasite resistance across the sheep genome, except for chromosomes 5 and 19. QTLs related to parasite resistance were mostly found on chromosome 3 (16 QTLs), followed by chromosome 14 (7 QTLs) and 44 of the 81 QTLs were reported on resistance to *Haemonchus spp.* (<http://www.animalgenome.org/QTLdb/>).

Benavides *et al.* (2015) conducted a study to identify novel loci associated with GI parasite resistance in a Red Maasai x Dorper backcross population and in this study, 126 markers across all chromosomes were found to be associated with FEC and other related traits of interest for *H. contortus*. Most of the QTL studies on GIN resistance traits based on both microsatellite markers as well as the Ovine SNP50 BeadChip have been conducted on

growing lambs, as GINs are mainly pathogenic to young animals (Atlíja *et al.*, 2016). In a study conducted by Moreno *et al.* (2014), QTL associated with *H. contortus* resistance were identified on chromosomes 5, 12, 13, 21. Sallé *et al.* (2012) studied QTL mapping for resistance to *H. contortus* in a 1000 Martinik Black-Belly × Romane back-cross lamb population using 160 microsatellite markers as well as the Illumina OvineSNP50 BeadChip and found five major QTL that affected FEC on OAR5, 7, 12, 13 and 21.

2.10.1.4 Genome-wide association studies (GWAS)

Genome-wide association studies (GWAS) aims to detect variants at genomic loci that are associated with complex traits (Visscher *et al.*, 2012). This is a powerful approach for identifying quantitative trait loci (QTL) without prior knowledge of location or function and increases the efficiency of animal breeding and selection (Gholizadeh *et al.*, 2015).

GWAS can be done in populations with pedigree information and also in case-control studies where the pedigree information is not available because it makes use of the polymorphic SNP markers spread evenly throughout the genome to reveal genomic regions associated with the trait of interest in an individual genome (Benavides *et al.*, 2016). Genomic regions or variants associated with a trait of interest are unlikely to be responsible for the observed phenotype, however, they are more likely to be in LD with a causative mutation and therefore can be used for selection (Riggio *et al.*, 2013). Markers in LD with mutations affecting the trait of interest are detected using GWAS and the identified significant markers are then incorporated into the prediction of breeding value (Hayes & Goddard, 2010).

There are limited GWA studies that have been reported in sheep in relation to GIN resistance traits and these studies have only been conducted in wool/meat-producing sheep breeds. (Kemper *et al.*, 2009; Riggio *et al.*, 2013; Benavides *et al.*, 2016; Atlíja *et al.*, 2016). Traditional QTL analysis and GWAS have led to the identification of a significant number of QTL or markers across the ovine genome that are associated with several resistance phenotypes including FEC, PCV and parasite-specific antibody titers (Guo *et al.*, 2016).

The Ovine SNP50 BeadChip has been used to map causal mutations for traits showing simple patterns of inheritance (Shariflou *et al.*, 2013), and to detect signatures of selection between/within sheep breeds (McRae *et al.*, 2014a; Mucha *et al.*, 2015). McRae *et al.* (2014a) studied signatures of selection in divergent lines of Romney and Perendale sheep, selectively bred for resistance or susceptibility to GIN infection. In the study they managed to

identify fourteen genomic regions associated with resistance or susceptibility to GINs i.e. Chitinase activity and cytokine response.

The development of the new high-throughput genomic tools have made it possible to determine the genetic variation of the host (Davies *et al.*, 2009). Candidate gene approach has been used to identify specific genes associated with host resistance and most of these are from the major MHC region on *Ovis aries* chromosome 20 (OAR20) and the interferon- γ (IFNG) gene on OAR3 (Janssen *et al.*, 2002; Matika *et al.*, 2011; Riggio *et al.*, 2013).

These genetic markers and QTLs have been reported to be associated with FEC reduction (Alba-Hurtado & Muñoz-Guzmán, 2013). Interferon- γ is a cytokine that is secreted by Th1 lymphocytes (Davies *et al.*, 2006) which is used as a candidate for nematode resistance, is correlated with host response following nematode challenge and also plays a role in determining whether a humoral or cell-mediated response predominates (McManus *et al.*, 2014). The QTL on OAR3 associated with immunoglobulin A (IgA) activity is very close to IFNG gene (Davies *et al.*, 2006).

2.10.2 Limitations of trait analysis with GWAS

Genome-wide association studies have identified hundreds of common genetic variants associated with complex diseases so far in humans and livestock animals (McManus *et al.*, 2014). The challenge of an undetectable “missing heritability” due to rare variants, epistasis, epigenetics and genotype-environment interactions has been reported (Zvinorova *et al.*, 2016). According to Korte & Farlow (2013), missing heritability refers to the portion of genetic variance that cannot be explained by all significant SNPs. This inconsistency might partly result from rare variants or due to incomplete linkage between causative variants and those genotyped (Zvinorova *et al.*, 2016).

Work done by Kemper *et al.* (2011) using a mixed-breed population of sheep, show that the detectable polymorphisms affecting resistance to nematode infections have relatively small effects. A meta-analysis of three independent populations was done, and it included those used by Sallé *et al.* (2012) and Riggio *et al.* (2013). Regions in common between the three populations were successfully identified; additional novel regions not identified before were identified in this meta-analysis, demonstrating the potential power of meta-analyses to address some of the challenges (Riggio *et al.*, 2014a). If SNPs are to be employed in genomic selection programs, however, the associations must be independently validated (McManus *et al.*, 2014).

A number of significant QTL regions have been detected across the entire genome and explored in several genetic marker and GWAS studies for nematode resistance (Beh *et al.*, 2002; Davies *et al.*, 2006; Riggio *et al.*, 2013; Guo *et al.*, 2016). However, results from these studies are difficult to employ in breeding programs (Goddard & Hayes, 2007; Periasamy *et al.*, 2014). The lack of consensus overlap among reported QTLs detected in different sheep breeds has delayed the identification of candidate genes and genetic markers for selection in sheep (Periasamy *et al.*, 2014).

2.10.3 The potential for implementation of genomic selection in sheep

Genomic selection (GS) is based on the genomic estimated breeding values (GEBVs) estimated from SNP markers covering the entire genome (Scheifers & Weigel, 2012). The GEBVs are calculated as the total of the effects of dense genetic markers within the whole genome in a large reference population with known genotypic and phenotypic information (Van der Werf, 2014), and this ensures that all the quantitative trait loci (QTL) contributing to polymorphism in a trait are captured (Hayes *et al.*, 2009).

Marker assisted selection (MAS) only makes use of a small number of SNPs in LD with QTL thus genetic gain is likely to be relatively small and, GS has been developed as an alternative technology for using dense SNP information (Hayes & Goddard, 2010). The availability of a high-quality reference genome assembly makes it possible to study and to better understand the genetic structure of complex traits in different sheep breeds (Atlija *et al.*, 2016), which will help develop breeding programs that will allow efficient selection of parasite-resistant animals (McRae *et al.*, 2014a).

GS normally makes an assumption that each QTL is in LD with at least one trait or SNP marker (Jonas & de Koning, 2015) and that markers provide all the information about the genetic differences for a trait in animals. This has become one of the focal points of attention in animal breeding and genetics in recent years (Badke *et al.*, 2014). The factors affecting the quality of genomic predictions include the number of phenotypes and the extent of LD in the reference population, genetic relatedness between animal, genetic markers, heritability of traits and the size of the reference population (Carillier *et al.*, 2013).

GS has been successfully integrated into dairy cattle breeding programs and the results from experimental implementation of GS studies in other livestock species are promising (De Mello *et al.*, 2014). However, the implementation of GS in other livestock species is hindered by the high genotyping costs (Fulton, 2012).

SNP genotyping arrays for both sheep and goats are now commercially available. Sheep and goats lack a proper recording scheme especially in many developing countries, which hinders the implementation of GS as developing a reference population is costly and estimating breeding values may be difficult (Todd, 2013; Van der Werf, 2014). The limited number of available phenotypes measured in sheep makes it difficult to get accurate breeding values (Carillier *et al.*, 2013). Other factors such as the short generation intervals, large effective population sizes, high genetic diversity between and within breeds in sheep and high genotyping costs may limit the genetic gain attained from GS (Ibañez-Escriche & Gonzalez-Recio, 2011). Therefore, genomic selection, at least with current technologies, is likely to be expensive and logistically difficult to implement in tropical sheep and goats (Bishop, 2012). The implementation of GS in small ruminants would require a marked change in the available genomic tools (Kemper *et al.*, 2011).

Genomics may have an added-value role to play in the optimal selection for animals with improved host resistance/resilience against GIN infections. Sheep can be selected for low FEC using GS even though it has been shown that there are many loci of small effect controlling the trait (Kemper *et al.*, 2011). Riggio *et al.* (2014a) evaluated the potential of GS to predict GEBV for nematode resistance traits both within and across populations and found moderate to good GEBV within-population, whereas across-population predictions were relatively low (accuracies close to zero) and then concluded that genomic predictions using the 50K SNP are unlikely to work across breeds. These authors also found that additive genetic relatedness between animals affected the accuracy estimates the most, rather than LD between SNP and QTL. The implementation of GS in small ruminants in future will depend on the existence of a reference population consisting of animals with both phenotypic and genotypic information, the structural organization of breeding programs and the efficiency of genotyping strategies (Ibañez-Escriche & Gonzalez-Recio, 2011).

Van der Werf (2014) stated that successful integration of GS into sheep breeding programs will require the use of low density SNP genotyping arrays together with imputation to genotype a large number of animals. The impact of GS on the rate of genetic gain will be greatest for complex traits such as sex limited, difficult or expensive to measure, low-heritable, postmortem and traits that are expressed late in life (Goddard & Hayes, 2007). The application of genomic selection in livestock will greatly benefit genetic improvement programs implemented in many animal species as GS ultimately increases the rate of genetic gain in a population by increasing the genetic prediction values and shortening the generation

interval (Ibañez-Escriche & Gonzalez-Recio, 2011).

2.11 Conclusion

Rapid advances made in genomic tools can speed up the process of making breeding for natural disease resistance a reality and also in ensuring that the use of chemical treatment decreases. Identification of genes under selection in animals selected for resistance to gastrointestinal parasites will help in our understanding of the biological processes underlying this trait. Investigating the genetic variation within specific regions of the genome or genes may assist in the identification of genetic markers associated with parasite resistance characteristics in different sheep populations. Breeding for improved resistance to nematode parasites may greatly facilitate and enhance parasite control. Indicator traits such as FEC, FAMACHA and BCS can be used to identify resistant and susceptible animals in a population. This study is designed as a case / control study aimed at investigating genetic differences in resistant and susceptible Dohne Merino lines and will provide information that can be used to develop a more sustainable, cost-effective and realistic long-term helminth management strategy.

Chapter 3: Materials and Methods

3.1 Introduction

This study was designed as a case-control study to determine genetic differences between resistant (control) and susceptible (case) sheep using high-throughput genome-wide SNP data. Resources (phenotypic data and blood samples) from the Wauldby Dohne Merino flock of Mr Robbie Blaine and the Grootfontein Dohne Merino flock of the Grootfontein Agricultural Development Institute (GADI) kept in the Grootfontein Biobank were used for this study.

Since 2011, animals at Wauldby have been selected for improved resistance against parasitic nematodes, specifically *H. contortus*. Animals that needed dosing were classified as Case and animals that did not need dosing were classified as Control animals. Whether an animal was dosed or not was determined by its FAMACHA[®] score (FAM), in combination with its BCS and FEC and these phenotypic traits were collected and recorded annually on the lambs from weaning in January until June/July. Animals for genotyping were selected on the basis of EBV-FEC. Animals with the lowest and highest EBV-FEC were selected within the Dosed and Not dosed groups over the experimental period. The Dohne Merino animals at GADI were never subjected to any selection for resistance against *H. Contortus* and were included as a reference population in the study.

Two hundred and forty Dohne Merino sheep from Wauldby (n=192) and GADI (n=48) were genotyped using the Illumina[®] Ovine SNP50 BeadChip. After genotyping, the data were merged and then subjected to quality control to prune low quality SNP genotypes. Genotype pruning is extremely important for meaningful downstream analysis. The set of SNPs meeting the quality control criteria were then used to calculate the basic population parameters, to determine the population genetic structure of the animals, to illustrate the relationship between the individuals of these South African Dohne Merino sheep populations, to determine the runs of homozygosity in the genomes of these animals and to determine if there were any differences in the available phenotypic traits among the Wauldby animals allocated to the different genetic clusters.

3.2 Description of resource flocks

3.2.1 Wauldby Dohne Merino flock

3.2.1.1 Location

The farm Wauldby is located in the Stutterheim district in the Eastern Cape Province (27° 37' East, 32° 35' South) in a high summer rainfall area. Rainfall and temperature averages are high from January to April and from October to December. The mean annual rainfall is 800 mm, with most rainfall occurring during summer. It receives the lowest rainfall (7 mm) in July and the highest (91 mm) in February. The average midday temperatures for Stutterheim ranges from 17.9 °C in June to 25.7 °C in February. The region is the coldest during July when the mercury drops to 4 °C on average during the night (source: http://www.saexplorer.co.za/south-africa/climate/stutterheim_climate.asp; <https://www.worldweatheronline.com/stutterheim-weather-history/eastern-cape/za.aspx>).

3.2.1.2 Management practices followed in the flock

The ewe flock of the Wauldby Dohne Merino flock consisted of 335 ewes. The animals were kept on natural grazing. Single sire mating was done, where a sire was run with approximately 40 ewes in a small camp from middle March until the first week of April for a three-week period. All lambs were identified and tagged at birth and sex, birth status and pedigree information were recorded. All ram and ewe lambs were retained until the age of 14 months. Ram and ewe lambs were separated after weaning into two flocks.

3.2.1.3 Selection procedures followed in the flock

Wauldby has a well-documented history of heavy *H. contortus* challenge and *Haemonchus* resistance to all five major anthelmintic groups on the market (Macrocyclic lactones, Imidazoles, Benzimidazoles, Halogenated salicylinalides and Organophosphates) prior to 2011 (Snyman, 2016a, 2016b). In the past, the farm was used for several trials related to resistance of *H. contortus* to anthelmintics. The severe anthelmintic resistance problem on the farm has inadvertently resulted in selection of sheep over many years with a high degree of resistance to internal parasites as drenching with anthelmintics has been largely ineffective (Snyman, 2016a, 2016b).

At the end of 2011, a project aimed at selection focussed on resistance to *H. contortus* was implemented. This project was a collaborative effort between the farmer, the Queenstown Provincial Veterinary Laboratory and the Grootfontein Agricultural

Development Institute (Snyman, 2016a, 2016b). The selection objective in the flock was to obtain sheep that are genetically resistant to *H. contortus*. Selection in the flock was aimed at increasing resistance to *H. contortus*, while maintaining reproductive performance, body weight, wool weight and fibre diameter and improving wool quality traits. Selection for the production traits was done on the basis of selection indices and BLUP of breeding values for the mentioned traits measured at 14 months of age, obtained from the Dohne Merino Breeders' Society. Selection for resistance to *H. contortus* was based on a selection index incorporating FEC, FAM and BCS, as well as BLUP-EBV for FEC (Snyman, 2016a, 2016b).

A selection line was established in 2012 as part of the project (Snyman, 2016a, 2016b). The aim with this selection line was to create a line in which the most resistant ewes were mated to the most resistant rams. These animals were run together with the rest of the flock animals, except during mating. Only ram and ewe lambs that had never been drenched were considered for selection into the selection line. Three rams and about 20 young ewes were selected annually for the selection line since 2012. Currently the selection line consists of 120 ewes, which are mated in three groups of 40 ewes each to the best three selected rams in single sire mating camps. All progeny born in both the selection line and the rest of the flock were evaluated together. Rams and ewes performing the best in terms of resistance could be selected for the selection line, whether their parents came from the selection line or the other flock animals (Snyman, 2016a, 2016b).

3.2.1.4 Collection of phenotypic data

Apart from full pedigree information, data on FEC, FAM and BCS were also collected annually on all lambs born since 2011. FEC, FAM and BCS of all lambs were recorded once in November or December before weaning, depending on the rainfall and climatic conditions. All lambs were drenched after this data collection. Lambs were weaned in December. FEC, FAM and BCS of all lambs were then recorded from the middle of January onwards. FAM was recorded weekly and FEC and BCS every 14 days until the first week of July when *Haemonchus* challenge had decreased. Lambs were only drenched when they had a FAM of 2.5 or more, in conjunction with a BCS of less than 1.5. Any lamb that was drenched was recorded as "Dosed". Data on all lambs were recorded throughout until July, irrespective whether they needed drenching or not. Any remedies or supplements supplied to the animals were also noted.

Faecal sampling and faecal egg counts

At least 5 g of faeces was collected directly from the rectum of each lamb. Faecal samples were placed in individual numbered plastic bags and immediately placed on ice for transportation to the Queenstown Provincial Veterinary Laboratory for faecal egg counts. Faecal egg counts were done with the McMaster procedure (Venter, 2011).

FAMACHA[®]-score

FAMACHA[®]-score was done according to the method described by Van Wyk *et al.* (1997) and Malan *et al.* (2000). Only one assessor did the FAM in order to eliminate operator variance.

Body condition score

Body condition score was assessed on a scale of one to five, with one being an emaciated sheep, three being a sheep in average condition and five being an obese sheep (Table 3.1). Only one assessor did the BCS in order to eliminate operator variance.

Table 3.1: Scale for body condition score (BCS) in sheep

BCS	Scale	Description
BCS 1	Emaciated	Spinous processes are sharp and prominent. Transverse processes are sharp; one can pass fingers under ends. It is possible to feel between each process. Loin muscle is shallow with no fat cover.
BCS 2	Thin	Spinous processes are sharp and prominent. Transverse processes are smooth and slightly rounded. It is possible to pass fingers under the ends of the transverse processes with a little pressure. Loin muscle has little fat cover, but is full.
BCS 3	Average	Spinous processes are smooth. Transverse processes are smooth and well covered and firm pressure is needed to feel over the ends. Loin muscle is full with some fat cover.
BCS 4	Fat	Spinous processes can be detected only with pressure as a hard line.

BCS	Scale	Description
		Transverse processes cannot be felt. Loin muscle is full with thick fat cover.
BCS 5	Obese	Spinous processes cannot be detected. Transverse processes cannot be detected. Loin muscle is very full with very thick fat cover.

Source: Thompson & Meyer (1994).

Phenotypic data

All phenotypic data were captured after collection and stored in the GADI Biobank.

Blood sampling

Blood samples were collected annually in November from the lambs born during the September lambing season by personnel of GADI. From the 2011- and 2012-born lambs, samples from only 40 animals were taken. These included 20 Dosed animals with high and low EBV for FEC and 20 not-dosed animals with high and low EBV for FEC. Since 2013, blood samples from all lambs born were collected each year. Blood samples were also collected from any sires brought in from outside sources.

Blood samples were collected by GADI personnel via veni-puncture in the left jugular vein into 10 ml EDTA plastic vacutainer blood collection tubes. Collected blood samples were put on ice directly after sampling and kept on ice while being transported to GADI from Wauldby. At GADI, all the blood samples were stored in aliquots of 2 ml each in the minus 80 °C freezers of the GADI-Biobank.

3.2.1 Grootfontein Dohne Merino flock

3.2.1.1 Location

The Grootfontein Dohne Merino flock is kept at Grootfontein Agricultural Development Institute (GADI) near Middelburg (31° 28' South, 25° 1' East) in the Eastern Cape Province under veld conditions. GADI lies on the eastern edge of the Karoo biome. The non-mountainous vegetation, which comprises 89% of the farm, is described as the Eastern Upper Karoo (Vegetation Type NKu 4; Mucina *et al.*, 2006). Mean annual rainfall is 373 mm (du Toit & O'Connor, 2014), with 22% occurring in spring, 39% in summer, 30% in autumn and 9% in winter. The average minimum temperature (July) is -0.4 °C and the average

maximum temperature (January) is 30.3 °C. Frost occurs from April to September (Worldweatheronline, 2014; Weatherbase, 2015).

3.2.1.2 Management practices followed in the flock

The ewe flock of the Grootfontein Dohne Merino flock at GADI consists of 400 ewes. All the ewes were managed as one flock on the veld, except during mating. Single sire mating was done in order to facilitate complete pedigree recording. Ewes were mated on the veld in small paddocks in groups of approximately 30 to an allocated sire during March each year for a 35-day mating period. The ewes were kept in individual pens during lambing for a few days and received a pelleted ration during this time. Afterwards they were kept for a maximum of two weeks in bigger kraal facilities. Three weeks after lambing, the ewes and lambs were taken back to the veld (Olivier, 2016a, 2016b).

All lambs were identified and tagged at birth and birth weight as well as sex, birth status and pedigree information were recorded. All ram and ewe lambs were retained until selection age at 12 months. All lambs and young two-tooth animals were kept on the veld throughout the year, where they received an energy-protein production lick, depending on the prevailing veld conditions.

3.2.1.3 Selection procedures followed in the flock

The Grootfontein Dohne Merino flock was established in 2001 following a request from the wool industry to establish a genetic pool of dual-purpose sheep with premium quality meat and super fine wool (Herselman, 2001; Olivier et al., 2010). During 2001 and 2002, a total of 217 ewes were bought from 25 Dohne Merino breeders. Only ewes with above average body weight and below average fibre diameter in the various flocks were bought (Herselman, 2002; Olivier et al., 2010). During 2006, a further 20 Dohne Merino flock ewes were bought from Dohne Merino breeders. Ewe numbers in the flock were let to increase. A further 130 ewes were bought from a Dohne Merino breeder in 2011, which brought the number of ewes available for mating during the 2012 mating season to 470 ewes. Since 2008, mostly own-bred rams were used as sires in the flock, with the exceptions of 2011 (2 sires), 2014 (2 sires), 2015 (1 sire) and 2016 (1 sire) when outside sires were used (Olivier, 2016b).

From 2001 until 2010, selection of replacement ewes and sires was based on BLUP of breeding values for body weight, fibre diameter and clean fleece weight, provided the

animals passed the breed inspection. Emphasis was placed on increasing body weight and decreasing fibre diameter. No specific selection for reproduction was carried out until 2010. From 2011 until 2015, rams were selected from dams with good reproduction values. No strategic drenching program was followed and animals were only drenched according to faecal egg counts; no specific selection for helminth resistance was done in the Grootfontein Dohne Merino flock (Olivier, 2016a, 2016b).

3.2.1.4 Collection of data

During 2015 it was decided to make the Grootfontein Dohne Merino flock a part of the Wauldby parasite project. The Grootfontein Dohne Merino flock has been part of the GADI-Biobank project since 2009. Together with the Dohne Merino flock from the Dohne Agricultural Development Institute (DADI) and the Dohne Merino flock of Wauldby, this flock forms the basis of a reference population for Dohne Merino sheep in South Africa. The DADI has been part of the GADI-Biobank since 2008 and the Wauldby flock since 2011 (Snyman, 2016a, 2016b). For this flock to be considered as a reference flock for the South African Dohne Merino breed, it is important that suitable genetic links with industry flocks are present. The origin of the flock leads itself ideally to this function. Therefore animals from the Grootfontein Dohne Merino flock were included in this study for comparison with the Wauldby Dohne Merino animals, which have been selected for resistance against *H. contortus* for some years.

Apart from full pedigree information, the following phenotypes associated with GIN resistance were collected on the Grootfontein Dohne Merino flock:

2014-born lambs

Faecal egg counts during December, March and May.

2015-born lambs

No FEC were recorded, as monthly FEC sampling of marker group animals indicated zero FEC due to dry conditions.

2016-born lambs

FEC were recorded during December, March and May, while FAM was recorded during March and May.

Faecal sampling and faecal egg counts

At least 5 g of faeces was collected directly from the rectum of each lamb. Faecal samples were placed in individual numbered plastic bags and immediately placed on ice for transportation to the Middelburg Provincial Veterinary Laboratory for faecal egg counts. Faecal egg counts were done with the McMaster procedure (Venter, 2011).

FAMACHA[©]-score

FAMACHA[©]-score was done according to the method described by Van Wyk *et al.* (1997) and Malan *et al.* (2000). Only one assessor did the FAM in order to eliminate operator variance.

Phenotypic data

All phenotypic data were captured after collection and stored in the GADI Biobank.

Blood sampling

Blood samples were collected annually in November from the lambs born during the August/September lambing season. Blood samples were collected via veni-puncture in the left jugular vein into 10 ml EDTA plastic vacutainer blood collection tubes. Collected blood samples were put on ice directly after sampling and then stored in aliquots of 2 ml each in the minus 80 °C freezers of the GADI-Biobank.

3.3 Animal welfare

The study complied with relevant Animal Welfare legislation and generally accepted norms regarding animal care and welfare. The Wauldby part of the study was carried out under the auspices of the state veterinarian of the Queenstown Provincial Veterinary Laboratory, Dr Alan Fisher. The Grootfontein state veterinarians, Dr Johan Van Rooyen and Dr Johan Viljoen were responsible for overseeing the animals' health and welfare aspects of the Grootfontein part of the study. All three veterinarians are registered with the South African Veterinary Council.

Animals on both farms received the standard vaccinations as prescribed by the veterinarians. Any diseases or conditions observed during the study periods were treated and noted. The project protocol was approved by the Ethical Committees of the Grootfontein Agricultural Development Institute (GVE/AP2/21/1) and the Animal Ethics Committee of the Faculty of Natural and Agricultural Sciences at the University of Pretoria (EC161205-088).

3.4 Selection of animals for genotyping

Breeding values (EBV) for FEC were estimated for the data available on the Wauldby animals born from 2011 to 2014. Within years, animals with the highest and lowest EBV for FEC were selected among the Dosed (n=48, Low EBV FEC; n= 48, High EBV FEC), as well as the Not dosed (n=52, Low EBV FEC; n=48, High EBV FEC) groups. Animals were selected within years to account for any possible genetic trends (Table 3.2).

Table 3.2: Selection of Wauldby animals for genotyping based on EBV for FEC

Year of birth	Number of animals			
	Dosed		Not dosed	
	Low EBV FEC	High EBV FEC	Low EBV FEC	High EBV FEC
2011	7	7	7	7
2012	6	6	13	10
2013	18	18	17	16
2014	17	17	15	15
Total (196)	48	48	52	48

In the case of the Grootfontein Dohne Merino animals, FEC data for the 2014- and 2016-born lambs were available. As data from only two years were available, no EBV were estimated, but animals with the highest and lowest FEC within each year were selected for genotyping (n = 24/year) (Table 3.3).

Table 3.3: Selection of Grootfontein Dohne Merino animals for genotyping based on FEC.

Year of birth	Number of animals			
	Low FEC		High FEC	
	Ram lambs	Ewe lambs	Ram lambs	Ewe lambs
2014	6	6	6	6
2016	6	6	6	6

Total (48)	12	12	12	12
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3.5 Wauldby data

3.5.1 Data editing

The available data were recorded over a four year period from 2012 (2011-born lambs) to 2015 (2014-born lambs) from January until June/July each year. Between 10 and 12 two-weekly recordings of FEC were performed per year. The number of individual data records available per year for the recorded resistance traits are summarised in Table 3.4.

Table 3.4: Number of individual data records available per year for the recorded resistance traits (FAM, BCS and FEC).

Recording	Number of records				Total
	2011	2012	2013	2014	
1	221	231	273	215	940
2	221	231	273	215	940
3	221	231	273	215	940
4	221	231	273	215	940
5	221	231	-	-	452
6	221	231	273	215	940
7	221	231	273	215	940
8	221	231	273	215	940
9	221	231	273	215	940
10	221	231	273	215	940
11	221	231	273	215	940
12	221	231	273	215	940
Total	2652	2772	3003	2365	10792

3.5.2 Statistical analysis

Data on FEC were transformed to logarithms to the base of 10 (after adding 10 to each value to account for zero counts) to improve the distribution of the data. Both the untransformed data (FEC) and the log-transformed data (LFEC) were used in all the analyses.

Description of the resistance traits

The minimum, maximum, mean, standard deviation (SD) and coefficient of variation (CV) for FAM, BCS, FEC and LFEC for the different recordings (averaged over years) were

obtained with PROC MEANS of SAS (SAS, 2016). Recording 5 was excluded from the analyses due to incomplete or missing data.

Phenotypic trends in resistance traits

Phenotypic trends in FEC, FAM and BCS for the Not dosed and Dosed ram and ewe lambs for the pooled data for 2011 to 2014 were generated from the raw data.

Influence of non-genetic effects on phenotypic traits

For these analyses, the average FAM, BCS, FEC and LFEC over all recordings was calculated for each animal for each year. The non-genetic effects tested for significance were year of birth, sex, birth status (1 = lambs born as singlets, 2 = lambs born as twins, 3 = lambs born as triplets), whether the animal was dosed (1) or not (2) and how many times (1 to 4) it was dosed (included as a concatenation), if it were selected as a case or control animal for genotyping and the respective two way interactions. Age of the animal at recording was included as a covariate. The PROC GLM procedure of the SAS statistical package was used to determine which of these fixed effects had a significant influence on the average of the resistance traits recorded over the experimental period (SAS, 2016). None of the two way interactions, nor age at recording had a significant influence on any of the resistance traits.

The following final fixed effect model was applied for FAM, BCS, FEC and LFEC, firstly to the entire data set, and secondly to the data set including only the genotyped animals:

$$Y_{ijklmn} = \mu + j_i + s_j + b_k + d_l + g_m + e_{ijklmn}$$

Where

Y_{ijklmn} = trait of the n^{th} animal of the m^{th} group of the l^{th} dosing status of the k^{th} birth status of the j^{th} sex of the i^{th} year of birth,

μ = overall mean,

j_i = fixed effect of the i^{th} year of birth (2011 to 2014),

s_j = fixed effect of the j^{th} sex (ram, ewe),

b_k = fixed effect of the k^{th} birth status (1, 2, 3),

d_l = fixed effect of the l^{th} dosing status (10, 21, 22, 23, 24),

g_m = fixed effect of the m^{th} group (Case or Control; fitted only for the second data set),

e_{ijklmn} = random error with zero mean and variance $1\sigma^2_e$.

Genetic trends in resistance traits

The data editing and analyses for EBV estimation is described in detail. However, the EBVs were estimated at GADI and provided to the student. For these analyses, the average FAM, BCS, FEC and LFEC over all recordings for each animal were used. Breeding values for FAM, BCS, FEC and LFEC were estimated with the ASReml program, employing univariate models including only direct additive genetic effects (Gilmour *et al.*, 2009). Fixed effects included for all traits were year of birth, sex, birth status and dosing status. Genetic trends in FEC, FAM and BCS over the study period for all the animals were obtained by regression of breeding values for the respective traits on birth year.

3.6 Grootfontein data

3.6.1 Data editing

The available resistance data were recorded over a three year period from 2015 (2014-born lambs) to 2017 (2016-born lambs) from December until May each year. It was planned to do three recordings of FEC on each group of lambs. However, no FEC recordings were done on the 2015 lambs, as monthly FEC sampling of marker group animals indicated zero FEC due to the prevailing dry conditions. Furthermore, as it was only decided in 2015 to make the Grootfontein Dohne Merino flock a part of the Wauldby parasite project, no FAM were recorded on the 2014-born lambs. The number of individual data records available per year for the recorded resistance traits are summarised in Table 3.5.

Table 3.5: Number of individual data records available per year for the recorded resistance traits (FAM and FEC).

Recording	Number of records			Total
	2014 - FEC	2016 - FEC	2016 - FAM	
1	614	461	-	1075
2	619	469	469	1557
3	294	450	450	1194
Total	1527	1380	919	3826

3.6.2 Statistical analysis

Data on FEC were transformed to logarithms to the base of 10 (after adding 10 to each value to account for zero counts) to improve the distribution of the data. Both the untransformed data (FEC) and the log-transformed data (LFEC) were used in all the analyses.

3.6.2.1 Description of the resistance traits

The minimum, maximum, mean, standard deviation (SD) and coefficient of variation (CV) for FAM, FEC and LFEC for the different recordings (averaged over years) were obtained with the PROC MEANS of SAS (SAS, 2016).

3.6.2.2 Phenotypic trends in resistance traits

As only three recordings were done for the Grootfontein Dohne Merino animals, no phenotypic trends were generated from the raw data.

3.6.2.3 Influence of non-genetic effects on phenotypic traits

For these analyses, the average FAM, FEC and LFEC over all recordings was calculated for each animal for each year. The non-genetic effects tested for significance were year of birth, sex, birth status, if it was selected as a high FEC or low FEC animal for genotyping and the respective two way interactions. Age of the animal at recording was included as a covariate. The PROC GLM procedure of the SAS statistical package was used to determine which of these fixed effects had a significant influence on the average of the resistance traits recorded over the experimental period (SAS, 2016). None of the two way interactions, nor age at recording had a significant influence on any of the resistance traits.

The following final fixed effect model was applied for FAM, FEC and LFEC, firstly to the entire data set, and secondly to the data set including only the genotyped animals:

$$Y_{ijklm} = \mu + j_i + s_j + b_k + g_l + e_{ijklm}$$

Where

Y_{ijklm} = trait of the m^{th} animal of the l^{th} group of the k^{th} birth status of the j^{th} sex of the i^{th} year of birth,

μ = overall mean,

j_i = fixed effect of the i^{th} year of birth (2011 to 2014),

s_j = fixed effect of the j^{th} sex (ram, ewe),

b_k = fixed effect of the k^{th} birth status (1, 2, 3),

g_l = fixed effect of the l^{th} group (High FEC or Low FEC; fitted only for the second data set),

e_{ijklm} = random error with zero mean and variance $\text{I}\sigma_e^2$.

3.7 Genotype data

3.7.1 DNA isolation and Genotyping

DNA was isolated using the DNA isolation NucleoMag[®] VET kit (NucleoMag - MACHEREY-NAGEL GmbH & Co KG, Düren, Germany) at Agricultural Research Council, Biotechnology Platform (ARC-BTP). Ethidium bromide-based agarose gel electrophoresis was used to visualize the extracted DNA. High concentration DNA (≥ 25 ng/ul) obtained from the blood samples and visually evaluated by 1% agarose gel electrophoresis, were genotyped at ARC-BTP using the Illumina[®] Ovine SNP50 BeadChip (Illumina Inc., San Diego, CA), which contains over 54 000 SNPs distributed over the 27 autosomes and sex chromosomes. SNP-calling was done using the Illumina[®] Genome Studio software v2.0 (Illumina, San Diego, California 92122 U.S.A). The resulting genotype input file was converted into a PLINK (ped and map files) input file using a plug-in in Genome Studio software v2.0.

3.7.2 Quality control

Genotype data for the Wauldby Dohne Merino (2 batches - cases/controls, n = 192 animals) and GADI Dohne Merino (1 batch, n = 48 animals) sheep populations were merged to prune SNP genotypes for downstream analyses. The SNP genotype data were subjected to quality control measures using PLINK v1.07 software (Purcell *et al.*, 2007) as follows: SNPs were removed if they had a call rate of less than 90%, a minor allele frequency (MAF) of < 2%, as well as missing genotypes and genotyping failure of more than 10%. SNPs that were out of Hardy-Weinberg equilibrium (HWE, $P < 0.001$), SNPs that were found on unknown chromosomes, mtDNA, linkage groups and sex chromosomes were also removed from the data. Before quality control, there were 54 241 markers and 240 animals. After data quality control, 2 of 240 (115 males, 125 females) individuals were removed for low genotyping (MIND>0.1) and were excluded from further downstream analysis. Four hundred and eighty markers were excluded based on HWE test ($P \leq 0.001$), 3 260 SNPs failed missingness test (GENO>0.1) and 6 166 SNPs failed frequency test (MAF<0.02). After frequency and genotyping pruning, there were 47 518 SNPs with a total genotyping rate of 0.94071 available for further analyses.

3.7.3 PCA Genetic Clustering

A principal component analysis (PCA) was performed to illustrate the relationship between these individuals of the South African Dohne Merino sheep population using the SVS software program (GoldenHelix Inc., Bozeman, MT, USA). PCA is a computationally well-organized approach, which can handle large numbers of markers, and is useful for visualizing population structure. Combined with a clustering tool, it can also be used for inferring population clusters and allocating individuals to subpopulations. Using PCA, genetic clusters were defined and their composition and basic parameters were investigated.

3.7.4 Flock structure

3.7.4.1 ADMIXTURE analysis

The model-based clustering ADMIXTURE 1.23 software (Alexander *et al.*, 2009) was used to determine the population genetic structure of the animals. ADMIXTURE was run using the --cv command to select the correct number of clusters or putative populations (i.e. K). ADMIXTURE is a software tool for maximum likelihood estimation of individual ancestries from multi-locus SNP genotype datasets. ADMIXTURE uses the same statistical model as STRUCTURE software (Pritchard *et al.*, 2009), but calculates estimates much more quickly using a fast numerical optimization algorithm. Genesis software (Buchmann & Hazelhurst, 2014) was used to generate the admixture plots from data outputted by ADMIXTURE 1.23 (Alexander *et al.*, 2009).

3.7.4.2 Wright's measure of population differentiation

Wright's measure of population differentiation (autosomal F_{ST}) was calculated using GoldenHelix SNP & Variation Suite (SVS) software (GoldenHelix Inc., Bozeman, MT, USA). Fixation index (F_{ST}) represents the shared ancestry within a population relative to the metapopulation and is normally used to measure genetic differentiation among populations. F_{ST} evaluates the reduction in genotypic heterozygosity and can range from zero (no genetic divergence between the subpopulations) to one (complete isolation of the subpopulations from each other). F_{ST} was estimated between the PCA based genetic clusters 1 to 4.

3.7.5 Basic Population parameters

Basic parameters such as inbreeding coefficient (F_{IS}), number of alleles, minor allele frequency (MAF), observed heterozygosity (H_o), and expected heterozygosity (H_e) were estimated for each of the PCA based clusters using PLINK V1.07 (Purcell *et al.*, 2007). The average H_o and H_e were calculated using all SNPs, based on the observed genotype frequencies. Basic population parameters were calculated using the --het command on PLINK V1.07 (Purcell *et al.*, 2007) which created the output file: plink.het, which contains the fields, one row per animal in the file:

FID = Family ID

IID = Individual ID

O(HOM) = Observed number of homozygotes

E(HOM) = Expected number of homozygotes

N(NM) = Number of non-missing genotypes

F = inbreeding coefficient estimate

The averages of the basic population parameters were calculated in Microsoft Excel (2013). The number of alleles and minor allele frequency were calculated on PLINK using the --freq command. The heterozygosity rate for the two sheep populations was estimated using --hardy command on PLINK V1.07 (Purcell *et al.*, 2007).

3.7.6 Runs of Homozygosity calling and Statistical analysis

ROHs were identified for each PCA based genetic cluster using PLINK V1.07 (Purcell *et al.*, 2007) and SVS from Golden Helix (GoldenHelix Inc., Bozeman, MT, USA) software programs. The programs use statistical algorithms to detect stretches of consecutive homozygous SNPs in the genome using genotype data. Table 3.6 shows the parameter settings that were used for both PLINK and SVS software programs to detect ROH regions in the genomes of both the Wauldby and GADI Dohne Merino sheep populations. The PLINK algorithm defines runs of homozygosity as regions of homozygous genotypes that were greater than 1000 kb in length identified in the genome with a sliding window of 50 SNPs. The algorithm allowed one heterozygous SNP per run and no more than 3 missing genotypes within a window (Table 3.6). No pruning was performed based on linkage disequilibrium (LD), however ROH detection parameters were used to exclude short and common ROHs that derived from LD.

Table 3.6: ROH Detection Parameters.

Program	Parameters	Code	Parameters used
PLINK	Heterozygote allowance	--homozyg-window-het	1
	SNP threshold to call a ROH	--homozyg-snp	50
	Sliding window size in SNPs	--homozyg-window-kb	1000
	Missing SNP allowance	--homozyg-window-missing	3
	Window threshold to call a ROH	--homozyg-window-threshold	0.05
	Allelic matching	--homozyg-match	0.98
	Allowed distance between SNPs	--homozyg-gap	100 kb
	Overlapping ROH	--homozyg --homozyg-group	-
SNP&Variation suite (SVS) Golden Helix	Distance	-	1000 kb with minimum no. of 50 SNPs
	Heterozygote allowance	-	1
	Missing genotypes	-	5
	Maximum gap between SNPs in a run	-	100
	Minimum no. of samples that must contain a run	-	5

3.7.7 Annotation of ROHs and association with QTL associated with parasite resistance

A search for QTLs associated with parasite resistance on chromosomes that had a high number of runs of homozygosity was undertaken. The Sheep Quantitative Trait Loci Database (QTLdb) was used (<http://www.animalgenome.org/QTLdb/sheep>) for this purpose.

3.7.8 Genetic cluster differentiation based on phenotypic data

Animals in the data set were allocated to the specific genetic cluster (based on PCA clustering) into which they were placed. Genetic clusters were described in terms of year of birth, sex, birth status, dosing status, Case/Control groups, High/Low FEC and the number of animals with each clusters that had selected sires/dams as parents. Least squares means for the various available phenotypic traits and combinations of the traits were estimated for the different genetic clusters of the Wauldby animals.

3.7.8.1 Least squares means for resistance traits for the different genetic clusters of the Wauldby animals

Least squares means for the various available resistance traits and combinations of the traits were obtained for the different PCA clusters of the Wauldby animals. The PROC GLM of SAS (2016) was used to obtain least-squares means for FEC, LFEC, FAM and BCS for the different clusters.

The following model was applied for each individual recording of, as well as the average FAM, BCS, FEC and LFEC:

$$Y_{ijklmn} = \mu + j_i + s_j + b_k + d_l + c_m + e_{ijklmn}$$

Where

Y_{ijklmn} = trait of the n^{th} animal of the m^{th} cluster of the l^{th} dosing status of the k^{th} birth status of the j^{th} sex of the i^{th} year of birth,

μ = overall mean,

j_i = fixed effect of the i^{th} year of birth (2011 to 2014),

s_j = fixed effect of the j^{th} sex (ram, ewe),

b_k = fixed effect of the k^{th} birth status (1, 2, 3),

d_l = fixed effect of the l^{th} dosing status (10, 21, 22, 23, 24),

c_m = fixed effect of the m^{th} cluster (2,3,4),

e_{ijklmn} = random error with zero mean and variance σ^2_e .

Analyses of the resistance data recorded on the Wauldby animals indicated that a combination of information recorded at the first, seventh and ninth recordings (FEC179, BCS179, FAM179 etc.) could be used as basis for selection for resistance against *H. contortus*. Therefore, these combinations for FEC, FAM and BCS were also compared

amongst the three Wauldby clusters, applying the above model. Furthermore, a selection index, incorporating LFEC, FAM and BCS, as well as EBVs for FEC, LFEC, FAM and BCS were also compared amongst the clusters. Average EBV for FEC, LFEC, FAM and BCS for the sires of the animals clustering into each cluster were also compared using GLM procedures (SAS, 2016).

3.7.8.2 Least squares means for resistance traits for the different genetic clusters of the Grootfontein animals

As all the Grootfontein Dohne Merino animals clustered into one cluster, this part of the analysis was not performed for the Grootfontein data.

Chapter 4: Results

4.1 Introduction

The aim of this study was to investigate phenotypic and genetic differences in resistance to *Haemonchus contortus* between resistant and susceptible animals in the Wauldby Dohne Merino sheep flock, which has been selected for resistance against *H. contortus* for some years. The Grootfontein Dohne Merino flock, which has never been subjected to selection for resistance to *H. contortus*, was used as a reference population for the Wauldby Dohne Merino animals. Analyses of the phenotypic indicator traits, FEC, FAM and BCS were done. Genomic data analyses including PCA clustering, flock structure, genetic diversity and runs of homozygosity were performed. Results of the analyses of the phenotypic indicator traits, as well as the SNP genotypes, are reported in this chapter.

4.2 Phenotypic data

4.2.1 Descriptive statistics of phenotypic traits

Descriptive statistics for the phenotypic traits recorded over the experimental period (2011 - 2014) was estimated for the Wauldby animals. The minimum, maximum, mean, standard deviation (SD) and coefficient of variation (CV) of the monthly recordings of FEC, FAM and BCS (averaged over the years) are presented in Table 4.1 for all the available Wauldby animals. Data on FEC were transformed to logarithms to the base of 10 (after adding 10 to each value to account for zero counts) to obtain LFEC. The Average for FAM was calculated as the average FAM over all 12 recordings per animal for each year. Average BCS, FEC and LFEC were calculated the same way.

A minimum FAM of 1.0 was recorded for all the recordings and a maximum FAM of 4.0, observed in the 1st, 2nd and 3rd recordings averaged over the experimental period. The average FAM was 1.26 with a SD of 0.47 and a CV of 36.76. The lowest BCS recorded was 1.0 and was observed on the 2nd, 3rd, 7th and 11th recordings. The highest BCS was observed for the 1st and 7th recordings. The minimum FEC value was 0 and the maximum value was 52 500 epg. The average FEC was 3281 with a SD of 4156 and CV of 131. The minimum LFEC recorded was 1.0 for all recordings and the maximum LFEC was 4.72 and was observed for the 11th recording.

Coefficient of variation (CV) is a measure of dispersion of the variables. CV was lower for BCS, LFEC and FAM and was higher for FEC. CV was higher (above 100) for

FEC compared to LFEC, and the SD for FEC was greater than the data mean. The level of variation in each recording for individual phenotypic traits was low except for FEC recordings.

Table 4.1: Descriptive statistics of the phenotypic data recorded on the Wauldby animals from 2011 until 2014 averaged per recording over years.

Trait	Recording	Minimum	Maximum	Mean	SD	CV
FAM	1	1.0	4.0	1.31	0.58	44.35
	2	1.0	4.0	1.29	0.50	38.82
	3	1.0	4.0	1.40	0.59	42.02
	4	1.0	3.0	1.29	0.51	39.80
	6	1.0	3.0	1.41	0.58	41.17
	7	1.0	3.0	1.38	0.55	39.59
	8	1.0	3.0	1.30	0.49	38.01
	9	1.0	3.0	1.21	0.42	34.67
	10	1.0	2.0	1.11	0.31	27.79
	11	1.0	3.0	1.08	0.30	27.87
	12	1.0	3.0	1.13	0.34	30.30
		Average ^a	1.0	3.18	1.26	0.47
BCS	1	1.2	3.5	2.31	0.39	17.02
	2	1.0	3.0	2.14	0.35	16.28
	3	1.0	3.0	2.09	0.35	16.80
	4	1.4	3.0	2.09	0.33	15.61
	6	1.3	3.0	2.13	0.31	14.75
	7	1.0	3.5	2.14	0.31	14.26
	8	1.4	3.0	2.15	0.29	13.39
	9	1.4	3.0	2.12	0.30	14.29
	10	1.3	3.0	2.13	0.27	12.47
	11	1.0	3.0	2.10	0.27	13.07
	12	1.4	3.0	2.06	0.27	13.13
		Average ^a	1.85	3.09	2.13	0.35
FEC	1	0	28600	3672	4260	116
	2	0	36900	3376	4335	128
	3	0	38100	5150	5450	106
	4	0	45800	3727	4890	131
	6	0	35900	4022	4924	122
	7	0	37900	3996	4657	117
	8	0	29000	3212	3992	124
	9	0	37600	2853	3934	138
	10	0	40100	2500	3941	158
	11	0	52500	1991	3027	152
	12	0	26700	1596	2303	144
		Average ^a	0	37191	3281	4156
LFEC	1	1.00	4.46	3.17	0.77	24.41
	2	1.00	4.57	3.12	0.76	24.34

Trait	Recording	Minimum	Maximum	Mean	SD	CV
	3	1.00	4.58	3.37	0.74	21.93
	4	1.00	4.66	3.09	0.87	28.30
	6	1.00	4.56	3.13	0.87	27.66
	7	1.00	4.58	3.22	0.76	23.67
	8	1.00	4.46	3.10	0.76	24.66
	9	1.00	4.58	3.03	0.77	25.42
	10	1.00	4.60	3.00	0.71	23.60
	11	1.00	4.72	2.91	0.73	25.04
	12	1.00	4.43	2.77	0.76	27.48
	Average ^a	1.00	4.58	3.08	0.77	25.14

Recording 1 to 12 = Average of different recordings for each trait e.g. FAM1, FAM2, FAM3 etc.

^aThe Average for FAM was calculated as the average FAM over all 12 recordings per animal for each year over the experimental period. These average FAM values obtained for 2011 to 2014 were averaged to obtain the Average FAM presented in the table. Average BCS, FEC and LFEC were calculated the same way.

The minimum, maximum, mean, standard deviation (SD) and coefficient of variation (CV) for FAM and FEC for the GADI animals are presented in Table 4.2. The minimum FAM observed was 1 and the maximum FAM was 5. The minimum FEC was 0 and the maximum FEC was 147000 observed in the 2nd recording.

Table 4.2: Descriptive statistics of the phenotypic data recorded on the GADI animals during 2014 and 2016.

Trait	Recording	Minimum	Maximum	Mean	SD	CV
FAM	2	1	5	2.3	0.83	36.0
	3	1	3	1.4	0.54	37.8
FEC	1	0	5600	106	341	321
	2	0	147000	9937	8388	84
	3	0	30200	2176	3888	179

Recording 1 to 3 = Different recordings for each trait e.g. FEC1, FEC2, FEC3 etc.

4.2.2 Trends in phenotypic traits

Phenotypic trends in FEC, FAM and BCS were obtained from the raw data collected on the Dosed and Not dosed Wauldby Dohne Merino animals from 2011 to 2014. The phenotypic trends in FEC, FAM and BCS for the pooled data from 2011 to 2014 for the ram and ewe lambs are depicted in Figures 4.1 to 4.3 respectively.

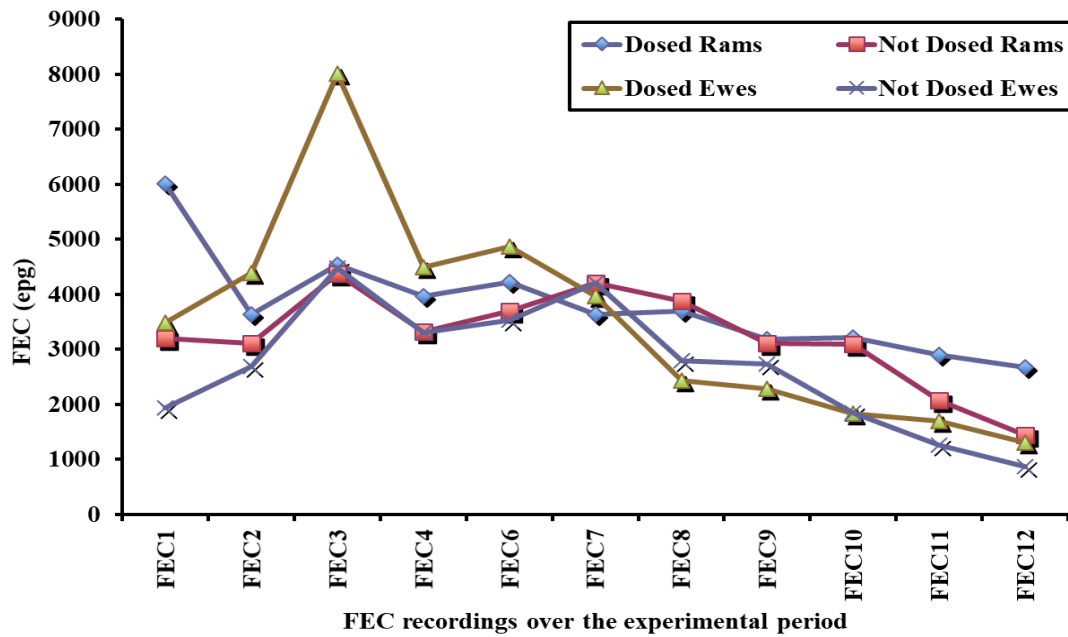


Figure 4.1: Phenotypic trends in FEC of Not dosed and Dosed lambs over the experimental period-pooled data for 2011 to 2014.

The FEC of Not dosed ram lambs for all years combined was highest in the 3rd recording and the lowest FEC was observed on the 12th recording. The FEC of Dosed ram lambs for all years combined was highest on the 1st FEC recording and lowest on the 12th FEC recording. The ram lambs that were not dosed had overall the lowest FEC compared to the Dosed ram lambs. There was a decrease in FEC from the 7th to the 12th recording, for both Dosed and Not dosed ram lambs. The FEC of both the Not dosed and Dosed ewe lambs for all years combined were the highest on the 3rd recording and the lowest FEC were observed on the 12th recording. Average FEC for the Dosed ewe lambs decreased from 6th to the 12th recording and from the 7th to the 12th recording for the Not dosed ewe lambs.

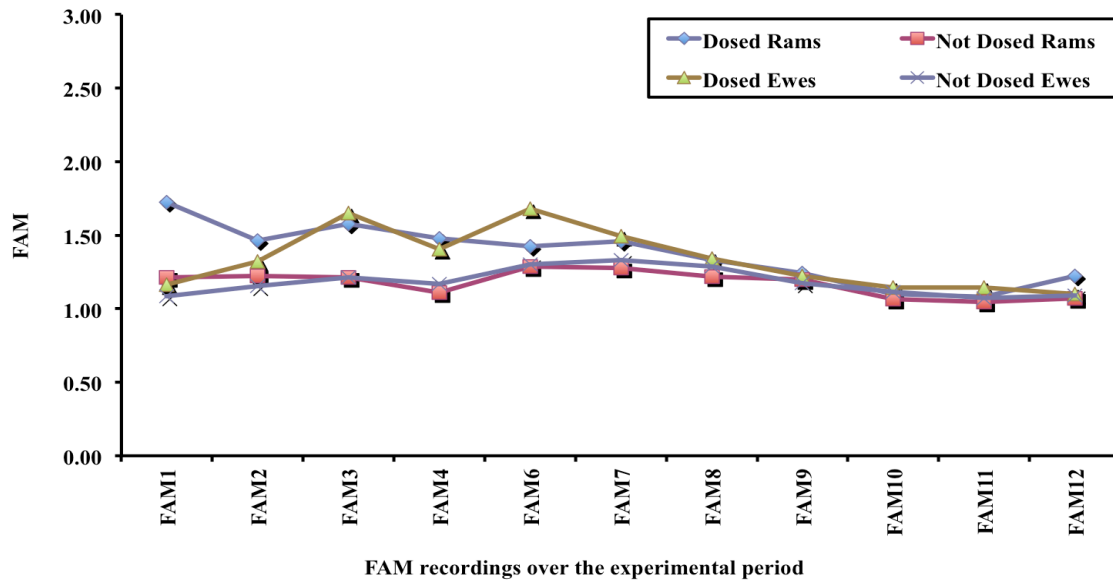


Figure 4.2: FAM of Not dosed and Dosed lambs over the experimental period-pooled data for 2011 to 2014.

The FAM of Not dosed ram lambs for all years combined was lower than that of Dosed ram lambs for all years combined. The FAM of Not dosed ewe lambs for all years combined was lower than that of Dosed ewe lambs for all years combined. The average FAM on the 1st and the 2nd recordings of the Dosed ewes was high compared to the other recordings.

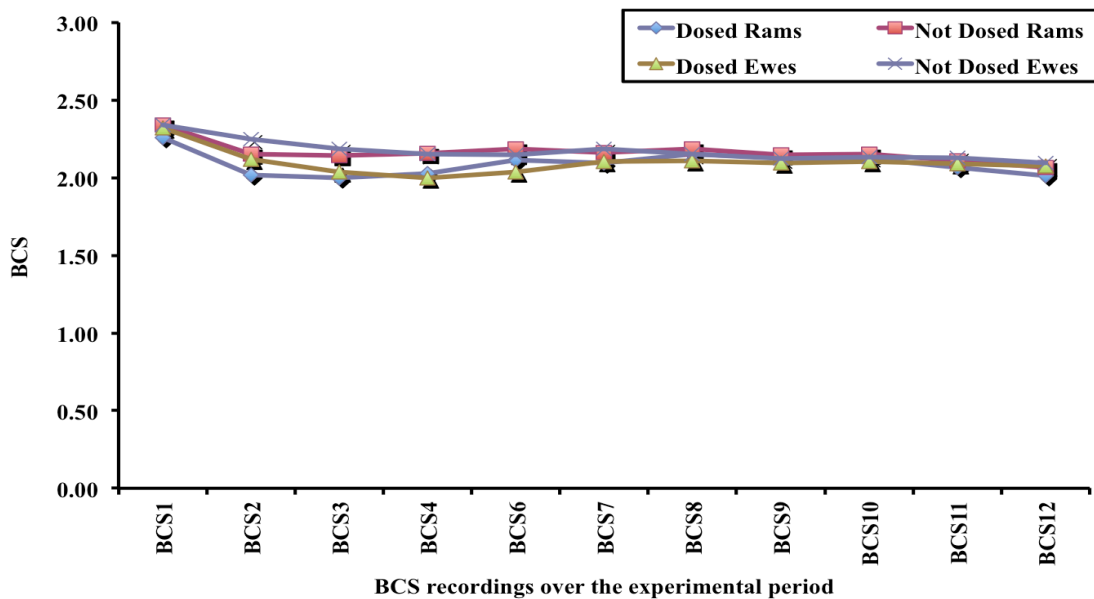


Figure 4.3: BCS of Not dosed and Dosed lambs over the experimental period-pooled data for 2011 to 2014.

There was no difference in BCS recordings of both Dosed and Not dosed ewes and rams over the experimental period.

4.2.3 Influence of non-genetic effects on phenotypic traits

The effect of year of birth, sex, birth status and dosing status on the average of the phenotypic traits recorded over the experimental period are summarised in Table 4.3 for all the Wauldby animals and in Table 4.4 for the genotyped Wauldby animals.

Table 4.3: Effect of year of birth, sex, birth status and dosing status on the average of the phenotypic traits recorded over the experimental period for all the Wauldby animals.

Effect	FAM	BCS	FEC	LFEC
Year of birth				
2011	1.41 ^a ± 0.10	2.55 ^a ± 0.06	6543 ^a ± 771	3.67 ^a ± 0.14
2012	1.66 ^b ± 0.10	2.06 ^b ± 0.05	4297 ^b ± 766	2.96 ^b ± 0.14
2013	1.80 ^c ± 0.10	1.92 ^c ± 0.05	5330 ^c ± 760	3.37 ^c ± 0.14
2014	1.79 ^c ± 0.10	1.77 ^d ± 0.06	5604 ^c ± 793	3.58 ^a ± 0.14
Sex				
Male	1.82 ^a ± 0.10	2.08 ^a ± 0.05	6359 ^a ± 749	3.54 ^a ± 0.13
Female	1.51 ^b ± 0.10	2.07 ^a ± 0.05	4528 ^b ± 755	3.25 ^b ± 0.13
Birth status				
1	1.80 ^a ± 0.06	2.11 ^a ± 0.04	6609 ^a ± 506	3.54 ^a ± 0.09
2	1.78 ^a ± 0.06	2.04 ^b ± 0.04	5889 ^b ± 500	3.41 ^b ± 0.09
3	1.42 ^a ± 0.23	2.08 ^{ab} ± 0.13	3831 ^b ± 1763	3.23 ^{ab} ± 0.31
Dosing status				
10	1.01 ^a ± 0.08	2.39 ^a ± 0.04	1862 ^a ± 584	2.92 ^a ± 0.10
21	1.24 ^b ± 0.08	2.28 ^b ± 0.04	3291 ^b ± 600	3.20 ^b ± 0.11
22	1.68 ^c ± 0.09	2.16 ^c ± 0.05	5469 ^c ± 682	3.47 ^c ± 0.12
23	1.79 ^c ± 0.14	1.83 ^d ± 0.08	7392 ^c ± 1085	3.48 ^{bc} ± 0.19

^{a,b,c,d} Values with different superscripts within effects and traits differed significantly ($P < 0.01$)

Year of birth had a significant effect at $P < 0.01$ on all traits. Sex had no effect on BCS and birth status had no effect on FAM. Sex had a significant effect on FEC, where male lambs had a higher average FEC than female lambs. Birth status had a significant effect on FEC and single lambs had a higher average FEC than twins and triplets. Dosing status had a significant effect on all phenotypic traits. Lambs that were not dosed had lower FEC and LFEC than lambs that were dosed.

Table 4.4: Effect of year of birth, sex, birth status, dosing status and group on the average of the phenotypic traits recorded over the experimental period for the genotyped Wauldby animals.

Effect	FAM	BCS	FEC	LFEC
Year of birth				
2011	1.46 ^a ± 0.16	2.78 ^a ± 0.09	7447 ^a ± 1178	3.86 ^a ± 0.25
2012	1.41 ^a ± 0.17	2.18 ^b ± 0.09	3877 ^b ± 1249	2.92 ^b ± 0.27
2013	1.59 ^a ± 0.15	2.09 ^b ± 0.08	5674 ^a ± 1087	3.45 ^c ± 0.23
2014	1.61 ^a ± 0.15	1.84 ^c ± 0.08	6039 ^a ± 1130	3.57 ^{ac} ± 0.24
Sex				
Male	1.73 ^a ± 0.15	2.24 ^a ± 0.08	6955 ^a ± 1081	3.63 ^a ± 0.23
Female	1.30 ^b ± 0.15	2.20 ^a ± 0.08	4564 ^b ± 1116	3.27 ^b ± 0.24
Birth status				
1	1.53 ^a ± 0.15	2.25 ^a ± 0.08	6302 ^a ± 1088	3.51 ^a ± 0.23
2	1.50 ^a ± 0.15	2.20 ^a ± 0.08	5216 ^a ± 1104	3.39 ^a ± 0.23
Dosing status				
10	1.21 ^{ab} ± 0.23	2.39 ^{ab} ± 0.12	2796 ^{ab} ± 1683	2.86 ^a ± 0.36
21	1.20 ^a ± 0.23	2.41 ^a ± 0.12	3129 ^a ± 1729	3.30 ^a ± 0.37
22	1.75 ^b ± 0.25	2.30 ^b ± 0.14	5553 ^b ± 1871	3.61 ^a ± 0.40
23	1.90 ^{ab} ± 0.39	1.79 ^b ± 0.21	11559 ^{ab} ± 2892	4.03 ^a ± 0.62
Group				
Case	1.61 ^a ± 0.14	2.17 ^a ± 0.07	5599 ^a ± 1038	3.27 ^a ± 0.22
Control	1.42 ^a ± 0.35	2.29 ^a ± 0.19	5919 ^a ± 2609	3.63 ^a ± 0.55

^{a,b,c} Values with different superscripts within effects and traits differed significantly ($P < 0.01$)

Year of birth had no significant effect on FAM and sex had no significant effect on BCS. Birth status had no significant effect on any of the traits. Dosing status had no significant effect on LFEC and group (Case/Control) had no significant effect on any of the traits. Male lambs had a higher average FEC than female lambs. The only difference recorded among the genotyped animals for dosing status for FEC was the higher FEC of the animals that were dosed once, compared to those who were dosed twice.

The effect of year of birth, sex and birth status on the average FAM, FEC and LFEC recorded for all the GADI animals are presented in Table 4.5 and those for the genotyped GADI animals in Table 4.6.

Table 4.5: Effect of year of birth, sex and birth status on the average of the phenotypic traits recorded over the experimental period for all the GADI animals.

Effect	FAM	FEC	LFEC
Year of birth			
2014	-	9103 ^a ± 323	3.79 ^a ± 0.02
2016	1.94 ± 0.04	6402 ^b ± 364	3.65 ^a ± 0.02
Sex			
Male	1.93 ^a ± 0.05	5677 ^a ± 335	3.60 ^a ± 0.02
Female	1.96 ^a ± 0.05	9828 ^b ± 345	3.84 ^b ± 0.02
Birth status			
1	1.85 ^a ± 0.05	7665 ^a ± 324	3.73 ^a ± 0.02
2	1.88 ^{ab} ± 0.03	7416 ^a ± 234	3.73 ^a ± 0.01
3	2.10 ^b ± 0.11	8177 ^a ± 763	3.71 ^a ± 0.05

^{a,b} Values with different superscripts within effects and traits differed significantly ($P < 0.05$)

Year of birth had no effect on LFEC, sex had no significant effect on FAM and birth status had no significant effect on FEC and LFEC. Year of birth had a significant effect on FEC and 2014-born lambs had a higher FEC than the 2016-born lambs. Lambs born as triplets had a higher FAM than single lambs. Year of birth and birth status had different effects on the phenotypic traits recorded for the Wauldby animals compared to the GADI animals. The same effect was observed for sex on phenotypic traits recorded for both GADI and Wauldby animals.

Table 4.6: Effect of year of birth, sex, birth status and group on the average of the phenotypic traits recorded over the experimental period for the genotyped GADI animals.

Effect	FAM	FEC	LFEC
Year of birth			
2014	-	14589 ^a ± 1485	3.76 ^a ± 0.05
2016	2.10 ± 0.15	6717 ^b ± 1594	3.41 ^b ± 0.05
Sex			
Male	2.02 ^a ± 0.19	9016 ^a ± 1486	3.59 ^a ± 0.05
Female	2.18 ^a ± 0.19	12290 ^a ± 1610	3.58 ^a ± 0.05
Birth status			
1	2.03 ^a ± 0.22	10638 ^a ± 1942	3.62 ^a ± 0.06
2	2.03 ^a ± 0.14	10071 ^a ± 1237	3.59 ^a ± 0.04

3	2.25 ^a ± 0.37	11250 ^a ± 2766	3.55 ^a ± 0.09
Category			
High	2.47 ^a ± 0.18	20181 ^a ± 1501	4.24 ^a ± 0.05
Low	1.73 ^b ± 0.18	1125 ^b ± 1573	2.93 ^b ± 0.05

^{a,b} Values with different superscripts within effects and traits differed significantly ($P < 0.01$)

Year of birth had a significant effect on FEC and LFEC, and lambs born in 2014 had a higher FEC than 2016-born lambs. Sex and birth status had no significant effect on all the phenotypic traits that were recorded. Category (High/Low FEC) had a significant effect on all traits and lambs in the high FEC category had a higher FEC than the lambs in the lower FEC category.

4.2.4 Genetic trends in FEC, FAM and BCS for all Wauldby animals

Genetic trends in FEC, FAM and BCS were obtained from estimated breeding values regressed on birth year for the Wauldby animals. These trends in FEC, FAM and BCS from 2011 until 2014 are depicted in Figures 4.4 to 4.6.

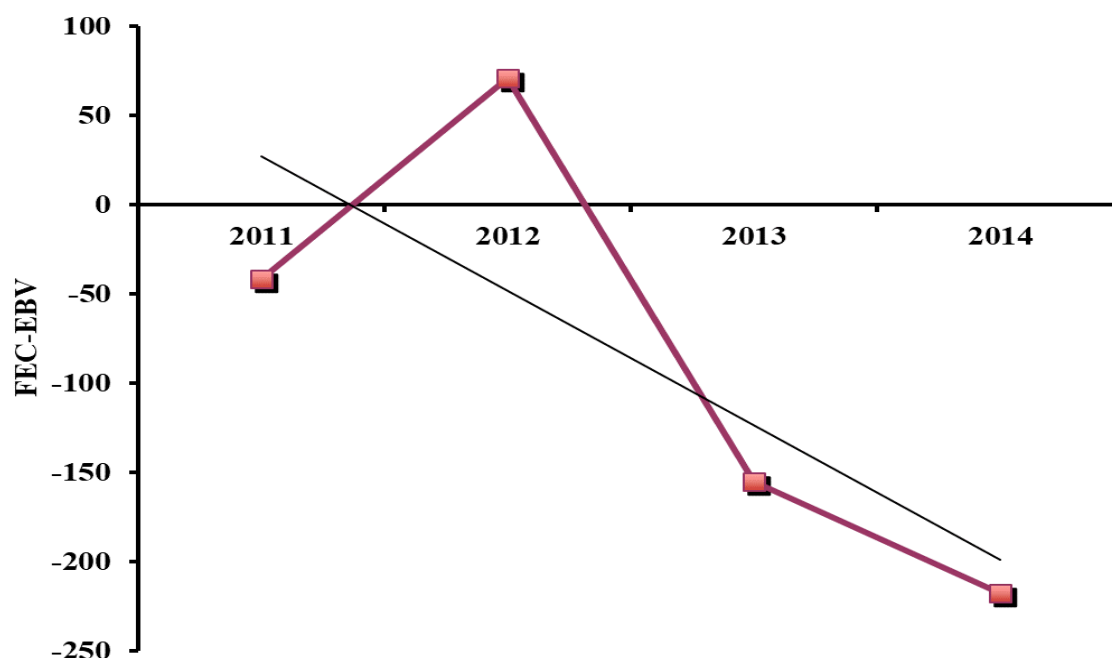


Figure 4.4: Genetic trend in FEC over the experimental period for Wauldby animals.

The EBV FEC was low in 2011 lambs and was very high in 2012 lambs. However, it was very low in 2013 and 2014-born lambs. Negative FEC EBV values observed in 2013 and 2014 show that there was genetic progress in FEC over the experimental period.

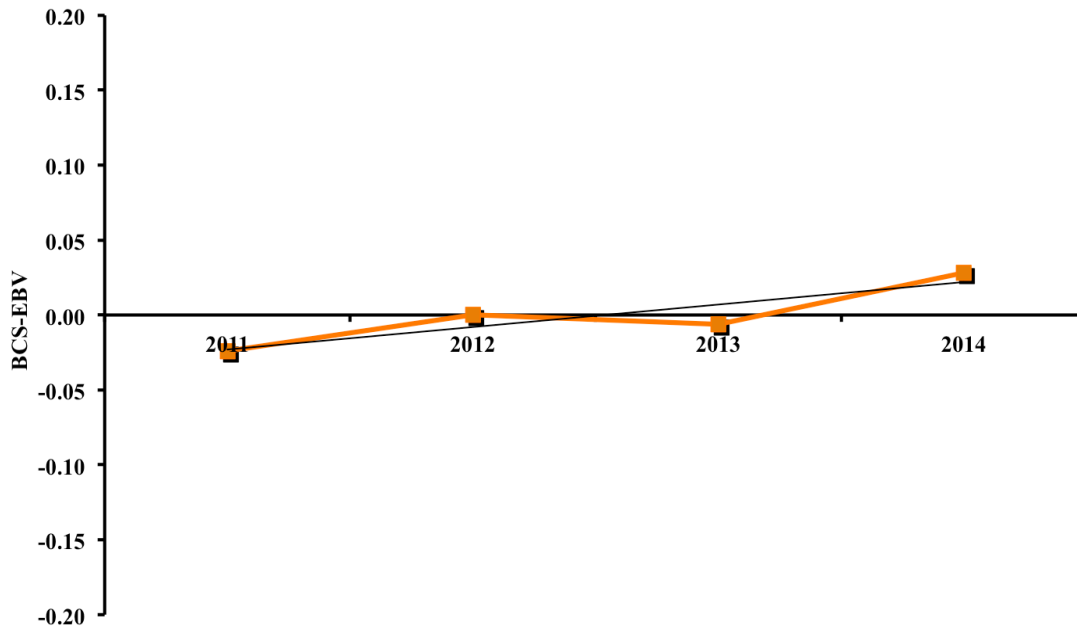


Figure 4.5: Genetic trend in BCS over the experimental period for Wauldby animals.

The breeding values for BCS were low (close to zero) over the experimental period. Therefore, there was no genetic progress made in BCS from 2011 to 2014.

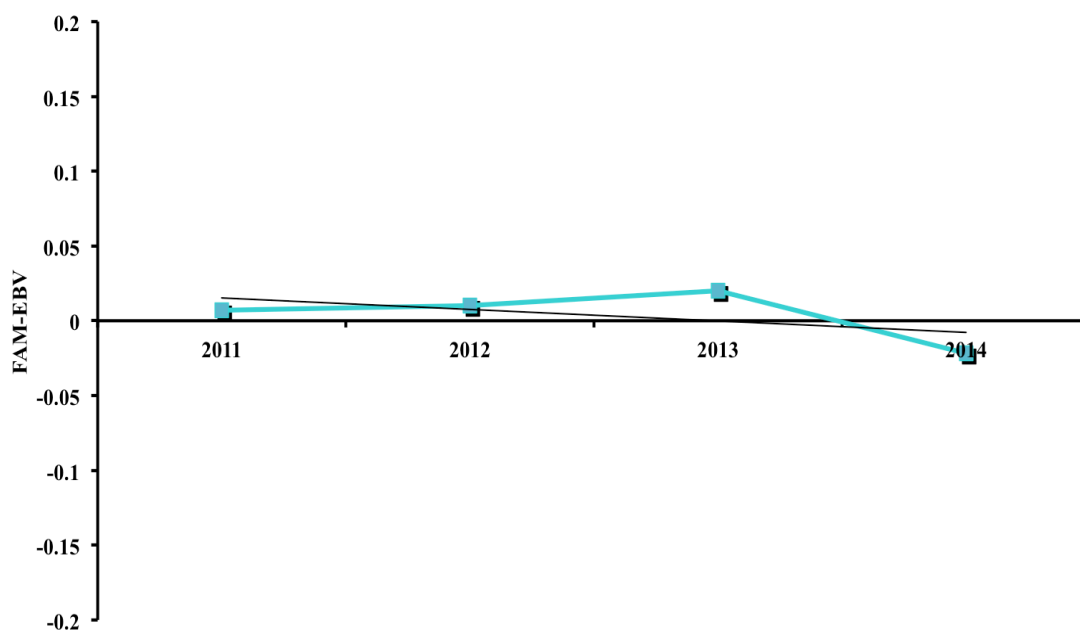


Figure 4.6: Genetic trend in FAM over the experimental period for Wauldby animals.

There was no trend or genetic progress made on FAM over the experimental period. As with BCS, this could be expected, as these traits were not under selection.

4.3 Genomic data

4.3.1 PCA genetic clustering

The principal component analysis (PCA) plot was performed using GoldenHelix SNP & Variation Suite (SVS) software (GoldenHelix Inc., Bozeman, MT, USA) to illustrate the relationship between animals within the Wauldby Dohne Merino and GADI Dohne Merino sheep populations. The first PCA plot of the Wauldby Dohne Merino animals was done to determine whether animals cluster according to Cases and Controls (Figure 4.7). The PCA plot of the Grootfontein animals was done based on High and Low FEC (Figure 4.8).

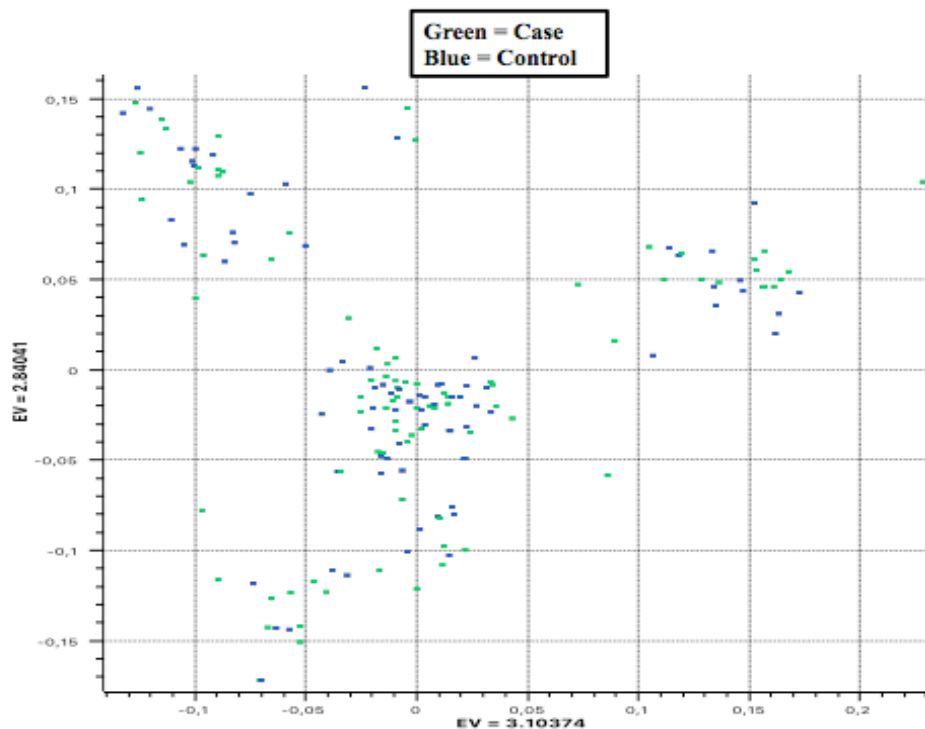


Figure 4.7: PCA based clustering of Wauldby Dohne Merino animals based on Cases and Controls.

Figure 4.7 illustrates that Cases and Controls of the Wauldby Dohne Merino animals clustered irrationally and were fairly dispersed; there were no clear groupings based on Case/Control.

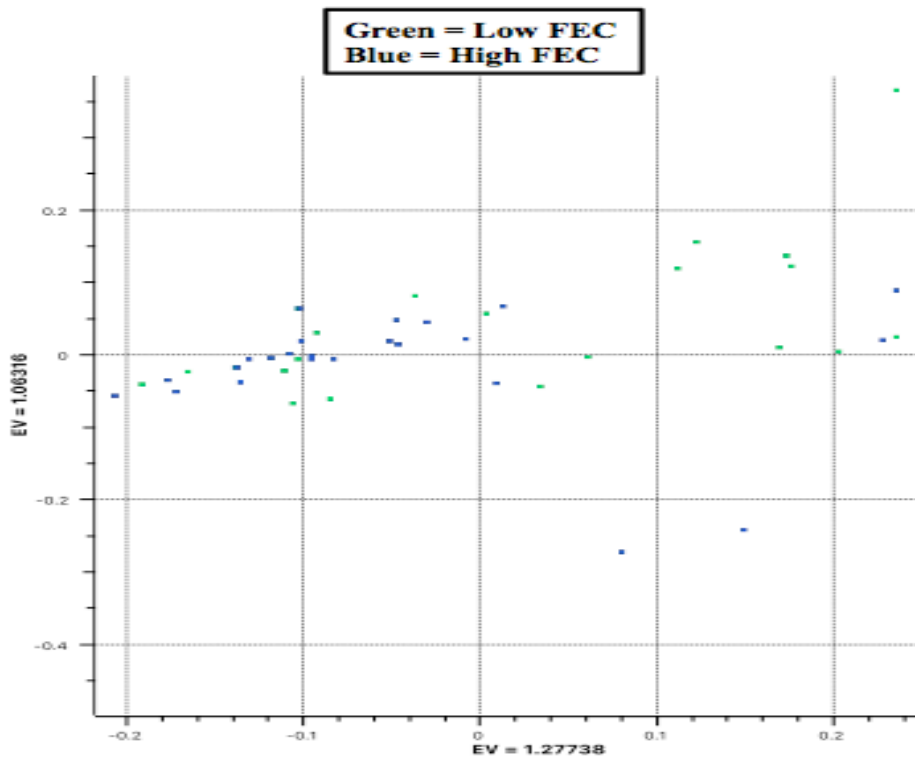


Figure 4.8: PCA based clustering of GADI Dohne Merino sheep population.

It can be observed from Figure 4.8 that animals within this flock were fairly dispersed and animals with low and high FEC values clustered together with no differentiation based on phenotype.

It was evident from Figures 4.7 and 4.8 that there was no clear-cut separate grouping of the Case and Control animals in the Wauldby or of the High and Low FEC animals in the Grootfontein flock. Consequently, principal component analysis (PCA) was done on the genotypes of all animals (without pre-defining any possible groups) to investigate the genetic differentiation of the Wauldby Dohne Merino and GADI Dohne Merino sheep populations. The resulting plot can be seen in Figure 4.9.

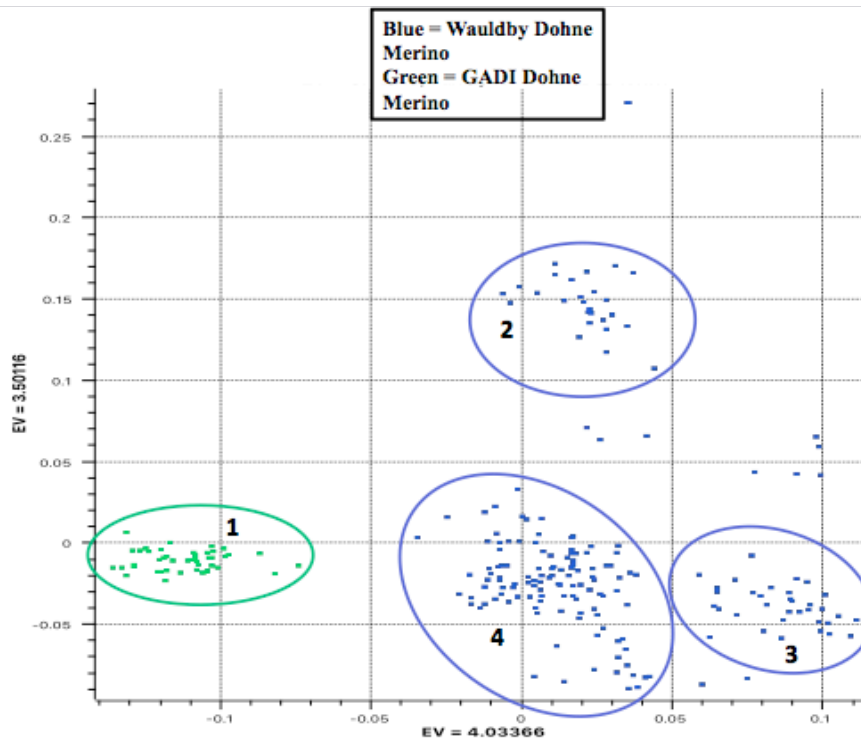


Figure 4.9: PCA based clustering of Wauldby Dohne Merino and GADI Dohne Merino sheep populations.

Four distinct genetic clusters were observed from the PCA, and the GADI Dohne Merino sheep population had its own separate genetic cluster. The Wauldby Dohne Merino had 3 distinct genetic clusters consisting of a mixture of lambs born between 2011 and 2014 (Cases/Controls). The specific allocation of animals per genetic cluster in Figure 4.9 is indicated in Table 4.7.

Table 4.7: Percentage of GADI and Wauldby animals in each genetic cluster.

Genetic Cluster	% of animals per population	
	GADI Population	Wauldby Population
Cluster 1	100%	-
Cluster 2	-	13.4%
Cluster 3	-	18.1%
Cluster 4	-	62.0%
Outliers	-	6.5%

All the animals in Cluster 1 were from the GADI population and animals in Cluster 2 to 4 were from the Wauldby population.

4.3.2 Flock structure

An ADMIXTURE plot of the GADI and Wauldby farm animals was generated to investigate flock structure. Cross-validation error estimates were obtained and then used to identify the most probable number of genetic groups (K value with the lowest cross-validation score). The cross-validation scores for each K value from 2-13 were plotted to determine the correct K value and the resulting plot can be seen in Figure 4.10. Representative results are shown for clustering using K=9, 10, and 11 (Figure 4.11).

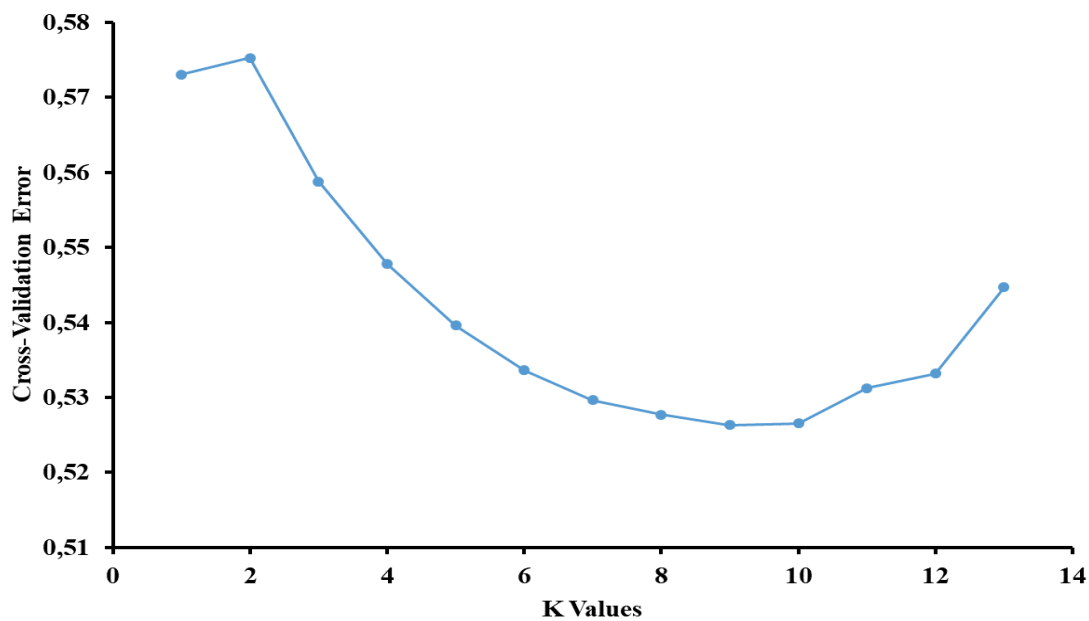


Figure 4.10: A cross-validation plot, indicating the cross-validation error rate for different K values.

The most probable number of inferred populations was chosen as ten, based on the cross-validation plot. K=10 had a lower cross-validation score than the other K values.

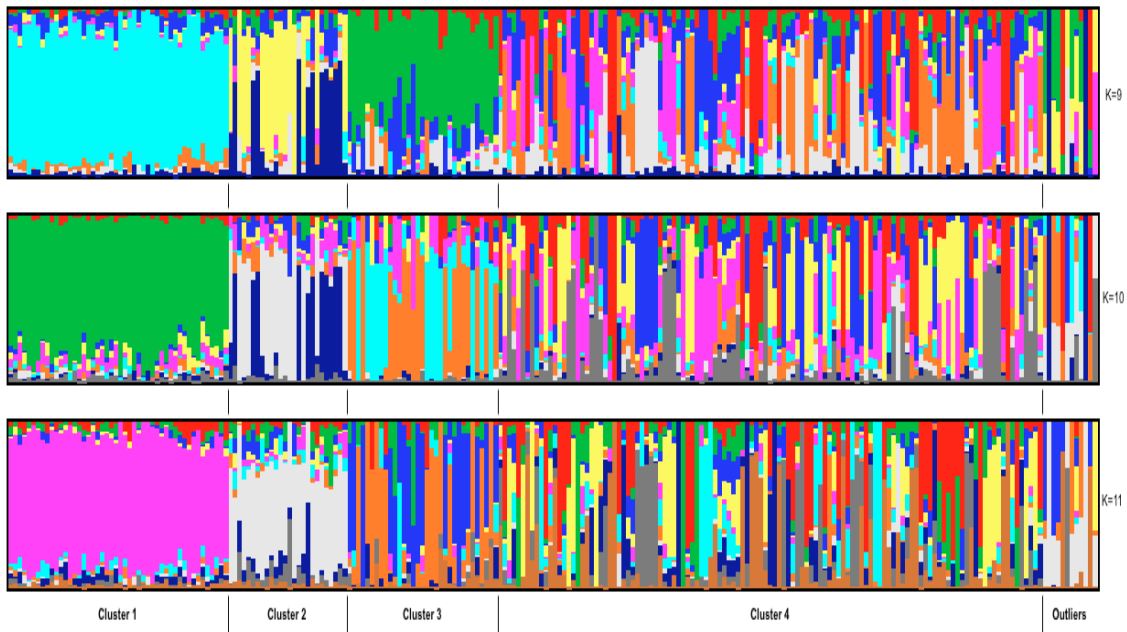


Figure 4.11: The admixture of the Wauldby Dohne Merino and GADI Dohne Merino sheep populations based on $K = 9$ to $K = 11$

Figure 4.11 shows the ancestry of sheep from the Wauldby Dohne Merino and GADI Dohne Merino sheep populations, and admixture is apparent within the Wauldby Dohne Merino flock and less admixture is observed in the GADI Dohne Merino population. The admixture results ($K=9$, $K=10$ and $K=11$) show that there was high variation within the Wauldby Dohne Merino animals, probably due to the use of specific selected sire lines. Sires with good FEC EBVs (Highly negative values) for each year were selected and then used in the following year. PCA analysis also show that there is high genetic diversity within the Wauldby Dohne Merino animals (Figure 4.7). The GADI samples clustered together in Cluster 1.

To measure the population differentiation within the Wauldby Dohne Merino and GADI Dohne Merino sheep populations, fixation index (F_{ST}) per genetic cluster was calculated. The genetic clusters were defined on the basis of the PCA clustering (Figure 4.9) and the analysis was performed to investigate the differences between genetic clusters. The results are summarized in Table 4.8.

The lowest F_{ST} was observed between Cluster 1 and Cluster 4 ($F_{ST} = 0.040$), indicating close relations between these two clusters relative to the others. The highest level of population differentiation was observed between genetic clusters 2 and 3 ($F_{ST} = 0.091$). Generally, the observed genetic diversity between genetic clusters ranged from low to

moderate.

Table 4.8: Wright’s population differentiation (F_{ST}) between the Wauldby Dohne Merino and GADI Dohne Merino sheep populations.

Genetic Cluster	Cluster 1	Cluster 2	Cluster 3	Cluster 4
Cluster 1	-			
Cluster 2	0.074	-		
Cluster 3	0.079	0.091	-	
Cluster 4	0.041	0.052	0.046	-

Genetic cluster 1 = GADI Dohne Merino sheep population

Genetic cluster 2, 3 and 4 = Mixture of Wauldby Dohne Merino animals, cases and controls

4.3.3 Population parameters

Population parameters such as observed heterozygosity (H_o), expected heterozygosity (H_e), minor allele frequency (MAF) and inbreeding coefficient (F_{IS}) were estimated for the four genetic clusters generated by the PCA (Figure 4.9). Outliers were excluded from further analysis. The levels of genetic diversity in the Wauldby Dohne Merino and GADI Dohne sheep population was evaluated using expected heterozygosity, observed heterozygosity and inbreeding coefficient. The heterozygosity rate for the two sheep populations was estimated using --hardy command in PLINK, while the MAF frequency was calculated using the --freq command. The average observed and expected heterozygosity and MAF values for the Wauldby Dohne Merino population (Genetic cluster 2, 3 and 4) and the GADI Dohne Merino population (Genetic cluster 1) can be observed in Table 4.9.

The H_e and H_o values estimated for the Wauldby and GADI populations were similar (Table 4.9).

The obtained MAF for both Wauldby Dohne Merino (28.1%) and GADI Dohne Merino (27.8%) sheep populations were high and similar to each other.

Table 4.9: Average MAF and Heterozygosity statistics for the Wauldby Dohne Merino and GADI Dohne Merino sheep populations.

Merino sheep Populations	Mean H_e	Mean H_o	Average MAF
Wauldby Dohne Merino (n=190)	0.368 ± 0.1285	0.373 ± 0.1341	0.281 ± 0.1345
GADI Dohne Merino (n=48)	0.364 ± 0.1310	0.373 ± 0.1468	0.278 ± 0.1355

The average genomic inbreeding coefficients (F_{IS}) calculated for the Wauldby Dohne Merino and GADI Dohne Merino sheep populations (based on genetic clusters) differed between genetic clusters with Cluster 3 attaining a higher average inbreeding coefficient of 0.0418 ± 0.0285 compared to the lambs in the other genetic clusters (Table 4.10). The calculated average F_{IS} values were low in all genetic clusters and this shows that inbreeding is not a problem either at Wauldby farm or GADI animals.

Table 4.10: The inbreeding coefficients (F_{IS}) of the Wauldby Dohne Merino and GADI Dohne Merino lambs per genetic cluster.

Genetic cluster	Average F_{IS}
Cluster 1	0.0088 ± 0.0296
Cluster 2	0.0022 ± 0.0387
Cluster 3	0.0418 ± 0.0285
Cluster 4	0.0112 ± 0.0284
Average	0.016 ± 0.0313

4.3.4 Runs of homozygosity

4.3.4.1 Distribution of runs of homozygosity

The average lengths of runs of homozygosity was calculated for both the Wauldby Dohne Merino and GADI Dohne Merino populations and were categorized based on their length. The results are summarized in Table 4.11. Five categories of ROH lengths were defined as follows 1-2, 2-5, 5-7, 7-10 and >10 Mb.

Table 4.11: Average length of runs of homozygosity in lambs per genetic cluster within the defined categories.

Genetic cluster	ROH averaged per genetic cluster				
	1-2 Mb	2-5 Mb	5-7 Mb	7-10 Mb	>10 Mb
Cluster 1	1.94 ± 0.032	3.42 ± 0.773	5.82 ± 0.552	8.19 ± 0.777	15.65 ± 7.728
Cluster 2	1.94 ± 0.006	3.46 ± 0.794	5.89 ± 0.554	8.20 ± 0.755	15.77 ± 7.836
Cluster 3	1.96 ± 0.043	3.12 ± 0.802	5.82 ± 0.558	8.25 ± 0.817	15.60 ± 7.547
Cluster 4	1.96 ± 0.054	3.43 ± 0.788	5.85 ± 0.575	8.13 ± 0.790	15.32 ± 7.042

The number of runs of homozygosity was calculated for each genetic cluster within the defined ROH categories and the results are depicted in Figure 4.12.

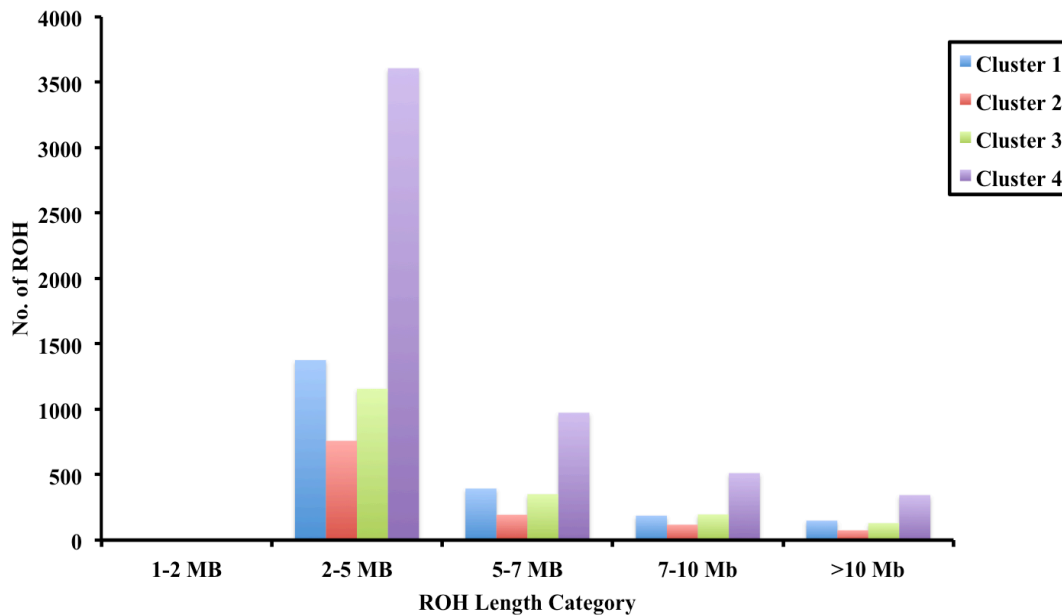


Figure 4.12: The number of runs of homozygosity per genetic cluster within the defined ROH length categories.

A total of 10546 ROH were observed, 5447 (51.65%) of which were found in the lambs in Genetic cluster 4 (116 animals), 2113 (20.04%) in the lambs in Genetic cluster 1 (48 animals), 1838 (17.43%) in the lambs in Genetic cluster 3 (34 animals), and 1148 (10.89%) were found in lambs in Genetic cluster 2 (25 animals). The number of runs of homozygosity is related to the total number of samples in each genetic cluster. The highest number of runs was observed in the category of 2-5 Mb for all the genetic clusters. The highest number of

long ROH was found in Genetic cluster 4. Number of ROH in the 1-2 MB category were not visible on the graph due to the scale as the number of ROHs in this category was low for all the genetic clusters.

4.3.4.2 The distribution of runs of homozygosity per chromosome

The number of ROH was obtained for each chromosome in the genomes of the of Wauldby Dohne Merino and GADI Dohne Merino populations. The distribution of runs of homozygosity per chromosome for each genetic cluster is depicted in Figures 4.13.

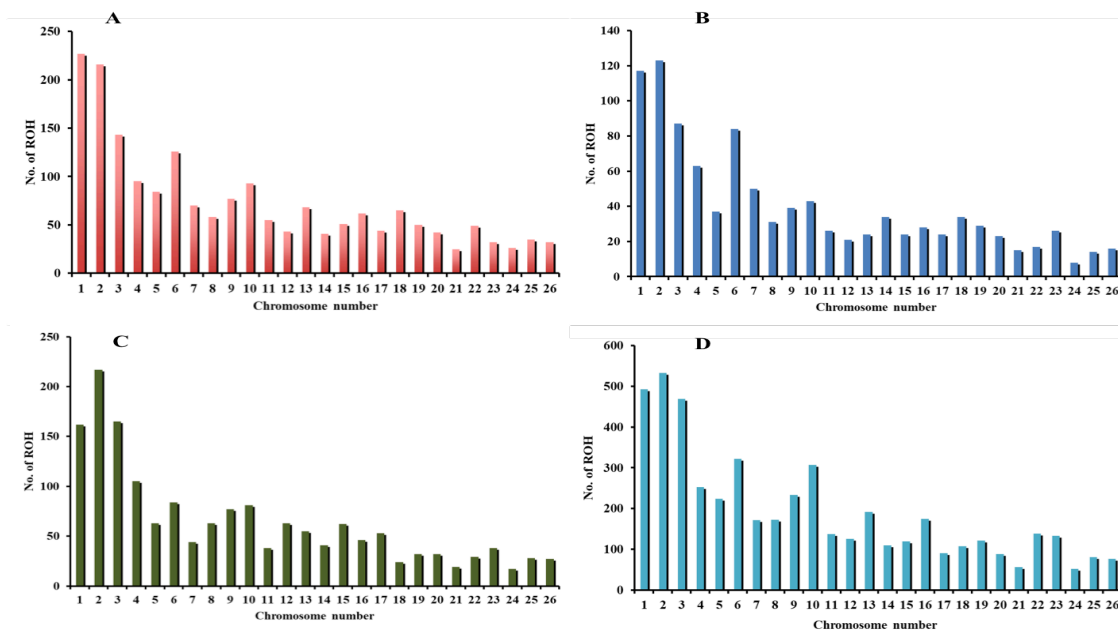


Figure 4.13: The number of runs of homozygosity (ROH) per chromosome for lambs in Genetic cluster 1 (A), Cluster 2 (B), Cluster 3 (C) and Cluster 4 (D).

Chromosome 1, 2, 3, 4, 6 and 10 had the highest number of ROH in the genomes of animals in Genetic cluster 1 (GADI Dohne Merino lambs). Chromosome 1, 2, 3, 4, 6, 7, 10 and Chromosome 1, 2, 3, 4, 6, 9 and 10 had more ROH in Genetic cluster 2 and 3 respectively (Wauldby farm Dohne Merino lambs). The smallest number of ROH was found in chromosome 21 and 24 in all Genetic clusters. Chromosome 1, 2, 3, 4, 6, 9 and 10 had the highest number of ROH in the genomes of animals in Genetic cluster 4 (Wauldby farm Dohne Merino lambs). Therefore, all clusters showed a significant number of ROH on OAR 1, 2, 3, 4, 6, 9 and 10.

4.3.5 Association of ROH with QTLs reported in sheep

The Sheep Quantitative Trait Loci Database (QTLdb) (<http://www.animalgenome.org/QTLdb/sheep>) was used to search for QTLs associated to parasite resistance in chromosomes with high number of ROH. The highest number of QTLs in the sheep genome are located in chromosome 1 (124), chromosome 2 (244), chromosome 3 (174) and chromosome 6 (182). The highest number of ROH was also found in these chromosomes. The positions of parasite resistance traits in the sheep genome are presented in Table 4.12.

Table 4.12: The location of different parasite resistance traits in the sheep genome. The location of different parasite resistance traits in the sheep genome.

Parasite resistance traits	QTL location
Fecal egg count (FECGEN)	Chr 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 13, 14, 15, 16, 17, 19, 21, 22, 23, 24, 25 and 26
Eggs per worm (EPW)	Chr 26
<i>Haemonchus contortus</i> FEC (HFEC)	Chr 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 15, 16, 18, 20, 21, 22, 23, 24, 25 and 26
<i>Haemonchus contortus</i> FEC (HFEC_2)	Chr 12, 13, 16, 20, 23
Worm count (WORMCT)	Chr 2, 12, 16, 18, 22, 23, 24, 25 and 26
Strongyle FEC (SFEC)	Chr 3 and 20
Nematodirus FEC (NFEC)	Chr 2, 3, 11, 14, 15
<i>Trichostrongylus colubriformis</i> FEC (TFEC_1)	Chr 1, 3, 6, 11, 12, 18, 22
Change in hematocrit (DHCT)	Chr 2, 3, 11, 13 and 26
Hematocrit (HCT)	Chr 1, 3, 5, 8, 14, 15, 18, 20, 22 and 25

Source: <http://www.animalgenome.org/QTLdb/sheep>

Parasite resistance traits are found throughout the sheep genome and traits such as fecal egg count (FECGEN) and *Haemonchus contortus* FEC (HFEC) are found almost in all chromosomes. EPW is only found on chromosome 26 in the sheep genome.

4.3.6 Genetic cluster differentiation based on phenotypic information

Genetic clusters were described in terms of year of birth, sex, birth status, dosing status, Case/Control groups, High/Low FEC and selected sires/dams as parents' results of which are presented in Table 4.13.

Table 4.13: Composition of Clusters in terms of fixed effect classes, group and parentage of animals in each cluster.

Effect	No of animals per cluster				Outliers
	1	2	3	4	
No of animals per cluster	48	25	34	116	12
Year of birth					
2011	-	5	0	22	0
2012	-	3	9	21	2
2013	-	6	0	55	4
2014	24	11	25	18	6
2016	24	-	-	-	-
Sex					
Male	24	11	23	50	5
Female	24	14	11	66	7
Birth status					
1	12	15	20	70	12
2	30	10	14	46	0
3	6	-	-	-	-
Dosing status					
10		13	19	62	4
21		8	11	43	7
22		2	4	11	1
23		2	0	0	0
Case/Control					
Case		12	15	55	8
Control		13	19	61	4
FEC					
High	25				
Low	23				
Selected sires/dams as parents					
Selected sire / selected dam		1	27	9	6
Selected sire / other dam		0	3	0	0
Other sire / selected dam		9	2	35	3
Other sire / other dam		15	2	72	3

Genetic cluster 1 = GADI Dohne Merino sheep population

Genetic cluster 2, 3 and 4 = Mixture of Wauldby Dohne Merino animals, cases and controls

Outliers = Mixture of Wauldby Dohne Merino animals, cases and controls

Genetic cluster 4 had the most animals (116) comprising of a mixture of cases and controls born between 2011 and 2014. Genetic clusters 2, 3 and 4 had more controls than

cases. One of the animals in Genetic cluster 2, 30 in Genetic cluster 3, and 9 in Genetic cluster 4 were from sires that were selected for the resistant line.

4.3.7 Least-square means (LSmeans) and combinations of the traits by cluster for the genotyped Wauldby animals

Least squares means for the various available phenotypic traits and combinations of the traits for all phenotypic traits were obtained for the different genetic clusters of the genotyped Wauldby animals. The results are summarized in Tables 4.14 to 4.18.

Table 4.14: LFEC averages (\pm SD) for each of the individual recordings for the different genetic clusters of the Wauldby Dohne Merino sheep.

Trait	Genetic clusters		
	2	3	4
LFEC1	3.602 ^a \pm 0.170	3.111 ^b \pm 0.186	3.278 ^{ab} \pm 0.154
LFEC2	3.231 ^a \pm 0.177	2.978 ^a \pm 0.194	3.280 ^a \pm 0.161
LFEC3	3.335 ^a \pm 0.177	3.179 ^a \pm 0.194	3.342 ^a \pm 0.160
LFEC4	2.986 ^a \pm 0.198	2.654 ^a \pm 0.217	2.856 ^a \pm 0.179
LFEC6	3.458 ^a \pm 0.172	2.944 ^b \pm 0.188	3.358 ^a \pm 0.156
LFEC7	2.835 ^{ab} \pm 0.216	2.423 ^a \pm 0.236	2.939 ^b \pm 0.196
LFEC8	3.152 ^a \pm 0.195	2.505 ^b \pm 0.213	3.076 ^a \pm 0.176
LFEC9	3.010 ^a \pm 0.204	2.337 ^b \pm 0.224	3.081 ^a \pm 0.185
LFEC10	2.926 ^{ab} \pm 0.183	2.574 ^a \pm 0.200	3.080 ^b \pm 0.166
LFEC11	2.996 ^a \pm 0.172	2.493 ^b \pm 0.188	2.954 ^a \pm 0.156
LFEC12	2.820 ^a \pm 0.171	2.424 ^b \pm 0.186	2.880 ^a \pm 0.155
LFECA	3.602 ^a \pm 0.170	3.111 ^b \pm 0.186	3.278 ^{ab} \pm 0.154

^{a,b} Values with different superscripts differ significantly ($P < 0.05$) between clusters within rows; Values with the same superscripts did not differ significantly ($P > 0.05$) between clusters within rows; LFEC1 = Log-transformed Faecal egg count of 1st recording, etc.; LFECA = Average Log-transformed Faecal egg count averaged over all recordings per year.

There was no significant difference between genetic clusters based on the 2nd, 3rd and 4th LFEC recordings. The LFEC values at all other recordings differed significantly between the 3 genetic clusters.

Table 4.15: FEC averages (\pm SD) for each of the individual recordings for the different genetic clusters of the Wauldby Dohne Merino sheep.

Trait	Genetic clusters		
	2	3	4
FEC1	7270 ^a \pm 788	3936 ^b \pm 862	5339 ^b \pm 714
FEC2	3655 ^a \pm 958	4142 ^a \pm 1048	3077 ^a \pm 869

FEC3	4021 ^a ± 1306	4238 ^a ± 1429	4467 ^a ± 1184
FEC4	3383 ^a ± 1157	2264 ^a ± 1265	2467 ^a ± 1049
FEC6	5311 ^{ab} ± 1160	3141 ^a ± 1269	5731 ^b ± 1052
FEC7	3999 ^a ± 1192	80 ^b ± 1304	3521 ^a ± 1081
FEC8	4151 ^a ± 998	270 ^b ± 1092	3399 ^a ± 905
FEC9	2715 ^a ± 765	70 ^b ± 837	2601 ^a ± 694
FEC10	2731 ^a ± 1054	871 ^a ± 1153	2241 ^a ± 956
FEC11	2076 ^a ± 445	819 ^b ± 486	1981 ^a ± 403
FEC12	1993 ^a ± 458	1102 ^a ± 501	1625 ^a ± 416
FECA	7270 ^a ± 788	3936 ^b ± 862	5339 ^b ± 714

^{a,b} Values with different superscripts differ significantly ($P < 0.05$) between clusters within rows; Values with the same superscripts did not differ significantly ($P > 0.05$) between clusters within rows; FEC1 = Faecal egg count of 1st recording, etc.; FECA = Faecal egg count averaged over all recordings per year.

Similarly to the results on LFEC, showed in Table 4.14, there was no significant difference between the groups for recordings 2-4. Additionally, no differences were found for recordings 10 and 12.

Table 4.16: BCS averages (±SD) for each of the individual recordings for the different genetic clusters of the Wauldby Dohne Merino sheep.

Trait	Genetic clusters		
	2	3	4
BCS1	2.17 ^{ab} ± 0.06	2.29 ^a ± 0.06	2.16 ^b ± 0.05
BCS2	2.05 ^a ± 0.05	2.08 ^a ± 0.06	2.02 ^a ± 0.05
BCS3	2.00 ^a ± 0.05	2.06 ^a ± 0.06	1.98 ^a ± 0.05
BCS4	1.96 ^a ± 0.06	2.05 ^a ± 0.07	1.98 ^a ± 0.06
BCS6	1.93 ^a ± 0.06	1.99 ^a ± 0.06	2.00 ^a ± 0.05
BCS7	2.04 ^a ± 0.05	2.22 ^b ± 0.06	2.13 ^a ± 0.05
BCS8	2.04 ^a ± 0.05	2.22 ^b ± 0.05	2.13 ^a ± 0.04
BCS9	2.03 ^{ab} ± 0.05	2.11 ^a ± 0.05	2.01 ^b ± 0.04
BCS10	2.05 ^a ± 0.04	2.16 ^b ± 0.05	2.13 ^{ab} ± 0.04
BCS11	2.06 ^a ± 0.04	2.14 ^a ± 0.05	2.07 ^a ± 0.04
BCS12	2.06 ^a ± 0.04	2.14 ^a ± 0.05	2.12 ^a ± 0.04
BCSA	2.17 ^a ± 0.06	2.29 ^b ± 0.06	2.16 ^a ± 0.05

^{a,b} Values with different superscripts differ significantly ($P < 0.05$) between clusters within rows; Values with the same superscripts did not differ significantly ($P > 0.05$) between clusters within rows; BCS1 = Body condition score for the 1st recording; BCSA = Body condition score averaged over all recordings per year.

BCSA differed significantly between Genetic cluster 2 and 4, and Genetic cluster 3 had a higher BCS than genetic cluster 2 and 4. The BCS phenotypes followed the same trend as the previous two traits, with no significant difference detected for recordings 2-4, and additionally for recordings 6, 11 and 12.

Table 4.17: FAM averages (\pm SD) for each of the individual recordings for the different genetic clusters of the Wauldby Dohne Merino sheep.

Trait	Genetic clusters		
	2	3	4
FAM1	1.53 ^a \pm 0.11	1.59 ^a \pm 0.12	1.60 ^a \pm 0.10
FAM2	1.29 ^a \pm 0.11	1.59 ^b \pm 0.12	1.41 ^{ab} \pm 0.10
FAM3	1.66 ^a \pm 0.13	1.87 ^a \pm 0.14	1.88 ^a \pm 0.12
FAM4	1.22 ^a \pm 0.11	1.29 ^a \pm 0.12	1.29 ^a \pm 0.10
FAM6	1.59 ^a \pm 0.14	1.70 ^a \pm 0.16	1.80 ^a \pm 0.13
FAM7	1.44 ^a \pm 0.12	1.49 ^a \pm 0.13	1.44 ^a \pm 0.10
FAM8	1.32 ^{ab} \pm 0.13	1.17 ^a \pm 0.14	1.41 ^b \pm 0.11
FAM9	1.15 ^{ab} \pm 0.09	1.00 ^a \pm 0.10	1.20 ^b \pm 0.08
FAM10	1.30 ^a \pm 0.07	1.10 ^b \pm 0.08	1.29 ^a \pm 0.07
FAM11	1.18 ^a \pm 0.05	1.09 ^a \pm 0.05	1.17 ^a \pm 0.04
FAM12	1.42 ^a \pm 0.08	1.37 ^a \pm 0.09	1.34 ^a \pm 0.07
FAMA	1.53 ^a \pm 0.11	1.59 ^a \pm 0.12	1.60 ^a \pm 0.10

^{a,b} Values with different superscripts differ significantly ($P < 0.05$) between clusters within rows; Values with the same superscripts did not differ significantly ($P > 0.05$) between clusters within rows; FAM1 = Famacha[©] score for the 1st recording; FAMA = Famacha[©] score averaged over all recordings per year.

There were only 4 recordings which showed significant differences between the genetic clusters for FAM, namely recordings 2, and 8-10.

Combinations of the traits were estimated for the different genetic clusters of the Wauldby animals. Analyses of the resistance data recorded on the Wauldby animals indicated that a combination of information recorded at the first, sixth and ninth recordings (FEC169, BCS169, FAM169 etc.) could be used to select animals with improved resistance against *H. contortus*. Selection index, incorporating LFEC, FAM and BCS was estimated and was also compared amongst the clusters. The results are summarized in Table 4.18.

Table 4.18: Averages (\pm SD) for resistance trait combinations for the different genetic clusters of the Wauldby Dohne Merino sheep.

Trait	Genetic clusters		
	2	3	4
FEC169	5099 ^a \pm 599	2382 ^b \pm 655	4557 ^a \pm 543
FEC179	4661 ^a \pm 678	1362 ^b \pm 742	3821 ^a \pm 615
LFEC179	3.15 ^a \pm 0.14	2.62 ^b \pm 0.15	3.10 ^a \pm 0.13
BCS179	2.07 ^a \pm 0.04	2.20 ^b \pm 0.04	2.09 ^a \pm 0.04

FAM179	1.38 ^a ± 0.07	1.36 ^a ± 0.07	1.41 ^a ± 0.06
SI179	7.54 ^a ± 0.19	8.22 ^b ± 0.21	7.58 ^a ± 0.17

^{a,b} Values with different superscripts differ significantly ($P < 0.05$) between clusters within rows; Values with the same superscripts did not differ significantly ($P > 0.05$) between clusters within rows; FEC169 = Average faecal egg count for the 1st, 6th and 9th recordings, etc; FAM179 = Average Famacha[©] score for the 1st, 7th and 9th recordings; BCS179 = Average body condition score for the 1st, 7th and 9th recordings; LFEC179 = Average Log-transformed Faecal egg count for the 1st, 7th and 9th recordings; FAM179 = Average Famacha[©] score for the 1st, 7th and 9th recordings. Selection index = Body condition score – Log-transformed FEC – Famacha[©] score.

Genetic cluster 3 differed significantly from Clusters 2 and 4 for all traits except for FAM179. Genetic cluster 3 had a lower FEC169, FEC179, LFEC17 and higher BCS179 and SI179 trait combinations compared to the other genetic clusters.

4.3.8 Estimated breeding values (EBVs) for recorded phenotypic traits

Breeding values were estimated for all the phenotypic traits for the genotyped Wauldby animals in the three different genetic clusters. The differences in EBVs of the sires of the animals between genetic clusters for all phenotypic traits are summarized in Table 4.19 and Table 4.20.

Table 4.19: Averages for estimated breeding values (\pm SD) for the phenotypic traits of animals in the different genetic clusters of the genotyped Wauldby Dohne Merino sheep.

Trait	Genetic clusters		
	2	3	4
EBV-FEC	114 ^a ± 97	-629 ^b ± 84	-2 ^a ± 45
EBV-LFEC	0.037 ^a ± 0.025	-0.209 ^b ± 0.022	0.005 ^a ± 0.012
EBV-FAM	-0.029 ^a ± 0.011	-0.025 ^a ± 0.010	0.015 ^b ± 0.005
EBV-BCS	-0.024 ^a ± 0.009	0.058 ^b ± 0.008	0.005 ^c ± 0.004

^{a,b,c} Values with different superscripts differ significantly ($P < 0.05$) between clusters within rows; Values with the same superscripts did not differ significantly ($P > 0.05$) between clusters within rows; EBV-FEC = Estimated breeding value for faecal egg count, etc.

Genetic cluster 3 differed significantly from Genetic cluster 2 and 4, on both EBV-FEC and EBV-LFEC, with lower EBVs for both traits. There was a significant difference between Genetic cluster 2 and 4 and between Cluster 3 and 4 for the EBV-FAM recording and Genetic cluster 2 had a lower EBV. Genetic clusters 2 and 3 and 4 differed significantly for EBV-BCS.

Table 4.20: Averages for estimated breeding values (\pm SD) for the phenotypic traits of the sires in the different genetic clusters of the Wauldby Dohne Merino sheep.

Trait	Genetic clusters		
	2	3	4
EBV-FEC	-328 ^{ab} \pm 300	-790 ^a \pm 300	-51 ^b \pm 146
EBV-LFEC	-0.060 ^{ab} \pm 0.077	-0.227 ^a \pm 0.077	-0.002 ^b \pm 0.038
EBV-FAM	-0.036 ^a \pm 0.025	-0.040 ^a \pm 0.025	0.024 ^b \pm 0.012
EBV-BCS	-0.002 ^{ab} \pm 0.035	0.072 ^a \pm 0.035	-0.009 ^b \pm 0.017

^{a,b} Values with different superscripts differ significantly ($P < 0.05$) between clusters within rows; Values with the same superscripts did not differ significantly ($P > 0.05$) between clusters within rows; EBV-FEC = Estimated breeding value for faecal egg count, etc.

Genetic cluster 3 and 4 differed significantly, for all traits except EBV-FAM where genetic cluster 2 and 3 did not differ and both genetic clusters were significantly different from genetic cluster 4. Genetic cluster 3 had a lower EBV-FEC, EBV-LFEC, EBV-FAM and higher EBV-BCS.

Chapter 5: Discussion

The aim of this study was to investigate phenotypic and genetic differences in resistance to *Haemonchus contortus* between resistant and susceptible animals in the Wauldby Dohne Merino sheep flock, which has been selected for resistance against *H. contortus* for some years. To achieve this, we firstly investigated phenotypic differences in terms of FEC, BCS and FAM amongst resistant and susceptible sheep. Subsequently, the genetic diversity and flock clustering of the Wauldby and GADI Dohne Merino flocks and its association with resistance to *H. contortus* were investigated on a genomic level.

5.1 Description of phenotypic traits associated with resistance to gastrointestinal nematodes

Internal parasites remain a major problem to the health and productivity of sheep worldwide (Cornelius *et al.*, 2014). The increasing incidence of AR and the need to minimize chemical residues in animal products, as well as the high cost associated with anthelmintic drugs, call for alternative nematode control strategies (Morris *et al.*, 2010). According to Pollott & Greeff (2004), breeding animals with inherent resistance to parasites is one of the options available to farmers. According to Riggio *et al.* (2013), selection for nematode resistance has mainly been based on the use of indicator traits such as BCS, body weight, FEC, PCV and FAMACHA[©] scoring. Some of these traits (e.g. BCS, FAM and FEC) were routinely recorded in the Wauldby flock over a period of 5 years.

Descriptive statistics for the phenotypic traits (FEC, FAM, BCS and LFEC) recorded over the experimental period (2011-2014) were estimated for all the Wauldby animals (Table 4.1). There were fewer animals that had a FAM score ≥ 3 among the Not dosed than among the Dosed animals over the experimental period. According to Riley & Van Wyk (2009), only animals with high FAMACHA[©] scores (3-5), with increasing pale eye membrane color are subjected to anthelmintic treatment. FAM was high (3.0 and 4.0) from the first recording up until the 8th recording after which there is a decrease in average FAM. The results show that some animals in these recordings were severely infected.

A mean FAM (coefficients of variation in brackets) of 1.23 (33%), 1.40 (43%) and 1.90 (42%) were reported under conditions of low, moderate and peak nematode challenge (Riley & Van Wyk, 2009). Riley and Van Wyk (2011) also reported a mean FAM of 1.92 (43%) in a South African Merino flock. In our study, FAM means for each recording were

lower than the FAM average recorded by Riley and Van Wyk (2011) in their study.

The overall average BCS recorded for all the years was 2.13 with a SD of 0.35 and a CV of 13.73. Some of the BCS scores recorded from 2011 to 2014 were critically low (1.0). Gallidis *et al.* (2009) stated that animals with BCS ≤ 2 should be treated with anthelmintics. The average BCS of each recording and the overall BCS average were higher than 2. The control animals had better BCS scores compared to case animals over the experimental period. Cornelius *et al.* (2014) recommended that sheep below a pre-determined BCS be included in the treatment group to minimize the risk of losses in sheep with low BCS. Vatta *et al.* (2002) didn't find a clear relationship between FEC and BCS and suggested that BCS is probably more closely related to the nutrition of the sheep.

The maximum FEC values recorded from 2011 to 2014 were very high. This shows that *Haemonchus* challenge was very high at Wauldby and that some animals were highly infected. According to Bowie (2014), FAM and FEC scores are expected to decrease steadily over time as a result of anthelmintic treatment. However, they may increase slightly before treatments lower them down further. The results provided in this study (Figure 4.1 and 4.2) are consistent with the findings of Bowie (2014) as far as the Dosed animals are concerned.

In this study, the observed CVs for FEC were very high (above 100%), suggestive of a very high variation and a non-normal distribution of FEC recordings over the experimental period. The distribution of FEC recorded over the experimental period was heavily skewed towards the lower end of the range. Cloete *et al.* (2007) and Pollott & Greeff (2004) also found similar distribution and variation in FEC. Large individual variation is expected in untransformed FEC data and is commonly reported in literature. The results provided in this study are consistent with those reported in the literature. Khusro *et al.* (2004) reported FEC in yearling and hogget Australian Merinos ranging from 0 to 51895 and 0 to 53583 epg respectively.

The untransformed FEC for a mixture of *Ostertagia* and *Trychostrongylus* spp. reported by Cloete *et al.* (2007) in the Merino flock at Tygerhoek ranged from 0 to 13667 epg. Faecal worm egg (FWEC) for a mixture of helminthic nematodes with the genera *Teladorsagia* (*Ostertagia*) and *Trychostrongylus* spp. present as major species and *Haemonchus contortus* as a minor species ranged from 0 to 27600 epg in a Merino flock at Elsenburg (Mpetile *et al.*, 2015).

In a study by Cloete *et al.* (2016), the FECs for *H. contortus* in Dorper and SAMM lambs were extremely variable ranging from 0 to 34100 epg in wet faeces at Elsenburg and

from 0 to 32700 epg in wet faeces at Tygerhoek. In this study, the GADI sheep's FAM ranged from 1 to 5 and FEC ranged from 0 to 147000 epg (Table 4.2). The observed FAM and FEC ranges in the GADI population were higher than those recorded for the Wauldby population. The differences in the range of FECs or FWECs observed in different studies are due to breed, nematode species and environmental differences.

FEC is known to be exceedingly variable and skewed, needing transformation prior to analysis to normalize the data (Cloete *et al.*, 2007). Data on FEC were transformed to logarithms to the base of 10 (after adding 10 to each value to account for zero counts) to normalize the skewed distribution. The application of transformations on FEC data improved the data as indicated by lower CVs in the log-transformed data. Cloete *et al.* (2007) and Matebesi-Ranthimo *et al.* (2014) reported a CV in FWEC exceeding 100% before transformation, which reduced to below 20% after log transformation. Similar results were found in this study, however, the level of CV was slightly higher (up to 28% after transformation) than in their studies. According to Mpetile *et al.* (2015) high CV values are indicative of significant phenotypic variation, which could lead to improved genetic gains and response to selection.

The genetic variation in resistance to nematode infection between and within breed genotypes is well documented (Woolaston & Piper, 1996; McManus *et al.*, 2014; Vijayasarithi *et al.*, 2016) and is influenced by genetic and environmental factors (Rout *et al.*, 2011). The difference in FAM and FEC observed between the Wauldby Dohne Merino and GADI Dohne Merino sheep populations may be due to genetic and environmental factors. Factors such as host age, sex, climate, nutrition, grazing management, physiological conditions and immunity influence the susceptibility of animals to nematode parasites (Van Wyk & Reynecke, 2011; Abuargob *et al.*, 2014). The environmental conditions in which the Wauldby and GADI Dohne Merino sheep populations were bred and maintained were completely different as Wauldby receives much higher rainfall per annum compared to GADI and the *H. contortus* challenge in the two regions is different. The GADI population was also not selected for *H. contortus* resistance.

According to Traoré *et al.* (2017), positive associations of FEC and FAMACHA[©] scores means that FAMACHA[©] scores could be used to identify animals that would benefit from anthelmintic treatment. In the current study the same trend was observed, the FAM averages were high when high FEC averages were recorded e.g. recordings 3, 6 and 7 for both phenotypic traits were high.

5.2 Genetic trends in FEC, FAM and BCS over the experimental period

The pattern of higher FECs observed in the first 3 or 4 recordings (January to April) from both Dosed and Not dosed ram/ewe lambs (Figure 4.1) agrees with studies in sheep raised under commercial farming conditions in the summer rainfall region of South Africa (Vatta *et al.*, 2002). In their study they found that FECs for *Haemonchus* were high from October to March at Rust de Winter and from September/October to February or April at Kraaipan. The lowest FECs were recorded in February at Rust de Winter and during October at Kraaipan. They also found that BCSs of the sheep at Rust de Winter were lower during August 1999 to mid-February 2000 (1.2 and 2.7) and that BCSs at Kraaipan were higher during the summer months but lower during July to December 1999 (1.3 to 2.0).

Greeff *et al.* (1995) stated that FEC is influenced by various factors, which include seasonal variation, management practice and geographical area. In their study, aimed at estimating the genetic composition of FWEC at various times of the year in Merino lambs born in a Mediterranean environment, they found that the heritability estimates for FWEC under natural worm challenge (*Ostertagia*, *H. contortus* and *Trychostrongylus spp.*) was high from June to October (0.21 to 0.25), and low from February to April (0.00 to 0.03), while the highest heritability of 0.51 was reached in July. Seasonal rainfall events and temperature fluctuations play a significant role in an environment to which sheep is subjected, and the development of infective larvae varies between environments (Vatta *et al.*, 2002).

The mean average FAM of Dosed ram/ewe lambs recorded from 2011 to 2014 was higher than that of Not dosed ram/ewe lambs recorded over the experimental period (Figure 4.2). These results were consistent with the findings of Vatta *et al.* (2002) where they observed that the lambs with high FECs (Dosed) had higher FAM scores compared to the not dosed or resistant/resilient sheep. Van Wyk (2001), also found a very good relationship between FEC and FAM scores and suggested that FAM can safely and cheaply be substituted for FEC's if *H. contortus* is the dominant species. Each animal has a different threshold between worm load carried and resulting anaemia.

The difference between the average BCS of Not dosed ewe/ram lambs and that of Dosed lambs over the experimental period was very small (Figure 4.3). There was no clear seasonal pattern in the BCSs of the Dosed or Not dosed ewe/ram lambs over the experimental period. A better understanding of the relationship between the appearance of symptoms normally associated with nematode parasitism, individual infection levels, and level of host response to infection is required (McManus *et al.*, 2014).

5.3 Influence of non-genetic effects on phenotypic traits

Sex had a significant ($P < 0.01$) effect on FAM, FEC and LFEC (Table 4.3) and the same trend was observed in genotyped Wauldby animals (Table 4.4). Males had a higher average FEC compared to females (Figure 4.1). The FEC of Dosed ewe lambs recorded over the experimental period in Figure 4.1 was high in the 2nd, 3rd, 4th, 5th and 6th recordings compared to the FEC of Not dosed ram lambs. The FEC of Not dosed rams over the experimental period was higher than the FEC of Not dosed ewe lambs from the 8th to the 12th recordings. Hence, the Not dosed ewe lambs were more resistant/resilient than the Not dosed ram lambs over the experimental period.

In a study by Khusro *et al.* (2004), the results of sex effects on FEC were variable. In yearling animals, ewes had a higher FEC than rams, while the opposite was true in hoggets. Greeff & Karlsson (2006) reported that some rams have a genetic tendency to acquire a higher FEC (more susceptible) in an environment with low GIN challenge and that some rams have the ability to resist the GIN infections more than others in an environment with high GIN challenge. Sex had a significant effect on FEC and LFEC in all GADI animals and females had higher FEC averages than males (Table 4.5) and these results are consistent with those of Khusro *et al.* (2004). Cloete *et al.* (2007) and Matebesi-Ranthimo *et al.* (2014), observed lower FEC in ewes than in rams, however, the magnitude was different between years. Mpetile *et al.* (2015) in their study found that the overall log-transformed FEC of ram lambs was almost double that of ewe lambs ($P < 0.001$). These results are consistent with results reported in the present study and also with Barger (1993) who found that females were more resistant than males after exposure to gastrointestinal strongyle infection.

Abuargob *et al.* (2014) stated that differences between female and males are due to difference in behavior, morphology or physiological status of sex. The consistent and clear sex differences in favor of females regarding response to GIN infections suggest that the male flock should be given more attention in order to maintain lower worm burdens or FEC values and mortality rates (Haile *et al.*, 2007).

Year of birth had a significant ($P < 0.01$) effect on all phenotypic traits and average FEC was lower in 2012 than in other years in all as well as the genotyped Wauldby animals (Tables 4.3 and 4.4). In a study by Burke *et al.* (2016), year had a significant effect on all GIN measures and in some models, gender and age influenced GIN measures. Mpetile *et al.* (2015) also found that birth year had a significant ($P < 0.001$) effect on Log-transformed FEC and our results are consistent with their findings.

In the current study, birth status had a significant ($P < 0.01$) effect on BCS, FEC and LFEC, and singles had a higher FEC than twins or triplets (Table 4.3). Mpetile *et al.* (2015) in their study, found higher FECs in single (766 ± 68) than in multiple (731 ± 67) birth types and the results from the current study are consistent with their findings. Cloete *et al.* (2007) reported similar results. These results show that singles are more susceptible to GIN infections. Our results are inconsistent with the study of Haile *et al.* (2007) where they found higher FECs in twins than in singles and concluded that singles are better in tolerating the pathogenic effect of parasites than twins.

In the present study, dosing status had a significant effect on all phenotypic traits. Lambs that were not dosed had a lower FEC (1862 ± 584), higher BCS (2.39 ± 0.04) and lower FAM (1.01 ± 0.08) compared to the lambs that were dosed. FEC, LFEC and FAM increased with the number of doses while BCS kept on decreasing. These results were expected since the Not dosed lambs were the resistant line and were expected to have low FEC, high BCS and low FAM. Case/Control group had no significant effect on any of the traits (Table 4.4). Year of birth and category (High/low FEC) had a significant effect on FAM, FEC and LFEC in the genotyped GADI animals (Table 4.6). These results support the PCA plot of the genotyped Wauldby Dohne Merino lambs (Figure 4.7) where cases and controls clustered together.

5.4 Genetic trends of the estimated breeding values for phenotypic traits associated with GIN resistance

Genetic resistance to GIN infection is one of the most promising means to control worms in a flock (Heckendorn *et al.*, 2017). According to Mpetile *et al.* (2015), breeding animals for improved GIN resistance would result in increasing and permanent improvements in desired traits. However, application of breeding for host resistance against GINs requires genetic variation of GIN resistance traits to be present and it also requires knowledge and understanding of the genetic and phenotypic relationships between parasite resistance and production traits (Greeff & Karlsson, 2006). Breeds and individuals that are resistant to GIN infections can be identified by recording traits that are indicative of host resistance to GINs (Periasamy *et al.*, 2014).

Selection of resistant sires using EBVs leads to lower FEC and FAM scores and higher BCS in offspring (Silva *et al.*, 2012). In the present investigation, genetic trends in FEC, FAM and BCS were obtained from average estimated breeding values (EBV) regressed

on birth year for the Wauldby animals. EBV for FEC is an important genetic selection tool that provides a great opportunity to help improve genetic resistance to internal parasites (Riggio *et al.*, 2014a).

Gray (1991) identified FEC as a practical method of indirectly measuring parasite resistance. As a result, FEC has been used extensively to estimate genetic variation in resistance to GIN infection in sheep (Morris *et al.*, 1996; Khusro *et al.*, 2004; Cloete *et al.*, 2007; Pickering *et al.*, 2012; Matebesi-Ranthimo *et al.*, 2014). These authors found that after a number of years of selection, the selection line had a reduced FEC compared to the control animals. Figure 4.4 shows the genetic trend in FEC over the experimental period and the FEC EBV was low in 2011 (-41.680 ± 63.734), high in 2012 (70.416 ± 66.612) and low in 2013 (-155.279 ± 62.606) and 2014 (-218.077 ± 67.555) (Table 4.1A). These results show that there was genetic progress in FEC over the experimental period. Therefore, it is possible to breed sheep that are more resistant to internal parasites using FEC over a period of time.

Animals that have a low or negative FEC EBV can be expected to be more resistance to parasites than animals with higher EBVs for FEC (Kemper *et al.*, 2011). Selecting animals with the lowest FEC would result in improved parasite resistance in a flock. Woolaston *et al.* (1997) reported that genetic selection reduced FEC markedly in an Australian Merino resource flock. This is in line with the result obtained in the current study over a period of 5 years. In a study that was aimed at selection for reduced FEC, genetic selection of animals with improved host resistance resulted in increased profit in Australia and similar results could be achieved in SA provided that comprehensive data collection methods are followed and that the timing of sampling for FEC is optimized (Pollott & Greeff, 2004; Greeff & Karlsson, 2006).

The genetic trend in BCS over the experimental period is shown in Figure 4.5. The BCS EBV was low in 2011 (-0.024 ± 0.008), 2012 (-0.0001 ± 0.008) and 2013 (-0.006 ± 0.008), and in 2014 (0.028 ± 0.008) (Table 4.1A). These results show that there was no genetic progress in BCS over the experimental period. Figure 4.6 shows the genetic trend in FAM over the experimental period and FAM EBV was low in 2011 (0.007 ± 0.008), low in 2012 (0.010 ± 0.009), low in 2013 (0.020 ± 0.008) and low in 2014 (-0.022 ± 0.009) (Table 4.1A). There was no genetic progress in FAM over the experimental period. FAM scores could offer producers the ability to select from candidate sires and dams using ranked predicted breeding values for FAM scores, to improve GIN resistance and/or resilience (McManus *et al.*, 2014).

The ultimate objective of breeding for nematode resistance is to have sheep that are able to live and produce better under conditions of relatively severe internal parasite challenge (De Souza Chagas *et al.*, 2016). Burke *et al.* (2016) in their study observed a decrease in EBV FEC and EBV FAM (indicating greater GIN resistance) over the experimental period. The findings presented in the current study followed the same trend although there were very small differences observed in EBV FAM recorded per year. Genetic resistance to parasite resistance is possibly the best way of GIN control, and can be achieved through selection of sires with favorable EBVs, which is shown by a lower FEC, lower FAM and higher BCS in offspring. According to the genetic trends observed in the current investigation, the selection criteria followed for selecting sheep that are resistant to *H. contortus* did result in genetic change in the FEC.

5.5 Genetic population structure of the Wauldby Dohne Merino and GADI Dohne Merino sheep populations

Studying population structure and genetic relationships within/between populations can reveal useful information within and between populations and this information can be used for breed improvement programs (Molotsi *et al.*, 2012). Characterizing the population structure and genetic diversity within/between the Wauldby Dohne Merino and GADI Dohne Merino sheep populations will help with an understanding of the genetics of host resistance or susceptibility to *H. contortus*. In order to understand the relationship within and between the Wauldby Dohne Merino and GADI Dohne Merino sheep populations, Principal Components Analysis (PCA), ADMIXTURE and fixation index (F_{ST}) analyses were performed.

There was no separation of cases and controls observed in a PCA analysis of the Wauldby Dohne Merino flock (Figure 4.7). The animals were expected to cluster according to cases and controls, with cases forming their group and control animals forming their own cluster as well. However, the results obtained in the present study did not indicate any clear-cut correlation between the Case/Control groupings. The control group also included animals with very high FECs that did not need dosing. The observed clustering may also be due to intensive selection pressure imposed by the farmer on the Wauldby Dohne Merino flock, which may have caused genetic divergence and high genetic diversity in a population. Small sample size (192 samples) could also be the other reason for the observed but unexpected clustering. If more animals were included in the study, maybe different results would've been

obtained from the current investigation. It is also possible that there was not enough genetic variation in GIN resistance traits within the Wauldby Dohne Merino flock. Increasing the experimental period could also improve the results, because the PCA and ADMIXTURE plots did demonstrate that the Wauldby Dohne Merino flock is under selection although not along the traits predefined.

Four distinct clusters were observed in the PCA analysis of both the Wauldby and GADI Dohne Merino flocks (Figure 4.9), with the GADI Dohne Merino sheep population clustering on its own (Cluster 1). Three distinct clusters (Clusters 2 to 4) were observed for the Wauldby Dohne Merinos consisting of a mixture of animals from the case and control groups (lambs born between 2011 and 2014). A separate tight cluster observed for the GADI Dohne Merinos during PCA analysis indicate that this flock is genetically distinct from the Wauldby population that is being selected in a certain direction. This PCA plot shows that there is high genetic diversity within the Wauldby Dohne Merinos and that the Wauldby Dohne Merino and GADI Dohne Merinos are two distinct populations. It also shows that there is low to moderate genetic diversity between the Wauldby Dohne Merino and GADI Dohne Merino sheep populations. PCA and ADMIXTURE results were generally in agreement and separated the individuals by population.

The most likely number of populations was $K=10$ for the current studied populations. A clear division was observed between the Wauldby Dohne Merino flock and the GADI Dohne Merino flock (Figure 4.11), corresponding to PCA (Figure 4.9). Figure 4.11 revealed high genetic differentiation within the Wauldby Dohne Merinos (Cluster 2 to 4). However, some individuals within the cases and controls clustered together, these animals shared some genomic components. There was a consistent pattern of within-population genetic subdivision in the Wauldby Dohne Merino flock.

The Wauldby Dohne Merino animals were clearly more diverse than animals from the GADI population. The GADI Dohne Merino sheep flock clustered together and there was very little divergence in the population which shows that the population is not being selected for disease resistance. The population differentiation results indicated that the Wauldby Dohne Merino and GADI Dohne Merino populations are two genetically different populations with different breeding objectives. These results support the genetic clustering observed on the PCA plot (Figure 4.9).

GoldenHelix SNP & Variation Suite (SVS) software (GoldenHelix Inc., Bozeman, MT, USA) was used to evaluate population relatedness using pair-wise estimates of F_{ST} . The

observed genetic differentiation between genetic clusters ranged from low to moderate. The F_{ST} values obtained from this study show that we sampled within a local population (Wauldby Dohne Merino population) in which mating is controlled and that there is low to moderate genetic differentiation between/within subpopulations. Genetic cluster 2 and 3 showed the highest genetic differentiation between any of the subpopulations tested ($F_{ST} = 0.091004398$).

The least genetic diversity was observed between genetic cluster 1 (GADI Dohne Merino population) and genetic cluster 4 (Wauldby Dohne Merino population) (0.040563653) (Table 4.8). This might be due to the fact that compared to other genetic clusters, genetic cluster 4 had more 2013-born lambs. Population differentiation between the Wauldby Dohne Merino population (genetic cluster 2, 3 and 4) and the GADI suggest high genetic diversity within the Wauldby Dohne Merino population and low/moderate genetic diversity between the Wauldby Dohne Merino and the GADI Dohne Merino sheep populations. The observed F_{ST} values between genetic clusters suggest that Wauldby Dohne Merino flock and the GADI Dohne Merino flock are genetically different. Results of the admixture plot are in agreement with the low F_{ST} values reported between genetic clusters.

5.6 Basic Population parameters estimated for the Wauldby Dohne Merino and GADI Dohne Merino sheep populations

Gene diversity in the Wauldby Dohne Merino flock was similar (mean $H_e = 0.3680$, mean $H_o = 0.3733$) to that observed in the GADI Dohne Merino flock (mean $H_e = 0.3647$, mean $H_o = 0.3736$) (Table 4.9). The H_e and H_o values of the Wauldby Dohne Merino and GADI Dohne Merino sheep populations were high and very similar. The observed heterozygosity within the Wauldby Dohne Merino and the GADI Dohne Merino sheep populations suggest that selection and genetic progress in resistance traits can be made without immediate concerns of inbreeding especially in the Wauldby Dohne Merino flock which was selected for improved host resistance against *H. contortus*.

According to Woolaston & Piper (1996), high level of genetic variation in populations could be due to factors such as management and breeding systems under which selection was done. The Wauldby Dohne Merino flock has been selected for resistance to parasites whereas the GADI population was not selected for parasite resistance. High genetic diversity within the Wauldby Dohne Merino and GADI Dohne Merino sheep populations is important for future selection for GIN resistance. According to Ganz & Ebert (2010), the genetically

diverse host populations are expected to have lower levels of infection.

Estimates of H_e of Wauldby Dohne Merino and GADI Dohne Merino sheep populations agree with literature estimates that indicate high levels ($H_e = 0.321$) of genetic diversity in Merino breeds (Kijas *et al.*, 2012). Grasso *et al.* (2014) studied the genetic diversity between Corriedale ($H_o = 0.3549$, $H_e = 0.3549$) and Merino breeds ($H_o = 0.3772$, $H_e = 0.3618$) and found generally high levels of polymorphism between the breeds. In their study, they concluded that the results for the Merino sheep were expected since this breed was included in the design/validation of OvineSNP50.

The Wauldby Dohne Merino flock exhibited a higher average minor allele frequency (MAF) (28.1%) than the GADI Dohne Merino flock (27.8%), although the MAF averages were close to each other. Sandenbergh *et al.* (2016) found average MAF of 22% for the South African Mutton Merino (SAMM), 23% for the Dorper and 26% for the SA Merino. They also found $H_e = 0.33$ for SAMM, and 0.34 and 0.35 for the Dorper and SA Merino respectively. Results found in the current study compare favorably with the mean MAF and heterozygosity estimates reported in the literature for other commercial sheep breeds (Kijas *et al.*, 2009; Molotsi *et al.*, 2012; Sandenbergh *et al.*, 2016). According to Kijas *et al.* (2009), the Merino breed is one of the most genetically diverse livestock breeds. Meadows *et al.* (2008) reported that the analysis of genetic diversity within five sheep breeds indicated that the Merino contained the highest genetic diversity. It is therefore not surprising that the Wauldby Dohne Merino and GADI Dohne Merino sheep populations included in the current study exhibited the high mean MAF and high levels of genetic diversity.

Inbreeding is the mating of closely related individuals, individuals that share a common ancestor (Ojango *et al.*, 2011). The genetic effects of inbreeding are well documented; inbreeding results in an increase in average homozygosity in a population and its effects commonly referred to as inbreeding depression include impaired health, loss of genetic variation, fertility and productivity in livestock species (Ballou, 1983; Purfield *et al.*, 2012). Inbreeding coefficient is defined as the likelihood that two alleles at any locus in an individual are identical by descent (Szulkin *et al.*, 2013) and is used to estimate levels of inbreeding in a population.

The calculated F_{IS} were low in all genetic clusters and these results strongly suggest that the Wauldby Dohne Merino and GADI Dohne Merino sheep flocks are outbred populations where breeding is controlled (Table 4.10). Swanepoel (2006) reported that the level of inbreeding in the South African Dohne Merino sheep population is very low.

Sandenbergh *et al.* (2016) also found low F_{IS} (0.06) in SA Merino population. The F_{IS} observed in the current study are in line with the results of Swanepoel (2006) and these results suggest that inbreeding is not a serious problem in the Wauldby Dohne Merino and GADI Dohne Merino sheep populations.

5.7 Runs of homozygosity per genetic cluster

One of the aims of this study was to determine the prevalence and distribution of runs of homozygosity in the Wauldby and GADI Dohne Merino sheep populations and their association with parasite resistance traits. Runs of homozygosity (ROH) are lengthy, unceasing segments of identical genotypes which are without heterozygosity in the diploid state (Ferenčaković *et al.*, 2013a). These regions are present in different individuals of the same species due to parents passing on identical haplotypes to their progeny. The availability of high density SNP arrays provides an opportunity to screen the genomes of both the Wauldby Dohne Merino and GADI Dohne Merino sheep populations for ROH.

According to Peripolli *et al.* (2017), selection increases homozygosity around the target region. Therefore, long or high ROHs are expected in regions under selection. Runs of homozygosity are signatures of inbreeding and their location in the genome may reveal trait affected by recent selection in the population (Mastrangelo *et al.*, 2017). The Wauldby farm animals are being selected for parasite resistance and QTLs associated with parasite resistance are expected to be located in regions with long consecutive ROHs / high ROH regions. In the present study, we were able to identify regions of homozygosity of different lengths in the genomes of the Wauldby Dohne Merino and GADI Dohne Merino sheep populations.

The presence of long ROH at relatively high frequency in a population could also indicate the presence of genetic substructure, with consanguineous mating (individuals who are related as second cousins or closer) occurring only within some subpopulations (Iacolina *et al.*, 2016). Genetic cluster 4 had the highest mean length of ROH in category >10 Mb (Table 4.11) compared to the other genetic clusters. The number of ROHs was greatest on chromosomes 1, 2, 3, 4, 6 and 10 in Genetic cluster 1 and 4 (Figure 4.13A and 4.13D), and was greatest on chromosome 1, 2, 3, 4, 6 and 7 in Genetic cluster 2 (Figure 4.13B). Only chromosome 1, 2, 3 and 4 had the highest number of ROH in Genetic cluster 3 (Figure 4.13C).

The highest number of quantitative trait loci (QTL) in the sheep genome is found on chromosome 1 (124), 2 (244), 3 (174) and 6 (182) (www.animalgenome.org/cgi-bin/QTLdb/OA/browse). This might explain the highest number of ROH observed in these chromosomes. There is quite a number of parasite resistance traits in the sheep genome and these include fecal egg count (FECGEN) which is found in all chromosomes except for Chr 12 and 18, *Haemonchus contortus* FEC (HFEC) found in all chromosomes except for Chr 14, 17 and 19, worm count (WORMCT) located on Chr 2, 12, 16, 18, 22, 23, 24, 25 and 26 and Strongyle FEC (SFEC) which is located on Chr 3 and 20 (www.animalgenome.org/cgi-bin/QTLdb/OA/browse). Yan *et al.* (2017) found 9 SNPs located within the FECGEN QTL (Chr 13) and were associated with FEC. Marshall *et al.* (2009) located HFEC on Chr 1, 3, 4, 7, 11, 16, 18, 21, 22 and 25.

QTL for Eggs per worm (EPW) is located on chromosome 26 and Nematodirus FEC (NFEC) is only found on Chr 2, 3, 11, 14, 15 in the sheep genome. Putative QTL for *Haemonchus contortus* FEC (HFEC_2) has been identified on Chr 12, 13, 16, 20, 23. The blood parameter traits for which QTL have been observed, include change in hematocrit (DHCT) which is located on Chr 2, 3, 11, 13 and 26 and hematocrit (HCT) which is found on Chr 1, 3, 5, 8, 14, 15, 18, 20, 22 and 25 (www.animalgenome.org/cgi-bin/QTLdb/OA/browse). Davies *et al.* (2006) located NFEC on Chr 2, 3 and 14 in the Scottish Blackface sheep breed. Marshall *et al.* (2009) detected HFEC_2 on Chr 1, 3, 6, 7, 8, 9, 10, 12, 15, 16, 20, 21, 22, 24, 25 and 26 in the Merino sheep breed. According to Gutiérrez-Gil *et al.* (2009), most studies on the detection of QTL for parasite resistance in sheep have been carried out in different sheep populations and that has resulted in little consensus among the results reported.

According to Peripolli *et al.* (2017), strong selection pressure reduces genetic diversity in a population and this is characterized by lengthy, continuous stretches of homozygous genotypes in the genome. The manner in which different parasite resistant traits are located in the sheep genome suggest that the indicator traits (FEC, FAM and BCS) used in the current study are also likely to be located in genomic regions with high number of ROHs since the Wauldby population is under selection for improved parasite resistance.

5.8 Least-square means (LSmeans) by cluster for Wauldby animals

An aim of this study was to investigate the genetic differences between the resistant and susceptible Wauldby animals (Cases/Controls). However, Case and Control animals

clustered together and created three genetic clusters in a PCA analysis. Significant differences between the three genetic clusters on a phenotypic level were then investigated for the genotyped Wauldby farm animals. LSmeans for the various available phenotypic traits, combinations of the traits and EBVs for all phenotypic traits were obtained for the different genetic clusters (2, 3 and 4) of the genotyped Wauldby farm animals.

Genetic clusters were described in terms of lamb's year of birth, birth status, sex, dosing status, case/control, and selected sires/dams. Animals in Cluster 3 had lower FEC, lower FAM, higher BCS and higher selection index values than the animals in Clusters 2 and 4. The majority of the sires (88%) of the animals in Cluster 3 was selected for the resistant line, while only 4.0% and 7.8% of the sires in Clusters 2 and 4 respectively, were selected sires. The findings presented in the current study clearly show that Cluster 3 had the highest number of highly resistant animals and that is why they clustered together. This also shows genetic progress over the experimental period since 74% of the animals in Cluster 3 were born in 2014. These results indicate that selection for resistance has resulted in genetic differentiation between animals, and the establishment of a more resistant line of animals.

Genetic cluster 3 had the lowest overall average FEC (3936 ± 862). Genetic cluster 2 had a highest overall average FEC (7270 ± 788). However, Genetic cluster 2 had the least number of animals (25). These results show that animals in Genetic cluster 2 were heavily infected; hence Genetic cluster 2 is a susceptible line. FEC179, LFEC179, BCS179 and SI179 differed significantly ($P < 0.05$) between Genetic cluster 2 and 3 and between 3 and 4. Genetic cluster 3 had a lowest FEC and FAM combinations compared to the other clusters while Genetic cluster 2 had the highest FEC combination. These results confirm that Genetic cluster 2 was the most susceptible genetic cluster and that Genetic cluster 3 was highly resistant. The EBVs for phenotypic traits differed between genetic clusters and Genetic cluster 3 had the lowest EBV FEC (-629 ± 84).

Bisset *et al.* (1997) reported a significant reduction in FECs and improved growth rates in resistant lambs compared to susceptible Romney sheep that were managed separately. EBVs for different phenotypic traits of the sires differed significantly ($P < 0.05$) between the different genetic clusters. Sires in Genetic cluster 3 had the lowest EBV FEC (-328 ± 300) and EBV FAM (-0.040 ± 0.025). These results show that sires in Genetic cluster 3 have a great genetic potential and can be used in breeding for improved *Haemonchus* resistance as sires in this genetic cluster are highly resistant.

Chapter 6: Conclusion and Recommendations

6.1 Conclusion

In this study we were able to investigate differences in FEC, BCS and FAM amongst resistant and susceptible sheep and also to investigate genetic diversity and flock clustering of the Wauldby and GADI Dohne Merino sheep and its association with resistance / resilience to *H. contortus*. Results pertaining to the sex effect on FEC were variable in the current investigation. Gender effects differed between years, in some years; rams had a lower mean for FEC than ewes, while the opposite was true in other years. The studied populations of the Wauldby and GADI Dohne Merino sheep revealed a high level of genetic diversity expressed by MAF, F_{IS} , H_e and H_o estimates at population levels. At population level, MAF, H_e and H_o estimates were high and close to each other which explains the observed level of genetic differentiation between the Wauldby and GADI Dohne Merino sheep populations. These results were comparable with international commercial sheep breeds.

ADMIXTURE and PCA plots also unambiguously revealed the degree of differentiation in the populations. Genetic differentiation between genetic clusters or populations ranged from low to moderate and this was also expressed by F_{ST} values. In a PCA analysis, GADI animals clustered together (Cluster 1) and the Wauldby flock had 3 separate clusters (Cluster 2 to 4) each consisting of the mixture of cases and control. These results support the hypotheses that resilient/resistant and susceptible animals at Wauldby have diverged significantly to constitute distinct genetic clusters. It was clear that the studied populations were two genetically distinct sheep populations. There were no clear Case/Control groupings in a PCA analysis.

The investigation to determine significant differences between the three genetic clusters on a phenotypic level for the genotyped Wauldby farm animals revealed that Genetic cluster 3 had the lowest FEC, FAM and the highest BCS compared to the other genetic clusters. Genetic cluster 3 had the highest number of sires that were selected for the resistant line compare to Cluster 2 and Cluster 4 and these results demonstrated that Cluster 3 was the most resistant group and that there was genetic progress. The EBVs for FAM, BCS and FEC differed between genetic clusters and Cluster 3 had the lowest EBV FEC compared to the other genetic clusters, while Cluster 2 had the highest EBV FEC. These results show that Genetic cluster 2 was the most susceptible genetic cluster and that Genetic cluster 3 was the resistant line. Most of the parasite resistance traits in the sheep genome were located in

regions with high number of ROHs.

The results obtained from this study show that there is genetic variation in host resistance against *H. contortus* in the Wauldby Dohne Merino sheep and breeding for worm resistance / low FEC is therefore feasible. Sires in Genetic cluster 3 were highly resistant and can be used in a breeding program to develop sheep that are resistant to GIN infections. The use of highly resistant sires into a breeding program will provide a practical, more sustainable, realistic long-term and cost-effective helminth management strategy in Wauldby farm. This will help alleviate problems associated with the use of anthelmintic drugs (high cost of anthelmintic drugs, consumer concerns about chemical residues and the development of AR) in the long term and this helminth management strategy can also be adopted by other sheep farmers in regions where *H. contortus* and AR are a major problem. Using highly resistant sires in a breeding program will result in decreased FEC which should then decrease pasture contamination, leading to additional benefits for all sheep grazing the same pasture.

6.2 Recommendations

In this study, genetic differences in FEC, BCS and FAM within the resistant/resilient Wauldby Dohne Merino sheep population was evident and this information can be used in a breeding program to develop sheep that are resistant to GIN infections. More positive results can be obtained if the experimental period is increased. Genetic clusters were differentiated by averages for phenotypic traits and EBVs for these phenotypic traits. This study demonstrated that selection for low FEC following natural *Haemonchus* challenge is effective for producing sheep which can reduce the number of GIN worms. However, before large scale commercial nematode resistance breeding programs are introduced, further research is required to assess host response mechanisms and also understand biological processes underlying host resistance to GIN infections.

It is necessary to do a genome-wide association (GWAS) study as this will help identify candidate immune variants for genes involved in host response to GIN infections. This information may provide additional information about genetic markers associated to sheep GIN resistance, which has potential to aid selection of resistance to GIN parasites in sheep. Investigations on these genes would also help to determine the immunological mechanisms responsible for resistance to GINs. These genetic markers will be combined with phenotypic information and will be used in commercial breeding programs. The development

of the new high-throughput genomic tools has made it possible to study host response mechanisms and these tools can be used in such studies.

References

- Abuargob, O., Stear, M., Mitchell, S. & Benothman M., 2014. Effect of Lamb Gender on Susceptibility to Natural Nematode Parasite Infection (in) Scottish Black Face Lambs. *J. Vet. Adv.* 1, 516-523.
- Al-Mamun, H.A., Clark, S., Kwan, P. & Gondro, C., 2015. Genome-wide linkage disequilibrium and genetic diversity in five populations of Australian domestic sheep. *Genet. Sel. Evol.* 47, 90-103.
- Alba-Hurtado, F., Romero-Escobedo, E., Muñoz-Guzmán, M., Torres-Hernández, G. & Becerril-Pérez C., 2010. Comparison of parasitological and productive traits of Criollo lambs native to the central Mexican Plateau and Suffolk lambs experimentally infected with *Haemonchus contortus*. *Vet. Parasitol.* 172, 277-282.
- Alba-Hurtado, F. & Muñoz-Guzmán, M.A., 2013. Immune responses associated with resistance to haemonchosis in sheep. *BioMed. Res. Int.* 2013, 1-11. Available Online: <http://dx.doi.org/10.1155/2013/162158>.
- Alexander, D.H., Novembre, J. & Lange, K., 2009. Fast model-based estimation of ancestry in unrelated individuals. *Genome Res.* 19, 1655-1664.
- Amarante, A. & Amarante, M., 2003. Breeding sheep for resistance to nematode infections. *J. Anim. Vet. Adv.* 2(3), 147-161.
- Andersson, L., Haley, C.S., Ellegren, H., Knott, S.A., Johansson, M., Andersson, K., Andersson-Eklund, L., Edfors-Lilja, I., Fredholm, M. & Hansson, I., 1994. Genetic mapping of quantitative trait loci for growth and fatness in pigs. *Science-AAAS-Weekly Paper Edition - Including Guide to Scientific Information*, 263, 1771-1773.
- Andersson, L., 2012. How selective sweeps in domestic animals provide new insight into biological mechanisms. *J. Intern. Med.* 271(1), 1-14.
- Arranz, J.J., Bayon, Y. & Primitivo, F.S., 2001. Genetic variation at microsatellite loci in Spanish sheep. *Small Rumin. Res.* 39, 3-10. Athanasiadou, S., Kyriazakis, I., Jackson, F. & Coop, R., 2001. Direct anthelmintic effects of condensed tannins towards different gastrointestinal nematodes of sheep: *in vitro* and *in vivo* studies. *Vet. Parasitol.* 99, 205-219.
- Atlija, M., Arranz, J.J., Martinez-Valladares, M. & Gutiérrez-Gil, B., 2016. Detection and replication of QTL underlying resistance to gastrointestinal nematodes in adult sheep using the ovine 50K SNP array. *Genet. Sel. Evol.* 48 (1), 4-19.

- Avery D., 2004. The distribution of macromammals in southern Africa over the past 30 000 years. South African Archaeological Soc. PO Box 15700, Vlaeberg 8018, South Africa.
- Badke, Y.M., Bates, R.O., Ernst, C.W., Fix, J. & Steibel, J.P., 2014. Accuracy of estimation of genomic breeding values in pigs using low-density genotypes and imputation. *G3: Genes. Genomes. Genetics* 4, 623-631.
- Bai, Y., Sartor, M. & Cavalcoli, J., 2012. Current status and future perspectives for sequencing livestock genomes. *J. Anim. Sci. Biotech.* 3 (1), 8-13.
- Baker, R. L., Mwamachi, D.M., Audho, J.O., Aduda, E.O. & Thorpe, W., 1999. Genetic resistance to gastro-intestinal nematode parasites in Red Massai, Dorper and Red Maasai x Droper ewes in sub-humid tropics. *Anim. Sci.* 69,335-334.
- Baker, R.L., Gibson, J.P., Irqi, F.A., Menge, D.M., Mugambi, J.M., Hanotte, O., Nagda, S., Wakelin, D., Behnke, J.M., 2003. Exploring the genetic control of resistance to gastrointestinal helminth infections in sheep and mice. *Proc. Assoc. Advmt. Anim. Breed. Genet.* 15, 183-190.
- Baker, R.L., Mugambi, J.M., Audho, J.O., Carles, A.B. & Thorpe, W., 2004. Genotype by environment interactions for productivity and resistance to gastro-intestinal nematode parasites in Red Maasai and Dorper sheep. *Anim. Sci.* 79, 343-353.
- Bakunzi, F., 2003. AR of nematodes in communally grazed goats in a semi-arid area of South Africa. *J. S. Afr. Vet. Assoc.* 74, 82-83.
- Ballou, J., 1983. Calculating inbreeding coefficients from pedigrees. *Genetics and Conservation: A Reference for Managing Wild Animal and Plant Populations* Menlo Park, California: Benjamin/Cummings, 509-520.
- Barger I., 1993. Influence of sex and reproductive status on susceptibility of ruminants to nematode parasitism. *Int. J. Parasitol.* 23, 463-946.
- Barillet, F., Andreoletti, O., Palhi`ere, I., Aguerre, X., Arranz, J.M., Minery, S., Soulas, C., Belloc, J.P., Briois, M., Frégeat, G., Teinturier, P., Amigues, Y., Astruc, J.M., Boscher, M.Y. & Schelcher, F., 2002. Breeding for scrapie resistance using PrP genotyping in the French dairy sheep breeds. *Proc. of the Seventh W. C. G. A. L. P.*, Montpellier, France, August 2002. Session 13, 1-4.
- Bath, G., & Van Wyk, J., 2009. The Five Point Check[©] for targeted selective treatment of internal parasites in small ruminants. *Small Rumin. Res.* 86, 6-13.
- Bath, G.F., 2014. The “BIG FIVE”—A South African perspective on sustainable holistic internal parasite management in sheep and goats. *Small Rumin. Res.* 118, 48-55.

- Beh, K., Hulme, D., Callaghan, M., Leish, Z., Lenane, I., Windon, R. & Maddox, J., 2002. A genome scan for quantitative trait loci affecting resistance to *Trichostrongylus colubriformis* in sheep. *Anim. Genet.* 33, 97-106.
- Belkaid, Y., Blank, R.B. & Suffia, I., 2006. Natural regulatory T cells and parasites: a common quest for host homeostasis. *Immunol. Rev.*, 212, 287-300.
- Benavides, M., Weimer, T., Borba, M., Berne, M. & Sacco, A., 2002. Association between microsatellite markers of sheep chromosome 5 and faecal egg counts. *Small Rumin. Res.* 46, 97-105.
- Benavides, M.V., Sonstegard, T.S., Kemp, S., Mugambi, J.M., Gibson, J.P., Baker, R.L., Hanotte, O., Marshall, K. & Van Tassell, C., 2015. Identification of novel loci associated with gastrointestinal parasite resistance in a Red Maasai x Dorper backcross population. *PloS one.* 10, e0122797.
- Benavides, M.V., Sonstegard, T.S. & Van Tassell, C., 2016. Genomic Regions Associated with Sheep Resistance to Gastrointestinal Nematodes. *Trends Parasitol.* 32, 470-480.
- Besier, R., 2012. Refugia-based strategies for sustainable worm control: factors affecting the acceptability to sheep and goat owners. *Vet. Parasitol.* 186, 2-9.
- Beynon, S.E., Slavov, G.T., Farré, M., Sunduimijid, B., Waddams, K., Davies, B., Haresign, W., Kijas, J., MacLeod, I.M. & Newbold, C.J., 2015. Population structure and history of the Welsh sheep breeds determined by whole genome genotyping. *BMC genetics* 16, 65-78.
- Bishop, S. & Morris, C., 2007. Genetics of disease resistance in sheep and goats. *Small Rumin. Res.* 70, 48-59.
- Bishop, S., 2012. Possibilities to breed for resistance to nematode parasite infections in small ruminants in tropical production systems. *Animal* 6, 741-747.
- Bishop, S.C. & Woolliams, J.A., 2014. Genomics and disease resistance studies in livestock. *Livest. Sci.* 166, 190-198.
- Bisset, S., Vlassoff, A., West, C. & Morrison, L., 1997. Epidemiology of nematodosis in Romney lambs selectively bred for resistance or susceptibility to nematode infection. *Vet. Parasitol.* 70, 255-269.
- Bisset, S., Morris, C., McEwan, J. & Vlassof, A., 2001. Breeding sheep in New Zealand that are less reliant on anthelmintics to maintain health and productivity. *N. Zeal. Vet. J.* 49, 236-246.

- Blackburn, H., Paiva, S., Wildeus, S., Getz, W., Waldron, D., Stobart, R., Bixby, D., Purdy, P., Welsh, C. & Spiller, S., 2011. Genetic structure and diversity among sheep breeds in the United States: identification of the major gene pools. *J Anim Sci.* 89, 2336–48.
- Blasco, A. & Toro, M.A., 2014. A short critical history of the application of genomics to animal breeding. *Livest. Sci.* 166, 4-9.
- Bowie, E.A., 2014. Alternative treatments for *Haemonchus Contortus* in sheep: Testing of a natural dewormer and literature review of management methods. Dickinson College Honors Theses. Paper 163.
- Bozkaya, F. & Kurar, E., 2005. Linkage disequilibrium between MHC-linked microsatellite loci in White Karaman, Awassi and Merinolandschaf sheep breeds. *Fyrat Univ. Saglik Bilimlery Dergisi* 19, 57-61.
- Brito, L.F., McEwan, J.C., Miller, S.P., Pickering, N.K., Bain, W.E., Dodds, K.G., Schenkel, F.S. & Clarke, S.M., 2017. Genetic diversity of a New Zealand multi-breed sheep population and composite breeds' history revealed by a high-density SNP chip. *BMC Genet.* 18, 25-35.
- Buchmann, R. & Hazelhurst, S., 2014. Genesis manual. University of the Witwatersrand, Johannesburg. (Available at: <http://www.bioinf.wits.ac.za/software/genesis/Genesis.pdf>).
- Buduram, P., 2004. Genetic characterization of Southern African sheep breeds using DNA markers. M.Sc. (Agric.) thesis, University of the Free State, Bloemfontein, South Africa.
- Burke, J., Kaplan, R., Miller, J., Terrill, T., Getz, W., Mobini, S., Valencia, E., Williams, M., Williamson, L. & Vatta, A., 2007. Accuracy of the FAMACHA system for on-farm use by sheep and goat producers in the southeastern United States. *Vet. Parasitol.* 147, 89-95.
- Burke, J., Notter, D. & Miller, J., 2016. Sire's Estimated Breeding Value (EBV) for Fecal Egg Count (FEC) Influences BW, FEC, and Anemia Measures of Offspring in Katahdin Sheep. *J. Animal Sci.* 95, 52-52.
- Capewools.co.za. (2015). Cape Wools SA | The Story of Wool. [online] Available at: <http://www.capewools.co.za/default> [Accessed 20 September 2017].
- Carillier, C., Larroque, H., Palhière, I., Clément, V., Rupp, R. & Robert-Granié, C., 2013. A first step toward genomic selection in the multi-breed French dairy goat population. *J. Dairy Sci.* 96, 7294-7305.
- Cavalli-Sforza, L., Menozzi, P. & Piazza, A., 1994. The history and geography of human genes. Princeton: Princeton University Press. 428 pp. Chylinski, C., Cortet, J., Neveu, C.

- & Cabaret, J., 2015. Exploring the limitations of pathophysiological indicators used for targeted selective treatment in sheep experimentally infected with *Haemonchus contortus*. *Vet. Parasitol.* 207, 85-93.
- Cloete, S., Olivier, J., Du Toit, E., & Dreyer F., 2007. Genetic analysis of faecal worm egg count in South African Merinos under natural challenge. *S. Afr. J. Anim. Sci.* 37, 237-247.
- Cloete, S., Olivier, J., Sandenbergh, L. & Snyman, M., 2014. The adaption of the South Africa sheep industry to new trends in animal breeding and genetics: A review. *S. Afr. J. Anim. Sci.* 44, 307-321.
- Cloete, S., Mpetile, Z. & Dzama, K., 2016. Genetic parameters involving subjective FAMACHA© scores and faecal worm egg counts on two farms in the Mediterranean region of South Africa. *Small Rumin. Res.* 145, 33-43.
- Cockett, N.E., Jackson, S.P., Shay, T.L., Nielsen, D., Moore, S.S., Steele, M.R., Barendse, W., Green, R.D. & Georges, M., 1994. Chromosomal localization of the callipyge gene in sheep (*Ovis aries*) using bovine markers. *Proc. Natl. Acad. Sci. U.S.A.* 91, 3019–3023.
- Cockett, N.E., Shay, T.L., Beever, J.E., Nielsen, D., Albretsen, J., Georges, M., Peterson, K., Stephens, A., Vernon, W., Timofeevskaja, O., South, S., Mork, J., Maciulis, A. & Bunch, T.D., 1999. Localization of the locus causing Spider Lamb Syndrome to the distal end of ovine Chromosome 6. *Mammalian Genome.* 10, 35-38.
- Collins, F.S., Brooks, L.D. & Chakravarti, A., 1998. A DNA polymorphism discovery resource for research on human genetic variation. *Genome Res.* 8, 1229-1231.
- Colvin, A., Walkden-Brown, S. & Knox, M., 2012. Role of host and environment in mediating reduced gastrointestinal nematode infections in sheep due to intensive rotational grazing. *Vet. Parasitol.* 184, 180-192.
- Cornelius, M., Jacobson, C. & Besier, R., 2014. Body condition score as a selection tool for targeted selective treatment-based nematode control strategies in Merino ewes. *Vet. Parasitol.* 206, 173-181.
- Cornelius, M., Jacobson, C. & Besier, R., 2015. Application of a body condition score index for targeted selective treatment in adult Merino sheep - A modelling study. *Vet. Parasitol.* 214, 125-131.
- Courtney, C., Parker, C., McClure, K. & Herd, R., 1984. A comparison of the periparturient rise in fecal egg counts of exotic and domestic ewes. *Int. J. Parasitol.* 14, 377-381.

- Courtney, C., Parker, C., McClure, K. & Herd, R., 1985. Resistance of nonlambing exotic and domestic ewes to naturally acquired gastrointestinal nematodes. *Int. J. Parasitol.* 15, 239-243.
- Courtney, C.H., Gessner, R., Sholz, S.R. & Loggins P.E., 1986. The peri-parturient rise in fecal egg counts in the three strains of the Florida Native ewes and its value in predicting resistance of the lambs to *Haemonchus contortus*. *Int. J. Parasitol.* 16, 185-189.
- Crawford, A.M., Paterson, K.A., Dodds, K.G., Tascon, C.D., Williamson, P.A., Thomson, M.R., Bisset, S.A., Beattie, A.E., Greer, G.J. & Green, R.S., 2006. Discovery of quantitative trait loci for resistance to parasitic nematode infection in sheep: I. Analysis of outcross pedigrees. *BMC Genomics.* 7, 178-187.
- Davies, G., Stear, M.J., Benothman, M., Abuagob, O., Kerr, A., Mitchell, S. & Bishop, SC., 2006. Quantitative trait loci associated with parasitic infection in Scottish blackface sheep. *Heredit.* 96(3), 252-258.
- Davies, G., Genini, S., Bishop, S. & Giuffra, E., 2009. An assessment of opportunities to dissect host genetic variation in resistance to infectious diseases in livestock. *Anim.* 3, 415-436.
- De Graef, J., Claerebout, E. & Geldhof, P., 2013. Anthelmintic resistance of gastrointestinal cattle nematodes. *Vlaams Diergen Tijds.* 82, 113-123.
- De Mello, F., Kern, E. & Bertoli, C., 2014. Progress in dairy cattle selection. *Adv. Dairy Res.* 1-2.
- De Souza Chagas, A.C., Domingues, L.F., Gaínza, Y.A., Barioni-Júnior, W., Esteves, S.N. & Niciura, S.C.M., 2016. Target selected treatment with levamisole to control the development of AR in a sheep flock. *Parasitol. Res.* 115, 1131-1139.
- Decker, J.E., McKay, S.D., Rolf, M.M., Kim, J., Alcalá, A.M., Sonstegard, T.S., Hanotte, O., Götherström, A., Seabury, C.M., Praharani, L., Babar, M.E., de Almeida Regitano, L.C., Yildiz, M.A., Heaton, M.P., Liu, W., Lei, C., Reecy, J.M., Saif-Ur-Rehman, M. Schnabel, R.D. & Taylor, J.F., 2014. Worldwide patterns of ancestry, divergence, and admixture in domesticated cattle. *PLOS Genetics* 10, e1004254.
- Dekkers, J.C.M., 2004. Commercial application of marker-and gene-assisted selection in livestock: Strategies and lessons. *American. Soc. Anim.Sci.* 82, 313-328.
- Department of Agriculture, Forestry and Fisheries, 2017. Newsletter: livestock statistics, estimated livestock numbers in RSA (August 2016 and February 2017) [Online].

- Pretoria. Available: [http://www.daff.gov.za/daffweb3/Home/Crop-Estimates/Statistical Information/Livestock](http://www.daff.gov.za/daffweb3/Home/Crop-Estimates/Statistical%20Information/Livestock) (accessed 04.10.17).
- Devlin, B. & Roeder, K., 1999. Genomic control for association studies. *Biometrics*. 55, 997-1004.
- Di Loria, A., Veneziano, V., Piantedosi, D., Rinaldi, L., Cortese, L., Mezzino, L., Cringoli, G. & Ciaramella, P., 2009. Evaluation of the FAMACHA system for detecting the severity of anaemia in sheep from southern Italy. *Vet. Parasitol.* 161, 53-59.
- Ding, L., Wiener, H., Abebe, T., Altaye, M., Go, R.C., Kercksmar, C., Grabowski, G., Martin, L.J., Hershey, G.K.K. & Chakorborty, R., 2011. Comparison of measures of marker informativeness for ancestry and admixture mapping. *BMC Genomics*. 12, 622-639.
- Dominik, S., 2005. Quantitative trait loci for internal nematode resistance in sheep: a review. *Genet. Select. Evol.* 37 (Suppl. 1), 83-96.
- Dominik, S., Henshall, J., O'Grady, J. & Marshall, K., 2007. Factors influencing the efficiency of a marker-assisted introgression programme in Merino sheep. *Genet. Sel. Evol.* 39, 495-511.
- Du Toit, J.C.O. & O'Connor, T.G., 2014. Changes in rainfall pattern in the eastern Karoo, South Africa, over the past 123 years. *Water SA*, 40, 453-460.
- Duforet-Frebourg, N., Luu, K., Laval, G., Bazin, E. & Blum, M.G., 2015. Detecting genomic signatures of natural selection with principal component analysis: application to the 1000 Genomes data. *Mol. Biol. Evol.* 33, 1082-1093.
- Dukkipati, V., Blair, H., Garrick, D. & Murray, A., 2006. 'Ovar-Mhc'—Ovine major histocompatibility complex: Role in genetic resistance to diseases. *N. Zeal. Vet. J.* 54, 153-160.
- Eck, S.H., Benet-Pagès, A., Flisikowski, K., Meitinger, T., Fries, R. & Strom, T.M., 2009. Whole-genome sequencing of a single *Bos taurus* animal for single nucleotide polymorphism discovery. *Genome Biol.* 10, R82.
- Falzon, L.C., O'Neill, T., Menzies, P., Peregrine, A., Jones-Bitton, A. & Mederos, A., 2014. A systematic review and meta-analysis of factors associated with AR in sheep. *Prev. Vet. Med.* 117, 388-402.
- Ferenčaković, M., Hamzić, E., Gredler, B., Solberg, T., Klemetsdal, G., Curik, I., & Sölkner, J., 2013a. Estimates of autozygosity derived from runs of homozygosity: empirical evidence from selected cattle populations. *J. Anim. Breed. Genet.* 130, 286-293.

- Ferenčaković, M., Sölkner, J. & Curik, I., 2013b. Estimating autozygosity from high-throughput information: effects of SNP density and genotyping errors. *Genet. Sel. Evol.* 45, 42-50.
- Fleming, S.A., Craig, T., Kaplan, R.M., Miller, J.E., Navarre, C. & Rings, M., 2006. AR of gastrointestinal parasites in small ruminants. *J. Vet. Intern. Med.* 20, 435-444.
- Flury, C., Tapio, M., Sonstegard, T., Drögemüller, C., Leeb, T., Simianer, H., Hanotte, O. & Rieder, S., 2010. Effective population size of an indigenous Swiss cattle breed estimated from linkage disequilibrium. *J. Anim. Breed. Genet.* 127, 339-347.
- Fulton, J., 2012. Genomic selection for poultry breeding. *Anim. Frontiers.* 2, 30-36.
- Gallidis, E., Papadopoulos, E., Ptochos, S. & Arsenos, G., 2009. The use of targeted selective treatments against gastrointestinal nematodes in milking sheep and goats in Greece based on parasitological and performance criteria. *Vet. Parasitol.* 164, 53-58.
- Galloway, S.M., McNatty, K.P., Cambridge, L.M., Laitinen, M.P., Juengel, J.L., Jokiranta, T.S., McLaren, R.J., Luiro, K., Dodds, K.G. & Montgomery, G.W., 2000. Mutations in an oocyte-derived growth factor gene (BMP15) cause increased ovulation rate and infertility in a dosage-sensitive manner. *Nature Genet.* 25, 279-284.
- Ganz, H.H. & Ebert, D., 2010. Benefits of host genetic diversity for resistance to infection depend on parasite diversity. *Ecol.* 91, 1263-1268.
- Gaouar, S.B.S., Da Silva, A., Ciani, E., Kdidi, S., Aouissat, M., Dhimi, L., Lafri, M., Maftah, A. & Mehtar, N., 2015. Admixture and local breed marginalization threaten Algerian sheep diversity. *PloS one* 10, e0122667.
- Gasser, R., Schwarz, E., Korhonen, P. & Young, N., 2016. Chapter Twelve-Understanding *Haemonchus contortus* Better Through Genomics and Transcriptomics. *Adv. parasitol.* 93, 519-567.
- Gauly, M., Schackert, M., Hoffmann, B. & Erhardt, G., 2006. Influence of sex on the resistance of sheep lambs to an experimental *Haemonchus contortus* infection. *Deut Tierarztl Woch.*, 113, 178-181.
- Gautier, M., Laloë, D. & Moazami-Goudarzi, K., 2010. Insights into the genetic history of French cattle from dense SNP data on 47 worldwide breeds. *PloS one* 5, e13038.
- Georges, M., Nielsen, D., Mackinnon, M., Mishra, A., Okimoto, R., Pasquino, A.T., Sargeant, L.S., Sorensen, A., Steele, M.R. & Zhao, X., 1995. Mapping quantitative trait loci controlling milk production in dairy cattle by exploiting progeny testing. *Genet.* 139, 907-920.

- Geurden, T., Hoste, H., Jacquet, P., Traversa, D., Sotiraki, S., Di Regalbono, A.F., Tzanidakis, N., Kostopoulou, D., Gaillac, C. & Privat, S., 2014. AR and multidrug resistance in sheep gastro-intestinal nematodes in France, Greece and Italy. *Vet. Parasitol.* 201, 59-66.
- Gholizadeh, M., Rahimi-Mianji, G. & Nejati-Javaremi, A., 2015. Genomewide association study of body weight traits in Baluchi sheep. *J. Genet.* 94, 143-146.
- Gilmour, A.R., Gogel, B.J., Cullis, B.R. & Thompson, R., 2009. ASReml User Guide Release 3.0 VSN International Ltd, Hemel Hempstead, HPI 1ES, UK.
- Goddard, M.E. & Hayes, B., 2007. Genomic selection. *J. Anim. Breed. Genet.* 124, 323-330.
- Goddard, M.E. & Hayes, B.J., 2009. Mapping genes for complex traits in domestic animals and their use in breeding programmes. *Nature Rev. Genet.* 10, 381-391.
- Grasso, A.N., Goldberg, V., Navajas, E.A., Iriarte, W., Gimeno, D., Aguilar, I., Medrano, J.F., Rincón, G. & Ciappesoni, G., 2014. Genomic variation and population structure detected by single nucleotide polymorphism arrays in Corriedale, Merino and Creole sheep. *Genet. Mol. Biol.* 37, 389-395.
- Gray, G.D., 1991. Breeding for resistance to trichostrongyle nematodes in sheep. In: *Breeding for diseases resistance in farm animals*. Eds. Owen, J.B. & Axford, R.F. Proceedings of an International Symposium, Bangor, Wales, Sept. 1990. Wallingford, U.K., CAB International, pp 100-125.
- Gray, G.D., 1995. Genetic variation in resistance to parasites. In: *Breeding for resistance to infectious diseases in small ruminants*. Eds. Gray, G.D., Woolaston, R.R. & Eaton, B.T. pp. 43-52. (ACIAR: Canberra).
- Greeff, J.C., Karlsson, L.J.E. & Harris, J.F., 1995. Heritability of faecal worm egg count at different times of the year in a Mediterranean environment. *Proc. Aust. Assoc. Anim. Breed. Gen.* 11, 117-121.
- Greeff, J.C. & Karlsson, L.J.E., 2006. Breeding for worm resistance – whole farm benefits. In ‘Wool meets meat - tools for a modern sheep enterprise. Proceedings of the 2006 Australian sheep industry CRC conference, Orange, Australia. Eds. Cronje, P.B. & Maxwell, D. pp. 102-108. (The Australian Sheep Industry CRC: Armidale, NSW).
- Greer, A., 2008. Trade-offs and benefits: implications of promoting a strong immunity to gastrointestinal parasites in sheep. *Parasite Immunol.* 30, 123-132.
- Greer, A.W., Kenyon, F., Bartley, D.J., Jackson, E.B., Gordon, Y., Donnan, A.A., McBean, D.W., Jackson, F., 2009. Development and field evaluation of a decision support model

- for anthelmintic treatments as part of a targeted selective treatment (TST) regime in lambs. *Vet. Parasitol.* 164, 12-20.
- Greer, A.W. & Hamie, J.C., 2016. Relative maturity and the development of immunity to gastrointestinal nematodes in sheep: an overlooked paradigm? *Parasite Immunol.* 38, 263-272.
- Gruner, L., Aumont, G., Getachew, T., Brunel, J.C., Pery, C.Y. & Guerin, Y., 2003. Experimental infection of Black Belly and INRA 401 straight and crossbred sheep with trichostrongyle nematode parasites. *Vet. Parasitol.* 116, 239–249.
- Guo, Z., González, J.F., Hernandez, J.N., McNeilly, T.N., Corripio-Miyar, Y., Frew, D., Morrison, T., Yu, P. & Li, R.W., 2016. Possible mechanisms of host resistance to *Haemonchus contortus* infection in sheep breeds native to the Canary Islands. *Scientific reports* 6.
- Gurgul, A., Semik, E., Pawlina, K., Szmatoła, T., Jasielczuk, I. & Bugno-Poniewierska, M., 2014. The application of genome-wide SNP genotyping methods in studies on livestock genomes. *J. Appl. Genet.* 55, 197-208.
- Gutiérrez-Gil, B., Pérez, J., Álvarez, L., Martínez-Valladares, M., de la Fuente, L.F., Bayón, Y., Meana, A., San Primitivo, F., Rojo-Vázquez, F.A. & Arranz, J.J., 2009. Quantitative trait loci for resistance to trichostrongylid infection in Spanish Churra sheep. *Genet. Select. Evol.* 41, 46-55.
- Haile, A., Tibbo, M., Baker, R. & Rege, J.O., 2007. Effects of non-genetic factors on responses to gastro-intestinal nematode infections in Ethiopian sheep. *Trop. Anim. Health Prod.* 39, 411-417.
- Haraksingh, R.R. & Snyder M.P., 2013. Impacts of variation in the human genome on gene regulation. *J. Mol. Biol.* 425, 3970-3977.
- Hayes, B., Bowman, P., Chamberlain, A. & Goddard, M., 2009. Invited review: Genomic selection in dairy cattle: Progress and challenges. *J. Dairy Sci.* 92, 433-443.
- Hayes, B. & Goddard, M., 2010. Genome-wide association and genomic selection in animal breeding. *Genome* 53, 876-883.
- Heckendorn F., Bieber A., Werne S., Saratsis A., Maurer V. & Stricker C., 2017. The genetic basis for the selection of dairy goats with enhanced resistance to gastrointestinal nematodes. *Parasite.* 24, 32-42.

- Henry, H.M., Dodds, K.G., Wuliji, T., Jenkins, Z.A., Beattie, A.E. & Montgomery, G.W., 1998. A genome screen for QTL for wool traits in a Merino × Romney backcross flock, Wool Tech. Sheep Breed. 46, 213-217.
- Herselman, M.J., 2001. Progress report. AP1/12: Establishment of a genetic pool of dual purpose sheep with premium quality meat and super fine wool under extensive conditions. Animal Production Unit. Grootfontein Agricultural Development Institute.
- Herselman, M.J., 2002. Progress report. AP1/12: Establishment of a genetic pool of dual purpose sheep with premium quality meat and super fine wool under extensive conditions. Animal Production Unit. Grootfontein Agricultural Development Institute.
- Hooda, V., Yadav C., Chaudhri S. & Rajpurohit B., 1999. Variation in resistance to haemonchosis: selection of female sheep resistant to *Haemonchus contortus*. J. Helminthol. 73, 137-142.
- Hoste, H., Jackson, F., Athanasiadou, S., Thamsborg, S.M. & Hoskin, S.O., 2006. The effects of tannin-rich plants on parasitic nematodes in ruminants. Trends Parasitol. 22, 253-261.
- Houston, E., Halliday, S., Jeffrey, M., Goldmann, W. & Hunter, N., 2002. New Zealand sheep with scrapie-susceptible PrP genotypes succumb to experimental challenge with a sheep-passaged scrapie isolate (SSBP/1). J. Gen. Virol. 83, 1247-1250.
- Howrigan, D.P., Simonson, M.A., & Keller, M.C., 2011. Detecting autozygosity through runs of homozygosity: a comparison of three autozygosity detection algorithms. BMC Genomics. 12, 460-474.
- <http://www.animalgenome.org/QTLdb/sheep> (accessed 15.06.17).
- http://www.saexplorer.co.za/south-africa/climate/stutterheim_climate.asp. (Accessed on 29.09.17).
- <https://www.worldweatheronline.com/stutterheim-weather-history/eastern-cape/za.aspx> (accessed on 29.09.17).
- Iacolina, L., Stronen, A.V., Pertoldi, C., Tokarska, M., Nørgaard, L.S., Muñoz, J., Kjærsgaard, A., Ruiz-Gonzalez, A., Kamiński, S. & Purfield, D.C., 2016. Novel Graphical Analyses of Runs of Homozygosity among Species and Livestock Breeds. Int. J. Plant. Genomics. 2016, 1-8.
- Ibañez-Escriche, N. & Gonzalez-Recio, O., 2011. Review. Promises, pitfalls and challenges of genomic selection in breeding programs. Spanish J. Agric. Res. 9, 404-413.

- Iqbal, Z., Sarwar, M., Jabbar, A., Ahmed, S., Nisa, M., Sajid, M.S., Khan, M.N., Mufti, K.A. & Yaseen, M. 2007. Direct and indirect anthelmintic effects of condensed tannins in sheep. *Vet. Parasitol.* 144, 125-131.
- Jackson, F. & Coop, R., 2000. The development of AR in sheep nematodes. *Parasitol.* 120, 95-107.
- Jackson, F., Varady, M. & Bartley, D., 2012. Managing AR in goats - Can we learn lessons from sheep? *Small Rumin. Res.* 103, 3-9.
- Jakobsson, M., Edge, M.D. & Rosenberg, N.A., 2013. The relationship between FST and the frequency of the most frequent allele. *Genet.* 193, 515-528.
- Jann, O.C., King, A., Lopez Corrales, N., Anderson, S.I., Jensen, K., Ait-Ali T., Tang, H., Wu, C., Cockett, N.E., Archibald, A.L. & Glass, E.J., 2009. Comparative genomics of Toll-like receptor signalling in five species. *BMC Genomics.* 10, 216-230.
- Janssen, M., Weimann, C., Gauly, M. & Erhardt, G., 2002. Associations between infections with *Haemonchus contortus* and genetic markers on ovine chromosome 20. *Proc. 7th World Cong. Genet. Appl. Livest. Prod. Montpellier*, 19-23.
- Johnson, P., McEwan, J., Dodds, K., Purchas, R. & Blair, H., 2005. A directed search in the region of GDF8 for quantitative trait loci affecting carcass traits in Texel sheep. *J. Anim. Sci.* 83, 1988-2000.
- Jonas, E. & de Koning, D.J., 2015. Genomic selection needs to be carefully assessed to meet specific requirements in livestock breeding programs. *Frontiers Genet.* 6, 49-56.
- Jones, L., Sakkas, P., Houdijk, J., Knox, D. & Kyriazakis, I., 2012. Amelioration of the periparturient relaxation of immunity to parasites through a reduction in mammalian reproductive effort. *Int. J. Parasitol.* 42, 1127-1134.
- Jovanović, S., Savić, M. & Živković, D., 2009. Genetic variation in disease resistance among farm animals. *Biotech. Anim. Husband.* 25, 339-347.
- Kahn, L. & Woodgate, R., 2012. Integrated parasite management: products for adoption by the Australian sheep industry. *Vet. Parasitol.* 186, 58-64.
- Kaplan, R., Burke, J., Terrill, T., Miller, J., Getz, W., Mobini, S., Valencia, E., Williams, M., Williamson, L. & Larsen, M., 2004. Validation of the FAMACHA[®] eye color chart for detecting clinical anemia in sheep and goats on farms in the southern United States. *Vet. Parasitol.* 123, 105-120.
- Karlsson, L. & Greeff, J., 2012. Genetic aspects of sheep parasitic diseases. *Vet. Parasitol.* 189, 104-112.

- Karrow, N.A., Goliboski, K., Stonos, N., Schenkel, F. & Peregrine, A., 2013. Genetics of helminth resistance in sheep. *Can. J. Anim.Sci.* 94, 1-9.
- Kearney, P., Murray, P., Hoy, J., Hohenhaus, M. & Kotze, A., 2016. The ‘Toolbox’ of strategies for managing *Haemonchus contortus* in goats: what’s in and what’s out. *Vet. Parasitol.* 220, 93-107.
- Kemper, K.E., Elwin, R.L., Bishop, S.C., Goddard, M.E. & Woolaston, R.R., 2009. *Haemonchus contortus* and *Trichostrongylus colubriformis* did not adapt to long-term exposure to sheep that were genetically resistant or susceptible to nematode infections. *Int. J. Parasitol.* 39(5), 607-614.
- Kemper, K.E., Emery, D.L., Bishop, S.C., Oddy, H., Hayes, B.J., Dominik, S., Henshall, J.M., and Goddard, M.E., 2011. The distribution of SNP marker effects for faecal worm egg count in sheep, and the feasibility of using these markers to predict genetic merit for resistance to worm infections. *Genet Res.* 93(3), 203-219.
- Kemper, K., Goddard, M., Bishop, S., 2013. Adaptation of gastrointestinal nematode parasites to host genotype: single locus simulation models. *Genet. Sel. Evol.* 45(1), 14.
- Kenyon, F., Greer, A., Coles, G., Cringoli, G., Papadopoulos, E., Cabaret, J., Berrag, B., Varady, M., Van Wyk, J.A. & Thomas, E., 2009. The role of targeted selective treatments in the development of refugia-based approaches to the control of gastrointestinal nematodes of small ruminants. *Vet. Parasitol.* 164, 3-11.
- Kevoorkian, S.E.M., Georgescu, S., Manea, M.A., Gavrilă, M.G., Hrinca, G. & Costache, M., 2010. The Spider Lamb Syndrome absence in five Romanian Sheep Breeds. *Lucrări Științifice* 53, 254-258.
- Khusro, M., Van der Werf, J.H.J., Brown, D.J. & Ball, A., 2004. Across flock (co)variance components for faecal worm egg count, live weight, and fleece traits for Australian Merinos. *Livest. Prod. Sci.* 91, 35-43.
- Kijas, J.W., Townley, D., Dalrymple, B.P., Heaton, M.P., Maddox, J.F., McGrath, A., Wilson, P., Ingersoll, R.G., McCulloch, R. & McWilliam, S., 2009. A genome wide survey of SNP variation reveals the genetic structure of sheep breeds. *PloS one* 4, e4668.
- Kijas, J.W., Lenstra, J.A., Hayes, B., Boitard, S., Neto, L.R.P., San Cristobal, M., Servin, B., McCulloch, R., Whan, V. & Gietzen, K., 2012. Genome-wide analysis of the world's sheep breeds reveals high levels of historic mixture and strong recent selection. *PLoS Biol* 10, e1001258.

- Kloosterman, A., Parmentier, H. & Ploeger, H., 1992. Breeding cattle and sheep for resistance to gastrointestinal nematodes. *Parasitol. Today* 8, 330-335.
- Knight, R.A. & Rodgers, D., 1974. Age resistance of lambs to single inoculation with *Haemonchus contortus*. *Proc. Helminthol. Soc. Wash.* p. 116.
- Knox, M., Torres-Acosta, J. & Aguilar-Caballero, A., 2006. Exploiting the effect of dietary supplementation of small ruminants on resilience and resistance against gastrointestinal nematodes. *Vet. Parasitol.* 139, 385-393.
- Köhler, K. & Bickeböller, H., 2006. Case-Control Association Tests Correcting for Population Stratification. *Ann. Hum. Genet.* 70, 98-115.
- Korte, A. & Farlow, A. 2013. The advantages and limitations of trait analysis with GWAS: a review. *Plant methods* 9, 29-37.
- Kunene, N.W., 2010. Characterisation of indigenous Zulu (Nguni) sheep for utilisation, improvement and conservation. Ph.D thesis, University of KwaZulu-Natal, Pietermaritzburg, South Africa.
- Laegreid, W., Clawson, M., Heaton, M., Green, B., O'Rourke, K.I. & Knowles, D., 2008. Scrapie resistance in ARQ sheep. *J. Virol.* 82, 10318-10320.
- Landman, A.M., 2013. Economic analysis of intensive sheep production systems in central South Africa. MSc (Agric) thesis, University of the Free State, Bloemfontein, South Africa.
- Leask, R., Van Wyk, J.A., Thompson, P. & Bath, G., 2013. The effect of application of the FAMACHA[®] system on selected production parameters in sheep. *Small Rumin. Res.* 110, 1-8.
- Leathwick, D. & Besier, R., 2014. The management of AR in grazing ruminants in Australasia-strategies and experiences. *Vet. Parasitol.* 204, 44-54.
- Louvandini, H., Veloso, C.F.M., Paludo, GR., 2006. Influence of protein supplementation on the resistance and resilience on young hair sheep naturally infected with gastrointestinal nematodes during rainy and dry seasons. *Vet. Parasitol.* 137(4), 103-111.
- Luca, D., 2007. Cluster analysis and PCA for modelling population structure. Ph.D Thesis proposal, Carnegie Mellon University, Pittsburgh, Pennsylvania, United States of America.
- Macfarlane, J.M., Lambe, N.R., Haresign, W. & Bunger, L., 2012. The effect of the Texel Muscling QTL on live and carcass weight in Texel lambs. *Small Rumin. Res.* 105, 117-121.

- Malan, F.S., Van Wyk, J.A. & Wessels, C.D., 2000. Clinical Evaluation of Anaemia in Sheep; Early Trials. FAO TCP Workshop: Sustainable Worm Control Programmes for Sheep and Goats, Onderstepoort Veterinary Institute.
- Marchini, J., Cardon, L.R., Phillips, M.S. & Donnelly, P., 2004. The effects of human population structure on large genetic association studies. *Nat. Genet.* 36, 512-517.
- Marshall, K., Maddox, J., Lee, S., Zhang, Y., Kahn, L., Graser, H.U., Gondro, C., Walkden-Brown, S. & Van Der Werf, J., 2009. Genetic mapping of quantitative trait loci for resistance to *Haemonchus contortus* in sheep. *Anim. Genet.* 40, 262-272.
- Marshall, K., Mugambi, J., Nagda, S., Sonstegard, T., Tassell, C., Baker, R. & Gibson J., 2013. Quantitative trait loci for resistance to *Haemonchus contortus* artificial challenge in Red Maasai and Dorper sheep of East Africa. *Anim. Genet.* 44, 285-295.
- Mastrangelo, S., Tolone, M., Sardina, M.T., Sottile, G., Sutura, A.M., Di Gerlando, R. & Portolano B., 2017. Genome-wide scan for runs of homozygosity identifies potential candidate genes associated with local adaptation in Valle del Belice sheep. *Genet. Sel. Evol.* 49, 84-93.
- Matebesi-Ranthimo, P., Cloete, S., Van Wyk, J. & Olivier, J., 2014. Genetic parameters and relationships of faecal worm egg count with objectively measured wool traits in the Tygerhoek Merino flock. *S. Afr. J. Anim. Sci.* 44, 178-188.
- Matika, O., Pong-Wong, R., Woolliams, J. & Bishop, S., 2011. Confirmation of two quantitative trait loci regions for nematode resistance in commercial British terminal sire breeds. *Anim.* 5, 1149-1156.
- McManus, C., Do Prado Paim, T., De Melo, C.B., Brasil, B.S. & Paiva, S.R., 2014. Selection methods for resistance to and tolerance of helminths in livestock. *Parasit.* 21, 56-70.
- McQuillan, R., Leutenegger, A.L., Abdel-Rahman, R., Franklin, C.S., Pericic, M., Barac-Lauc, L., Smolej-Narancic, N., Janicijevic, B., Polasek, O. & Tenesa, A., 2008. Runs of homozygosity in European populations. *Am. J. Hum. Genet.* 83, 359-372.
- McRae, K.M., McEwan, J.C., Dodds, K.G. & Gemmell, N.J., 2014a. Signatures of selection in sheep bred for resistance or susceptibility to gastrointestinal nematodes. *BMC Genomics.* 15, 637-649.
- McRae, K.M., Good, B., Hanrahan, J.P., Glynn, A., O'Connell, M.J. & Keane, O.M., 2014b. Response to *Teladorsagia circumcincta* infection in Scottish Blackface lambs with divergent phenotypes for nematode resistance. *Vet. Parasitol.* 206, 200-207.

- Meadows, J.R., Chan, E.K. & Kijas, J.W., 2008. Linkage disequilibrium compared between five populations of domestic sheep. *BMC Genet.* 9, 61-70.
- Mederos, A., Kelton, D., Peregrine, A., VanLeeuwen, J., Fernández, S., LeBoeuf, A., Menzies, P. & Martin R., 2014. Evaluation of the utility of subjective clinical parameters for estimating fecal egg counts and packed cell volume in Canadian sheep flocks. *Vet. Parasitol.* 205, 568-574.
- Miller, J. & Horohov, D., 2006. Immunological aspects of nematode parasite control in sheep. *J. Anim. Sci.* 84, E124-E32.
- Miller, J., Bishop, S., Cockett, N. & McGraw, R., 2006. Segregation of natural and experimental gastrointestinal nematode infection in F 2 progeny of susceptible Suffolk and resistant Gulf Coast Native sheep and its usefulness in assessment of genetic variation. *Vet. Parasitol.* 140, 83-89.
- Miller, J., Burke, J., Terrill, T. & Kearney, M. 2011. A comparison of two integrated approaches of controlling nematode parasites in small ruminants. *Vet. Parasitol.* 178, 300-310.
- Molotsi, A.H., Taylor, J.F., Cloete, S.W., Muchadeyi, F., Decker, J.E., Whitacre, L.K., Sandenbergh, L. & Dzama, K., 2017. Genetic diversity and population structure of South African smallholder farmer sheep breeds determined using the OvineSNP50 beadchip. *Trop. Anim. Health. Prod.* 49(8), 1771-1777.
- Moniruzzaman, M., Khatun, R. & Minto, A., 2015. Application of marker assisted selection for livestock improvement in Bangladesh. *Bangladesh Veterinarian* 31, 1-11.
- Moors, E. & Gauly, M., 2009. Is the FAMACHA[©] chart suitable for every breed? Correlations between FAMACHA[©] scores and different traits of mucosa colour in naturally parasite infected sheep breeds. *Vet. Parasitol.* 166, 108-111.
- Moradi, M.H., Nejati-Javaremi, A., Moradi-Shahrbabak, M., Dodds, K.G., and McEwan, J.C., 2012. Genomic scan of selective sweeps in thin and fat tail sheep breeds for identifying of candidate regions associated with fat deposition. *BMC Genet.* 13, 10-24.
- Moreno, C.R., Gruner, L., Scala, A., Mura, L., Schibler, L., Amigues, Y., Sechi, T., Jacquiet, P., Francois, D., Sechi, S., Roig, A., Casu, S., Barillet, F., Brunel, J.C., Bouix, J., Carta, A & Rupp, R., 2006. QTL for resistance to internal parasites in two designs based on natural and experimental conditions of infection. *Proc. 7th World Congress in Genetics Applied to Livestock Production*, Communication 15-5.

- Moreno, C.R., Jacquiet, P., Bouvier, F., Cortet, J., Blanchard-Letort, A., Guégnard, F., Francois, D., Bourdillon, Y., Grisez, C. & Prevot, F., 2014. Validation of QTL affecting resistance to nematodes in sheep identified in a back-cross design in a pure breed population. Proc. 10th World Congress on Genetics Applied to Animal Production, Vancouver, Canada.
- Morris, C. & Bisset, S., 1996. Progress on genetic studies of resilience to nematode parasites in sheep. Proc. New Zeal. Soc. Anim. Prod. 56, 91-93.
- Morris, C., Clarke J., Watson T., Wrigglesworth A. & Dobbie J., 1996. Faecal egg count and food intake comparisons of Romney single-trait selection and control lines. New Zeal. J. Agr. Res. 39, 371-378.
- Morris, C.A., Vlassoff, A., Bisset, S., Baker, R., Watson, T.G., West, C.J., and Wheeler, M., 2000. Continued selection of Romney sheep for resistance or susceptibility to nematode infection: estimates of direct and correlated responses. Anim. Sci. 70, 17-27.
- Morris, C., Bisset, S., Vlassoff, A., Wheeler, M., West, C., Devantier, B. & Mackay A., 2010. Selecting for resilience in Romney sheep under nematode parasite challenge, 1994-2007. New Zeal. J. Agr. Res. 53, 245-261
- Mpetile, Z., Cloete, S., Kruger, A. & Dzama, K., 2015. Environmental and genetic factors affecting faecal worm egg counts in Merinos divergently selected for reproduction. S. Afr. J. Anim. Sci. 45, 510-520.
- Mucha, S., Bungler, L. & Conington, J., 2015. Genome-wide association study of footrot in Texel sheep. Genet. Sel. Evol. 47, 35-44.
- Mucina, L., Rutherford, M.C., Palmer, A.R., Milton, S.J., Scott, L., Lloyd, J.W., der Merwe, B., Hoare, D.B., Bezuidenhout, H., Vlok, J.H.J., Euston-Brown, D.I.W., Powrie, L.W. & Dold, A.P., 2006. Nama-Karoo Biome. In: The vegetation of South Africa, Lesotho and Swaziland. Eds. L. Mucina & M.C. Rutherford. SANBI, Pretoria. pp. 324-347.
- Mugambi, J.M., Bain, R.K., Wanyangu, S.W., Ihiga, M.A., Duncan, J.L., Murray, M., Stear, M.J., 1997. Resistance of four sheep breeds to natural and subsequent artificial *Haemonchus contortus* infection. Vet. Parasitol. 69, 265-273.
- Napolitano, F., De Rosa, G. & Sevi, A., 2008. Welfare implications of artificial rearing and early weaning in sheep. Appl. Anim. Behav. Sci. 110, 58-72.
- Nieuwoudt, S., Theron, H. & Kruger, L., 2002. Genetic parameters for resistance to *Haemonchus contortus* in Merino sheep in South Africa. J. S. Afr. Vet. Assoc. 73, 4-7.

- Nimbkar, C., Ghalsasi, P.M., Swan, A.A., Walkden-Brown, S.W., Kahn, L.P., 2003. Evaluation of growth rates and resistance to nematodes of Deccani and Bannur lambs and their crosses with Garole. *Anim. Sci.* 76, 503-515.
- Nothnagel, M., Lu, T.T., Kayser, M. & Krawczak, M., 2010. Genomic and geographic distribution of SNP-defined runs of homozygosity in Europeans. *Hum. Mol. Genet.* 19, 2927-2935.
- O'Sullivan, B. & Donald, A., 1973. Responses to infection with *Haemonchus contortus* and *Trichostrongylus colubriformis* in ewes of different reproductive status. *Int. J. Parasitol.* 3, 521-30.
- Ojango, J., Mporu, N., Marshall, K. & Andersson-Eklund, L., 2011. Quantitative methods to improve the understanding and utilization of animal genetic resources. *Animal Genetics Training Resources*. International Livestock Research Institute, Nairobi, Kenya and Swedish University of Agricultural Science, Uppsala, Sweden.
- Olivier, W.J., Herselman, M.J. & Van Heerden, M., 2010. Production norms of the Grootfontein Dohne Merino flock. *Grootfontein Agric* 10(1), 55-66.
- Olivier, W.J., 2016a. Project proposal AP1/19: Evaluation of maternal breeding values for early body weights as selection criteria in Dohne Merino sheep. Animal Production Unit. Grootfontein Agricultural Development Institute.
- Olivier, W.J., 2016b. Project proposal AP10/1/6: Maintenance of a Dohne Merino flock as resource for research and as reference flock for a biological bank for Dohne Merino sheep in South Africa. Animal Production Unit. Grootfontein Agricultural Development Institute.
- Paolini, V., Bergeaud, J., Grisez, C., Prevot, F., Dorchies, P. & Hoste, H., 2003. Effects of condensed tannins on goats experimentally infected with *Haemonchus contortus*. *Vet. Parasitol.* 113, 253-61.
- Paschou, P., Ziv E., Burchard, E.G., Choudhry, S., Rodriguez-Cintron, W., Mahoney, M.W. & Drineas, P., 2007. PCA-correlated SNPs for structure identification in worldwide human populations. *PLoS genetics* 3, e160.
- Patterson, N., Price, A.L. & Reich, D., 2006. Population structure and eigenanalysis. *PLoS genetics* 2, e190.
- Periasamy, K., Pichler, R., Poli, M., Cristel, S., Cetrá, B., Medus, D., Basar, M., Thiruvankadan, A., Ramasamy, S. & Ellahi, M.B., 2014. Candidate gene approach for

- parasite resistance in sheep - variation in immune pathway genes and association with fecal egg count. *PloS one* 9, e88337.
- Peripolli, E., Munari, D., Silva, M., Lima, A., Irgang, R. & Baldi, F., 2017. Runs of homozygosity: current knowledge and applications in livestock. *Anim. Genet.* 48, 255-271.
- Pernthaner, A., Vlassoff, A., Douch, P.G.C., Maas, D., 1997. Cytokine mRNA expression and IFN-production in nematode resistant and susceptible line lambs artificially infected with gastro-intestinal nematodes. *Acta Parasitol.* 42, 55- 61.
- Peter, B.M., 2016. Admixture, population structure, and F-statistics. *Genet.* 202, 1485-1501.
- Pickering, N.K., Dodds, K.G., Blair, H., Hickson, R., Johnson, P. & McEwan, J., 2012. Genetic parameters for production traits in New Zealand dual-purpose sheep, with an emphasis on dagginess. *J. Anim. Sci.* 90, 1411-1420.
- Polat, M., Aida, Y., Takeshima, S.N., Aniwashi, J. & Halik, M., 2015. The diversity of major histocompatibility complex class II DRB1 gene in sheep breeds from Xinjiang, China. *Tissue antigens* 85, 50-57.
- Pollott, G. & Greeff, J., 2004. Genetic relationships between faecal egg count and production traits in commercial Merino sheep flocks. *Anim. Sci.* 79, 21-32.
- Ponz, R., Moreno, C., Allain, D., Elsen, J.M., Lantier, F., Lantier, I., Brunel, J.C. & Perez-Enciso, M., 2001. Assessment of genetic variation explained by markers for wool traits in sheep via a segment mapping approach, *Mamm. Genome.* 12, 569-572.
- Preston, S., Jabbar, A. & Gasser, R.B., 2016. A perspective on genomic-guided anthelmintic discovery and repurposing using *Haemonchus contortus*. *Infect. Genet. Evol.* 40, 368-373.
- Price, A.L., Patterson, N.J., Plenge, R.M., Weinblatt, M.E., Shadick, N.A. & Reich, D., 2006. Principal components analysis corrects for stratification in genome-wide association studies. *Nature genetics* 38, 904-909.
- Pritchard, J.K., Wena, X. & Falush, D., 2009. Documentation for structure software: Version 2.3.
- Purcell, S., Neale, B., Todd-Brown, K., Thomas, L., Ferreira, M.A., Bender, D., Maller, J., Sklar, P., De Bakker, P.I. & Daly, M.J., 2007. PLINK: a tool set for whole-genome association and population-based linkage analyses. *Am. J. Hum. Genet.* 81, 559-575.
- Purfield, D.C., Berry, D.P., McParland, S. & Bradley, D.G., 2012. Runs of homozygosity and population history in cattle. *BMC Genet.* 13, 70-80.

- Purfield, D.C., McParland, S., Wall, E. & Berry D.P., 2017. The distribution of runs of homozygosity and selection signatures in six commercial meat sheep breeds. *PloS one* 12 (5), e0176780. <https://doi.org/10.1371/journal.pone.0176780>.
- Qamar, M.F., Maqbool, A., Khan, M.S., Ahmad, N. & Muneer, M.A., 2009. Epidemiology of Haemonchosis in sheep and goats under different managemental conditions. *Vet. World.* 2, 413-417.
- Reese, T.A., Liang, H.E., Tager, A.M., Luster, A.D., Van Rooijen, N., Voehringer, D. & Locksley, R.M., 2007. Chitin induces accumulation in tissue of innate immune cells associated with allergy. *Nature*, 447(7140), 92-96.
- Riggio, V., Matika, O., Pong-Wong, R., Stear, M. & Bishop, S., 2013. Genome-wide association and regional heritability mapping to identify loci underlying variation in nematode resistance and body weight in Scottish Blackface lambs. *Heredit.* 110, 420-429.
- Riggio V., Abdel-Aziz M., Matika O., Moreno C., Carta A. & Bishop S., 2014a. Accuracy of genomic prediction within and across populations for nematode resistance and body weight traits in sheep. *Anim.* 8, 520-528.
- Riggio, V., Pong-Wong, R., Sallé, G., Usai, M.G., Casu, S., Moreno, C., Matika, O. & Bishop, S.C., 2014b. A joint analysis to identify loci underlying variation in nematode resistance in three European sheep populations. *J. Anim. Breed. Genet.* 131, 426-436.
- Riley, D. & Van Wyk, J.A., 2009. Genetic parameters for FAMACHA[®] score and related traits for host resistance/resilience and production at differing severities of worm challenge in a Merino flock in South Africa. *Vet. Parasitol.* 164, 44-52.
- Riley, D.G., Van Wyk, J.A., 2011. The effects of penalization of FAMACHA[®] scores of lambs treated for internal parasites on the estimation of genetic parameters and prediction of breeding values. *Small Rum. Res.* 99, 122-129.
- Rout, P., Chauhan, K., Matika, O. & Bishop, S., 2011. Exploring the genetic resistance to natural gastrointestinal nematode infection in Indian goats. *Vet. Parasitol.* 180, 315-322.
- Saddiqi, H.A., Jabbar, A., Sarwar, M., Iqbal, Z., Muhammad, G., Nisa, M. & Shahzad, A., 2011. Small ruminant resistance against gastrointestinal nematodes: a case of *Haemonchus contortus*. *Parasitol. Res.* 109, 1483-1500.
- Sallé, G., Jacquiet, P., Gruner, L., Cortet, J., Sauvé, C., Prevot, F., Grisez, C., Bergeaud, J. P., Schibler, L., Tircazes, A., Francois, D., Pery, C., Bouvier, F., Thouly, J. C., Brunel, J. C., Legarra, A., Elsen, J. M., Bouix, J., Rupp, R., and Moreno, C. R., 2012. A genome scan

- for QTL affecting resistance to *Haemonchus contortus* in sheep. J Anim. Sci. 90(13), 4690-4705.
- Sallé, G., Moreno, C., Boitard, S., Ruesche, J., Tircazes-Secula, A., Bouvier, F., Aletru, M., Weisbecker, J.L., Prévot, F. & Bergeaud, J.P., 2014. Functional investigation of a QTL affecting resistance to *Haemonchus contortus* in sheep. Vet. Res. 45, 68-79.
- Sandenbergh, L., Cloete, S., Roodt-Wilding, R., Snyman, M. & Bester-van der Merwe, A., 2016. Evaluation of the OvineSNP50 chip for use in four South African sheep breeds. S. Afr. J. Anim. Sci. 46, 89-93.
- Sankararaman, S., Sridhar, S., Kimmel, G. & Halperin, E., 2008. Estimating local ancestry in admixed populations. Am. J. Hum. Genet. 82, 290-303.
- Sargison, N.D., 2016. Keys to solving health problems in small ruminants: AR as a threat to sustainable nematode control. Small Rumin. Res. 142, 11-15.
- SAS Institute Inc., 2016. SAS OnlineDoc® 9.3. Cary, NC, SAS Institute Inc.
- Sayers, G., Good, B., Hanrahan, J., Ryan, M., Angles, J. & Sweeney, T., 2005. Major histocompatibility complex DRB1 gene: its role in nematode resistance in Suffolk and Texel sheep breeds. Parasitol. 131, 403-409.
- Schallig, H.D.F.H., 2000. Immunological responses of sheep to *Haemonchus contortus*. Parasitol. 120, S63-S72.
- Schefers, J.M. & Weigel, K.A., 2012. Genomic selection in dairy cattle: Integration of DNA testing into breeding programs. Anim. Frontiers 2, 4-9.
- Schichowski, C., Moors, E. & Gauly, M., 2010. Influence of weaning age and an experimental *Haemonchus contortus* infection on behaviour and growth rates of lambs. Appl Anim Behav Sci. 125, 103-108.
- Schwerin, M., Brockmann, G., Vanselow, J. & Seyfert, H.M., 1995. Perspectives of molecular genome analysis in livestock improvement. Arch. Tierz. Dummerstorf. 38, 21-31.
- Sebastiano, R.S., Sweeney, T., Keady, T.W., Hanrahan, J.P. & Good B., 2017. Can the amount of digestible undegraded protein offered to ewes in late pregnancy affect the periparturient change in resistance to gastrointestinal nematodes? Vet. Parasitol. 235, 8-16.
- Shalaby, H.A., 2013. Anthelmintics resistance: How to overcome it? Iran. J. Parasitol. 8 (1), 18-32.

- Shariflou, M., Wade, C., Kijas, J., McCulloch, R., Windsor, P., Tammen, I. & Nicholas, F., 2013. Brachygnathia, cardiomegaly and renal hypoplasia syndrome (BCRHS) in Merino sheep maps to a 1.1-megabase region on ovine chromosome OAR2. *Anim. Genet.* 44, 231-233.
- Silva, M., Sonstegard, T., Hanotte, O., Mugambi, J., Garcia, J., Nagda, S., Gibson, J., Iraqi, F., McClintock, A. & Kemp, S., 2012. Identification of quantitative trait loci affecting resistance to gastrointestinal parasites in a double backcross population of Red Maasai and Dorper sheep. *Anim. Genet.* 43, 63-71.
- Smith, J.A., Wilson, K., Pilkington, J.G. & Pemberton, J.M., 1999. Heritable variation in resistance to gastro-intestinal nematodes in an unmanaged mammal population. *Proc. Royal Soc. Lond. B: Biol. Sci.* 266, 1283-90.
- Snyman, M.A., 2016a. Project proposal AP2/21: Genome wide association study to identify genetic markers associated with resistance to *Haemonchus contortus* in sheep. Animal Production Unit. Grootfontein Agricultural Development Institute.
- Snyman, M.A., 2016b. Project proposal AP2/21/1: A case-control investigation to evaluate resistance to *Haemonchus contortus* in South African Dohne Merino sheep. Animal Production Unit. Grootfontein Agricultural Development Institute.
- Soma, P., Kotze, A., Grobler, J. & Van Wyk, J., 2012. South African sheep breeds: Population genetic structure and conservation implications. *Small Rumin. Res.* 103, 112-119.
- Sommer, S., Courtiol, A., & Mazzoni, C.J., 2013. MHC genotyping of non-model organisms using next-generation sequencing: a new methodology to deal with artefacts and allelic dropout. *BMC Genomics.* 14, 542-558.
- Southey, B.R., Thomas, D.L., Gottfredson, R.G. & Zelinsky, R.D., 2002. Ewe productivity of Booroola Merino-Rambouillet crossbred sheep during early stages of intro-gression of the Fec(B) allele into a Rambouillet population, *Livest. Prod. Sci.* 75, 33-44.
- Stafford, K., Morgan, E. & Coles, G., 2009. Weight-based targeted selective treatment of gastrointestinal nematodes in a commercial sheep flock. *Vet. Parasitol.* 164, 59-65.
- Stear, M. & Wakelin, D., 1998. Genetic resistance to parasitic infection. *Revue scientifique et technique (International Office of Epizootics)* 17, 143-153.
- Stear, M., Boag, B., Cattadori, I. & Murphy, L., 2009. Genetic variation in resistance to mixed, predominantly *Teladorsagia circumcincta* nematode infections of sheep: from heritabilities to gene identification. *Parasite Immunol.* 31, 274-282.

- Subramaniam, N.S., Morgan, E., Wetherall, J., Stear, M. & Groth, D., 2015. A comprehensive mapping of the structure and gene organisation in the sheep MHC class I region. *BMC Genomics*. 16, 810-826.
- Swanepoel, J.W., 2006. A genetic evaluation of the Dohne Merino breed in South Africa. MSc (Agric) thesis, University of the Free State, Bloemfontein, South Africa.
- Szmatola, T., Gurgul, A., Ropka-Molik, K., Jasielczuck, I., Zabek, T. & Bugno-Poniewierska M., 2016. Characteristics of runs of homozygosity in selected cattle breeds maintained in Poland. *Livest. Sci.* 188, 72-80.
- Szulkin, M., Stopher, K.V., Pemberton, J.M. & Reid, J.M., 2013. Inbreeding avoidance, tolerance, or preference in animals? *Trends Ecol. & Evol.* 28, 205-211.
- Tariq, K.A., 2015. A review of the epidemiology and control of gastrointestinal nematode infections of small ruminants. *Proceedings of the National Academy of Sciences, India Section B: Biological Sciences* 85, 693-703.
- Tenesa, A., Navarro, P., Hayes, B.J., Duffy, D.L., Clarke, G.M., Goddard, M.E. & Visscher, P.M., 2007. Recent human effective population size estimated from linkage disequilibrium. *Genome Res.* 17. 520-526.
- Thomaz-Soccol, V., Souza, F.P.D., Sotomaior, C., Castro, E.A., Milczewski, V. & Mocelin, G., 2004. Resistance of gastrointestinal nematodes to anthelmintics in sheep (*Ovis aries*). *Braz. Arch. Biol. Technol.* 47, 41-47.
- Thompson, J. & Meyer, H., 1994. Body condition scoring of sheep. Extension Service, Oregon State University, US Department of Agriculture, 1-4.
- Todd, D.L., 2013. Implementation of genomic selection in UK beef and sheep breeding. Ph.D thesis, The University of Edinburgh, Scotland.
- Toro, M.A. & Caballero, A., 2005. Characterization and conservation of genetic diversity in subdivided populations. *Philos. Trans. R. Soc. Lond. B. Biol Sci.* 360, 1367-1378.
- Torres-Acosta, J., Sandoval-Castro, C., Hoste, H., Aguilar-Caballero, A., Cámara-Sarmiento, R. & Alonso-Díaz M., 2012. Nutritional manipulation of sheep and goats for the control of gastrointestinal nematodes under hot humid and subhumid tropical conditions. *Small Rumin. Res.* 103, 28-40.
- Traoré, A., Notter, D.R., Soudre, A., Kaboré, A., Álvarez, I., Fernández, I., Sanou, M., Shamshuddin, M., Periasamy, K. & Tamboura, H.H., 2017. Resistance to gastrointestinal parasite infection in Djallonké sheep. *Anim.* 11(8), 1354-1362.

- Tsotetsi, A.M., Njiro, S., Katsande, T.C., Moyo, G. & Mpofu, J., 2013. Prevalence of gastrointestinal helminths and AR on small-scale farms in Gauteng Province, South Africa. *Trop. Anim. Health Prod.* 45, 751-761.
- Vadlejch, J., Kopecký, O., Kudrnáčová, M., Čadková, Z., Jankovská, I. & Langrová, I., 2014. The effect of risk factors of sheep flock management practices on the development of AR in the Czech Republic. *Small Rumin. Res.* 117, 183-190.
- Vagenas, D., Jackson, F., Russel, A., Merchant, M., Wright, I. & Bishop, S., 2002. Genetic control of resistance to gastro-intestinal parasites in crossbred cashmere-producing goats: responses to selection, genetic parameters and relationships with production traits. *Anim. Sci.* 74, 199-208.
- Valilou, R.H., Rafat, S.A., Notter, D.R., Shojda, D., Moghaddam, G. & Nematollahi, A., 2015. Fecal egg counts for gastrointestinal nematodes are associated with a polymorphism in the MHC-DRB1 gene in the Iranian Ghezel sheep breed. *Front. Genet.* 6, 105-115.
- Van Burgel, A., Oldham, C., Behrendt, R., Curnow, M., Gordon, D. & Thompson, A., 2011. The merit of condition score and fat score as alternatives to liveweight for managing the nutrition of ewes. *Anim. Prod. Sci.* 51, 834-841.
- Van der Werf, J.H., 2007. Marker assisted selection in sheep and goats. In: *Marker-Assisted Selection: Current status and future perspectives in crops, livestock, forestry and fish.* Eds. Guimarães, E., Ruane, J., Scherf, B., Sonnino, A. & Dargie, J. FAO, Rome, Italy. pp. 230-247.
- Van der Werf, J., Banks, R., Clark, S., Lee, S., Daetwyler, H., Hayes, B. & Swan, A., 2014. Genomic selection in sheep breeding programs. In: *Proceedings of the 10th world congress of genetics applied to livestock production*, pp 17-22.
- Van Marle-Köster, E., Visser, C. & Berry, D., 2013. A review of genomic selection- Implications for the South African beef and dairy cattle industries. *S. Afr. J. Anim. Sci.* 43, 1-17.
- Van Wyk, J.A., Malan, F.S., Gerber, H.M. & Alves, R.M.R., 1989. The problem of escalating resistance to *H. contortus* to the modern anthelmintics in South Africa. *Onderstepoort J. Vet. Res.* 56, 41-49.
- Van Wyk, J. & Van Schalkwyk, P., 1990. A novel approach to the control of anthelmintic-resistant *Haemonchus contortus* in sheep. *Vet. Parasitol.* 35, 61-69.

- Van Wyk, J.A., Malan, F.S., Bath, G.F., 1997. Rampant anthelmintic resistance in sheep in South Africa - What are the options? In: Proceedings of the 16th International Conference of the WAAVP, 1997, South Africa: Workshop on managing anthelmintic resistance in endoparasites, pp 51-63.
- Van Wyk, J.A., 2001. Refugia - overlooked as perhaps the most potent factor concerning the development of anthelmintic resistance. *Onderstepoort J. Vet. Res.* 68, 55-67.
- Van Wyk, J.A. & Bath, G.F., 2002. The FAMACHA system for managing haemonchosis in sheep and goats by clinically identifying individual animals for treatment. *Vet. Res.* 33, 509-529.
- Van Wyk, J., Swanepoel, J., Cloete, S., Olivier, J. & Delpont, G. 2008. Across flock genetic parameter estimation for yearling body weight and fleece traits in the South African Dohne Merino population. *S. Afr. J. Anim. Sci.* 38, 31-37.
- Van Wyk, J. & Reynecke, D., 2011. Blueprint for an automated specific decision support system for countering AR in *Haemonchus* spp. at farm level. *Vet. Parasitol.* 177, 212-223.
- Vanimiseti H., Andrew S., Zajac A. & Notter D., 2004. Inheritance of fecal egg count and packed cell volume and their relationship with production traits in sheep infected with. *J. Anim. Sci.* 82, 1602-1611.
- Várady, M., Papadopoulos, E., Dolinská, M. & Königová, A., 2011. AR in parasites of small ruminants: sheep versus goats. *Helminthologia* 48, 137-144.
- Vatta, A., Krecek, R., Van der Linde, M., Motswatswe, P., Grimbeek, R., Van Wijk, E. & Hansen, J., 2002. *Haemonchus* spp. in sheep farmed under resource-poor conditions in South Africa-effect on haematocrit, conjunctival mucous membrane colour and body condition. *J. S. Afr. Vet. Assoc.* 73, 119-123.
- Venter, I., 2011. Quantitative techniques for separating and concentrating eggs/larvae. Provincial Veterinary Laboratory, Middelburg, Eastern Cape Province. DOC -P- PAR-04. 5th Ed. 04/10/2011.
- Vijayasarithi, M., Sreekumar, C., Venkataramanan, R. & Raman, M., 2016. Influence of sustained deworming pressure on the AR status in strongyles of sheep under field conditions. *Trop. Anim. Health Prod.* 48 (7), 55-62.
- Visser, P.M., Brown, M.A., McCarthy, M.I. & Yang, J., 2012. Five years of GWAS discovery. *Am. J. Hum. Genet.* 90, 7-24.

- Wakchaure, R., Ganguly, S., Praveen, P., Kumar, A. & Sharma, S., 2015. Marker Assisted Selection (MAS) in Animal Breeding: A Review. *J Drug Metab Toxicol* 6, e127.
- Wang, J., 2005. Estimation of effective population sizes from data on genetic markers. *Philos. Trans. R. Soc. Lond. B. Biol. Sci.* 360, 1395-1409.
- Wanyangu, S.W., Mugambi, J.M., Bain, R.K.D.J.L., Murray, M., Stear, M.J., 1997. Response to artificial and subsequent natural infestation with *Haemonchus contortus* in Red Maasai and Dorper ewes. *Vet. Parasitol.* 69, 275-282.
- Weatherbase, 2015. Middelburg, South Africa. [Online] Available: www.weatherbase.com.
- Wolstenholme, A.J., Fairweather, I., Prichard, R., von Samson-Himmelstjerna, G. & Sangster, N.C., 2004. Drug resistance in veterinary helminths. *Trends Parasitol.* 20, 469-476.
- Woolaston, R., 1992. Selection of Merino sheep for increased and decreased resistance to *Haemonchus contortus*: peri-parturient effects on faecal egg counts. *Int. J. Parasitol.* 22, 947-953.
- Woolaston, R. & Piper, L., 1996. Selection of Merino sheep for resistance to *Haemonchus contortus*: genetic variation. *Anim. Sci.* 62, 451-460.
- Woolaston, R., Barger, I. & Eady, S., 1997. The relative effectiveness of alternative worm control measures and their interactions. *Proc. Assoc. Advmt. Anim. Breed. Genet.* pp. 45-49.
- Worldweatheronline, 2014. Middelburg historical weather, South Africa. [Online] Available: <http://www.worldweatheronline.com/middelburg-weather-history/eastern-cape/za.aspx>.
- Wright, S., 1951. The genetical structure of populations. *Ann Eugenics.* 15, 323-354.
- Wu, C., DeWan, A., Hoh, J. & Wang, Z., 2011. A comparison of association methods correcting for population stratification in case-control studies. *Ann. Hum. Genet.* 75, 418-427.
- www.animalgenome.org/cgi-bin/QTLdb/OA/browse (accessed 15.06.17).
- Yan, J., Dukkupati, V., Blair, H., Biggs, P., Hamie, J. & Greer, A., 2017. A genome-wide scan of positive selection signature using Ovine Infinium® HD SNP BeadChip in two Romney lines, selected for resistance or resilience to nematodes. *Proc. Assoc. Advmt. Anim. Breed. Genet.* 22, 585-588
- Yasmeen, A., Sherwani, S.K., Ur Rehman, Z., Babar, M.E., Ahmad, N., Ullah, Z., Hussain, S.M., Intzar, M., Tahir, M.Z. & Hussain, T., 2014. Major Histocompatibility Complex

- Class-I Genes: Role in Marker Assisted Selection in Caprine and Ovine Species. *World Appl. Sci. J.* 31, 741-744.
- Zajac, A., Herd, R. & McClure K., 1988. Trichostrongylid parasite populations in pregnant or lactating and unmated Florida Native and Dorset/Rambouillet ewes. *Int. J. Parasitol.* 18, 981-985.
- Zenger, K.R., Khatkar, M.S., Cavanagh, J.A., Hawken, R.J. & Raadsma, H.W., 2007. Genome wide genetic diversity of Holstein Friesian cattle reveals new insights into Australian and global population variability, including impact of selection. *Anim. Genet.* 38, 7-14.
- Zhang, H., Wang, Z., Wang, S. & Li, H., 2012. Progress of genome wide association study in domestic animals. *J. Anim. Sci. Technol.* 3 (1), 26-35.
- Zohary, D., Tchernov, E. & Horwitz, L.K., 1998. The role of unconscious selection in the domestication of sheep and goats. *J. Zool.* 245, 129-135.
- Zvinorova, P., Halimani, T., Muchadeyi, F., Matika, O., Riggio, V. & Dzama, K., 2016. Breeding for resistance to gastrointestinal nematodes—the potential in low-input/output small ruminant production systems. *Vet. Parasitol.* 225, 19-28.

Appendix

The following Figures depict the phenotypic trends in FEC, FAM and BCS for Dosed and Not dosed ram and ewe lambs per year.

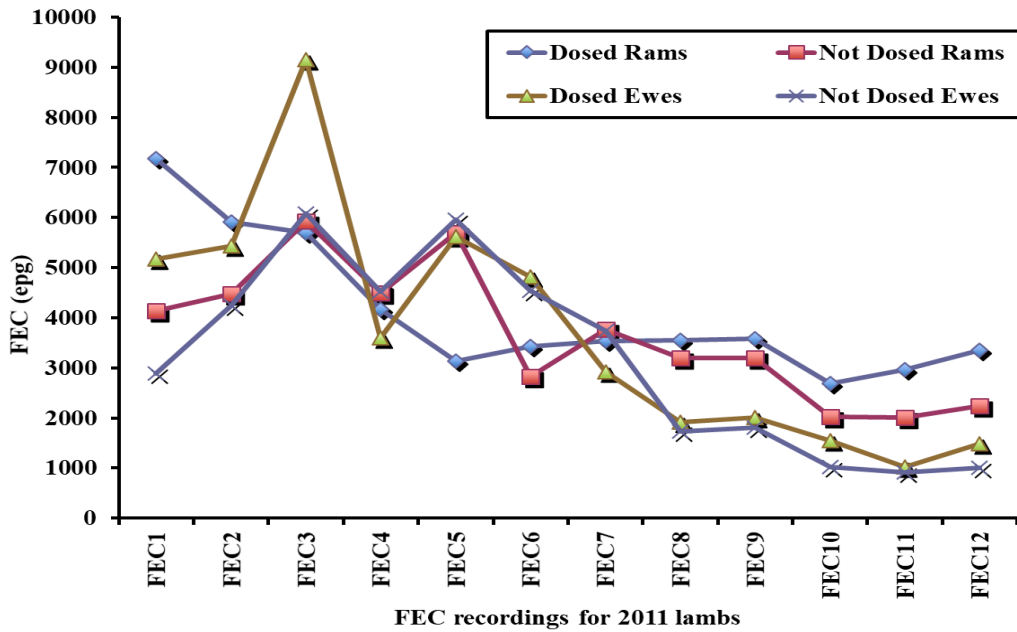


Figure 4.1A: FEC of Not dosed and Dosed 2011-born lambs of the Wauldby animals.

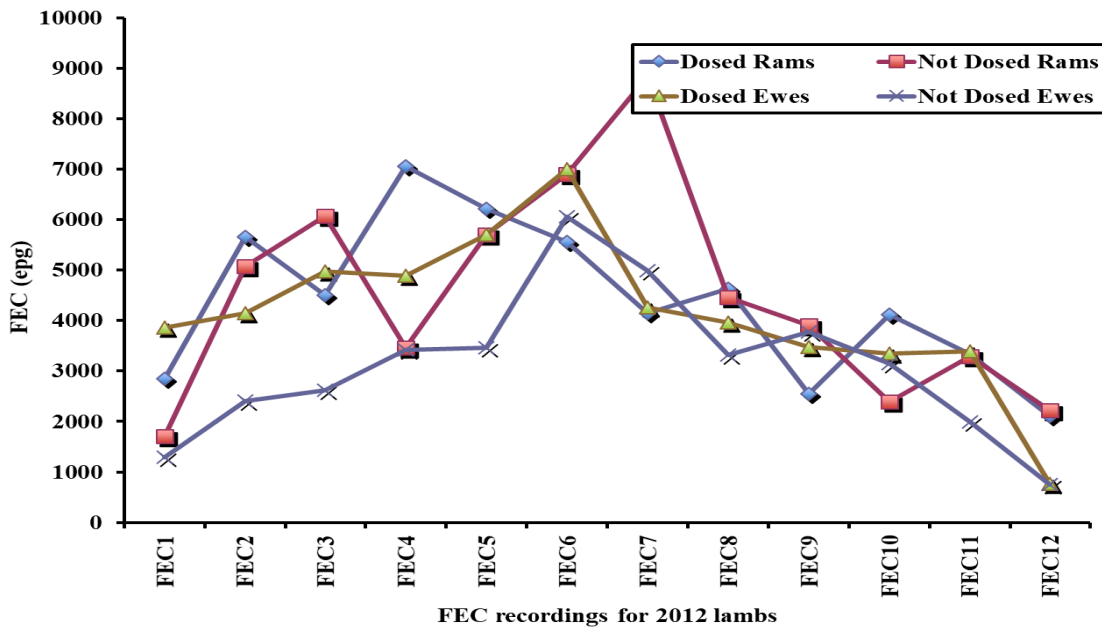


Figure 4.2 A: FEC of Not dosed and Dosed 2012-born lambs of the Wauldby animals.

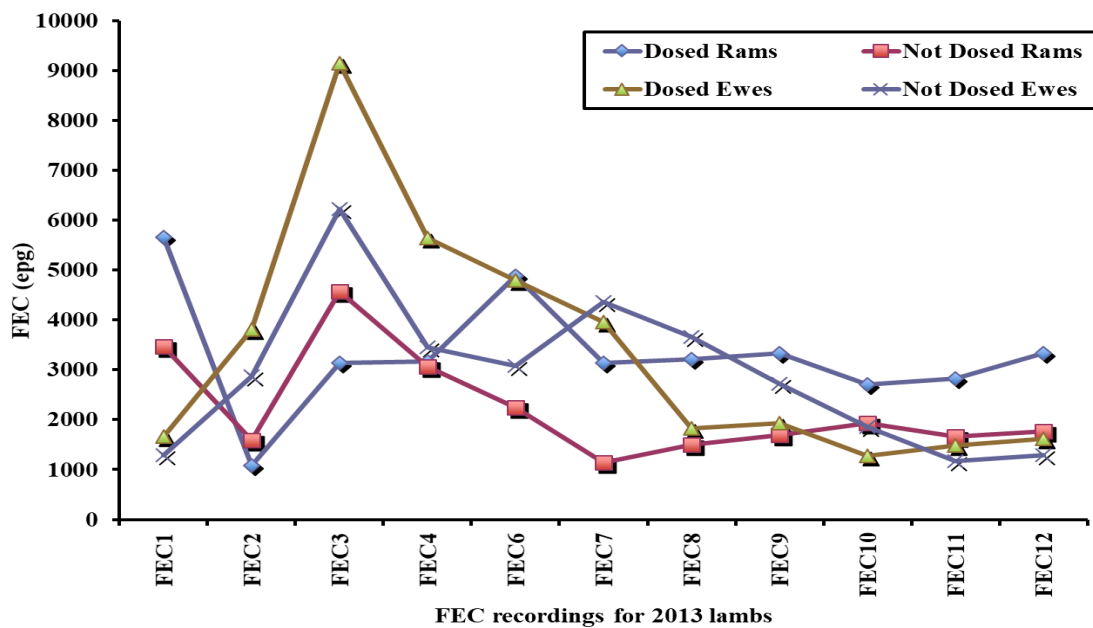


Figure 4.3A: FEC of Not dosed and Dosed 2013-born lambs of the Wauldby animals.

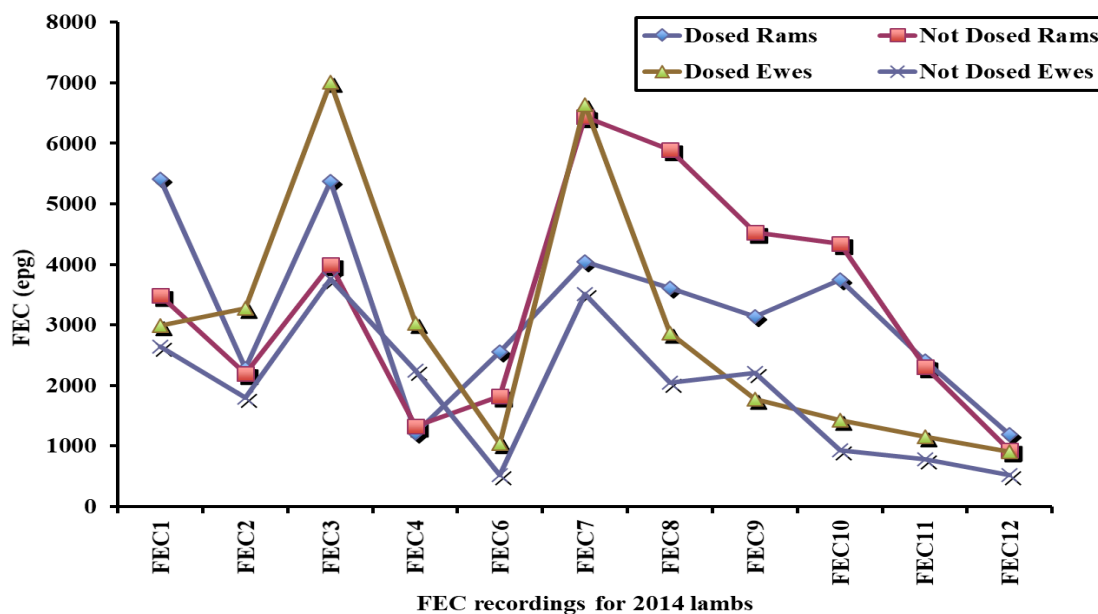


Figure 4.4A: FEC of Not dosed and Dosed 2014-born lambs of the Wauldby animals.

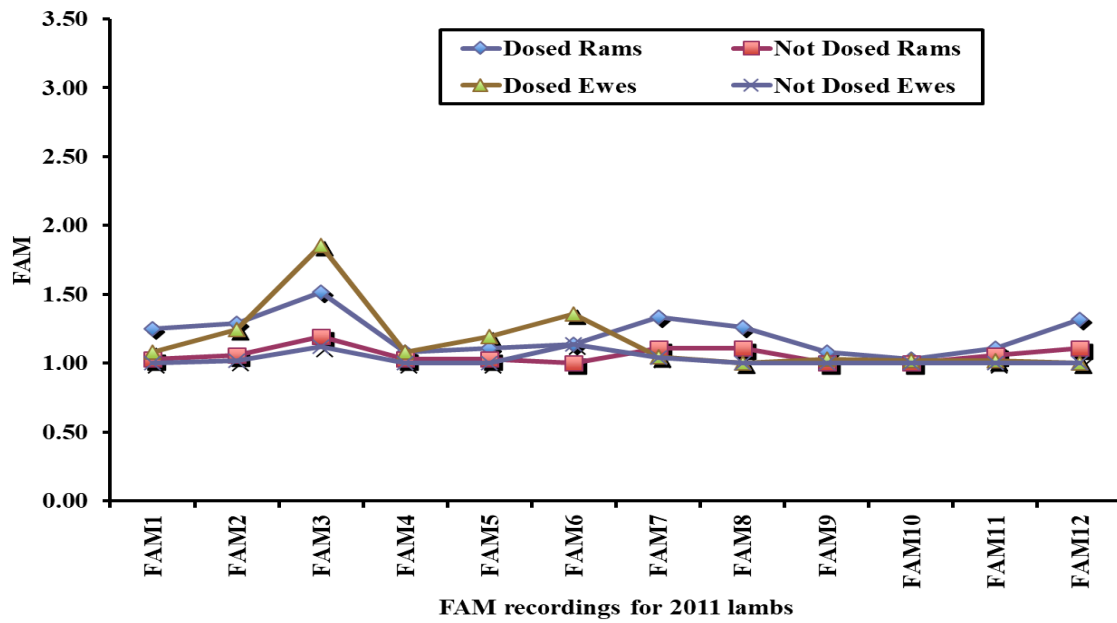


Figure 4.5A: FAM of Not dosed and Dosed 2011-born lambs of the Wauldby animals.

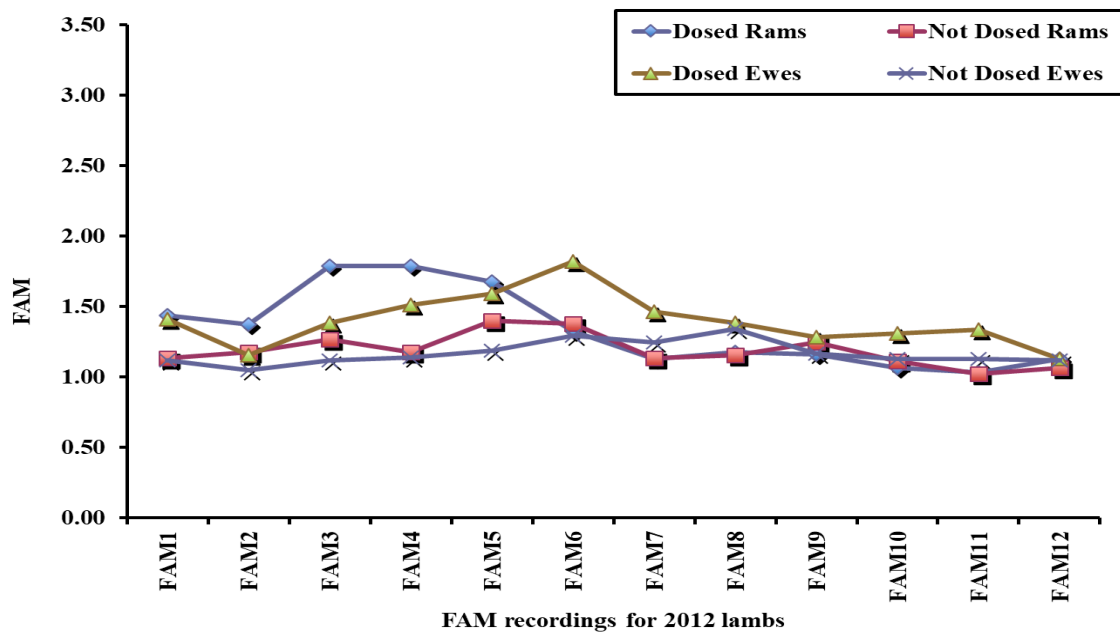


Figure 4.6A: FAM of Not dosed and Dosed 2012-born lambs of the Wauldby animals.

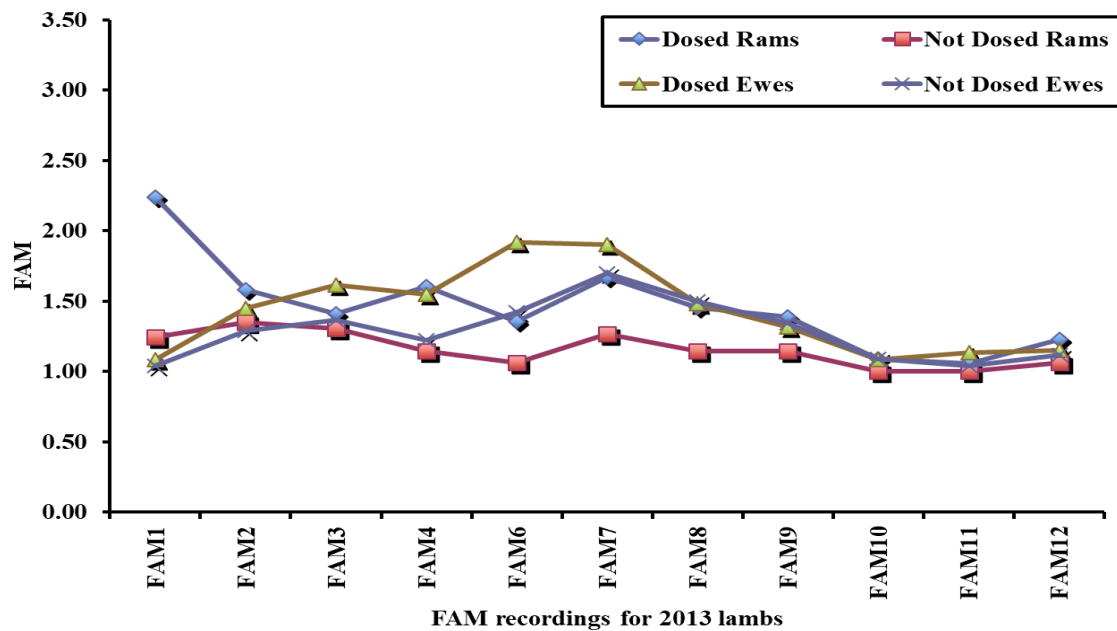


Figure 4.7: FAM of Not dosed and Dosed 2013-born lambs of the Wauldby animals.

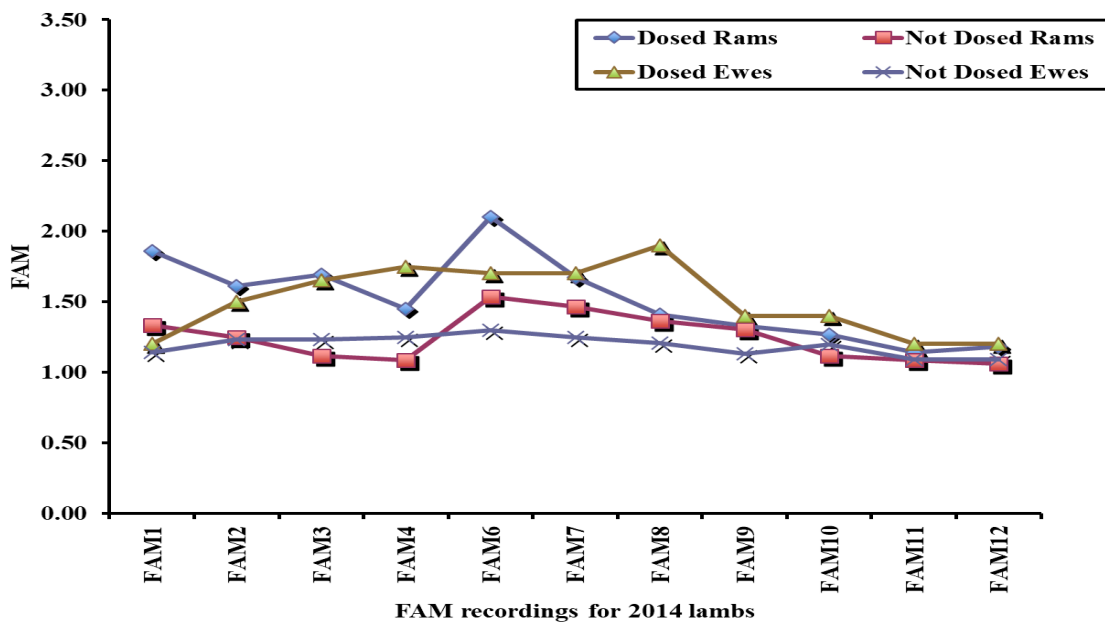


Figure 4.8: FAM of Not dosed and Dosed 2014-born lambs of the Wauldby animals.

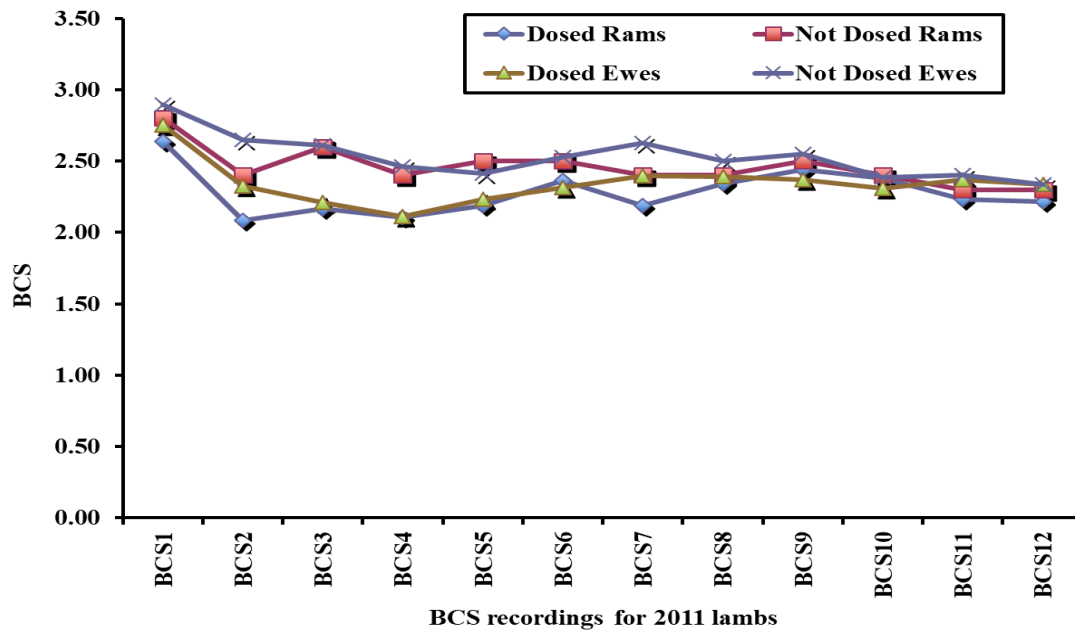


Figure 4.9: BCS of Not dosed and Dosed 2011-born lambs of the Wauldby animals.

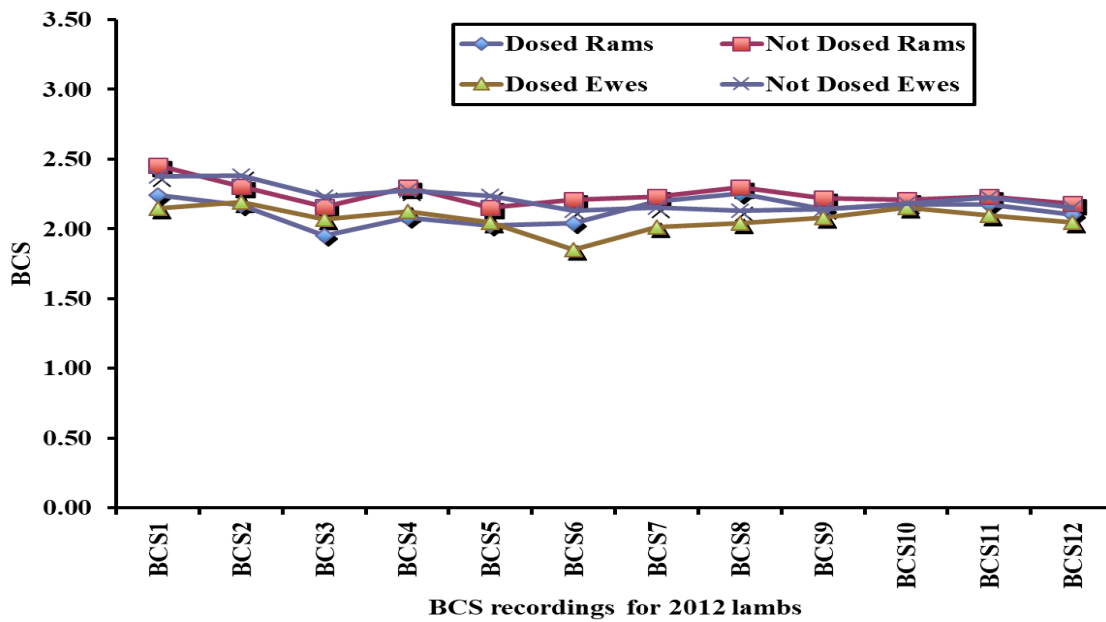


Figure 4.10A: BCS of Not dosed and Dosed 2012-born lambs of the Wauldby animals.

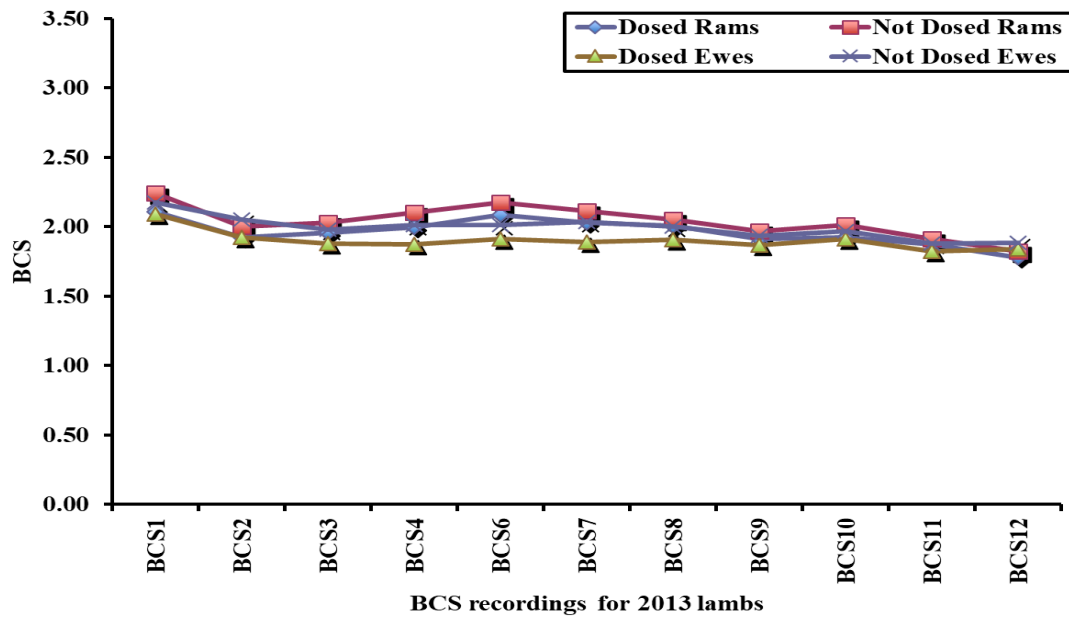


Figure 4.11A: BCS of Not dosed and Dosed 2013-born lambs of the Wauldby animals.

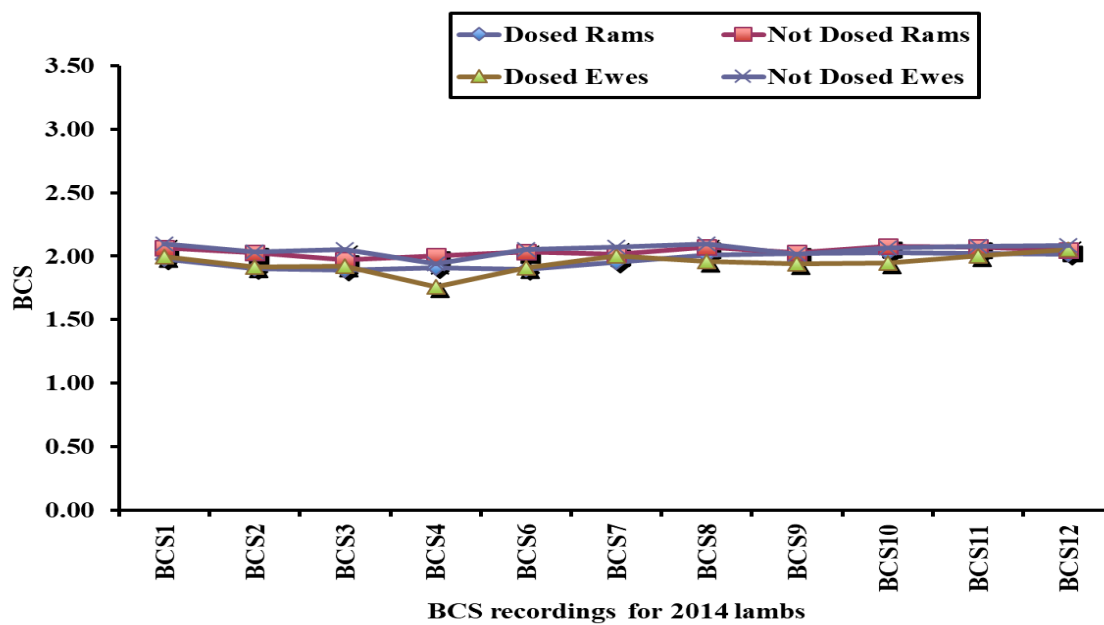


Figure 4.12A: BCS of Not dosed and Dosed 2014-born lambs of the Wauldby animals.

Table 4.1A: Effect of year of birth and dosing status on the average EBV of the phenotypic traits recorded over the experimental period for all the Wauldby animals.

Effect	EBV-FAM	EBV-BCS	EBV-FEC	EBV-LFEC
Year of birth				
2011	0.007 ^a ± 0.008	-0.024 ^a ± 0.008	-41.680 ^a ± 63.734	0.005 ^a ± 0.015
2012	0.010 ^{ab} ± 0.009	-0.0001 ^b ± 0.008	70.416 ^b ± 66.612	-0.001 ^a ± 0.015
2013	0.020 ^b ± 0.008	-0.006 ^b ± 0.008	-155.279 ^c ± 62.606	-0.017 ^a ± 0.014
2014	-0.022 ^c ± 0.009	0.028 ^c ± 0.008	-218.077 ^c ± 67.555	-0.072 ^b ± 0.016
Dosing status				
10	0.004 ^a ± 0.003	-0.002 ^a ± 0.003	-109.003 ^a ± 21.102	-0.015 ^a ± 0.005
21	0.005 ^a ± 0.003	-0.005 ^a ± 0.003	-30.676 ^b ± 25.166	-0.007 ^a ± 0.006
22	0.007 ^a ± 0.006	-0.010 ^a ± 0.006	10.291 ^{ab} ± 49.147	-0.004 ^a ± 0.011
23	0.004 ^a ± 0.015	-0.023 ^a ± 0.014	5.089 ^{ab} ± 113.348	0.002 ^a ± 0.026
24	0.001 ^a ± 0.035	0.037 ^a ± 0.032	-306.476 ^{ab} ± 267.199	-0.083 ^a ± 0.062

^{a,b,c} Values with different superscripts within effects and traits differed significantly ($P < 0.01$)

Table 4.2A: Effect of year of birth and dosing status on the average EBV of the phenotypic traits recorded over the experimental period for the genotyped Wauldby animals.

Effect	EBV-FAM	EBV-BCS	EBV-FEC	EBV-LFEC
Year of birth				
2011	0.014 ^a ± 0.015	-0.022 ^a ± 0.012	115.326 ^a ± 138.798	0.040 ^a ± 0.038
2012	-0.010 ^{ab} ± 0.015	-0.016 ^a ± 0.013	-73.191 ^a ± 139.625	-0.021 ^{ab} ± 0.038
2013	0.018 ^a ± 0.013	-0.012 ^a ± 0.010	-54.205 ^a ± 116.376	0.007 ^a ± 0.032
2014	-0.022 ^b ± 0.013	0.010 ^b ± 0.011	-109.002 ^a ± 123.756	-0.067 ^b ± 0.034
Dosing status				
10	-0.006 ^a ± 0.006	0.009 ^{ab} ± 0.005	-97.599 ^a ± 57.277	-0.025 ^a ± 0.016
21	0.007 ^a ± 0.007	0.008 ^{ab} ± 0.006	-102.283 ^a ± 68.338	-0.035 ^a ± 0.019
22	-0.003 ^a ± 0.014	0.021 ^a ± 0.012	-132.563 ^a ± 133.177	-0.053 ^a ± 0.037
23	0.003 ^a ± 0.042	-0.079 ^b ± 0.035	211.372 ^a ± 391.298	0.072 ^a ± 0.107

^{a,b} Values with different superscripts within effects and traits differed significantly ($P < 0.01$)