A Genome wide association study of carcass traits based on Real Time Ultrasound in South African Nguni cattle

by

Jani de Vos

Submitted in partial fulfilment of the requirements for the degree

Magister Scientiae Agriculturae

MSc Agric Animal Science: Animal Breeding and Genetics

in the Faculty of Natural and Agricultural Sciences

Department of Animal and Wildlife Sciences

University of Pretoria

Pretoria

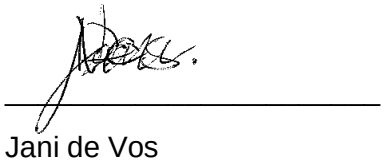
December 2018

Supervisor: Prof. E. van Marle-Köster

Co-supervisor: Dr. H. Theron

Declaration

I, Jani de Vos hereby declare that this thesis, submitted for the MSc(Agric) Animal Science: Animal Breeding and Genetics degree at the University of Pretoria, is my own work and has not previously been submitted by me for a degree at any other University.



Jani de Vos

Pretoria

November 2018

“When one dream burns to ash, you don’t crumble beneath it. You get on your hands and knees, and you sift through those ashes until you find the very last ember, the very last spark. Then you breathe. You breathe, and you make a new fire.”

-The Summer of chasing mermaids by Sarah Ockler

Acknowledgements

The financial assistance of the National Research Foundation (NRF) towards this research is hereby acknowledged. Opinions expressed and conclusions arrived at, are those of the author and are not necessarily to be attributed to the NRF. A very big thank you to the Bovine Genomics Project (BGP) for the funding of this project!

Most importantly, I would like to thank my parents for their support in all my years of study and for many encouraging words during my MSc. To my Dad thank you for always believing in me, sometimes even more than I believed in myself. Thank you for always listening patiently to all my stories! To my Mother and biggest confidant, thanks for all the phone conversations, quick lunches, care packages and encouragement. You have always been such an inspiration of what I can achieve as a woman! You both are the best and I have been truly blessed!

To my supervisor and mentor, Prof E. van Marle-Köster, you are the biggest inspiration for all I want to achieve in my life. Thank you for our 'small' cups of coffee while working. Thank you for teaching me that even when you are working hard and have deadlines, you should still live your life. I have been so blessed to have you in my life and I can never thank you enough!

To the rest of my 'Pretoria family', thank you for many lunches, dinners and cups of coffee! Leo, we had many conversations from serious to totally ridiculous; you lifted my spirits on days when it was totally difficult! Emma, thanks for being my un-biological sister. We spent many days on the porch or in the kitchen working and had many coffee dates where we talked about all things in life! Oom Heiko, for always taking an interest in my studies and giving an encouraging word.

Thank you to my co-supervisor Dr. H Theron, for patiently helping me to figure out SAS and helping with analyses and the statistics!

To my family, Marina, Pete, Taryn, Rob, Amy, Arran, Neil, Tammy, Wendy, Peter, Ouma, Oupa Hannes and Oupa Dirk, for always supporting me in everything I do and encouraging me to follow my dreams.

Rulien and Amelia. Rulien you always gave an encouraging word and many cups of coffee when both of us needed the energy. You helped me climb the figurative Kilimanjaro! Amelia, I could come over to your house anytime day or night and you would be ready with a cup of tea and willing listen to all my problems. My friends, all of you gave me support throughout this journey. Whether it be coffee breaks, tea breaks, having a quick lunch, many phone calls and adventures throughout the past two years! Karla, Christelle, Jomi, Danah and Sonja. Thanks for all the support and encouragement throughout this journey, I have been so blessed! In the department of Animal Sciences all my colleagues, especially Cheryl and Simon!

Most importantly to my God. I love Animal Sciences, especially Animal Breeding and Genetics; everything I have learnt just made my religion stronger. How amazing is this life and the way everything works. (Psalm 121)

I can't wait to see what is next on this amazing journey!

Abstract

The purpose of this study was to identify genomic regions associated with carcass traits using real-time ultrasound measurements in South African Nguni cattle. The dataset contained measurements from 200 Nguni steers finished in a growth trial. The following carcass traits were measured: ultrasound measurements of eye muscle area (EMA), rump fat thickness (RF) and backfat thickness (BF), slaughter weight (SW), dressing percentage (DP). The ultrasound measurements were measured at two separate dates during the growth trial. The 150k GGP HD SNP array (Geneseek) was used for genotyping 141 of the 200 cattle from the trial. The technical quality of the genomic data was investigated using SNP and individual call rates of 90%. Thereafter a genome-wide association study (GWAS) was performed on the data without genotypic data quality control and after genotypic quality control with MAF = 0.02 and HWE = 0.0001. After technical (SNP and individual call rate) and genetic (MAF and HWE) quality control, 137 789 SNPs and 124 178 SNPs remained in each dataset, respectively, with 139 animals remaining in both datasets. PLINK as well as EMMAX software was used to perform the GWAS and a 5% confidence interval was applied. SNPs at a threshold of $p<10^{-5}$ were identified for BF (BTA1, BTA16, BTA25); EMA (BTA2, BTA7, BTA8, BTA9, BTA13, BTA20, BTA25) and RF (BTA5, BTA9, BTA16) at 72 days on trial. Similar chromosomes were detected with putative SNPs for BF, EMA and RF at 91 days on trial. Additional SNPs were observed for BF on BTA2, BTA3, BTA5, BTA28 and X-chromosome and EMA on BTA12, BTA23, BTA29. Furthermore, SNPs with a threshold of $p<10^{-}5$ were identified for SW (BTA4, BTA9, BTA19) and DP (BTA16, X-chromosome). Of the 14 genes associated with the traits, *NIPA1, SYNE1, NT5C3B, SMYD3* were the most applicable to the traits studied and involved in binding function. This study is the first GWAS in SA Sanga cattle on carcass traits and provides insight on the genes involved in carcass traits. Novel SNPs were observed with associations for BF on BTA3, BTA5, BTA25, BTA28 and X-chromosome; SW on BTA9; EMA on BTA25; RF on BTA1, BTA16 and for DP on the X-chromosome. Further studies on larger datasets will be required for confirmation.

Table of Contents

List of Abbreviations

BF	Backfat thickness
BF72	Backfat thickness measured at 72 days on test
BF91	Backfat thickness measured at 91 days on test
BGP	Bovine genomics programme
BTA	Bos Taurus genome
BP	Base pairs
CT	X-Ray based Computer Tomography
CFAP54	Cilia and Flagella associated protein 54
DAFF	Department of Agriculture Forestry and Fisheries
DCAF15	DDB1 and CUL4 associated factor15
DDR2	Discoidin Domain Receptor Tyrosine Kinase 2
DMI	Dry matter intake
DNA	Deoxyribonucleic Acids
DP	Dressing percentage
DXA	Dual energy X-ray absorptiometry studies
EBV	Estimated Breeding Value
EMA	Eye muscle area
EMA72	Eye muscle area measured at 72 days on test
EMA91	Eye muscle area measured at 91 days on test
FAO	Food and Agriculture Organisation of the United Nations
g	Grams
GB	Giga base pair
GDP	Gross Domestic Product
GS	Genomic selection
GWAS	Genome-wide association study
HDAC9	Histone Deacetylase 9
IMF	Intramuscular fat
kg	Kilogram
MAS	Marker assisted selection
mm	Millimetres
mRNA	messenger Ribonucleic Acid

MRI	Magnetic Resonance Imaging
n	Number
NIPA1	Magnesium Transporter 1
NRXN3	Neurexin 3
NT3C3B	5'-nucleotidase, cytosolic IIIB
P2RY11	Purinergic receptor 11 P2Y11
PPEF1	Protein phosphatase with EF-hand domain 1
PCR	Polymerase Chain Reaction
QC	Quality Control
QTL	Quantitative trait loci
RF	Rump fat thickness
RF72	Rump fat thickness measured at 72 days on test
RF91	Rump fat thickness measured at 91 days on test
RFI	Residual Feed Intake
RS1	Retinoschisin 1
RTU	Real time Ultrasound
SA	South Africa
SAS	Statistical Analysis Software
SMYD3	SET and MYND domain containing 3
SNP	Single Nucleotide Polymorphism
SW	Slaughter Weight
SYNE1	Spectrin Repeat containing nuclear envelope protein 1
TIA	Technology Innovations Agency
WCM	Warm carcass weight
WGS	Whole genome sequencing

List of tables

List of figures

Chapter 1 Introduction and Literature review

1.1 Introduction

The South African (SA) human population has been growing at an average rate of 1.5 % per year over the past ten years and has a current population size of approximately 56.5 million (Department of Agriculture, Forestry and Fisheries, 2017). The expected population by 2040 is 64.4 million (StatsSA, 2018). The demand for sufficient animal protein especially beef, is increasing and it has been reported that global livestock production will have to double by 2050 in order to satisfy the demands for animal products (Ilea, 2009; Garnett *et al.* 2013; Webb & Erasmus, 2013; Dawkins, 2017). Livestock production is a major contributor to food security in South Africa (Meissner *et al.*, 2013) and contributed R127 288 million to the Gross Domestic Product in SA during 2016 – 2017 (van Marle-Köster & Visser, 2018). Developing countries such as SA are envisaged to contribute significantly to the increase in animal production in Africa (Webb & Erasmus, 2013).

There are 13.0 million head of cattle in SA with beef cattle encompassing 80% of the population (ARC Annual report, 2016; DAFF, 2017). It is estimated that 3 678 000 cattle are slaughtered per year in SA with a per capita beef consumption of 19.2 kg/year (ARC Annual report, 2016; DAFF, 2017). Livestock and game utilize 70% of agricultural land in SA and intensification of agricultural production systems will be necessary to supply in the growing demand for animal protein (Meissner *et al*, 2013). Beef cattle are mostly raised on natural pastures (extensive production systems), thereafter 70% of weaner calves are finished in feedlots (Scholtz *et al.*, 2008; Webb & Erasmus, 2013). Weaners are fattened in the feedlots before slaughtering at target weights of between 400 to 450kg. There are several benefits for finishing weaner calves in the feedlot such as decreased stocking rates on natural grazing and the cattle obtain the desired carcass weight when finished at the feedlot (Webb & Erasmus, 2013).

In South Africa there are approximately 30 registered cattle breeds that include *Bos indicus* (Zebu), Sanga and *Bos taurus* (European) types. Historically Nguni cattle were used as a multi-purpose breed with the production of meat, milk, skin and the hide (Musemwa *et al*., 2008). The occurrence of Nguni cattle in South Africa for farming purposes dates back 2000 years (Schoeman, 1989). The Nguni breed is classified as Sanga cattle, which originated from the Bos *indicus (Zebu)* and *taurine* Longhorn cattle (Bos *taurus* cattle) (Rege, 1999; Scholtz *et al*., 2011). Nguni cattle are well adapted to the sub-tropical environment in SA and farmed in both the commercial and developing sector (small holder) in SA (van Marle-Köster & Visser, 2018). Bos *indicus* cattle tend to have lower beef tenderness, higher age at slaughter and lower fat in the carcass (de Oliveira Silva *et al.*, 2017). In contrast the Sanga cattle have good meat quality which is comparable to Bos *taurus* cattle breeds (Frylinck *et al.,*2009). However, in comparison with Bos *taurus* cattle the Sanga types

have less desirable growth, performance and carcass characteristics due to a small frame size (Wheeler *et al*., 2001; Frylinck *et al.*, 2009).

In the South African feedlots 67% of cattle are British, European or crossbred types (Soji *et al.*, 2015). More than 14 cattle breeds are found in the South African feedlot with Bonsmara (16%) and Hereford (12%) cattle having a higher prevalence compared to Sanga breeds such as Nguni and non-descript cattle. The Nguni and non-descript cattle only comprise 5% of feedlot cattle. This is due to the Nguni cattle having lower feed efficiency compared to exotic breeds as well as low weaning weight and slow post-weaning growth (Strydom, 2008).

Beef is classified as a red meat, which is required as part of a balanced diet. Health problems such as obesity has been associated with a high intake of saturated fats from animal products (Hall *et al.*, 2015; Mann, 2018). Healthy, good quality meat is required by consumers, especially as people are more conscious about the effect of diet on health (Vermeulen *et al.,* 2015; Soji et al., 2015). The amount of fat in meat is decreasing with more fat trimming at abattoirs or leaner carcasses at slaughter (Hall *et al.,* 2015). The cut of meat together with the meat quality is an important price determinant. Different cuts of meat in the same carcass differs more in the fat content compared with fat content in beef carcasses of different ages and fatness level (Schönfeldt & Gibson, 2008). Fats are often perceived as unhealthy causing consumers to prefer leaner meat (Wood *et al*., 2008; Soji *et al*., 2015).

There are intrinsic and extrinsic factors that influence carcass quality such as tenderness, nutrition, age of the animal, sex, breed of animal, fat content: intramuscular fat (IMF), sub-cutaneous fat (SCF), firmness and colour of the meat as well as nutritional content of the meat (Schönfeldt & Strydom, 2011; Bureš & Bartoň, 2012; Caetano *et al*., 2013). Older animals typically have a higher fat content, which means there is a lower proportion of meat that is of a high quality to be marketed at a high price. Fat measurements can also indicate the tenderness, juiciness and flavour of the meat (Bureš & Bartoň, 2012; Ribeiro & Tedeschi, 2012; Caetano *et al*., 2013). The tenderness of a cut of meat can be measured by tasting panels or the Warner-Bratzler shearforce. It is important to measure the trait as it influences the eating quality of the meat, which in turn influences the price that the consumer is willing to pay for the meat. This influences the economic returns that can be achieved by the farmer (Kause *et al*., 2015).

Carcass classification systems are used throughout the world as a tool to classify the quality of the meat based on different factors (Soji *et al.*, 2015; Strydom, 2016). The SA carcass grading has been developed in 1932 (Strydom, 2016), based on seven classes of fatness, measured in millimetres and four age classes based on the number of incisors present. Detail of the grading system has been attached as Addendum A. Age and fatness are the primary criteria used in the system (Agricultural Products Standard Act, 1990; SAMIC, 2006; Strydom, 2011; Soji *et al.*, 2015; Strydom, 2016).

The evaluation of the live animal during the feedlot phase is necessary in determining the carcass quality of the animal at slaughter. Real-time ultrasound scans are a non-invasive method of evaluating the body composition of beef cattle, in particular the fat and lean yields, as well as fat to lean ratio can be visualised (Polák *et al.*, *et al.* 2007; Gupta *et al*., 2013). These RTU measurements are relatively easy to incorporate in the feedlot practices and is a useful tool to evaluate and predict the carcass quality of the animal (Crews & Carstens, 2012; Kause *et al*., 2015). In SA there has been limited use of RTU measurements for prediction of carcass traits and has not been studied on a genomic level in Sanga breeds.

Genomics provided the opportunity for investigating traits of economic importance on a genomic level. The first quantitative trait loci (QTL) studies in beef cattle was limited to microsatellite markers (Pollak, 2005). An example of QTL discovered with the use of a microsatellite panel of 213 markers is the myostatin gene. Muscle hypertrophy (myostatin) was identified as locus *mh* on chromosome two (Charlier *et al.,* 1995).

The bovine genome was completed in 2009 with a size of 2.9 Gb (Fan *et al*., 2010), which provided the opportunity for SNP discovery (Matukumalli *et al.*, 2009). Development of high-density SNP arrays enabled genome wide scans. Genome wide association studies (GWAS) have been shown to be useful for identification of chromosomal fragments which are linked to the traits of economic importance (Matukumalli *et al.*, 2009). GWAS utilizing SNP markers enable higher precision in detecting QTL and identification of chromosomal regions associated with a trait of interest (Meuwissen & Goddard, 2000, Hill, 2014). Breeding programs for polygenic traits can incorporate whole genome marker data, as the dense SNP arrays are becoming more affordable and available (Meuwissen *et al*., 2011). This holds the potential for increased genetic progress in carcass traits in beef cattle (Schaeffer *et al.*, 2006; van Eenenaam, 2006; van Eenenaam & Drake, 2012; Hill, 2014). The genetic progress in successive generations of animals would increase causing the breeder goals to be reached quicker. GWAS can be applied for the identification of QTL and genes responsible for traits.

Aim of the study

This project was funded by Technology Innovations Agency (TIA) through the bovine genomics programme (BGP). The aim of this study was to perform a genome-wide association study for carcass traits in South African indigenous Nguni cattle based on real time ultrasound data. A growth trial with 200 Nguni bulls were conducted at a private commercial feedlot and RTU measurements were recorded. Available funding made provision for genotyping of 141 cattle using a 150 K SNP array

The objectives to reach the aim for this study were as follows:

1. Estimate descriptive genomic statistics for the animals with available genotypes
2. To perform a GWAS to determine genetic variants significantly associated with RTU traits and growth traits.
3. Gene annotation for the biological processes and molecular functions of genes

1.2 Literature review

1.2.1 Introduction

The genetic improvement of carcass traits poses certain challenges to the beef farmer, as these traits are difficult and costly to measure (Hocquette *et al.*, 2007). Carcass traits such as fat distribution, tenderness and yield are however important in determining the price and eating quality of meat (Bureš & Bartoň, 2012). RTU scans are a non-invasive method available for measuring the traits in live animals (Gupta *et al.*, 2013; Ribeiro *et al.,* 2014). This is a relatively affordable measurement which can be implemented by stud breeders or in the feedlot (MacNeil & Northcutt, 2008; Seroba *et al.*, 2011) and can be used for prediction of beef carcass traits.

Genomic technology holds potential to study carcass traitson a genome level. GWAS studies provide the opportunity to identify genomic regions involved with traits of economic importance and diseases (Matukumalli *et al.*, 2009). The aim of this section was to review relevant literature regarding genetic improvement of carcass traits in beef cattle and the use of genomic tools for genetic improvement with special reference to genome wide association studies (GWAS).

1.2.2 Selection for growth and carcass traits

Growth traits are relatively easy to measure, making it easy to select for improved growth in the animal. Growth traits such as birth weight, weaning weight, shoulder height, mature weight and carcass weight (Arnold *et al.*, 1991; Seabury *et al*., 2017) all have a moderate to high heritability, and selection for these traits should result in genetic progress. The heritability values of the growth traits were summarised in Table 1.1.

Table 1.1 Summary of heritability estimates for growth traits in beef cattle from literature

Trait	Heritability	Reference
Birth weight		
	0.35	Koots *et al.* (1994)
	0.42	Saatchi *et al*., (2011); Weng *et al.* (2016)
	0.53	Lopes *et al.* (2016)
	0.27	de Oliveira *et al.* (2018)
Weaning weight		
	0.27	Koots *et al.* (1994)
	0.10	van Marle-Köster *et al*. (2000)
Mature weight		
	0.5	Koots *et al.* (1994)
	0.55	Saatchi *et al*., (2011)
Slaughter weight		
	0.45	Koots *et al.* (1994)
	0.24	Arnold *et al.* (1991)
	0.4	Saatchi *et al*., (2011)
	0.38 to 0.51	Kause *et al.* (2015)

The ages at which cattle mature vary within breeds and this rate of maturity within an animal is determined by the pattern of organ development and order of maturation of the organs. The muscle and bone development in the body will increase to adult age and then reach a plateau, followed by an increased rate of gain of fat (Berg & Butterfield, 1968). Muscle and bone formation are the first phases of growth and at birth the bone will be at a higher proportion compared to later stages of growth (Thonney, 2014). Thereafter muscle has a higher growth rate than bone and during the fattening phase the proportion of muscle decreases and fat is deposited at a higher growth impetus than muscle (Guenther *et al.*, 1965; Owens *et al.*, 1995). The degree of maturity is closely linked to the ratios of muscle, bone and fat and this is important for the value of the carcass. Larger type of cattle will also mature at a later stage (Thonney, 2014).

Angus and Hereford cattle are British breeds typically characterised as late maturing animals. These animals have a good growth potential and desirable carcass traits (Frylinck *et al.,* 2009). In comparison with the British breeds, the Sanga cattle are early maturing and have a medium frame size. Indigenous breeds such as the Sanga breeds have lower feed requirements, are adapted to the harsh South African climate and have good meat quality characteristics (Bonsma, 1980; Scholtz, 1988; Strydom, 2008). Bonsmara cattle are a composite breed consisting of 5/8 Afrikaner and 3/8

Hereford / Shorthorn breeds developed in South Africa (Bonsma, 1980; Makina *et al.,* 2016). Bonsmara cattle is a breed that is used commonly in feedlots as this breed is late maturing with a large frame size. This breed has good growth and is profitable in the feedlot (Esterhuizen *et al.*, 2008). The medium sized Drakensberger cattle are typically characterized with good performance and adaptation for many different grazing conditions (Bisschoff & Lotriet, 2013). These cattle are early maturing and there will be more fat deposition at an earlier stage during the feedlot period compared to Bonsmara cattle. The Tuli cattle are early maturing type cattle with similar body composition and performance as Drakensberger cattle.

Nguni cattle typically reach puberty at a significantly earlier age as well as lower weight compared to Bonsmara and Drakenberger cattle (Schoeman, 1989). The growth rate of the Nguni is slower in comparison to the Bonsmara and Drakensberger cattle, as breeds with larger frame size typically have a higher growth rate. Table 1.2 summarises characteristics of beef cattle breeds found in SA.

Table 1.2 A summary of early and late maturing beef cattle breeds in South Africa (SA Studbook annual report, 2016)

Breed	Maturity	Frame size	Shoulder height (mm)	Final weight (kg)
Nguni	Early	Medium	1124	268.4
Tuli	Early	Medium	1164	342.6
Afrikaner	Late	Large	1188 -1213	328 -346.9
Drakensberger	Early	Medium	1161 -1176	307.9 -329.8
Bonsmara	Late	Large	1172	410 -418.4
Hereford	Late	Medium	1218	401.3
Beefmaster	Late	Large	1192 -1202	438.7 -456.5
Angus	Early	Medium	1222 -1236	413 -442.9
Boran	Early	Medium	1099 -1167	307 -322.7

A larger frame size in cattle is linked to a leaner carcass due to a longer growth period for bone and muscle growth (Owens *et al.*, 1995). After growth, fat is deposited at a much later stage in large framed cattle when compared to smaller framed cattle (Berg & Butterfield, 1968; Owens *et al.*, 1995). Early maturing cattle have a higher maintenance requirement while consuming the same amount of feed compared to late maturing cattle (Thonney, 2014).

Later maturing animals tend to have a lower feed intake together with improved carcass conformation and a higher growth rate (Batt, 1980; Hosner, 2005; Moloney & McGee, 2017). Evidence has shown that through selection for increased cow size there can be changes in the carcass conformation, rate of growth and efficiency of feed intake between the different breeds (Thonney, 2014). Maturity types influence the distribution of the body fat between the animals. Therefore, the slaughter end points differ between maturity types. Moloney & McGee (2017) compared slaughter weight and body composition of different maturity types when slaughtered at the same chronological age, as well as slaughtered at the same fat level. Late maturing animals were leaner when the animals were slaughtered at the same age, in contrast to being heavier when slaughtered at the same fat level. This is due to the later maturing animals having fewer fat deposits when slaughtered at the same age (Batt, 1980; Hosner, 2005).

The need for non-invasive methods to study body composition dates back to as early as 1936 and 1938 with the development of X-radiography by Kronacher & Hogreve (Scholz *et al.*, 2015). It was tested for the use in determination of the pelvis shape and adipose tissue deposition in pigs. This was followed by the testing of a specific velocity of ultrasound in different body tissues in 1956 (Temple *et al.,* 1956), followed by the development of X-ray based computer tomography (CT) in 1981 and nuclear magnetic resonance imaging (MRI) in 1983. Finally, dual energy X-ray absorptiometry studies (DXA) in 1996 for evaluation of body composition was developed. After these advances, there have been constant progress in this technology for the determination of body composition.

Realtime ultrasound scans are a non-invasive method of evaluating the body composition of beef cattle (Lazzaroni, 2007; Polák *et al.,* 2007; Drennan *et al.*, 2009; Gupta *et al*., 2013). It measures the fat ratio, lean ratio and the ratio between fat and lean tissue in cattle. There are several ultrasound recording devices available, but special transducers are mostly used (Lazzaroni, 2007; Polák *et al.,* 2007; Gupta *et al*., 2013). Size of the animal such as a larger sized animal (cattle) compared to a smaller sized animal (sheep) is not a problem when using RTU for measurements. One advantage of the use of RTU scans is there is no size limit for the equipment as with other methods. Transducers are portable devices and this method causes no radiation making it safer. Measurements are in real time and this method is less expensive than other methods (Lazzaroni, 2007; Polák *et al.,* 2007; Crews & Carstens, 2012; Gupta *et al*., 2013).

There are only a few disadvantages of using this method such as the images not being able to display the whole-body conformation of the animal. Image analysis of this kind will not easily be automated, and the anatomical resolution may be less accurate when compared to CT/MIRS (Kallweit, 1992). Table 1.3 summarises the points of measurement when using ultrasound scans for the prediction of fat depth in cattle.

Table 1.3 Description of RTU measurements on different areas of the body

RTU measurement	Description	Reference
Backfat thickness (BF)	Measured as the subcutaneous fat layer over the l*ongissimus dorsi* muscle between the 12th and 13th rib.	Greiner *et al.* (2003); Caetano *et al*. (2013); Gupta *et al*. (2013)
Rump fat thickness (RF)	Measured at the junction of the *biceps femoris* and *gluteus medium* between the hook and pin bones. This is more commonly known as subcutaneous fat depth at the P8 site.	Caetano *et al*. (2013) Gupta *et al*. (2013)
Eye muscle area (EMA)	Measured, at the *longissimus thoracicus et lumborum* area. Lean to fat ratio is predicted by the eye muscle area.	Reverter *et al.* (2003) Caetano *et al*. (2013) Gupta *et al*. (2013)
Marbling	The percentage intra-muscular fat which is a contributor to the meat quality especially tenderness, palatability and juiciness.	Lazzaroni (2007) Gupta *et al*. (2013)

Backfat thickness is an important measurement when used with other live measurements in predicting the carcass finish and the trims obtained from the carcass (Reverter *et al.*, 2000; Realini *et al*., 2001). Rump fat measurements have a higher repeatability, accuracy and can be used to improve the accuracy of the total external fat cover. The eye muscle area is a valuable measurement in prediction of meat yield and amount of external fat.

Points of measurement are shown in Figure 1.1, where 1 is the percentage intramuscular fat (marbling), 2 is the eye muscle area (EMA) and backfat thickness (BF) and 3 is the rump fat thickness (Hicks, 2011). The eye muscle are is an indication of the amount of fat in the animal. Backfat thickness and rump fat thickness are subcutaneous fat, which is found as the layer between the muscle and skin. Intramuscular fat is the fat between muscle cells and is the last fat depot in animals (Batt, 1980; Hosner, 2005; Moloney & McGee, 2017).

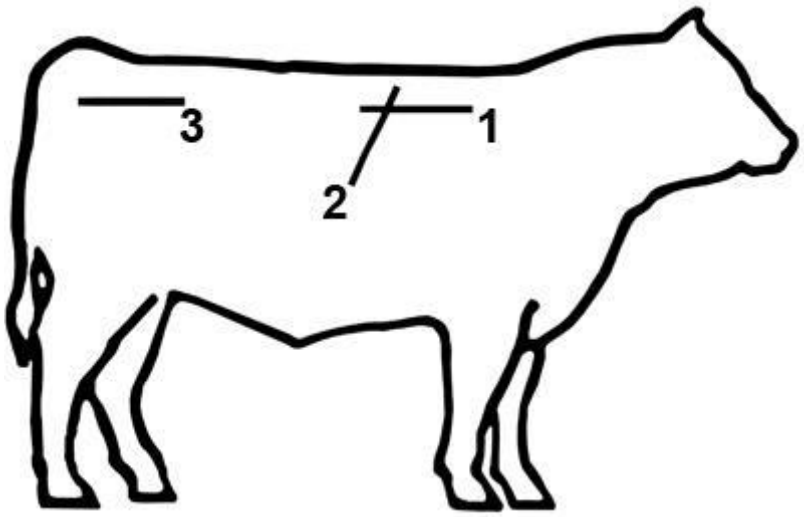


Figure 1.1 Graphic presentation of where the RTU scans are taken for the different measurements (Hicks, 2011).

Video image analysis is a recent technology assessing the body composition of the animal post mortem. It is used in the grading and classification of animals with the EUROP system. It uses a video camera to take images of a carcass, which is then converted into an electrical map (Beriain *et al.*, 2007; Menesatti *et al.*, 2007; Gupta *et al*., 2013). The principle of differences in colours, volume, curvature and angles are used to distinguish between fat and lean tissue. This is an adequate method that is accurate and can be used rapidly by graders, but is a more expensive method (Lazzaroni, 2007; Beriain *et al.*, 2007; Menesatti *et al.*, 2007; Gupta *et al*., 2013). Figure 1.2 shows an image generated with RTU scanning on the rump fat area to measure rump fat thickness.

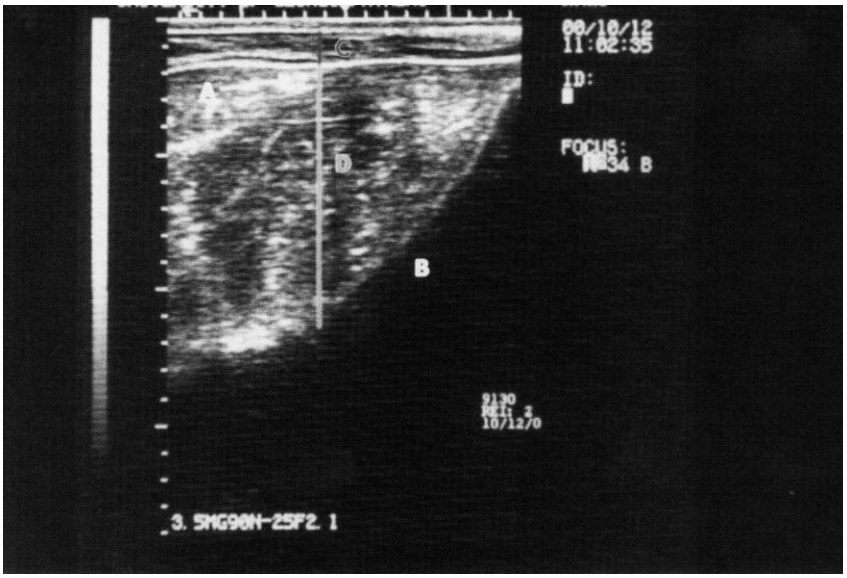


Figure 1.2 Ultrasound image of the rump fat thickness (Realini *et al*., 2001)

The heritability of ultrasound scans as a measurement of the fat has a low to moderate heritability. This indicates selection for these traits will show some genetic progress and there is potential in selecting for these traits.

Table 1.4 Heritability estimates for carcass traits in beef cattle from literature

Trait	Heritability	Reference
Backfat		
	0.49	Arnold *et al.* (1991)
	0.39	Weng *et al.* (2016)
	0.31	Miar *et al*. (2014); Bolormaa *et al.* (2014)
	0.07	Lopes *et al.* (2017)
	0.29	Su *et al*. (2017); de Oliveira *et al*. (2018)
Eye muscle area		
	0.46	Arnold *et al.* (1991)
	0.17	Miar *et al*. (2014)
	0.39	Weng *et al.* (2016)
	0.37	Lopes *et al.* (2017)
	0.31	Su *et al*. (2017)
	0.3	de Oliveira *et al*. (2018)
Marbling		
	0.35	Arnold *et al.* (1991)
	0.37	Miar *et al*. (2014)
	0.23	Weng *et al.* (2016)
	0.4	Bolormaa *et al.* (2014)
	0.45	Su *et al*. (2017)

The benefits of the use of RTU are plentiful and support the argument for using this as an indicator of the carcass quality and indirectly of the growth of the animal. These measurements are rapid with accurate and objective results (Polák *et al.,* 2007; Crews & Carstens, 2012; Gupta *et al*., 2013). There is no need for the expenses and time required to evaluate the carcass after slaughter, making it more cost effective. Genetic (r_g) and phenotypic (r_p) correlations between slaughter weight and EMA are moderate (0.3 and 0.45) as reported by Miar *et al*. (2014); Su *et al*. (2017). Slaughter weight and back fat have a moderate r_g (0.36 and 0.19) as reported by Arnold *et al.* (1991) and Su *et al*. (2017). EMA and back fat have low r_g (0.09 to 0.2) as reported in Devitt & Wilton (2001); Robinson & Oddy (2004); Seroba *et al.* (2011); Ceacero *et al.* (2016); Su *et al*. (2017). Correlations (r_g) for EMA and rump fat are low (0.11 to 0.18) (Robinson & Oddy, 2004; Ceacero *et al.*, 2016), while rump fat and back fat are highly correlated (r_g) (0.63 to 0.99) (Reverter *et al.* 2000; Robinson & Oddy 2004; Seroba *et al.*, 2011; Ceacero *et al*., 2016). Back fat has a moderate correlation (r_g)with slaughter weight (0.36) and weight traits are moderately correlated (r_g) (0.25) to EMA and back fat (Arnold *et al.,*1991). This indicates the usefulness of ultrasound scans as an indicator or predictor of weight traits and the slaughter weight.

There are more opportunities for RTU measurements for animals at central testing stations in comparison with on farm testing (SA Studbook annual report, 2016). Similarly, there is more

measurements in Hereford, Drakensberger and Boran cattle breeds. There are no records of RTU measurements for Nguni cattle at central testing stations or on farm (SA Studbook annual report, 2016). In SA RTU is not a routine measurement for most of the cattle breeds on farm or in the feedlots (van Marle-Köster & Visser, 2018). There is a need for the recording of this phenotype for the use in selection indices or genomic selection. RTU measurements in SA Sanga cattle are even less compared to composite breeds (Bonsmara) or British breeds (Herefords), despite these breeds comprising a large part of the SA beef cattle population (Nguni: 11.3%, Boran:10.9%, Drakensberger: 3.9%, Tuli: 2.9%) (van Marle-Köster & Visser, 2018). These cattle are adapted to the tropical environment is SA and recordings of these breeds are important.

1.2.3 Tools for genetic improvement

A brief history on development of markers and molecular genetics

Historically, selection was based on the phenotype of the animal which was mostly qualitative traits due to no recordings done (Dekkers & Hospital, 2002; van Marle-Köster *et al.*, 2013). The development of quantitative theory provided the concept of heritability in 1937 by J. Lush, correlations in 1943 by L.N. Hazel and later (1947) selection indices (Hill, 2014), which enabled genetic improvement in these traits (Walsh, 2000). However, most traits of economic importance are influenced by many genes with small effects (additive traits) and is influenced by the environment resulting in the complex nature of the traits (Dekkers & Hospital, 2002). Quantitative genetics has certain limitations such as in cases of sex-limited traits and traits that are expressed later in life. Quantitative genetics has limitations in resolving negative correlations between genes due to linkage or epistasis. Furthermore, not all traits have a high heritability, especially traits of economic importance which usually has a low to moderate heritability (Dekkers & Hospital, 2002).

Developments in molecular genetic technology resulted in studies focussed on the genomes of various species. In 1983 the polymerase chain reaction (PCR) was developed which allowed reliable and accurate amplification of small segments of DNA and most importantly specific regions on the genome of livestock species (Mullis *et al.,* 1986; Fore *et al.*, 2006). In 1989 Hypervariable regions in the human DNA was discovered which led to further discovery of markers such as Variable Number Tandem Repeats (VNTRs), Randomly Amplified Polymorphic DNA (RAPD), Amplified Fragment Length Polymorphisms (AFLPs) and Restricted Fragment Length Polymorphisms (RFLPs) (Tautz, 1989; Beuzen *et al.*, 2000)

Of the first DNA markers, microsatellites are short tandem nucleotide sequences (1-6bp), usually di, tri or tetra nucleotide repeats on the DNA sequence which can occur up to 60 times in a genome (McClure *et al*., 2013). Genetic variance was originally detected using the markers for identification of causal mutations. It also contributed to understanding the genetics of the traits under

investigation using the process of linkage analysis and contributed to the development of linkage maps (van Arendonk *et al.*, 1999; van Marle-Köster *et al.*, 2013; Berry *et al.*, 2017). Microsatellite markers were widely applied in the identification of genetic defects (bovine leukocyte adhesion deficiency (BLAD)*,* major genes (*CAST* and *CAPN1* genes for meat tenderness) and parentage verification (Williams *et al*., 1997; Cavanagh *et al.*, 2007; van Marle-Köster *et al.*, 2013). Microsatellites occur at a low frequency which limits the potential of the marker in detecting all possible variants influencing a trait of interest.

Early mapping (1990's) of quantitative trait loci (QTL) were based on linkage mapping of microsatellite markers (Lipkin *et al.*, 1988; Picard *et al.*, 2015). Two methods for the identification of QTL was used namely the genotyping of a large number of markers spread out across the whole genome together with the phenotype of the animal. The genetic and phenotypic information were then combined using statistical methods to predict the regions on chromosomes which were most likely involved with the trait. The second method for determining the QTL was the candidate gene approach based on either association or resequencing approaches (Hirschhorn & Daly, 2005). After identification of QTL further fine mapping is done with the main aim in finding genes influencing the trait (Dekkers & Hospital, 2002).

Potential QTLs have been identified in a number of studies for various traits. QTLs for birthweight have been reported on BTA1 (Stone *et al.*, 1999; Casas *et al.,* 2003), BTA2 (Casas *et al.,* 2003), BTA3 (Casas *et al.,* 2003), BTA5 (Casas *et al.,* 2003); BTA20 (Casas *et al.*, 2004; Kim *et al.*, 2003) and BTA21 (Casas *et al.*, 2004). Identification of potential QTL for EMA has been reported by Stone *et al*. (1999) (BTA14) and Casas *et al.* (2004) (BTA5 and BTA6). Fat distribution have QTL identified on BTA2, BTA3, BTA7 and BTA14 (Casas *et al.*, 2003) and BTA1 and BTA17 have suggestive QTL reported by Casas *et al.* (2004). An important factor to consider when using a single QTL, is that it often explains only a small portion of variation for a trait, limiting the potential impact on the genetic improvement for the trait (Meuwissen *et al.*, 2016). The difficulty in identifying QTL for lowly heritable, complex traits is another disadvantage of QTL (Pausch *et* al., 2012). Further limitations for QTL mapping using microsatellite markers is the family-based nature of the markers, as well as QTLs identified due to haplotype effects. The haplotype effects are due to several linked polymorphisms and higher LD within families (Andersson, 2013).

The human genome was the first genome mapping project which started in 1989 and was completed in 2003 (www.ornl.gov.hgmis). This was constructed using whole genome shotgun and BAC-to-BAC sequencing (Adams, 2008; Andersson, 2009). Mapping of the human genome was important to enable the mapping of genomes of other species (Eggen, 2012; van Marle-Köster *et al*., 2013). A partially inbred Hereford cow was used in the construction of the bovine genome map that was completed in 2009 (The Bovine Genome Sequencing and Analysis Consortium, 2009; Fan *et al*. 2010).

After completion of the bovine genome sequence, more than 2.3 million putative SNPs were identified (Williams *et al.,* 2009*)*. Single nucleotide polymorphisms (SNP) are defined as a difference at the same position on a genome between individuals or between individual chromosome pairs (Hayes & Goddard, 2010). SNP markers are typically bi-allelic and easy to interpret (Fan *et al.,* 2010). The SNP markers are spread out widely across the genome of animals and this is advantageous in identifying traits which may be influenced by many genes (Fan *et al.,* 2010). Validation of the identified SNPs were necessary especially for the development of a high-density SNP array, which could be used for genome wide association studies in cattle. Matukumalli *et al.* (2009) reported on the SNPs selected for the development of the 54 000 SNP array. The SNP haplotype alleles are determined by examining segregation patterns and establishing the locus position on the bovine linkage map. Thereafter the positions of loci are compared to the position in human ortholog sequences (Grosse *et al.,*1999). Selection of the SNPs for an assay requires the allele frequency of the SNPs, as well as a large number of evenly spaced validated SNPs (Matukumalli *et al.*, 2009).

Table 1.5 shows the development of SNP array in different species and includes the density of the SNPs spread throughout the genome. From Table 1.5 it can be seen the chicken genome was the first livestock genome to be developed in 2004 followed by the bovine genome in 2009. The bovine genome was identified to contain 2.2 million SNPs and the genome has a size of 2.91 GB.

Table 1.5 Summary of SNPs identified in different animal species (Fan *et al*., 2010)

Species	Year assembled	Number of SNPs identified	Frequency of SNPs	Genome size
Dog	2003	2.5 million	1 SNP/0.9kb (between breeds) 1 SNP/1.5kb (within breeds)	2.3 – 2.4GB
Chicken	2004	2.8 million	5 SNPs/kb	1.05GB
Bovine	2009	2.2 million	1 SNP/kb	2.91GB
Equine	2009	1.1 million	1 SNP/2kb	2.47GB

The development of SNP panels in cattle and other livestock species was an important milestone and low-cost panels are now readily available for the genotyping of livestock species using these DNA markers (Hayes & Goddard, 2010). These SNPs are used as markers in the animal's genome as a method of investigation of the animal's genetic make-up. SNP genotyping has been influenced by the emergence of the SNP panels and this has enabled theoretical and applied studies of quantitative genetics, population genetics and molecular evolution (Fan *et al.,* 2010). Table 1.6 summarizes the diversity of bovine SNP chips commercially available.

Table 1.6 Some of the commercial bovine SNP chips available

Platform	SNP chips	Size (SNPs)
Affymetrix®	Axiom® Genome wide BOS1	648 875
Geneseek®	GGP Bovine LD v4	30 125
	GGP HD	76 879
	GGP150K	139 480
	70k Indicus chip	74 000
Ilumina®	Bovine LD	7931
	Bovine SNP 50	53 714
	Bovine HD	777 962

(http://www.affymetrix.com/products_services/arrays/specific/axiom_gwas_bovine.affx; https://genomics.neogen.com/en/ggp-beef; https://www.illumina.com/products/all-products.html)

The majority of traits of economic importance are complex traits and the benefits of identifying most of the genes influencing the expression of the traits are multiple. Selection is improved when incorporating SNP marker technology and a much higher accuracy as well as faster genetic progress will be observed. Genomic selection (GS) is a method in which the entire genome of the animal is investigated and genomic regions that appear to be associated with a desirable trait are selected (Meuwissen *et al*., 2001). A larger improvement of the trait under selection will be observed as most of the genomic regions associated with the trait are identified and included for selection. Figure 1.3 illustrates the discovery of DNA markers for potential use in genome wide-association studies.

Figure 1.3 Timeline illustrating DNA marker discovery for potential application in GWAS

1.2.4 Genome-wide association studies

A genome-wide association study (GWAS) can be defined as a genotypic study that aims to distinguish common genetic variants which are associated with variation in a certain trait. This is achieved by genotyping the genetic variant in a population where the phenotypic information is available (Ali *et al.*, 2015). A correlation between the genotype and phenotype is then identified; this indicates an association between the variant and the trait (Hirschhorn & Daly, 2005; Bush & Moore, 2012). The main goal of GWAS is to gain insight into the relationship between a genotype with polymorphic sites that cause variation in complex traits. It can contribute to useful information in the selection potential for the traits in a population (Barendse, 2009; Bolormaa *et al*., 2014; Santiago *et al.* 2017; Xia *et al.*, 2017).

GWAS was first used in human genetics followed by the mouse, model organisms such as the *Arabidopsis* and finally in cattle (Korte & Farlow, 2013). This is enabled by the use of high-density chip-based micro-array technology for assaying more than one million SNPs in a genome (Bush & Moore, 2012; Gurgul *et al*., 2014; Berry *et al.*, 2017). A major benefit of GWAS is the understanding on the underlying genetics of the trait and when used in conjunction with QTL mapping it holds potential for improved interpretation (Korte & Farlow, 2013). One of the advantages of a GWAS is that it considers a large number of genes that influences a single trait, potentially explaining a large proportion of the variation in the expression of the trait (Plastow, 2016). This is in contrast to the use of marker assisted selection (MAS), which only considers the effect of one or a few genes influencing a complex trait (Hayes & Goddard, 2010).

A number of statistical methods are available to exploit the associations between markers and causative mutations. The simplest form of a GWAS is a marker-by marker approach where a single marker regression is used (Hayes *et al.* 2013). A linear model (which is often additive) is used, with the marker having a fixed effect. In the case of two alleles identified, the second allele will have double the effect compared to only one copy and no copies have zero effect (Hayes *et al.* 2013). The regression coefficient is a statistical parameter predicting the amount of linkage disequilibrium (LD) between markers (Hill & Robertson, 1968). GWAS is based on the assumption that causative mutations for a trait and SNPs are in LD causing significant associations to emerge (Hayes & Goddard, 2010). Certain factors should be accounted for such as the choice of significance level, confidence intervals and population parameters. A 5% significance level and 95% confidence interval are generally used with corrections made when designing the study. Population structure needs to be accounted for to avoid inflated associations or false positives.

Another method is a GWAS using haplotypes rather than individual SNP markers. This method tests the association of haplotype windows across a genome with the phenotype. In GWAS, fitting all markers can be investigated simultaneously, similar to the model suggested for genomic prediction (Meuwissen *et al*., 2001) and in this model the SNPs are fit as random effects (Hayes *et*

al. 2013). Table 1.7 summarises the primary methods of GWAS analysis and some software that can be used to perform the GWAS.

Table 1.7 Summary of the primary methods for GWAS (Hayes, 2013)

Method	Software/ Approach	Description	Advantages	Disadvantages
Single locus analysis/ SNP by SNP basis: (Allelic and genotypic association test)	PLINK Linear model ASReml Single SNP Regression using WOMBAT EMMAX EMMA R packages (GenABLE and qq-man) GCTA.	Association between one allele of a SNP and the phenotype is investigated. OR The association between the genotypes and phenotype is investigated.	Straightforward and easy to conduct the analysis. Works for small datasets.	Statistical approaches differ between quantitative and case / control traits.
Multi locus analysis	Bayesian approach (BayesA) WOMBAT GCTA 2-Step Bayesian approach (BayesB+BayesC) Bayesian mixture model (BayesR) GCTA.	All SNPs are fit simultaneously.	No need for multiple testing. Potential to improve the mapping precision. Performs unbiased analysis for interactions within a selected set of SNPs. Investigate the interactions among the genetic variants throughout the genome.	There are computational, statistical and logistical challenges involved with this method. Need for large amount of memory for calculations and the computational time is very long.

The number of SNPs detected through a GWAS is dependent on the sample size, as well as the density of the SNP array. A larger sample size and higher density SNP array would increase the amount of SNPs identified by a GWAS (Visscher *et al.*, 2012; Hill, 2014). SNPs identified through a GWAS usually accounts for only a small percentage of the variance in a trait that limits the wider application (Yang *et al.,* 2011; Hill, 2014). Rare alleles are difficult to identify by a GWAS and this causes utilization of the mutations to be quite ineffective (Hill, 2014). A GWAS does not typically account for epistatic variance which is a possible explanation of why the GWAS does not identify a portion of the genetic variance for a trait (Zuk *et al.*, 2012).

The results from a GWAS are often plotted on a Manhattan plot, therewith it can be analysed and visualised. Significance level for associations are shown on the Manhattan plot as a Bonferroni corrected significance levels, in the case of levels >0.05 trait is significantly associated SNPs. Figure 1.4 depicts an example of a Manhattan plot for the eye muscle area. From the plot it can clearly be seen that there is significant association on chromosome 10 (Santiago *et al.*, 2017).

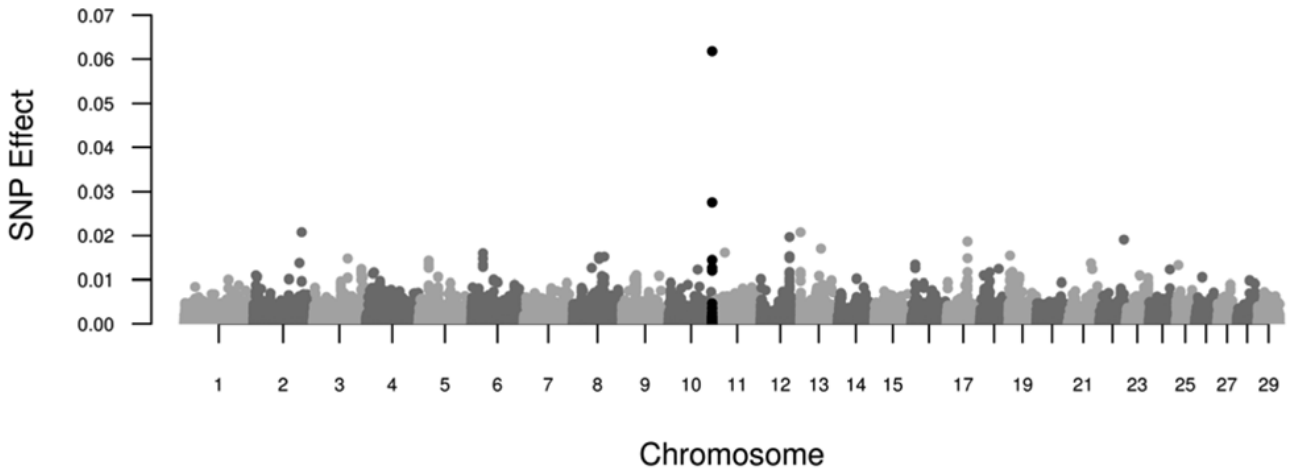


Figure 1.4 A Manhattan plot for EMA from a study by Santiago *et al.* (2017)

Application of GWAS in beef cattle

Application of GWAS in beef cattle has been extensive for growth, carcass and meat quality traits (Hayes & Goddard, 2010; Berry *et al*., 2017). Table 1.8 summarises the number of SNPs identified for traits of economic importance using a GWAS to identify significant associations. A large number of SNPs have been identified on different chromosomes for growth and carcass traits in beef cattle. There has however been limited research in GWAS applied to RTU scans in beef cattle. De Oliveira-Silva observed SNPs associated with RTU measurements in Nellore cattle using a GWAS. There is much potential in using GWAS for the identification of carcass traits and RTU measurements as can be seen from Table 1.8.

Table 1.8 Summary of traits of economic importance reported in literature on the chromosomes identified by GWAS

Trait	Chromosome (BTA)	Reference
Calving Ease	14, 21	Pausch *et al.* (2012)
Birth weight	14	Sharma *et al.* (2015)
ADG (ADG)	1, 2, 3, 4, 5, 6, 7, 8, 9, 11, 13, 14, 15,16, 17, 18, 19, 20, 27, 28	Nkrumah *et al.* (2007); Seabury *et al.* (2017)
Dry matter intake (DMI)	1, 2, 3, 6, 7, 8, 10, 11, 14, 15, 17, 18, 19, 20, 21, 26	Nkrumah *et al.* (2007); Seabury *et al.* (2017)
Residual feed intake (RFI)	1, 2, 3, 5, 6, 7, 8, 11, 12, 13, 14, 15, 16, 17, 19, 22, 26, 29	Nkrumah *et al.* (2007); Seabury *et al.* *(*2017)
Slaughter weight (SW)	1, 2, 4, 5, 6, 8, 14, 18, 23	Bolormaa *et al*. (2013); Lee *et al.* (2013); Li *et al.* (2017); Sorbolini *et al*. (2017); Bhuiyan *et al.* (2018)
Back fat thickness (BF)	1, 2, 6, 7, 9, 10, 11, 13, 14, 16, 17, 21, 22, 29	Kim *et al*. (2011); de Oliveira Silva *et al.* (2017); Hay & Roberts (2018)
Eye muscle area (EMA)	1, 4, 6, 7, 8, 12, 13, 14, 15, 16, 17, 18, 20, 21, 24, 28	Casas *et al.* (2005); Xia *et al.* (2016); Santiago *et al*. (2017); de Oliveira Silva *et al*. (2017); Bhuiyan *et al.* (2018); Hay & Roberts (2018)
Rump fat thickness (RF)	2, 5, 6, 8, 9, 13, 14, 15, 19, 20	Bolormaa *et al.* (2011); de Oliveira Silva *et al*. (2017)
Marbling	6, 17, 22, 29	Park *et al.* (2012)
Dressing percentage (DP)	2, 9	Sorbolini *et al.* (2017)

The carcass traits: SW, BF and EMA have been investigated in more studies compared to the rump fat thickness and dressing percentage. The study performed by de Oliveira Silva *et al.* (2017) measured the sub-cutaneous fat deposition with ultrasound measurements. Chromosome 14 has been identified in a number of studies as being associated with the SW of cattle. SW and EMA have been identified as being associated to the same chromosome regions (Bhuiyan *et al.*, 2018). SW and EMA are associated (0.45) as an increase in the slaughter weight caused by increased muscle yield will be observed by increase in the EMA (Miar *et al.,* 2014). The EMA can be used as an indicator of the carcass yield and carcass weight (de Oliveira Silva *eta al.*, 2017).

Genes identified for carcass traits

Once the QTL has been identified with GWAS it is useful to identify the gene encoded within that region of the QTL. Gene ontology explains the molecular and biological functions of the genes, which assists with understanding the physiological mechanisms involved with a trait. There are several databases available for identification of genes and gene ontology which is briefly summarised in Table 1.9.

Table 1.9 Databases for QTL comparison and gene ontology

Database	Uses	Access	Reference
NCBI	Different species genome maps Gene description and functions of genes Protein functions	http://www.ncbi.nlm.nih.gov/home/literature.shtml http://www.ncbi.nlm.nih.gov/home/genes.shtml http://www.ncbi.nlm.nih.gov/home/proteins.shtml	NCBI Research Co-ordinators (2018)
PANTHER (Protein Analysis Through Evolutionary Relationships)	Analyse gene lists Information about the evolution and function of protein coding genes Gene ontology and biological pathways	http://pantherdb.org	Mi *et al.* (2017)
CattleQTLdb	Database of all QTL identified from studies for different traits	https://www.animalgenome.org/cgi-bin/QTLdb/BT/index	
Gene Ontology Annotation (GOA) database	Gene Ontology annotations to proteins in the UniProt Knowledgebas (UniProtKB)	http://www.ebi.ac.uk/GOA	Huntley *et al.* (2014)
Ensembl release 94	Genome browser for vertebrate genomes. Gene annotations, functions, computes multiple alignments, predicts regulatory functions and collects disease data	https://www.ensembl.org/index.html	Aken *et al.* (2016)

Several major genes associated with carcass traits include Myostatin, Leptin, Calpain, Calpastatin and Thyroglobulin. The *MSTN* gene is found on BTA2 and is partially recessive (Mateescu, 2014). Seven mutations in this gene have been identified. This gene is responsible for

an increase in size of skeletal muscles which is caused by growth in size of component cells. Hyperplasia rather than hypertrophy of the muscle cells occur (Aiello *et al.,* 2018; Baile *et al.,* 2018). Some of these mutations are beneficial as there is an increase in the lean yield, higher muscle mass with less fat, tenderness and the longissimus muscle area (Mateescu, 2014). The disadvantage of some of the mutations are the occurrence of dystocia due to calves expressing this gene having a higher birthweight (Casas *et al.*, 2000).

The *CAPN* (Calpain genes) are responsible for the tenderness of the meat which in turn influences the eating quality of the meat. This gene is responsible for the degradation of muscle fibers (Leal-Gutiérrez *et al*., 2018). Genetic markers identified *CAPN* on BTA10 and BTA29 (Mateescu, 2014). Smith *et al*. (2000) reported the *CAPN1* candidate gene in the region of BTA29 which is associated with the meat tenderness (Casas *et al.*, 2003). Tenderness is influenced by the *CAST* gene which is responsible for the expression of Calpastatin. This gene works similarly to the Calpain system in the degradation of muscle fibers. The *CAST T1* gene is identified on chromosome 7 (Mateescu, 2014). Thyroglobulin (*TG*) is a precursor for the thyroid hormones and plays a role in the fat metabolism as it influences the adipocyte development. The QTL region on BTA14 associated with this gene has been identified for back fat thickness and marbling score (Mateescu, 2014). Casas *et al.* (2003) reported 2 candidate genes associated with the intra muscular fat deposition: *DGAT1* and *TG* genes.

Leptin has been identified as being associated with carcass composition and fat thickness. Markers for leptin have been identified on BTA4. Associations between the growth hormone and fat distribution have been identified (Mateescu, 2014). The gene for expression of the growth hormone have been identified with BTA19. This gene has been associated with the rump fat and eye muscle area and this influences the body composition of cattle. The fatty acid composition is also influenced by the growth hormone. Genes affecting the fat composition and metabolism are: *FABP4, SCD, FASN* and *SREBP1*. *FABP4* (adipose fatty acid binding protein) is located on BTA14 and the QTL within this region is associated with fat thickness, yield grade, marbling and slaughter weight (Mateescu, 2014). *SCD* (stearoyl-coAdesaturase) is responsible for the fatty acid composition and the marbling. *FASN* (fatty acid synthase) is located on BTA19 and synthesizes long chain fatty acids. This region on BTA19 is associated with fatty acid composition in adipose tissue in beef cattle and the milk fat composition in dairy cattle. *SREBP1* (sterol regulatory element-binding protein1) is related to the slaughter weight of beef cattle in addition to the fatty acid composition and fat quality (Mateescu, 2014).

The abovementioned genes are major genes that have been identified for the carcass composition and meat quality of beef cattle. There are many genes with small effects that have been identified as being associated to carcass quality. Genes with smaller effects on the carcass composition and meat quality are shown in Table 1.10.

Table 1.10 Summary of genes identified for carcass traits (Xia *et al.,* 2015; Sasago *et al.*, 2017; Bhuiyan *et al.,*2018)

Genes	Trait	Function
SLIT2, FAM13A, MED28, PLAG1, TOX, SDCBP, DCAF16, NCPAGCHCD7, FAM110B, CYP7A1	Slaughter weight	Cellular function and maintenance, Skeletal and muscle development and function, Skeletal muscle mass
COL1A2	SW	Osteogenesis imperfecta and collagen formation
KNCIP	Yearling weight	Calcium ion binding
DCAF PPARGC1A, CRH	EMA	Regulatory genes involved in lipid and glucose metabolism
TPM1	Muscle and fat tissue	Actin-binding protein in contractile system of striated and smooth muscle, and in cytoskelton of non-muscle cells
FASW	FA composition	-
SCD	C14:1	Stearoyl Acetyl CoA desaturase gene which encodes enzyme synthesizing oleic acid
CNNM2	C18:0 and Mg reabsorption in the kidney	Gene encodes protein responsible for Mg homeostasis
EDG1	Marbling, EMA, RF, BF	Encodes protein involved in differentiation of endothelial cells
GH1	Growth, SW	Gene is part of Somatotropin family which is responsible for growth control

These genes all contribute to important biological and functional processes of growth and development.

1.2.6 Conclusion

Real-time ultrasound scans are useful to determine the body composition and fat distribution in the live animal. Using RTU scans as a prediction of the carcass quality will increase rate of genetic gain following selection. This method is not as expensive or time consuming in comparison to other post slaughter methods. The literature that has been reviewed supports the statement that there is an opportunity for implementing RTU measurements as a test for beef cattle in feedlots. RTU measurements is not common-practice in South Africa and there is much potential in including it as regular feedlot practice.

Nguni cattle is a type of Sanga breed and there is limited research in the genetic architecture of these type of cattle. SNP markers are useful tool for identification of variation within the genome of individual animals. Literature has shown GWAS as a useful method for investigating the underlying genetic mechanisms for traits of interest. GWAS has the potential for a better understanding of the genetic architecture of variants within the genome associated with specific traits.

Chapter 2 Materials and Methods

2.1 Introduction

For this study an external data set was used, which was generated from a growth trial funded by the Nguni Breeders Society. This growth trial was performed at a private commercial feedlot and included a total of 200 diverse weaner calves from 23 Nguni breeders. The external data set consisted of various carcass trait measurements (weight of cattle at different growth stages, ultrasound measurements of fat distribution in carcass, slaughter weight, dressing percentage and rumen damage). Ethical approval was obtained from University of Pretoria Ethics Committee (EC170627-135) to use this data set. From the 200 weaner calves a total of 146 animals were selected for SNP genotyping with the 150k SNP chip to perform a genome-wide association study for the different carcass traits. Additional funding for genotyping was provided by the Beef Genomic Program (BGP) funded by TIA (Technology Innovation Agency) for genotyping.

2.2 Materials

2.2.1 Growth trial

Upon arrival at the feedlot, with an average age of 278 days of age, the animals were identified with an eartag, received growth hormone implant and vaccinated. After 30 days of backgrounding on veld during which the animals received ad lib Eragrostis grass as well as the starter ration, the feedlot trail commenced (Venter, 2017).

The animals were randomly divided into four groups of 50 animals each, where each group was fed a different ration containing different levels of roughage (low, medium, high roughage & commercial diet). Standard feedlot practices were followed for the feeding and treatment of the animals. Weight measurements were recorded for the 200 bull calves upon arrival at the feedlot, before backgrounding, at the start of the feedlot phase, at 42 days on trial and before slaughter (Venter, 2017). Figure 2.1 shows the distribution of the 200 bull calves received for the trail from different breeders and provinces.

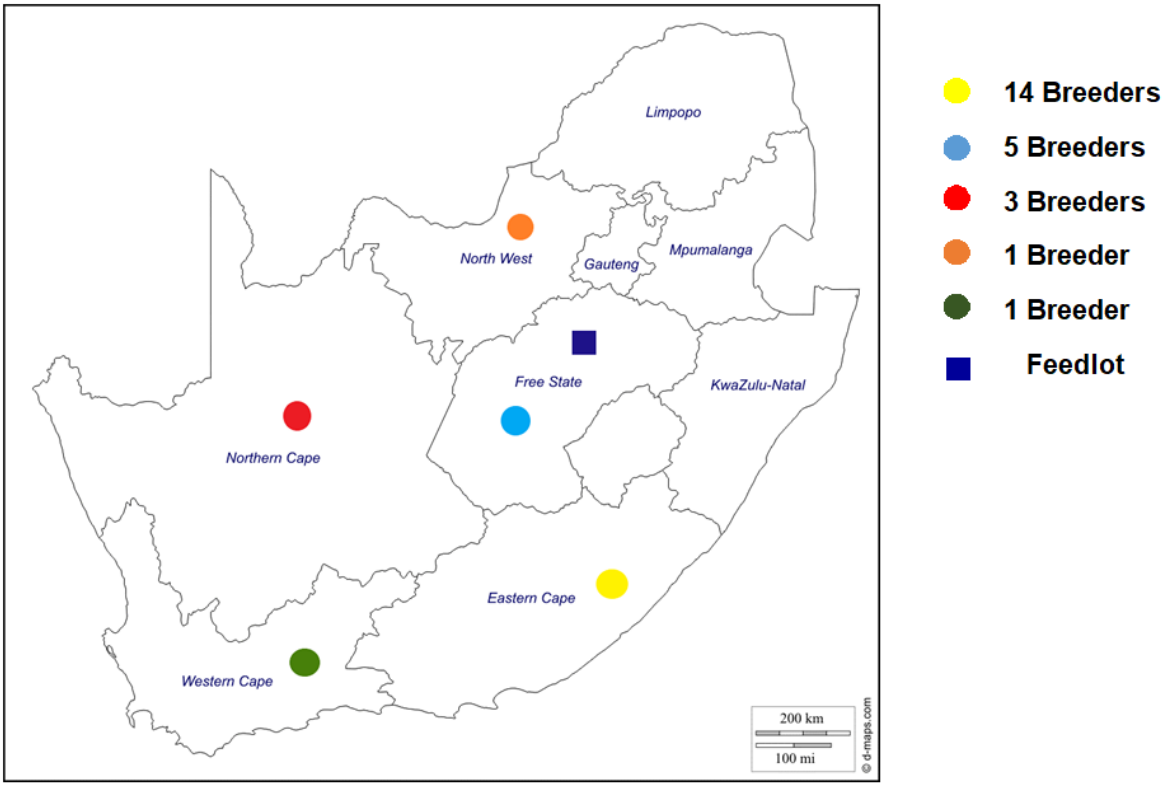


Figure 2.1 A map of South Africa indicating the distribution of the animals used in this study

Real time ultrasound (RTU) measurements were taken during the feedlot phase at 72 days on test for eye muscle area, rump fat, back fat thickness as well as at two weeks before slaughter (91 days on test) the measurements were recorded. The RTU scans were carried out at the feedlot's medical facility which allowed the mature cattle to move through the crush and neck clamp. A real-time ultrasound scanner (MyLab™OneVET) was used for the measurements and this was achieved through manual recording. The process of scanning was as follows: cattle entered the crush, the area of measurement is oiled (using corn oil) to improve the acoustic contact of the device. The scanning of the area of interest can then commence. It was not required that animals were fasted before the RTU measurements were taken during this trial.

The following RTU measurements were recorded: eye muscle area (EMA), back fat thickness (BF) and rump fat thickness. The cattle were slaughtered after visual judging according to body weight and body condition for the determination of when a carcass grading of A2 (1–3 mm) was achieved. Calves were slaughtered in three groups at 105, 120 and 135 days after entering the feedlot. The times of measurement for the different weight traits, RTU measurements and carcass traits during the growth trial are shown in Table 2.1.

Table 2.1 A summary of measurements recorded during the growth trial

Days on test	Measurements
-32	Weigh animals: Arrival weight
0	Weigh animals: Start weight
9	Weigh animals
44	Weigh animals RTU measurements: Rump fat, Rib fat
72	Weigh animals RTU measurements: EMA, Rump fat, Rib fat
91	RTU measurements: EMA, Rump fat, Rib fat,
99	Weigh animals
105	Slaughtered at A2 (1–3 mm) carcass grade. Slaughter weights
120	Slaughtered at A2 (1–3 mm) carcass grade. Slaughter weights
135	Slaughtered at A2 (1–3 mm) carcass grade. Slaughter weights

Different post-mortem slaughter measurements were also recorded such as the slaughter weight, dressing percentage and other traits of economic importance as indicated in Table 2.2.

Table 2.2 Measurements recorded during growth on the live animal and post slaughter

Measurements	Description
(Measurements on the live animal)	
Eye muscle area (EMA) scanned	Measured at the cross-sectional area of the *longissimus dorsi* muscle and should be taken between the ribs not over the ribs.
Rump fat scanned	Measured between the hook and pin bones of the animal or measured at the apex of the *biceps femoris* muscle
Back fat scanned	Measurements of the l*ongisimus dorsi* muscle between the 12th and 13th rib.
Marbling scanned	The percentage intra-muscular fat. Longitudinal image in the region of the 11th, 12th, and 13th rib, approximately 2/3rd of the distance from the medial to the dorsal end of the *longissimus dorsi* muscle.
Weight of the cattle during trial	Weights of the animals were measured at arrival, start of trial, 9 days, 44 days, 72 days, 91 days, 99 days, 105 days, 120 days and 135 days
(Post Slaughter measurements)	
Slaughter weights	Measured in kg after slaughter
Warm carcass weight	Measured in kg as the hot weight of the carcass with head, hide and intestinal tract and internal organs removed
Cold carcass weight	Calculated as 2% less than the warm carcass weight
Dressing percentage (%)	Calculated from the live weight and slaughter weight

2.2.2 Animal selection and genotyping

From the 200 animals that were included in the growth trial, a subset of 139 representative animals were selected for genotyping. Animals with missing animal IDs or that were not slaughtered at an A2 (0 teeth, 1-3mm) carcass grade were excluded from selection. These animals were selected to achieve a normal distribution of all traits measured. Hair samples of 141 animals were submitted to the Agricultural Research Centre Biotechnology (ARC BTP) platform for genotyping using the Geneseek GGP-HD Bovine 150 K SNP array. The distribution of animals per breeder and per province is displayed in Table 2.3

Table 2.3 Summary of number of animals for the different variables

Variable		Number of animals	Number of breeders
Ration	Commercial	24	23
	High roughage	38	24
	Medium roughage	39	24
	Low roughage	43	23
Province	Eastern Cape	72	14
	Free State	24	5
	Northern Cape	24	3
	North West	14	1
	Western Cape	10	1

2.3 Methods

Phenotypic data from the growth data was received in Excell and the structure of the data was edited for statistical analysis. This data was statistically analysed using SAS enterprise guide, version 9.4 (2013). Data was firstly edited to remove missing animal identification numbers and animals with an A1 (<1mm) or A3 (>3 and <5 mm) carcass grading. Summary statistics of this data was calculated using the MEANS procedure of SAS enterprise guide 9.4 (2013). Effect of diet and province on the traits were calculated using the ANOVA procedure (Theron, 2018).

Raw data was received from Genome Studio® 1.9.0 software and converted from Illumina® final report format to PLINK input file (MAP and PED files) format using SNP convert (Nicolazzi *et al*., 2015). PLINK (v1.9) (Purcell *et al*., 2007) was used to perform technical (sample based, and marker based) quality control (QC) to filter out uninformative individuals and SNPs from the dataset.

Individual and SNP call rates of 90% was implemented. This resulted in the removal of two animals as well as 3198 SNPs.

The genotypic data was analysed firstly by applying no further genetic quality control (minor allele frequency and Hardy- Weinberg equilibrium) to assess whether any SNPs that will eventually be filtered were indeed associated with the traits of interest. Genetic quality control may introduce bias to the data, as certain SNPs that may show association, as well as individuals are removed from the data set (Uiterlinden, 2016). Secondly, the dataset was further analysed after genetic QC parameters were applied. The QC thresholds that was applied to the dataset was as follows: SNPs were removed based on a minor allele frequency of 0.02 (12 232SNPs removed) and a Hardy Weinberg Equilibrium (HWE) threshold of less than 0.0001 (1 SNP removed). The total number of SNPs and animals that were in the two datasets (one with technical QC applied and the second with genetic QC filtering), are summarised in Table 2.4.

Table 2.4 Summary of data set with technical QC filters and with additional genetic QC filters

	Technical QC (SNP and individual call rates = 0.1)	**Genetic QC** (MAF = 0.02, HWE = 0.0001)
SNPs	137 798	124 178
Animals	139	139

2.3.1 Principal component analysis (PCA)

To account and avoid false positives the SNP-based genetic relatedness between individual cattle were evaluated using GCTA (v 1.24) (Genome-wide Complex Trait Analysis; Yang et al., 2011) A genetic relationship matrix was firstly calculated, thereafter eigenvalues and eigenvectors were generated for the first three principal components. Microsoft Excel (2013) was used to plot the eigenvector values for the principal components. A PCA was constructed for the relatedness between the animals as well as for four different rations that were used, and for the different provinces which animals originated from. This was done to identify a possible effect of ration or province on the results. The steps followed for GWAS is illustrated in Figure 2.2.

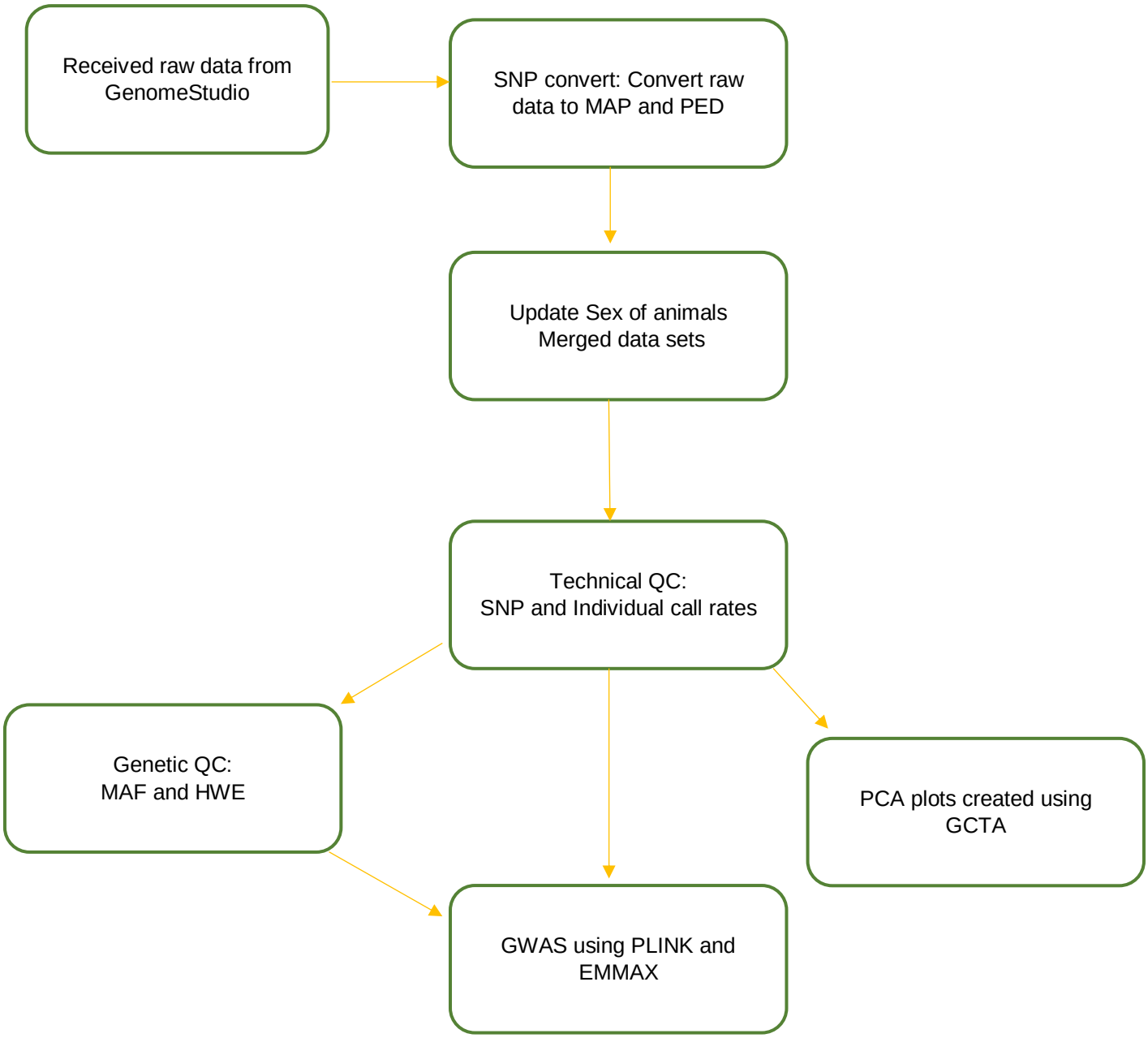


Figure 2.2 Flow diagram illustrating the approach followed for conducting the GWAS studies

2.3.2 Genome-wide association studies

A genome-wide association study (GWAS) was performed on both datasets (dataset 1: technical QC applied, dataset 2: genetic QC filters applied). *PLINK* software (v1.9) was initially used to perform a genetic association of nine carcass traits (quantitative traits) (Rentería *et al.,* 2013). The following commands were used in *PLINK (*Purcel *et al.*, 2007) for the GWAS:

The *--pheno* command in PLINK was used to replace the missing phenotypes in the original PED/FAM files. This command detects whether the phenotypes are quantitative or case/control. A

confidence interval of 95% was included for the beta coefficient, this also generates standard error values for each estimate. The following command was used in *PLINK* to perform a GWAS for each individual trait and for the combination of traits: *plink --noweb --cow --bfile[filename] --allow-no-sex --pheno [file.txt] --assoc --adjust --ci 0.95 --out [filename]*. *PLINK* generates a **.qassoc* and **.qassoc.adjusted* files. *.qassoc.adjusted output is useful as it contains adjusted p-values and the most significant tested SNPs are listed at the top of the file. *PLINK* software detects quantitative compared to case/control data sets (Rentería *et al.,* 2013).

Additional software, efficient mixed model association eXpedited (EMMAX) was used for GWAS (Kang *et al*., 2010). Files generated in PLINK were then used to create an identity by state (IBS) kinship matrix and creating a transposed Ped and Fam file. Output files from the kinship matrix are *.mibs and the transposed files are in the *.tfam and *.tped fromat. These files together with a phenotype file containing the family ID, individual ID and respective phenotypes for the different traits. To perform the GWAS using EMMAX the following command is used: *emmax -v -d 10 -t [tped filename] -p [phenotype file.txt] -k [kinship file] -o [output file name]*.

R Studio

Results from the association analysis was visualised by creating a Manhattan plot in R-studio (v1.1.456) (2015). The following packages were downloaded and installed in R suited for GWAS analysis and plotting of the data: *readr*, *plotly* and *manhattanly*. Output files for each trait generated in PLINK are imported into R studio and results are displayed in R-studio. The results obtained were then used to identify most significant ($p<10^{-7}$) and suggestive ($p<10^{-5}$) SNPs, from which putative genes can be identified.

2.3.3 Putative gene identification

A gene search was performed on the chromosomal regions that were defined by the positions of suggestive and significant SNPs using the Bos Taurus: UMD_3.1.1 (GCF_000003055.6) reference genome assembly on NCBI (https://www.ncbi.nlm.nih.gov/genome/gdv/?org=bos-taurus). Gene names and symbols were identified within a window of 5000 bp upstream and downstream from the SNP position. Identified genes were listed and uploaded to Panther (Mi *et al.*, 2017), for metabolic function and biological processes of the genes. The QTL database (https://www.animalgenome.org/cgi-bin/QTLdb/BT/index) was used to identify previously discovered QTL within a window of 5000bp upstream and downstream of the identified SNPs.

Chapter 3 Results

3.1 Descriptive statistics of growth trial

There were 200 animals in the feedlot trial with only 141 genotyped. The averages and standard deviations for all traits for animals phenotyped and genotyped are shown in Table 3.1 and Table 3.2. In Table 3.1 the averages for the growth traits measured throughout the trial is shown. Post-slaughter measurements and the average time spent on test is included in this table. The average age of the cattle at the start of the trial was 309.5 ± 45 days of age with an average starting weight of 188.7kg ± 34.2 kg. The average time of the animals in the feedlot till slaughter was 120 ± 12 days on test. The average slaughter weight for the animals in this trial was 346.4 ± 33.2kg. The animals selected for genotyping had an average starting weight of 189.0±367 kg and slaughter weight of 343.9±36.2.

Table 3.1 Average for the growth traits and post slaughter measurements for the cattle on test

	Total number of animals (200)	**Number of animals genotyped (141)**		
Variable	**Mean ± SD**	**Mean ± SD**	**Minimum**	**Maximum**
Arrival weight (kg)	164.0 ±29.9	165.0 ±31.2	94.0	242.0
Start weight (kg)	188.7 ±34.2	189.0 ±36.7	106.0	288.0
Weight at 9 days (kg)	203.9 ±36.2	204.3 ±39	114.0	308.0
Weight at 44 days (kg)	253.6 ±40.3	254.7 ±44.3	154.0	372.0
Weight at 72 days (kg)	299.5 ±41.7	299.8 ±46	196.0	416.0
Weight at 99 days (kg)	375.4 ±27.6	378.6 ±28.9	316.0	440.0
Slaughter weight (kg)	346.4 ±33.2	343.9 ±36.2	244.0	444.0
Warm carcass weight (kg)	198.2 ±20.9	197.4 ±23.3	132.6	258.8
CM2%	194.3 ±20.5	193.4 ±22.8	129.9	253.6
Dressing percentage (%)	56.1 ±1.7	56.2 ±1.7	50.9	62.6
Slaughter date (days)	120.9 ±12.1	120 ±12.4	105.0	135.0

Table 3.1 shows the variation of animals that were used in this trial, as well as the animals selected for genotyping, especially when considering the minimum and maximum values for the weight measurements. In Table 3.2 the ultrasound measurements for the cattle on the growth trial is shown. The means for the different traits under investigation was fairly similar between the population of animals included in the trial and the animals selected for genotyping.

Table 3.2 The average of the ultrasound measurements at 72, 91 and 120 days on test

	Total number of animals (200)	**Number of animals genotyped (141)**		
Variable	**Mean ± SD**	**Mean ± SD**	**Minimum**	**Maximum**
RF72 (mm)	5.1 ±1.7	5.2 ±1.8	2.5	21.0
RF 91 (mm)	5.4 ±1.3	5.5 ±1.4	2.8	9.9
RF120 (mm)	6.0 ±1.4	5.9 ±1.4	3.2	9.9
BF 44 (mm)	2.4 ±0.5	2.5 ±0.6	1.3	3.8
BF 72 (mm)	3.1 ±0.8	3.2 ±0.8	1.8	5.5
BF 91 (mm)	3.5 ±0.9	3.5 ±0.9	1.8	6.6
BF 120 (mm)	3.8 ±0.7	3.8 ±0.8	2.1	5.8
EMA 72 (cm^2)	49.1 ±6.2	49.1 ±6.6	31.0	66.0
EMA 91 (cm^2)	52.3 ±5.6	52.0 ±6.0	40.0	69.0

RF: Rump fat thickness, BF: Backfat thickness, EMA: Eye muscle area

Relatively large differences were observed in the group for rump fat, back fat thickness and eye muscle area at 72 and 91 days on test, as well as for rump fat and backfat thickness at 120 days.

3.2 Principal component analysis (PCA)

The principal component analysis (PCA) results are based on 141 genotypes that passed technical QC and 141 716 SNPs. The relatedness between animals are shown in figure 3.1, which shows three outliers in the upper and lower left quadrants.

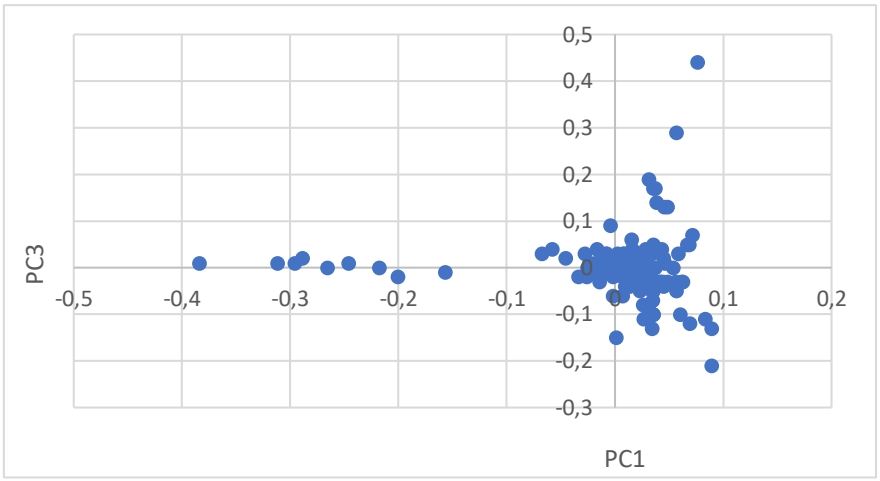


Figure 3.1 Genetic relationships among the 139 cattle for the first and third principal components (PC1 and PC3)

In Figure 3.1 a and b the results are displayed and analysed for diet and province respectively.

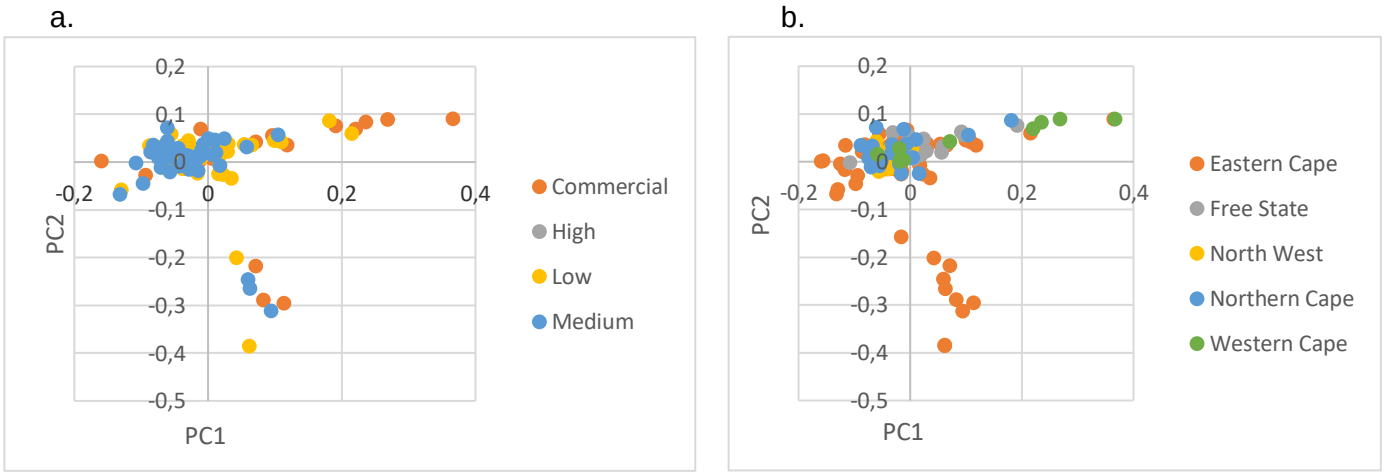


Figure 3.2 Genetic relationships among the 139 cattle for the first and second principal components (PC1 and PC2). a: Animals grouped according to different diets. b: Animals grouped according to different provinces before entering the feedlot.

Figure 3.2a illustrates that the majority of individuals clustered tightly together irrespective of diet. A number of outliers were observed that included animals on commercial, low and medium roughage diet. Outliers are defined as animals in the upper and lower right quadrant of the PCA plot. The outliers that were observed can mainly be assigned to the animals that were fed either a commercial diet, medium or a low roughage ration. In Figure 3.2b represented all different provinces except for a few outliers that originated mostly from the Eastern Cape and the Western Cape provinces. The effects of the diet and province on the carcass traits was tested and reported by Theron *et al.* (2017). Province had a non-significant ($p< 0.05$) effect on the dressing percentage

(DP), slaughter weight and the end weight. However, there was a significant difference ($p<0.05$) in the start weight of the calves and the different provinces. There was a significant difference in the slaughter weight of the calves fed the commercial diet compared to the high, medium and low roughage diets. The North West and Eastern Cape provinces had a significant effect ($p<0.05$) on the ultrasound measurement for the eye muscle area. Diet had no significant effect on the EMA and back fat measurements, however it did have a significant effect ($p<0.05$) on the rump fat thickness.

3.3 Genome-wide association studies (GWAS)

A total of 38 SNPs was found to be associated with all traits (Slaughter weight, Dressing percentage, EMA72, EMA91, BF72, BF91, RF72, RF91). In the first analyses the data with only technical QC was used for the eight traits. The results for the GWAS using RTU traits (EMA (a), RF (b) and BF (c)) measured at 72 days are shown in Figure 3.3. In Figure 3.3 (a) five suggestive SNPs ($p<10^{-5}$) were identified for the ultrasound measurement of EMA at 72 days on test. One suggestive SNP is identified on BTA2 and four suggestive SNPs on BTA 25. GWAS. The GWAS for RF72 (Figure 3.3 b) shows three suggestive ($p<10^{-5}$) SNPs are identified, with one suggestive SNP each on BTA 5, BTA 9 and BTA 16 respectively. Figure 3.3 (c) displays the GWAS for back fat thickness measured at 72 days on test, which identified no SNPs.

a.

b.

c.

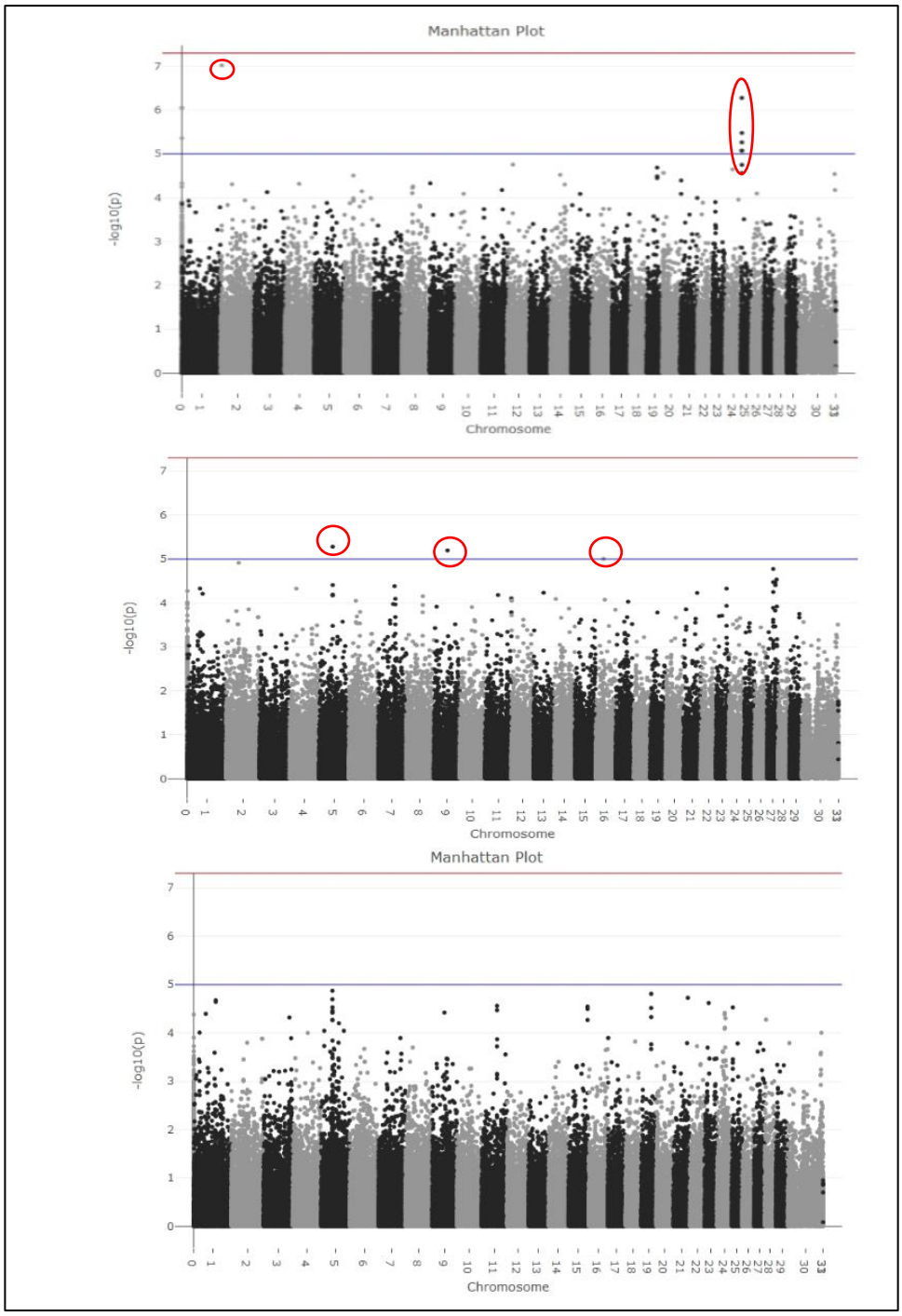


Figure 3.3 Manhattan plot displaying the results (-log 10 of p-values) for SNPs detected for a: EMA72, b: Rump fat72, c: Back fat72

The results of the GWAS for RTU measured at 91 days on test were displayed in Figure 3.4. For EMA five SNPs ($p<10^{-5}$) were identified (Figure 3.4 a) on BTA2 (1SNP), BTA12 (1SNP), BTA25 (1SNP) and BTA28 (1SNP), while only two suggestive SNPs ($p<10^{-5}$) were identified for rump fat on BTA1 (1SNP) and BTA 16 (1SNP). In Figure 3.4 c six suggestive ($p<10^{-5}$) SNPs were identified using GWAS for backfat on BTA2, BTA3, BTA 5, BTA 25, BTA28 and the X-chromosome.

a.

b.

c.

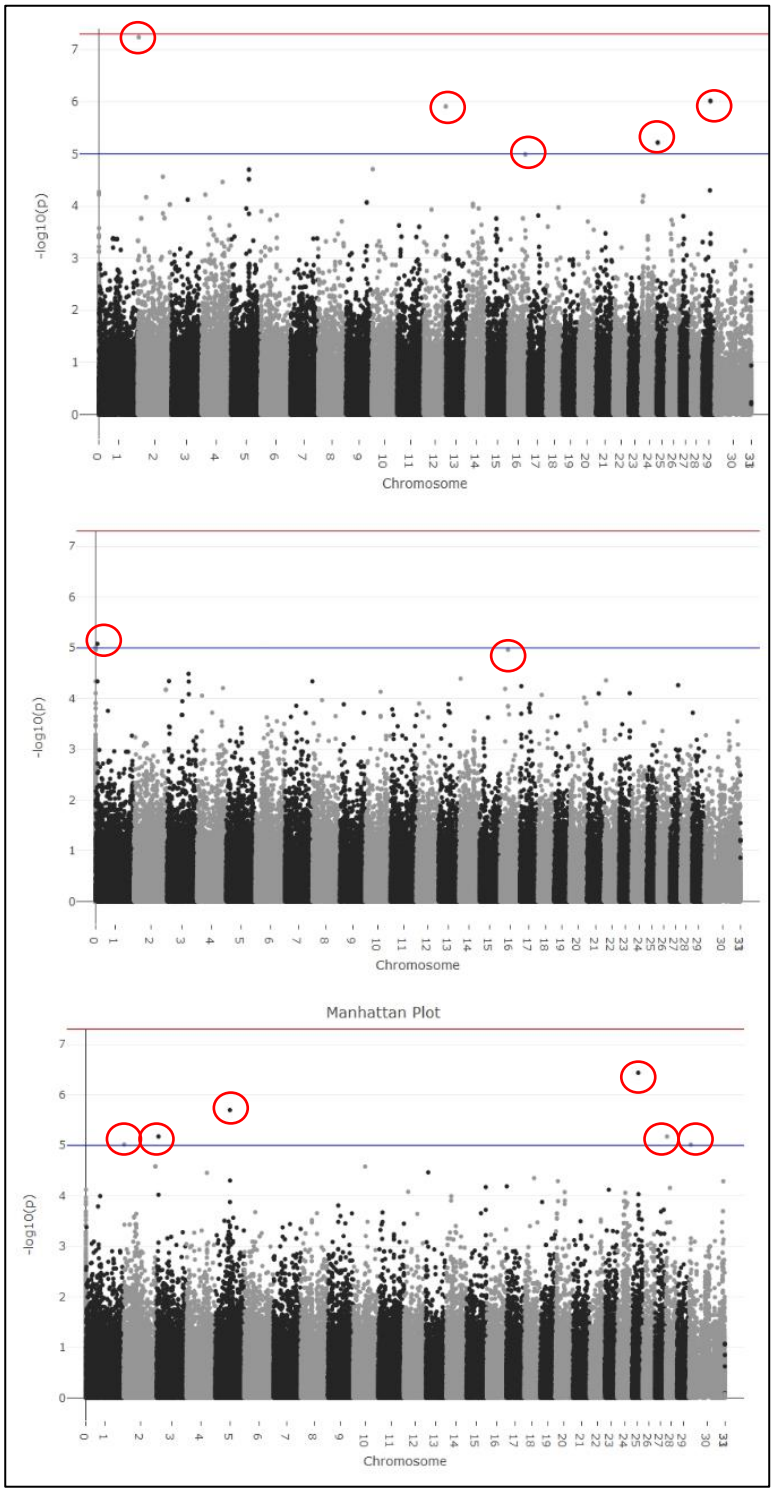


Figure 3.4 Manhattan plot displaying the results (-log 10 of p-values) for the SNPs detected for a: EMA91, b: Rump fat91, c: Back fat91

Figure 3.5 displays the GWAS for the slaughter weight of the animals in this trial with six SNP identified ($p<10^{-5}$) on BTA4 (1SNP), BTA 9 (2 SNPs) and BTA 19 (3 SNPs).

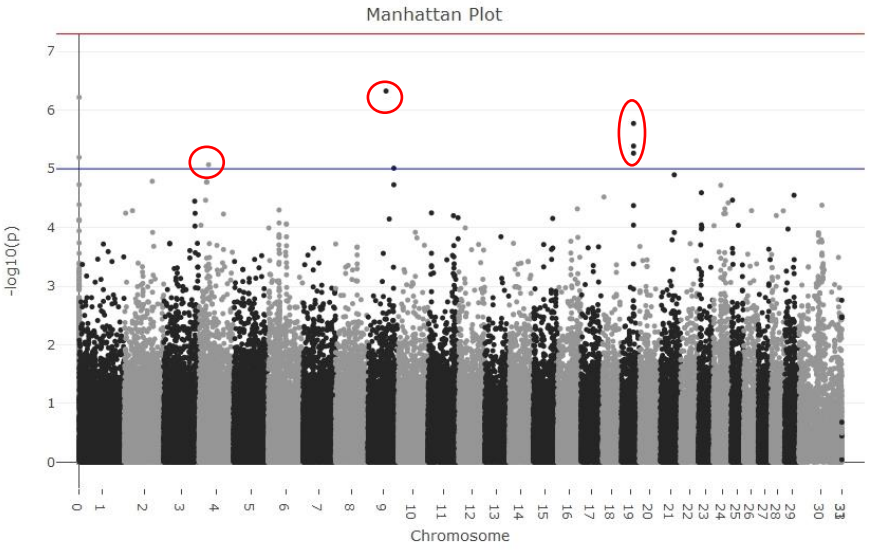


Figure 3.5 Manhattan plot displaying the results for SNPs detected for the slaughter weight (-log 10 of p-values)

Results for the GWAS performed for the dressing percentage are displayed in figure 3.6. On the X chromosome there are three suggestive SNPs identified for this trait.

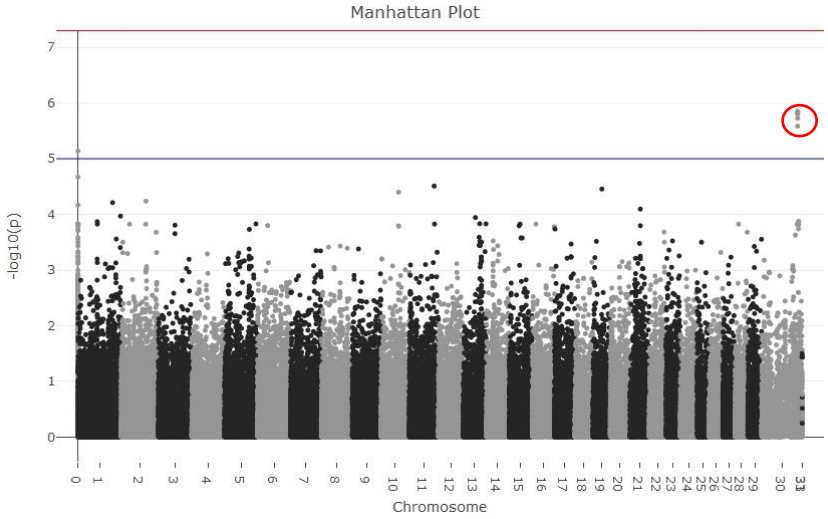


Figure 3.6 Manhattan plot displaying the results for SNPs detected for dressing percentage (-log 10 of p-values)

The GWAS was repeated for all the genotypic data after genotypic QC (MAF and HWE) was applied. Manhattan plots from the results of the GWAS are shown in addendum C. The SNPs identified through this association was identical to the results found for the data set with only technical QC. This was found for all traits except for the dressing percentage, where no SNPs were identified to be associated.

The results from EMMAX software for the dataset with technical QC (SNP and individual call rates) as well as dataset with additional genetic QC (MAF and HWE) are shown in Addendum C. Results using EMMAX gave similar results for some SNPs such as: BTA4 for SW, X-chromosome for DP, BTA2 for BF72, BTA25 for BF91 and BTA28 for BF91. Additional SNPs were identified for EMA72 on BTA7, BTA8, BTA9. BTA13, BTA20 and for EMA91 on BTA23. Results of GWAS for BF at 72 days identified an additional SNP on BTA3 and two SNPs for BF at 91 days on BTA23 and BTA25.

Table 3.3 compares the SNPs identified for ultrasound measurements of the eye muscle area using PLINK and EMMAX software for the GWAS. For this trait EMMAX identified additional SNPs associated with the EMA measured at 72 days on test. The SNPs were identified on BTA7, BTA8, BTA9, BTA13 and BTA20. On chromosome 2 there is a SNP identified by both software and EMMAX identified BTA 23 in addition to the SNPs identified by PLINK.

Table 3.3 Chromosomes and SNPs identified for ultrasound measurements of eye muscle area (EMA) using different software and data quality control measures

Trait	Chromosome and SNP			
	PLINK		EMMAX	
	Technical QC	Genetic QC	Technical QC	Genetic QC
EMA72	BTA2 BovineHD0200001009	BTA2 BovineHD0200001009	None	None
	None	None	BTA7 ARS-BFGL-NGS-102773, BovineHD0700004386, ARS-BFGL-NGS-95757	BTA7 ARS-BFGL-NGS-102773, BovineHD0700004386, ARS-BFGL-NGS-95757
	None	None	BTA8 BovineHD0800007273	BTA8 BovineHD0800007273
	None	None	BTA9 BovineHD0900026853	BTA9 BovineHD0900026853
	None	None	BTA13 BTB-01124378	BTA13 BTB-01124378
	None	None	BTA20 BovineHD2000004268	BTA20 BovineHD2000004268
	BTA25 BovineHD2500000886, BovineHD2500000904, BovineHD2500000912, ARS-BFGL-NGS-62236	BTA25 BovineHD2500000886, BovineHD2500000904, BovineHD2500000912, ARS-BFGL-NGS-62236	None	None
EMA91	BTA2 BovineHD0200001009	BTA2 BovineHD0200001009	None	None
	BTA12 BovineHD1200026405	BTA12 BovineHD1200026405	None	None
	BTA16 BovineHD1600009129	BTA16 BovineHD1600009129	None	None
	None	None	BTA23 BovineHD2300003974	BTA23 BovineHD2300003974
	BTA25 BovineHD2500000979	BTA25 BovineHD2500000979	None	None
	BTA29 ARS-BFGL-NGS-16031	BTA29 ARS-BFGL-NGS-16031	None	None

SW: Slaughter weight, DP: Dressing percentage

The SNPs identified for the ultrasound measurement of rump fat thickness using PLINK and EMMAX for the GWAS is shown in Table 3.4. EMMAX did not identify any SNPs to be associated with this trait.

Table 3.4 Chromosomes and SNPs identified for ultrasound measurements rump fat thickness (RF) using different software and data quality control measures

Trait	Chromosome and SNP			
	PLINK		EMMAX	
	Technical QC	Genetic QC	Technical QC	Genetic QC
RF72	BTA5 BovineHD0500015865	BTA5 BovineHD0500015865	None	None
	BTA9 BTB-00393138	BTA9 BTB-00393138	None	None
	BTA16 BovineHD1600009129	BTA16 BovineHD1600009129	None	None
RF91	BTA1 BovineHD0100002202	BTA1 BovineHD0100002202	None	None
	BTA16 BovineHD1600009129	BTA16 BovineHD1600009129	None	None

SW: Slaughter weight, DP: Dressing percentage

Table 3.5 shows the different SNPs identified for the ultrasound back fat thickness using the different software. The same SNPs are identified for BF72 on chromosome one and for BF91 on chromosome three and chromosome 25.

Table 3.5 Chromosomes and SNPs identified for ultrasound measurements of back fat thickness from different software and data quality control measures

Trait	Chromosome and SNP			
	PLINK		EMMAX	
	Technical QC	**Genetic QC**	**Technical QC**	**Genetic QC**
BF72	None	None	BTA1 BovineHD0100026449 BovineHD0100026452	BTA1 BovineHD0100026449 BovineHD0100026452
BF91	BTA2 BovineHD0200000238 BTA3 BovineHD0300002167 BTA5 BTA-73733-no-rs BTA25 ARS-BFGL-NGS-1148 BTA28 Hapmap40383-BTA-100914 X BovineHD3000002170	BTA2 BovineHD0200000238 BTA3 BovineHD0300002167 BTA5 BTA-73733-no-rs BTA25 ARS-BFGL-NGS-1148 BTA28 Hapmap40383-BTA-100914 X BovineHD3000002170	None BTA3 BovineHD0300002167 None BTA25 ARS-BFGL-NGS-1148 None None	None BTA3 BovineHD0300002167 None BTA25 ARS-BFGL-NGS-1148 None None

SW: Slaughter weight, DP: Dressing percentage

Table 3.6 shows the SNPs identified with slaughter weight and dressing percentage as identified with the different software. This indicates the SNPs on chromosome 4 is associated when using both software. For dressing percentage, the same chromosome is identified using both software for the same data set.

Table 3.6 Chromosomes and SNPs identified for slaughter weight and dressing percentage from different software and data quality control

Trait	Chromosome and SNP			
	PLINK		EMMAX	
	Technical QC	Genetic QC	Technical QC	Genetic QC
SW	BTA4 BovineHD0400007766	BTA4 BovineHD0400007766	BTA4 BovineHD0400007766	None
	BTA9 BovineHD0900017304, ARS-BFGL-NGS-83811	BTA9 BovineHD0900017304 ARS-BFGL-NGS-83811	None	None
	BTA19 Hapmap54526-ss46526755, ARS-BFGL-NGS-82930, BovineHD1900012186	BTA19 Hapmap54526-ss46526755, ARS-BFGL-NGS-82930, BovineHD1900012186	None	None
DP	BTA30 BovineHD3000037488 BovineHD3000037479 BovineHD3000037443	None	BTA30 BovineHD3000037488	None

SW: Slaughter weight, DP: Dressing percentage

Table 3.7 shows the minor allele frequency (MAF) and the frequency of the favourable allele for the SNPs identified for the different traits. From the 36 SNPs identified from the GWAS for carcass traits, the major allele occurs at a frequency of 90% and higher in 16 of these SNPs. The major allele frequency is very high (>60%) in most of the SNPs identified. A high MAF (>40%) is seen in three SNPs namely: BovineHD1300009087, BovineHD2300003974 and BovineHD0100026449. These two SNPs are associated with the EMA91, BF72, RF72 and RF91.

Table 3.7 Minor allele frequencies (MAF) of the alleles for the SNPs identified

Trait	CHR	SNP	A1	A2	MAF	Frequency of the favourable allele
BF72	1	BovineHD0100026449	B	A	0.12	0.88
	1	BovineHD0100026449	A	B	0.94	0.06
BF91	2	BovineHD0200000238	B	A	0.35	0.65
	3	BovineHD0300002167	A	B	0.16	0.84
	5	BTA-73733-no-rs	B	A	0.04	0.96
	28	Hapmap40383-BTA-100914	B	A	0.18	0.82
	30	BovineHD3000002170	B	A	0.19	0.81
RF72, RF91, SW	16	BovineHD1600009129	A	B	0.32	0.68
EMA72	7	ARS-BFGL-NGS-95757	A	B	0.03	0.97
		ARS-BFGL-NGS-102773	A	B	0.03	0.98
		BovineHD0700004386	A	B	0.03	0.98
	8	BovineHD0800007273	B	A	0.03	0.97
	9	BovineHD0900026853	B	A	0.03	0.97
	13	BTB-01124378	A	B	0.08	0.92
	20	BovineHD2000004268	A	B	0.02	0.98
	25	BovineHD2500000886	B	A	0.37	0.63
	25	BovineHD2500000904	B	A	0.3	0.70
	25	BovineHD2500000912	B	A	0.29	0.71
	25	ARS-BFGL-NGS-62236	B	A	0.31	0.69
EMA72, EMA91	2	BovineHD0200001009	A	B	0.05	0.95
EMA91	12	BovineHD1200026405	A	B	0.21	0.79
	23	BovineHD2300003974	A	B	0.43	0.57
	25	BovineHD2500000979	A	B	0.13	0.87
	29	ARS-BFGL-NGS-16031	A	B	0.09	0.91
RF72	5	BovineHD0500015865	A	B	0.36	0.64
	9	BTB-00393138	B	A	0.02	0.97
RF91	1	BovineHD0100002202	A	B	0.09	0.91
SW	4	BovineHD0400007766	A	B	0.01	0.99
	9	BovineHD0900017304	B	A	0.30	0.7
		ARS-BFGL-NGS-83811	A	B	0.26	0.74
	19	ARS-BFGL-NGS-82930	A	B	0.27	0.73
		Hapmap54526-ss46526755	A	B	0.23	0.77
		BovineHD1900012186	A	B	0.26	0.74
DP	30	BovineHD3000037443	B	A	0.01	0.99
		BovineHD3000037479	A	B	0.01	0.99
		BovineHD3000037488	B	A	0.01	0.99

*CHR: Chromosome

The genotypic frequencies based on the identified SNPs are useful for showing the number of homozygotes and heterozygotes in a population. The SNPs identified for carcass traits in the Nguni population showed 17 SNPs where the homozygous (BB) genotype occurred the most. Eight SNPs showed more homozygous (AA) animals in the population compared with heterozygotes (AB) and homozygous (BB) animals. Heterozygote genotypes occurred in most of the animals for the following five SNPs: BovineHD0200000238, BovineHD0500015865, BovineHD2300003974 and

BovineHD2500000886. These SNPs are associated with BF91, RF72, EMA72. Table 3.8 shows the genotypic frequency for each SNP identified in this study for carcass traits.

Table 3.8 Genotypic frequencies of the identified SNPs for the population

Trait	CHR	SNP	AA	AB	BB
EMA72	7	ARS-BFGL-NGS-95757	0	0,05	0,95
	7	ARS-BFGL-NGS-102773	0	0,04	0,96
	7	BovineHD0700004386	0	0,04	0,96
	8	BovineHD0800007273	0,94	0,06	0
	9	BovineHD0900026853	0,93	0,04	0,01
	13	BTB-01124378	0,01	0,14	0,86
	20	BovineHD2000004268	0	0,04	0,96
	25	BovineHD2500000886	0,37	0,51	0,11
	25	BovineHD2500000904	0,52	0,37	0,12
	25	BovineHD2500000912	0,5	0,38	0,09
	25	ARS-BFGL-NGS-62236	0,52	0,34	0,14
EMA72, EMA91	2	BovineHD0200001009	0	0,11	0,89
EMA91	12	BovineHD1200026405	0,04	0,32	0,63
	23	BovineHD2300003974	0,17	0,52	0,3
	25	BovineHD2500000979	0,01	0,23	0,76
	29	ARS-BFGL-NGS-16031	0,01	0,17	0,81
RF72	5	BovineHD0500015865	0,12	0,47	0,4
	9	BTB-00393138	0,94	0,06	0
RF91	1	BovineHD0100002202	0	0,19	0,81
RF72, RF91, SW	16	BovineHD1600009129	0,09	0,45	0,45
BF72	1	BovineHD0100026452	0	0,19	0,81
	1	BovineHD0100026449	0.80	0.16	0.03
BF91	2	BovineHD0200000238	0,4	0,51	0,09
	3	BovineHD0300002167	0,06	0,2	0,74
	5	BTA-73733-no-rs	0,94	0,04	0,01
	28	Hapmap40383-BTA-100914	0,65	0,32	0,02
SW	4	BovineHD0400007766	0	0,03	0,97
	9	BovineHD0900017304	0,46	0,46	0,07
	9	ARS-BFGL-NGS-83811	0,06	0,38	0,53
	19	ARS-BFGL-NGS-82930	0,09	0,36	0,55
	19	Hapmap54526-ss46526755	0,08	0,29	0,56
	19	BovineHD1900012186	0,09	0,35	0,56

*CHR: Chromosome

The genotypic frequencies for the population is further illustrated in Figure 3.7. Each genotype is displayed for each of the SNPs identified in this study. It is seen from this graph the

homozygous genotypes occur more frequently for the respective SNPs. In some of the SNPs the distribution of the genotypes is equal for the two homozygous genotypes.

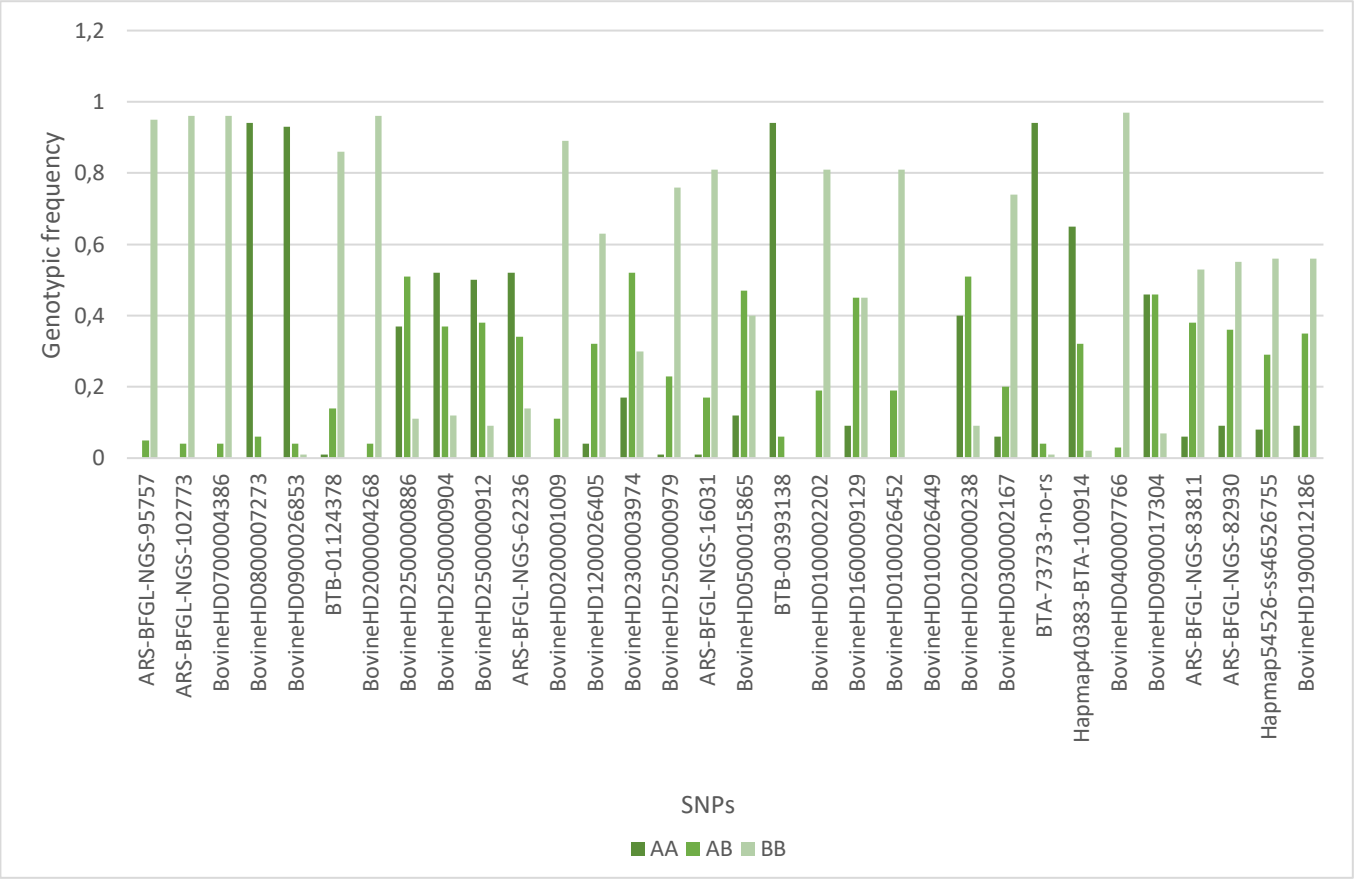


Figure 3.7 Graph depicting the genotypic frequencies for the SNPs identified for the population

3.4 Gene Ontology

Identification of suggestive and significant SNPs enabled identification of the gene associated with these SNPs. Table 3.9 summarises the SNPs for the respective carcass traits and the accompanying genes. For all SNPs identified as being associated with the slaughter weight, genes were detected within the chromosomal regions. In a total of 25 chromosomal regions identified as associated, no genes were detected. One gene (*SMYD3*) was associated with the BF72, EMA91, RF72 and RF91. *LOC107131809* gene is associated with the EMA for both measurements (72 and 91 days on test).

Table 3.9 Summary of genes identified for carcass traits in this study

Trait	Gene	Chromosome	SNP location (bp)
BF91	*NIPA1*	2	934 912
	DDR2	3	6 833 523
	CFAP54	5	61 183 506
BF72	*NRXN3*	1	91 769 482
EMA91, RF72, RF91	*SMYD3*	16	32 161 861
EMA72	*DCAF15*	7	12 833 745
	P2RY11		15 895 135
EMA72	*LOC107131809*	25	4 354 023
EMA91			4 370 698
SW	*HDAC9*	4	26 839 566
	SYNE1	9	62 996 226
	TTC25	19	42 749 182
	NT5C3B		42 660 383
	FKBP10		42636853
DP	*PPEF1*	X	131 925 131
			132 021 752
	RS1		132 049 552

SW: Slaughter weight, BF: Backfat thickness, EMA: Eye muscle area, RF: Rump fat thickness

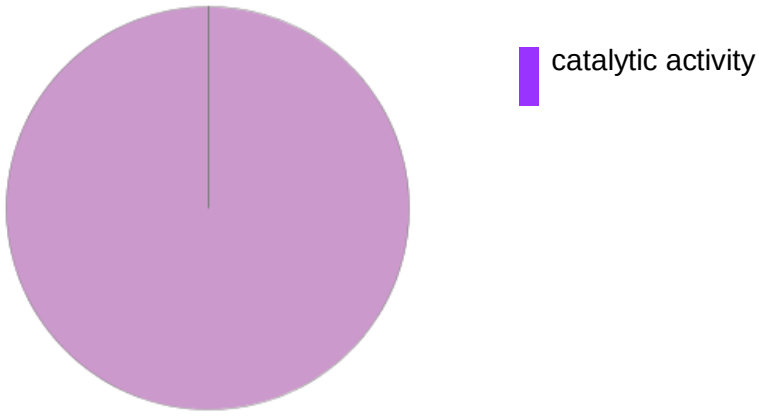
Five genes were identified for slaughter weight and two genes for the dressing percentage. The gene PPEF1 is found within the chromosomal region of two of the SNPs associated with the dressing percentage. There was no identification of the TTCD gene associated with slaughter weight when investigated using the Panther database. The different molecular functions and biological processes of the genes associated with slaughter weight and dressing percentage is shown in Table 3.10.

Table 3.10 Summary of relevant function of genes identified for slaughter weight and dressing percentage (Panther, 2018)

Gene	Molecular function	Biological process
HDAC9	• Protein kinase C binding; • NAD-dependent histone deacetylase activity (H3-K14 specific); • Histone deacetylase binding; • Metal ion binding; • Repressing transcription factor binding	• Negative regulation of transcription by RNA polymerase II; • Cellular response to insulin stimulus; • Peptidyl-lysine deacetylation; • Histone H3 deacetylation; • Histone H4 deacetylation; • Positive regulation of cell migration involved in sprouting angiogenesis
SYNE1	min binding; zyme binding; tin filament binding	lgi organization; uscle cell differentiation; toskeletal anchoring at nuclear membrane; clear matrix anchoring at nuclear membrane
NT5C3B	• Nucleotide binding; • Magnesium ion binding	• Nucleotide metabolic process; • Dephosphorylation
FKBP10	• Peptidyl-prolyl cis-trans isomerase activity; • Calcium ion binding; • Fk506 binding	• Protein peptidyl-prolyl isomerization
PPEF1	• Phosphoprotein phosphatase activity; • Iron ion binding; • Calcium ion binding; • Manganese ion binding	• Protein dephosphorylation; • Detection of stimulus involved in sensory perception
RS1	• Phosphatidylserine binding; • Phosphatidylinositol-4	• 5-bisphosphate binding; • Phosphatidylinositol-3

Figure 3.8 shows the frequencies of the biological processes and molecular functions for the genes associated with the slaughter weight and dressing percentage. The molecular functions for these genes are only catalytic (100%) and the cellular process (28.6%) occurs in most of the genes as a biological process. The other biological processes occur equally as processes for the different genes (14.3%).

a.



b.

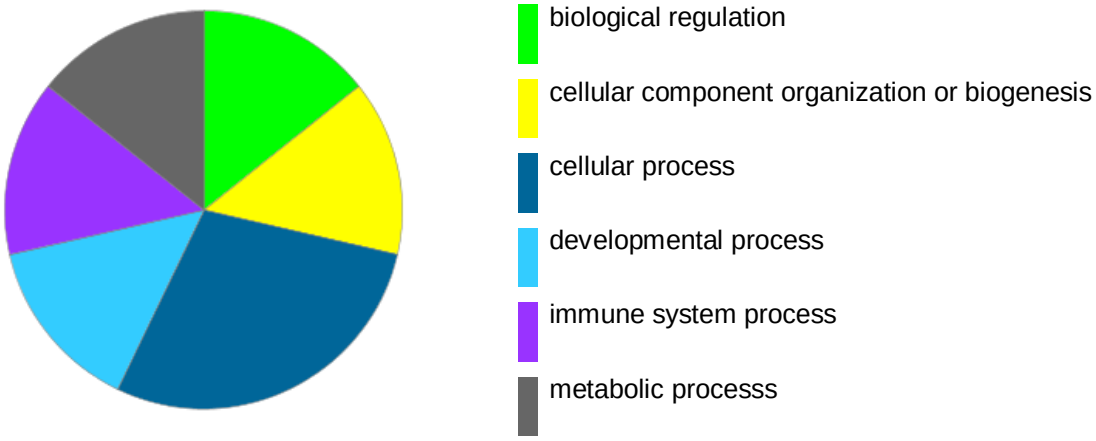


Figure 3.8 Distribution of the (a) metabolic function and (b) biological processes for slaughter weight and dressing percentage

Although seven genes have been identified for possible association with ultrasound measurements, for only five genes molecular/ biological processes were available on the Panther database. *CFAP54* gene molecular functions and biological processes was not available on the database for this gene. The *LOC107131809* gene is an uncharacterized protein and the pathway was not available. Table 3.11 shows the molecular functions and biological processes for each gene identified for the ultrasound measurements.

Table 3.11 Summary of relevant function of genes identified for ultrasound measurements (Panther, 2018)

Gene	Molecular function	Biological process
NIPA1	Magnesium ion transmembrane transporter activity	Magnesium ion transmembrane transport
DDR2	ATP Binding	• Endochondral bone growth, positive regulation of fibroblast migration; • Peptidyl-tyrosine phosphorylation; • Collagen fibril organization; • Regulation of bone mineralization; • Biomineral tissue development; • Chondrocyte proliferatio; • Collagen-activated tyrosine kinase receptor signaling pathway; • Positive regulation of osteoblast differentiation; • Positive regulation of protein kinase activity; • Protein autophosphorylation; • Positive regulation of fibroblast proliferation; • Positive regulation of DNA-binding transcription factor activity; • Positive regulation of extracellular matrix disassembly
SMYD3	• RNA polymerase II proximal promoter sequence-specific DNA binding; • RNA polymerase II complex binding; • RNA polymerase II intronic transcription regulatory region sequence-specific DNA binding; • Histone-lysine N-methyltransferase activity	• Nucleosome assembly; • Negative regulation of protein kinase activity; • Myotube cell development; • Positive regulation of peptidyl-serine phosphorylation; • Histone lysine methylation; • Establishment of protein localization; • Positive regulation of transcription by RNA polymerase II; • Cellular response to dexamethasone stimulus
DCAF15	-	• Protein ubiquitination
P2RY11	G protein-coupled purinergic nucleotide receptor activity	• Adenosine receptor signaling pathway; • Calcium-mediated signaling; • G protein-coupled purinergic nucleotide receptor signaling pathway; • Cellular response to ATP

The binding function is seen as a molecular function in most of the genes (50%). Receptor activity and signal transducer activity occurs equally (25%) as molecular function in the genes. There are many biological processes identified for the genes associated with ultrasound measurements. The biological process that occurs for most of the genes is a

metabolic process (25%). These remaining biological processes occur at the same frequency (12.5%) as a function for all genes. Figure 3.9 shows the frequency at which the molecular functions and biological processes occur for genes.

a.

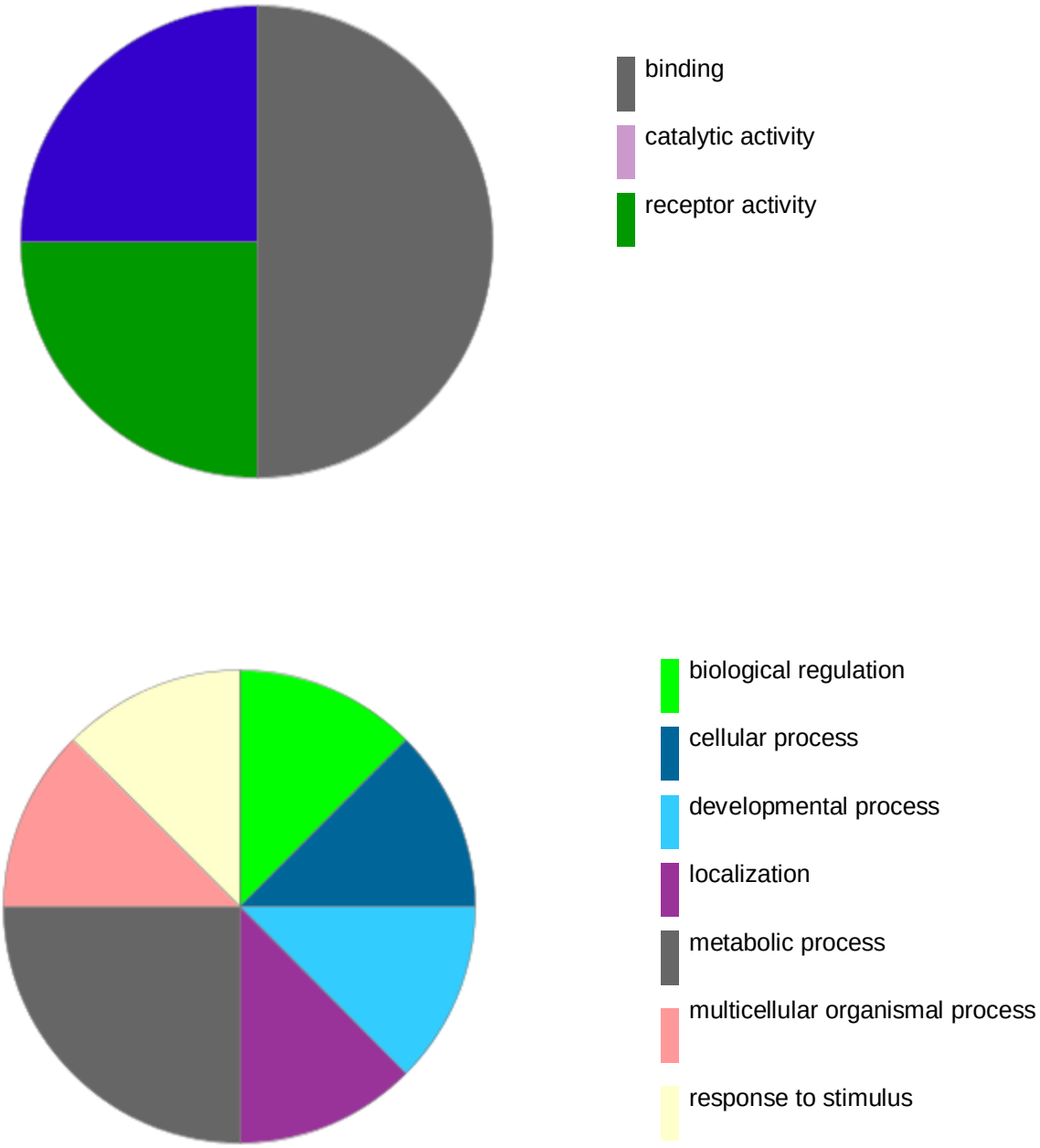


Figure 3.9 Distribution of the (a) metabolic function and (b) biological processes for ultrasound measurements

Chapter 4 Discussion

The overall aim of this study was to perform a genome wide association study (GWAS) on carcass traits in SA Nguni cattle. Carcass traits are important to beef cattle breeders, as these traits ultimately determine the yield and the quality of the end product. RTU measurements have been shown to be a non-invasive and effective selection tool (Polák *et al.,* 2007; Drennan *et al.*, 2009; Gupta *et al*., 2013). In SA beef cattle, RTU scans are not a routine tool used for prediction of carcass traits. A limited number of large stud herds perform RTU scans during intensive growth tests or on farm (SA Studbook annual report, 2016). SA Nguni cattle are not a preferred breed for feedlot finishing due to its smaller frame size and slower growth compared to breeds such as SA Bonsmara (composite) or British developed breeds and Angus cattle. Sanga types are however widely used in the developing farming sector and these farmers have interest in the potential of the breed under feedlot conditions as well as the carcass potential.

Nguni cattle in this study reached an average slaughter weight (SW) of 346.4 ± 33.2 kg at 428 days of age, which is comparable to similar studies where Nguni cattle was finished under feedlot conditions, with weights of 349.4 kg (Strydom *et al*., 2001) and 320 kg (Mapiye *et al.,* 2007) respectively. The average dressing percentage (DP) for the Nguni cattle on this trial was 56.1 ±1.7% which is slightly higher than 54.8 to 55.6 % reported for Nguni cattle, but comparable to SA studies in Bonsmara cattle (57.1 to 57.8 %) (Strydom *et al.*, 2008).

The eye muscle area (EMA) in this study (49.1 to 52.3 cm^2) was, however, lower compared to SA studies on Nguni cattle (68.1 to 70.2 cm^2) and Bonsmara cattle (75.9cm^2 to 84.8 cm^2) respectively (Strydom *et al.*, 2001; Strydom, 2008). The rump fat (RF) measurements recorded in this study (5.1 to 5.4 mm) was similar to Bonsmara cattle under feedlot conditions (5.6 mm) (Strydom *et al.*, 2008).

In this study lower SW were observed for the Nguni cattle, as expected for small framed breeds. However, despite the slower growth rate and smaller carcasses of the Nguni, the RTU results indicated that the desired carcass traits can be obtained. Research from Strydom *et al.* (2001) and Strydom *et al.* (2008) indicated that late maturing breeds, such as the Bonsmara, had higher growth rates and higher slaughter weights.

Genomic technology provides opportunity for studying genetic mechanisms for different traits and was applied in this study to provide insight on carcass traits based on RTU measurements in SA Nguni cattle. In order to study the carcass traits on a genome level a genome wide association study was performed based on genotypes generated with a Geneseek 150K GGP HD SNP chip. The first step to determine the relatedness of the animals in the population was to investigate the population stratification using principal component analysis (PCA). The tight clustering seen in the PCA plots indicated the relatedness between

the animals and the few outliers were from different herds. From this PCA it was concluded that there was no population sub-structure causing population stratification within the sample of animals.

The genetic clustering was further divided to determine whether diet and province of origin had a significant effect on the performance of these animals. The tight clustering seen on both PCA plots to investigate the effect of diet and province firstly indicated an effect for the Eastern Cape (most animals originated from) and Western Cape provinces. The effects of different diet levels and effect of province of origin was reported by Theron *et al*. (2017) based on phenotypic data. Province had a significant ($p < 0.05$) effect on the arrival weight of the calves due to the different environmental conditions between the provinces. There are different temperatures, rainfall conditions and feeding conditions between the different herds in different provinces. The Eastern Cape and Western Cape are very different climatic regions in comparison to the Free State, North West and Northern Cape. This explains the differences in arrival weight of the calves. No significant effect ($p < 0.05$) of the province on end weight, slaughter weight or the dressing percentage were observed.

The commercial diet was the only diet to have a significant effect ($p < 0.05$) on the growth of the animals and on the rump fat thickness. The different diets had no significant effect ($p < 0.05$) on the EMA and backfat thickness measured in the animals (Theron *et al*., 2017). The backfat thickness and EMA are the fat measurements that have been investigated more frequently in literature (Drennan, *et al.,* 2009).

This indicates that there is a small possibility of bias and the results from the GWAS will have no / low number of false positives. However, Dekkers & Hospital (2002) concluded a high number of false positives are more desirable than a high number of false negatives. For this study no QC was therefore applied including results from potential false positives. These may have a larger effect on traits selected for, in comparison to selection with a high number of false negatives and possible genes or chromosomal regions not included in selection.

In this study the quality control was done on an individual and marker-based level (technical and genetic QC) and two animals were removed with poor quality (<90%). Results for GWAS based only on technical QC observed suggestive SNPs for DP not observed with genetic QC. All other GWAS results based on technical and genetic QC were similar.

Li *et al.* (2017); Sasago *et al.* (2017) and Bhuiyan *et al.* (2018) reported associations with chromosomes 2, 4, 6,18 and 19 for the slaughter weight. The results from this study also found SNPs on BTA4 and BTA19, similar to the abovementioned studies. Additional SNPs were identified on chromosome nine which has not been identified in the literature for association with slaughter weight. A possible reason for this would be that the results found in previous studies are for slaughter weight applied to taurine breeds. There is also a possibility of ascertainment bias for the SNP chip used, as this was developed for Taurine breeds. The

additional SNP associations on the specific chromosomes observed in this study could be unique to the Sanga breed.

A number of QTL have been identified on chromosome 2 for EMA (CattleQTLdb, 2011). The following QTL was identified for EMA on chromosome 2 within the 5000bp window of SNP observed in this study: QTL18423, QTL11643, QTL11687, QTL11688, QTL11882, QTL2753. Four SNPs were associated with SNPs on chromosome 25 for EMA72 in this study, while no QTL has been previously reported within the same regions as the observed SNPs on chromosome 25. Further studies should be conducted to investigate the association of this chromosome with the EMA. QTL 10935 has been associated with EMA on chromosome nine, where a SNP was observed in this study (CattleQTLdb, 2011). No QTL within the regions on chromosomes 12 and 25 has been reported previously for association with EMA as observed for EMA91 in this study. Additional SNPs was observed with EMMAX software for the EMA on BTA7, BTA8, BTA9, BTA13, BTA20, BTA23. de Oliveira Silva *et al.* (2017) reported an association of EMA with chromosome 2 and chromosome 29, which support the chromosomes identified for the EMA91 in this study. Nellore cattle was used in the study and there is a possibility of the genetic architecture of these animals being more similar to Nguni cattle. Previous studies identified the following chromosomes associated with the EMA namely: BTA7, BTA8, BTA9, BTA10, BTA12, BTA13, BTA20, BTA23 in*Taurine* breeds and BTA15 for an *Indicus breed* (Saatchi *et al.*, 2014; Xia *et al.,* 2016; de Oliveira Silva *et al.*, 2017; Li *et al.*, 2017; Bhuiyan *et al.*, 2018; Hay & Roberts, 2018). Most of the SNPs observed in this study was identified in the above-mentioned chromosomes.

In this study significant associations ($p<10^{-5}$) were observed for rump fat measured at 72 days (three SNPs) and 91 days (two SNPs). QTL for fatty acids were previously identified within the regions observed in this study (CattleQTLdb, 2011). A study by de Oliveira Silva *et al.* (2017) reported associations with rump fat on BTA2, BTA5, BTA6, BTA9, BTA13, BTA14, BTA15, BTA19 and BTA20. Association of BTA5 and BTA9 with rump fat thickness supports the association identified in this study. PLINK software identified SNPs associated with the trait, whereas no SNP associations with rump fat was seen using EMMAX software. This difference between the two software programmes may be due to the differences in the methodology of each software.

Back fat thickness had more SNPs associated when the trait was measured at 91 days on test, with three SNPs for 72day measurement and six SNPs identified for 91 day measurement. QTL for backfat thickness measured at 91 days was seen on QTL10751 within the region identified by the SNP identified on BTA5 (CattleQTLdb, 2011). BTA1, BTA2, BTA7, BTA9, BTA10, BTA11, BTA14, BTA16, BTA21, BTA29 were reported as having associations with backfat thickness by Hay & Roberts (2018) and de Oliveira Silva *et al.* (2017). The associations on BTA1, BTA2 and BTA16 with backfat thickness supports the association found

in this study. In contrast, Bhuiyan *et al*. (2018) reported no identification of SNPs associated to the backfat thickness.

Allele frequencies that were calculated for the SNPs associated with the carcass traits are useful for the selection of the traits. The high frequencies of the favourable alleles as well as the high number of homozygotes indicate there has been selection for all traits. It has possibly been indirect selection that have caused fixation of the alleles for carcass traits within this population. There are fewer heterozygotes (AB) compared with the homozygotes (BB) and homozygotes (AA). Traits with high homozygosity for the AA alleles were BF91, EMA72 and rump fat 72. Nguni cattle have good meat quality (Frylinck *et al.*, 2009), and this is explained by the fixation of the favourable alleles for the carcass traits.

In this study several SNPs were identified where no genes to date have been reported in the data bank (NCBI). Several genes could be associated with the carcass traits and a brief discussion on the most applicable genes were included. All genes associated with SW and DP has a catalytic activity (100%) as the molecular function. Biological processes for the slaughter weight occur equally as cellular processes (25%), metabolic processes (25%), biological regulation (25%) and cellular component organization or biogenesis (25%).

Four genes were identified for BF. Binding function (50%) especially protein binding occurs mostly as a molecular function in all the genes involved in back fat. Molecular functions of the genes identified for EMA is binding which would explain the binding of muscle for an increase in the EMA. Metabolic processes furthermore occur mostly as the biological process for the genes identified for EMA. Only *SMYD3* gene on BTA16 was identified for the rump fat thickness measured at 72 and 91 days on test, as well as for EMA91 and BF72. Binding function occurs in this gene identified for RF.

Receptor activity and signal transducer activity occurs in only one of the fourteen genes as a molecular function. The binding function occurs the most frequently as a function of the genes associated with carcass traits. The biological process which is a function of many genes are cellular processes (20%), metabolic processes (20%) and this is followed by the developmental processes (13.3%) and biological regulation for all genes identified. The characterisation of the genes in terms of molecular function and biological processes supports the genes associated with carcass traits. The binding function could possibly indicate the binding of muscle and adipose cells relating to the slaughter weight and fat measurements (Mi *et al.*, 2017).

Many of the genes are responsible for various protein functions, mineral binding, as well as ATP binding and function. *NIPA1, P2RY11, HDAC9, NT5C3B* and *FKBP10* genes are responsible for mineral binding such as calcium binding, manganese ion binding, iron ion binding and magnesium ion activity. Calcium is an important mineral for many cellular processes, one such a process is muscle functioning. Muscle excitation and contraction by

calcium has been well established, however this mineral has a role in muscle formation, growth and regeneration. These functions are still under investigation (Tu *et al.,* 2016). This could possibly relate to carcass traits and the minerals involved in muscle formation, muscle health and contraction which influences the tenderness and meat quality.

The gene *HDAC9* is part of the histone deacetylase family. Within this family of genes there are two classes, of which this gene is part of class II. This gene is involved in the negative feedback cascade in the myogenic process (https://www.ncbi.nlm.nih.gov/gene/). Myogenesis is the muscle development during the embryonic phase where muscle regulating factors regulate the growth (Bentzinger *et al.*, 2012). Transcription factors MEF2 plays a role in early skeletal muscle development, as well as the expression of the *HDAC9* gene (Haberland *et al.*, 2007). The MEF2 transcription factors are responsible for muscle formation and the *HDAC9* controls the amount of muscle differentiation that takes place. Calcium has a role in this mechanism which influences the amount of muscle formation (Tu *et al.*, 2016). *HDAC3* which is a part of the same gene family as *HDAC9* is responsible for lipid browning (Cao *et al.*, 2017). Slaughter weight is associated with the *HDAC9* gene and the function of this gene is associated with muscle formation which would increase the slaughter weight

SMYD3 has been found to be involved as a signal transducer and Spurlock *et al.* (2014) has identified this gene as a candidate gene for feed efficiency traits in dairy cattle. In the mouse ortholog this gene is responsible to modulate myostatin and c-Met transcription of primary skeletal muscle cells (Proserpio *et al.*, 2013). C-Met is involved in the migration of the muscle satellite cells and muscle atrophy (Zou *et al.,*2009). This is important in regulating the size of the myotube and the skeletal muscle cells. The myostatin causes down-regulation of differentiation related genes such as MyoD which prevents the myogenesis process (Huang *et al.,* 2007). This gene regulates the maintenance of skeletal muscle mass and is an effector of skeletal muscle atrophy. In this study the gene is associated with the BF72, EMA91, RF72 and RF91 which can be related to the function of this gene, as muscle cell formation is finished when fat deposition starts taking place during the feedlot phase.

NIPA1 gene is responsible for magnesium transportation and magnesium is an important mineral found in muscle tissue. This mineral improves the muscle strength and performance (van Dronkelaar *et al.*, 2017). In the human ortholog this gene is responsible for muscle spastic paraplegia (Arkadir *et al.*, 2014), and recently it has been identified in a patient with epilepsy and muscle neurone disease (Tanti *et al.*, 2017). In this study the gene is associated with the BF91, which is not directly related to muscle diseases or the functioning of muscles.

SYNE1 gene is Nesprin 1 gene which is responsible for muscular dystrophy, specifically Emery-Dreifuss muscular dystrophy (EDMD) in human orthologs. This gene has a binding function and specifically binds emerin and lamins (inner nuclear membranes). The binding

enables the muscle to link the nucleo-skeleton to the inner muscle membranes (Zhang *et al.*, 2007). The *SYNE1* gene was associated with the EMA91 in this study, which could possibly relate to the functions as reported in PANTHER and Zhang *et al*., (2007). EMA is the area measured at the *longissimus thoracicus et lumborum* and functions relating to the muscle formation and the binding of muscle and fat would explain the association between this gene and the EMA.

RS1 gene is involved in the function of the retina and a mutation would lead to defects in the retina. In knockout rodents it was shown there is a difference in the function of this gene in the retina and in the pineal gland (Takada *et al.,* 2006). A different function in the pineal gland could influence the circadian rhythms in animals and indirectly influence puberty and growth. As this gene was associated with the dressing percentage in this study a growth function could be a possible explanation.

The function of the *DDR2* gene has been reported in knockout mice where the pituitary gland was significantly smaller in the mutants and this gene was found in the pituitary gland of the wild type. The knockout mice had an increased lean mass as well as a decreased body mass in comparison with the wild type (Kano *et al.*, 2008). This gene influences growth which explains the association of this gene with BF91 in this study. Further investigation into the applicability of the abovementioned genes identified in this study, to the specific traits are required.

From this study it can be concluded that there is potential for RTU measurements in feedlots to predict the carcass quality. The GWAS provided information regarding the genetics underlying carcass characteristics in Nguni cattle. Further investigation into the gene ontology for the observed SNPs associated with each trait was useful to understand the biological and metabolic processes involved with each trait.

Chapter 5 General conclusion and recommendations

5.1 Conclusion

The aim of this study was to perform a genome-wide association study for carcass traits in South African indigenous Nguni cattle based on real time ultrasound data. Real time ultrasound (RTU) measurements are an important measure to determine the carcass quality on the live animal. The use of RTU measurements are limited in all breeds in SA, especially for the Nguni cattle. These measurements should be applied in Nguni cattle, to further improve the carcass quality of this breed.

To conduct the genome-wide association study, 141 animals were genotyped with the Geneseek 150K GGP HD SNP chip. The genotyping of the animals in this study formed a part of the Bovine Genomics Project. Two data sets, with technical QC and genetic QC, were used to perform a GWAS using PLINK and EMMAX software. Results from the software identified 43 SNPs in total, from which PLINK identified 28 SNPs and EMMAX identified 15 SNPs. Seven SNPs were identified in both software programmes, which could possibly validate the SNPs associated with the traits. The data set with only technical QC identified four additional SNPs for the dressing percentage. This confirms the theory that the application of MAF and HWE could possibly exclude valuable SNPs on rare alleles with a low MAF. These SNPs could provide valuable information concerning the traits under investigation and it is concluded that only technical QC should be applied to a dataset.

The frequency of the favourable allele as well as genotypic frequencies for suggestive SNPs observed were calculated. From the results it was clear most of the frequencies of the major allele is high which indicates the possibility of indirect selection for carcass traits in the Nguni breed. Another explanation of this occurrence is all animals used in this study was slaughtered at an A2 slaughter weight. The high frequencies of the favourable alleles are beneficial as selection will be possible and genetic progress will be possible. The genotypic frequencies further supported these conclusions, as homozygous genotypes were identified for most of the SNPs. Three SNPs were identified for more than one trait under investigation which indicates the polygenic nature of these traits.

Gene ontology of the identified chromosomal regions identified 15 genes. The biological process of these genes were mostly metabolic and cellular processes which can be related to carcass traits. Genes identified had a binding function and this is responsible for meat production.

In conclusion farmers and cattle breeders should use RTU scans as a measurement of the carcass yield and quality in the feedlot. A GWAS is useful for the detection of associations between carcass traits and SNPs. The results presented here improves understanding of the

genetic mechanisms regulating the muscle tissue deposition and subcutaneous fat cover deposition of an indigenous Sanga cattle breed such as the Nguni. Novel associations with chromosomes and genes identified are possibly unique to Sanga breeds in SA and require further investigation. There are many chromosomal regions responsible for carcass traits and selection implementing this information will possible show genetic progress in Nguni cattle for carcass traits.

5.2 Recommendations

It is recommended that estimated breeding values (EBV) of the carcass traits should be included in further studies using GWAS. Further studies to validate the genes and QTL identified in this study for Nguni cattle would be recommended. It would be useful to replicate this study with a few Sanga breeds indigenous to South Africa. This would enable the validation of SNPs observed in this study for Sanga breeds as well as, identify additional genes and chromosomal regions unique to Sanga cattle in SA would also be possible.

The study should investigate growth and efficiency traits such as the average daily gain (ADG). This would possibly assist in understanding the genetic mechanisms of growth in Nguni cattle and the reason these cattle have slower growth. Comparison of the gene expression in indigenous Sanga type cattle and Taurine cattle would be recommended to understand why the Taurine breeds have better growth in the feedlot. The sample size used in the growth trial as well as the number of animals genotyped should be increased for improvement of the power and accuracy of this study.

It is further recommended that the chromosomal regions identified by the SNPs in this study should be used for selection of carcass traits in Nguni and possibly Sanga breeds. In particular SNPs identified by both software programmes should be used for selection. In future if a SNP array is developed specifically for Sanga cattle these SNPs should be included.

References

Adams, J., 2008. Sequencing the human genome: The contributions of Francis Collins and Craig Venter. *Nature Education.* 1.

Agricultural Products Standards Act, Act No. 119 of 1990. Regulations regarding the classification and marking of meat intended for sale in the Republic of South Africa. Government Notice No. R863.

ARC, 2016, Agricultural Research Council Annual Report 2016/17. Accessed: http://www.arc.agric.za/Documents/Annual%20Reports/ARC%20Annual%20Report%202016-2017.pdf

Aiello, D., Patel, K. and Lasagna, E., 2018. The myostatin gene: an overview of mechanisms of action and its relevance to livestock animals. *Animal genetics*. 49. 505 – 519.

Aken, B.L., Ayling, S., Barrell, D., Clarke, L., Curwen, V., Fairley, S., Fernandez Banet, J., Billis, K., García Girón, C., Hourlier, T. and Howe, K., 2016. The Ensembl gene annotation system. *Database*. 2016.

Ali, A.A., Khatkar, M.S., Kadarmideen, H.N. and Thomson, P.C., 2015. Additive and epistatic genome-wide association for growth and ultrasound scan measures of carcass-related traits in Brahman cattle. *Journal of Animal Breeding and Genetics*. 132, 187-197.

Atwell S, Huang YS, Vilhjalmsson BJ, Willems G, Horton M, Li Y, Meng D, Platt A, Tarone AM, Hu TT., Jiang, R., Muliyati, NW., Zhang, X., Amer, MA., Baxter, I., Brachi, B., Chory, J., Dean, C., Debieu, M., de Meaux, J., Ecker, JR., Faure, N., Kniskern, JM., Jones, JDG., Michael, T. Nemri,J., Roux, F., Salt, DE., Tang, C., Todesco, M., Traw, BM., Weigel, D., Marjoram, P., Borevitz, JO., Bergelson, J., and Nordborg M., 2010. Genome-wide association study of 107 phenotypes in Arabidopsis thaliana inbred lines. *Nature*. 465, 627–631.

Andersson, L. 2009. Genome-wide association analysis in domestic animals: a powerful approach for genetic dissection of trait loci. *Genetica*. 136, 341-349.

Arkadir, D., Noreau, A., Goldman, J.S., Rouleau, G.A. and Alcalay, R.N., 2014. Pure hereditary spastic paraplegia due to a de novo mutation in the NIPA 1 gene. *European Journal of Neurology*. 21, e2.

Arnold, J.W., Bertrand, J.K., Benyshek, L.L. and Ludwig, C., 1991. Estimates of genetic parameters for live animal ultrasound, actual carcass data, and growth traits in beef cattle. *Journal of Animal Science*. 69*,* 985-992.

Baile, C.A., Della-Fera, M.A., Yang, J.Y. and Rayalam, S., 2018. Myostatin: Physiology and Applications. *Encyclopedia of Animal Science. CRC Press.* 806-809.

Barendse, W., 2009. Genetic-based diagnostic tools for predicting meat quality. *Improving the Sensory and Nutritional Quality of Fresh Meat*. 292-317.

Batt, R.A., 1980. What are growth and development. In: Influences on animal growth and development. Ed. Arnold, E.

Bentzinger, C.F., Wang, Y.X. and Rudnicki, M.A., 2012. Building muscle: molecular regulation of myogenesis. *Cold Spring Harbor Perspectives in Biology*. 4, a008342.

Berg, R.T. and Butterfield, R.M., 1968. Growth patterns of bovine muscle, fat and bone. *Journal of Animal Science.* 27, 611-619.

Beriain, M.J., Insauti, K., and Indurain, G., 2007. Image analysis, ultrasounds and colour reflectance for beef quality estimations. In: Evaluation of carcass and meat quality in cattle and sheep. Ed. Lazzaroni, C., Gigli, S. and Gabiña, D., Wageningen Academic Pub, Netherlands, pp. 13-18.

Berry, D.P., Conroy, S., Pabiou, T. and Cromie, A.R., 2017. Animal breeding strategies can improve meat quality attributes within entire populations. *Meat Science*, 132, 6-18.

Beuzen, N.D., Stear, M.J. and Chang, K.C., 2000. Molecular markers and their use in animal breeding. *Veterinary Journal*. 160, 42-52.

Bhuiyan, M.S., Lim, D., Park, M., Lee, S., Kim, Y., Gondro, C., Park, B. and Lee, S., 2018. Functional Partitioning of Genomic Variance and Genome-Wide Association Study for Carcass Traits in Korean Hanwoo Cattle Using Imputed Sequence Level SNP Data. *Frontiers in Genetics*. 9, 1-14.

Bisschoff, C. and Lotriet, R., 2013. The Drakensberger as competitive breed of cattle in the South African beef industry. In *19th International Farm Management Congress*. 39-49.

Bolormaa, S., Neto, L.P., Zhang, Y.D., Bunch, R.J., Harrison, B.E., Goddard, M.E. and Barendse, W., 2011. A genome-wide association study of meat and carcass traits in Australian cattle. *Journal of animal science*. 89, 2297-2309.

Bolormaa, S., Pryce, J.E., Kemper, K., Savin, K., Hayes, B.J., Barendse, W., Zhang, Y., Reich, C.M., Mason, B.A., Bunch, R.J. and Harrison, B.E., 2013. Accuracy of prediction of genomic breeding values for residual feed intake and carcass and meat quality traits in Bos taurus, Bos indicus, and composite beef cattle. *Journal of animal science.* 91, 3088-3104.

Bolormaa, S., Pryce, J.E., Reverter, A., Zhang, Y., Barendse, W., Kemper, K., Tier, B., Savin, K., Hayes, B.J., and Goddard, M.E., 2014. A Multi-Trait, Meta-Analysis for Detecting Pleiotropic Polymorphisms for Stature, Fatness and Reproduction in Beef Cattle. *PLoS Genetics*. 10, 1-23.

Bonsma JC., 1980. Cross-breeding, breed creation and the genesis of the Bonsmara. In: Livestock production: a global approach. Tafelberg, Cape Town: Publishers Ltd. 126–36.

Bureš, D. and Bartoň, L., 2012. Growth performance, carcass traits and meat quality of bulls and heifers slaughtered at different ages. *Czech Journal of Animal Science*. 57, 34-43.

Bush, W.S. and Moore, J.H., 2012. Genome-wide association studies. *PLoS Computational Biology*. 8, e1002822.

Caetano, S. L., Savegnago, R. P., Boligon, A. A., Ramos, S. B., Chud, T. C. S., Lôbo, R. B., and Munari, D. P., 2013. Estimates of genetic parameters for carcass, growth and reproductive traits in Nellore cattle. *Livestock Science.* 155, 1–7.

Cao, K.X., Hao, D., Wang, J., Peng, W.W., Yan, Y.J., Cao, H.X., Sun, F. and Chen, H., 2017. Cold exposure induces the acquisition of brown adipocyte gene expression profiles in cattle inguinal fat normalized with a new set of reference genes for qRT-PCR. *Research in Veterinary Science*. 114, 1-5.

Casas, E., Shackelford, S.D., Keele, J.W., Stone, R.T., Kappes, S.M. and Koohmaraie, M., 2000. Quantitative trait loci affecting growth and carcass composition of cattle segregating alternate forms of myostatin. *Journal of animal öience*. 78, 560-569.

Casas, E., Shackelford, S.D., Keele, J.W., Koohmaraie, M., Smith, T.P.L. and Stone, R.T., 2003. Detection of quantitative trait loci for growth and carcass composition in cattle. *Journal of animal science*. 81, 2976-2983.

Casas, E., Keele, J.W., Shackelford, S.D., Koohmaraie, M. and Stone, R.T., 2004. Identification of quantitative trait loci for growth and carcass composition in cattle. *Animal Genetics*. 35, 2-6.

Casas, E., White, S.N., Riley, D.G., Smith, T.P.L., Brenneman, R.A., Olson, T.A., Johnson, D.D., Coleman, S.W., Bennett, G.L. and Chase Jr, C.C., 2005. Assessment of single nucleotide polymorphisms in genes residing on chromosomes 14 and 29 for association with carcass composition traits in Bos indicus cattle. *Journal of Animal Science*. 83, 13-19.

CattleQTLdb, 2003-2018, NAGRP - Bioinformatics Coordination Program, accessed 5 October 2018, https://www.animalgenome.org/cgi-bin/QTLdb/BT/index.

Cavanagh, J.A., Tammen, I., Windsor, P.A., Bateman, J.F., Savarirayan, R., Nicholas, F.W. and Raadsma, H.W., 2007. Bulldog dwarfism in Dexter cattle is caused by mutations in ACAN. *Mammalian Genome*. 18, 808-814.

Ceacero, T.M., Mercadante, M.E.Z., Cyrillo, J.N.D.S.G., Canesin, R.C., Bonilha, S.F.M. and de Albuquerque, L.G., 2016. Phenotypic and genetic correlations of feed efficiency traits with growth and carcass traits in Nellore cattle selected for postweaning weight. *PloS one*. 11, e0161366.

Charlier, C., Coppieters, W., Farnir, F., Grobet, L., Leroy, P.L., Michaux, C., Mni, M., Schwers, A., Vanmanshoven, P., Hanset, R. and Georges, M., 1995. The mh gene causing double-muscling in cattle maps to bovine chromosome 2. *Mammalian Genome*. 6, 788-792.

Crews, D.H. and Carstens, G.E., 2012. Measuring individual feed intake and utilization in growing cattle. In: Feed efficiency in the beef industry, Ed. Hill, R.A., John Wiley & Sons, Oxford, UK, pp. 21-28.

DAFF, 2017. Abstract of Agricultural statistics 2017, Department of Agriculture Forestry and Fisheries, South Africa. 51–67.

Dawkins, M.S., 2017. Animal welfare and efficient farming: is conflict inevitable?. *Animal Production Science.* 57*,* 201-208.

de Oliveira, H.R., Ventura, H.T., Costa, E.V., Pereira, M.A., Veroneze, R., de Souza Duarte, M. and e Silva, F.F., 2018. Meta-analysis of genetic-parameter estimates for reproduction, growth and carcass traits in Nellore cattle by using a random-effects model. *Animal Production Science*. 58, 1575-1583.

de Oliveira Silva, R.M., Stafuzza, N.B., de Oliveira Fragomeni, B., de Camargo, G.M.F., Ceacero, T.M., Cyrillo, J.N.D.S.G., Baldi, F., Boligon, A.A., Mercadante, M.E.Z., Lourenco, D.L. and Misztal, I., 2017. Genome-wide association study for carcass traits in an experimental Nelore cattle population. *PloS one*. 12, 1-10.

Dekkers, J.C. and Hospital, F., 2002. Multifactorial genetics: The use of molecular genetics in the improvement of agricultural populations. *Nature Reviews Genetics*. *3*, 22-32.

Devitt, G. J. B., and Wilton, J. W. 2001. Genetic correlation estimates between ultrasound measurements on yearling bulls and carcass measurements on finished steers. *Journal of Animal Science*. 79, 2790–2797

Drennan MJ, McGee M, Conroy SB, Keane MG, Kenny DA, and Berry DP, 2009. The relationship between various live animal scores/measurements and carcass classification for conformation and fatness with meat yield and distribution, and ultimate carcass value. *Beef Production Series*. 89, 1-40.

Eggen, A., 2012. The development and application of genomic selection as a new breeding paradigm. *Animal Frontiers*. 2, 10-15.

Elsik CG, Unni DR, Diesh CM, Tayal A, Emery ML, Nguyen HN, Hagen DE., 2016. Bovine Genome Database: new tools for gleaning function from the Bos taurus genome. *Nucleic Acids Research*. 44, 834-9

Esterhuizen, J., Groenewald, I.B., Strydom, P.E. and Hugo, A., 2008. The performance and meat quality of Bonsmara steers raised in a feedlot, on conventional pastures or on organic pastures. *South African Journal of Animal Science*. 38, 303-314.

Fan, B., Du, Z-Q., Gorbach, D.M. and Rothschild, M.F., 2010. Development and application of high-density SNP arrays in genomic studies of domestic animals. *Asian-Australian Journal of Animal Science.* 7, 833-847.

Flint, J., and Eskin, E., 2012. Genome-wide association studies in mice. *Natural Revolutionary Genetics*. 13, 807–817.

Fore, J., Wiechers, I.R. and Cook-Deegan, R., 2006. The effects of business practices, licensing, and intellectual property on development and dissemination of the polymerase chain reaction: case study. *Journal of Biomedical Discovery and Collaboration*. *1,* 7-24.

Frylinck, L., Van Wyk, G.L., Smith, T.P.L., Strydom, P.E., van Marle-Köster, E., Webb, E.C., Koohmaraie, M. and Smith, M.F., 2009. Evaluation of biochemical parameters and genetic markers for association with meat tenderness in South African feedlot cattle. *Meat Science*. 83, 657-665.

Garnett, T., Appleby, M.C., Balmford, A., Bateman, I.J., Benton, T.G., Bloomer, P., Burlingame, B., Dawkins, M., Dolan, L., Fraser, D. and Herrero, M., 2013. Sustainable intensification in agriculture: premises and policies. *Science*, 341*,* 33-34.

Greiner, S.P., Rouse, G.H., Wilson, D.E., Cundiff, L.V. and Wheeler, T.L., 2003. The relationship between ultrasound measurements and carcass fat thickness and longissimus muscle area in beef cattle. *Journal of Animal Science*. 81, 676-682.

Grosse, W.M., Kappes, S.M., Laegreid, W.W., Keele, J.W., Chitko-McKown, C.G. and Heaton, M.P., 1999. Single nucleotide polymorphism (SNP) discovery and linkage mapping of bovine cytokine genes. *Mammalian Genome*. 10, 1062-1069.

Guenther, J.J., Bushman, D.H., Pope, L.S. and Morrison, R.D., 1965. Growth and development of the major carcass tissues in beef calves from weaning to slaughter weight, with reference to the effect of plane of nutrition. *Journal of Animal Science*. *24,* 1184-1191.

Gupta, S., Kumar, A., Kumar, S., Bhat, Z. F., Hakeem, H. R., and Abrol, A. P. S., 2013. Recent trends in carcass evaluation techniques - a review. *Journal of Meat Science Technology*. 1, 50–55.

Gurgul, A., Semik, E., Pawlina, K., Szmatoła, T., Jasielczuk, I. and Bugno-Poniewierska, M., 2014. The application of genome-wide SNP genotyping methods in studies on livestock genomes. *Journal of Applied Genetics*. *55*, 197-208.

Haberland, M., Arnold, M.A., McAnally, J., Phan, D., Kim, Y. and Olson, E.N., 2007. Regulation of HDAC9 gene expression by MEF2 establishes a negative-feedback loop in the transcriptional circuitry of muscle differentiation. *Molecular and Cellular Biology*. 27, 518-525.

Hall, N., Schonfeldt, H.C. and Pretorius, B., 2015. Changes in the composition of South African red meat. *South African Journal of Animal Science*. 45, 325-338.

Hay, EH., and Roberts, A., 2018. Genome-wide association study for carcass traits in a composite beef cattle breed. *Livestock Science.* 213, 35-43.

Hayes, B.J., and Goddard, M.E., 2001. The distribution of the effects of genes affecting quantitative traits in livestock. *Genetics Selection Evolution*. *33*, 209.

Hayes, B.J., Bowman, P.J., Chamberlain, A.J. and Goddard, M.E., 2009. Invited review: Genomic selection in dairy cattle: Progress and challenges. *Journal of dairy science*. *92*, 433-443.

Hayes, B.J., and Goddard, M., 2010. Genome-wide association and genomic selection in animal breeding. *Genome*. 53, 876-883.

Hayes, B., 2013. Overview of statistical methods for genome-wide association studies (GWAS). In Genome-wide association studies and genomic prediction. Ed. Gondoro, C., van der Werf, J., Hayes, B., Springer Humana, London, pp. 149-169.

Hayes, B.J., Lewin, H.A. and Goddard, M.E., 2013. The future of livestock breeding: genomic selection for efficiency, reduced emissions intensity, and adaptation. *Trends in Genetics*. *29,* 206-214.

Hicks, C., 2011. Using Live Animal Carcass Ultrasound in Beef Cattle, *Bulletin*. 1337, 1-4.

Hill, W.G., and Robertson, A., 1968. Linkage disequilibrium in finite populations. *Theoretical and Applied Genetics.* 38, 226–231

Hill, W.G., 2014. Applications of population genetics to animal breeding, from Wright, Fisher and Lush to genomic prediction. *Genetics*. 196, 1-16.

Hirschhorn, J.N. and Daly, M.J., 2005. Genome-wide association studies for common diseases and complex traits. *Nature Reviews Genetics*. 6, 95.

Hocquette, J.f., Renand, G., Dufour, E., Lepetit, J., and Nute, G.R.., 2007. Traditional and new methods to assess beef quality. In: Evaluation of carcass and meat quality in cattle and sheep. Ed. Lazzaroni, C., Gigli, S. and Gabiña, D., Wageningen Academic Pub, Netherlands, pp. 13-18.

Hossner, K.L., 2005. Development of Muscle, Skeletal System and Adipose Tissue. In: Hormonal regulation of farm animal growth. CABI, Oxfordshire, UK, pp. 55-93.

Huang, Z., Chen, D., Zhang, K., Yu, B., Chen, X. and Meng, J., 2007. Regulation of myostatin signaling by c-Jun N-terminal kinase in C2C12 cells. *Cellular signalling*. 19, 2286-2295.

Huntley, R.P., Sawford, T., Mutowo-Meullenet, P., Shypitsyna, A., Bonilla, C., Martin, M.J. and O'donovan, C., 2014. The GOA database: gene ontology annotation updates for 2015. *Nucleic Acids Research*. 43, D1057-D1063.

Ilea, R.C., 2009. Intensive livestock farming: Global trends, increased environmental concerns, and ethical solutions. *Journal of Agriculture and Environmental Ethics.* 22, 153-167.

Kallweit, E., 1992. Measurement of body, carcass, and tissue composition in meat animals by non-invasive methods. *Pig News and Information*. 13, 147N-148N.

Kang, H.M., Sul, J.H., Service, S.K., Zaitlen, N.A., Kong, S.Y., Freimer, N.B., Sabatti, C. and Eskin, E., 2010. Variance component model to account for sample structure in genome-wide association studies. *Nature Genetics*. 42, 348-366.

Kano, K., Marin de Evsikova, C., Young, J., Wnek, C., Maddatu, T.P., Nishina, P.M. and Naggert, J.K., 2008. A novel dwarfism with gonadal dysfunction due to loss-of-function allele of the collagen receptor gene, Ddr2, in the mouse. *Molecular Endocrinology*. 22, 1866-1880.

Kause, A., Mikkola, L., Strandén, I., Sirkko, K. 2015. Genetic parameters for carcass weight, conformation and fat in five beef cattle breeds. *Animal.* 9, 35–42

Kim J.J., Farnir F., Savell J. and Taylor J.F., 2003. Detection of quantitative trait loci for growth and beef carcass fatness traits in a cross between Bos taurus (Angus) and Bos indicus (Brahman) cattle. *Journal of Animal Science*. 81, 1933–1942.

Kim, Y., Ryu, J., Woo, J., Kim, J.B., Kim, C.Y. and Lee, C., 2011. Genome-wide association study reveals five nucleotide sequence variants for carcass traits in beef cattle. *Animal Genetics*, *42*, 361-365.

Koots, K. R., J. P. Gibson, C. Smith, and J. W. Wilton. 1994. Analyses of published genetic parameter estimates for beef production traits. 1. Heritability. *Animal Breeding Abstracts*. 62, 309−338.

Korte, A. and Farlow, A., 2013. The advantages and limitations of trait analysis with GWAS: a review. *Plant Methods*. 9, .29-38.

Lander, E.S. and Botstein, D., 1989. Mapping mendelian factors underlying quantitative traits using RFLP linkage maps. *Genetics*. 121, 185-199.

Lazzaroni, C., 2007. Evaluation of carcass and meat quality in the EAAP. In: Evaluation of carcass and meat quality in cattle and sheep. Ed. Lazzaroni, C., Gigli, S. and Gabiña, D., Wageningen Academic Pub, Netherlands, pp. 13-18.

Leal-Gutiérrez, J.D., Elzo, M.A., Johnson, D.D., Scheffler, T.L., Scheffler, J.M. and Mateescu, R.G., 2018. Association of μ-Calpain and Calpastatin Polymorphisms with Meat Tenderness in a Brahman–Angus Population. *Frontiers in Genetics. 9*, 56-66.

Lee, S.H., Choi, B.H., Lim, D., Gondro, C., Cho, Y.M., Dang, C.G., Sharma, A., Jang, G.W., Lee, K.T., Yoon, D. and Lee, H.K., 2013. Genome-wide association study identifies major loci for carcass weight on BTA14 in Hanwoo (Korean cattle). *PloS one. 8,* e74677.

Li, Y., Gao, Y., Kim, Y.S., Iqbal, A. and Kim, J.J., 2017. A whole genome association study to detect additive and dominant single nucleotide polymorphisms for growth and carcass traits in Korean native cattle, Hanwoo. *Asian-Australasian Journal of Animal Sciences*. 30, 8-19.

Lipkin, E., M. O. Mosig, A. Darvasi, E. Ezra, A. Shalom, A. Friedmann, and M. Soller. 1988. Quantitative trait locus mapping in dairy cattle by means of selective milk DNA pooling using dinucleotide microsatellite markers: Analysis of milk protein percentage. *Genetics*. 149, 1557-1567.

Lopes, F. B., Corr, M., and Magnabosco, C. U. 2016. Selection Indices and Multivariate Analysis Show Similar Results in the Evaluation of Growth and Carcass Traits in Beef Cattle, *Plos one.* 11, 1–21.

MacNeil, M.D. and Northcutt, S.L., 2008. National cattle evaluation system for combined analysis of carcass characteristics and indicator traits recorded by using ultrasound in Angus cattle. *Journal of Animal Science.* 86, 2518 – 2524.

Makina, S.O., Whitacre, L.K., Decker, J.E., Taylor, J.F., MacNeil, M.D., Scholtz, M.M., Marle-Köster, E., Muchadeyi, F.C., Makgahlela, M.L. and Maiwashe, A., 2016. Insight into the genetic composition of South African Sanga cattle using SNP data from cattle breeds worldwide. *Genetics Selection Evolution*. *48*, 88-95.

Mann, N.J., 2018. A brief history of meat in the human diet and current health implications. *Meat Science*. 144, 169-179.

Mapiye, C., Chimonyo, M., Muchenje, V., Dzama, K., Munyaradzi, C., and Raats, J. G. 2007. Potential for value-addition of Nguni cattle products in the communal areas of South Africa: a review. *African Journal of Agricultural Research.* 2, 488–495.

Mateescu, R.G., 2014. Genetics of meat quality. In: The genetics of cattle. Ed. Garrick, D. and Ruvinsky, A, CABI, UK Croyden, pp.544-570.

Matukumalli, L.K., Lawley, C.T., Schnabel, R.D., Taylor, J.F., Allan, M.F., Heaton, M.P., O'connell, J., Moore, S.S., Smith, T.P., Sonstegard, T.S. and Van Tassell, C.P., 2009. Development and characterization of a high-density SNP genotyping assay for cattle. *PloS one*. 4, e5350.

McClure, M. C., Sonstegard, T. S., Wiggans, G. R., Van Eenennaam, A. L., Weber, K. L., Penedo, C. T., Berry, D., Flynn, J., Garcia, J.F., Carmo, A.S. and Regitano, L.C., 2013. Imputation of microsatellite alleles from dense SNP genotypes for parentage verification across multiple Bos taurus and Bos indicus breeds. *Frontiers in Genetics*. 4, 1–11.

Menesatti, P., Andrea, S.D., and Costa, C., 2007. Spectral and thermo imaging for meat quality evaluations. In: Evaluation of carcass and meat quality in cattle and sheep. Ed. Lazzaroni, C., Gigli, S. and Gabiña, D., Wageningen Academic Pub, Netherlands, pp. 13-18.

Meissner, H.H., Scholtz, M.M. and Palmer, A.R., 2013. Sustainability of the South African livestock sector towards 2050 Part 1: Worth and impact of the sector. *South African Journal of Animal Science*. 43, 282-297.

Meuwissen, T. H. E., and M. E. Goddard, 2000 Fine mapping of quantitative trait loci using linkage disequilibria with closely linked marker loci. *Genetics*. 155, 421–430

Meuwissen T.H.E, Hayes B, and Goddard ME, 2001. Prediction of total genetic value using genome-wide dense marker maps. *Genetics*. 157, 1819–1829

Meuwissen, T.H.E., Luan, T. and Woolliams, J.A., 2011. The unified approach to the use of genomic and pedigree information in genomic evaluations revisited. *Journal of Animal Breeding and Genetics*. 128, 429-439.

Meuwissen, T.H.E, Hayes, B. and Goddard, M., 2016. Genomic selection: A paradigm shift in animal breeding. *Animal frontiers*. 6, 6-14.

Mi, H, Huang, X., Muruganujan, A., Tang, H., Mills, C., Kang, D. and Thomas, P.D., 2017. PANTHER version 11: expanded annotation data from Gene Ontology and Reactome pathways, and data analysis tool enhancements. *Nucleic Acids Res*earch, 45, D183-D189.

Miar, Y., Plastow, G.S., Bruce, H.L., Moore, S.S., Durunna, O.N., Nkrumah, J.D. and Wang, Z., 2014. Estimation of genetic and phenotypic parameters for ultrasound and carcass merit traits in crossbred beef cattle. *Canadian Journal of Animal Science*. *94,* 273-280.

Moloney, A.P. and McGee, M., 2017. Factors Influencing the Growth of Meat Animals. In: Lawrie´ s Meat Science, Ed. Toldra, F., Woodhead Publishing, Cambridge, pp. 19-47.

Mullis, K., Faloona, F., Scharf, S., Saiki, R.K., Horn, G.T. and Erlich, H., 1986. Specific enzymatic amplification of DNA in vitro: the polymerase chain reaction. *Cold Spring Harbor Symposia on QuantitativeBbiology,* 51, 263-27.

Musemwa, L., Mushunje, A., Chimonyo, M., Fraser, G., Mapiye, C. and Muchenje, V., 2008. Nguni cattle marketing constraints and opportunities in the communal areas of South Africa. *African Journal of Agricultural Research*. 3*,* 239-245.

Neimann-Soressen, A., and Robertson, A., 1961. The association between blood groups and several production characters in three Danish cattle breeds. *Acta Agriculturae Scandinavica*. 11, 163,196.

Nicolazzi, E.L., Caprera, A., Nazzicari, N., Cozzi, P., Strozzi, F., Lawley, C., Pirani, A., Soans, C., Brew, F., Jorjani, H. and Evans, G., 2015. SNPchiMp v. 3: integrating and standardizing single nucleotide polymorphism data for livestock species. *BMC genomics*. 16, 283.

Nkrumah, J.D., Crews Jr, D.H., Basarab, J.A., Price, M.A., Okine, E.K., Wang, Z., Li, C. and Moore, S.S., 2007. Genetic and phenotypic relationships of feeding behavior and temperament with performance, feed efficiency, ultrasound, and carcass merit of beef cattle. *Journal of Animal Science*. 85*,* 2382-2390.

Olsen, H.G., Hayes, B.J., Kent, M.P., Nome, T., Svendsen, M., Larsgard, A.G., and Lien S., 2011. Genome-wide association mapping in Norwegian Red cattle identifies quantitative trait loci for fertility and milk production on BTA12. *Animal Genetics*. 42, 466–474.

Owens, F.N., Gill, D.R., Secrist, D.S. and Coleman, S.W., 1995. Review of some aspects of growth and development of feedlot cattle. *Journal of Animal Science*. 73, 3152-3172.

Pausch, H., Wang, X., Jung, S., Krogmeier, D., Edel, C., Emmerling, R., Götz, K.U. and Fries, R., 2012. Identification of QTL for UV-protective eye area pigmentation in cattle by progeny phenotyping and genome-wide association analysis. *PloS one*. 7*,* e36346.

Park, H., Seo, S., Min, C.Y., Oh, S.J., seong, H., Lee, S.H. and Lim, D., 2012. Identification of Candidate genes associated with beef marbling using QTL and Pathway analysis in Hanwoo (Korean cattle). *Asian-Australian Journal of Animal Science*. 5, 613-620.

Picard, B., Lebret, B., Cassar-Malek, I., Liaubet, L., Berri, C., Le Bihan-Duval, E., Hocquette, J.F. and Renand, G., 2015. Recent advances in omic technologies for meat quality management. *Meat Science*. 109, 18-26.

Plastow, G.S., 2016. Genomics to benefit livestock production: improving animal health. *Revista Brasileira de Zootecnia.* 45*,* 349-354.

Polák, P., Daňo, J., Oravcová, M., and Krupa, E., 2007. The use of ultrasound measurements in estimation of valuable cut content in fattened bulls. In: Evaluation of carcass and meat quality in cattle and sheep. Ed. Lazzaroni, C., Gigli, S. and Gabiña, D., Wageningen Academic Pub, Netherlands, pp. 13-18.

Pollak, E.J., 2005. Application and impact of new genetic technologies on beef cattle breeding: a 'real world'perspective. *Australian Journal of Experimental Agriculture*. 45*,* 739-748.

Proserpio, V., Fittipaldi, R., Ryall, J.G., Sartorelli, V. and Caretti, G., 2013. The methyltransferase SMYD3 mediates the recruitment of transcriptional cofactors at the myostatin and c-Met genes and regulates skeletal muscle atrophy. *Genes and Development*. 27, 1299-1312.

Purcell, S., Neale, B., and Todd-Brown, K., 2007. PLINK: a tool set for whole-genome association and population-based linkage analyses*. American Journal of Human Genetics*. 81, 559–575.

Realini, C.E., Williams, R.E., Pringle, T.D. and Bertrand, J.K., 2001. Gluteus medius and rump fat depths as additional live animal ultrasound measurements for predicting retail product and trimmable fat in beef carcasses. *Journal of Animal Science*. 79, 1378-1385.

Rege, J.E.O., 1999. The state of African cattle genetic resources I. Classification framework and identification of threatened and extinct breeds. *Animal Genetic Resources*. 25, 1-25.

Rentería, M.E., Cortes, A. and Medland, S.E., 2013. Using PLINK for genome-wide association studies (GWAS) and data analysis. In: Genome-Wide Association Studies and Genomic Prediction. Ed. Gondoro, C., van der Werf, J., Hayes, B., Springer Humana, London, pp. 193-213.

Reverter, A., Johnston, D.J., Ferguson, D.M., Perry, D., Goddard, M.E., Burrow, H.M., Oddy, V.H., Thompson, J.M. and Bindon, B.M., 2003. Genetic and phenotypic characterisation of animal, carcass, and meat quality traits from temperate and tropically adapted beef

breeds. 4. Correlations among animal, carcass, and meat quality traits. *Australian Journal of Agricultural Research*. 54, 149-158.

Reverter, A., Johnston, D.J., Graser, H.U., Wolcott, M.L. and Upton, W.H., 2000. Genetic analyses of live-animal ultrasound and abattoir carcass traits in Australian Angus and Hereford cattle. *Journal of Animal Science*. 78, 786-1795.

Ribeiro, F.R.B. and Tedeschi, L.O., 2012. Using real-time ultrasound and carcass measurements to estimate total internal fat in beef cattle over different breed types and managements. *Journal of Animal Science*. 90, 3259-3265.

Ribeiro, F.R.B., Thompson, A.J., Aragon, S.N., Hosford, A.D., Hergenreder, J.E., Jennings, M.A. and Johnson, B.J., 2014. Comparison of real-time ultrasound measurements for body composition traits to carcass and camera data in feedlot steers. *The Professional Animal Scientist*. 30, 597-601.

Robinson, D.L. and Oddy, V.H., 2004. Genetic parameters for feed efficiency, fatness, muscle area and feeding behaviour of feedlot finished beef cattle. *Livestock Production Science*. 90*,* 255-270.

Saatchi, M., Beever, J.E., Decker, J.E., Faulkner, D.B., Freetly, H.C., Hansen, S.L., Yampara-Iquise, H., Johnson, K.A., Kachman, S.D., Kerley, M.S. and Kim, J., 2014. QTLs associated with dry matter intake, metabolic mid-test weight, growth and feed efficiency have little overlap across 4 beef cattle studies. *BMC genomics.* 15, 1004-1018.

Saatchi, M., McClure, M.C., McKay, S.D., Rolf, M.M., Kim, J., Decker, J.E., Taxis, T.M., Chapple, R.H., Ramey, H.R., Northcutt, S.L. and Bauck, S., 2011. Accuracies of genomic breeding values in American Angus beef cattle using K-means clustering for cross-validation. *Genetics Selection Evolution*. 43, 40-56.

SAMIC, 2006. Classification of red meat. Retrieved on 3 October 2018 from www.samic.co.za.

Santiago, G.G., Siqueira, F., Cardoso, F.F., Regitano, L.C.A., Ventura, R., Sollero, B.P., Souza, M.D., Mokry, F.B., Ferreira, A.B.R. and Torres, R.A.A., 2017. Genomewide association study for production and meat quality traits in Canchim beef cattle. *Journal of Animal Science*. 95, 3381-3390.

Sasago, N., Abe, T., Sakuma, H., Kojima, T. and Uemoto, Y., 2017. Genome-wide association study for carcass traits, fatty acid composition, chemical composition, sugar, and the effects of related candidate genes in Japanese Black cattle. *Animal Science Journal*. 88, 33-44.

SA Stud Book Annual Report (2016). SA Stud Book Annual Report. Bloemfontein. Available online at: http:// www.sastudbook.co.za

Schaeffer, L. R., 2006 Strategy for applying genome-wide selection in dairy cattle. *Journal of Animal Breeding and Genetics*. 123, 218–223.

Schoeman, S.J., 1989. Recent research into the production potential of indigenous cattle with special reference to the Sanga. *South African Journal of Animal Science*. 19, 55-61.

Scholtz, M.M., 1988. Selection possibilities of hardy beef breeds in Africa: The Nguni example. In *3. Congres Mondial de Reproduction et Selection des Ovins et Bovins a Viande,* Paris (France), 19-23 Jun 1988. INRA.

Scholtz, M.M., Bester, J., Mamabolo, J.M. and Ramsay, K.A., 2008. Results of the national cattle survey undertaken in South Africa, with emphasis on beef. *Applied Animal Husbandry in Rural Development.* 1, 1-9.

Scholtz, M.M., Gertenbach, W. and Hallowell, G., 2011. The Nguni breed of cattle: past, present and future. Pretoria: The Nguni Cattle Breeders' Society.

Scholz, A.M., Bünger, L., Kongsro, J., Baulain, U. and Mitchell, A.D., 2015. Non-invasive methods for the determination of body and carcass composition in livestock: dual-energy X-ray absorptiometry, computed tomography, magnetic resonance imaging and ultrasound: invited review. *Animal*. 9*,* 1250-1264.

Schönfeldt, H.C. and Gibson, N., 2008. Changes in the nutrient quality of meat in an obesity context. *Meat Science*. 80*,* 20-27.

Schönfeldt, H.C. and Strydom, P.E., 2011. Effect of age and cut on cooking loss, juiciness and flavour of South African beef. *Meat science*. 87*,* 180-190.

Seabury, C.M., Oldeschulte, D.L., Saatchi, M., Beever, J.E., Decker, J.E., Halley, Y.A., Bhattarai, E.K., Molaei, M., Freetly, H.C., Hansen, S.L. and Yampara-Iquise, H., 2017. Genome-wide association study for feed efficiency and growth traits in US beef cattle. *BMC Genomics*. 18*,* 386-411.

Seroba, M.M., Maiwashe, A., Nephawe, K.A. and Norris, D., 2011. Genetic parameter estimates for live animal ultrasound measures of carcass traits in South African Angus cattle. South African *Journal of Animal Science*. 41, 243 – 249.

Sharma, A., Lee, J.S., Dang, C.G., Sudrajad, P., Kim, H.C., Yeon, S.H., Kang, H.S. and Lee, S.H., 2015. Stories and challenges of genome wide association studies in livestock—A review. *Asian-Australasian Journal of Animal Sciences*. 28, 1371.

Shen, X., Kan, S., Liu, Z., Lu, G., Zhang, X., Chen, Y. and Bai, Y., 2017. EVA1A inhibits GBM cell proliferation by inducing autophagy and apoptosis. *Experimental Cell Research*. 352, 130-138.

Smith, T. P. L., Casas, E., Rexroad III, C.E., Kappes, S.M., and Keele, J. W., 2000. Bovine CAPN1 maps to a region of BTA29 containing a quantitative trait locus for meat tenderness. *Journal Animal of Science*. 78, 2589–2594.

Soji, Z., Chikwanda, D., Jaja, I.F., Mushonga, B. and Muchenje, V., 2015. Relevance of the formal red meat classification system to the South African informal livestock sector. *South African Journal of Animal Science*. 45, 263-277.

Sorbolini, S., Bongiorni, S., Cellesi, M., Gaspa, G., Dimauro, C., Valentini, A. and Macciotta, N.P.P., 2017. Genome wide association study on beef production traits in Marchigiana cattle breed. *Journal of Animal Breeding and Genetics*. 134*,* 43-48.

Spurlock, D.M., Tempelman, R.J., Weigel, K.A., Veerkamp, R.F. and de Haas, Y., 2014. Genetic architecture and biological basis of feed efficiency in dairy cattle. *Proceedings 10th World Congress of Genetics Applied to Livestock Production*. 16-21 August 2014, Vancouver.

StatsSA, 2018. Mid-year population estimates 2018, Statistics, South Africa.

Stone, R.T., Keele, J.W., Shackelford, S.D., Kappes, S.M. and Koohmaraie, M., 1999. A primary screen of the bovine genome for quantitative trait loci affecting carcass and growth traits. *Journal of Animal Science*. 77,1379-1384.

Strydom, P. E., Naudé, R. T., Smith, M. F., Kotzé, A., Scholtz, M. M., and Van Wyk, J. B. 2001. Relationships between production and product traits in subpopulations of Bonsmara and Nguni cattle. *South African Journal of Animal Science*. 31, 181–194.

Strydom, P.E., 2008. Do indigenous Southern African cattle breeds have the right genetics for commercial production of quality meat?. *Meat Science*. 80*,* 86-93.

Strydom, P.E., Frylinck, L., Van der Westhuizen, J. and Burrow, H.M., 2008. Growth performance, feed efficiency and carcass and meat quality of tropically adapted breed types from different farming systems in South Africa. *Australian Journal of Experimental Agriculture*. 48, 599-607.

Strydom, P.E., Frylinck, L., Montgomery, J.L. and Smith, M.F., 2009. The comparison of three β-agonists for growth performance, carcass characteristics and meat quality of feedlot cattle. *Meat Science*. 81*,* 557-564.

Strydom, P.E., 2011. Quality related principles of the South African beef classification system in relation to grading and classification systems of the world. *South African Journal of Animal Science*. 41,177-193.

Strydom, P.E., 2016. Performance-enhancing technologies of beef production. *Animal Frontiers*. 6, 22-30.

Su, H., Golden, B., Hyde, L., Sanders, S. and Garrick, D., 2017. Genetic parameters for carcass and ultrasound traits in Hereford and admixed Simmental beef cattle: Accuracy of evaluating carcass traits. *Journal of Animal Science*. 95, 4718-4727.

Takada, Y., Fariss, R.N., Muller, M., Bush, R.A., Rushing, E.J. and Sieving, P.A., 2006. Retinoschisin expression and localization in rodent and human pineal and consequences of mouse RS1 gene knockout. *Molecular Vision*. 12, 1108-1116.

Tanti, M., Mirza, N. and Young, C., 2017. Hereditary spastic paraplegia, epilepsy and motor neurone disease in a family with nipa1 mutation. *Journal of Neurology, Neurosurgery and Psychiatry*. 88, A61.

Tautz, D., 1989. Hypervariabflity of simple sequences as a general source for polymorphic DNA markers. *Nucleic Acids Research*. 17, 6463-6471.

Temple, R.S., 1956. Ultrasonic and conductivity methods for estimating fat thickness in live cattle. *Procedings of the Western Section of American Society for Animal Production.* 7, 477-481.

The Bovine Genome Sequencing and Analysis Consortium, Elsik, C.G., and Tellam, R.L., Worley, K.C., 2009. The genome sequence of Taurine cattle: A window to ruminant biology and evolution. *Science*. 324, 522–528

Theron, H., van der Westhuizen, J. and Venter, Y., 2017. Nguni feedlot project report.

Thonney, M.L., 2014 Genetics of growth and body composition. In: The Genetics of Cattle. Ed. Garrick, D. and Ruvinsky, A, CABI, UK Croyden, 523-543.

Tu, M.K., Levin, J.B., Hamilton, A.M. and Borodinsky, L.N., 2016. Calcium signaling in skeletal muscle development, maintenance and regeneration. *Cell Calcium*. 59, 91-97.

Uitterlinden, A.G., 2016. An introduction to genome-wide association studies: GWAS for dummies. *Seminars in Reproductive Medicine*. 34, 196-204

van Arendonk, J.A.M., Bink, M.C., Bijma, P., Bovenhuis, H., De Koning, D.J. and Brascamp, E.W., 1999. Use of phenotypic and molecular data for genetic evaluation of livestock. *From Jay L. Lush to genomics: Visions for Animal Breeding and Genetics*. 60-69.

van Dronkelaar, C., van Velzen, A., Abdelrazek, M., van der Steen, A., Weijs, P.J. and Tieland, M., 2017. Minerals and sarcopenia; the role of calcium, iron, magnesium, phosphorus, potassium, selenium, sodium, and zinc on muscle mass, muscle strength, and physical performance in older adults: a systematic review. *Journal of the American Medical Directors Association*. 1, 6-11.

Van Eenennaam, A., 2006. What is the future of animal biotechnology?. *California Agriculture*. 60, 132-139.

Van Eenennaam, A., 2010. DNA-based biotechnologies. *Beef Sire Selection Manual.* 68-78.

Van Eenennaam, A.L. and Drake, D.J., 2012. Where in the beef-cattle supply chain might DNA tests generate value?. *Animal Production Science*. 52, 185-196.

van Marle-Köster, E., Mostert, B.E. and van der Westhuizen, J., 2000. Body measurements as selection criteria for growth in South African Hereford cattle. *Archives Animal Breeding*. 43, 5-16.

van Marle-Köster, E., Visser, C. and Berry, D.P., 2013. A review of genomic selection-Implications for the South African beef and dairy cattle industries. *South African Journal of Animal Science*. 43, 1-17.

van Marle-Köster, E. and Visser, C., 2018. Genetic improvement in South African livestock: can genomics bridge the gap between the developed and developing sectors?. *Frontiers in Genetics*. 9.

Venter, Y., 2017. Investigation into the precision feeding of Nguni steers under feedlot conditions. Report. 1-3.

Vermeulen, H., Schonfeldt, H.C. and Pretorius, B., 2015. A consumer perspective of the South African red meat classification system. *South African Journal of Animal Science*. 45, 339-354.

Visscher, P. M., M. A. Brown, M. I. McCarthy, and J. Yang, 2012 Five years of GWAS discovery. *American Journal of Human Genetics*. 90, 7–24.

Walsh, B., 2000. Minireview: Quantitative genetics in the age of genomics. *Theoretical Population Biology*. 59, 175-184.

Webb, E.C. and Erasmus, L.J., 2013. The effect of production system and management practices on the quality of meat products from ruminant livestock. *South African Journal of Animal Science*. 43, 413-423.

Weng, Z., Su, H., Saatchi, M., Lee, J., Thomas, M.G., Dunkelberger, J.R. and Garrick, D.J., 2016. Genome-wide association study of growth and body composition traits in Brangus beef cattle. *Livestock Science*. 183, 4-11.

Wheeler, T.L., Cundiff, L.V., Shackelford, S.D. and Koohmaraie, M., 2001. Characterization of biological types of cattle (Cycle V): carcass traits and longissimus palatability. *Journal of Animal Science*. 79, 1209-1222.

Wheeler, D.L., Barrett, T., Benson, D.A., Bryant, S.H., Canese, K., Chetvernin, V., Church, D.M., DiCuccio, M., Edgar, R., Federhen, S. and Geer, L.Y., 2006. Database resources of the national center for biotechnology information. *Nucleic Acids Research*. 35, D5-D12.

Williams, J.L., Usha, A.P., Urquhart, B.G.D. and Kilroy, M., 1997. Verification of the identity of bovine semen using DNA microsatellite markers. *Veterinary Record*. 140, 446-449.

Williams, J.L., Dunner, S., Valentini, A., Mazza, R., Amarger, V., Checa, M.L., Crisa, A., Razzaq, N., Delourme, D., Grandjean, F. and Marchitelli, C., 2009. Discovery, characterization and validation of single nucleotide polymorphisms within 206 bovine genes that may be considered as candidate genes for beef production and quality. *Animal Genetics*. 40, 486-491.

Wood, J.D., Enser, M., Fisher, A.V., Nute, G.R., Sheared, P.R., Richardson R.I., Hughes, S.I. and Whittington, F.M., 2008. Fat deposition, fatty acid composition and meat quality: A review. *Meat Science*. 78, 343-358.

Xia, J., Qi, X., Wu, Y., Zhu, B., Xu, L., Zhang, L., Gao, X., Chen, Y., Li, J. and Gao, H., 2016. Genome-wide association study identifies loci and candidate genes for meat quality traits in Simmental beef cattle. *Mammalian Genome*. 27, 246-255.

Xia, J., Fan, H., Chang, T., Xu, L., Zhang, W., Song, Y., Zhu, B., Zhang, L., Gao, X., Chen, Y. and Li, J., 2017. Searching for new loci and candidate genes for economically important

traits through gene-based association analysis of Simmental cattle. *Scientific Reports*. 7, 42048.

Yang, J., Benyamin, B., McEvoy, B.P., Gordon, S., Henders, A.K., Nyholt, D.R., Madden, P.A., Heath, A.C., Martin, N.G., Montgomery, G.W. and Goddard, M.E., 2010. Common SNPs explain a large proportion of the heritability for human height. *Nature Genetics*. 42, 565–569.

Yang, J., Hong Lee, S., Goddard, M.E., and Visscher, P.M., 2011. GCTA: a tool for genome-wide complex trait analysis. *American Journal of Human Genetics.* 88, 76–82.

Zhang, Q., Bethmann, C., Worth, N.F., Davies, J.D., Wasner, C., Feuer, A., Ragnauth, C.D., Yi, Q., Mellad, J.A., Warren, D.T. and Wheeler, M.A., 2007. Nesprin-1 and-2 are involved in the pathogenesis of Emery–Dreifuss muscular dystrophy and are critical for nuclear envelope integrity. *Human Molecular Genetics*. 16, 2816-2833.

Zou, J.N., Wang, S.Z., Yang, J.S., Luo, X.G., Xie, J.H., Xi T., 2009. Knockdown of SMYD3 by RNA interference down-regulates c-Met expression and inhibits cells migration and invasion induced by HGF. *Cancer Letters*. 280, 78–85.

Zuk, O., E. Hechter, S. R. Sunyaev, and E. S. Lander, 2012 The mystery of missing heritability: Genetic interactions create phantom heritability. Proceedings of the National Academy of Sciences of the United States of America. 109, 1193–1198.

Addenda

Addendum A: Carcass classification

Table A1 Description of the classification of the carcass

Measurement	Classification
Age	0 teeth: A
	1–2 teeth: AB
	3–6 teeth: B
	more than 6 teeth: C
Fat thickness	0: no fat (0mm)
	1: very lean (<1mm)
	2: lean (1-3mm)
	3: medium (>3 and ≤5mm)
	4: fat (>5 and ≤7mm)
	5: slightly overfat (>7 and ≤ 10mm)
	6: extremely overfat (>10mm)
Conformation / Roundness	1: very flat
	2: flat
	3: medium
	4: round
	5: very round
Sex	Male, Female, Castrated
Bruising	1: slight
	2: moderate
	3: severe

Addendum B: Genome-wide association studies (GWAS) using PLINK software for data with genetic quality control

a.

b.

c.

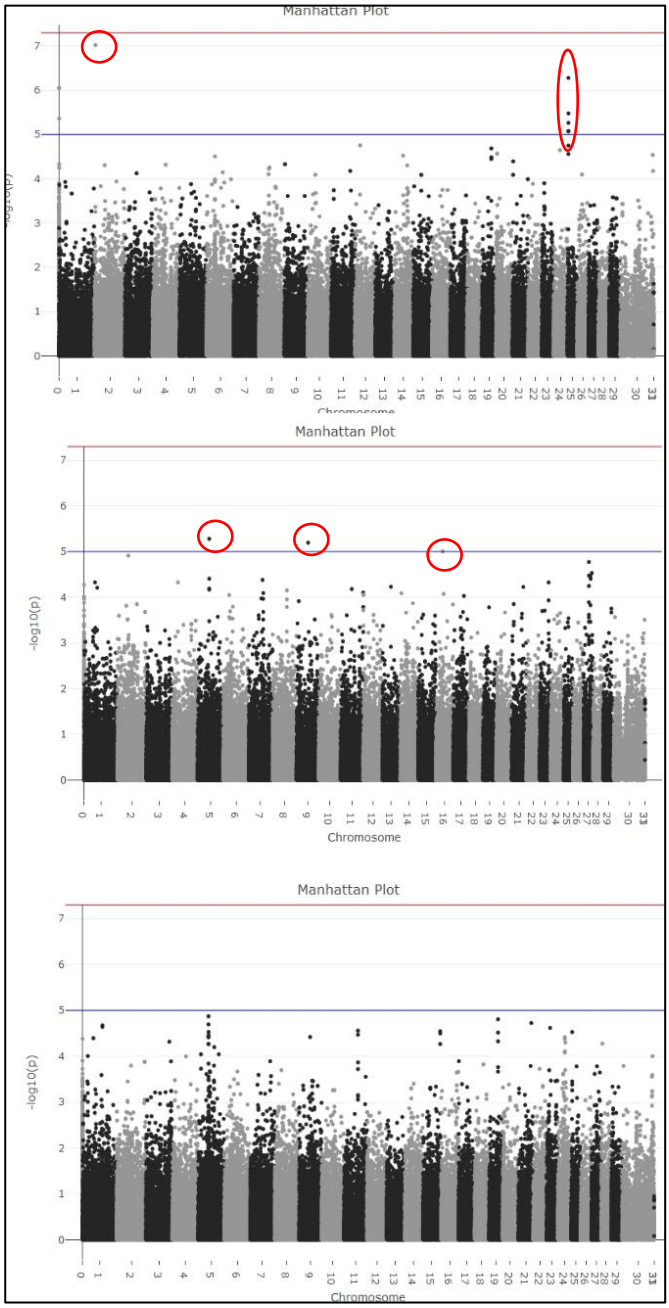


Figure B1 Manhattan plot displaying the results (-log 10 of p-values) for SNPs detected for a: EMA72, b: Rump fat72, c: Back fat72

a.

b.

c.

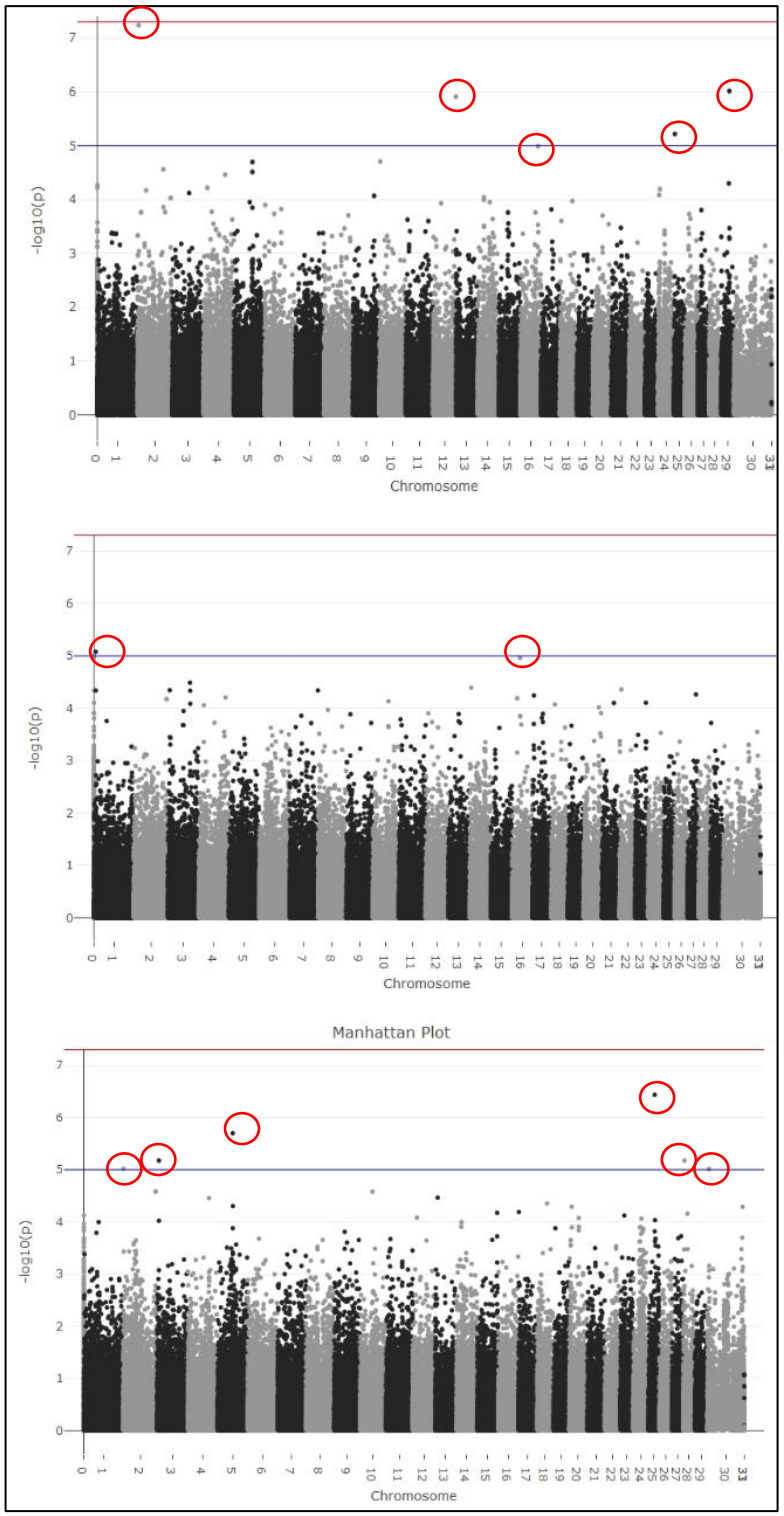


Figure B2 Manhattan plot displaying the results (-log 10 of p-values) for SNPs detected for a: EMA91, b: Rump fat91, c: Back fat91

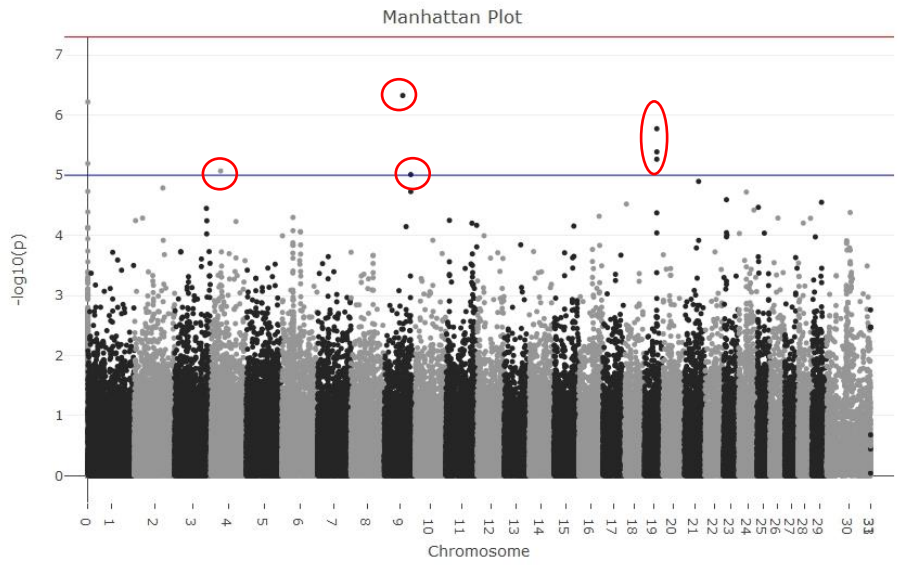


Figure B3 Manhattan plot displaying the results (-log 10 of p-values) for SNPs detected for slaughter weight

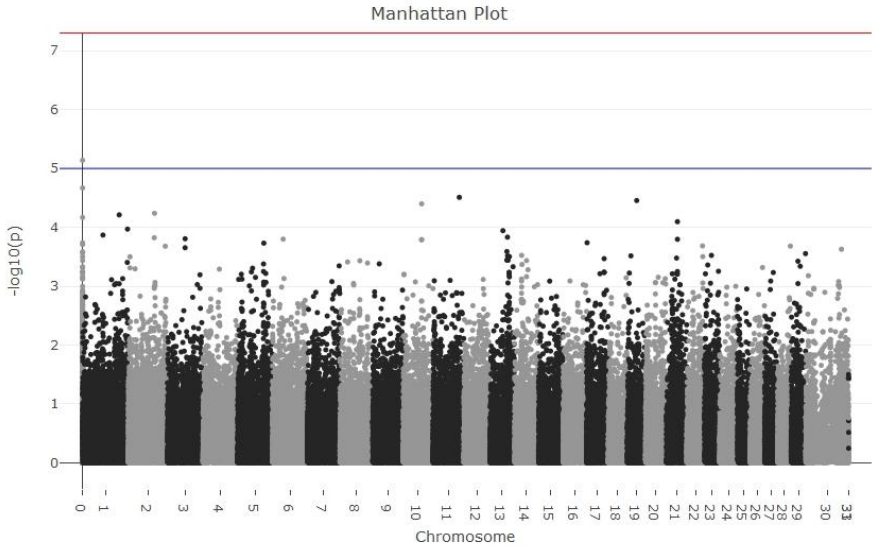
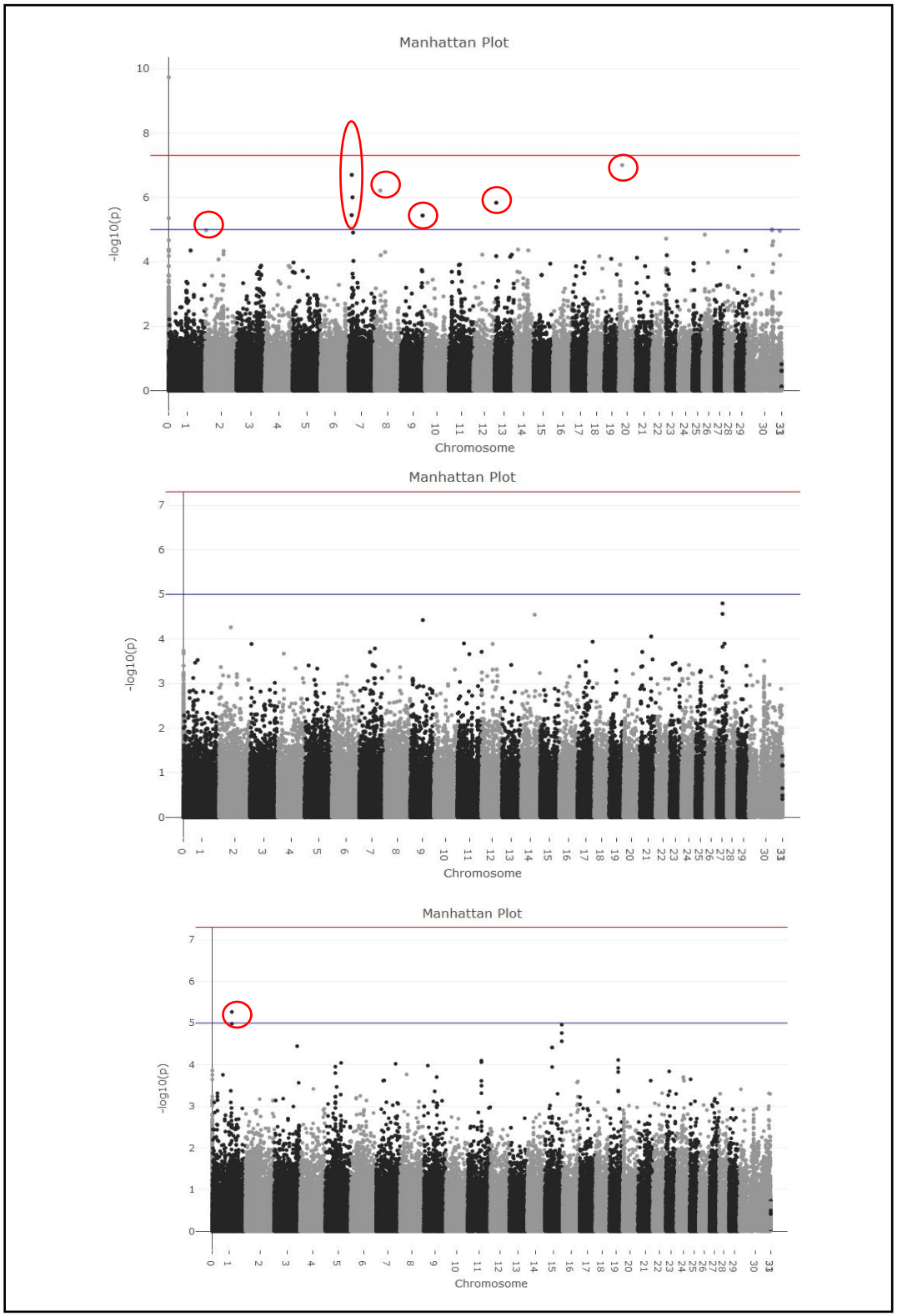


Figure B4 Manhattan plot displaying the results (-log 10 of p-values) for SNPs detected for dressing percentage

Addendum C: Genome-wide association studies (GWAS) using EMMAX software for data with technical and quality control

a.



d.

e.

Figure C1 Manhattan plot displaying the results (-log 10 of p-values) for SNPs detected for a: EMA72, b: Rump fat72, c: Back fat72 (Data with only technical QC)

a.

b.

c.

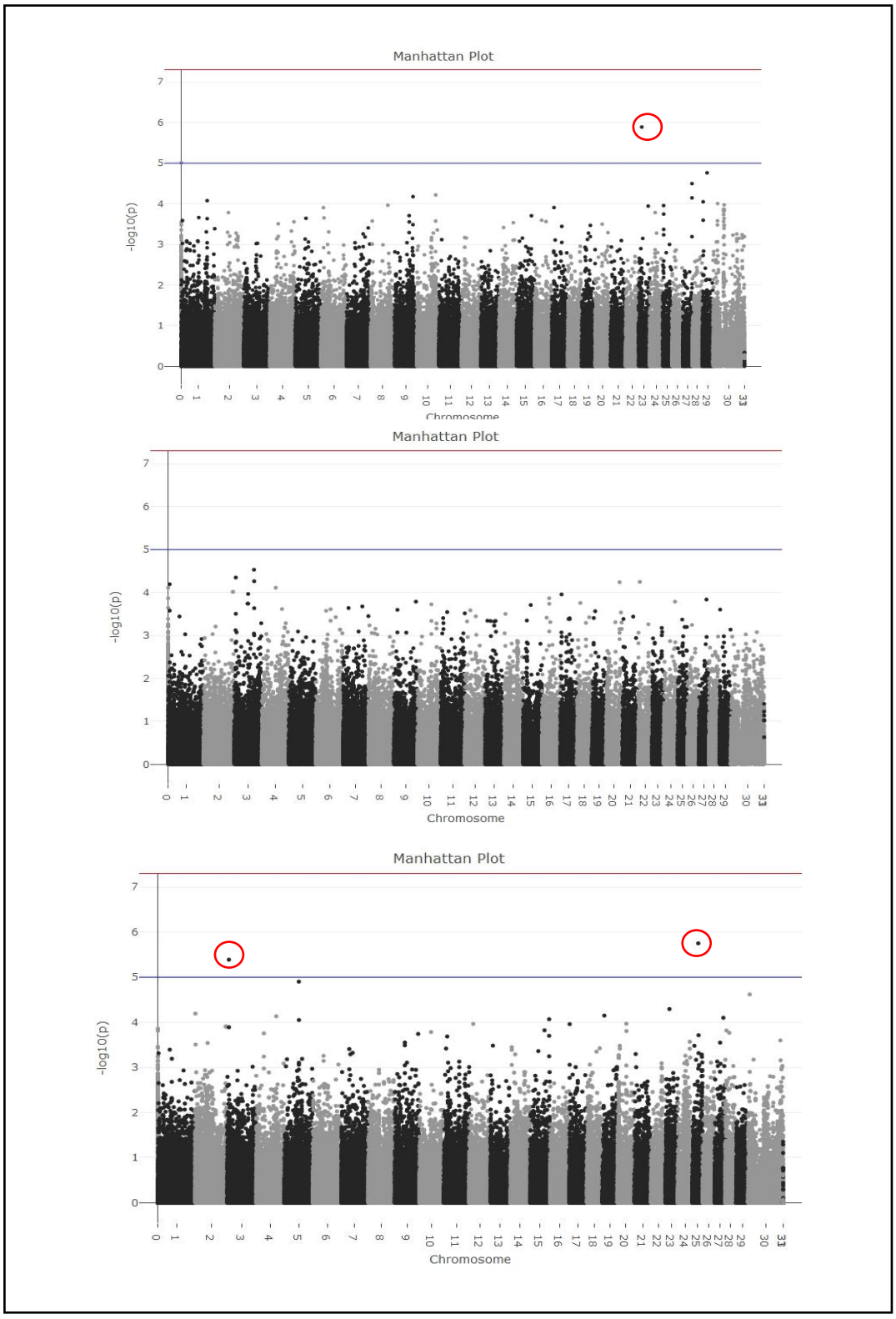


Figure C2 Manhattan plot displaying the results (-log 10 of p-values) for SNPs detected for a: EMA91, b: Rump fat91, c: Back fat91 (Data with only technical QC)

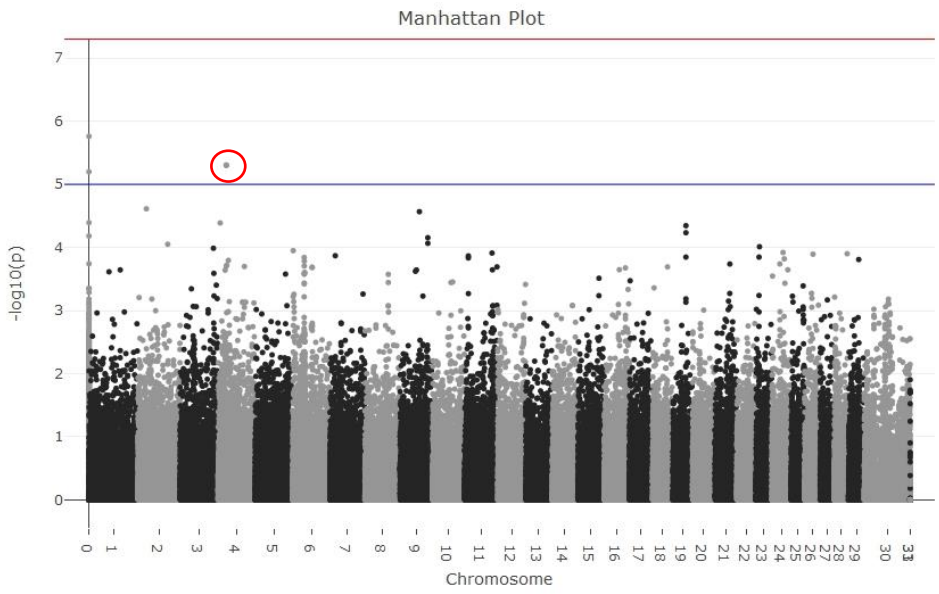


Figure C3 Manhattan plot displaying the results (-log 10 of p-values) for SNPs detected for slaughter weight (Data set with technical QC)

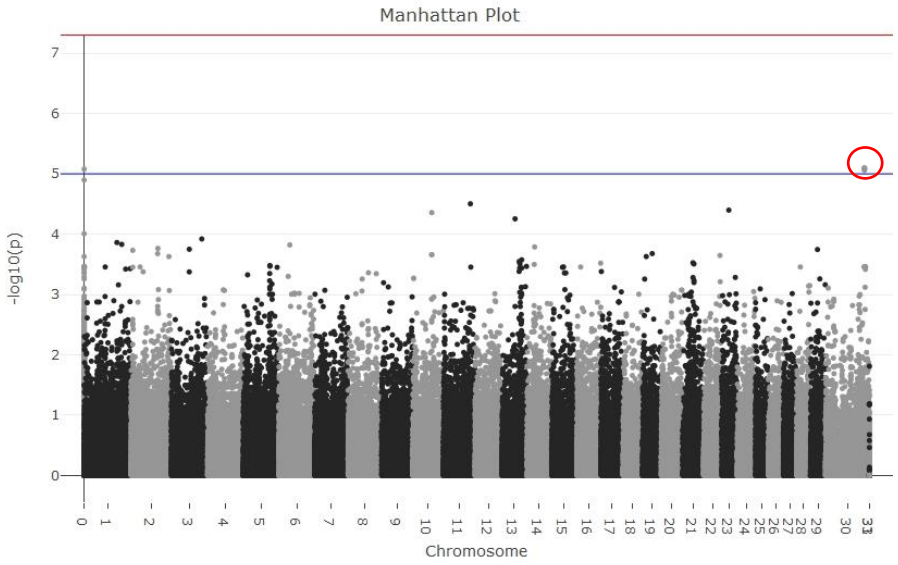


Figure C4 Manhattan plot displaying the results (-log 10 of p-values) for SNPs detected for dressing percentage (Data set with technical QC)

a.

b.

c.

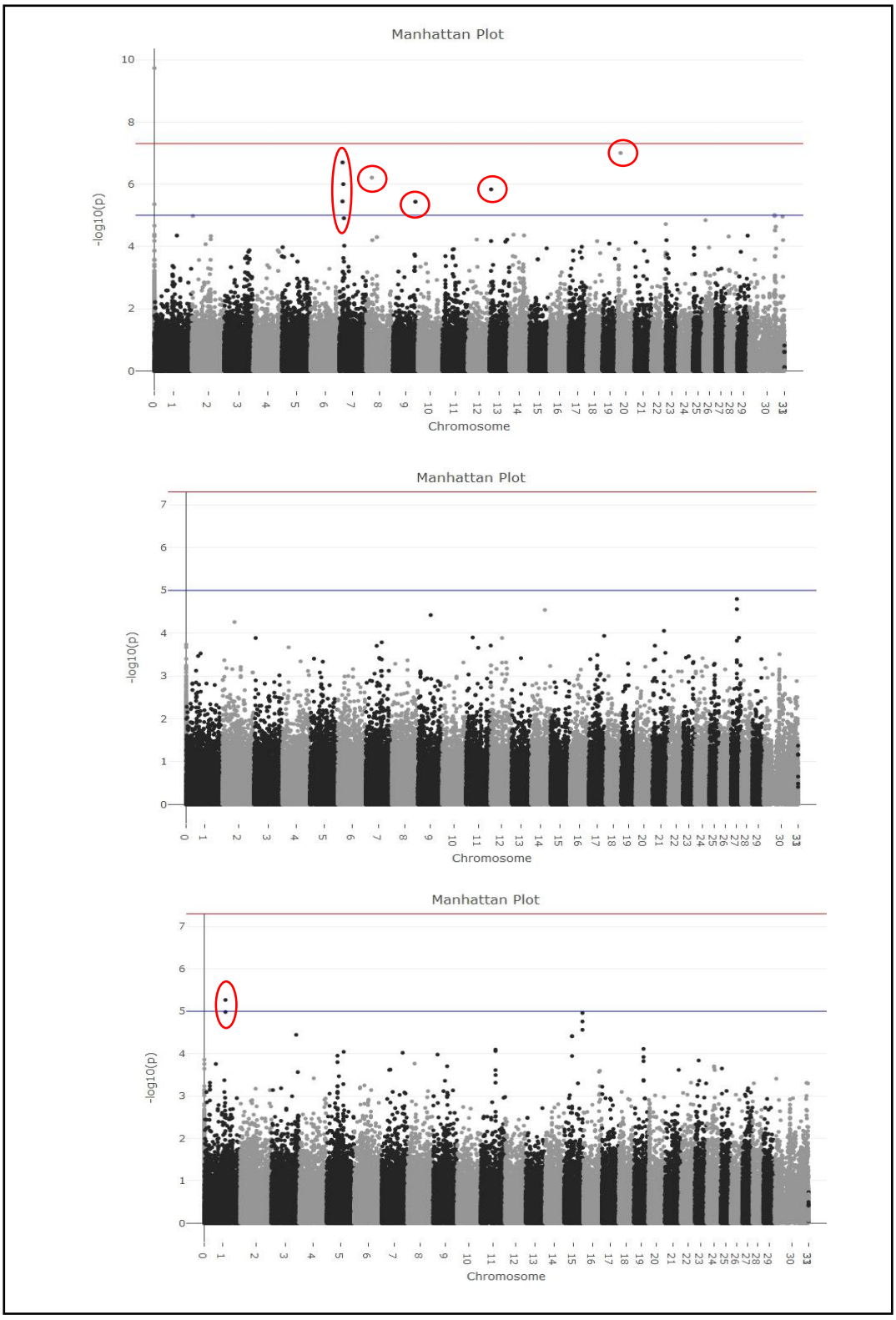


Figure C5 Manhattan plot displaying the results (-log 10 of p-values) for SNPs detected for a: EMA72, b: Rump fat72, c: Back fat72 (Data set with technical and genetic QC)

a.

b.

c.

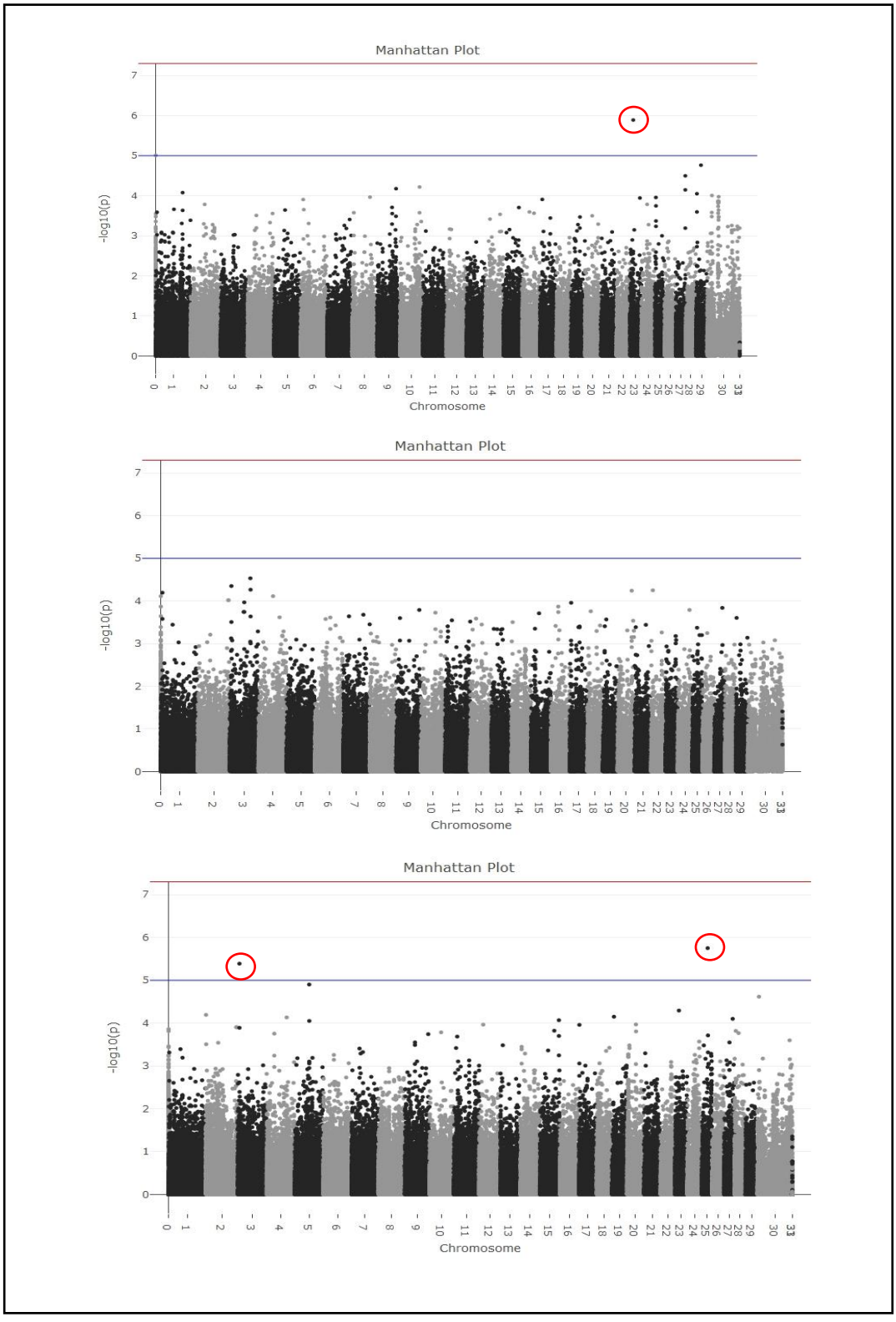


Figure C6 Manhattan plot displaying the results (-log 10 of p-values) for SNPs detected for a: EMA91, b: Rump fat91, c: Back fat91 (Data set with technical and genetic QC)

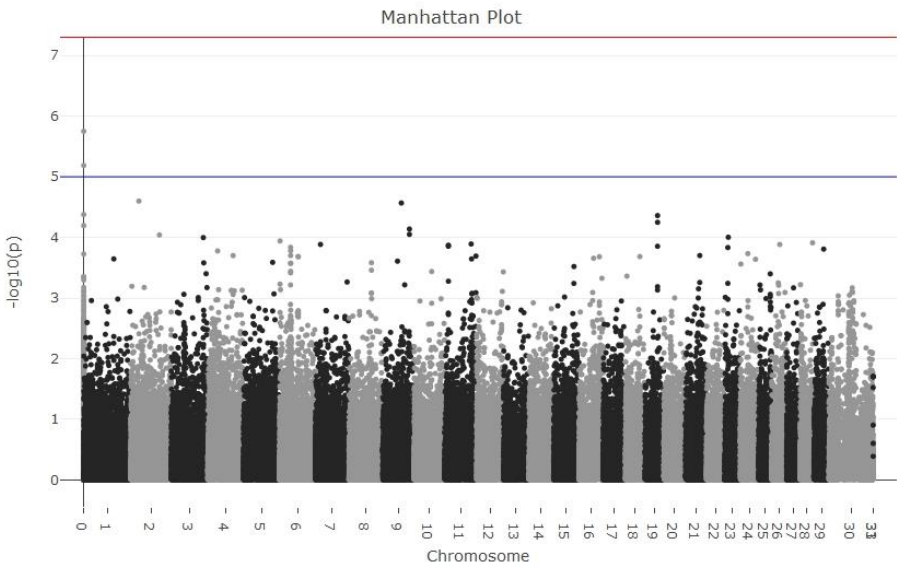


Figure C7 Manhattan plot displaying the results (-log 10 of p-values) for SNPs detected for slaughter weight (Data set with technical and genetic QC)

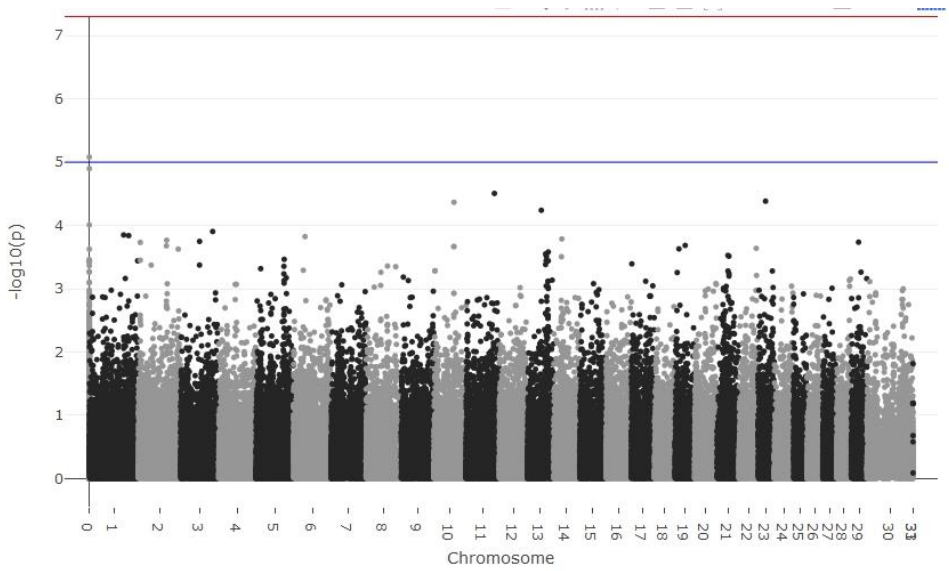


Figure C8 Manhattan plot displaying the results (-log 10 of p-values) for SNPs detected for dressing percentage (Data set with technical and genetic QC)