INVESTIGATION INTO THE RELATIONSHIP BETWEEN LEPTIN GENOTYPES, BODY CONDITION AND CARCASS TRAITS OF NGUNI AND HEREFORD CATTLE

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PREFACE

The experiment work described in this dissertation was conducted at the University of KwaZulu-Natal, Pietermaritzburg, under the supervision of Mr Edgar Dzomba / Professor Annabel Fossey.

The results have not been submitted in any other form to another University and except where the work of others is acknowledged in the text, are the results of my own investigation.

. . . . . .

October 2010

I certify the above statement is correct.

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Mr Edgar Dzomba

Supervisor

DECLARATION

I, Kerry-Lee Etsebeth, declare that

1. The research reported in this thesis, except where otherwise indicated, is my original research.

2. This thesis has not been submitted for any degree or examination at any other university.

3. This thesis does not contain other persons' data, pictures, graphs or other information, unless specifically acknowledged as being sourced from other persons.

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Signed: ……

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ABBREVIATIONS

AFLP	amplified fragment length polymorphism
AI	artificial insemination
Amp	amperes
APS	aminopropyl silica
Arg	arginine
BCS	body condition score
BLASTn	nucleotide Basic Local Alignment Search Tool Program
BLUP	best linear unbiased prediction
bp	base pair/s
BST	bovine somatotropin
°C	degrees Celsius
C	cytosine
CAPN	calpain
CAST	calpastatin
CFN	carcass conformation
COOH	Carboxylic acid
CRH	corticotrophin-releasing hormone
Cys	cysteine
d.	distilled
DNA	deoxyribonucleic acid
dNTP	deoxyribonucleotide triphosphate
EBV	estimated breeding value

EDTA	ethylenediaminetetraacetic acid
FAT	carcass fat content / fatness
g	gramme/s
G	genotype
GABA	gamma-aminobutyric acid
HCl	hydrochloric acid
H_2O	water
hrs	hours
HWE	Hardy-Weinberg Equilibrium
IGF1	insulin-like growth factor 1
ILW	initial live weight
INDEL	insertion deletion length polymorphism
ITS	internal spacer region
kb	kilobase/s
kDa	kilo Dalton
kg	kilogramme/s
Lep	leptin gene
LEPR	leptin receptor
M	Mol/s
MAS	marker assisted selection
Max	maximum value
mg	milligramme/s
$MgCl_2$	Magnesium chloride
Mh	myostatin

Min	minimum value
min	minute/s
ml	millilitre/s
mm	millimetre/s
mM	millimol/s
mRNA	messenger ribonucleic acid
MSH	melanocyte-stimulating hormone
MSTN	myostatin
mw	molecular weight
MWM	molecular weight marker
n	number
NaCl	Sodium chloride
NaOH	Sodium hydroxide
ng	nanogramme/s
NPY	neuropeptide Y
PCR	polymerase chain reaction
pmol	picomol
POMC	pro-opiomelancortin
QTL	quantitative trait loci/locus
RAPD	random amplification of polymorphic DNA
RDA	redundancy analysis
RFLP	restriction fragment length polymorphism
rpm	rotations per minute
s	second/s

SAMIC	South African Meat Industry Company
SDS	Sodium dodecyl sulfate
SLW	slaughter live weight
SNP	single nucleotide polymorphism
SSR	simple sequence repeat length polymorphism
Stat	single transducer and activator of transcription
T	thymine
T_a	annealing temperature
TAE	Tris-acetate-EDTA
TBE	Tris-borate-EDTA
TEMED	tetramethylethylenediamine
Tris	tris(hydroxymethyl)aminomethane
U	unit
UKZN	University of KwaZulu-Natal
V	volt/s
WCM	warm carcass mass
WG	weight gain

ABSTRACT

Leptin, a 16 (kilo Dalton) kDa hormone secreted predominantly by white adipocytes, regulates reproduction, energy intake and expenditure, and is involved in immune system function. Previous studies have identified associations between polymorphism E2FB in the leptin gene (*lep*) of cattle and milk quality and quantity, feed intake, and fat deposition in dairy and beef cattle though further studies have shown inconclusive results. Furthermore, indigenous South African cattle have not been involved in *lep* investigations or the applicability of the marker in South African beef grading systems. An investigation was conducted into the association of an SNP of a cytosine (C) to thymine (T) SNP (single nucleotide polymorphism) mutation in exon 2 of the bovine *lep* (leptin) gene with weight gain, body condition, carcass fat content and quality in a population of indigenous Nguni cattle (n = 70) as well as a population of exotic British Hereford cattle (n = 54). The Hereford population had higher T-allele frequencies and a lower *P*-value (*P* = 0.172) for the *E2FB* genotypes than the Nguni population (*P* = 0.958). The resulting E2FB *lep* genotypes *CC*, *CT* and *TT* did not show an association with the pre- and post-slaughter traits initial live weight (ILW), body condition score (BCS), slaughter live weight (SLW), carcass fat content (FAT), carcass conformation (CFN) or warm carcass mass (WCM) for either population though t-tests revealed an association with the CT genotype with increased ILW than TT and a significantly higher WG in the *TT* genotypes than the CT (*P*<0.05). Subsequently, differences in pre- and post-slaughter traits in both populations were largely attributable to breed differences. The Hereford population exhibited significantly higher WG, CFN, SLW, WCM and CCM (*P*<0.05) than the Nguni population. The Nguni displayed significantly higher ILW and BCS values when graded in terms of the commercial South African AAA feedlot system.

CHAPTER 1

LITERATURE REVIEW

1.1 Introduction

Cattle have become one of the most economically significant domestic animal species in the world. Since the domestication of cattle thousands of years ago, cattle have had a respected formative association with human society with respect to agriculture, commercial products, and cultural and religious functions (Bradley *et al*., 1996; Loftus *et al*., 1999; Hanotte *et al*., 2002). It has therefore been recognized that cattle have greatly profited world agriculture, in beef as well as dairy production. In turn these industries have played significant roles in modern society as cattle produce the majority of animal protein for human consumption as well as providing other commodities such as hides, traction and dung (Mac Hugh *et al*., 1997; Casas *et al*., 2000). The surge of the world's human population exerts enormous demands on agriculture for increased food production (United States department of agriculture, USDA, 2002). Agricultural resources and especially the livestock industry are strained and there is a need for more effective methods of producing adequate supplies of animal protein (Scholtz *et al*., 1999a). Society's considerable dependence on cattle products necessitates the need to develop these resources, to anticipate future demands, and to mitigate the risks of not conserving genetic resources (Martín-Burriel *et al*., 1999; Casas *et al*., 2000).

With the introduction of domestic cattle into southern Africa several thousand years ago, pastoralism is practiced extensively and remains the livelihood of millions in contemporary South Africa (Hanotte *et al*., 2002). Cattle fulfil social, economic and cultural roles in rural communities in the developing areas (Casas *et al*., 2000). South Africa has a dual agricultural economy consisting of a commercial, as well as a subsistence-orientated sector (Raath, 2001). The

commercial livestock sector in South Africa plays a key role in agriculture. Livestock products account for approximately 40% of total agricultural production (Food and agriculture organization, FAO, 2008). In South Africa 80% of agricultural land is not fit for crop production, though it can support livestock (Scholtz *et al*., 1999a). Consequently, many projects have been launched to address the improvement of livestock populations, of which a number have been targeted at the beef and dairy industries with the aim of augmenting biological and economical efficiency through genetic advancement and improved management practices (Scholtz *et al*., 1999a).

1.2 Cattle breeds

Currently there are approximately 800 different cattle breeds worldwide, though many face the threat of extinction (Porter, 1991; Fries and Ruvinsky, 1999). There are three major types of cattle, *Bos taurus* (humpless), commonly referred to as taurine, *Bos indicus* (humped) which is known as Zebu, and the hybrid Bos *taurus africanus* which includes the Sanga cattle breeds (Loftus *et al*., 1994).

Taurine breeds are the major beef and dairy producers used worldwide (Wheeler *et al*., 1994). The most noteworthy dairy breeds include Holstein-Friesian, Ayrshire and Jersey, whilst major beef breeds include Angus, Hereford, Charlois and Brahman though many composite breeds have been developed in pursuit of specific economic traits valued by different groups nationwide (Porter, 1991; Casas *et al*., 2000).

In South Africa, indigenous and locally developed breeds such as the Nguni, Tuli, Afrikaner, Bonsmara, Drakensburger, Huguenot and Sanganer are primarily considered beef breeds (Porter, 1991; Scholtz *et al*., 1999a; Ramsay *et al.*, 2000).

1.3 Beef industry in South Africa

1.3.1 Introduction

On a commercial scale, South Africa provides 0.7% of the world's meat supply and is one of the world's leading indigenous beef suppliers (FAO, 2008). Furthermore, beef and dairy production make up South Africa's third largest commodity after sugarcane and maize (FAO, 2008).

Breeding practices in the South African beef industry have undergone many changes in recent years. In the past, authorities and breeders regarded indigenous cattle as inferior, they were replaced or used only in crossbreeding programmes with exotic breeds to make them more acceptable to farmers who were accustomed to stocky European and British breeds (Hunlun, 2000; Ramsay, 2000). Furthermore, local breeds have been decimated due to government decree in the 70's thereby accelerating the dilution of the indigenous gene pool (South African stud book and livestock improvement association, SASBA, 2001; Strydom, 2008). However, local breeders and scientists, as well as commercial farmers from abroad, are slowly recognizing the intrinsic qualities of indigenous cattle and they are now more readily included in improvement programmes (Poland *et al*., 2003). In recognition of this increased role, strategies are being implemented to preserve the gene pool (Loftus *et al*., 1994; Kotze *et al*., 2000). The limitations of the exotic breeds introduced into South Africa's harsh environment brought about an appreciation for the valuable attributes of indigenous cattle in commercial agriculture (Ramsay, 2000; Poland *et al*., 2003).

1.3.2 Beef breeds

South Africa has a wide variety of genetically diverse indigenous beef breeds that have adapted to a wide range of environmental conditions (Ramsay *et al.,* 2000). These cattle breeds have adapted over many years to various biomes which have periodic droughts, seasonal dry periods, nutritional shortages in the natural

veldt and a variety of parasites and diseases which resulted in the evolution of hardy ecotypes (Kotze *et al*., 2000; Ramsay *et al.,* 2000).

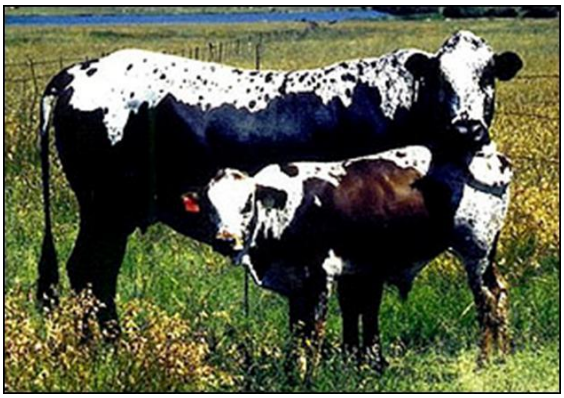

Figure 1.1 Example of the indigenous Nguni breed.

Photo: courtesy Nguni Cattle cc.

In addition to the effect of harsh environmental conditions, the development of the indigenous cattle breeds has also been attributed to the influence by human migrations and customs (Porter, 1991).

One of South Africa's prominent indigenous Sanga beef breeds is the Nguni (Bos *taurus africanus*. Nguni are characterized by their short glossy coat and the pigmentation around their eyes (Figure 1.1). They exhibit an assortment of coat colours such as white, brown, black, red, dun, tan, and yellow and their uniquely patterned hides have become a substantial export commodity (Porter, 1991; Scholtz *et al*., 1999a). Nguni have intrinsic adaptability to the harsh climate and the diverse agricultural systems practiced in South Africa (Scholtz *et al*., 1999a). This small-framed animal is renowned for its fertility, calving ease, and excellent mothering ability under harsh conditions (Morgan, 2001). Their resilience in facing severe environmental conditions and resistance to drought and tick-borne diseases is remarkable (Ramsay *et al.,* 2000; Poland, 2003). Nguni hides enable

them to dissipate heat and reflect light making them heat tolerant and allowing them to perform well in trying conditions (Poland, 2003). Furthermore, Nguni have good foraging ability as well as a placid temperament, longevity, acceptable meat quality, and a thin hide that yields superior quality leather (Poland, 2003). Nguni are perceived as a low maintenance (minimum care) breed with high performance, making them very profitable animals (Ramsay *et al.,* 2000). These adapted traits have been selected and utilized in crossbreeding programmes with exotic livestock (Porter, 1991).

Despite these advantages, Nguni performance has previously been limited in the beef industry due to its characteristically small frame. However, it boasts a range of other favourable qualities for beef production, namely, meat tenderness and quality, high fertility, low inter-calving period, calving ease, cow efficiency, hardiness, as well as tick and disease resistance (Spickett *et al*., 1989; Rechav *et al*., 1991). The ability of Nguni to adapt to harsh conditions as well as its inherent capacity to produce meat of a quality comparable to that of commercial breeds has made it popular abroad (Strydom *et al*., 2000; Musemwa *et al*., 2008).

Traditionally, the beef industry in South Africa has been served by a variety of introduced exotic breeds, mostly of British and European origin. Hereford, a widely popular beef breed both locally and globally, was introduced to South Africa from west England in the 1900s, and is characterized by a white face and brown coat (Porter, 1991) (Figure 1.2). The Hereford breed has been genetically developed by strategic artificial selection practices for many years and is well known in the beef industry for their large frames, meat tenderness and quality, fertility, short gestation period, short inter-calving period, early maturity and calving ease (Porter, 1991; Sherbeck *et al*., 1995; SASBA, 2001). Furthermore, they are known for their extensive foraging ability (Porter, 1991). However, in tropical areas this breed is prone to eye cancer (Porter, 1991). Due to substantial genetic modification Hereford is able to perform well on a commercial scale.

Figure 1.2 Example of the exotic Hereford breed.

1.3.3 Economically important traits in the beef industry

Economically important traits in the beef industry vary from country to country. Consumer behaviour and current market requirements dictate what traits are most valued. Worldwide, growing health awareness has predisposed consumer preferences towards leaner beef (Perry and Fox, 1997; Brester, 2003). Currently, the beef market is inclined towards faster growing leaner cattle as opposed to an early maturing and compact cattle type (SAMIC, 2007). The ideal beef carcass is composed of high proportion of muscle, a low proportion of bone and optimum levels of fatness (Berg and Butterfield, 1976).

Globally, one of the main economically important traits determining beef grade and quality is that of intramuscular fat or marbling (Nishimura *et al*., 1999; Strydom *et al*., 2000; Brester *et al*., 2003). Marbling is associated with juiciness, tenderness and flavour (Barkhouse *et al*., 1996; Nishimura *et al*., 1999; Reverter *et al*., 2003; Gosey, 2004). Factors such as age, gender and developmental stage influence fat deposition and are also taken in account during grading procedures (Kempster, 1980).

Beef is graded in several ways worldwide. Major beef producers such as the United States, use an in-depth though costly system to measure and grade carcasses. Based on the USDA guidelines, these carcasses are graded according to marbling, age and yield grade of carcass (USDA, 2004).

South Africa utilizes a classification system rather than a grading system for beef carcasses. Where grading entails a generalized merit rating system that assumes all consumer preferences are alike, a classification system rates carcasses by class. This alternative system emphasizes subcutaneous fat rather than intramuscular fat, as subcutaneous fat levels are used to predict meat quality, tenderness and palatability (Dolezal *et al*., 1982; Scholtz *et al.*, 1999b; SAMIC, 2007). The grading of carcasses involves carcass measurements as well as visual appraisals conducted by trained officials. The carcasses are classified according to the following features (SAMIC, 2007):

1. Carcass mass (kg),
2. Age of the animal (A, AB, B or C),
3. Fat content of the carcass (0 to 6),
4. Carcass conformation (1 to 5),
5. Damage to the carcass (1 to 3), and
6. In the case of oxen in the B and C grades the sex of the animal is also recorded.

In addition to the above beef grading and classification system outlined by SAMIC, accurate determination of the economic viability of cattle requires an in-depth investigation involving measurements and data records, growth evaluation, reproductive performance and carcass quality of individuals in a given constant environment, which is accomplished through beef performance testing. Measurements and data records include age, sex, body condition score (BCS) and mature weight (as per SAMIC guidelines). Furthermore, growth evaluation entails weaning weight or pre-weaning rate of gain, post-weaning rate of gain and efficiency of feed conversion/growth rate. Essential reproductive traits

selected for in beef breeding include age of onset of puberty, conception, mothering ability and low birth weight for ease of calving. Carcass quality, and ultimately profitability, is determined by the following traits (Koots *et al*., 1994a; O'Connor *et al.*, 1997):

1. Backfat,
2. Intramuscular fat,
3. Subcutaneous fat,
4. Rib eye area,
5. Dressing %,
6. Lean: bone ratio (% of carcass yield from slaughter weight),
7. Warner-Bratzler force (indication of tenderness),
8. Cutability, and
9. Carcass mass.

1.3.4 Beef breeding

Breeding schemes, including farm management systems, should formulate a suitable strategy whereby appropriate animals are inducted into breeding procedures. The fundamental principles of beef breeding are based upon an understanding of the interactions involving the phenotype, genotype and the environmental influence as follows:

$$P = G + E$$

P = phenotype;

G = genotype; and

E = non-heritable environmental components.

Therefore, before implementing a breeding strategy, a breeder should develop clear and realistic breeding goals of desirable phenotypes (P), have a thorough understanding of the principles underlying inheritance (G), and ensure general management and nutrition in the herd is of an acceptable standard (E). Assuming that managerial and environmental conditions are optimal and consistent, breeding practices then focus on the genetic selection of economically important beef traits as outlined in section 1.3.3. In order to improve a beef breed and increase the response to selection, a number of factors are taken into account (Koots *et al*., 1994b; Green *et al*., 2000):

1. Whether the trait under consideration is discrete or continuous/ quantitative: Most carcass traits are quantitative in that they are determined by many genes that have an additive effect and these are known as quantitative trait loci or QTL.
2. The number of traits to consider for selection: This impacts the rate of genetic improvement. Response to selection takes longer when more traits are selected for.
3. The amount of available genetic variation of the traits under consideration: The response to selection is most effective if there is a large amount of variation within the economically important traits.
4. The measurability of the traits under consideration: Some traits are phenotypically measurable whereas others may be difficult or expensive to measure. These include carcass traits, traits only observed in one gender or traits that are dependent on the developmental stage of the animal. When dealing with traits that are difficult to measure, data from progeny testing are utilized.
5. The heritability of the trait under consideration: Traits with a relatively high heritability (0.4 - 1.0) in a given population respond favourably to selection (DAEARD, 2007). On the other hand, traits with a low heritability should rather be improved by cross breeding to utilize heterosis (the superiority of a hybrid when compared with a pure breed) (Bergen, 2005; Golden *et al*.,

2008). Table 1.1 provides a summary of heritability estimates for a number of economically important traits:

Table 1.1 Heritabilities for economically important traits.

Economically important trait	Heritability	Reference
Backfat	0.36 - 0.44	Koots *et al*., 1994a; Utrera and Van Vleck, 2004
Slaughter weight	0.3 - 0.5	Koots *et al*., 1994a; Utrera and Van Vleck, 2004
Carcass weight	0.23 - 0.4	Wilson *et al*., 1993; Utrera and Van Vleck, 2004
Dressing %	0.38 - 0.39	Utrera and Van Vleck, 2004; Golden *et al*., 2008
Marbling	0.31 - 0.38	Koots *et al.*, 1994a; Utrera and Van Vleck, 2004
Lean : bone ratio	0.61 - 0.63	Utrera and Van Vleck, 2004
Rib eye area	0.38 - 0.44	Wilson *et al*., 1993; Utrera and van Vleck, 2004

6. Genetic correlations between traits under consideration: Breeders need to consider the complexities of genetic interactions. These interactions are either cooperative or antagonistic in nature. It is therefore essential to consider the strength and direction of the correlation between traits. These values give an indication of how the traits will respond simultaneously to selection (Golden *et al*., 2008). Some traits such as growth rate and feed conversion behave synergistically. Feed conversion is costly and difficult to measure, but the beneficial correlation with growth rate means that selection for growth rate will also result in improvement in feed conversion. Other traits behave antagonistically, for example carcass traits such as marbling and leanness. These antagonistic genetic correlations make progress from selection more difficult and the response to selection smaller and slower to achieve (Golden *et al*., 2008). Table 1.2 provides a

summary of correlation estimates of a number of economically important trait pairs:

Table 1.2 Correlations for economically important traits.

Economically important trait	Correlation	Reference
Calving ease/birth weight	-0.74 - 0.56	Golden *et al*., 2008
Marbling/cutability	-0.25 - 0.17	Aaabg Genetic Parameters, 2008
Marbling/yearling weight	-0.33 - 0.14	MacNeil *et al.*, 1984; Koots *et al*., 1994b
Marbling/rib eye area	-0.4 - 0.21	Koots *et al*., 1994b
Marbling/backfat	0.22 - 0.35	Aaabg Genetic Parameters, 2008
Marbling/shear force	-0.31	Koots *et al*., 1994b

7. Selection indexes: Selection indexes are devised by combining performance records from several traits into a single value for each individual, taking into account weighted economic value, heritability and genetic associations with other traits.
8. Generation interval: The longer the generation interval, the slower the response to selection.

Breeding strategies have become more sophisticated in recent years with the implementation of progeny testing, collection of performance data, development of technologies such as artificial insemination (AI) and molecular techniques as well as the application of advanced statistical analysis. The latest advanced molecular techniques enable breeders to efficiently evaluate desirable genotypes and significantly decrease the generation interval (Kinghorn *et al*., 2000). This is especially relevant to evaluate an individual's genetic predisposition for carcass traits, as this is usually difficult to measure. DNA (deoxyribonucleic acid) markers for major gene effects have the potential to supplement traditional selection tools to yield more precise selection for specific carcass traits (Kinghorn *et al*., 2000).

1.4 Molecular markers in beef breeding

Most of the economically important traits in beef cattle are influenced by a number of genes as well as environmental factors. Evaluation procedures like best linear unbiased prediction (BLUP), a type of selection index, have been developed to estimate breeding values (EBVs) of animals in order to identify animals with superior breeding value. EBV is a component of the individual's genotype (G) that describes the genetic value of an individual and gives an indication of the transmittable gene effects (Falconer, 1989; Kinghorn *et al*., 2000). Until recently, breeders have utilized phenotypic and pedigree information to calculate EBVs to identify animals with desirable genotypes. This approach to selection has several limitations as phenotype is an imperfect predictor of the breeding value of an individual (Lande and Thompson, 1990). The evaluation of breeding value can be enhanced by molecular genotyping and marker assisted selection as DNA can be obtained from an individual, male or female, at any age, and more specifically before reproductive age. This molecular approach is most useful for selection of traits that are difficult to improve through conventional means due to low heritability or difficulty or expense in recording the phenotype. These techniques locate and utilize genes or genetic markers (known DNA sequences) which are tightly linked genes that have a major effect on quantitative traits (known as quantitative trait loci or QTL) (Kinghorn *et al*., 2000; Switonski, 2002).

1.4.1 Molecular markers

A marker can be used as a ‘flag’ to observe inheritance at a QTL and can be either phenotypic or genotypic. Genotypic markers are used to detect polymorphisms through analysis of proteins known as allozymes or alternatively at the DNA level using DNA markers (Figure 1.3). DNA markers can be further classified into dominant or co-dominant (Figure 1.4). Co-dominant markers can identify heterozygotes from homozygotes whereas with dominant markers it is not possible to distinguish between a locus that is heterozygous or homozygous (Kinghorn *et al*., 2000). Genetic markers can be direct or linked. Direct markers actually measure the relevant polymorphism in the gene that causes the effect because there is never any recombination between the marker and the QTL. Hence, the marker genotype identifies the exact genotype of the QTL genotype. However, if a marker is situated near to or linked to a QTL there is a possibility that recombination can take place between the two and result in a break up at meiosis. The consequence of the linked marker is that there is no guarantee of identifying the exact QTL (Kinghorn *et al*., 2000).

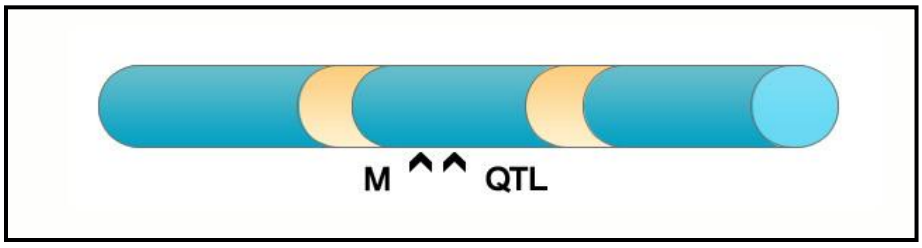

Figure 1.3 Diagrammatic representation of a genetic marker.

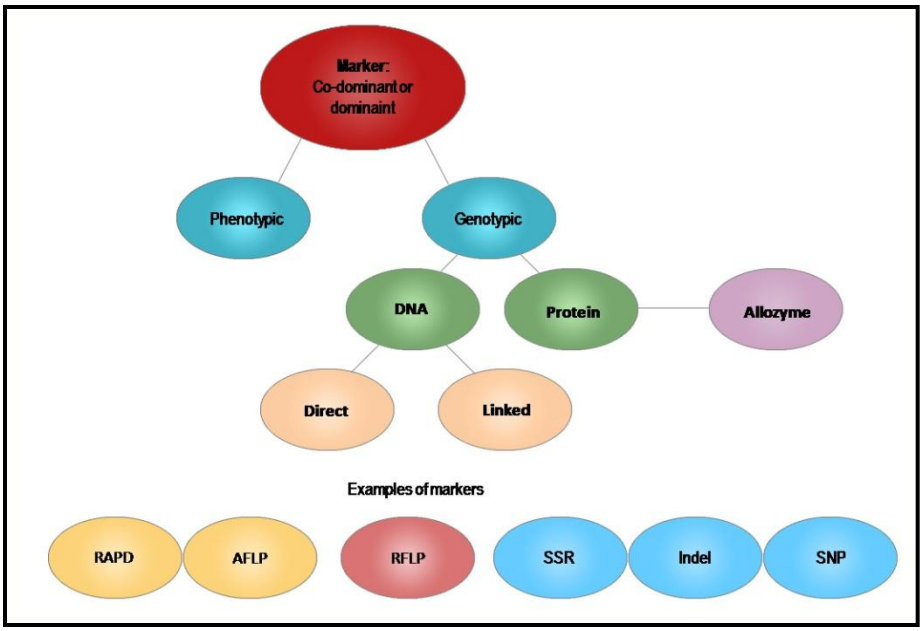

Figure 1.4 Classification of molecular markers.

There are numerous types of molecular markers including: random amplification of polymorphic DNA (RAPD), amplified fragment length polymorphisms (AFLPs), restriction fragment length polymorphisms (RFLPs), insertion deletion length polymorphisms (INDELs), simple sequence repeat length polymorphisms (SSRs) and single nucleotide polymorphisms (SNPs).

RAPDs are DNA polymorphisms produced by rearrangements or deletions at or between oligonucleotide primer binding sites in the genome. RAPDs are typically dominant markers. Assays are developed via the amplification of random segments of DNA using several short primers of 8 - 12 oligonucleotides. No knowledge of the DNA sequence of the target gene is required and the primers bind randomly to the DNA sequence (Williams *et al.,* 1990). The fragments are visualized on denaturing polyacrylamide gels either through autoradiography or fluorescence methodologies.

AFLPs are PCR-based (polymerase chain reaction) random markers that are used in genetics research, DNA fingerprinting, and in the process of genetic engineering. AFLP involves the restriction of genomic DNA followed by ligation of

complementary double stranded adaptors to the ends of the restriction fragments (Zabeau and Vos, 1993). A subset of the restriction fragments is then amplified using two primers complementary to the adaptor and restriction site fragments. AFLP technique does not require DNA sequence information prior to amplification. The amplified fragments are visualized on denaturing polyacrylamide gels either through autoradiography or fluorescence methodologies. AFLP is a highly sensitive method for the detection of various polymorphisms in DNA and has become widely used for the identification of genetic variation in strains or closely related species of plants, fungi, animals, and bacteria. AFLP technique has been used in criminal and paternity tests, in population genetics to determine slight differences within populations, and in linkage studies to generate maps for QTL analysis (Meudth and Clarke, 2007).

RFLPs are variations in DNA sequences of a genome that create or abolish restriction endonuclease sites. These variations are caused by SNPs or INDELs. SNP refers to a change in a single nucleotide in a given DNA sequence whereas INDEL is a portmanteau of ‘insertion’ and ‘deletion’ (Sherry *et al.,* 2001). RFLPs are easy to interpret and score by analyzing the size of the resulting restricted fragments by gel electrophoresis. RFLP occurs when the size of a detected fragment varies between individuals. Each fragment size is considered an allele and is therefore locus-specific and can be used in genetic analysis. RFLPs are co-dominant markers meaning that heterozygotes can be distinguished from dominant homozygotes. RFLPs are vital tools in genome mapping and genetic disease analysis though prior knowledge of the DNA sequence is required to carry out this assay (Saiki *et al*., 1985).

SSRs or microsatellites are DNA sequences composed of polymorphic tandem repeats of 1 - 6 bp in length present in nuclear and organellar DNA (Novoa and Usaquén, 2010). The simple sequence can be repeated 10 to 100 times in a genome. These microsatellites are typically neutral and co-dominant and are used as molecular markers with wide-ranging application in the field of

population studies, recombination mapping and paternity testing (Novoa and Usaquén, 2010). They can be amplified for identification by PCR using primers that are unique to one locus in the genome and the base pair on either side of the repeated portion. Therefore, a single pair of PCR primers will work for every individual in the species and produce different sized products for each of the microsatellites. The amplified fragments are visualized on denaturing polyacrylamide gels through either autoradiography or fluorescence methodologies.

1.4.2 Marker assisted selection (MAS) in beef cattle

Markers are instrumental in the development of biotechnology as they have the potential to increase disease resistance, productivity and product quality as well as reproductive efficiency in cattle (Pollak, 2005; Montaldo, 2006). Biotechnology is broadly defined as the application of technology in the production or modification of agri-products, processes or living organisms in order to produce new products or processes (Bunders *et al*., 1996). This includes a range of technologies relating to *in vitro* production process: manipulation, cryopreservation and transfer of cattle embryos, embryo and sperm sexing, AI, marker assisted selection (MAS), cloning and biopharming to increase the efficiency of cattle reproduction (Morris and Sreenan, 2001). The importance of biotechnology to the agricultural industry cannot be overestimated. It has the potential to impact production and processing technologies not only to increase efficiency but also to meet consumer demand for food safety and quality while at the same time ensuring sustainability and the protection of the environment.

MAS is an indirect selection process whereby the QTL or trait of interest is selected not based on the trait itself, but on the marker (morphological, biochemical or one based on DNA/RNA variation) that is linked to it (Zhang *et al.,* 1997). MAS is the application of technology gained from identifying a QTL and is only effective under the assumption that the marker is tightly linked with the QTL.

MAS is useful for traits that are difficult or expensive to measure, have low heritability, are gender specific or are dependent on developmental stage (Kinghorn *et al*., 2000). This technology greatly improves response to selection and decreases the generation interval, especially in traits that are not easily targeted with classical methods. One of the potential major benefits of selection based upon marker information is that marker genotypes can be determined based on easily collected samples (e.g. saliva or blood) that can be taken from an individual at birth. Economically important factors such as meat and milk quality as well as disease resistance are among the traits that are manipulated by MAS. Although the effects of the QTL on all relevant traits must be somewhat known before commencing with breeding strategies (Kinghorn *et al*., 2000). Unless genetic markers can capture most of the genetic variation for a trait, which is far from the case at the present, selection must be based on a combination of marker and phenotypic data. Although several useful genes (primarily linked markers) have been identified in various livestock species, their application has been limited and their success inconsistent, because the genes were not identified in breeding populations, or because they interact with other genes or with the environment. Until complex traits can be fully dissected, the application of MAS will be limited to genes of moderate to major effect (Pollak, 2005).

Several useful genes identified in the bovine genome have markers in the form of polymorphisms associated with QTL (indirect marker) or causal mutations (direct marker) (Fries *et al*., 1990). The bovine genome map contains over 3600 identified marker loci, which aid the investigation of genes that determine economically important quantitative traits (Kinghorn *et al.*, 2000; Switonski, 2002). Amidst numerous potential candidate genes, research has targeted myostatin (*Mh*), calpastatin (CAST), calpain (CAPN), growth hormone, growth hormone receptor, insulin-like growth factor-1 (IGF1), the pituitary-specific transcription factor 1, corticotrophin-releasing hormone (CRH), pro-

opiomelancortin (POMC) and leptin (*Lep*) (Switonski, 2002; Buchanan *et al*., 2005; Vankan *et al*., 2010) (Table 1.3).

In addition, a candidate gene approach has also been applied to the hormone leptin. Recent literature has described a possible causative mutation in the leptin gene (*lep*) associated with economically important traits in beef cattle (Buchanan *et al*., 2002; Schenkel *et al*., 2005). A detailed examination of leptin could provide an understanding of the significance this hormone plays in fat deposition and subsequently its influence on carcass traits.

Table 1.3 Summary of polymorphisms discovered for several candidate genes.

Gene	Chromosome location	Function	Polymorphism	Reference
Myostatin (MSTN / Mh)	2	Protein that limits muscle tissue growth.	*Mh* polymorphism results in 20% increase in muscle mass i.e. double muscling effect.	Marchitelli *et al.* , 2003; Grobet et al., 1997; Casas et al., 2000.
Calpain (CAPN)	7	Enzyme that lyses myofibril proteins thereby changing the structure of meat and increasing its tenderness and palatability.	3 Markers developed have been linked with increased meat tenderness.	Page *et al.,* 2002; Wheeler and Koohmaraie, 1994.
Calpastatin (CAST)	7	Enzyme that inhibits calpain and thereby regulates post-mortem proteolysis.	Several markers with loss of function mutations result in increased meat tenderness and palatability.	Schenkel *et al.,* 2006; Casas et al., 2006.
Somatotropin (BST) and Somatotropin receptors	20	Stimulates growth and cell reproduction in cattle.	Several polymorphisms developed have shown an increase in protein and fat % in milk production i.e. F279Y polymorphism.	Viitala *et al.* , 2006; Maj et al., 2005.
Insulin-like growth factor 1 (IGF1)	5	Regulates growth, differentiation, and the maintenance of differentiated function in numerous tissues and in specific cell types of mammals through binding to a family of specific membrane-associated glycoprotein receptors.	Several polymorphisms and causative mutations have been found to be associated with birth weight, pre-weaning average daily gain, average daily gain on feed, live weight and carcass weight.	Li *et al.,* 2004.
Signal transducer and activator of transcription (Stat)	2, 19 and 5	Several bovine stat genes that have been identified are involved in signal pathways regulating milk protein gene expression.	*Stat* mutations or deficiencies result in failure to lactate. SNPs were associated with increased milk, fat and protein yields.	Yang *et al* ., 2000; Seyfert et al., 2000; Cobanoglu et al., 2006.
Corticotrophin-releasing hormone (CRH)	14	Hormone that indirectly causes the release of glucocorticoids (growth inhibitors).	SNPs were associated with post-natal growth in cattle, rib-eye area and carcass weight.	Barendse *et al.* , 1997; Buchanan et al., 2005.
Pro-opiomelancortin (POMC)	11	Increases the production of CRH.	SNPs were associated with average daily gain and carcass weight.	Buchanan *et al.* , 2005.

1.5 Role of leptin in the beef industry

1.5.1 Introduction

Leptin is a 16-kid protein hormone that maintains body weight through regulation of appetite, energy expenditure and metabolism (Friedman, 1997; Zhang *et al.*, 1997; Bell *et al.,* 2005). Furthermore, leptin is also involved in the regulation of haematopoiesis, immune and inflammatory response (OMIM, 2010). Leptin was identified and cloned in 1994 at the Rockefeller University by Jeffrey Friedman during experiments conducted on diabetic and obese mice (Friedman, 1998; Soares and e Guimarães, 2001; Margetic, 2002). The resultant obese phenotype of the mice was due to a genotype that was homozygous (*ob/ob*) for the mutant leptin gene (*lep*) and hence produced inert leptin (Figure 1.5) (Breslow *et al*., 1999).

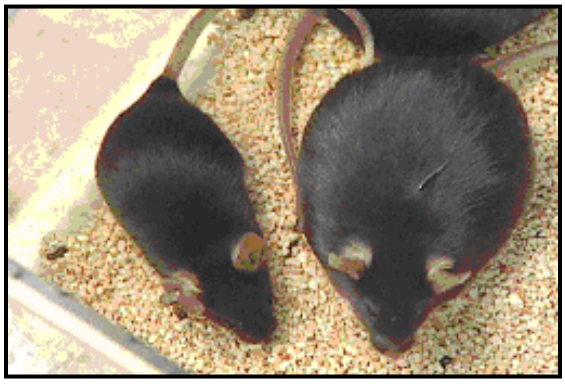

Figure 1.5 Obese mouse with *ob/ob* genotype (right) with normal genotype (left).

Photo: courtesy Daniel Eitzman.

In order to better understand the biological activity and physical properties of leptin, the structure of the *lep* gene and that of the hormone itself must be examined.

1.5.2 The biology of leptin

The *lep* gene is highly conserved between species. Since 1994, leptin has been cloned and characterized in several species such as humans, cattle, sheep, swine, gorillas, chimpanzees, orang-utans, rhesus monkeys and canines with a surprisingly high conservation between species sequence homology of 67% between the diverse species (Zhang *et al.*, 1997; Margetic, 2002; Switonski, 2002). Thus leptin has been recognized as a potential candidate for therapeutic developments in the treatment of human obesity as well as marker assisted selection in the agricultural industry. The bovine *lep* gene was mapped at position 4q32 on chromosome 4 in cattle (Figure 1.6) (Stone *et al., 1996;* Pomp *et al.*, 1997). It is 3090 bp in length, comprised of three exons and two introns that translate into a 146 amino acid peptide (Switonski, 2002).

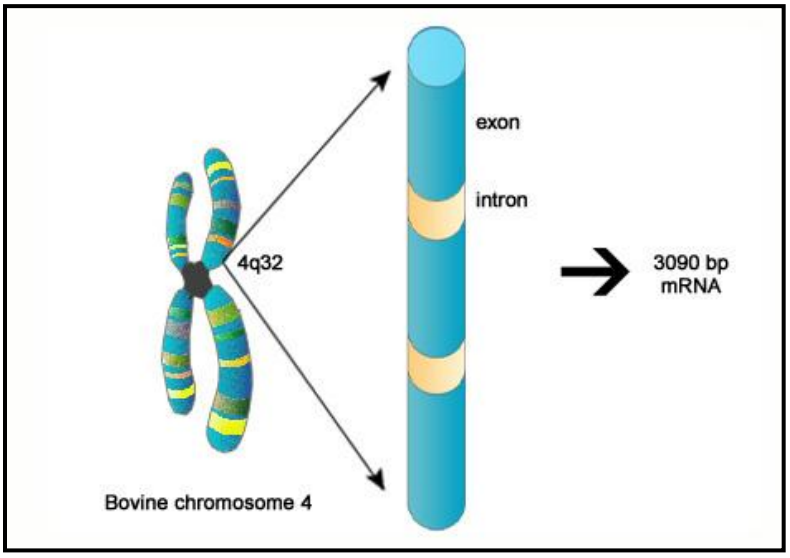

Figure 1.6 Diagrammatic structure of the bovine *lep* gene.

Leptin belongs to the haemopoeitic cytokine family (Rock *et al*., 1996; Margetic, 2002). Cytokines are soluble signal proteins that are secreted into the blood and are distinguished by their distinctive three-dimensional folding even though they share no sequence similarity (Bruno *et al*., 2005). The leptin molecule itself is comprised of 167 amino acids that undergo a number of post-translational modifications such as the cleaving of the amino-terminal secretory signal sequence of 21 amino acids, which

enables leptin to be exported outside the cell and function peripherally. The final protein product of leptin is a mature non-glycosylated peptide consisting of 146 amino acids. The three dimensional structure of leptin is comprised of 4-α-helices named A, B, C and D. These helices are connected via crossover links thereby resulting in a structure AB and CD arranged in a left-hand twisted helical bundle (Zhang *et al.,* 1997) (Figure 1.7). Two cysteine residues (Cys 146 and Cys 96) form an intra-chain disulphide bridge between the C-terminus, COOH, and the beginning of the CD structure of the protein (Zhang *et al., 1997*). It is these cysteine residues that are critical to the structural integrity and stability of leptin therefore mutations in these cysteine residues leads to a loss in structure and ultimately loss of leptin activity (Xie *et al.,* 1999).

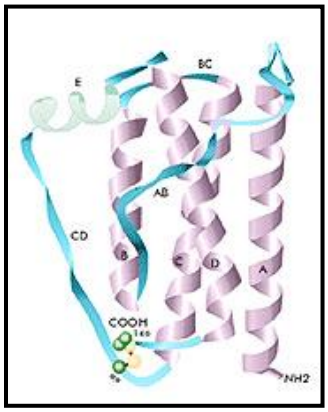

Figure 1.7 Quarternary helical structure of leptin.

Leptin is synthesized and expressed primarily by adipocytes, though it is found to a lesser extent in brown adipose tissue, the placenta, the ovaries, skeletal muscle, the stomach, mammary epithelial cells, bone marrow, the pituitary gland and the liver (Houseknecht *et al*., 1998; Bell *et al.,* 2005, Zieba, 2005). Leptin acts both centrally and peripherally as a messenger, informing the brain and various tissues about the body's energy status. Leptin and its six receptors (LepRa-LepRf) form integral components of a homeostatic system that regulates satiety, lipid partitioning, fertility, immune functioning, lactation and other metabolic pathways in mammals (Friedman 1998; Hossner 1998; Houseknecht *et al.,* 1998; Ren *et al.,* 1999; Soares and e Guimarães, 2001). Peripherally, leptin is secreted from adipose tissue, circulating at levels that are

proportional to body adipose stores, and exerts its effects through the leptin receptor (LEPR) through (Figure 1.8):

1. counteracting the effects of orexigenic signals which promote food intake such as neuropeptide Y (NPY) neurons,
2. promoting anorexigenic signals which inhibit food intake such as pro-opiomelancortin (POMC) neurons by decreasing the inhibitory action of neurotransmitter GABA (gamma-aminobutyric acid),
3. activating POMC which produces alpha-MSH (melanocyte-stimulating hormone - an appetite suppressant),
4. stimulating the secretion of reproductive hormones such as gonadotropin-releasing hormone (GnRH) which in turn stimulates the pituitary to produce luteinising and follicle stimulating hormone, and
5. raising body temperature to increase energy expenditure (Bell *et al.,* 2005).

Furthermore, leptin acts directly on liver and muscle cells where it stimulates the oxidation of fatty acids inside the mitochondria thereby causing a reduction in the storage of fat in the specific tissues.

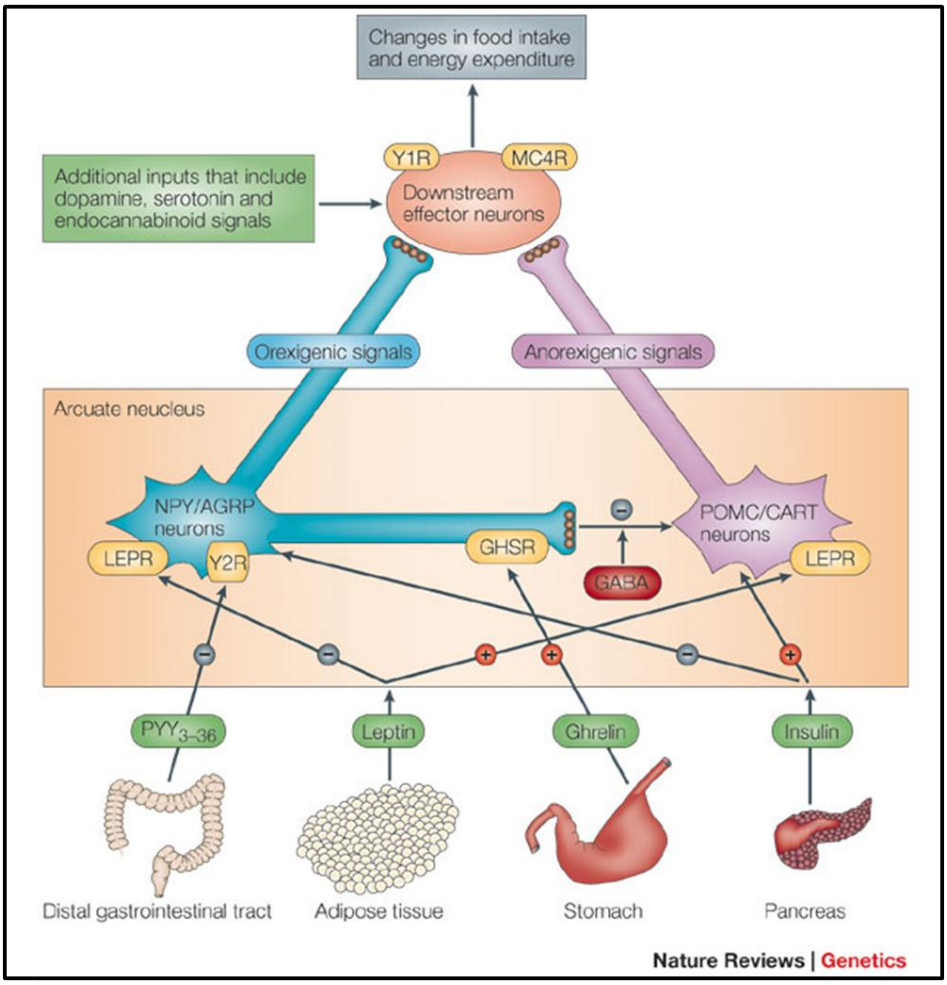

Figure 1.8 Physiological regulation of energy balance (adapted from Bell *et al.*, 2005)

1.5.3 Leptin locus as a molecular marker in the beef industry

Research data from swine, sheep and cattle indicate that serum and plasma leptin concentrations are highly correlated with feed intake, carcass quality as well as adipose tissue mass (Nkrumah *et al*., 2004b). Since the bovine leptin gene has been identified on chromosome 4, several polymorphisms and SNPs have been identified in introns and exons among different breeds of cattle (Pomp *et al.,* 1997; Fitzsimmons *et al*., 1998; Haegeman *et al*., 2000; Geary *et al.,* 2003, Schenkel *et al*., 2005). Furthermore, Fitzsimmons *et al.* (1998) reported that the alleles of the microsatellite marker (*BM1500*), located 3.6 kb (kilo bases) away from the bovine leptin gene, showed an

association with body fatness in cattle. Tessanne *et al*. (1999) studied the relationship of polymorphisms in bovine leptin gene with differences in beef carcass traits. The effect of genetic variation at several loci (leptin, growth hormone, kappa casein, beta lactoglobulin, and Pit-1) and their allelic effects on growth and carcass traits have been reported in beef cattle (Zwierzchowski *et al*., 2001). The leptin gene polymorphism was shown to affect feed intake, conversion as well as some carcass traits.

In addition, Buchanan *et al*. (2001) identified a cytosine (C) to thymine (T) transition that encoded an amino acid change from an argenine to a cysteine in exon two of the leptin gene. A PCR-RFLP was designed and allele frequencies were correlated with carcass fat which resulted in the T-allele showing a strong correlation with an increased carcass fat than that of the C-allele. The research conducted indicated that the T-allele, which encoded the amino acid transition, resulted in partial loss of biological function of the leptin hormone and could hence be the causative mutation. Further investigation and application of the SNP (E2FB) has extended to positive associations with feed intake, intramuscular fat, milk yield and composition (Buchanan *et al*., 2002).

Lagonigro *et al*. (2003) screened an experimental cattle population for polymorphisms in the *lep* gene and five SNPs were found in the regions containing the coding sequences. The results suggested an association between a polymorphism in exon 2 and feed intake. Barendse *et al*. (2004) studied the association of E2FB with marbling, fatness (including backfat thickness), and efficiency of production as well as milk and milk protein yield. However, no associations were found between the *lep* genotypes and fatness. Schenkel *et al*. (2005) evaluated the association of several SNPs in the *lep* gene with carcass and meat quality traits from a large sample of crossbred beef cattle. Five SNPs (UASMS1, UASMS2, UASMS3, E2JW, and E2FB) were genotyped on 1,111 crossbred bulls, heifers, and steers. Associations between the SNPs within the *lep* gene were found with lean yield, fatness, and tenderness.

1.6 Aim

Leptin polymorphisms may be associated with differences in carcass traits and body composition. Research conducted on the causative mutation in *lep* exon 2 (E2FB) is not conclusive due to inconsistent evidence presented by several researchers (Buchanan *et al*., 2002; Nkrumah *et al*., 2004b; Barendse *et al*., 2005; Schenkel *et al*., 2005). The principle aim of this investigation was to determine whether the SNP, E2FB, could be used as a direct marker for selecting beef attributes.

Furthermore, two populations of different beef cattle breeds were included in the investigation, namely Nguni, an indigenous breed, and Hereford, an exotic breed. The E2FB SNP detected by Buchanan *et al*. (2002) has not yet been conducted on the indigenous Nguni breed or the usefulness of its application determined within South African meat quality standards. This research was partitioned into four separate investigations to determine the suitability of E2FB as a direct marker:

Investigation 1: Assessment of phenotypic variation in pre- and post-slaughter beef traits within Nguni and Hereford breeds.

Investigation 2: Assessment of genetic variation at the *lep* locus in Nguni and Hereford breeds.

Investigation 3: Assessment of the relationship between the E2FB genotypes and pre- and post-slaughter phenotypes in Nguni and Hereford breeds.

Investigation 4: A comparative analysis of Nguni and Hereford breeds in terms of feedlot performance.

The relationships between the different investigations are illustrated in Figure 1.9.

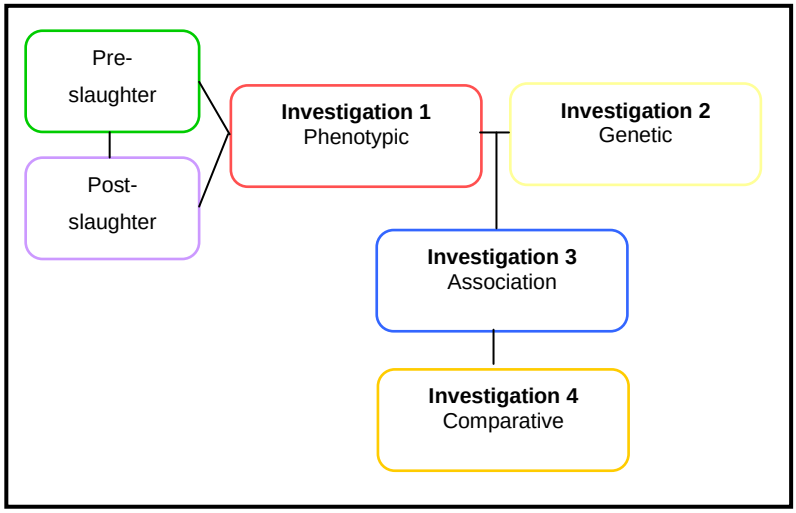

Figure 1.9 Flow diagram of the different investigations and analyses implemented in this research project.

CHAPTER 2

MATERIALS AND METHODS

2.1 Selection of sample cattle populations

In this investigation cattle were selected from feedlots, analysed and compared. The one population comprised Nguni, an indigenous breed, and the other population comprised Hereford, an exotic breed.

Both Indigenous and exotic cattle breeds are used in the South African commercial beef industry. An investigation of the incidence of advantageous alleles within the different breeds is valuable in the genetic improvement of beef cattle. It is possible to compare exotic and indigenous breeds in order to assess the natural heritage of the *lep* gene in indigenous cattle and subsequently employ these breeds in crossbreeding schemes. Feedlots within the beef industry rarely utilize purebred animals as crossbreeding schemes are utilized in order to maximize hybrid vigour (Annandale, personal communication, 2004). Furthermore, purebred Nguni are especially difficult to locate in feedlots as their small frames limit their potential in the beef industry (Scholtz *et al*., 1999a). However, Nguni are widely employed in crossbreeding schemes in order to incorporate advantageous alleles acquired through years of selection and adaptation to local conditions. As a result only a few feedlots have populations of pure breeds. Consequently, populations of purebred Nguni and Hereford could only be obtained from two different feedlots for this investigation.

Individuals for this investigation were randomly selected from the individuals in the two feedlots who qualified for the study based on the following criteria:

1. Easily obtainable blood samples for genotyping,
2. Access to animals for phenotypic measurements,
3. Accessibility of carcass data after slaughter,

4. Availability of sizeable populations comprised of relatively pure bred (based on the discernment of qualified animal scientists present) unrelated individuals of similar age (1 yr old) and predominantly male, and
5. Standardized feedlot conditions and management practices. This included similar environment, duration in feedlot (120 days) and diet programme.

Two feedlots that conformed to the selection criteria were identified, both of which were subdivisions of Crafcor Farming (Pty) Ltd. The Nguni population comprised 70 cattle of which 55 were oxen and 15 heifers, obtained from Mr Wimpie Annandale, from the CCC Feedlot located on the Maybole farm, in the Dundee district, KwaZulu-Natal. The Hereford population comprised 48 oxen obtained from Mr Danie van Huysting, from the CCC Riversdale Feedlot along the R614 road, in the Wartburg district, KwaZulu-Natal. Animals were randomly selected from these large sized populations of up to 5000 (in accordance with the above mentioned specifications), therefore ensuring that they were more than likely unrelated (generally no pedigree data is available for oxen entering feedlots). Both feedlotting periods ran concurrently from May till August over the winter period whereby the animals were contained in an enclosure outside (average temperatures ranged from 4-22°C). The diet programme is specified in Appendix B. Table 2.1 provides identification information of the two respective populations. It should be noted that in the Nguni population, 23 of the 55 oxen were treated with a hormone called Zulmax, whilst the remaining 22 oxen remained on an untreated regular diet. This information was only made known to the researcher after sample selection had taken place and hence subsequent modification of statistical analyses to account for variation due to hormone treatment in the Nguni population.

Table 2.1 Cattle populations selected for this research project.

Population	Identification tag	No. of individuals	Sex	Location
Nguni	N1-N23	23	Ox	Dundee
	N24-N55	22	Ox	Dundee
	NH1-NH15	15	Heifer	Dundee
Hereford	H1-H48	48	Ox	Riversdale

2.2 Investigation 1: Assessment of phenotypic variation

2.2.1 Introduction

Fat levels influence many of the economically important carcass traits, and it is therefore expected that leptin could play a major role in carcass quality. Furthermore, age, sex and breed are additional attributes to be taken into account when investigating carcass quality as these factors have great bearing on carcass fat distribution, tenderness and overall meat quality (Kempster, 1980; Strydom *et al*., 2000). The value-based marketing system of the South African beef industry is partial to carcasses without excess fat cover and carcasses are graded according to the legislation set forth by the government organization of SAMIC (Perry and Fox, 1997). South African grading is focused on subcutaneous fat, unlike the official standards for grades of carcass beef in the USA, Canada and Japan, where intramuscular fat is valued (Nishimura *et al*., 1999; SAMIC, 2007). The objective of this investigation was thus to assess the phenotypic variation of carcass traits in Nguni and Hereford cattle in order to facilitate subsequent genetic analyses and comparisons.

2.2.2 Methods

The measurements of pre-slaughter traits for each animal, live weight and body composition were recorded over the 120 day feedlot period. A body composition score (BCS) was assigned to each animal on the first day of the feedlotting period, while the live weight was recorded on day 1 and day 113. The BCS was determined according to the guidelines specified by Webster (1987), the tailhead area and loin area of the animal was assigned a grade according to conformation and fatness levels. The conformation score ranged from zero to five where a score of zero was indicative of poor condition, whereas a value of five indicated a morbidly obese animal. The live weight readings (kg) were taken on standard feedlot weighing scales for each individual on the two respective days, 1 and 113.

The measurements of post-slaughter traits for each animal, carcass classification and carcass weights, were taken at the Cato Ridge Abattoir, KwaZulu-Natal. All procedures and measurements were conducted according to SAMIC grading standards (Table 2.2). The classification of carcasses produced the following data: age (in years), sex, fatness, conformation and carcass damage. Additional data provided by the abattoir included warm carcass mass (kg) and cold carcass mass (kg).

Table 2.2 Classification of beef carcasses according to SAMIC

Age		Class	Symbol
0 Teeth	(<2yrs)	A	
1-2 Teeth	(2-$2^{1/2}$yrs)	AB	
3-6 Teeth	($2^{1/2}$-3yrs)	B	
>6 Teeth	(4yrs)	C	

Fatness	Class
No Fat	0
Very lean	1
Lean	2
Medium	3
Fat	4
Overfat	5
Excessively overfat	6

Eg. Class 3

Conformation	Class	Symbol
Very flat	1	
Flat	2	
Medium	3	Eg. Class 3
Round	4	
Very round	5	

Damage	Class
Slight	1
Moderate	2
Severe	3

Sex

Only for oxen and sheep carcasses in the AB, B and C groups

Standard summary statistics: mean, standard deviation and variance, were calculated to assess the overall phenotypic performance of the various traits measured in the two breeds using the software program SPSS version 11.5.1 (LEAD Technologies Inc., 2002). A further analysis within the Nguni breed was conducted in order to assess the effect of hormone treatment on the pre-slaughter and post-slaughter traits by means of a General linear model (GLM). According to the following equation:

$$Y_{jklmn} = u + b_1 L_j + S_k + A_l + G_m + T_n + (G \times T)_{mn} + (G \times S \times A \times T)_{mkln} + e \qquad [1]$$

Where Y_{jklmn} is the trait measured for jth individual; u= overall mean for the trait; $b_1 L_j$ = regression coefficient (b_1) of the covariate initial live weight (L_j) for the jth animal; S_k = fixed effects of sex where k denotes male or female; A_l= age where l indicates 1, 2, or 3

years of age; G_m = genotype whereby m represents genotypes *CC*, *CT* or *TT*; T_n = where n denotes whether the individual was hormone treated or not $(G \times T)_{mn}$ = interaction between genotype and treatment; $(G \times S \times A \times T)_{mkln}$ = interaction between genotype, sex, age and treatment; and e = environment (Cato ridge feedlot).

A comparison between the different feedlots, Cato Ridge and Dundee, could not be carried out due to the fact that feedlot effect is confounded within breed effects i.e. the effect of breed is essentially the effect of the two different feedlots.

2.3 Investigation 2: Assessment of genetic variation at the *lep* locus

2.3.1 Introduction

The *lep* gene has been implicated as a potential candidate gene that influences beef carcass characteristics, especially in terms of fat deposition (Kononoff *et al*., 2005). Consequently, the *lep* locus possesses great potential for MAS in the beef industry; however, the effect of the *lep* locus on specific economically important carcass traits in the South African beef industry has not yet been investigated.

The *lep* gene consists of three exons and two introns. Buchanan *et al*. (2002) described an association between polymorphisms in exon 2 of the *lep* gene with carcass fat levels due to a causative mutation. This polymorphismhas been identified as an SNP mutation (E2FB) located in the 1st position of the 25th codon (U50365). The SNP is characterized by a cytosine (C) to thymine (T) transition which subsequently results in an amino acid change of arginine (Arg) to cysteine (Cys) (Oprządek *et al*., 2003). The resultant E2FB allelomorphs have been termed T- and C-alleles, with the ensuing genotypes being homozygous *TT* and *CC* or heterozygous *CT*. Investigations have demonstrated that the T-allele is associated with an increased fat deposition in terms of intramuscular and subcutaneous fat as well as increased leptin mRNA levels (Buchanan *et al*., 2002). Therefore, individuals that are homozygous or heterozygous for the T-

allele have been genetically earmarked as ‘superior’, in compliance with the hypothesis that states that the phenotypic outcome of the mutation results in favourable carcass characteristics (Nkrumah *et al.*, 2004b).

Veerkamp *et al*. (2000) suggested that PCR-RFLP technology is suitable for the identification of different allelomorphs at a particular SNP site in a DNA sequence. The allelomorphs of E2FB are also identifiable through the application of this technology by employing the restriction endonuclease *Aci*I (Figure 2.1). This enzyme recognizes a recognition site that spans the *lep* SNP. The SNP in exon 2 is thus distinguishable after the amplification of the exon region and digestion with *Aci*I endonuclease. The allelomorphs can then be identified by the presence or absence of the different nucleotides (T or C) at the SNP position.

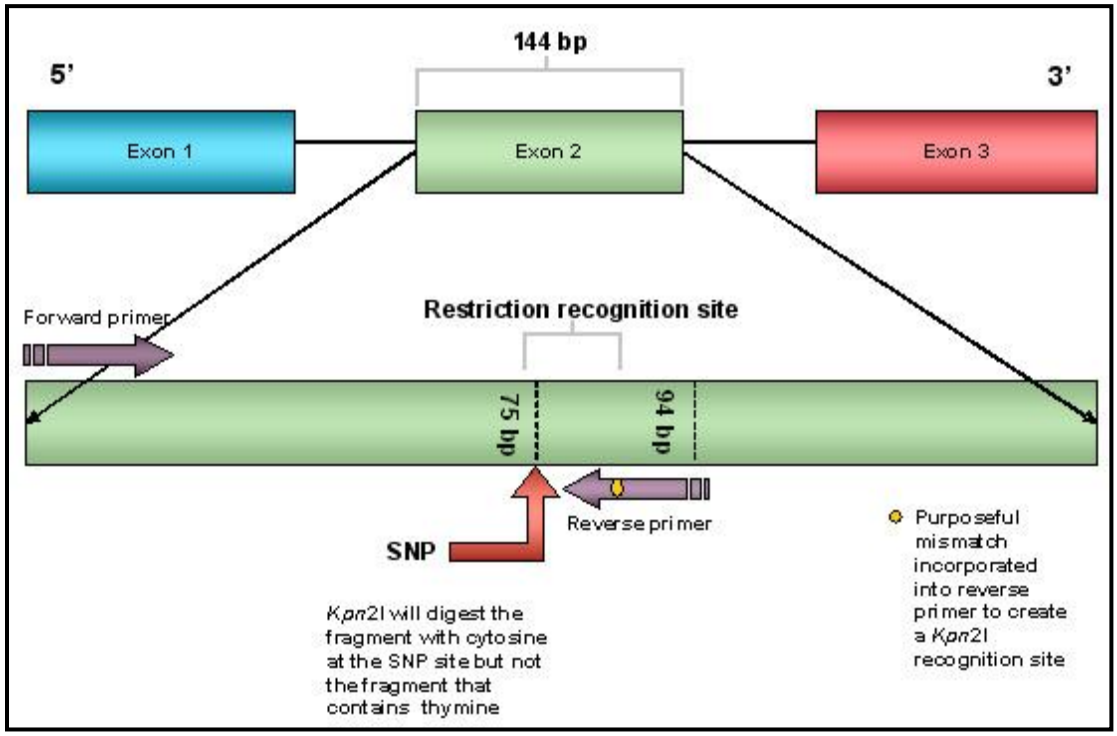

Figure 2.1 Amplification of *lep* exon 2 with adapted primers.

It is known that *Aci*I has an inhibitory effect on PCR reagents and would therefore obstruct the amplification process of exon 2, thus an alternative method was designed

by Buchanan *et al*. (2003) to facilitate the successful identification of the *lep* alleles with PCR-RFLP. Primers that amplify a 94 base pair (bp) segment of exon 2 in the region where the SNP occurs, were specifically designed to incorporate a purposeful mismatch mutation (**G**) in the reverse primer so that an alternative restriction site is created which is recognized by the restriction endonuclease *Kpn*2I (Figures 2.2 and 2.3). The amplified product subsequently would contain a *Kpn*2I recognition site that upon digestion with the endonuclease *Kpn2*I would cleave C-allele amplification product into 2 fragments of 75 and 19 bp, whilst the amplification product of the T-allele would remain as an undigested 94 bp fragment (Figure 2.3).

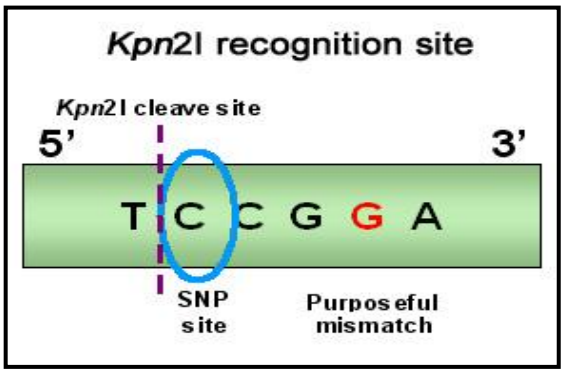

Figure 2.3 Restriction site of lep exon 2.

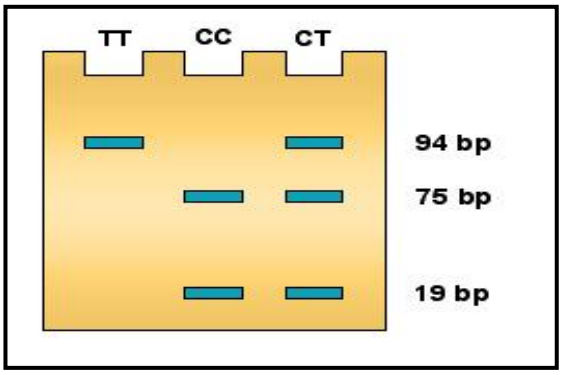

Figure 2.2 SNP genotypic identification. RFLP products can be visualized using gel electrophoresis in order to discriminate between alleles and determine genotypes.

The adapted primers designed by Buchanan *et al*. (2002) were employed to determine the genotypic constitution of the *lep* locus of all the animals that participated in this investigation to establish possible linkages with carcass traits. DNA was isolated from cattle blood for subsequent genotyping and the identification of the various *lep* SNP genotypes was carried out with PCR-RFLP. This technique required a number of procedures such as DNA isolation, *lep* gene amplification, verification of amplification product, *lep* gene restriction, determination of *lep* alleles and genotypes as well as population analysis of the *lep* locus.

All recipes of chemical solutions used in this investigation can be found in Appendix A.

2.3.2 Methods: DNA source and isolation

Blood was used as a source of DNA for genotypic analyses, as it is practical, readily obtainable and easy to extract from cattle. Cattle blood erythrocytes are enucleated; therefore nucleated leukocytes are usually used as the primary source of nuclear DNA. Venous blood was collected from each animal of each population on day 1 of the 120 day feedlotting period by qualified animal scientists using sterile Becton-Dickinson precision glide Vacutainer Systems needles (1.2 X 38 mm). The venous blood was extracted from either the tail or jugular vein into a 4 ml (millilitre) vacutainer tube containing EDTA (ethylenediaminetetraacetic acid) to prevent coagulation and thereafter placed on ice until transferred to a -70°C freezer within 24 hours (Sambrook *et al*., 1989). The blood samples were stored at -70°C until the DNA was isolated (Polakova *et al*., 1989).

DNA was isolated from the venous blood using a protocol adapted from Sambrook *et al*. (1989) and Bruford *et al*. (1992). Successful isolation of DNA involved a number of sequential steps, namely:

1. Leukocyte nuclei isolation,
2. DNA isolation,
3. DNA precipitation and
4. DNA verification and quantification.

1. *Leukocyte nuclei isolation*

1. A vacutainer tube containing EDTA and blood was removed from the freezer and placed in a 37°C water bath for 10 minutes (min) to defrost.
2. The tube was then gently finger-tapped to dislodge the blood clump that had accumulated at the bottom of the vacutainer tube.
3. 400 μl blood was removed from the vacutainer tube using a micropipette and placed in a sterile 1.5 ml Eppendorftube.

4. The Eppendorftube was centrifuged at 2 000 rotations per minute (rpm) for 15 min at a temperature of 0°C in a Beckman Centrifuge using rotor 18.1. The supernatant containing the blood plasma was removed by gentle decantation and discarded. Only the white buffy coat containing leukocytes remained in the Eppendorftube.
5. The pellet containing leukocytes was re-suspended in 1 ml ice-cold 1 X blood cell lysis buffer and 14 μl 10% Triton X-100. The tube was then vortexed using a Vortex mixer until the bottom of the tube was clean.
6. The Eppendorftube was centrifuged at 2 000 rpm for 20 min at 2°C in a Beckman Centrifuge using rotor 18.1.
7. The supernatant containing blood plasma was gently decanted and discarded. The residual pellet in the Eppendorftube was briefly drained.
8. The pellet was again re-suspended in 1 ml ice-cold 1 X blood cell lysis buffer and 14 μl 10% Triton X-100. The tube was then vortexed until the bottom of the tube no longer contained debris.
9. The Eppendorftube was centrifuged at 2 000 rpm for 15 min at 2°C.
10. The supernatant in the Eppendorftube was discarded by decantation and the remaining pellet was briefly drained for 10 min. The pellet was opaque and white in appearance, as it consisted only of leukocyte nuclei.
11. With the use of a micropipette, the following was added to the pellet: 500 μl 1 X TNE, 50 μl 1 M Tris-HCl pH 8, 7.5 μl 25% SDS, and 7.5 μl Triton X-100. Lastly, 1 μl of 10 mg/ml Proteinase K was added to the pellet. Proteinase K, in the presence of EDTA, digests leukocytes as it seizes divalent cations and inhibits DNases rendering membranes soluble and proteins denatured.
12. The Eppendorftube containing the suspended pellet was incubated overnight in a 37°C water bath.

2. *DNA isolation*

1. The Eppendorftube was removed from the 37°C water bath and 400 μl 5 M NaCl was added to the pellet using a micropipette.
2. The tube was then shaken gently for 15 min with caution to avoid fragmentation of the DNA.
3. The Eppendorftube was centrifuged at 5 000 rpm for 15 min at room temperature in a Bench top Microfuge.
4. The supernatant containing DNA was removed with caution to avoid the foam, using a micropipette and transferred into a fresh sterile Eppendorftube.
5. Steps 1 to 3 were then repeated in order to obtain a clear supernatant.

3. *DNA precipitation*

1. The supernatant was removed with a micropipette and transferred to a fresh sterile Eppendorftube.
2. 800 µl of ice cold 100% ethanol was added to the supernatant using a micropipette.
3. The Eppendorftube was mixed by inversion until the white string-like DNA precipitate became visible. Thereafter, the tube was placed in a -20°C freezer for 30 min to increase the DNA yield.
4. The Eppendorftube was centrifuged at 13 000 rpm for 15 min at room temperature in a Bench top Microfuge. Thereafter, the ethanol in the Eppendorftube was decanted and the tube was gently blotted on tissue paper.
5. The remaining DNA pellet was washed in 400 µl 70% ethanol by gentle inversion of the Eppendorftube for 5 min.
6. The Eppendorftube was centrifuged at 13 000 rpm for 10 min at room temperature in a Bench top Microfuge.
7. Steps 5 to 6 were then repeated. The ethanol was gently decanted and remaining pellet in the tube was air-dried for 30 min to remove as much ethanol as possible.
8. The pellet was re-suspended in 50 µl 10 mM Tris-HCl (pH8) in a 37°C water bath overnight.

4. *DNA verification and quantification*

Verification of DNA

Agarose gel electrophoresis, a technique to isolate, detect and quantify DNA fragments obtained after isolation, amplification or restriction digestion, was used to verify the presence of successful cattle DNA isolates. Agarose gels have lower resolution than polyacrylamide gels and are used to separate double-stranded DNA fragments of high molecular weight. In appropriate concentration and configuration, agarose gels separate DNA fragments at a velocity inversely proportional to the length of the fragment and proportional to the electric field strength. Hence, the successful isolation of high molecular weight cattle DNA was verified through agarose gel electrophoresis (1%) using an adapted protocol from Sambrook *et al*. (1989):

1. 2 g of agarose was weighed out on a balance and placed in a clean Erlynmeyer flask that contained 200 ml 1 X TAE (Tris-acetate-EDTA) buffer. The contents of the flask were swirled to mix.
2. The solution in the flask was heated in a microwave for 2 min until clear and transparent. Caution was taken not to let the solution boil over.
3. The agarose solution was cooled to a temperature of 50°C. Prior to the gel pouring, 12.5 μl of 0.5 μg/ml ethidium bromide solution was added to the solution with a micropipette and the flask was swirled to mix the contents.
4. The melted solution was carefully poured into a clean gel casting tray with an appropriate sized comb and allowed to set for 45 min.
5. The solidified gel was placed into an electrophoresis tank that contained 1.5 l 1 X TAE buffer ensuring the buffer submerged the gel by 2 mm. The gel comb was carefully removed and 12.5 μl of 0.5 μg/ml ethidium bromide solution was pipetted into the electrophoresis tank to ensure even staining of the gel.
6. The DNA containing sample was thawed at room temperature. 5 μl of the sample was transferred to a fresh, sterile Eppendorftube with a micropipette. 5 μl sterile distilled water and 2 μl bromophenol blue loading buffer was added to the tube with a micropipette.
7. A molecular weight marker (MWM) sample was prepared in a sterile Eppendorftube that contained 0.6 μl MWM 50 bp ladder, 10 μl distilled sterile water and 2 μl bromophenol blue loading buffer.
8. 12 μl of the sample as well as the marker were carefully loaded into separate wells in the agarose gel with a micropipette.
9. The lid of the apparatus was replaced and the power leads were connected. The gel initially electrophoresed at 120 V for 5 min to aid the outflow of the samples from the wells and thereafter at 100 V for 3 hrs.
10. The agarose gel containing the DNA was then visualized and captured with BioRad Versa Doc Imaging system (Model 4000) and analyzed with Quantity One 4.5.1 computer software program.

Determination of DNA quantity and quality

DNA quantity and quality was assessed through spectrophotometry and agarose gel electrophoresis. Spectrophotometry analysis was conducted on a large number of samples, however, DNA samples isolated at a later period in the investigation could not be analyzed, as it was established that the apparatus' calibration was unreliable after repeated readings did not yield the same result. Thus, quantification of the remainder of samples was undertaken with agarose gel electrophoresis, and the quality and quantity

of the DNA sample was estimated through visual observation by comparison with a DNA sample of known concentration of 1 μg/μl.

1. 500 μl 1 X TAE was added to a sterile semi-micro quartz curvette with a micropipette. The curvette was then placed into a Beckman Du 640 Spectrophotometer and used as a blank to calibrate the instrument.
2. 10 μl of the DNA sample and 490 μl 1 X TAE were added to a sterile semi-micro quartz curvette with a micropipette. The curvette was then placed into the spectrophotometer where A_{260} and A_{280} measurements were recorded. The procedure was repeated for each DNA sample ensuring the instrument was blanked every 7^{th} sample in order to give consistent and accurate readings.
3. The concentration of DNA for each sample was determined by the calculation:
   A_{260} X 50 (dilution factor) X 50 (constant) = *x* μg/ml

   The purity of each sample was assessed by calculation of the ratio A_{260} / A_{280} (Sambrook *et al*., 1989) where a ratio of 1.8 is indicative of optimal DNA quality.

2.3.3 Methods: *Lep* gene amplification

PCR, an iterative process that allows for the synthesis of many copies of a desired fragment of DNA, was used to amplify the alleles of the respective *lep* exon 2 SNP genotypes. The DNA, isolated from all the individuals of the sample populations, underwent amplification with the adapted primers in order to generate a 94 bp fragment of *lep* exon 2 that harboured the SNP.

Successful amplification of the *lep* gene thus required the synthesis of the primer set for amplification of *lep* gene exon 2, amplification of *lep* gene exon 2 as well as verification of the amplification product.

2.3.3.1 Primer set of *lep* gene exon 2

The primer set described by Buchanan *et al*. (2002) for amplification of a 94 bp DNA segment of *lep* exon 2 is presented in Table 2.3. This primer set, designed with a mismatch mutation in the reverse primer to present a *Kpn*2I restriction site (represented in bold), was synthesized by Roche (Pty) Ltd.

Table 2.3 Primers for amplification of *lep* exon 2.

Primer (No. bp)	Sequence
Forward (22)	ATG CGC TGT GGA CCC CTG TAT C
Reverse (21)	TGG TGT CAT CCT GGA CCT T**C**C

2.3.3.2 Amplification of *lep* gene exon 2

The initial PCR profile applied was taken from a known protocol by Buchanan *et al*. (2003). The profile conditions and the controls used are presented in Table 2.4.

The controls used for the amplification process were as follows:

- Control 1, a negative control in which Taq polymerase was excluded to ensure no contamination had taken place,
- Control 2, a negative control in which DNA template was excluded to ensure no contamination had taken place,
- Control 3, a negative control that consisted of plant DNA template with *lep* primers to ensure the specificity of the *lep* primers for cattle DNA template, and
- Control 4, a control that consisted of plant DNA template with corresponding primers that amplified a 750 bp nuclear ribosomal DNA internal spacer region (ITS) to ensure the cycling conditions and reagents were in order.

The preparation of the samples for amplification took place at a sterile laminar flow bench where reagents were placed in a sterile 1.5 ml microfuge tube with a micropipette according to the quantities specified in Table 2.4. Taq polymerase was added to the mixture last, after the initial denaturation step to prevent non-specific primer-template complexes from forming. Amplification of all the samples was carried out with an Applied Biosystems Gene Amp PCR system 9700 according to the following profile (Buchanan *et al*., 2003):

2 min 94°C
35 x (45s 94°C; 45s 52°C; 55s 72°C)
3 min 72°C
Hold 4°C

Table 2.4 Preliminary PCR protocol.

Reagents (μl)	Control 1	Control 2	Control 3	Control 4	Sample	Final [ ]
10 X PCR Buffer (with 1.5 mM $MgCl_2$)	5	5	5	5	5	1X
$MgCl_2$ (25 mM)	3	3	3	3	3	1.5 mM
dNTP mix (100 mM each)	1	1	1	1	1	200 μM
Forward primer	5	5	5	3.75	5	10 pmol
Reverse primer	5	5	5	3.75	5	10 pmol
DNA template	2.5	0	2.5	2.5	2.5	> 100 ng
Taq polymerase (5 U/μl)	0	0.5	0.5	0.5	0.5	1 U/μl
Sterile H_2O	28.5	30.5	28	31	28	-
Total volume (μl)	50	50	50	50	50	-

The preliminary PCR yielded inadequate amplification product for further genotypic analyses, probably due to the unpredictable nature of the untested DNA template, as well as newly synthesized reagents and different laboratory conditions. Therefore, optimization of reagent concentrations and cycling conditions was required to enhance the quality and yield of the amplification product. A number of optimization attempts were undertaken by following directives from Sambrook *et al*. (1989), Don *et al*. (1991), Buchanan *et al*. (2002), Madeja *et al*. (2004); Nkrumah *et al*. (2004b). These included the modification of PCR reagent concentrations and PCR cycling conditions in the order presented in Table 2.5:

Table 2.5 PCR troubleshooting optimization attempts.

Modification	Reason	Result
$MgCl_2$ concentration (mM) 0.5, 1.5, 2.5.	Increase stringency of primer annealing.	Superior amplification product with 1.5 mM.
Annealing temperature (T_a) 54°C.	Increase specificity of primer annealing.	Laddered amplification product.
Touchdown profile 5 X (T_a = 58°C, 57°C, 56°C, 55°C, 54°C) 30 X (T_a = 53°C).	Increase stringency and specificity of primer annealing.	Faint amplification product.
Annealing temperature (T_a) 56°C (initial PCR profile).	Increase specificity of primer annealing.	Faint amplification product.
Primer concentration 5 pmol (T_a = 54°C).	Decrease spurious priming and avoidance of primer-dimer.	Faint laddered amplification product.
dNTP concentration 100 μM.	Enhance accuracy of extension.	Faint amplification product.
Taq Polymerase concentration 0.5 U/μl.	Increase stringency and eliminate unnecessary cost.	Equivalent to 1 U/μl concentration.
Annealing time 30s.	Decrease spurious priming.	Faint amplification product.
Extension time 15s.	Eliminate unnecessary time expenditure.	Faint amplification product.
Annealing temperature (T_a) T_a = 46°C, 48°C, 50°C, 52°C respectively.	To attain amplification product then increase the stringency of the reaction.	Poor amplification product.
No. of cycles 40.	Increase amplification product yield.	Smeared amplification product.
Primer set Working stock dilutions were reconstructed.	Avoid contamination and primer-dimer formation.	Intense amplification product lacking clarity.

Optimization troubleshooting attempts yielded little improvement of the amplification product. An alternative publication by Madeja *et al*. (2004) employed a modified profile with the same primer set. This profile was then attempted along with the reconstitution of the PCR reagents and working stock solutions (Table 2.6). Satisfactory amplification was attained by modifying the annealing temperature by one degree Celsius. Amplification was carried out according to the following profile:

2 min 94°C
35 x (45s 94°C; 45s 60°C; 1 min 72°C)
5 min 72°C
Hold 4°C

Table 2.6 Optimized PCR protocol.

Reagents (μl)	Control 1	Control 2	Control 3	Control 4	Sample	Final [ ]
10 X PCR Buffer (with 1.5 mM $MgCl_2$)	5	5	5	5	5	1X
$MgCl_2$ (25 mM)	3	3	3	3	3	1.5 mM
dNTP mix (100 mM each)	1	1	1	1	1	200 μM
Forward primer	5	5	5	3.75	5	10 pmol
Reverse primer	5	5	5	3.75	5	10 pmol
DNA template	2.5	0	2.5	2.5	2.5	>100 ng
Taq polymerase (5 U/μl)	0	0.25	0.25	0.25	0.25	0.5 U/μl
Sterile H_2O	28.5	30.75	28.25	30.75	28.25	-
Total volume (μl)	50	50	50	50	50	-

2.3.3.3 Verification of amplification product

Successful amplification was verified through 3% agarose gel electrophoresis in a manner similar to that outlined in section 2.3.2.

Once it had been established that amplification product had successfully been produced, further confirmation was undertaken to ensure that the amplification product was the expected product size of 94 bp. As agarose is not able to distinguish between fragments that differ in size of up to 20 bp, polyacrylamide gel electrophoresis was used

to estimate the size of the apparent 94 bp DNA fragment as its resolving power is up to 1 bp. Hence, the successful confirmation of amplification product size was verified through polyacrylamide gel electrophoresis (20%) using an adapted protocol from Sambrook *et al*. (1989):

1. The glass plates and spacers for casting the polyacrylamide gel were thoroughly cleaned and dried ensuring no grease spots were deposited on the working surface. The plates and spacers were then carefully assembled together and placed into a vertical gel casting apparatus and reinforced with 1% agarose to avoid gel leakage.
2. A 10 ml 20% Polyacrylamide gel was prepared by means of adding 6.66 ml of 29% acrylamide and 1% bisacrylamide solution, 1.27 ml d.H_2O and 2 ml 5 X TBE buffer into a clean glass beaker with the use of a micropipette. The contents of the beaker were swirled to mix.
3. Prior to the gel pouring, 85 μl of 10% APS (aminopropyl silica) and 10 ml TEMED (tetramethylethylenediamine) was added to the solution in the beaker with a micropipette and the contents swirled to mix.
4. The gel solution was carefully poured into the vertical gel casting plates. An appropriate-sized comb was carefully placed into the top of the gel and allowed to set for 75 min.
5. The solidified gel, still in the casting plates, was placed into a vertical electrophoresis tank that contained 0.5 l 1 X TBE buffer ensuring the buffer submerged the wells of the gel by 2 mm. The gel comb was then carefully removed.
6. The lid of the apparatus was replaced and the power leads were connected. The gel was pre-electrophoresed at 150 V for 30 min to equilibrate the gel.
7. The amplification product sample was thawed at room temperature. 5 μl of the sample and 2 μl bromophenol blue loading buffer were micropipetted into a fresh, sterile Eppendorftube.
8. A 50 bp MWM sample was prepared in a sterile Eppendorftube that contained 0.6 μl MWM 50 bp ladder, 5 μl d.H_2O and 2 μl bromophenol blue loading buffer.
9. 7 μl of the sample as well as the marker were carefully loaded into separate wells in the polyacrylamide gel with a micropipette.
10. The lid of the apparatus was replaced and the power leads were connected. The gel was electrophoresed at 150 V for 2 hrs.
11. The polyacrylamide gel containing the amplification products was then carefully removed from the electrophoresis apparatus as well as from the glass plate cast and placed in a flat dish containing 12.5 μl of 0.5 μg/ml ethidium bromide solution dissolved in 250 ml 0.5 X TBE buffer and allowed to stain for 30 min.

12. The polyacrylamide gel containing the amplification products was then visualized and captured with BioRad Versa Doc Imaging system (Model 4000) and analyzed with Quantity One 4.5.1 computer software program.

Confirmation that the amplified product was the expected *lep* exon 2 was required, and the amplified product was sequenced by Inqaba Biotechnical Industries (Pty) Ltd. The sequencing was performed according to the Sanger dideoxy method and involved the forward and reverse sequencing of the amplification product (Sambrook *et al*., 1989). An electronic copy of the sequenced output of the amplification product was then analyzed and edited with Chromas version 2.3. The sequence identity was further validated by conducting an alignment between the *lep* gene sequence taken from the Genbank database (Accession No. U5036) and the edited sequence using *BLASTn* (nucleotide Basic Local Alignment Search Tool Program) version 2.2.10 (Claverie and Notredame, 2003).

2.3.4 Methods: *Lep* gene restriction

The amplification product of *lep* exon 2 was digested with restriction endonuclease *Mro*I, an isoschizomer of *Kpn*2I, in order to generate RFLPs for the subsequent identification of the E2FB allelomorphs. The restriction digestion protocol was taken from a known protocol by Buchanan *et al*. (2003).

The controls used for the restriction process were as follows:

- Control 1, a negative control from which *Mro*I restriction endonuclease was excluded to ensure no contamination had taken place,
- Control 2, a negative control from which DNA product was excluded to ensure no contamination had taken place,
- Control 3, a positive control in which λ DNA was digested to ensure that the restriction endonuclease was active and,
- Control 4, a positive control in which eukaryotic DNA was digested.

The preparation of the samples and controls for restriction digestion took place on ice. The reagents were placed in a sterile 1.5 ml Microfuge tube with a micropipette whilst care was taken to ensure that the restriction endonuclease *Mro*I was added last to each tube. The samples were then incubated in a water bath at 37°C for 2 hrs and the product verification of the restriction digestion conducted using polyacrylamide gel electrophoresis as outlined in section 2.3.3.3.

2.3.5 Methods: Determination of *lep* alleles and genotypes

RFLP products for every individual in each sample population were visualized using polyacrylamide gel electrophoresis in order to discriminate and identify the *lep* T- and C-alleles, in order to determine the genotypic constitution of each individual in a similar manner to that illustrated in Figure 2.4

2.3.6 Methods: Population analysis of the *lep* locus

Standard summary statistics - allele frequencies, genotypic frequencies, Hardy-Weinberg Equilibrium (HWE), mean, standard deviation, and variance - were calculated to assess the overall genotypic variation at the *lep* locus for the two populations investigated using the software program SPSS version 11.5.1 (LEAD Technologies Inc., 2002). Examples of the raw data and computer printouts of the summary statistics can be found in Appendix C.

2.4 Investigation 3 and 4: Assessment of associations between *lep* genotypes with phenotype and a comparative analysis between Nguni and Hereford

2.4.1 Introduction

Buchanan *et al*. (2002) demonstrated that significant associations existed between E2FB genotypes with carcass fat levels and marbling within a range of commercial beef breeds. An association was found between the *TT* genotype and an increase in carcass fat levels and increased marbling, while the *CC* genotype was associated with leaner carcasses and decreased marbling. This investigation was limited to a range of commercial exotic cattle breeds of which only a few are used in South Africa. At the time of the inception of this investigation, no information on E2FB existed for indigenous South African breeds such as Nguni. Nguni have historically not been as extensively bred as Hereford for specific traits deemed important to the beef industry. Therefore, the genetic constitution and allelic distribution of these cattle breeds are expected to differ in terms of economically important traits. Thus, an investigation and comparative analysis was undertaken to examine whether an association existed between the E2FB genotypes of the *lep* gene with body condition and carcass quality, with the major effects of breed taken into account.

2.4.2 Methods

General trends and relationships between the *lep* genotypes (*CC, TT* and *CT*) and individual phenotypic response variables (BCS, WG, SLW, FAT, CFN, WCM and CCM) were assessed in the Nguni and Hereford populations. Due to the multivariate nature of the data, a general linear model was used to calculate significant trends using ILW and hormone treatment as a covariate and breed, sex, age and genotype as dependant variables. The data was pooled and analyzed using S.A.S edition 8.1 (SAS Institute Inc., 2007) according to the following equation:

$$Y_{ijklmno} = u + b_1 L_i + B_j + S_k + A_l + G_m + T_n + (B \times G)_{jm} + (B \times G \times T)_{jmn} + (B \times T \times S \times A \times G)_{jklmn} + e_o \quad [2]$$

Where $Y_{ijklmno}$ is the trait measured for ith individual; u= overall mean for the trait; $b_1 L_i$= regression coefficient (b_1) of the covariate initial live weight (L_i) for the ith animal; B_j= breed whereby j is Nguni or Hereford; S_k= fixed effects of sex where k denotes male or female; A_l= age where l indicates 1, 2, or 3 years of age; G_m= genotype whereby m represents genotypes *CC*, *CT* or *TT*; T_n= where n denotes whether the individual was hormone treated or not $(B \times G)_{jm}$= interaction between breed and genotype; $(B \times G \times T)_{jmn}$= interaction between breed, genotype and treatment; $(B \times T \times S \times A \times G)_{jklmn}$= interaction between breed, sex, age and genotype; and e_o= environment where o represents the feedlot in Dundee or Cato ridge.

Subsequently, the phenotypic response variables were individually assessed by means of a t-test for associations with the various genotypes, breed, age and sex using the program SPSS version 11.5.1 (LEAD Technologies Inc., 2002) to obtain significance levels and correlation estimates.

In addition, data that was not normally distributed was further analyzed by redundancy analysis (RDA) with the program Canoco for Windows version 4.51 (ter Braak and Smilauer, 1998). RDA employs a Monte Carlo permutation test in order to calculate F-statistics which are not distribution driven (Palmer, 1993; Okland, 1999). The nature of the relationship between the genotypes (*CC*, *CT* and *TT*) and the phenotypic response variables for each breed, including the fixed effects of age, sex, breed and treatment, were illustrated using an ordination graph constructed with the program Canodraw (ter Braak and Smilauer, 1998). In an ordination graph, RDA results are described in the form of triplot (Figure 2.4).

An ordination graph is a pictorial representation of measures of association between variables and samples (Figure 2.4). If no association is present, it is expected that the

individual sample data ought to be randomly scattered on the plot (H_o) (Lepš and Smilauer, 1999). However, if the individual sample data in the ordination graph demonstrated distortion from random scatter, the null hypothesis would be rejected. The measure of association is expressed as an eigenvalue which demonstrates the strength of the ordination axes and quantifies the significance of the variables (Lepš and Smilauer, 1999; Okland, 1999). The ordination axis is denoted by (▲). It describes the variables and covariates, such as genotype, sex, age and feeding regime. When different treatment groups of a variable such as oxen and heifer of the variable sex, appear close to one another in the graph, it indicates that the differences between the treatment groups are small. However, when they are distant from one another, differences between the oxen and heifer group are large as indicated in the example. Individual sample data is denoted by (○). The response variables WG, BCS, SLW, FAT, CFN and WCM are represented by colorful lines and describe the responses to the fixed effects and covariates. Response variables are described in terms of length of arrow as well as the angle of the arrow and the ordination axis. The longer the arrow and smaller the angle, the stronger the association with the independent variable. For example, the heifer group is strongly associated with the response variable FAT. Arrows pointing in a similar direction indicate a positive correlation, while arrows that are more or less perpendicular to one another demonstrate no correlation (Leps and Smilauer 1999).

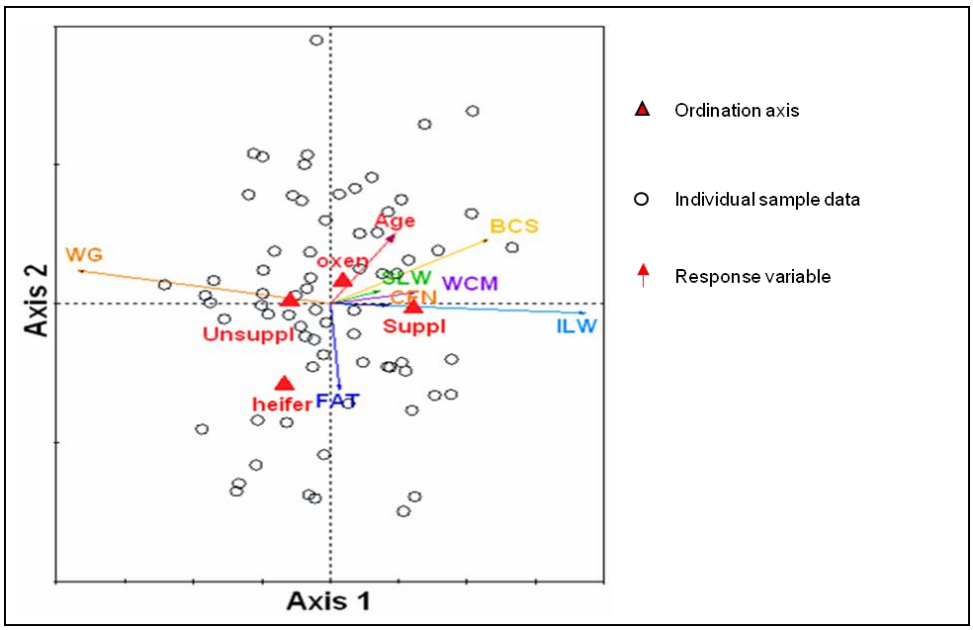

Figure 2.4 RDA ordination guide graph and key.

RDA analysis of the associations of genotype with phenotypic response variables for the Hereford population was subdivided according to the availability of phenotypic measurements. The first subset consisted of 14 individuals for which a complete set of data for all the phenotypic response measurements were available (ILW, BCS, WG, SLW, FAT, CFN and WCM), whilst the second subset consisted of the remainder of the individuals (n = 40) for which the measurements ILW, SLW, and BCS were available. A separate RDA analysis for breed differences compared the untreated Nguni oxen group with the Hereford oxen in order to eliminate the effects of treatment and sex. The RDA of the two breeds was initially carried out using selected phenotypic response measurements. The Nguni population consisted of 32 individuals, whereas the Hereford population consisted of 40 individuals for whom phenotypic measurements could be obtained for ILW, BCS, WG and SLW only. Thereafter, an RDA was performed using the same Nguni subset (n = 32) and the remaining Hereford (n = 14) for which measurement data for all the phenotypic response variables (ILW, BCS, WG, SLW, FAT, CFN and WCM) was included.

CHAPTER 3

RESULTS

3.1 Investigation 1: Assessment of phenotypic variation

3.1.1 Introduction

The phenotypic variation of pre-slaughter and post-slaughter carcass traits in the Nguni and Hereford populations was assessed to facilitate subsequent genetic analyses and comparisons. Standard summary statistics of these traits were calculated for each of the two populations to obtain a better understanding of the overall phenotypic performance of the various traits investigated (Table 3.1). The measurement data of the various traits were supplied by the respective feedlots and Cato Ridge abattoir. As the collection of the measurements of the various traits was dependent upon the time available and cooperation of the management at the feedlots and abattoir, it was not possible to obtain full data sets for both populations. The data set of the Hereford population had a number of missing values for some of the traits, whilst the Nguni population data set was complete.

The pre-slaughter traits in this investigation included initial live weight ILW, SLW, WG and BCS. WG was calculated as the difference between ILW (day 1 of feedlot period) and SLW (day 120 of feedlot period) readings. This measurement provides an indication of growth response of a particular individual over 120 day feedlot period. The post-slaughter traits in this investigation comprised of CFN, FAT, WCM and CCM.

Table 3.1 Summary statistics for the phenotypic response variables for the Nguni and Hereford populations.

Breed	Sex	n	Variable	μ	Std. Dev
Nguni	Oxen	55	BCS	4.3	0.6
		55	ILW	345.3	63.5
		54	SLW	403.4	49.8
		54	WG	59.1	32.5
		55	FAT	2.9	0.7
		55	CFN	3.4	0.5
		55	WCM	242.6	33.1
		55	CCM	237.1	32.3
	Heifer	15	BCS	3.6	0.6
		15	ILW	307.3	30.4
		15	SLW	383.5	38.0
		15	WG	76.2	45.8
		15	FAT	3.3	0.65
		15	CFN	3.3	0.5
		15	WCM	234.5	27.6
		15	CCM	229.3	27.0
Hereford	Oxen	48	BCS	3.9	0.9
		48	ILW	254.4	69.2
		40	SLW	418.2	38.1
		40	WG	184.3	28.7
		14	FAT	3.4	1.1
		14	CFN	4.1	0.4
		14	WCM	259.9	19.9
		14	CCM	254.1	19.5

ILW= Initial live weight (kg); SLW= Slaughter live weight (kg); WG= Weight gain (feedlot response) (kg); BCS= Body composition score; CFN= Conformation; FAT= Fatness; WCM= Warm carcass mass (kg); CCM= Cold carcass mass (kg); n= No. of individuals; μ= mean; Std. Dev= standard deviation.

3.1.2 Analysis of the effect of hormone treatment in the Nguni population

The Nguni population was divided into subgroups according to sex and feeding regime. Firstly, the animals were divided into two groups, namely, oxen (Ox) and heifers (NH). The oxen were further divided into two subgroups according to their feeding regime; one group was selected owing to their significantly heavier ILW (50 kg) upon entering the feedlot and provided with a growth hormone treatment called Zulmax (Ox^+), whereas the remaining Nguni oxen that had lighter ILW values remained untreated with growth hormone (Ox^-). The fact that a select few of the Nguni oxen were hormone treated was only revealed after the sample and data collection. Hence all analyses of the Nguni data had to account for treatment in order to prevent skewed results. Summary statistics for the phenotypic variables can be found in Table 3.2 for both Ox^+ and Ox groups.

Table 3.2 Comparative analysis of the Nguni subgroups for pre-slaughter and post-slaughter traits in order to identify the effects of hormone treatment.

Treatment group	n	Variable	μ	Std. Dev
Ox^+	23	BCS	4.6	0.5
	23	ILW	381.6	43.4
	22	SLW	423.4	43.4
	22	WG	24.3	8.8
	23	FAT	3.0	0.8
	23	CFN	4.0	0.5
	23	WCM	251.3	28.2
	23	CCM	245.7	27.5
Ox^-	32	BCS	4.1	0.6
	32	ILW	306.6	45.1
	32	SLW	389.7	49.9
	32	WG	83.0	17.3
	32	FAT	2.8	0.6
	32	CFN	3.4	0.5
	32	WCM	236.7	35.8
	32	CCM	231.4	35.0

In order to assess the efficacy of hormone treatment in the Nguni population, the phenotypic traits of Ox^+ and Ox^- Nguni oxen subgroups were compared using a GLM whereby treatment, sex, genotype and age (and their respective interactions) were independent variables and ILW, a covariate (Table 3.3). Highly significant differences were obtained for the response variables SLW, WG, WCM and CCM for the different treatment groups ($P \leq 0.01$). Furthermore, no significant differences in the variation of the response variables BCS, FAT and CFN could be accounted for by hormone treatment.

Table 3.3 Pooled GLM summarized results for Nguni hormone treated and non-treated animals.

Dependant variable	**n**	**Independent variables**	**Type III SS**	**F value**	**Pr>F**
BCS	118	ILW (covariate)	0.773	2.09	0.15
		Treatment	0.273	0.74	0.39
		Genotype	0.513	0.69	0.50
		Sex	1.554	4.20	0.05*
		Age	0.220	0.30	0.74
		G x T	0.159	0.43	0.51
		G x S x A x T	1.100	0.99	0.40
SLW	109	ILW	84655.782	131.19	<0.0001***
		Treatment	9387.638	14.55	0.0003***
		Genotype	410.711	0.32	0.73
		Sex	184.145	0.29	0.60
		Age	524.059	0.41	0.67
		G x T	539.321	0.84	0.36
		G x S x A x T	1997.575	1.03	0.39
WG	109	ILW	611.548	0.95	0.33
		Treatment	9473.993	14.70	0.0003***
		Genotype	407.997	0.32	0.73
		Sex	182.151	0.28	0.60
		Age	529.824	0.41	0.67

		G x T	519.732	0.81	0.37
		G x S x A x T	2011.622	1.04	0.38
FAT	84	ILW	0.001	0.00	0.96
		Treatment	0.356	0.74	0.39
		Genotype	1.951	2.02	0.14
		Sex	2.505	5.19	0.03*
		Age	1.166	1.21	0.31
		G x T	0.072	0.15	0.70
		G x S x A x T	0.625	0.43	0.73
CFN	84	ILW	1.004	4.26	0.04*
		Treatment	0.036	0.15	0.70
		Genotype	0.487	1.03	0.36
		Sex	0.213	0.91	0.35
		Age	0.403	0.86	0.43
		G x T	0.041	0.17	0.68
		G x S x A x T	1.055	1.49	0.23
WCM	84	ILW	38719.953	109.07	<0.0001***
		Treatment	7191.698	20.26	<0.0001***
		Genotype	237.925	0.34	0.72
		Sex	1.867	0.01	0.94
		Age	942.503	1.33	0.27
		G x T	94.792	0.27	0.61
		G x S x A x T	2188.871	2.06	0.12
CCM	84	ILW	36983.404	109.02	<0.0001***
		Treatment	6866.481	20.24	<0.0001***
		Genotype	227.302	0.34	0.72
		Sex	1.763	0.01	0.94
		Age	901.851	1.33	0.27
		G x T	90.717	0.27	0.60
		G x S x A x T	2985.194	2.05	0.12

*significant P≤ 0.05; *** highly significant P≤ 0.01; SS= sum of squares; Pr>F= probability for F value obtained; T= Treatment; G= genotype; S= sex and A= age.

An in-depth analysis of the significant effect of hormone treatment on the response variables SLW, WG, WCM and CCM was carried out by means of a t-test (Table 3.4).The Ox^+ subgroup displayed a mean SLW 35.8 kg greater than the Ox^- group, a mean WCM of 13.98 kg greater than the Ox^- group, and as expected, a CCM 14.38kg heavier than the mean of the Ox^- group. However, the Ox^- group displayed a significantly higher WG (56.53 kg) than that of the Ox^+ group. No significant differences could be identified for the remaining traits BCS, CFN, and FAT, assessment of the means of these remaining traits showed that the two oxen groups (Ox^+ and Ox^-) displayed a similar fat distribution.

Table 3.4 Summary of significant t-test results for treatment with phenotypic response variables.

Dependant variable	**SLW (kg)**	**WG (kg)**	**WCM (kg)**	**CCM (kg)**
Treated (T) vs non-treated (NT)	423.5 > 387.70 T > NT	80.85 > 24.32* NT > T	250.74 > 236.02*** T > NT	245.10 > 230.72*** T > NT

*significant $P \leq 0.05$; *** highly significant $P \leq 0.01$.

3.2 Investigation 2: Assessment of genetic variation at the *lep* locus

This investigation required the establishment of the genotypic composition of all the animals within the two populations. After DNA isolation and verification, exon 2 of the *lep* locus was amplified as the initial step to reveal the presence of one of the two alleles at the SNP site. The amplification product was then restricted to reveal which nucleotide resided at the SNP site, where the presence of the C nucleotide resulted in the restriction of the amplification product, while the presence of T prohibited restriction, thereby producing different fingerprint profiles which were then used to identify the *lep* alleles.

3.2.1 DNA source and isolation

DNA was successfully isolated through a salting out technique (Sambrook *et al*., 1989; Bruford *et al*., 1998) and verified through 3% agarose gel electrophoresis (Figure 3.1). The intensity of the DNA bands verified the presence of high molecular weight DNA.

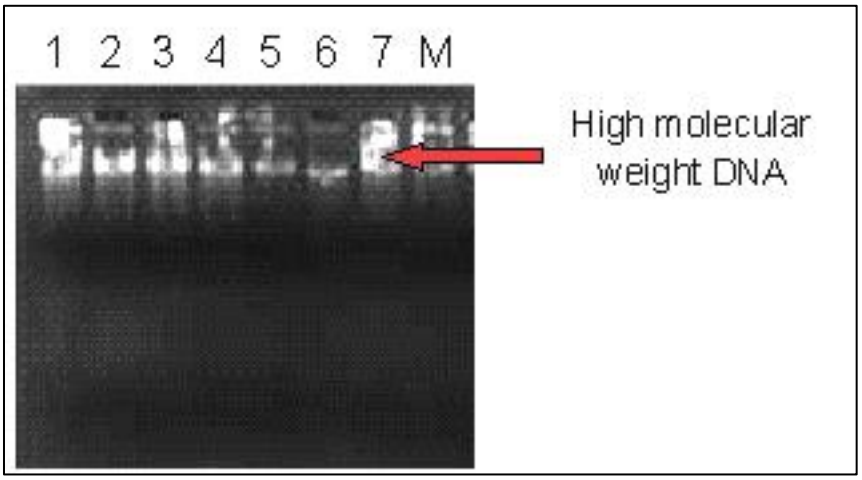

Figure 3.1 Agarose gel displaying high molecular weight bovine genomic DNA. Lanes 1-7: Bovine DNA; Lane M: MWM.

The quantity and quality of the isolated DNA that was measured by an accurate spectrophotometer, was found to be of high molecular weight and within an acceptable range of quantity and purity for subsequent analyses by amplification; the calculated concentrations of the samples ranged from 11 to 548.5 μg/ml and the purity ratios ranged from 0.7 to 2.1. The DNA concentrations were then used to determine the amount of sample required for DNA amplification.

3.2.2 *Lep* gene amplification and restriction

The Buchanan *et al*. (2002) PCR profile was used to carry out an initial amplification of *lep* exon 2. Although this profile successfully produced amplification product of the expected 94 bp size, the quantity of the product yielded faint bands on 3% agarose gel and was thus unsatisfactory for subsequent restriction (Figure 3.2).

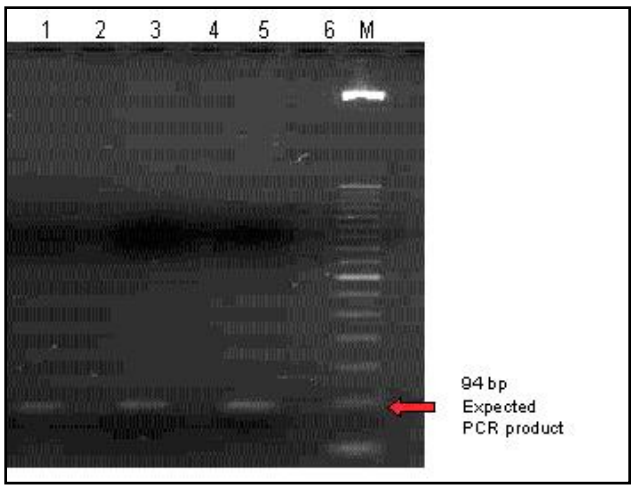

Figure 3.2 Agarose gel displaying an amplified segment of *lep* exon 2.
Lanes 1, 3, 5: Amplification product.
Lane M: MWM.

After a series of unsuccessful optimization attempts, amplification was re-attempted according to the conditions and cycling conditions described by Madeja *et al*. (2004) that employed a modified profile utilizing the same primer sets as Buchanan *et al*. (2002). Reagent working stock and primer solutions were also reconstituted. A modification of the annealing temperature by only one degree Celsius yielded satisfactory amplification results as indicated by a 3% agarose gel (Figure 3.3 left). The size of the amplification product was thereafter verified by 20% polyacrylamide gel electrophoresis (Figure 3.3 right).

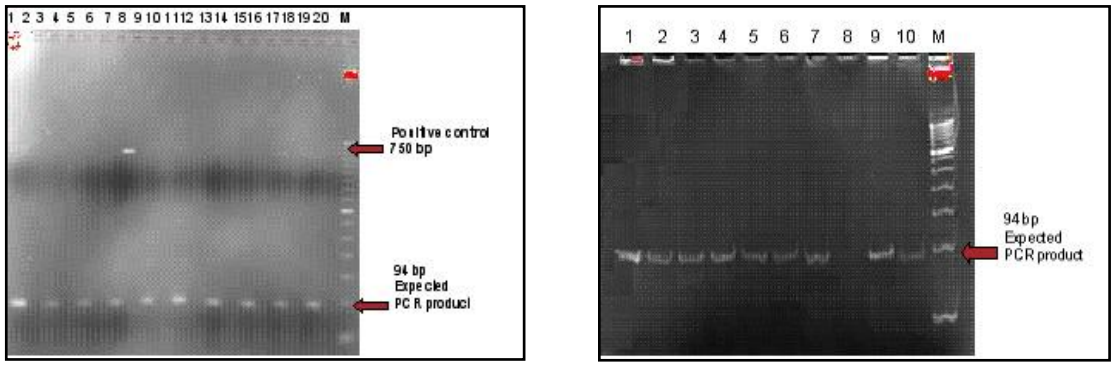

Figure 3.3 Gels displaying amplification product on Agarose gel (left) and Polyacrylamide gel (right).

(a) Lanes 1, 3, 5, 7, 9-20: Amplification product.
Lanes 2, 4, 6, 8: Controls 1-4.
Lane M: MWM.

(b) Lanes 1-10: Amplification product.
Lane M: MWM.

The sequence content of the amplification product further confirmed that the amplified product was the desired *lep* exon 2. A *BLASTn* analysis using the *lep* forward primer confirmed a 98% sequence identity with that of the *lep* exon 2 gene provided by Genbank (Accession No. U5036). Similarly, the *BLASTn* output using the *lep* reverse primer confirmed a 96% sequence identity (Figure 3.4).

```
Query: 39  ggctttggcccatctgtcttacgtggaggctgtgcccatccgcaaggtccaggatgaca
98
           ||||||||||||||||||||||||||||| |||||||||||| ||||||||||||||||
Sbjct: 9   ggctttggcccatctgtcttacgtggaggctgtgcccatccggaaggtccaggatgaca
68

                   Score =  116 bits (60), Expect = 1e-23
                   Identities = 62/63 (98%)
                    Strand = Plus / Plus
```

```
Query  1   GCGCTGTGGACCCCTGTATCGATTCCTGTGGCTTTGGCCTATC-TGTCTTACGTGGAGG  58
           |||||||| |||| | ||||||||||||||||||||||||| || ||||||||||||||
Sbjct  1   GCGCTGTGGACCC-TGTATCGATTCCTGTGGCTTTGGCCTATCTTGTCTTACGTGGAGG  59

                   Score = 83.4 bits (43),  Expect = 5e-14
                   Identities = 57/59 (96%), Gaps = 2/59 (3%)
                   Strand=Plus/Plus
```

Figure 3.4 BLASTn sequence identities of lep exon 2, (above) from lep forward primer end, and (below) from lep reverse primer end.

3.2.3 Determination of *lep* alleles and genotypes

Restriction of *lep* exon 2 amplification product allowed for subsequent discrimination between the SNP harbouring the T nucleotide or the C nucleotide. If the C nucleotide were present, the restriction enzyme *Mro*I would restrict the amplification product into two shortened fragments of 75 and 19 bp long. Only the 75 bp fragment would be visible on a gel, as the 19 bp fragment would run off the gel. The presence of the T nucleotide would result in no restriction of the amplification product (94 bp). It was therefore possible to separate the T- and C- alleles and distinguish the three genotypes, namely, the two homozygotes *TT* and *CC*, and the heterozygote *CT* on a 20% polyacrylamide gel (Figure 3.5).

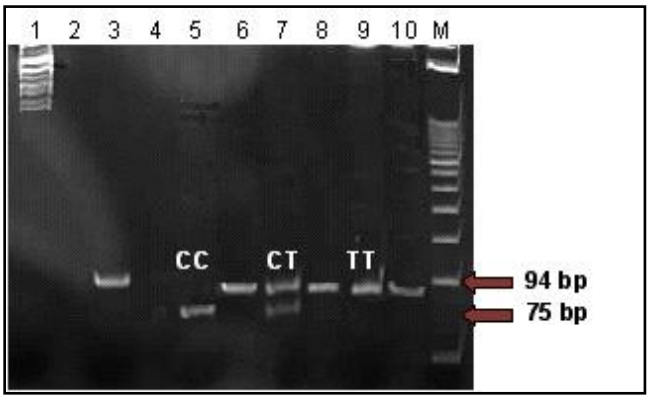

Figure 3.5 Polyacrylamide gel displaying *lep* genotypes generated by RFLP.

Lane 1: Control 4; Lane 2: Control 2.

Lane 3: Control 1; Lanes 5,7,9: *Lep* genotypes *CC, CT* and *TT* respectively;

Lane M: MWM; Lanes 6,8,10: Undigested PCR product.

3.2.4 Population analysis of the *lep* locus

Both the SNP alleles T and C were present in both the Nguni and Hereford populations as well as in the different subgroups of the Nguni. All three SNP genotypes, on the other hand, were only present in the Ox^+ subgroup of the Nguni population (Table 3.5). The remaining two subgroups, Ox^- and NH only revealed the genotypes *CT* and *TT*, which was also the case with the Hereford population.

Table 3.5 Nguni and Hereford *lep* genotypes.

Nguni population (n = 70)				Hereford population (n = 48)			
Individual	**Genotype**	**Individual**	**Genotype**	**Individual**	**Genotype**	**Individual**	**Genotype**
N1	*TT*	N37	*TT*	H1	*TT*	H41	*TT*
N2	*TT*	N38	*CT*	H2	*CT*	H42	*TT*
N3	*CT*	N39	*CT*	H3	*TT*	H43	*TT*
N4	*CT*	N40	*CT*	H4	*CT*	H44	*TT*
N5	*CT*	N41	*CT*	H5	*CT*	H45	*TT*
N6	*CC*	N42	*CT*	H6	*CT*	H47	*TT*
N7	*CT*	N43	*CT*	H7	*TT*	H48	*TT*
N8	*CT*	N45	*CT*	H9	*TT*	H49	*CT*
N9	*TT*	N46	*CT*	H10	*CT*	H51	*CT*
N10	*CT*	N47	*CT*	H11	*TT*	H52	*CT*
N11	*CT*	N48	*CT*	H12	*TT*	H53	*TT*
N12	*CT*	N49	*CT*	H13	*CT*	H54	*CT*
N13	*CT*	N50	*CT*	H14	*TT*	H55	*CT*
N14	*TT*	N51	*CT*	H15	*CT*	H56	*TT*
N15	*CT*	N52	*TT*	H16	*CT*		
N16	*CT*	N53	*TT*	H17	*TT*		
N17	*CT*	N54	*TT*	H18	*CT*		
N18	*TT*	N55	*TT*	H19	*TT*		
N20	*CT*	N56	*CT*	H20	*TT*		
N21	*TT*	NH1	*CT*	H21	*CT*		
N22	*TT*	NH2	*TT*	H22	*TT*		
N23	*TT*	NH3	*TT*	H24	*CT*		
N24	*TT*	NH4	*CT*	H25	*TT*		
N25	*TT*	NH5	*CT*	H26	*TT*		
N26	*CT*	NH6	*CT*	H29	*CT*		
N27	*TT*	NH7	*CT*	H30	*CT*		
N28	*CT*	NH8	*CT*	H31	*CT*		
N29	*TT*	NH9	*TT*	H33	*TT*		
N30	*TT*	NH10	*CT*	H34	*CT*		
N31	*TT*	NH11	*CT*	H35	*TT*		
N32	*TT*	NH12	*CT*	H36	*CT*		
N33	*TT*	NH13	*TT*	H37	*CT*		
N34	*TT*	NH14	*CT*	H38	*CT*		
N35	*TT*	NH15	*TT*	H40	*TT*		
N36	*TT*						

The distribution trends of the genotypes *TT, CT* and *CC* for the Nguni and Hereford populations are demonstrated by the histograms in Figure 3.6. One *CC* genotype was present in the Nguni population, whereas none was found in the Hereford population. The heterozygote *CT* and homozygote *TT* genotypes were approximately evenly distributed in both the Nguni and Hereford populations.

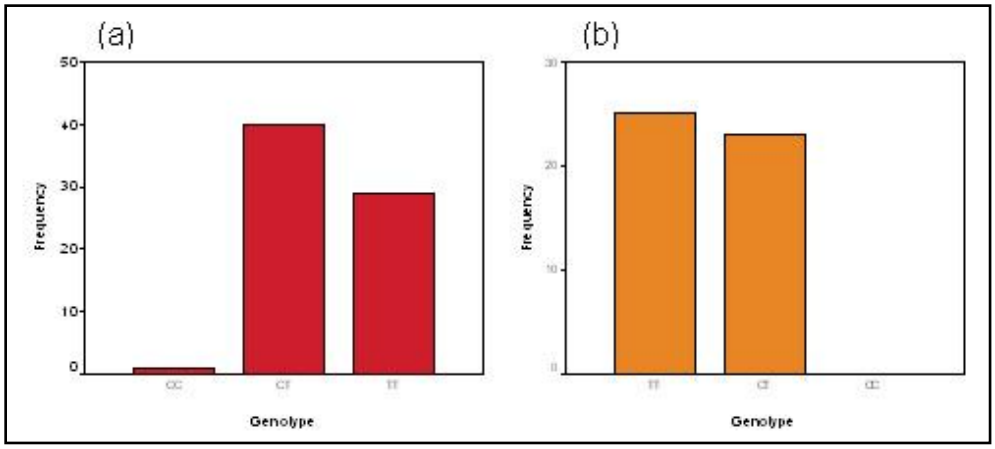

Figure 3.6 Histogram of genotypic frequency distribution of (a) Nguni population and (b) Hereford population.

The C-allele was found to be in the minority in both the Nguni (total population) and Hereford populations, ranging between 24-30% (Table 3.6). Although the C-allele appeared less frequently than the T-alleles, it was found to be marginally greater within the two Nguni subgroups Ox^- and NH, where the frequency of C was greater than 30%. Although the C-allele exceeded 20%, the homozygous genotype *CC* was absent in the two Nguni subgroups (Ox^- and NH) as well as in the entire Hereford population, while a single *CC* individual was encountered in the Ox^+ subgroup. A chi-square test revealed that both the Nguni and Hereford populations were not in HWE with the exception of subgroup NH, which was found to be in HWE. No significant difference was found for the allele frequencies in the Nguni and Hereford population.

Table 3.6 Nguni and Hereford genotypic analysis.

Population	n	*Allele frequency*		*Genotypic frequency*			*HWE Genotypic frequency*			χ^2	*P*- value
		T	C	*TT*	*CT*	*CC*	p^2	2pq	q^2		
Nguni											
***Subgroup: Ox*$^+$**	23	0.75	0.25	0.35	0.61	0.04	0.56	0.38	0.06	5.16	<0.05*
***Subgroup: Ox*$^-$**	32	0.65	0.35	0.50	0.50	0.00	0.42	0.46	0.12	4.44	<0.05*
Subgroup: NH	15	0.67	0.33	0.33	0.67	0.00	0.45	0.44	0.10	3.78	>0.05
Total Nguni	70	0.70	0.30	0.41	0.57	0.01	0.49	0.42	0.09	10.55	<0.01***
Total Hereford	48	0.76	0.24	0.52	0.48	0.00	0.58	0.36	0.06	26.21	<0.05*

* Significant value where *P*≤0.05; *** Significant value where *P*≤0.01.

3.3 Investigation 3 and 4: Assessment of associations between *lep* genotypes and phenotypes and a comparative analysis between Nguni and Hereford

3.3.1 General Linear model

The E2FB genotypes were identified and summarized in accordance with the corresponding response variables namely BCS, ILW (kg), SLW (kg), WG (kg), FAT, CFN, WCM (kg) and CCM (kg) for both the Nguni and Hereford populations (Table 3.7). Discrepancies in the number of data sets for the Hereford individuals were due to errors in data collection at the abattoir hence some readings had to be excluded for the analyses. Furthermore, standard deviation estimates could not be obtained for the Nguni CC individual due to a single sample size.

Table 3.7 Genotypic summary statistics for Nguni and Hereford populations.

Breed	Genotype	n	Variable	μ	Std. Dev
Nguni	*CC*	1	BCS	4.0	-
		1	ILW	393.0	-
		1	SLW	435.0	-
		1	WG	42.0	-
		1	FAT	2.0	-
		1	CFN	4.0	-
		1	WCM	258.0	-
		1	CCM	252.2	-
	CT	40	BCS	4.2	0.7
		40	ILW	343.7	65.0
		39	SLW	403.4	47.9
		39	WG	61.1	39.2
		40	FAT	3.0	0.7
		40	CFN	3.4	0.5
		40	WCM	242.9	31.6
		40	CCM	237.4	30.8
	TT	29	BCS	4.1	0.7
		29	ILW	326.3	51.8
		29	SLW	392.1	48.6
		29	WG	65.8	32.5
		29	FAT	3.1	0.8
		29	CFN	3.4	0.5
		29	WCM	237.5	33.3
		29	CCM	232.2	32.5
Hereford	*CT*	23	BCS	3.8	0.9
		23	ILW	265.4	80.8
		19	SLW	408.6	43.4
		19	WG	174.5	30.3
		8	FAT	3.8	1.3

	8	CFN	4.1	0.4
	8	WCM	261.6	15.8
	8	CCM	255.8	15.5
TT	25	BCS	3.9	1.0
	25	ILW	244.2	56.4
	21	SLW	426.9	31.2
	21	WG	193.2	24.7
	6	FAT	3.0	0.6
	6	CFN	4.2	0.4
	6	WCM	257.7	25.9
	6	CCM	251.9	25.3

The relationship between the E2FB genotypes (*TT*, *CT* and *CC*) and the phenotypic carcass traits (BCS, WG, SLW, FAT, CFN, WCM and CCM) in Nguni and Hereford cattle were investigated using a pooled GLM with ILW as a covariate and sex, treatment, age, breed and genotype as independent variables (Table 3.8). In order to avoid redundancy, the results for the covariate treatment were excluded in the table though the effect of treatment was included in the GLM analysis itself in order to prevent skewed results. Highly significant differences were obtained for the response variable BCS with ILW ($P<0.0001$) and sex had a low *P*-value (0.074) though not significant. ILW and breed differences had significantly high influences on the response variable SLW ($P<0.0001$) whereas an interestingly low *P*-value for breed-genotype interaction. Weight gain was significantly influenced by ILW and breed ($P< 0.024$ and 0.0001 respectively) whilst breed-genotype interaction also displayed a low though non-significant *P*-value. Sex and breed-genotype interaction produced significant values for the response variable FAT. ILW and breed resulted in significantly different CFN scores. As expected, WCM and CCM yielded similar results whereby ILW and breed accounted for highly significant differences in the response variables ($P< 0.0001$). Furthermore, when the covariate ILW was analyzed, breed was a significant factor that accounted for variation in the obtained initial weights.

Table 3.8 Pooled GLM summarized results for Nguni and Hereford populations.

Dependant variable	n	Independent variables	Type III SS	F value	Pr>F
BCS	118	ILW (covariate)	10.001	20.26	<0.001***
		Breed	0.008	0.02	0.867
		Genotype	0.206	0.42	0.660
		Sex	1.611	3.26	**0.074**
		Age	0.259	0.52	0.593
		B x G	0.284	0.57	0.450
		T x B x G	0.054	0.11	0.741
		T x B x G x S x A	0.362	0.73	0.534
SLW	109	ILW	112160.755	170.07	<0.0001***
		Breed	59371.473	90.02	<0.0001***
		Genotype	247.307	0.37	0.689
		Sex	169.237	0.26	0.614
		Age	206.028	0.31	0.732
		B x G	2142.166	3.25	**0.075**
		T x B x G	766.014	1.16	0.284
		T x B x G x S x A	656.596	1.00	0.399
WG	109	ILW	3466.761	5.26	<0.024*
		Breed	59496.370	90.24	<0.0001***
		Genotype	255.434	0.39	0.680
		Sex	167.219	0.25	0.616
		Age	208.583	0.32	0.730
		B x G	2107.108	3.20	**0.077**
		T x B x G	744.197	1.13	0.290
		T x B x G x S x A	1984.738	1.00	0.395
FAT	84	ILW	0.169	0.29	0.594
		Breed	1.807	1.13	0.291
		Genotype	0.634	1.07	0.348
		Sex	2.539	4.30	0.042*
		Age	0.528	0.89	0.413

		B x G	2.630	4.45	0.038*
		T x B x G	0.036	0.06	0.807
		T x B x G x S x A	0.192	0.32	0.808
CFN	84	ILW	1.300	6.01	0.017*
		Breed	6.981	32.38	<0.0001***
		Genotype	0.224	1.04	0.369
		Sex	0.212	0.98	0.325
		Age	0.209	0.97	0.384
		B x G	0.070	0.33	0.570
		T x B x G	0.035	0.16	0.689
		T x B x G x S x A	0.354	1.64	0.188
WCM	84	ILW	39492.598	110.92	<0.0001***
		Breed	12344.943	34.67	<0.0001***
		Genotype	101.057	0.28	0.754
		Sex	1.348	0.00	0.951
		Age	466.530	1.31	0.276
		B x G	11.395	0.03	0.859
		T x B x G	127.492	0.36	0.552
		T x B x G x S x A	721.363	2.03	0.119
CCM	84	ILW	37727.764	110.89	<0.0001***
		Breed	11803.631	34.69	<0.0001***
		Genotype	96.584	0.28	0.754
		Sex	1.271	0.00	0.951
		Age	446.386	1.31	0.276
		B x G	10.863	0.03	0.859
		T x B x G	121.913	0.36	0.551
		T x B x G x S x A	687.194	2.02	0.119
ILW	118	Breed	23434.596	7.70	0.007*
		Genotype	3858.802	1.27	0.286
		Sex	65.178	0.02	0.884
		Age	3836.394	1.26	0.288
		B x G	118.869	0.04	0.844

T x B x G	3775.120	1.24	0.268
T x B x G x S x A	198.368	0.07	0.978

*significant P≤ 0.05; *** highly significant P≤ 0.01; SS= sum of squares; Pr>F= probability for F value obtained; T= Treatment; B= breed; G= genotype; S= sex and A= age.

Furthermore, the trends identified with GLM were individually analyzed by means of a t-test to further investigate whether specific relationships were significant and to obtain significance levels. T-tests were carried out for each of the response variables with breed, genotype, sex, treatment and age and the significance levels obtained were summarized in Table 3.9.

Table 3.9 Summary of significant results obtained for t-tests carried out on phenotypic response variables (*P*≤ 0.05).

Dependant Variable	**Significant differences between the means of the following independent variables:**			
	Breed	**Genotype**	**Sex**	**Age**
BCS	N > H 4.16 > 3.88		O > NH 4.11 > 3.60	
SLW (kg)	H > N 418.18>399.17		O > NH 409.71 > 383.53	2 > 1 yrs 32.75
WG (kg)	H > N 184.33 > 62.83	*TT* > *CT* 21.044 *TT* > *CC* 77.32 *CT* > *CC* 56.28	O > NH 112.39 > 76.20	1 > 2 yrs 64. 65
FAT	-	-	-	-
CFN	H > N 4.14 > 3.40			
WCM (kg)	H > N 259.93 > 240.86		O > NH 246.10 > 234.53	2 > 1 yrs 29.96
CCM (kg)	H > N 254.09 > 235.44		O > NH 254.10 > 235.44	2 > 1 yrs 29.29
ILW (kg)	N > H 337.20 > 254.38	*CT* > *TT* 26. 80		2 > 1 yrs 92.149

N= Nguni; H= Hereford; O= Ox and; NH= Heifer.

Nguni was found to have significantly higher BCS than Hereford (Table 3.8 and 3.9). The oxen displayed significantly higher BCS values when compared with the heifers (Table 3.9), though the GLM analysis yielded a low though non-significant *P*-value. The significant effect of breed on SLW values obtained by GLM analysis was verified by a t-test that resulted in a significantly greater SLW in the Hereford population than the Nguni. However, unlike the GLM results from Table 3.8, the oxen significantly outweighed the heifers and the 1 yr old age group outperformed the 2 yr old age group by an average of 32.5 kg. When compared by means of a t-test; breed, genotype, sex and age were all significant influences on WG whereas only breed accounted for significant differences in WG in the GLM analysis. Furthermore, GLM analysis (Table 3.8) showed that FAT was significantly influenced by sex though this relationship did not result in any significant values when compared by t-test (Table 3.9). Though no significant results could be obtained for CFN by genotype, sex and age, breed effects showed that the Hereford population outperformed the Nguni significantly in the GLM and verified by t-test comparison of the group means. WCM and CCM, as expected, showed almost identical results whereby breed, sex and age proved to be significant factors accounting for variation in these response variables though genotype did not yield any significant results. Hereford significantly outperformed Nguni WCM and CCM, the oxen outperformed the heifers and the two-year old individuals outperformed the one year old individuals (Table 3.9). Similarly, GLM analysis showed significant results for breed though none for sex or age. ILW was significantly greater in the Nguni population than the Hereford (Table 3.9) as previously shown with the GLM analysis (Table 3.8). Individuals with a *CT* genotype displayed an average ILW significantly heavier than the TT individuals (26.80kg). Furthermore, the two-yr old age group was significantly heavier than the one-yr old age group by an average of 92.15kg.

3.3.2 Redundancy analysis

Furthermore, general indications of trends and significant relationships within these data were established using the canonical ordination technique of redundancy analysis

(RDA) and visually depicted by means of an ordination graph. This method was employed as it calculates F-values and is not distribution driven.

An RDA was performed on the Nguni population to determine whether significant associations existed between the *lep* genotypes *CC*, *CT* and *TT* and the phenotypic carcass traits using Canoco for Windows version 4.51 (ter Braak and Smilauer, 1998). No significant relationships could be established between the *lep* genotypes and the phenotypic carcass traits as previously shown by the GLM analysis. This outcome was further supported by a low eigenvalue and a high *P*-value which indicated that little variation in the phenotype was due to genotype (Table 3.10). A similar outcome was established when treatment (hormone-treated or non-treated), sex and age were included as independent variables in place of genotype in the RDA analysis (Table 3.10).

Table 3.10 Relationships between genotype and covariates with phenotypic response variables of the Nguni population.

	Monte Carlo test summary		
	Eigenvalue	**F-ratio**	***P*-value**
Nguni (genotype)	0.008	0.345	0.958
Nguni (treatment, age and sex)	0.102	6.580	0.786

In order to obtain a more detailed understanding of the nature of the relationships between the Nguni phenotypic response variables and the independent variables, an ordination graph was constructed (Figure 3.7). The graph illustrated the strong relationship between the response variables ILW, SLW, WG and BCS with treatment. The treated (suppl) group displayed a strong association with increased ILW, SLW and BCS values, while the untreated group (unsuppl) displayed a strong relationship with increased WG. Interestingly, the Heifer group was positively associated with FAT which was also the case for the GLM analysis. However, the GLM analysis showed significant

results for WCM and CCM with the treated Nguni and unlike the RDA, no significant results were obtained for BCS with GLM.

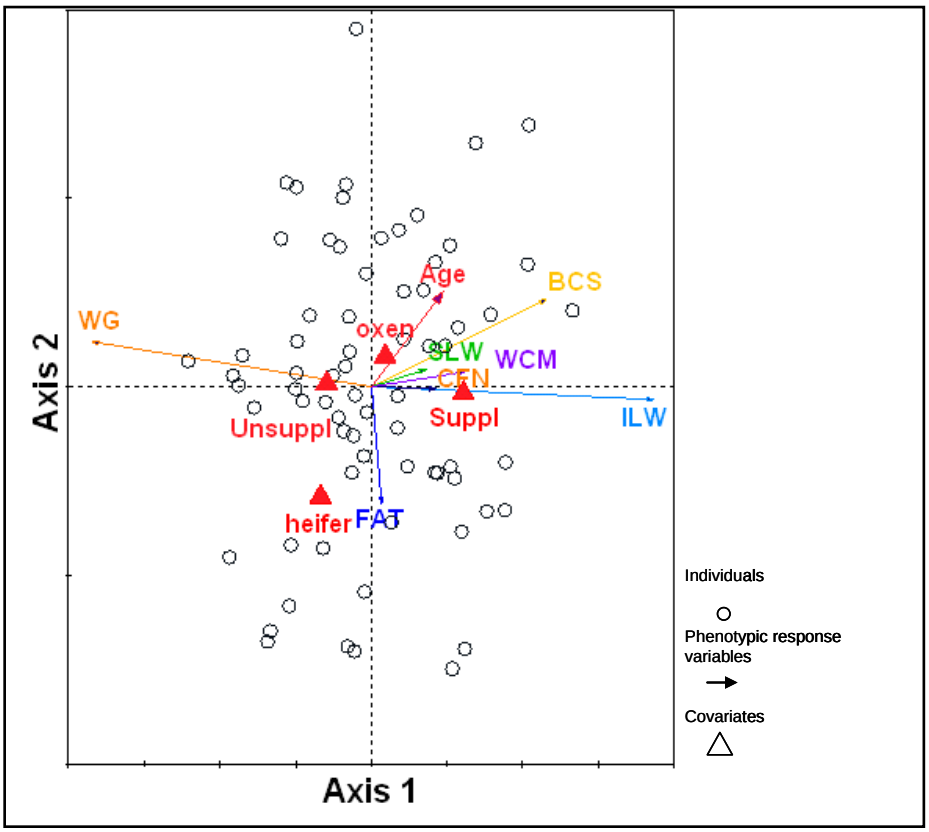

Figure 3.7 Ordination graph of fixed effects of treatment, sex and age with phenotypic response variables of the Nguni population.

An RDA was also performed on the Hereford population to determine whether significant associations existed between the *lep* genotypes and the phenotypic carcass traits. As the Hereford population comprised of non-hormone-treated oxen of similar age, no covariates were implemented in these analyses. Measurement data for the seven phenotypic response variables could not be obtained for all the individuals in the Hereford population. For only 14 individuals, a complete set of measurements were recorded, while for the remainder of the population (n = 40), measurements excluded the response variables FAT, CFN and WCM. No significant relationships could be established between the *lep* genotypes, *CT* and *TT*, with the phenotypic carcass traits in

both of the sample groups (Table 3.11). Although the different genotypes did not account for any significant variation in these phenotypic response variables, the *P*-value obtained for the Hereford data (*P* = 0.172) was much lower than that of the Nguni (*P* = 0.958). This outcome was further supported by a low eigenvalue which indicated that little variation in the phenotype was due to the E2FB genotypes.

Table 3.11 Analysis of Hereford population to determine the relationship between genotype and phenotypic response variables.

	Monte Carlo test summary		
	Eigenvalue	**F-ratio**	***P*-value**
Partial Hereford data (n = 40)	0.048	1.905	0.172
Complete Hereford data (n = 14)	0.082	1.074	0.390

A detailed understanding of the relationships between the phenotypic response variables and the covariates was obtained through the construction of an ordination graph (Figure 3.10). The graph further showed a strong relationship between WG and the *TT* genotype (also supported by t-test) as well as the lack of association between the remaining response variables with genotype. The graph clearly illustrates that variation in the phenotypic response variables traits is due to factors other than genotype, hence the arrows' association with the vertical axis, not the horizontal axis that represents genotype.

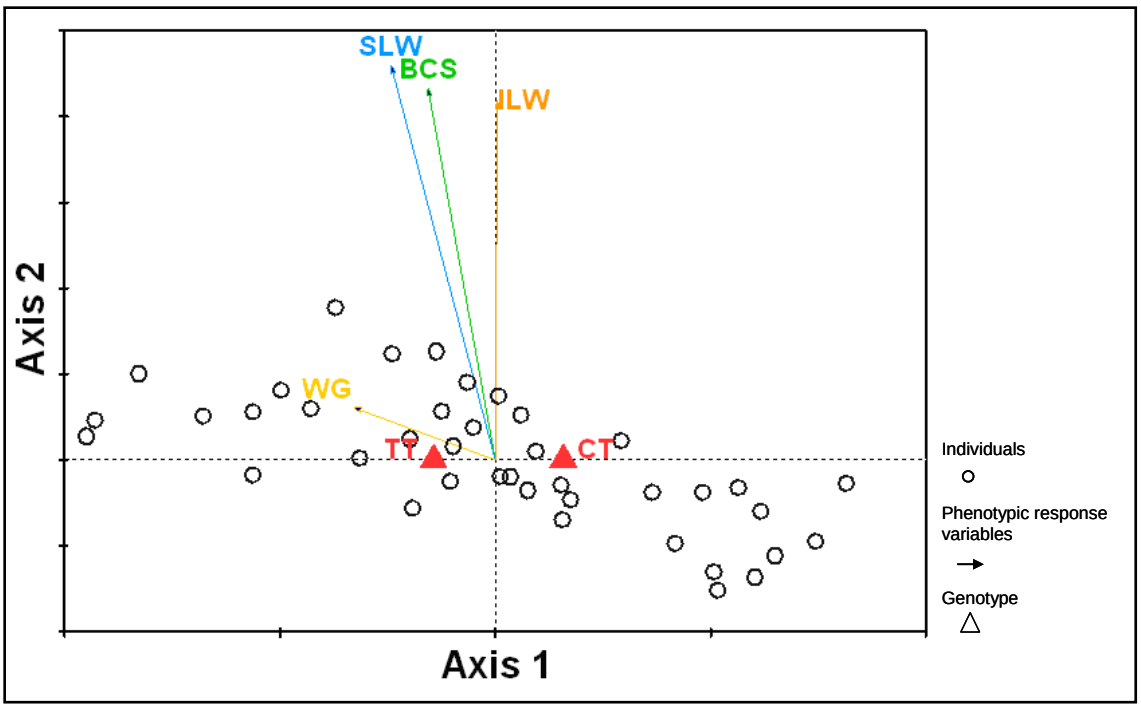

Figure 3.8 Ordination graph of genotype with phenotypic response variables ILW, BCS, WG and SLW of the Hereford population.

Lastly, the effect of breed on the response variables was further analysed by means of RDA and plotted in an ordination graph. The untreated Nguni oxen (n = 32) were compared with the Hereford oxen (n = 54) as these populations were similar in terms of feeding regime, sex and age. First, to establish whether there were any significant differences between breed and the selected phenotypic response variables ILW, SLW, WG and BCS; an RDA was performed on the Nguni and Hereford breeds. The untreated Nguni oxen subgroup (n = 32) was compared to the Hereford population (n = 40) for which the selected phenotypic response measurements were available. The results of the RDA were generated in the form of a Monte Carlo test which established a highly significant relationship between breed and phenotypic response variables (Table 3.12). The eigenvalue obtained was higher when compared to any of the values obtained for RDA, thereby further confirming the strength of the relationship between breed type and phenotypic response variable.

A similar outcome was obtained when the Nguni and the Hereford data set, containing measurements of all the phenotypic response variables (n = 14), were compared. A highly significant relationship was established between breed type and the phenotypic response variables which was also further supported by the eigenvalue (Table 3.12).

Table 3.12 Analysis of associations between breed and phenotypic response variables.

	Monte Carlo test summary		
	Eigenvalue	**F-ratio**	***P*-value**
Nguni oxen and partial Hereford data (n = 72)	0.348	12.103	0.002**
Nguni oxen and complete Hereford data (n = 46)	0.274	5.285	0.002**

* Significant value where $P \leq 0.05$.

** Significant value where $P \leq 0.01$.

Significant differences were found between breeds with the response variables ILW, SLW, WG, CFN FAT and WCM. No significant difference could be established for BCS (Table 3.12). Previous analysis by GLM yielded similar results though FAT was not significantly influenced by breed. The ordination graph also validated the strong relationship between the response variables ILW, SLW, and WG and breed (Figure 3.9). Nguni displayed higher ILW values than Hereford, whereas Hereford showed significantly higher SLW and WG values than Nguni. From the graph, it was noted that BCS was not associated with difference in breed.

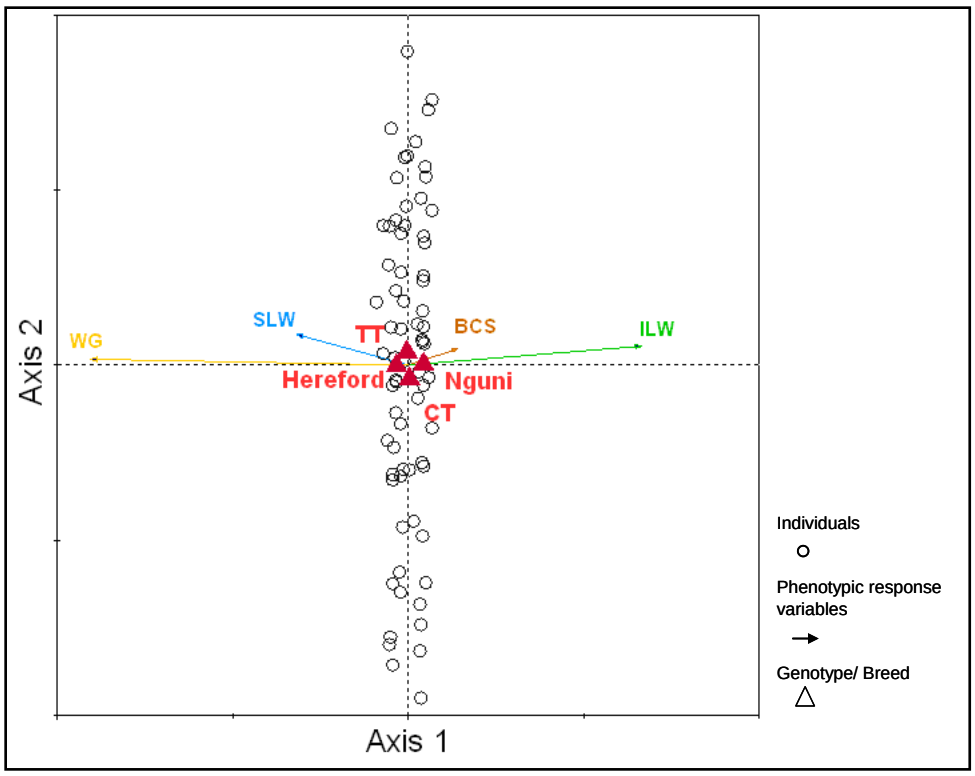

Figure 3.9 Ordination graph of Nguni and Hereford breeds with phenotypic response variables ILW, BCS, WG and SLW.

CHAPTER 4

DISCUSSION

In South Africa, commercial beef industries recognize the need to optimise traits that determine meat quality and marketability. The efficacy of beef production is largely appraised by phenotypic traits such as feedlotting ability, carcass weight, body composition and conformation, and fat distribution. More specifically, carcass weight and fat distribution are currently the main determinants of price for beef within the South African market.

When producers use inefficient breeding and managerial practices their profits are reduced. This is further amplified by the use of subjective measurements such as visual appraisal to determine body condition scores and ultimately predict carcass composition. The price of meat is therefore determined by decisions made 2 to 3 years before the product is sold to the consumer.

The phenotypic traits measured for both Nguni and Hereford population included live weight (LW), weight gain (WG), body composition score (BCS) conformation (CFN), fatness (FAT), warm carcass mass (WCM) and cold carcass mass (CCM). Standard summary statistics were calculated and recorded for each breed with each response variable.

Nguni oxen were earmarked for hormone treatment as they have a high capacity for compensatory growth, unlike Herefords (Goetsch *et al*., 1991). Farmers have been known to use hormones in beef cattle in order to enhance feedlotting performance as they are paid based on the weight of the animals sold for slaughter. There is much controversy in the beef industry due to the effect that these hormones have on consumers (Galbraith, 2002). Breeders need to consider these factors together with the complexity of gene combinations and trait correlations in order to circumvent the loss of other profitable traits.

It was expected that the hormone-treated individuals would have greater carcass values as a result of their larger and heavier frames compared with that of the non-treated individuals. The resultant GLM analysis yielded significant differences for an increased WG for untreated individuals (56.53 kg) than with the treated sample group. This is because WG is a measure of the response to the feedlotting period; the untreated individuals entered the feedlot at a significantly lower ILW and required much more weight gain than the treated individuals in preparation for slaughter. The resultant SLW of the treated group was 35.8 kg significantly heavier than the untreated group compared with the initial average LW difference of 50 kg between the sample groups. The treated group displayed a significantly higher WCM and CCM than the untreated group by an increase of 13.98 kg. Research has shown that untreated cattle display an increased WG compared with treated cattle, confirming the theory that every breed has a limit to their genetic potential for growth (Ray *et al.*, 1969; Adams *et al*., 1990; Hersom *et al*., 1994). Breeders should take note that it may be more profitable to maximize the growth (WG) of animals of lower ILW rather than heavier animals. This would increase WG and WCM of the majority of the population rather than exhausting the limit of a few treated animals for a 14kg difference in carcass weight. No significant differences for hormone treatment could be found for the response variables BCS, FAT and CFN.

Breeders aim to produce animals with specific alleles in order to achieve the desired combination of alleles for a suitable genotype in the offspring. The availability of information concerning these alleles allows insight into the complexity of gene combinations as well as trait correlations in order to circumvent the loss of other profitable traits. Breeders must consider the heritability of the trait they select for in order to select the appropriate strategy of selection. For example, intramuscular fat has a high heritability and will therefore respond rapidly to genetic selection (Porter, 1991). The most cost effective means of improving meat quality is to use appropriate genotypes, as this allows producers to minimize the time taken to reach a given market specification whilst maximizing the number of animals reaching that specification. In short, greater amounts of available genetic information regarding carcass and breed characteristics allow producers to better target specific markets and increase the

competitiveness of their beef operation. The Nguni, along with several other South African cattle breeds, are only recently being included in genetic research. However, the Nguni have not previously been considered in leptin studies.

The Nguni and Hereford populations were genotyped at the *lep* locus in order to distinguish *CC, CT, TT* individuals. Only one *CC* genotype was present in the Nguni population, whereas none were present in the Hereford population. The C-allele was found in marginal numbers in both the Nguni and Hereford populations. The heterozygote *CT* and homozygote *TT* genotypes were approximately evenly distributed in both the Nguni and Hereford populations resulting in Hardy-Weinberg disequilibrium. Buchanan *et al*. 2002, also found that there was a greater incidence of T-alleles in the British breeds (i.e. Hereford). Similarly, Schenkel *et al*. 2005, reported a lower (though not significant) frequency of the C allele in British breeds. Previous research also indicated a possible association of the T-allele (missense mutation) with fatter carcasses and the C-allele (normal) with leaner carcasses (Buchanan *et al.,* 2002). It would therefore be expected that with further analysis the T-allele would outperform the C-allele in terms of carcass traits.

Researchers have discovered various polymorphisms in the *lep* gene that have been found to impact economically important traits in cattle such as milk and protein yield (Liefers *et al*., 2002; Buchanan *et al*., 2003; Madeja *et al*., 2004), carcass fat content (Fitzsimmons *et al*., 1998; Buchanan *et al*., 2002), quality grade and carcass weight (Oprządek *et al*., 2003; Kononoff *et al*., 2005), backfat thickness, subcutaneous fat and yield grade (Nkrumah *et al*., 2004a), intramuscular fat (Geary *et al*., 2003), as well as growth rate and live weight (Nkrumah *et al*., 2005).

A GLM and RDA analysis of both the Nguni and Hereford in this investigation revealed that no significant relationship could be established between the various *lep* genotypes *TT*, *CT* and *CC* with the phenotypic carcass traits. This conflicts with the evidence of an association of an SNP in the *lep* exon 2 as identified by Buchanan *et al*. (2002), with carcass fat content and various carcass traits such as intramuscular fat, backfat and subcutaneous fat as well as carcass weight in Hereford cattle. Furthermore, Schenkel *et*

al. (2005) also identified an association of the *lep* genotypes with fat, lean yield and intramuscular fat in crossbred cattle. However, further evidence that supports the finding of the current research was reported by Nkrumah *et al*. (2004b), Barendse *et al*. (2005) and Almeida *et al*. (2007) who could not demonstrate any significant association between E2FB with weight gain, feed intake and intramuscular fat, though some evidence did suggest that carcass grade fat might be influenced by the SNP genotypes.

Furthermore, when the results were anyalized by means of a t-test, significant associations were found between specific E2FB genotypes with ILW. The *CT* genotype was found to have an average ILW (26.8 kg) more than the individuals with *TT* genotype across both breed types. Furthermore, the t-tests and ordination graph displayed a significant increased WG in individuals with a *TT* genotype (21.04 kg) as opposed to *CT* individuals. This inverse relationship was expected due to feedlotting response of lighter weight animals. It is interesting to note however, that the difference in weight of ILW was greater than the difference in WG during the feedlotting period which may suggest that the CT genotype would be favoured in beef breeding. Nkrumah *et al*. 2005 also reported an increased feed intake in cattle homozygous for *TT*. The *TT* and *CT* genotypes displayed significantly higher WG than *CC* individuals which was expected due to the skewed nature of the genotypic frequency whereby only one *CC* individual was present in the population. The results, however, represent an initial association of the *TT* and *CT* individuals with these traits in a South African grading system and further studies are necessary to validate these findings in other populations. Interestingly, however, the *P*-value obtained for the Hereford data was much lower than that of the Nguni which suggests that the two breeds are genetically different at the *lep* locus.

The current research was based on a South African system graded largely according to subcutaneous fat content and carcass mass compared with the USDA's emphasis on intramuscular fat and carcass mass. Therefore this research addresses the relevance of the *lep* SNP as a marker in the South African beef industry. Since there are no previous studies on *lep* associations in Nguni and Hereford with economically important traits

graded according to South African standards, further analysis would be required in order to fully investigate this claim.

Previous research has yielded inconsistent results across various cattle populations regarding SNP's in the leptin gene of livestock species and do not seem to have such drastic consequences; however, some more or less strong effects on body fat tissue have been observed (Altmann and Von Borell, 2007). Hence Leptin is, however, considered as a strong candidate gene, as serum levels of leptin have shown to affect carcass traits in beef cattle populations (Minton *et al*., 1998; Wegner *et al*., 2001; Geary *et al*., 2003; Altmann and Von Borell, 2007). However, it is has not conclusively been demonstrated that the SNP at *lep* exon 2 is indeed a causative mutation or a reliable marker that can be universally applied in the beef industry. In such circumstances, mutations with such effects may not be causal but may instead reflect linkage disequlibrium with mutations in other genes potentially at some distance from the lep gene (Banos *et al*., 2008). Studies across diverse and sizeable beef cattle populations are required to properly characterize the association of polymorphisms in the *lep* gene exon 2 with economically important traits before this information can be implemented efficiently in breeding practices in the South African beef industry. The SNP at exon 2 has only developed recently as indicated by the fact that this mutation was up until recently, only detected in taurine cattle (Choudhary *et al*., 2005). Variations at the DNA level contribute to the genetic characterization of livestock populations and this may help to identify possible hybridization events as well as past evolutionary trends (Altmann and Von Berell, 2007).

Since no significant relationship was established between the *lep* genotypes with differences in phenotypic traits, the variation of the phenotypic response variables would expectedly be the result of factors such as sex, age, environment, breed, genetic interactions and different grading systems. Since feedlot conditions as well as grading systems were standardized and therefore negligible, it was therefore necessary to investigate the independent variables, covariates and their interactions in order to describe any associations with the phenotypic response variables.

The independent variable sex significantly influenced the phenotypic response variable FAT as revealed by GLM analysis. The heifer group had significantly higher FAT scores than oxen. Zembayashi *et al.* (1995) also confirmed that heifers are known to have higher levels of subcutaneous and intramuscular fat than oxen. The influence of sex on carcass traits is well known, whereby the greatest effect of sex is related to fat deposition, in that heifers start fat deposition at lighter weights than castrated and intact males (Žgur *et al.,* 2003). Furthermore, t-tests showed that Oxen had significantly higher BCS than heifers, increased SLW, WG, WCM and CCM. The average weight difference in carcass mass between the sexes amounted to 12kg. The heifers also had lower ILW values due to the difference in frame size between the two sexes. It was interesting to note, however, that the treated oxen displayed no significant difference in fat distribution compared with the non-treated oxen suggesting that fat accretion is a complex entity that is highly attributable to sex and breed (Holloway *et al*., 1990).

Variation in the phenotypic response variables BCS, SLW, CFN and FAT were not significantly influenced by age according to GLM analyses. However, age did significantly affected traits such as ILW, WG, WCM and CCM. Animals that were 2 years old had significantly greater ILW, WCM and CCM whereas animals as young as a year of age showed significantly higher WG during the feedlotting period. This expected result was due to the larger frames of the older animals though research has also shown that younger individuals produce significantly more tender meat compared with their older counterparts (Morgan *et al*., 2002.)

The covariate ILW, as expected, significantly influenced traits BCS and WG. Animals with higher ILW values had significantly higher SLW, WCM and hence CCM whereas no difference could be found for FAT. As previously discussed, the main determinant of FAT values relies heavily on sex as well as breed.

Certain cattle breeds are favoured over others for exhibiting desirable carcass traits as major differences in slaughter traits can often be attributed to breed differences, as verified by Zembayashi *et al*. (1995). A comparison of the Nguni and Hereford phenotypic response variables by GLM yielded significant results for ILW, SLW, WG,

CFN, WCM and CCM whereas no differences were found for the remaining traits such as BCS and FAT. The Nguni had a significantly higher ILW than the Hereford (82.82 kg), and hence a higher BCS. The Hereford group had a significantly higher WG than the Nguni group (121.5 kg), greater SLW (19.01 kg), CFN and WCM and CCM (19.06 kg and 18.65 kg respectively). The GLM and t-test findings were further validated by the RDA results.

Interestingly, the Nguni population entered the feedlot at a higher ILW than the Hereford. Since the cattle that enter the feedlot were formerly on natural pasture, this further attests the findings of the Nguni's inherent ability to perform well on natural pasture and has even proven to produce meat of a quality comparable with that of established beef breeds when raised on natural pasture (Muchenje *et al*., 2007).

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Furthermore, the investigation to determine breed differences at the exotic and indigenous level yielded interesting results. A comparison between the overall phenotypic performances of the Nguni with the Hereford population revealed that the Hereford had significantly greater feedlotting capacity due to significantly higher WG values and SLW, WCM and CCM which could be attributed to genetic differences between the two breeds as confirmed by previous research in Hereford (Dean *et al*., 1976). The growth performance and carcass yield of the Nguni, under intensive feeding conditions has appeared to be less favourable when compared to exotic breeds (Strydom, 2008). Variation in frame size amongst the indigenous breeds has however limited their feedlotting capacity owing to the local beef industry's emphasis on profit comprised of price per carcass weight and feed margins (Strydom, 2008). Nguni is known for its characteristically small frame, in AAA commercial feedlot systems, these indigenous breeds compete with exotic breeds driven by global principles. Hence it would appear that the indigenous Nguni did not compare well with the exotic Hereford in the commercial AAA beef grading system whereby carcass weight largely determines the value of the product. Huerta-Liedenz *et al*. (1993) and Zembayashi *et al*. (1995) also found that breed differences accounted largely for differences in carcass traits and more specifically fat distribution and accretion. This further supported the evidence that there

is a genetic basis for differences in fatty acid composition between different breeds which would subsequently explain CFN differences between the Nguni and Hereford populations under investigation. According to the current research, the Hereford breed was superior to the Nguni in terms of carcass traits and feedlotting performance though further investigation is warranted to confirm the claim due to small conclusive data sets for the Hereford population. Although the carcass trait and feedlot performance data of the Nguni was not favourable compared to the Hereford in this study, it is however, important to note that Strydom *et al*. (2000) found that indigenous beef cattle breeds were equivalant to the exotic breeds in terms of feedlot performance. As previously mentioned, the Nguni's characteristically small frame has limited its success in the commercial beef sector though is not to be summarily dismissed as it has a formidable lineage of hardiness and productivity. As previously mentioned, the Nguni are primarily known as an outstanding beef breed due to their optimal production under severe conditions thereby making them very popular in the communal farming sector. In a commercial sector, however, the advantages of the stockier British breed, Hereford, are still evident. Therefore it can be concluded that breed accounts significantly for differences in performance traits in the AAA beef industry and that the Nguni and Hereford breeds are very different from one another at a genetic level.

However, outside of the AAA sector, lies a commercial trend toward beef quality and tenderness as well as the local consumer preference for organic beef. Consumer perception views beef as one of the tougher meats available. Subsequently local chefs and restaurants have steered away from AAA beef based on carcass weight in preference of organic beef with favourable tenderness and quality. Moreover, meat quality analyses indicate minimal to no difference between indigenous breeds and exotic British or European breeds but has indicated superiority in terms of quality when compared to *Bos indicus* breeds (Strydom, 2008). Random marker tests for genes related to tenderness confirmed high frequencies of advantageous genes in indigenous breeds thereby further supporting the favourability of indigenous beef quality (Strydom, 2008). Therefore, subsequent emphasis on indigenous beef quality in comparison to exotic beef breeds rather than carcass weight should be investigated further.

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APPENDIX A: LABORATORY REAGENTS AND CALCULATIONS

Adapted from: Bell et al. (1981) PNAS 78: 5879- 5832

Bruford *et al*., (1992). Single locus and multi locus DNA fingerprinting. Molecular Genetic Analysis of Populations: A Practical Approach. A. Hoelzel. Oxford, IRL Press: 225-269.

DNA Extraction: reagents and recipes

5 X BCL Buffer

- 57.4 g sucrose
- 2.5 ml 2 M Tris-Cl pH 7.5
- 2.5 ml $MgCl_2$

2 M Tris-Cl pH 7.5 (mw = 121.1 g / 1000 ml)
therefore 0.30275 g in 2.5 ml Millipore H_2O

$MgCl_2$. 6 H_2O (should ideally be anhydrous)
(mw = 203.31 g / 1000 ml)
therefore 0.508275 g in 2.5 ml Millipore H_2O

Dissolved Tris in 2.5 ml Millipore H_2O, then added to the sucrose, which was dissolved in 80 ml Millipore H_2O. Adjusted pH to 7.5 with HCl, then add $MgCl_2$. Made final volume of 100 ml. Autoclaved and stored at room temperature.

Before use, diluted 1: 5 with d.H_2O, added 0.05 vols 20% Triton X-100. Stored in a sterile Mc Cartney bottle, on ice.

Lysis Buffer: per reaction

- 500 μl 1 X TNE
- 50 μl 1 M Tris-HCl pH 8
- 7.5 μl 25% SDS
- 7.5 μl Triton X-100
- 1 μl Proteinase K (10 mg / ml)

1 X TNE

(0.4 M NaCl, 10 mM Tris-HCl, pH 8, 2 mM EDTA pH 8)

volume = 100 ml

- 0.4 M NaCl (mw = 58.443 g / 1000 ml = 1 M)
  therefore 0.23372 g used
- 10 mM Tris-HCl pH 8 (mw = 157.56 g / 1000 ml)
  therefore 0.1576 g used

Added NaCl and Tris-HCl to approximately 80 ml d.H_2O and adjusted to pH 8 with NaOH, before the addition of EDTA.

- 2 mM EDTA (mw = 292.25 g / 1000 ml)
  Therefore 0.05845 g used

Dissolved using stirrer, made up to 100 ml. Autoclaved and store at room temperature.

1 M Tris-HCl pH 8

(mw = 157.56 g / 1000 ml)

volume = 100 ml

therefore 15.756 g in approximately 80 ml d.H_2O

Adjusted to pH 8 with 3 M NaOH, made up to 100 ml. Autoclaved and stored at room temperature.

25% SDS

(25 g SDS in 100 ml d.H_2O)

therefore 6.25 g SDS in 25 ml d.H_2O. No autoclave. Stored at room temperature.

- Triton X-100

collected 2 ml

- Proteinase K

Added 0.01 g proteinase K into 1 ml d.H_2O. Stored in fridge.

- 5M NaCl

volume = 20 ml

(mw = 58.443 g / 1000 ml = 1 M)

Therefore 5.8443 g in 20 ml d.H_2O. Autoclaved and stored at room temperature

1 X BCL Buffer

volume = 30 ml

- 6 ml 5 X BCL
- 24 ml d.H_2O
- 1.5 ml 20% Triton X-100

Made in sterile Mc Cartney bottle and kept on ice.

20% Triton X-100

volume = 1.5 ml

- 300 μl Triton X-100
- 1200 μl d.H_2O

Made 1.5 ml in an Eppendorftube. Placed in beaker of H_2O and put on stirrer on low heat to mix.

10% Triton X-100

volume = 10 ml

- 1600 μl Triton X-100
- 8400 μl d.H_2O

Stirred 5 minutes on low heat to mix.

70% EtOH

70 ml 100% EtOH added to 30 ml d.H_2O

10 mM Tris-HCL pH 8

volume = 1 ml

- 10 μl Tris-HCl
- 990 μl d.H_2O

Agarose Gel Electrophoresis: reagents and recipes

1% Agarose gel: for genomic DNA

- volume = 200 ml
- 2 g agarose
- 200 ml 1 X TAE

3% Agarose gel

- volume = 200 ml
- 6 g agarose
- 200 ml 1 X TAE

10 X TAE

volume = 1.5 l

400 mM Tris-HCl – 72.6 g / l

200 mM Na-acetate – 24.6 g

10 mM EDTA – 4.35 g

Adjusted to pH 8 with acetic acid
(did not add lots of d.H_2O before adjusting pH - much acetic acid was required to reach pH 8)

1 X TAE
volume = 1.2 *l*
120 ml 10 X TAE
made up to 1.2 *l* with d.H_2O

Loading Buffer
volume = 40 ml

- 0.25% bromophenol blue- 0.1 g
- 0.25% xylene cyanol- 0.1 g
- 30% glycerol in H_2O –12 ml glycerol

Made up to 40 ml with d.H_2O. Vortexed before use.

Samples for gel electrophoresis

- 2 μl loading buffer
- 5 μl sample
- 5 μl d.H_2O

Quantification of DNA using Spectrophotometry: reagents and recipes

10 X TE Buffer
volume = 250 ml

- 100 mM Tris-HCl pH 7.5 – 3.0275 g
- 100 mM EDTA – 0.93 g

Adjusted pH of Tris with concentrated HCl, then added EDTA. Autoclaved. Used at 1 X concentration.

1. Diluted 10 μl DNA into 490 μl 1 X TE in a semi-micro quartz curvette, and determined A260 and A280:

A260 = x A280 = y

A260/A280 = purity ratio

Pure DNA = 1.8

RNA & proteins = <1.8

Phenolic compounds = >1.8

Calculations:

[DNA] = A260 X dilution factor (50) X 50 = Z μg / ml

Z / 1000 = μg / μl

Dilution factor = total spec sample (500 μl) / DNA sample (10 μl)

= 50

Sample individual	A_{260}	A_{280}	Purity ratio A_{260} / A_{280}	Concentration µg/ml
		Nguni Population		
N1	0.1	0.084	1.14	477.5
N2	0.023	0.021	1.07	113
N3	0.124	0.103	1.2	620
N4	0.022	0.021	1.07	110.5
N5	0.022	0.029	0.76	108.5
N6	0.027	0.027	0.99	132.5
N7	0.021	0.019	1.13	106
N8	0.016	0.02	0.79	80.5
N9	0.024	0.017	1.41	121.5
N10	0.026	0.022	1.2	130
N11	0.077	0.049	1.57	386
N12	0.032	0.027	1.16	158
N13	0.02	0.017	1.19	98.5
N14	0.017	0.014	1.24	86.5
N15	0.015	0.013	1.15	77
N16	0.018	0.017	1.06	91.5
N17	0.012	0.011	1.13	62
N18	0.006	0.005	1.25	29.5
N19	0.014	0.014	0.99	69.5
N20	0.02	0.015	1.38	104.5
N21	0.012	0.015	0.8	59.5
N22	0.005	0.006	0.88	24.5
N23	0.003	0.004	0.79	16.5
N24	0.0207	0.02	1.07	103.5
N25	0.0248	0.0194	1.28	124
N26	0.02	0.025	0.82	101.5
N27	0.023	0.021	1.07	113.5
N28	0.019	0.027	0.72	95.5
N29	0.008	0.01	0.88	42
N30	0.006	0.006	1.03	29.5
N31	0.024	0.02	1.16	118
N33	0.071	0.046	1.55	357
N34	0.117	0.07	1.68	584.5
N35	0.058	0.042	1.37	288
N35	0.019	0.024	0.77	93
Average	0.03	0.026	1.11	151.8

Sample individual	A_{260}	A_{280}	Purity ratio A_{260} / A_{280}	Concentration µg/ml
		Hereford Population		
H1	0.016	0.01	1.58	39
H2	0.01	0.006	1.6	23.3
H3	0.008	0.004	1.92	18.8
H4	0.005	0.002	2.05	11.3
H5	0.015	0.008	1.87	37.8
H6	0.046	0.024	1.9	115.5
H7	0.057	0.033	1.8	146.5
H9	0.048	0.024	1.78	119.3
H10	0.011	0.007	1.75	28.5
H11	0.004	0.002	2.1	11
H12	0.028	0.016	1.72	69.5
H13	0.009	0.006	1.41	21.5
H14	0.051	0.028	1.86	128.5
H15	0.118	0.061	1.94	295.5
H16	0.018	0.011	1.59	44.3
H17	0.014	0.008	1.85	34.8
H18	0.006	0.005	1.33	15.3
H19	0.139	0.07	1.97	346.3
H20	0.012	0.007	1.61	29.3
H21	0.012	0.007	1.85	30.5
H22	0.148	0.078	1.9	369.3
H24	0.022	0.012	1.84	55.8
Average	0.036	0.019	1.78	90.5

RFLPs: Amplification and digestion reagents and recipes

For life science research only. Not for use in diagnostic procedures.
FOR *IN VITRO* USE ONLY.

Restriction Endonuclease Mro I (Acc III)

From Micrococcus roseus

Cat. No. 11 102 982 001
100 units (1–5 U/µl)

Please see label for lot specific values.

T↓CCGGA
AGGCC↑T

Version July 2005

Store at −15 to −25°C

Stability/Storage The undiluted enzyme solution is stable when stored at −15 to −25°C until the control date printed on the label. Do not store below −25°C to avoid freezing.

Sequence Specificity *Mro* I recognizes the sequence T/CCGGA and generates fragments with 5'-cohesive ends.

Compatible ends *Mro* I generates compatible ends to *Cfr* 10I, *Sgr* AI, and *Xma* I.

Isoschizomers *Mro* I is an isoschizomer to *Acc* III, *Bse*A I, *Bsi*M I, *Bsp*E I, *Kpn*2 I.

Methylation sensitivity *Mro* I is not inhibited by overlapping *dam*-methylation (°) (1), in contrast to the isoschizomer *Bse*A I. *Mro* I is inhibited by 5-methylcytosine, as indicated (°).

Storage buffer 10 mM Tris-HCl, 200 mM NaCl, 0.1 mM EDTA, 5 mM 2-mercaptoethanol, 200 µg/ml bovine serum albumin; glycerol, 50% (v/v); pH ca. 7.5.

Suppl. Incubation buffer (10 x) 330 mM Tris-acetate, 660 mM K-acetate, 100 mM Mg-acetate, 5 mM dithiothreitol; pH 7.9 (at 37°C); (≙ SuRE/Cut Buffer **A**).

Activity in SuRE/Cut 5 Buffer System

A	B	L	M	H
100%	0–10%	50–75%	50–75%	0–10%

Incubation temp. 37°C

Unit definition One unit is the enzyme activity that completely cleaves 1 µg λDNA in 1 h at 37°C in the incubation buffer in a

Typical experiment

Component	Final concentration
DNA	1 µg
10 × SuRE/Cut Buffer **A**	2.5 µl
Repurified water	Up to a total volume of 25 µl
Restriction enzyme	1 unit

Incubate at **37°C** for 1 h.

PFGE tested *Mro* I has been tested in Pulsed-Field Gel Electrophoresis (test system bacterial chromosomes). For cleavage of genomic DNA (*E. coli* C 600) embedded in agarose for PFGE analysis 10 units of enzyme/µg DNA and 4 h incubation time are recommended.

Heat inactivation There is no information about *Mro* I and heat-inactivation available.

Number of cleavage sites on different DNA's (2):

λ	Ad2	SV40	ΦX174	M13mp7	pBR322	pBR328	pUC18
24	8	0	0	0	1	1	0

Troubleshooting A critical component is the DNA substrate. Many compounds used in the isolation of DNA *e.g.*, phenol, chloroform , EtOH, SDS, high levels of NaCl, metals (*e.g.*, Hg^{2+}, Mn^{2+}), inhibit or alter recognition specifity of many restriction enzymes. Such compounds should be removed by EtOH precipitation followed by drying, before the DNA is added to the restriction digest reaction. Appropriate mixing of the enzyme is recommended.

Check out the Restrictions Enzymes Frequently Asked Questions at http://www.roche-applied-science.com/support.

Quality control

See data label for lot-specific values.

Absence of unspecific endonuclease activities 1 µg λDNA is incubated for 16 h in 25 µl incubation buffer with excess of *Mro* I. The number of enzyme units which do not change the enzyme-specific pattern is stated under "Endo" printed on the label.

Absence of exonuclease activity Approx. 5 µg [^{3}H] labeled calf thymus DNA are incubated with 3 µl *Mro* I for 4 h at 37°C in a total volume of 100 µl 50 mM Tris-HCl, 10 mM $MgCl_2$, 1 mM dithioerythritol, pH approx. 7.5. The release of radioactivity is calculated as a percentage value of liberated to input radioactivity per unit of enzyme (stated under "Exo" as printed on the label).

Ligation and recutting assay *Mro* I fragments obtained by complete digestion of 1 µg λDNA are ligated with 1 U T4-DNA ligase (Cat. No. 10 481 220 001) in a volume of 10 µl by incubation for 16 h at 4°C in 66 mM Tris-HCl, 5 mM $MgCl_2$, 5 mM dithiothreitol, 1 mM ATP, pH 7.5 (at 20°C).

The percentage of ligation and subsequent recutting with *Mro* I which yields the typical pattern of λ × *Mro* I fragments are determined and stated under "Lig" and "Rec" printed on the label.

References

1 Suetake, T. et al., Nippon Nôgeikagakukai Agricultural Chemical Society.
2 Kessler, C. & Hähnke, H.-J. (1986) *Gene* **47**, 1–153.
3 Rebase The Restriction Enzyme Database: http://rebase.neb.com
4 Benchmate: http://www.roche-applied-science.com/benchmate

07/05 11105847

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Ordering Information

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For a complete overview of related products and manuals, please visit and bookmark our Special Interest Sites: http://www.roche-applied-science.com

Product	Application	Packsize	Cat. No.
Restriction Enzymes	DNA restriction digestion	Please refer to website or catalogue	
Rapid DNA Ligation Kit	Ligation of sticky-end or blunt-end DNA fragments in just 5 min at 15-25°C.	Kit (40 DNA ligations)	11 635 379 001
T4 DNA Ligase	Ligation of sticky- and blunt ended DNA fragments.	100 U 500 units (1 U/µl)	10 481 220 001 10 716 359 001
Alkaline Phosphatase, shrimp	Dephosphorylation of 5'-phosphate residues from nucleic acids. Heat inactivation: 15 min at 65° C.	1000 U	11 758 250 001
Alkaline Phosphatase (AP), special quality for molecular biology	Dephosphorylation of 5'-phosphate residues from nucleic acids.	1000 U (20 U/µl)	11 097 075 001
Agarose MP	Multipurpose agarose for analytical and preparative electrophoresis of nucleic acids	100 g 500 g	11 388 983 001 11 388 991 001
Agarose LM-MP	Low melting point agarose allows enzymatic manipulations	50 g 100 g	11 441 345 001 11 441 353 001
Agarose Gel DNA Extraction Kit	For the elution of DNA fragments from agarose gels.	1 Kit (max. 100 reactions)	11 696 505 001
High Pure PCR Product Purification Kit	Purification of PCR or enzymatic modification reaction (e.g. restriction digest)	50 purifications 250 purifications	11 732 668 001 11 732 676 001
SuRE/Cut Buffer Set for Restriction Enzymes	Incubation buffers A,B,L,M and H for restriction enzymes	1 ml each (10× conc. solutions)	11 082 035 001
SuRE/Cut Buffer A	Restriction enzyme incubation	5 × 1 ml (10× conc. solution)	11 417 959 001
SuRE/Cut Buffer B	Restriction enzyme incubation	5 × 1 ml (10× conc. solution)	11 417 967 001
SuRE/Cut Buffer H	Restriction enzyme incubation	5 × 1 ml (10× conc. solution)	11 417 991 001
SuRE/Cut Buffer L	Restriction enzyme incubation	5 × 1 ml (10× conc. solution)	11 417 975 001
SuRE/Cut Buffer M	Restriction enzyme incubation	5 × 1 ml (10× conc. solution)	11 417 983 001
Water, PCR Grade	Specially purified, double-distilled, deionized, and autoclaved	100 ml (4 vials of 25 ml) 25 ml (25 vials of 1 ml) 25 ml (1 vial of 25 ml)	03 315 843 001 03 315 932 001 03 315 959 001
BSA, special quality for molecular biology	Maintaining enzyme stability	20 mg (1 ml)	10 711 454 001

Printed Materials You can view the following manuals on our website:

Laminated Buffer Chart
Lab FAQS "Find a Quick Solution"
Restriction Enzyme FAQS and Ordering Guide
Molecular Weight Markers for Nucleic Acids
Poster "Rec. Sequences of Restriction Enzymes"

SuRE/Cut is a trademark of a member of the Roche group

Commonly used bacterial strains

Strain	Genotype
BL21	*E. coli B F⁻ dcm ompT hsdS(r_B⁻ m_B⁻) gal* (Studier, F.W. et al (1986) *J. Mol. Biol.*, **189**, 113.)
C600[1]	*supE44 hsdR2 thi-1 thr-1 leuB6 lacY1 tonA2 t;* (Hanahan, D. (1983) *J. Mol. Biol.* **166**, 557.)
DH5α	*supE44 Δ(lacU169 (ϕ80dlacZΔM15) hsdR17 recA1 endA1 gyrA96 thi-1 relA1;* (Hanahan, D. (1983) *J. Mol. Biol.* **166**, 557.)
HB101	*supE44 hsdS20 recA13 ara-14 proA2 lacY1 galK2 rpsL20 xyl-5 mtl-1;* (Hanahan, D., (1983) *J. Mol. Biol.* **166**, 557.)
JM108	*recA1 supE44 endA1 hsdR17 gyrA96 relA1 thi Δ(lac-proAB);* (Yanisch- Perron, C. et al., (1985) *Gene* **33**, 103.)
JM109	*recA1 supE44 endA1 hsdR17 gyrA96 relA1 thi Δ(lac-proAB) F'[traD36proAB⁺, lacIq lacZΔM15];* (Yanisch- Perron, C. et al., (1985) *Gene* **33**, 103.)
JM110	*rpsL (Str') thr leu thi-1 lacY galK galT ara tonA tsx dam dcm supE44 Δ(lac-proAB) F'[traD36proAB⁺, lacIq lacZΔM15];* (Yanisch- Perron, C. et al., (1985) *Gene* **33**, 103.)
K802	*supE hsdR gal metB;* (Raleigh, E. et al., (1986) Proc. Natl. Acad. Sci USA, 83, 9070.; Wood, W.B. (1966) J. Mol. Biol., **16**, 118.)
SURE[1]	*recB recJ sbc C201 uvrC umuC::Tn5(kanr) lac , Δ(hsdRMS) endA1 gyrA96 thi relA1 supE44 F'[proAB⁺ lacIq lacZΔM15 Tn10 (tetR);* (Greener, A. (1990) Stratagies, **3**, 5.)
TG1	*supE hsd Δ5 thi Δ(lac-proAB) F'[traD36proAB⁺, lacIq lacZΔM15];* (Gibson, T.J. (1984) PhD Theses. Cambridge University, U.K.)
XL1-Blue[1]	*supE44 hsdR17 recA1 endA1 gyrA46 thi relA1 lac F'[proAB⁺, lacIq lacZΔM15 Tn10 (tetr)];* (Bullock et al., (1987) BioTechniques, 5, 376.)

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Roche Diagnostics GmbH
Roche Applied Science
Nonnenwald 2
82372 Penzberg
Germany

*Kpn*2I Primer sequence used for restriction (adapted from Buchanan *et al*., 2002)

Forward primer:

5’ ATG-CGC-TGT-GGA-CCC-CTG-TAT-C 3’

Reverse primer:

5’ TGG-TGT-CAT-CCT-GGA-CCT-TCC 3’

PCR reaction mixture

volume = 20 μl

- 10 X PCR Buffer
- 1.5 mM $MgCl_2$
- 200 μM dNTPs
- 10 pmol each primer
- 1 U Taq
- 50-100 ng genomic DNA

Restriction digestion:

volume = 20 μl

- 15 μl PCR product
- 2 U *Kpn*2I
- 10 X Y Tango buffer
- 4 mM spermidine

Molecular Weight Marker used for restriction digestion:

For life science research only. Not for use in diagnostic procedures.
FOR *IN VITRO* USE ONLY.

DNA Molecular Weight Marker XIII (50–750 bp)

50 base pair ladder

Cat. No. 11 721 925 001

50 µg ≅ 1 A_{260} unit

50 µg (200 µl) for 50 gel lanes

Version July 2005

Store at −15 to −25° C

Product overview

Formulation	Solution in 10 mM Tris-HCl, 1 mM EDTA, pH 8.0.
Concentration	250 µg/ml
Size distribution	Fragment mixture prepared by cleavage of a specially constructed plasmid with restriction endonucleases. The mixture contains 15 double stranded DNA fragments with the following base pair lengths: 50, 100, 150, 200, 250, 300, 350, 400, 450, 500, 550, 600, 650, 700, 750 and an additional band of 2642 bp. The 250 bp and 500 bp banding pattern is two to three times brighter.
Application	The DNA Molecular Weight Marker XIII allows accurate sizing of DNA fragments generated by PCR or restriction digest separated on agarose gels. The DNA molecular weight marker can be used in conjunction with DNA Molecular Weight Marker XIV (100 bp ladder)* for precise size determination. Electrophoretic separation of this molecular weight marker results in a regular pattern. The fragments have 5'-protruding ends which can be labeled by filling in reactions using e.g. [^{32}P]-dTTP and [^{32}P]-dGTP, or using DIG-11-ddUTP* and Terminal Transferase*.
Typical analysis	The DNA fragment mixture shows the ladder with 15 bands and an additional band of 2642 bp in agarose gel electrophoresis as shown in the figure.
Separation conditions	Apply 1 µg DNA per lane on a 2% Agarose MP* gel.
Stability/ Stability	The unopened reagent is stable at -15 to -25°C until the expiration date printed on the label. ***Note:*** This product is shipped on dry ice. Once thawed we recommend further storage at +2 to +8°C. Repeated freezing and thawing should be avoided.
Printed Materials	LabFAQs „Find a Quick Solution" Molecular Weight Markers for Nucleic Acids or available at http://www.roche-applied-science.com/prod_inf/applic/mwm.pdf Restriction Enzyme Ordering Guide Restriction Enzyme Poster

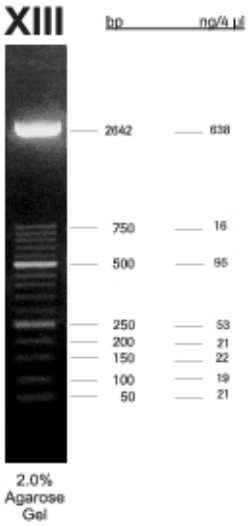

Fig.: Separation of 1 µg DNA molecular weight marker XIII on a 2% agarose gel. Ethidium bromide stain.

* available from Roche Applied Science

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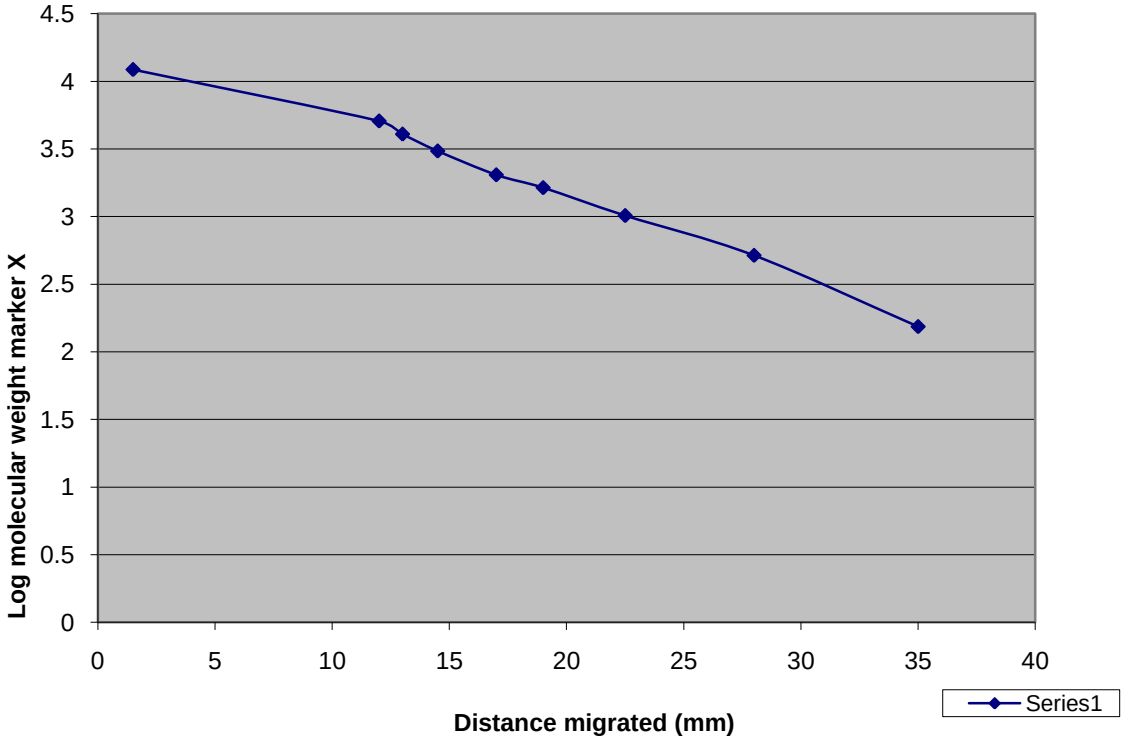
DNA 11727 281

Molecular weight marker - determination of size by distance migrated



Polyacrylamide gel electrophoresis of PCR-RFLP product

5 X TBE

volume = 100 ml

50 ml 10 X TBE in 50 ml d.H_2O

10 X TBE

volume = 100 ml

(1 M Tris, 1 M Boric Acid, 0.5 M EDTA, pH 8.3)

- 12.11 g Tris
- 6.183 g Boric acid
- 4.4 ml EDTA (Adjust to pH 8.0 with NaOH before adding to Tris and Boric solution)

(2.9225 g in 10 ml H_2O)

Adjusted to pH 8.3 with HCl. Autoclaved. (The solution may be re-autoclaved to dissolve precipitates that form.)

30% Acrylamide: Bisacrylamide solution

volume = 100 ml (made up with d.H_2O)

- 29 g Acrylamide
- 1 g Bisacrylamide

Stored in foil wrapped bottle at 4°C

- TEMED

(*N,N,N*',*N*'-tetramethylethylenediamine)

10% Ammonium Persulfate

0.0228 g in 1 ml d.H_2O

20 X TBE

Volume= 1 l

- 216 g Tris Base
- 110 g Boric Acid
- 80 mL 500 mM EDTA, pH 8.0
- 700 ml d.H2O

Autoclaved. Stored at room temperature.

Acrylamide Solution

volume = 1 l (made up with d.H_2O)

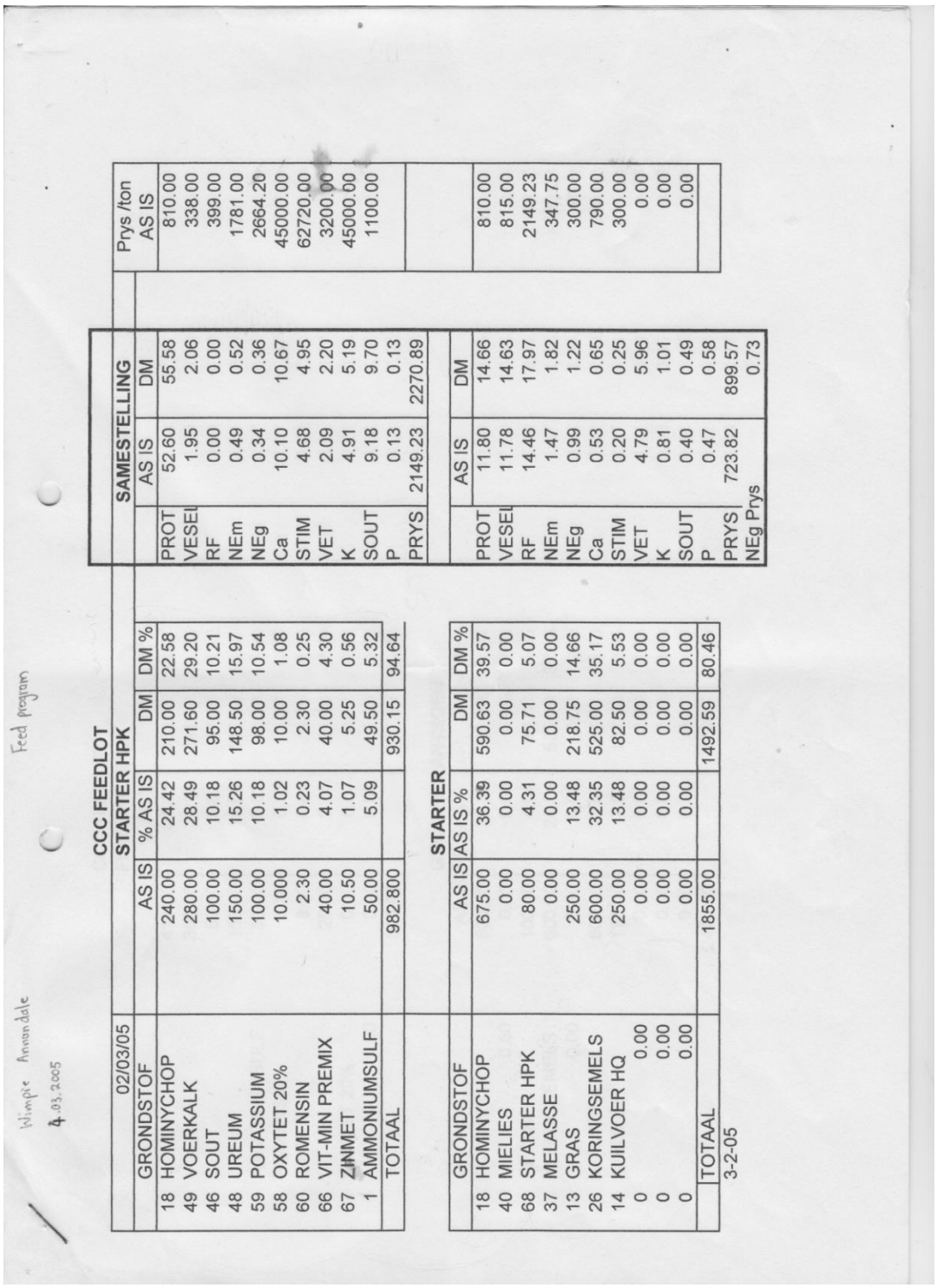
- 6% Acrylamide (60.0 g Acrylamide)

- 0.25% Bisacrylamide (2.5 g Bisacrylamide)
- 8 M Urea (422.0 g Urea)

- 25 ml 0.5 X TBE500 ml 20 X TBE
  Filtered. Stored at 4°C in the dark.

APPENDIX B: SAMPLE DATA

Diet Programme



Wimpie Annandale
4.03.2005

Feed program

CCC FEEDLOT

02/03/05	STARTER HPK			
GRONDSTOF	AS IS	% AS IS	DM	DM %
18 HOMINYCHOP	240.00	24.42	210.00	22.58
49 VOERKALK	280.00	28.49	271.60	29.20
46 SOUT	100.00	10.18	95.00	10.21
48 UREUM	150.00	15.26	148.50	15.97
59 POTASSIUM	100.00	10.18	98.00	10.54
58 OXYTET 20%	10.000	1.02	10.00	1.08
60 ROMENSIN	2.30	0.23	2.30	0.25
66 VIT-MIN PREMIX	40.00	4.07	40.00	4.30
67 ZINMET	10.50	1.07	5.25	0.56
1 AMMONIUMSULF	50.00	5.09	49.50	5.32
TOTAAL	982.800		930.15	94.64

STARTER				
GRONDSTOF	AS IS	AS IS %	DM	DM %
18 HOMINYCHOP	675.00	36.39	590.63	39.57
40 MIELIES	0.00	0.00	0.00	0.00
68 STARTER HPK	80.00	4.31	75.71	5.07
37 MELASSE	0.00	0.00	0.00	0.00
13 GRAS	250.00	13.48	218.75	14.66
26 KORINGSEMELS	600.00	32.35	525.00	35.17
14 KUILVOER HQ	250.00	13.48	82.50	5.53
0 0.00	0.00	0.00	0.00	0.00
0 0.00	0.00	0.00	0.00	0.00
0 0.00	0.00	0.00	0.00	0.00
TOTAAL	1855.00		1492.59	80.46

3-2-05

SAMESTELLING		
	AS IS	DM
PROT	52.60	55.58
VESEL	1.95	2.06
RF	0.00	0.00
NEm	0.49	0.52
NEg	0.34	0.36
Ca	10.10	10.67
STIM	4.68	4.95
VET	2.09	2.20
K	4.91	5.19
SOUT	9.18	9.70
P	0.13	0.13
PRYS	2149.23	2270.89

	AS IS	DM
PROT	11.80	14.66
VESEL	11.78	14.63
RF	14.46	17.97
NEm	1.47	1.82
NEg	0.99	1.22
Ca	0.53	0.65
STIM	0.20	0.25
VET	4.79	5.96
K	0.81	1.01
SOUT	0.40	0.49
P	0.47	0.58
PRYS	723.82	899.57
NEg Prys		0.73

Prys /ton AS IS
810.00
338.00
399.00
1781.00
2664.20
45000.00
62720.00
3200.00
45000.00
1100.00
810.00
815.00
2149.23
347.75
300.00
790.00
300.00
0.00
0.00
0.00

CCC FEEDLOT

02/03/05	FINISHER HPK				
	GRONDSTOF		% AS IS	DM	DM %
18	HOMINYCHOP	410.00	41.13	358.75	38.67
49	VOERKALK	300.00	30.10	291.00	31.36
46	SOUT	50.00	5.02	47.50	5.12
48	UREUM	150.00	15.05	148.50	16.01
1	AMMONIUMSULF	50.00	5.02	49.50	5.34
60	ROMENSIN	3.30	0.33	3.30	0.36
67	ZINMET	8.50	0.85	4.25	0.46
66	VIT-MIN PREMIX	25.00	2.51	25.00	2.69
58	OXYTET 20%	0.00	0.00	0.00	0.00
0	0.00	0.00	0.00	0.00	0.00
	TOTAAL	996.80		927.80	93.08

GROWER RANTSOEN

	GRONDSTOF	AS IS	AS IS %	DM	DM %
18	HOMINYCHOP	800.00	32.32	700.00	40.24
0	0.00	0.00	0.00	0.00	0.00
69	FINISHER HPK	100.00	4.04	93.08	5.35
26	KORINGSEMELS	600.00	24.24	525.00	30.18
0	0.00	0.00	0.00	0.00	0.00
14	KUILVOER HQ	800.00	32.32	264.00	15.18
40	MIELIES	175.00	7.07	157.50	9.05
0	0.00	0.00	0.00	0.00	0.00
0	0.00	0.00	0.00	0.00	0.00
0	0.00	0.00	0.00	0.00	0.00
	TOTAAL	2475.00		1739.6	70.29

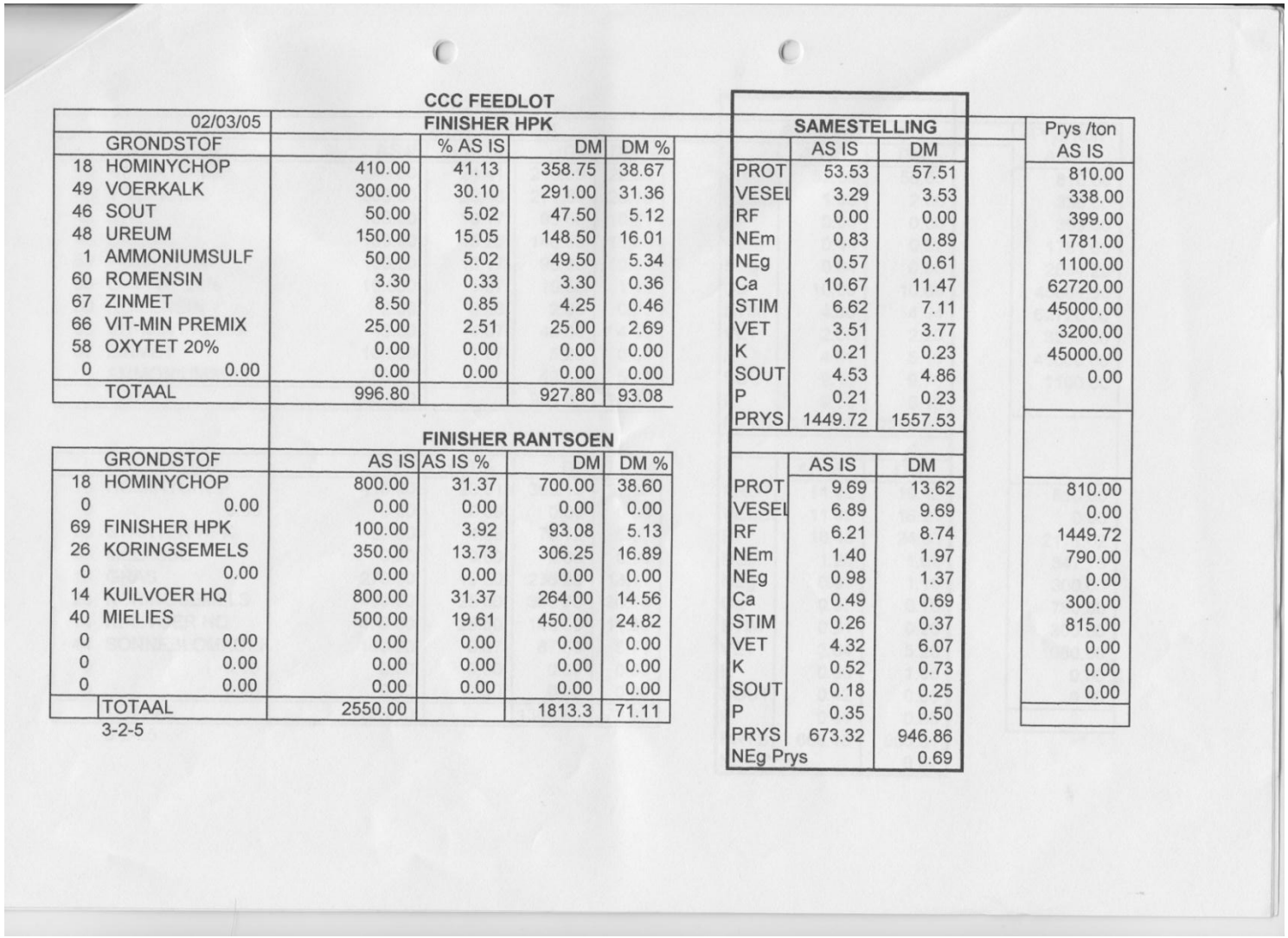
3-2-05

SAMESTELLING

	AS IS	DM
PROT	53.53	57.51
VESEL	3.29	3.53
RF	0.00	0.00
NEm	0.83	0.89
NEg	0.57	0.61
Ca	10.67	11.47
STIM	6.62	7.11
VET	3.51	3.77
K	0.21	0.23
SOUT	4.53	4.86
P	0.21	0.23
PRYS	1449.72	1557.53

	AS IS	DM
PROT	10.40	14.79
VESEL	7.69	10.94
RF	6.40	9.11
NEm	1.37	1.95
NEg	0.94	1.34
Ca	0.52	0.73
STIM	0.27	0.38
VET	4.24	6.03
K	0.60	0.85
SOUT	0.18	0.26
P	0.41	0.58
PRYS	666.50	948.27
NEg Prys		0.71

Prys /ton AS IS
810.00
338.00
399.00
1781.00
1100.00
62720.00
45000.00
3200.00
45000.00
0.00
810.00
0.00
1449.72
790.00
0.00
300.00
815.00
0.00
0.00
0.00



CCC FEEDLOT

02/03/05

FINISHER HPK

	GRONDSTOF		% AS IS	DM	DM %
18	HOMINYCHOP	410.00	41.13	358.75	38.67
49	VOERKALK	300.00	30.10	291.00	31.36
46	SOUT	50.00	5.02	47.50	5.12
48	UREUM	150.00	15.05	148.50	16.01
1	AMMONIUMSULF	50.00	5.02	49.50	5.34
60	ROMENSIN	3.30	0.33	3.30	0.36
67	ZINMET	8.50	0.85	4.25	0.46
66	VIT-MIN PREMIX	25.00	2.51	25.00	2.69
58	OXYTET 20%	0.00	0.00	0.00	0.00
0	0.00	0.00	0.00	0.00	0.00
	TOTAAL	996.80		927.80	93.08

FINISHER RANTSOEN

	GRONDSTOF	AS IS	AS IS %	DM	DM %
18	HOMINYCHOP	800.00	31.37	700.00	38.60
0	0.00	0.00	0.00	0.00	0.00
69	FINISHER HPK	100.00	3.92	93.08	5.13
26	KORINGSEMELS	350.00	13.73	306.25	16.89
0	0.00	0.00	0.00	0.00	0.00
14	KUILVOER HQ	800.00	31.37	264.00	14.56
40	MIELIES	500.00	19.61	450.00	24.82
0	0.00	0.00	0.00	0.00	0.00
0	0.00	0.00	0.00	0.00	0.00
0	0.00	0.00	0.00	0.00	0.00
	TOTAAL	2550.00		1813.3	71.11

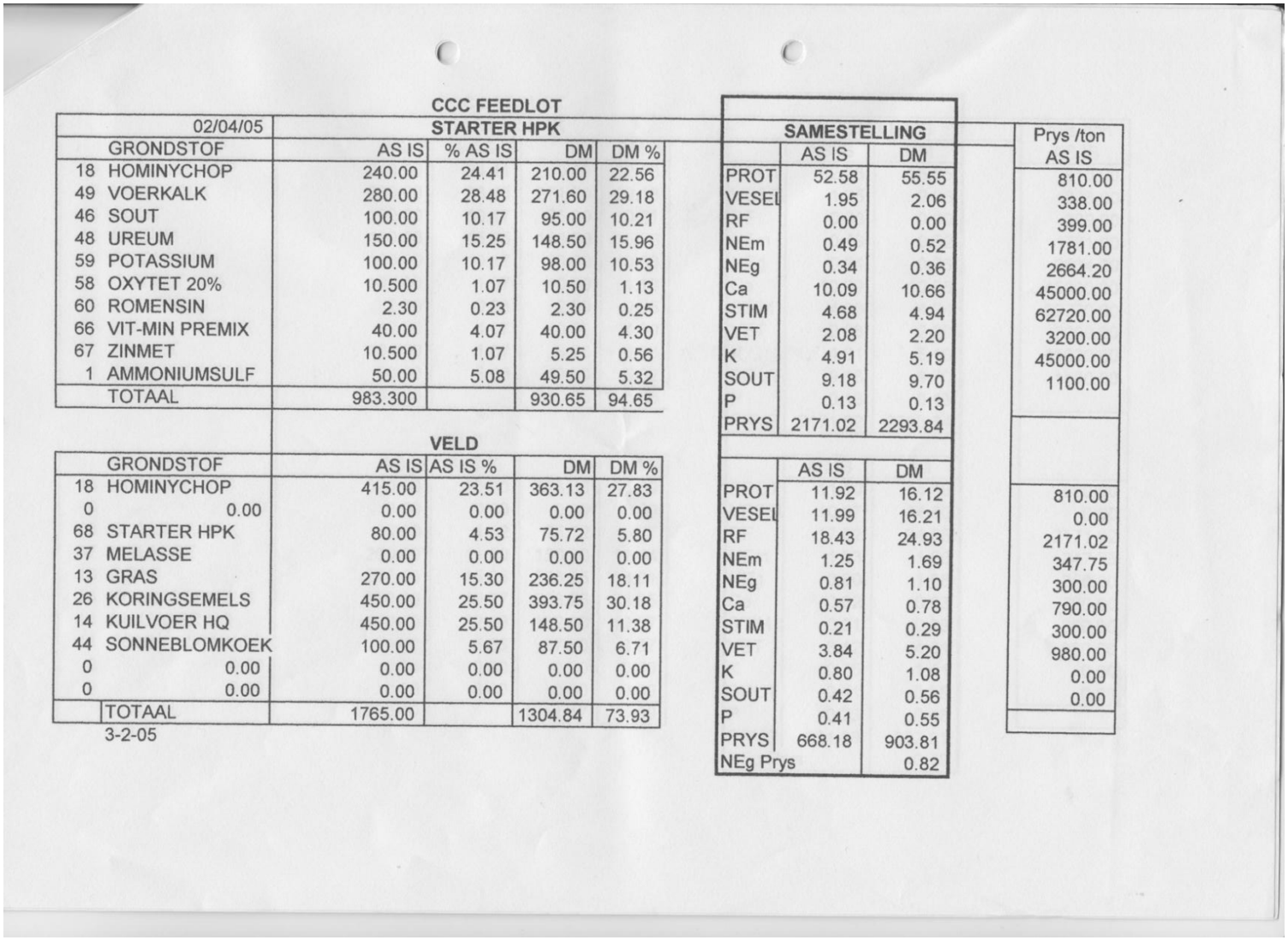
3-2-5

SAMESTELLING

	AS IS	DM
PROT	53.53	57.51
VESEL	3.29	3.53
RF	0.00	0.00
NEm	0.83	0.89
NEg	0.57	0.61
Ca	10.67	11.47
STIM	6.62	7.11
VET	3.51	3.77
K	0.21	0.23
SOUT	4.53	4.86
P	0.21	0.23
PRYS	1449.72	1557.53

	AS IS	DM
PROT	9.69	13.62
VESEL	6.89	9.69
RF	6.21	8.74
NEm	1.40	1.97
NEg	0.98	1.37
Ca	0.49	0.69
STIM	0.26	0.37
VET	4.32	6.07
K	0.52	0.73
SOUT	0.18	0.25
P	0.35	0.50
PRYS	673.32	946.86
NEg Prys		0.69

Prys /ton AS IS
810.00
338.00
399.00
1781.00
1100.00
62720.00
45000.00
3200.00
45000.00
0.00
810.00
0.00
1449.72
790.00
0.00
300.00
815.00
0.00
0.00
0.00



CCC FEEDLOT

02/04/05

STARTER HPK

	GRONDSTOF	AS IS	% AS IS	DM	DM %
18	HOMINYCHOP	240.00	24.41	210.00	22.56
49	VOERKALK	280.00	28.48	271.60	29.18
46	SOUT	100.00	10.17	95.00	10.21
48	UREUM	150.00	15.25	148.50	15.96
59	POTASSIUM	100.00	10.17	98.00	10.53
58	OXYTET 20%	10.500	1.07	10.50	1.13
60	ROMENSIN	2.30	0.23	2.30	0.25
66	VIT-MIN PREMIX	40.00	4.07	40.00	4.30
67	ZINMET	10.500	1.07	5.25	0.56
1	AMMONIUMSULF	50.00	5.08	49.50	5.32
	TOTAAL	983.300		930.65	94.65

VELD

	GRONDSTOF	AS IS	AS IS %	DM	DM %
18	HOMINYCHOP	415.00	23.51	363.13	27.83
0	0.00	0.00	0.00	0.00	0.00
68	STARTER HPK	80.00	4.53	75.72	5.80
37	MELASSE	0.00	0.00	0.00	0.00
13	GRAS	270.00	15.30	236.25	18.11
26	KORINGSEMELS	450.00	25.50	393.75	30.18
14	KUILVOER HQ	450.00	25.50	148.50	11.38
44	SONNEBLOMKOEK	100.00	5.67	87.50	6.71
0	0.00	0.00	0.00	0.00	0.00
0	0.00	0.00	0.00	0.00	0.00
	TOTAAL	1765.00		1304.84	73.93

3-2-05

SAMESTELLING

	AS IS	DM
PROT	52.58	55.55
VESEL	1.95	2.06
RF	0.00	0.00
NEm	0.49	0.52
NEg	0.34	0.36
Ca	10.09	10.66
STIM	4.68	4.94
VET	2.08	2.20
K	4.91	5.19
SOUT	9.18	9.70
P	0.13	0.13
PRYS	2171.02	2293.84

	AS IS	DM
PROT	11.92	16.12
VESEL	11.99	16.21
RF	18.43	24.93
NEm	1.25	1.69
NEg	0.81	1.10
Ca	0.57	0.78
STIM	0.21	0.29
VET	3.84	5.20
K	0.80	1.08
SOUT	0.42	0.56
P	0.41	0.55
PRYS	668.18	903.81
NEg Prys		0.82

Prys /ton AS IS
810.00
338.00
399.00
1781.00
2664.20
45000.00
62720.00
3200.00
45000.00
1100.00
810.00
0.00
2171.02
347.75
300.00
790.00
300.00
980.00
0.00
0.00

CCC FEEDLOT

02/03/05	STARTER HPK			
GRONDSTOF	AS IS	% AS IS	DM	DM %
18 HOMINYCHOP	240.00	24.41	210.00	22.56
49 VOERKALK	280.00	28.48	271.60	29.18
46 SOUT	100.00	10.17	95.00	10.21
48 UREUM	150.00	15.25	148.50	15.96
59 POTASSIUM	100.00	10.17	98.00	10.53
58 OXYTET 20%	10.500	1.07	10.50	1.13
60 ROMENSIN	2.30	0.23	2.30	0.25
66 VIT-MIN PREMIX	40.00	4.07	40.00	4.30
67 ZINMET	10.500	1.07	5.25	0.56
1 AMMONIUMSULF	50.00	5.08	49.50	5.32
TOTAAL	983.300		930.65	94.65

	SAMESTELLING		Prys /ton
	AS IS	DM	AS IS
PROT	52.58	55.55	810.00
VESEL	1.95	2.06	338.00
RF	0.00	0.00	399.00
NEm	0.49	0.52	1781.00
NEg	0.34	0.36	2664.20
Ca	10.09	10.66	45000.00
STIM	4.68	4.94	62720.00
VET	2.08	2.20	3200.00
K	4.91	5.19	45000.00
SOUT	9.18	9.70	1100.00
P	0.13	0.13	
PRYS	2171.02	2293.84	

VELD

GRONDSTOF	AS IS	AS IS %	DM	DM %
18 HOMINYCHOP	370.00	20.90	323.75	24.69
0 0.00	0.00	0.00	0.00	0.00
68 STARTER HPK	80.00	4.52	75.72	5.77
37 MELASSE	200.00	11.30	150.00	11.44
13 GRAS	270.00	15.25	236.25	18.01
26 KORINGSEMELS	350.00	19.77	306.25	23.35
14 KUILVOER HQ	400.00	22.60	132.00	10.07
44 SONNEBLOMKOEK	100.00	5.65	87.50	6.67
0 0.00	0.00	0.00	0.00	0.00
0 0.00	0.00	0.00	0.00	0.00
TOTAAL	1770.00		1311.47	74.09

	AS IS	DM	Prys /ton AS IS
PROT	11.10	14.98	810.00
VESEL	10.97	14.80	0.00
RF	17.82	24.05	2171.02
NEm	1.23	1.66	347.75
NEg	0.80	1.08	300.00
Ca	0.62	0.83	790.00
STIM	0.21	0.29	300.00
VET	3.42	4.61	980.00
K	0.75	1.01	0.00
SOUT	0.41	0.56	0.00
P	0.35	0.48	
PRYS	631.88	852.81	
NEg Prys		0.79	

3-2-05

Sample Data

Abbatoire information for each individual of the Nguni population:

Tag ID/ Individual ID (From jugular)	Sex: Oxen 1, Heifer 2	Age: A-1, AB-2, B-3	Genotype	BCS (0-5) 17.05.05	Initial live weight	Live weight	Weight gain- feedlot response	Grade/fatness	Conformation	Warm carcass mass	Cold carcass mass
N1	1	1	TT	4	417	436	19	3	3	243	237.5
N2	1	2	TT	4	388	401	13	3	4	250	244.4
N3	1	1	CT	5	416	449	33	3	4	259	253.2
N4	1	1	CT	4	386	411	25	2	3	243	237.5
N5	1	1	CT	4	382	405	23	3	4	229	223.8
N6	1	1	CC	4	393	435	42	2	4	258	252.2
N7	1	1	CT	4	482	505	23	2	4	307	300.1
N8	1	1	CT	4	371	397	26	3	3	224	219
N9	1	1	TT	5	380	405	26	5	3	238	232.6
N10	1	1	CT	5	388	419	25	3	3	245	239.5
N11	1	1	CT	4	370	399	29	2	3	228	222.9
N12	1	1	CT	5	424	461	37	4	4	290	283.5
N13	1	2	CT	5	456	490	34	2	4	298	291.3
N14	1	1	TT	5	398	433	35	2	4	261	255.1
N15	1	1	CT	5	433	445	12	4	3	258	252.2
N16	1	1	CT	5	511	525	14	3	3	312	305
N17	1	1	CT	5	407	419	12	4	3	257	251.2
N18	1	1	TT	4	392	407	15	3	3	244	238.5
N19	1	1	CT	5	405			4	4	238	232.6
N20	1	1	CT	5	361	373	12	4	4	227	221.9
N21	1	1	TT	5	358	383	25	3	4	222	217.0
N22	1	1	TT	5	331	361	30	3	3	212	207.2
N23	1	1	TT	5	333	358	25	3	4	224	219.0217
N24	1	1	TT	4	370	458	88	4	4	276	269.8
N25	1	1	TT	4	283	385	102	2	3	233	227.8
N26	1	1	CT	4	313	400	87	3	4	256	250.2
N27	1	1	TT	5	280	364	84	4	4	216	211.1
N28	1	1	CT	4	321	411	90	2	4	246	240.5
N29	1	1	TT	5	289	381	92	3	3	229	223.8
N30	1	1	TT	5	385	455	70	3	4	290	283.5
N31	1	1	TT	4	315	362	47	2	4	223	218
N32	1	1	TT	3	230	299	69	3	3	175	171.1
N33	1	1	TT	3	252	324	72	2	3	171	167.2
N34	1	1	TT	4	317	398	81	3	3	245	239.5
N35	1	1	TT	4	345	436	91	3	3	262	256.1
N36	1	1	TT	5	325	431	106	2	3	273	266.9
N37	1	1	TT	4	254	314	60	4	3	186	181.8
N38	1	1	CT	5	345	434	89	3	4	265	259

N39	1	1	CT	5	297	384	87	3	3	229	223.8
N40	1	1	CT	4	303	411	108	3	3	233	227.8
N41	1	2	CT	4	334	422	88	2	3	270	263.9
N42	1	1	CT	4	277	359	82	3	3	216	211.1
N43	1	1	CT	4	321	382	61	3	4	241	235.6
N45	1	1	CT	4	237	346	109	3	3	205	200.4
N46	1	1	CT	3	355	441	86	2	4	276	269.8
N47	1	1	CT	4	258	333	75	2	3	201	196.5
N48	1	1	CT	4	281	356	75	3	3	214	209.2
N49	1	1	CT	4	231	312	81	3	3	184	179.9
N50	1	1	CT	4	294	393	99	2	3	232	226.8
N51	1	1	CT	5	371	455	84	3	3	301	294.2
N52	1	3	TT	4	377	437	60	3	3	265	259
N53	1	1	TT	3	372	496	124	3	4	298	291.3
N54	1	1	TT	3	241	316	75	2	3	184	179.9
N55	1	1	TT	4	315	362	47	3	3	227	221.9
N56	1	1	CT	5	324	412	88	3	4	253	247.3
NH1	2	1	CT	3	252	425	173	3	3	260	254.2
NH2	2	1	TT	5	323	395	72	4	4	259	253.2
NH3	2	1	TT	4	342	460	118	4	4	288	281.5
NH4	2	1	CT	3	361	391	30	3	3	217	212.1
NH5	2	1	CT	4	337	406	69	3	3	241	235.6
NH6	2	1	CT	4	285	370	85	4	4	237	231.7
NH7	2	1	CT	4	305	336	31	4	3	188	183.8
NH8	2	1	CT	3	291	394	103	4	3	242	236.6
NH9	2	1	TT	3	295	402	107	4	3	252	246.3
NH10	2	1	CT	3	321	320	-1	3	3	193	188.7
NH11	2	1	CT	4	288	363	75	3	4	220	215
NH12	2	1	CT	4	313	428	115	3	3	265	259
NH13	2	1	TT	4	264	357	93	3	4	226	220.9
NH14	2	1	CT	3	340	350	10	2	3	214.0	209.2
NH15	2	1	TT	3	293	356	63	3	3	216	211.1

HYGRADE MEATS (PTY) LTD

SLAUGHTER SHEET

Rpt: R60201

Batch **520303** **Triple C Helfers Ngunis**
Creditor **CRA100** **CRAFCOR - TRIPLE C**

Slaughter Date 2005/05/20 Date 2005/05/23
Live Mass 5900 Page 1

Slaughter % 58.29 Ref #
Avg Cold Mass 229.3 Agent CHG
Quantity Batch 15 Date Received 2005/05/20

Serial #	Grade	Qty	Warm Kg	Cold Kg @2.3 %	EarTag Avg Kg	Sex	Conf %	Grade	Sick	Cut	Damage Right	Damage Left
303009	A2	1	214.0	209.2	NH14	H	3					
Sub Total A2		**1**	**214.0**	**209.2**	**209.2**			**6.7%**				
303001	A3	1	260.0	254.2	NH1	H	3					
303004	A3	1	220.0	215.0	NH11	H	4					
303005	A3	1	216.0	211.1	NH15	H	3					
303006	A3	1	193.0	188.7	NH10	H	3					
303007	A3	1	241.0	235.6	NH5	H	3					
303008	A3	1	265.0	259.0	NH12	H	3					
303011	A3	1	226.0	220.9	NH13	H	4				F1	
303012	A3	1	217.0	212.1	NH4	H	3					
Sub Total A3		**8**	**1,838.0**	**1,796.6**	**224.6**			**53.3%**				
303002	A4	1	242.0	236.6	NH8	H	3					
303003	A4	1	252.0	246.3	NH9	H	3					
303010	A4	1	237.0	231.7	NH6	H	4					
303013	A4	1	188.0	183.8	NH7	H	3					
303014	A4	1	259.0	253.2	NH2	H	4					
303015	A4	1	288.0	281.5	NH3	H	4					
Sub Total A4		**6**	**1,466.0**	**1,433.1**	**238.9**			**40.0%**				
BATCH TOTAL		**15**	**3,518.0**	**3,438.9**	**0.0**							

HYGRADE MEATS (PTY) LTD

SLAUGHTER SHEET

Rpt: R60201

Batch 520302 **Triple C Oxen Ngunis**
Creditor CRA100 **CRAFCOR - TRIPLE C**

Slaughter Date 2005/05/20 Date 2005/05/23
Live Mass 12960 Page 1

Slaughter % 58.49 Ref #
Avg Cold Mass 229.7 Agent CHG
Quantity Batch 33 Date Received 2005/05/20

Serial #	Grade	Qty	Warm Kg	Cold Kg @2.3 %	EarTag Avg Kg	Sex	Conf %	Grade	Sick	Cut	Damage Right		Damage Left	
302001	A2	1	171.0	167.2	N33	O	3				L1	F1	L1	F1
302002	A2	1	233.0	227.8	N25	O	3							
302006	A2	1	232.0	226.8	N50	O	3							
302007	A2	1	246.0	240.5	N28	O	4						L1	F1
302021	A2	1	184.0	179.9	N54	O	3					F1		
302022	A2	1	201.0	196.5	N47	O	3							
302029	A2	1	273.0	266.9	N36	O	3				L1	F1		
302032	A2	1	223.0	218.0	N31	O	4							
302033	A2	1	276.0	269.8	N46	O	4							
Sub Total A2		**9**	**2,039.0**	**1,993.4**	**221.5**			**27.3%**						
302003	A3	1	233.0	227.8	N40	O	3							
302004	A3	1	175.0	171.1	N32	O	3							
302005	A3	1	298.0	291.3	N53	O	4							
302008	A3	1	253.0	247.3	N56	O	4							
302009	A3	1	179.0	175.0	N44	O	3							
302010	A3	1	227.0	221.9	N55	O	3							
302011	A3	1	205.0	200.4	N45	O	3							
302012	A3	1	241.0	235.6	N43	O	4							
302013	A3	1	216.0	211.1	N42	O	3							
302014	A3	1	229.0	223.8	N29	O	3							
302015	A3	1	265.0	259.0	N38	O	4							
302016	A3	1	262.0	256.1	N35	O	3							
302017	A3	1	301.0	294.2	N51	O	3							
302018	A3	1	229.0	223.8	N39	O	3							
302019	A3	1	245.0	239.5	N34	O	3							
302026	A3	1	184.0	179.9	N49	O	3							
302028	A3	1	214.0	209.2	N48	O	3							
302030	A3	1	256.0	250.2	N26	O	4							
302031	A3	1	290.0	283.5	N30	O	4							
Sub Total A3		**19**	**4,502.0**	**4,400.7**	**231.6**			**57.6%**						
302020	A4	1	186.0	181.8	N37	O	3							
302023	A4	1	276.0	269.8	N24	O	4							
302024	A4	1	216.0	211.1	N27	O	4							
Sub Total A4		**3**	**678.0**	**662.7**	**220.9**			**9.1%**						
302027	AB2	1	270.0	263.9	N41	O	3					F1		
Sub Total AB2		**1**	**270.0**	**263.9**	**263.9**			**3.0%**						
302025	B3	1	265.0	259.0	N52	O	3					F1		
Sub Total B3		**1**	**265.0**	**259.0**	**259.0**			**3.0%**						
BATCH TOTAL		**33**	**7,754.0**	**7,579.7**	**0.0**									

Abbatoire information for each individual of the Hereford population:

Tag ID/ Individual ID (From jugular)	Sex: 1-Oxen, 2-Heifer	Age	Genotype	BCS	Initial Live Weight (Kg): 9-8-2004	Slaughter weight (Kg)	Weight gain-feedlot response	Fatness	Conformation	Warm carcass mass	Cold carcass mass
H1	1	1	TT	4	251	442	191				
H3	1	1	TT	3	212	402	190				
H4	1	1	CT	3	224	410	186				
H5	1	1	CT	3	211	400	189				
H7	1	1	TT	5	262	460	198				
H9	1	1	TT	5	248	446	198				
H10	1	1	CT	3	262	388	126				
H12	1	1	TT	2	183	368	185				
H13	1	1	CT	2	179	366	187				
H15	1	1	CT	4	204	354	150				
H17	1	1	TT	3	204	362	158				
H19	1	1	TT	4	196	432	236				
H21	1	1	CT	4	191	456	265				
H22	1	1	TT	4	227	410	183				
H26	1	1	TT	5	260	482	222				
H31	1	1	CT	4	236	458	222				
H34	1	1	CT	3	202	354	152				
H35	1	1	TT	4	226	434	208				
H36	1	1	CT	4	228	420	192				
H37	1	1	CT	3	159	336	177				
H41	1	1	TT	4	215	412	197				
H43	1	1	TT	4	184	398	214				
H44	1	1	TT	4	216	400	184				
H47	1	1	TT	5	209	456	247				
H48	1	1	TT	5	227	450	223				
H49	1	1	CT	3	182	364	182				
H2	O	1	CT	5	272	436	164	6	4	277	270.8
H6	O	1	CT	3	244	394	150	3	4	259	253.2
H14	O	1	TT	4	304	462	158	3	4	303	296.2
H16	O	1	CT	5	328	494	166	2	5	251	245.4
H18	O	1	CT	5	247	411	164	4	4	244	238.5
H20	O	1	TT	4	282	457	175	3	4	240	234.6
H24	O	1	CT	5	290	460	170	3	4	268	262
H25	O	1	TT	4	255	424	169	3	4	251	245.4
H29	O	1	CT	4	255	410	155	4	4	247	241.4
H30	O	1	CT	3	235	394	159	5	4	257	251.2
H33	O	1	TT	4	226	411	185	3	4	231	225.8
H38	O	1	CT	5	298	458	160	3	4	290	283.5
H40	O	1	TT	4	270	444	174	4	5	271	264.9
H45	O	1	TT	3	250	412	162	2	4	250	244.4
H51	O	1	CT	4	388						
H52	O	1	CT	4	433						
H53	O	1	TT	5	412						
H54	O	1	CT	4	416						
H55	O	1	CT	5	421						
H56	O	1	TT	5	389						
H42	O	1	TT	2	200						
H11	O	1	TT	2	197						

HYGRADE MEATS (PTY) LTD

SLAUGHTER SHEET

Rpt: R60201

Batch **442270** **Riversdale Oxen Tags**
Creditor **CRA200** **CRAFCOR - RIVERSDALE**

Slaughter Date 2004/10/15 Date 2004/11/08
Live Mass 3060 Page 1

Slaughter % 58.68 Ref #
Avg Cold Mass 256.5 Agent CHG
Quantity Batch 7 Date Received 2004/10/15

Serial #	Grade	Qty	Warm Kg	Cold Kg @2.3 %	EarTag Avg Kg	Sex	Conf %	Sick	Cut	Damage Right	Damage Left
270002	A2	1	251.0	245.4		O	5				
Sub Total A2		**1**	**251.0**	**245.4**	**245.4**		**14.3%**				
270003	A3	1	300.0	293.2		O	4				
270004	A3	1	268.0	262.0		O	4				
270007	A3	1	240.0	234.6		O	4				
Sub Total A3		**3**	**808.0**	**789.8**	**263.3**		**42.9%**				
270005	A4	1	244.0	238.5		O	4				
Sub Total A4		**1**	**244.0**	**238.5**	**238.5**		**14.3%**				
270006	A5	1	257.0	251.2		O	4				
Sub Total A5		**1**	**257.0**	**251.2**	**251.2**		**14.3%**				
270001	A6	1	277.0	270.8		O	4				
Sub Total A6		**1**	**277.0**	**270.8**	**270.8**		**14.3%**				
BATCH TOTAL		**7**	**1,837.0**	**1,795.7**	**0.0**						

HYGRADE MEATS (PTY) LTD

SLAUGHTER SHEET

Rpt: R60201

Batch **442271** **Riversdale Oxen Tags**
Creditor **CRA200** **CRAFCOR - RIVERSDALE**

Slaughter Date 2004/10/15 Date 2004/11/08
Live Mass 3800 Page 1

Slaughter % 60.51 Ref #
Avg Cold Mass 255.5 Agent CHG
Quantity Batch 9 Date Received 2004/10/15

Serial #	Grade	Qty	Warm Kg	Cold Kg @2.3 %	EarTag Avg Kg	Sex	Conf %	Sick Grade	Cut	Damage Right	Damage Left
271009	A2	1	250.0	244.4		O	4				
Sub Total A2		**1**	**250.0**	**244.4**	**244.4**			**11.1%**			
271001	A3	1	259.0	253.2		O	4				
271002	A3	1	290.0	283.5		O	4				
271003	A3	1	251.0	245.4		O	4				
271004	A3	1	231.0	225.8		O	4				
271005	A3	1	303.0	296.2		O	4				
271007	A3	1	250.0	244.4		O	4				
Sub Total A3		**6**	**1,584.0**	**1,548.5**	**258.1**			**66.7%**			
271006	A4	1	247.0	241.4		O	4				
271008	A4	1	271.0	264.9		O	5				
Sub Total A4		**2**	**518.0**	**506.3**	**253.1**			**22.2%**			
BATCH TOTAL		**9**	**2,352.0**	**2,299.2**	**0.0**						

APPENDIX C: STATISTICAL ANALYSIS

BLAST sequence results:

Blast 2 Sequences results

PubMed Entrez BLAST OMIM Taxonomy Structure

BLAST 2 SEQUENCES RESULTS VERSION BLASTN 2.2.10 [Oct-19-2004]

Match: 1 Mismatch: -2 gap open: 5 gap extension: 2

x_dropoff: 50 expect: 10.0 wordsize: 11 Filter Align

Sequence 1 lcl|Bos_taurus leptin (obese) gene, complete cds **Length** 101 (1 .. 101)

Sequence 2 lcl|H38 Lep-FW sequence exported from chromatogram file **Length** 72 (1 .. 72)

2

1

NOTE:The statistics (bitscore and expect value) is calculated based on the size of nr database

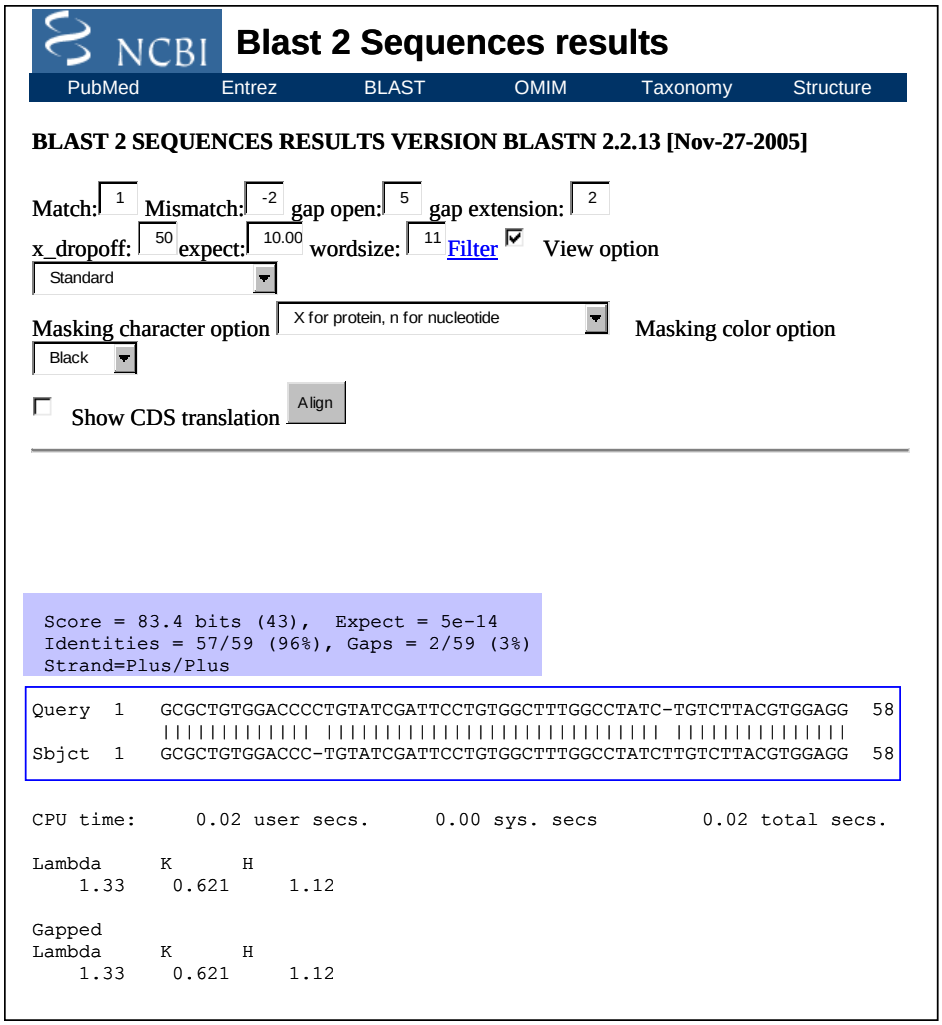
NOTE:If protein translation is reversed, please repeat the search with reverse strand of the query sequence

```
Score =  116 bits (60), Expect = 1e-23
Identities = 62/63 (98%)
 Strand = Plus / Plus
```

```
Query: 39  ggctttggccctatctgtcttacgtggaggctgtgcccatccgcaaggtccaggatgaca
98
           ||||||||||||||||||||||||||||||||||||||||||| ||||||||||||||||
Sbjct: 9   ggctttggccctatctgtcttacgtggaggctgtgcccatccggaaggtccaggatgaca
68


Query: 99  cca 101
           |||
Sbjct: 69  cca 71
CPU time:     0.01 user secs.      0.01 sys. secs         0.02 total
secs.
```

```
Lambda      K       H
```



NCBI **Blast 2 Sequences results**

PubMed Entrez BLAST OMIM Taxonomy Structure

BLAST 2 SEQUENCES RESULTS VERSION BLASTN 2.2.13 [Nov-27-2005]

Match: 1 Mismatch: -2 gap open: 5 gap extension: 2

x_dropoff: 50 expect: 10.00 wordsize: 11 Filter View option

Standard

Masking character option X for protein, n for nucleotide Masking color option

Black

Show CDS translation Align

```
 Score = 83.4 bits (43),  Expect = 5e-14
 Identities = 57/59 (96%), Gaps = 2/59 (3%)
 Strand=Plus/Plus

Query  1   GCGCTGTGGACCCCTGTATCGATTCCTGTGGCTTTGGCCTATC-TGTCTTACGTGGAGG  58
           ||||||||||||| ||||||||||||||||||||||||||||| ||||||||||||||
Sbjct  1   GCGCTGTGGACCC-TGTATCGATTCCTGTGGCTTTGGCCTATCTTGTCTTACGTGGAGG  58

CPU time:     0.02 user secs.      0.00 sys. secs         0.02 total secs.

Lambda      K       H
    1.33    0.621     1.12

Gapped
Lambda      K       H
    1.33    0.621     1.12
```

GLM SATISTICAL ANALYSES:

```
                          The SAS System        10:38 Monday, August 23, 2010   1

                        The GLM Procedure

                    Class Level Information

          Class         Levels    Values

          trt                2    1 2

          Breed              2    Hereford Nguni

          genotype           3    CC CT TT

          sex                2    1 2

          age                3    1 2 3

                 Data for Analysis of bcs
```

Number of Observations Read 118
Number of Observations Used 118

Data for Analysis of slw wg

Number of Observations Read 118
Number of Observations Used 109

Data for Analysis of fat cfn wcm ccm

Number of Observations Read 118
Number of Observations Used 84

NOTE: Variables in each group are consistent with respect to the presence or absence of missing values.

The GLM Procedure

Dependent Variable: bcs

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	13	23.43992119	1.80307086	3.65	<.0001
Error	104	51.34821440	0.49373283		
Corrected Total	117	74.78813559			

R-Square	Coeff Var	Root MSE	bcs Mean
0.313418	17.38240	0.702661	4.042373

Source	DF	Type I SS	Mean Square	F Value	Pr > F
ilw	1	17.08791556	17.08791556	34.61	<.0001
trt	1	0.31863875	0.31863875	0.65	0.4236
Breed	1	0.86765291	0.86765291	1.76	0.1879
genotype	2	0.80368171	0.40184086	0.81	0.4459
sex	1	2.34011658	2.34011658	4.74	0.0317
age	2	0.48241814	0.24120907	0.49	0.6149
Breed*genotype	1	0.21260376	0.21260376	0.43	0.5131
trt*Breed*genotype	1	0.23958873	0.23958873	0.49	0.4876
trt*Bre*geno*sex*age	3	1.08730505	0.36243502	0.73	0.5340

Source	DF	Type III SS	Mean Square	F Value	Pr > F
ilw	1	10.00162793	10.00162793	20.26	<.0001
trt	1	0.00715385	0.00715385	0.01	0.9044
Breed	1	0.00832854	0.00832854	0.02	0.8969
genotype	2	0.41202533	0.20601267	0.42	0.6600
sex	1	1.61136432	1.61136432	3.26	0.0737
age	2	0.51838569	0.25919285	0.52	0.5931
Breed*genotype	1	0.28357925	0.28357925	0.57	0.4502
trt*Breed*genotype	1	0.05405442	0.05405442	0.11	0.7414
trt*Bre*geno*sex*age	3	1.08730505	0.36243502	0.73	0.5340

The GLM Procedure
Least Squares Means

trt	bcs LSMEAN
1	Non-est
2	Non-est

Breed	bcs LSMEAN
Hereford	Non-est
Nguni	Non-est

genotype	bcs LSMEAN
CC	Non-est
CT	Non-est
TT	Non-est

sex	bcs LSMEAN
1	Non-est
2	Non-est

age	bcs LSMEAN
1	Non-est
2	Non-est
3	Non-est

The GLM Procedure

t Tests (LSD) for bcs

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	104
Error Mean Square	0.493733
Critical Value of t	1.98304
Least Significant Difference	0.3238
Harmonic Mean of Cell Sizes	37.0339

NOTE: Cell sizes are not equal.

Means with the same letter are not significantly different.

t Grouping	Mean	N	trt
A	4.6087	23	1
B	3.9053	95	2

The GLM Procedure

t Tests (LSD) for bcs

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	104
Error Mean Square	0.493733
Critical Value of t	1.98304
Least Significant Difference	0.2611
Harmonic Mean of Cell Sizes	56.94915

NOTE: Cell sizes are not equal.

Means with the same letter are not significantly different.

t Grouping	Mean	N	Breed
A	4.1571	70	Nguni
B	3.8750	48	Hereford

The GLM Procedure

t Tests (LSD) for bcs

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	104
Error Mean Square	0.493733
Critical Value of t	1.98304

Comparisons significant at the 0.05 level are indicated by ***.

genotype Comparison	Difference Between Means	95% Confidence Limits	
CT - TT	0.01058	-0.24782	0.26899
CT - CC	0.04762	-1.35680	1.45204
TT - CT	-0.01058	-0.26899	0.24782
TT - CC	0.03704	-1.36921	1.44328
CC - CT	-0.04762	-1.45204	1.35680
CC - TT	-0.03704	-1.44328	1.36921

The GLM Procedure

t Tests (LSD) for bcs

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	104
Error Mean Square	0.493733
Critical Value of t	1.98304
Least Significant Difference	0.3851
Harmonic Mean of Cell Sizes	26.18644

NOTE: Cell sizes are not equal.

Means with the same letter are not significantly different.

t Grouping	Mean	N	sex
A	4.1068	103	1
B	3.6000	15	2

The GLM Procedure

t Tests (LSD) for bcs

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	104
Error Mean Square	0.493733
Critical Value of t	1.98304

Comparisons significant at the 0.05 level are indicated by ***.

age Comparison	Difference Between Means	95% Confidence Limits	
2 - 1	0.29825	-0.51675	1.11324
2 - 3	0.33333	-1.27563	1.94230
1 - 2	-0.29825	-1.11324	0.51675
1 - 3	0.03509	-1.36441	1.43459
3 - 2	-0.33333	-1.94230	1.27563
3 - 1	-0.03509	-1.43459	1.36441

The GLM Procedure

Dependent Variable: slw

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	13	159765.2161	12289.6320	18.63	<.0001
Error	95	62653.4628	659.5101		
Corrected Total	108	222418.6789			

R-Square	Coeff Var	Root MSE	slw Mean
0.718308	6.323637	25.68093	406.1101

Source	DF	Type I SS	Mean Square	F Value	Pr > F
ilw	1	37916.1932	37916.1932	57.49	<.0001
trt	1	4026.8582	4026.8582	6.11	0.0153
Breed	1	111125.5145	111125.5145	168.50	<.0001
genotype	2	1523.9879	761.9939	1.16	0.3193
sex	1	322.6947	322.6947	0.49	0.4860
age	2	377.8472	188.9236	0.29	0.7516
Breed*genotype	1	2231.4586	2231.4586	3.38	0.0690
trt*Breed*genotype	1	270.8738	270.8738	0.41	0.5231
trt*Bre*geno*sex*age	3	1969.7881	656.5960	1.00	0.3984

Source	DF	Type III SS	Mean Square	F Value	Pr > F
ilw	1	112160.7545	112160.7545	170.07	<.0001
trt	1	8066.8926	8066.8926	12.23	0.0007
Breed	1	59371.4736	59371.4736	90.02	<.0001
genotype	2	494.6146	247.3073	0.37	0.6883
sex	1	169.2370	169.2370	0.26	0.6136
age	2	412.0563	206.0282	0.31	0.7324
Breed*genotype	1	2142.1655	2142.1655	3.25	0.0747
trt*Breed*genotype	1	766.0138	766.0138	1.16	0.2839
trt*Bre*geno*sex*age	3	1969.7881	656.5960	1.00	0.3984

The GLM Procedure

Dependent Variable: wg

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	13	432084.4245	33237.2634	50.41	<.0001
Error	95	62635.9975	659.3263		
Corrected Total	108	494720.4220			

R-Square	Coeff Var	Root MSE	wg Mean
0.873391	23.90529	25.67735	107.4128

Source	DF	Type I SS	Mean Square	F Value	Pr > F
ilw	1	310102.6129	310102.6129	470.33	<.0001
trt	1	4121.3551	4121.3551	6.25	0.0141
Breed	1	111173.2439	111173.2439	168.62	<.0001
genotype	2	1572.7911	786.3956	1.19	0.3079
sex	1	320.5742	320.5742	0.49	0.4873
age	2	386.2402	193.1201	0.29	0.7468
Breed*genotype	1	2187.3978	2187.3978	3.32	0.0717
trt*Breed*genotype	1	235.4716	235.4716	0.36	0.5515
trt*Bre*geno*sex*age	3	1984.7377	661.5792	1.00	0.3949

Source	DF	Type III SS	Mean Square	F Value	Pr > F
ilw	1	3466.76080	3466.76080	5.26	0.0241
trt	1	8135.33593	8135.33593	12.34	0.0007
Breed	1	59496.36992	59496.36992	90.24	<.0001
genotype	2	510.86896	255.43448	0.39	0.6799
sex	1	167.21910	167.21910	0.25	0.6157
age	2	417.16631	208.58315	0.32	0.7296
Breed*genotype	1	2107.10867	2107.10867	3.20	0.0770
trt*Breed*genotype	1	744.19682	744.19682	1.13	0.2907
trt*Bre*geno*sex*age	3	1984.73775	661.57925	1.00	0.3949

The GLM Procedure
Least Squares Means

trt	slw LSMEAN	wg LSMEAN
1	Non-est	Non-est
2	Non-est	Non-est

Breed	slw LSMEAN	wg LSMEAN
Hereford	Non-est	Non-est
Nguni	Non-est	Non-est

genotype	slw LSMEAN	wg LSMEAN
CC	Non-est	Non-est
CT	Non-est	Non-est
TT	Non-est	Non-est

sex	slw LSMEAN	wg LSMEAN
1	Non-est	Non-est
2	Non-est	Non-est

age	slw LSMEAN	wg LSMEAN
1	Non-est	Non-est
2	Non-est	Non-est
3	Non-est	Non-est

The GLM Procedure

t Tests (LSD) for slw

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	95
Error Mean Square	659.5101
Critical Value of t	1.98525
Least Significant Difference	12.167
Harmonic Mean of Cell Sizes	35.11927

NOTE: Cell sizes are not equal.

Means with the same letter are not significantly different.

t Grouping	Mean	N	trt
A	423.500	22	1
B	401.713	87	2

The GLM Procedure

t Tests (LSD) for wg

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	95
Error Mean Square	659.3263
Critical Value of t	1.98525
Least Significant Difference	12.165
Harmonic Mean of Cell Sizes	35.11927

NOTE: Cell sizes are not equal.

Means with the same letter are not significantly different.

t Grouping	Mean	N	trt
A	128.425	87	2
B	24.318	22	1

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The GLM Procedure

t Tests (LSD) for slw

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	95
Error Mean Square	659.5101
Critical Value of t	1.98525
Least Significant Difference	10.132
Harmonic Mean of Cell Sizes	50.6422

NOTE: Cell sizes are not equal.

Means with the same letter are not significantly different.

t Grouping	Mean	N	Breed
A	418.175	40	Hereford
B	399.116	69	Nguni

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The GLM Procedure

t Tests (LSD) for wg

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	95
Error Mean Square	659.3263
Critical Value of t	1.98525
Least Significant Difference	10.13
Harmonic Mean of Cell Sizes	50.6422

NOTE: Cell sizes are not equal.

Means with the same letter are not significantly different.

t Grouping	Mean	N	Breed
A	184.325	40	Hereford
B	62.826	69	Nguni

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The GLM Procedure

t Tests (LSD) for slw

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	95
Error Mean Square	659.5101
Critical Value of t	1.98525

Comparisons significant at the 0.05 level are indicated by ***.

genotype Comparison	Difference Between Means	95% Confidence Limits	
CC - TT	28.280	-23.210	79.770
CC - CT	29.914	-21.507	81.335
TT - CC	-28.280	-79.770	23.210
TT - CT	1.634	-8.205	11.473
CT - CC	-29.914	-81.335	21.507
CT - TT	-1.634	-11.473	8.205

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The GLM Procedure

t Tests (LSD) for wg

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	95
Error Mean Square	659.3263
Critical Value of t	1.98525

Comparisons significant at the 0.05 level are indicated by ***.

genotype Comparison	Difference Between Means	95% Confidence Limits		
TT - CT	21.044	11.207	30.881	***
TT - CC	77.320	25.837	128.803	***
CT - TT	-21.044	-30.881	-11.207	***
CT - CC	56.276	4.862	107.689	***
CC - TT	-77.320	-128.803	-25.837	***
CC - CT	-56.276	-107.689	-4.862	***

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The GLM Procedure

t Tests (LSD) for slw

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	95

```
                    Error Mean Square            659.5101
                    Critical Value of t           1.98525
                    Least Significant Difference   14.175
                    Harmonic Mean of Cell Sizes  25.87156

                       NOTE: Cell sizes are not equal.


                Means with the same letter are not significantly different.

                      t Grouping          Mean      N    sex

                               A       409.713     94    1

                               B       383.533     15    2

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                                     The GLM Procedure

                                    t Tests (LSD) for wg

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

                    Alpha                           0.05
                    Error Degrees of Freedom          95
                    Error Mean Square            659.3263
                    Critical Value of t           1.98525
                    Least Significant Difference   14.173
                    Harmonic Mean of Cell Sizes  25.87156

                       NOTE: Cell sizes are not equal.


                Means with the same letter are not significantly different.

                      t Grouping          Mean      N    sex

                               A       112.394     94    1

                               B        76.200     15    2

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                                     The GLM Procedure

                                    t Tests (LSD) for slw

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

                    Alpha                        0.05
                    Error Degrees of Freedom       95
                    Error Mean Square        659.5101
                    Critical Value of t       1.98525


                Comparisons significant at the 0.05 level are indicated by ***.

                                    Difference
                          age          Between      95% Confidence
                       Comparison        Means          Limits

                         2 - 3           0.667     -58.204   59.537
                         2 - 1          32.752       2.900   62.605  ***
```

3 - 2	-0.667	-59.537	58.204	
3 - 1	32.086	-19.140	83.311	
1 - 2	-32.752	-62.605	-2.900	***
1 - 3	-32.086	-83.311	19.140	

The GLM Procedure

t Tests (LSD) for wg

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	95
Error Mean Square	659.3263
Critical Value of t	1.98525

Comparisons significant at the 0.05 level are indicated by ***.

age Comparison	Difference Between Means	95% Confidence Limits		
1 - 3	49.648	-1.571	100.866	
1 - 2	64.648	34.799	94.496	***
3 - 1	-49.648	-100.866	1.571	
3 - 2	15.000	-43.862	73.862	
2 - 1	-64.648	-94.496	-34.799	***
2 - 3	-15.000	-73.862	43.862	

The GLM Procedure

Dependent Variable: fat

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	13	11.35554254	0.87350327	1.48	0.1475
Error	70	41.34683841	0.59066912		
Corrected Total	83	52.70238095			

R-Square	Coeff Var	Root MSE	fat Mean
0.215465	25.11992	0.768550	3.059524

Source	DF	Type I SS	Mean Square	F Value	Pr > F
ilw	1	0.55208075	0.55208075	0.93	0.3370
trt	1	0.46024872	0.46024872	0.78	0.3804
Breed	1	1.65407713	1.65407713	2.80	0.0987
genotype	2	1.18318763	0.59159381	1.00	0.3725
sex	1	3.10282378	3.10282378	5.25	0.0249
age	2	1.33055884	0.66527942	1.13	0.3300
Breed*genotype	1	2.38626459	2.38626459	4.04	0.0483
trt*Breed*genotype	1	0.11098080	0.11098080	0.19	0.6660
trt*Bre*geno*sex*age	3	0.57532031	0.19177344	0.32	0.8075

Source	DF	Type III SS	Mean Square	F Value	Pr > F

ilw	1	0.16927881	0.29	0.5941
trt	1	0.66946388	1.13	0.2907
Breed	1	1.80743520	3.06	0.0846
genotype	2	1.26728316	1.07	0.3476
sex	1	2.53944606	4.30	0.0418
age	2	1.05537941	0.52768971	0.89	0.4139
Breed*genotype	1	2.62959608	2.62959608	4.45	0.0384
trt*Breed*genotype	1	0.03559256	0.03559256	0.06	0.8068
trt*Bre*geno*sex*age	3	0.57532031	0.19177344	0.32	0.8075

The GLM Procedure

Dependent Variable: cfn

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	13	9.86229187	0.75863784	3.52	0.0003
Error	70	15.09008908	0.21557270		
Corrected Total	83	24.95238095			

R-Square	Coeff Var	Root MSE	cfn Mean
0.395245	13.17603	0.464298	3.523810

Source	DF	Type I SS	Mean Square	F Value	Pr > F
ilw	1	0.00148041	0.00148041	0.01	0.9342
trt	1	0.00351892	0.00351892	0.02	0.8987
Breed	1	7.96107339	7.96107339	36.93	<.0001
genotype	2	0.38012463	0.19006231	0.88	0.4186
sex	1	0.00000371	0.00000371	0.00	0.9967
age	2	0.44866188	0.22433094	1.04	0.3586
Breed*genotype	1	0.00546181	0.00546181	0.03	0.8740
trt*Breed*genotype	1	0.00133377	0.00133377	0.01	0.9375
trt*Bre*geno*sex*age	3	1.06063335	0.35354445	1.64	0.1880

Source	DF	Type III SS	Mean Square	F Value	Pr > F
ilw	1	1.29663253	1.29663253	6.01	0.0167
trt	1	0.02334797	0.02334797	0.11	0.7431
Breed	1	6.98116341	6.98116341	32.38	<.0001
genotype	2	0.44828945	0.22414473	1.04	0.3589
sex	1	0.21154323	0.21154323	0.98	0.3253
age	2	0.41831768	0.20915884	0.97	0.3840
Breed*genotype	1	0.07041725	0.07041725	0.33	0.5695
trt*Breed*genotype	1	0.03494540	0.03494540	0.16	0.6885
trt*Bre*geno*sex*age	3	1.06063335	0.35354445	1.64	0.1880

The GLM Procedure

Dependent Variable: wcm

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	13	55060.69993	4235.43846	11.90	<.0001
Error	70	24924.19293	356.05990		

Corrected Total	83	79984.89286			

R-Square	Coeff Var	Root MSE	wcm Mean
0.688389	7.732290	18.86955	244.0357

Source	DF	Type I SS	Mean Square	F Value	Pr > F
ilw	1	20740.59020	20740.59020	58.25	<.0001
trt	1	10309.04453	10309.04453	28.95	<.0001
Breed	1	20869.34979	20869.34979	58.61	<.0001
genotype	2	176.85506	88.42753	0.25	0.7808
sex	1	56.54111	56.54111	0.16	0.6915
age	2	714.74913	357.37457	1.00	0.3717
Breed*genotype	1	16.11053	16.11053	0.05	0.8322
trt*Breed*genotype	1	13.37059	13.37059	0.04	0.8469
trt*Bre*geno*sex*age	3	2164.08900	721.36300	2.03	0.1181

Source	DF	Type III SS	Mean Square	F Value	Pr > F
ilw	1	39492.59819	39492.59819	110.92	<.0001
trt	1	6689.08968	6689.08968	18.79	<.0001
Breed	1	12344.94295	12344.94295	34.67	<.0001
genotype	2	202.11522	101.05761	0.28	0.7538
sex	1	1.34827	1.34827	0.00	0.9511
age	2	933.06160	466.53080	1.31	0.2763
Breed*genotype	1	11.39597	11.39597	0.03	0.8585
trt*Breed*genotype	1	127.49160	127.49160	0.36	0.5515
trt*Bre*geno*sex*age	3	2164.08900	721.36300	2.03	0.1181

The GLM Procedure

Dependent Variable: ccm

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	13	52600.27596	4046.17507	11.89	<.0001
Error	70	23815.93392	340.22763		
Corrected Total	83	76416.20988			

R-Square	Coeff Var	Root MSE	ccm Mean
0.688339	7.732279	18.44526	238.5488

Source	DF	Type I SS	Mean Square	F Value	Pr > F
ilw	1	19805.96687	19805.96687	58.21	<.0001
trt	1	9850.77467	9850.77467	28.95	<.0001
Breed	1	19946.56157	19946.56157	58.63	<.0001
genotype	2	168.84796	84.42398	0.25	0.7809
sex	1	54.08905	54.08905	0.16	0.6913
age	2	684.17104	342.08552	1.01	0.3711
Breed*genotype	1	15.30100	15.30100	0.04	0.8327
trt*Breed*genotype	1	12.98123	12.98123	0.04	0.8457
trt*Bre*geno*sex*age	3	2061.58256	687.19419	2.02	0.1190

Source	DF	Type III SS	Mean Square	F Value	Pr > F

ilw	1	37727.76415	37727.76415	110.89	<.0001
trt	1	6387.73923	6387.73923	18.77	<.0001
Breed	1	11803.63154	11803.63154	34.69	<.0001
genotype	2	193.16717	96.58359	0.28	0.7537
sex	1	1.27107	1.27107	0.00	0.9514
age	2	892.77286	446.38643	1.31	0.2758
Breed*genotype	1	10.86367	10.86367	0.03	0.8587
trt*Breed*genotype	1	121.91288	121.91288	0.36	0.5514
trt*Bre*geno*sex*age	3	2061.58256	687.19419	2.02	0.1190

The GLM Procedure
Least Squares Means

trt	fat LSMEAN	cfn LSMEAN	wcm LSMEAN	ccm LSMEAN
1	Non-est	Non-est	Non-est	Non-est
2	Non-est	Non-est	Non-est	Non-est

Breed	fat LSMEAN	cfn LSMEAN	wcm LSMEAN	ccm LSMEAN
Hereford	Non-est	Non-est	Non-est	Non-est
Nguni	Non-est	Non-est	Non-est	Non-est

genotype	fat LSMEAN	cfn LSMEAN	wcm LSMEAN	ccm LSMEAN
CC	Non-est	Non-est	Non-est	Non-est
CT	Non-est	Non-est	Non-est	Non-est
TT	Non-est	Non-est	Non-est	Non-est

sex	fat LSMEAN	cfn LSMEAN	wcm LSMEAN	ccm LSMEAN
1	Non-est	Non-est	Non-est	Non-est
2	Non-est	Non-est	Non-est	Non-est

age	fat LSMEAN	cfn LSMEAN	wcm LSMEAN	ccm LSMEAN
1	Non-est	Non-est	Non-est	Non-est
2	Non-est	Non-est	Non-est	Non-est
3	Non-est	Non-est	Non-est	Non-est

The GLM Procedure

t Tests (LSD) for fat

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	70
Error Mean Square	0.590669
Critical Value of t	1.99444
Least Significant Difference	0.3751
Harmonic Mean of Cell Sizes	33.40476

NOTE: Cell sizes are not equal.

Means with the same letter are not significantly different.

t Grouping	Mean	N	trt
A	3.0656	61	2
A			
A	3.0435	23	1

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The GLM Procedure

t Tests (LSD) for cfn

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	70
Error Mean Square	0.215573
Critical Value of t	1.99444
Least Significant Difference	0.2266
Harmonic Mean of Cell Sizes	33.40476

NOTE: Cell sizes are not equal.

Means with the same letter are not significantly different.

t Grouping	Mean	N	trt
A	3.5246	61	2
A			
A	3.5217	23	1

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The GLM Procedure

t Tests (LSD) for wcm

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	70
Error Mean Square	356.0599
Critical Value of t	1.99444
Least Significant Difference	9.2086
Harmonic Mean of Cell Sizes	33.40476

NOTE: Cell sizes are not equal.

Means with the same letter are not significantly different.

t Grouping	Mean	N	trt
A	250.739	23	1
B	241.508	61	2

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The GLM Procedure

t Tests (LSD) for ccm

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	70
Error Mean Square	340.2276
Critical Value of t	1.99444
Least Significant Difference	9.0015
Harmonic Mean of Cell Sizes	33.40476

NOTE: Cell sizes are not equal.

Means with the same letter are not significantly different.

t Grouping	Mean	N	trt
A	245.096	23	1
B	236.080	61	2

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The GLM Procedure

t Tests (LSD) for fat

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	70
Error Mean Square	0.590669
Critical Value of t	1.99444
Least Significant Difference	0.4488
Harmonic Mean of Cell Sizes	23.33333

NOTE: Cell sizes are not equal.

Means with the same letter are not significantly different.

t Grouping	Mean	N	Breed
A	3.4286	14	Hereford
A			
A	2.9857	70	Nguni

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The GLM Procedure

t Tests (LSD) for cfn

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	70
Error Mean Square	0.215573
Critical Value of t	1.99444
Least Significant Difference	0.2711
Harmonic Mean of Cell Sizes	23.33333

NOTE: Cell sizes are not equal.

Means with the same letter are not significantly different.

t Grouping	Mean	N	Breed
A	4.1429	14	Hereford
B	3.4000	70	Nguni

The GLM Procedure

t Tests (LSD) for wcm

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	70
Error Mean Square	356.0599
Critical Value of t	1.99444
Least Significant Difference	11.018
Harmonic Mean of Cell Sizes	23.33333

NOTE: Cell sizes are not equal.

Means with the same letter are not significantly different.

t Grouping	Mean	N	Breed
A	259.929	14	Hereford
B	240.857	70	Nguni

The GLM Procedure

t Tests (LSD) for ccm

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	70
Error Mean Square	340.2276
Critical Value of t	1.99444
Least Significant Difference	10.77
Harmonic Mean of Cell Sizes	23.33333

NOTE: Cell sizes are not equal.

Means with the same letter are not significantly different.

t Grouping	Mean	N	Breed
A	254.093	14	Hereford
B	235.440	70	Nguni

The GLM Procedure

t Tests (LSD) for fat

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	70
Error Mean Square	0.590669
Critical Value of t	1.99444

Comparisons significant at the 0.05 level are indicated by ***.

genotype Comparison	Difference Between Means	95% Confidence Limits	
CT - TT	0.0262	-0.3145	0.3669
CT - CC	1.0833	-0.4654	2.6320
TT - CT	-0.0262	-0.3669	0.3145
TT - CC	1.0571	-0.4974	2.6117
CC - CT	-1.0833	-2.6320	0.4654
CC - TT	-1.0571	-2.6117	0.4974

The GLM Procedure

t Tests (LSD) for cfn

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	70
Error Mean Square	0.215573
Critical Value of t	1.99444

Comparisons significant at the 0.05 level are indicated by ***.

genotype Comparison	Difference Between Means	95% Confidence Limits	
CC - TT	0.45714	-0.48201	1.39629
CC - CT	0.50000	-0.43561	1.43561
TT - CC	-0.45714	-1.39629	0.48201
TT - CT	0.04286	-0.16297	0.24868
CT - CC	-0.50000	-1.43561	0.43561
CT - TT	-0.04286	-0.24868	0.16297

The GLM Procedure

t Tests (LSD) for wcm

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	70
Error Mean Square	356.0599
Critical Value of t	1.99444

Comparisons significant at the 0.05 level are indicated by ***.

genotype Comparison	Difference Between Means	95% Confidence Limits	
CC - CT	12.021	-26.003	50.045
CC - TT	17.029	-21.139	55.197
CT - CC	-12.021	-50.045	26.003
CT - TT	5.008	-3.357	13.373
TT - CC	-17.029	-55.197	21.139
TT - CT	-5.008	-13.373	3.357

The GLM Procedure

t Tests (LSD) for ccm

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	70
Error Mean Square	340.2276
Critical Value of t	1.99444

Comparisons significant at the 0.05 level are indicated by ***.

genotype Comparison	Difference Between Means	95% Confidence Limits	
CC - CT	11.750	-25.419	48.919
CC - TT	16.649	-20.661	53.958
CT - CC	-11.750	-48.919	25.419
CT - TT	4.899	-3.278	13.075
TT - CC	-16.649	-53.958	20.661
TT - CT	-4.899	-13.075	3.278

The GLM Procedure

t Tests (LSD) for fat

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	70
Error Mean Square	0.590669
Critical Value of t	1.99444
Least Significant Difference	0.4367
Harmonic Mean of Cell Sizes	24.64286

NOTE: Cell sizes are not equal.

Means with the same letter are not significantly different.

t Grouping	Mean	N	sex
A	3.3333	15	2
A			
A	3.0000	69	1

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The GLM Procedure

t Tests (LSD) for cfn

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	70
Error Mean Square	0.215573
Critical Value of t	1.99444
Least Significant Difference	0.2638
Harmonic Mean of Cell Sizes	24.64286

NOTE: Cell sizes are not equal.

Means with the same letter are not significantly different.

t Grouping	Mean	N	sex
A	3.5652	69	1
A			
A	3.3333	15	2

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The GLM Procedure

t Tests (LSD) for wcm

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	70
Error Mean Square	356.0599
Critical Value of t	1.99444
Least Significant Difference	10.721
Harmonic Mean of Cell Sizes	24.64286

NOTE: Cell sizes are not equal.

Means with the same letter are not significantly different.

t Grouping	Mean	N	sex
A	246.101	69	1
B	234.533	15	2

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The GLM Procedure

t Tests (LSD) for ccm

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	70
Error Mean Square	340.2276

Critical Value of t 1.99444
Least Significant Difference 10.48
Harmonic Mean of Cell Sizes 24.64286

NOTE: Cell sizes are not equal.

Means with the same letter are not significantly different.

t Grouping	Mean	N	sex
A	240.568	69	1
B	229.260	15	2

The GLM Procedure

t Tests (LSD) for fat

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha 0.05
Error Degrees of Freedom 70
Error Mean Square 0.590669
Critical Value of t 1.99444

Comparisons significant at the 0.05 level are indicated by ***.

age Comparison	Difference Between Means	95% Confidence Limits	
1 - 3	0.0875	-1.4549	1.6299
1 - 2	0.7542	-0.1473	1.6556
3 - 1	-0.0875	-1.6299	1.4549
3 - 2	0.6667	-1.1033	2.4366
2 - 1	-0.7542	-1.6556	0.1473
2 - 3	-0.6667	-2.4366	1.1033

The GLM Procedure

t Tests (LSD) for cfn

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha 0.05
Error Degrees of Freedom 70
Error Mean Square 0.215573
Critical Value of t 1.99444

Comparisons significant at the 0.05 level are indicated by ***.

age Comparison	Difference Between Means	95% Confidence Limits	
2 - 1	0.14167	-0.40290	0.68623
2 - 3	0.66667	-0.40260	1.73593

1 - 2	-0.14167	-0.68623	0.40290
1 - 3	0.52500	-0.40678	1.45678
3 - 2	-0.66667	-1.73593	0.40260
3 - 1	-0.52500	-1.45678	0.40678

The GLM Procedure

t Tests (LSD) for wcm

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	70
Error Mean Square	356.0599
Critical Value of t	1.99444

Comparisons significant at the 0.05 level are indicated by ***.

age Comparison	Difference Between Means	95% Confidence Limits		
2 - 3	7.667	-35.789	51.123	
2 - 1	29.967	7.835	52.098	***
3 - 2	-7.667	-51.123	35.789	
3 - 1	22.300	-15.569	60.169	
1 - 2	-29.967	-52.098	-7.835	***
1 - 3	-22.300	-60.169	15.569	

The GLM Procedure

t Tests (LSD) for ccm

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	70
Error Mean Square	340.2276
Critical Value of t	1.99444

Comparisons significant at the 0.05 level are indicated by ***.

age Comparison	Difference Between Means	95% Confidence Limits		
2 - 3	7.533	-34.946	50.012	
2 - 1	29.290	7.655	50.924	***
3 - 2	-7.533	-50.012	34.946	
3 - 1	21.756	-15.261	58.773	
1 - 2	-29.290	-50.924	-7.655	***
1 - 3	-21.756	-58.773	15.261	

The GLM Procedure

Class Level Information

Class	Levels	Values
trt	2	1 2
Breed	2	Hereford Nguni
genotype	3	CC CT TT
sex	2	1 2
age	3	1 2 3

Number of Observations Read 118
Number of Observations Used 118

The GLM Procedure

Dependent Variable: ilw

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	12	348646.4133	29053.8678	9.54	<.0001
Error	105	319705.0782	3044.8103		
Corrected Total	117	668351.4915			

R-Square	Coeff Var	Root MSE	ilw Mean
0.521651	18.18065	55.17980	303.5085

Source	DF	Type I SS	Mean Square	F Value	Pr > F
trt	1	261692.3574	261692.3574	85.95	<.0001
Breed	1	65394.0136	65394.0136	21.48	<.0001
genotype	2	6493.2365	3246.6183	1.07	0.3480
sex	1	33.0984	33.0984	0.01	0.9172
age	2	8144.4364	4072.2182	1.34	0.2670
Breed*genotype	1	499.3581	499.3581	0.16	0.6863
trt*Breed*genotype	1	5794.8087	5794.8087	1.90	0.1707
trt*Bre*geno*sex*age	3	595.1042	198.3681	0.07	0.9782

Source	DF	Type III SS	Mean Square	F Value	Pr > F
trt	1	33453.59636	33453.59636	10.99	0.0013
Breed	1	23434.55230	23434.55230	7.70	0.0065
genotype	2	7717.60423	3858.80212	1.27	0.2859
sex	1	65.17789	65.17789	0.02	0.8840
age	2	7672.78825	3836.39413	1.26	0.2879
Breed*genotype	1	118.86980	118.86980	0.04	0.8438
trt*Breed*genotype	1	3775.11950	3775.11950	1.24	0.2680
trt*Bre*geno*sex*age	3	595.10419	198.36806	0.07	0.9782

The GLM Procedure
Least Squares Means

trt	ilw LSMEAN
1	Non-est
2	Non-est

Breed	ilw LSMEAN
Hereford	Non-est
Nguni	Non-est

genotype	ilw LSMEAN
CC	Non-est
CT	Non-est
TT	Non-est

sex	ilw LSMEAN
1	Non-est
2	Non-est

age	ilw LSMEAN
1	Non-est
2	Non-est
3	Non-est

The GLM Procedure

t Tests (LSD) for ilw

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	105
Error Mean Square	3044.81
Critical Value of t	1.98282
Least Significant Difference	25.426
Harmonic Mean of Cell Sizes	37.0339

NOTE: Cell sizes are not equal.

Means with the same letter are not significantly different.

t Grouping	Mean	N	trt
A	399.22	23	1
B	280.34	95	2

The GLM Procedure

t Tests (LSD) for ilw

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	105
Error Mean Square	3044.81
Critical Value of t	1.98282
Least Significant Difference	20.504

Harmonic Mean of Cell Sizes 56.94915

NOTE: Cell sizes are not equal.

Means with the same letter are not significantly different.

t Grouping	Mean	N	Breed
A	337.20	70	Nguni
B	254.38	48	Hereford

The GLM Procedure

t Tests (LSD) for ilw

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	105
Error Mean Square	3044.81
Critical Value of t	1.98282

Comparisons significant at the 0.05 level are indicated by ***.

genotype Comparison	Difference Between Means	95% Confidence Limits		
CC - CT	77.889	-32.387	188.165	
CC - TT	104.685	-5.735	215.105	
CT - CC	-77.889	-188.165	32.387	
CT - TT	26.796	6.506	47.087	***
TT - CC	-104.685	-215.105	5.735	
TT - CT	-26.796	-47.087	-6.506	***

The GLM Procedure

t Tests (LSD) for ilw

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	105
Error Mean Square	3044.81
Critical Value of t	1.98282
Least Significant Difference	30.237
Harmonic Mean of Cell Sizes	26.18644

NOTE: Cell sizes are not equal.

Means with the same letter are not significantly different.

t Grouping	Mean	N	sex
A	307.33	15	2
A			
A	302.95	103	1

The GLM Procedure

t Tests (LSD) for ilw

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	105
Error Mean Square	3044.81
Critical Value of t	1.98282

Comparisons significant at the 0.05 level are indicated by ***.

age Comparison	Difference Between Means	95% Confidence Limits		
2 - 3	15.667	-110.671	142.004	
2 - 1	92.149	28.155	156.144	***
3 - 2	-15.667	-142.004	110.671	
3 - 1	76.482	-33.408	186.373	
1 - 2	-92.149	-156.144	-28.155	***
1 - 3	-76.482	-186.373	33.408	