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LIST OF ABBREVIATIONS

ABI	Applied Biosystems
AFABP	Adipocyte fatty acid-binding protein.
AI	Artificial insemination
AMSA	American Meat Science Association
A^{pat}	A allele, paternally inherited
ARC	Agricultural Research Council
ARC-API	Agricultural Research Council – Animal Production Institute
AUCC	Animal Use and Care Committee
BF	backfat
BFAP	Bureau for Food and Agricultural Policy
BFT	Backfat Thickness
BLUP	Best linear unbiased prediction
C10:0	Capric
C12:0	Lauric
C14:0	Myristic
C15:0	Pentadecylic
C16:0	Palmitic
C16:1c9	Palmitoleic
C17:0	Margaric
C17:1c10	Heptadecenoic
C18:0	Stearic acid
C18:1t9	Elaidic
C18:1c9	Oleic
C18:1c7	Vaccenic
C18:2c9,12 (n-6)	Linoleic
C20:0	Arachidic
C18:3c6,9,12 (n-3)	γ-Linolenic
C20:1c11	Eicosenoic
C18:3c9,12,15 (n-3)	α-Linolenic
C20:2c11,14 (n-6)	Eicosadienoic
C20:3c8,11,14 (n-6)	Eicosatrienoic
C20:3c11,14,17 (n-3)	Eicosatrienoic
C20:4c5,8,11,14 (n-6)	Arachidonic
C20:5c5,8,11,14,17 (n-3)	Eicosopentaenoic
C22:5c7,10,13,16,19 (n-3)	Docosapentaenoic
C22:6c4,7,10,13,16,19 (n-3)	Docosahexanoic
CPLG	Callipyge
°C	degree Celsius
DAFF	Department of Agriculture, Forestry and Fisheries
DBI	Double bond index
DFD	Dark, firm and dry
DNA	Deoxyribonucleic acid
dsDNA BR	Double-stranded DNA Broad range
EBV	Estimated breeding value
EFC	Extractable fat content
FA	Fatty acid
FABP	Fatty acid-binding protein
FAME	Fatty acid methyl esters
FFDM	Fat free dry matter

GC	Gas chromatographic
GDP	Gross domestic product
G^{pat}	G allele, paternally inherited
HAL	Halothane
HFABP	Heart fatty acid-binding protein
HGP	Hennessy Grading Probe
IMF	Intramuscular fat
IGF2	Insulin-like growth factor 2
IV	Iodine value
LD	*Longissimus dorsi*
LSD	Least Significant Difference
LW	South African Large White
M	Molar
MC4R	Melanocortin-4 receptor
MFL	Myofibrillar fragment length
Mg	Milligram
mg/mL	Milligram per microliter
MH	Malignant Hyperthermia
mm^2	square millimetre
mm^3	Cubic millimetre
MSTN	Myostatin
MUFA	Mono-unsaturated fatty acid
n-3	omega 3
n-6	omega 6
nm	nanomole
Nn	MH heterozygous
Nn	MH homozygous
NN	Non-carriers (Normal)
NTC	No template control
PCR	Polymerase chain reaction
%	percentage
pH_u	ultimate pH
PISSA	Pig Information System of South Africa
PRKAG3	Protein kinase, AMP-activated, gamma(3)-subunit
PSE	Pale, soft and exudative
psi	Pound per square inch
PRRS	Porcine Reproductive and Respiratory Syndrome
PUFA	Poly-unsaturated fatty acid
QTL	Quantitative Trait Loci
RN^-	Rendement Napole
RYR1	Ryanodine receptor 1
SA	South Africa
SAL	South African Landrace
SAPPO	South African Pork Producers' Organization
SFA	Saturated Fatty acid
SNP	Single Nucleotide Polymorphism
SSC	*Sus Scrofa* chromosome
SM	Supplementary material
TBARS	Thiobarbituric acid reactive substances
ug/mL	Micro gram per milliliter
V	Volts

VIA	Video image analyzer
w/w	weight of solute/total weight of solution
WBSF	Warner Bratzler shear force
WHC	Water-holding capacity
µL	Microlitre
µM	Micromolar

CHAPTER ONE: INTRODUCTION AND LITERATURE REVIEW

1.1 Introduction

Pigs were amongst the first animals to be domesticated with the common ancestor as the wild boar (*Sus scrofa*). Domestic pigs can be divided into the Asian and European types, which diverged from each other approximately 58 000 years ago (Groenen *et al*., 2012). European wild boars were domesticated in the 4th millennium BC in central Europe, Germany, and migrated to the rest of Europe (Larson *et al*., 2005). The Southern African pig population consists of a mixture of pigs introduced through diverse pathways. According to a single archaeological record (Plug & Badenhorst, 2001), pigs were introduced to South Africa between the 3rd and 7th centuries when Iron Age communities with domestic animals migrated from central Africa and entered South Africa via the eastern parts through present-day Zimbabwe and Mozambique. Pigs were most likely exchanged with indigenous communities during that time (Quin, 1959) by both Chinese and Portuguese trade ships passing the South African shores. There is no conclusive historical evidence of the presence of pigs (*Sus scrofa*) in Southern Africa before the mid-seventeenth century. The first reference to domestic pigs was recorded by J.A van Riebeeck, as an entry in his Daghregister (Diary) in 1652. It is believed that the first imported consignment of Large White pigs took place after the South African Boer War (1899 – 1902), when efforts were made to re-build the depleted local pig population. The first Landrace pigs were imported from Holland into South Africa in 1952 (Briggs, 1983), while the first Duroc pigs were brought from Canada in 1980 primarily for cross-breeding purposes (Visser *et al*., 1993). Thereafter over time, importations of live animals and semen took place primarily from England, Canada, Netherlands, Sweden, Germany and the United States of America (http://www.studbook.co.za). Through genetic improvement programs, the local population made satisfactory progress that the breed became known as the 'South African Landrace' and resulted in gaining entry into a herd-book register. The South African pig industry has grown over the last 80 years contributing a gross producer value of about R1 billion per annum (approximately 83 000 000 US$). The industry is relatively small in terms of overall South African agricultural sector (DAFF, 2012). The South African Department of Agriculture, Forestry and Fisheries (DAFF) indicates that animal production contributes about 50% to the gross value of South African agricultural production, yet the contribution of the pork industry to total animal production is only 4.4%. The pig breeding industry in South Africa is organized into two major sectors consisting of a small number of individual stud breeders, a well-developed commercial pig farming sector and two international breeding companies.

Globally, pork accounts for the majority of meat consumed. The European Union, China and the United States of America account for about 86% of the world's pork production. They are also the world's largest consumers of pork averaging between 27.8 to 42.6 kg per capita per year. According to the United Nations projections, the world population is set to increase to 9.1 billion by 2050 with almost all of the

population increase in developing countries (http://www.fao.org; Thornton & Gerber, 2010). The demand for food is expected to continue to increase as a result of both population growth and increased incomes. Meat production will have to grow by over 200 million tons in order to meet the population demands, with seventy two percent of the meat produced to be consumed in developing countries (http://www.fao.org; October 2009).

Urbanisation and economic growth are contributing to the increasing presence of international and local fast food restaurants across our continent. In addition to the entry of fast food brands like KFC and Dominoes, supermarket and retail giants like Walmart (Massmart in South Africa), Shoprite and SPAR are introducing a range of pork-dominated Western products to African consumers (Livestock Data Innovation in Africa Project, March 2013). Foreign tourists, workers, investors, and their families are also flocking to African shores for business and pleasure. Hotels and restaurants with a large customer base of foreigners need to add pork delicacies to the menus (BFAP, 2008). This creates an opportunity for local pig farmers to increase and maintain a large and regular stock of pork products to satisfy the growing demand. In Morocco, an overwhelmingly Muslim country, pig production is increasing to cater for the demands of millions of tourists (especially Europeans) who visit this north African country every year. The trend is sure to continue as Africa's cities grow to accommodate more foreign tastes (www.sth.au.com).

The South African Pork Producers' Organisation (SAPPO) is the mouthpiece of commercial pork producers in South Africa. The industry consists of approximately 97 532 sows and approximately 7 000 boars owned by an estimated 400 pig farmers. There are approximately 46 registered pig abattoirs where modern technology is applied and are responsible for slaughtering of about 2 million pigs annually, representing a total of 18 stud breeders (http://www.sapork.com). These producers average more than 180 000 tons of pork per annum (BFAP, 2012). The farms varied with 100 sows to larger commercial farms with 7 000 sows. Informal producers (non-registered producers) account for a further 40 000 sows countrywide. Despite the industry's small size, an average of 27 piglets is weaned per sow per year, allowing the small number of producers to compete with international counterparts. The industry is relatively concentrated, with 80% of the 2.65 million pigs slaughtered annually (http://www.agribenchmark.org; SAPPO 2012; DAFF, 2013). An increased production was noted in 2012 (Figure 1.1) in response to an increase in domestic consumption.

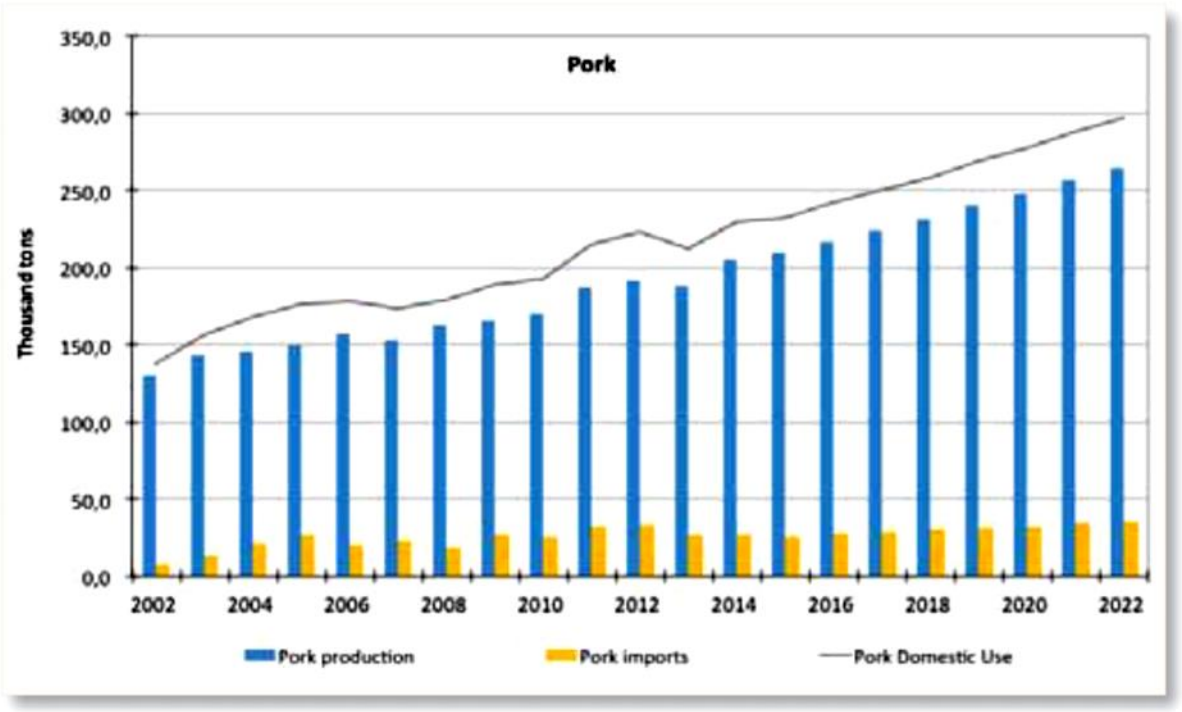

Figure 1.1 SA Pork Production, consumption and imports (BFAP, 2013-2022)

Traditionally, South African consumers have a strong preference for beef, while chicken provides a cheap and healthy alternative. In 2012, South Africans consumed only 4.2 kg of pork per capita, as compared to 34.6 kg chicken per capita. Agricultural statistics indicate that the consumption of pork has overtaken that of lamb or mutton (Figure 1.2) and pork consumption is projected to grow by 41% until 2022, as compared to 62% over the period 2002-2012. Pork consumption is influenced by religious factors, with a large share of the population not consuming any pork products due to religious beliefs. Consumer choice regarding meat quality depends on a number of factors including meat tenderness, fat content and colour. The challenge is therefore to produce an acceptable product for the segment of the community supporting pork products.

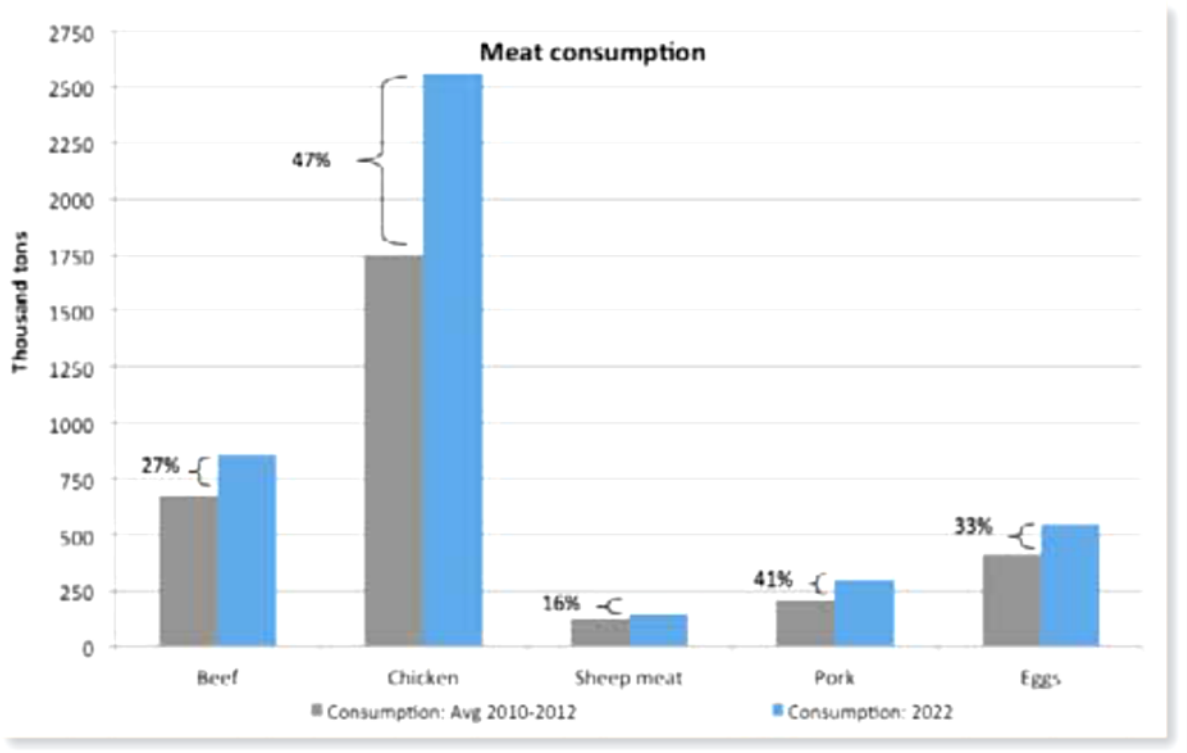

Figure 1.2 SA Meat consumption (BFAP, 2013-2022)

According to the Pig Information System of South Africa (PISSA, 1 October 2014) herd count report, there are thirteen pig breeds registered with South African Studbook and Animal Improvement Association. These breeds include the South African Large White (LW), Duroc, Large Black, South African Landrace (SAL), Hampshire, Chester White, Welsh, QM Hamline, Robuster, Pietrain, Berkshire, L08 Composite Dam Line and L65 composite sire line, comprising of individual breeders and breeding companies (PISSA, 2014, Table 1.1). The predominant South African pig breeds are the Landrace, Large White, Duroc and Pietrain breeds (Visser *et al*, 1993; Visser, 2014).

Table 1.1 Total number of pigs per breed participating in the National Pig performance and Progeny Testing Scheme (October 1, 2014) (PISSA Herd Count Report)

Breed	**Boar Total**	**Sow Total**	**Members**
SAL	104	661	10
Duroc	158	919	9
Large Black	158	919	-
Large White	255	2400	11
Hampshire	255	2400	3
Chester White	256	2400	4
Pietrain	256	2400	3
Welsh	104	661	-
QM Hamline	256	2400	-
Robuster	256	2400	-
Berkshire	256	2400	-
L08 Composite Dam Line	256	2400	1
L65 Composite Sire Line	263	2432	1
Total	**2985**	**26531**	

A major objective in livestock production since agricultural intensification has been to increase the meat yield and meat quality. Selection based on quantitative animal breeding has been the major tool and also successfully applied to reach this objective (Scholtz, 2007). Since the mapping of livestock genomes, including the pig genome, molecular genetic tools have become available to further improve selection strategies (Rothschild, 2003; http://www.animalgenome.org).

These advances in biotechnology offer possibilities of improving, utilising and conserving present pig populations. The genomes of livestock species, including pigs, contain an extensive number of mutations with phenotypic effects which have been sustained by selective breeding (Basrur & King, 2005). Since animal breeding is focused on economically important traits, livestock species are of particular interest in the search for genes that control growth, muscularity, reproduction, behavior, meat quality and other traits (Andersson, 2000). Traits such as growth and muscularity are complex phenomena controlled by an unknown number of genes as well as environmental factors. The RN^{-} gene was shown to have an effect on muscle glycolytic potential and related traits (Le Roy *et al*., 2000; Ciobanu *et al*., 2001)). The causative mutation for the RN^{-} gene is located in the protein kinase adenosine monophosphate-activated γ3 (*PRKAG3*) gene. The effect of the gene to have an effect on carcass leanness was shown in studies (Le Roy *et al*., 2000; Enfält *et al*., 2006), although other literature data showed no effect of the RN^{-} allele on backfat thickness (Hamilton *et al*., 2000). *PRKAG3* has also been shown to have positive effects on lean meat quality in pigs (Galve *et al*., 2013; Uimari & Sironen, 2014). A number of studies have reported quantitative trait loci (QTL) in both commercial and exotic pig breeds for growth, carcass and meat quality traits, as well as several chromosomes for reproduction (Bidanel *et al*., 2002; Harmegnies *et al*., 2006; http://www.genome.iastate.edu.cgi-bin/QTLdb/SS/index, 2013).

The pig genome (*Sus scrofa*) sequence was completed in 2012. The final assembly (*Sscrofa*10.2) has been deposited in the public sequence databases (GenBank/EMBL/DDBJ) under accession number AEMK01000000 (Groenen *et al*., 2012). DNA microarrays or 'chips' have been used in studies ranging from gene expression to identification of single nucleotide polymorphisms (SNP) or differences in DNA sequences amongst breeds (Wang *et al*., 1998; Kim *et al*., 2011). The application of SNP's for livestock species has already been identified (Altshuler *et al*., 2000). However, SNP's are rapidly becoming the marker of choice and could also be used to provide information on selected as well as neutral variation (Boettcher *et al*., 2010). SNP chips arrays (e.g. 60K) (Illumina, San Diego, CA), permits simultaneous high throughput examination of hundreds of thousands of loci with high accuracy and thus offers the opportunity of a diverse range of genetic research applications. The Porcine SNP60K Bead chip was developed by Illumina in collaboration with International Porcine SNP Chip Consortium in early 2009 (Ramos *et al*., 2009). The Porcine SNP60K is the most comprehensive genome-wide genotyping array for the porcine genome, providing intense power to exploit genetic variation across a number of pig breeds, including Pietrain, Landrace, Duroc and Large White. It allows for the simultaneous genotyping of 65 000 genetic markers in a single pig thereby reducing the cost per genotype per animal (Gorbach *et al*., 2010).

Genomic selection was first described by Meuwissen *et al*. (2001) and is based on the principle that breeding values can be estimated from the information generated by a large number of DNA (genetic) markers. With a large number of genetic markers spread across the entire genome there will always be a

genetic marker close to the gene(s) of interest. With the availability of the pig genome sequence, a number of SNP have been identified and form the basis of genomic selection. Through this technology, a large number of SNP's are selected across the genome to serve as an information source for breeding value estimation. Genomic selection has more added-value when used for traits with a low heritability, traits that are sex-linked, or the ones that can only be measured at *post mortem*. We are at the start of the genomic era; new applications to make most use of this new technology are being developed. A study by Boddicker *et al*. (2012), were a number of pigs were infected with the Porcine Reproductive and Respiratory Syndrome (PRRS) virus. A whole genome analysis was done to identify genomic regions associated with the ability to cope with a PRRS infection. The SNP markers identified could be used to select the most resilient animals in the breeding herd without having to infect them with the virus.

The availability of genetic-based selection tools does contribute to the improvement of performance traits in pigs and has to be accompanied by an evaluation of the correlated effect on pork quality. A commercial test was developed for identifying the favourable alleles for the Insulin-like growth factor 2 (*IGF2*) gene associated with leanness or fatness. The test for *IGF2* is a leading, cutting edge, affordable technology that allows for identification of the gene for leanness or fatness (Gentec NV, 2005). The gene has a unique mode of inheritance, "maternal imprinting". Only the gene inherited from the father is expressed while the one from the mother is suppressed (Buys, 2003; Van Laere *et al.*, 2003). In the pig a region on chromosome 2 was fine mapped and *IGF2* was implicated in causing a major effect on muscle mass (Georges *et al.*, 2003). The *IGF2* gene can also be used to produce pork with the desired level of leanness from fatter sows. Based on its physiological function, *IGF2* has been considered a candidate gene for a QTL in pigs affecting muscularity. Unlike the Ryanodine receptor 1 (*RYR1*) gene where there is a benefit to eliminate undesirable genotypes, the *IGF2* gene allows for pigs carrying either the lean or fat genotype to be useful. For lean growth, the effects of the gene on muscle mass and leanness are of the same magnitude as that reported for the *RYR1* gene (*HAL* or *MH* gene) but without the undesirable effects on meat quality.

1.2 Aims and objectives

There is an opportunity for South African pig producers to increase pork production to satisfy the growing demand. Worldwide trends indicate consumer preferences for leaner meat and pig producers need to address the challenge of producing leaner pork, without compromising the quality of the product. Studies on *IGF2*, indicates towards this gene as a useful candidate for selection of lines for leaner meat.

Several studies have reported that pigs carrying the paternal 'A' allele of *IGF2* have a higher lean growth and lower backfat compared to those that have the maternal 'G' allele (Estélle *et al.*, 2005; Oczkowicz *et al.*, 2009; Clark *et al.*, 2014). The paternally inherited 'G' allele has been reported to have

advantage in breeding fatter pigs. Both genotypes can be useful to South African breeders allowing them to make informed decisions for selection of breeding stock. The South African pig production industry has focused primarily on input parameters, namely, growth performance, with little emphasis on output parameters such as carcass yield and meat quality (Visser, 2004). Since the early 1990's, pig breeders have been using gene marker technology to eradicate genes like *RYR1* gene. Pork producers have relied on phenotypic evaluations to evaluate sires. Testing stations were then used in which boars' performance was measured and indexed. This information would guide producers towards sires that would improve herd productivity. Best linear unbiased prediction (BLUP), allowed breeders to estimate the genetic merit of animals. These conventional selection and breeding criteria have worked well in the local pig industry but it has limited application to improve carcass and meat quality traits (Visser *et al.*, 1993).

The effects of this gene on lean meat content and backfat thickness (BFT) of pigs were reported by several studies (Ruan *et al.*, 2013; Sheller *et al.*, 2002; Lee *et al.*, 2001), but the effects on meat tenderness, juiciness and taste within the LW and SAL populations in SA are unknown. A study to determine the frequency of the *IGF2* gene amongst the breeding boars in South Africa and the effect thereof on meat quality and carcass traits such as tenderness, juiciness and taste thus becomes imperative for the South African pork industry.

A previous study on the South African pig population indicated a low frequency of the *Ryanodine receptor (RYR1)* gene (Soma *et al.*, 2014). *IGF2* has potential to increase leanness of the carcass and provide the information required to make informed selection decisions with regard to breeding programs. This is the first attempt in South Africa to determine the *IGF2* gene status of the SAL and LW breeds and associate the specific *IGF2* genotypes with meat and carcass traits.

Aim

The aim of the study was to determine the association of the *IGF2* gene, presented at three genotypes, with pork carcass and meat quality traits. This research was addressed in two phases.

The **specific objectives** included:

To investigate the frequency of, and association between the *RYR1* gene /malignant hyperthermia (*MH*) and Insulin-like growth factor 2 (*IGF2*) gene status within the major pig breeds used in the South African industry, namely, the South African Landrace, Large White, Duroc, Pietrain, and Chester White (Phase 1).

To evaluate the effect of the *IGF2* gene on carcass and meat quality (% carcass fat and % lean meat, meat colour, water holding capacity and drip loss, meat tenderness, measured by Warner Bratzler shear force,

and fat and protein oxidative stability) in two populations of South African Large White and Landrace breeds (Phase 2).

To evaluate the effect of the *IGF2* gene on fat and fatty acid composition of the South African Large White and Landrace breeds (Phase 2).

1.3 Literature review

1.3.1 Introduction

Growth is a complex biological phenomenon controlled by a complex of endo-, para- and autocrine control mechanisms. This has been studied in depth in most farm animals and it has been shown that the insulin growth factor complex (IGF) plays a critical role in growth regulation, together with insulin, thyroid hormones, steroids and the growth hormone (Rejduch *et al.*, 2010). *IGF2*-gene plays an important role in muscle growth in pigs and has potential to assist pig breeders by using genotypic information in the selection programs.

Meat quality is a complex concept defined by different facets such as the compositional quality (lean to fat ratio) and the palatability quality such as meat colour, tenderness, juiciness, and flavour. The nutritional quality of meat is also considered by the consumer, as being very important. Meat quality traits are measured *post mortem*. A variety of over-lapping criteria such as chemical, morphological, nutritional, sensory and culinary measures can be used to measure meat quality. The properties of processed and fresh pork is dependent on factors related to the composition of the product including moisture, lipid and protein content. The characteristics of the protein, water and lipid are responsible for the differences in the colour, texture, water holding capacity and tenderness of pork.

This section will provide an overview of the role of *IGF2* and its association with meat and fat quality traits and highlight the general factors influencing pork quality.

1.3.2 *IGF2*, as a major gene

A gene can be considered a major gene when the difference between the mean value of the individual homozygous for the gene and that of individuals not carrying the gene, is equal to or greater than one phenotypic standard deviation of the trait of interest (Sellier and Monin, 1994). Insulin-like growth factors (*IGF*) are growth-promoting peptides, which are structurally homologous with insulin. Their biological effects are also similar to that of insulin that is synthesized only in the pancreatic islets of Langerhans, while *IGF*'s are synthesized in tissues throughout the body (Nedbel *et al*., 2000). *IGF*'s are a family of hormones which control hyperplasia and differentiation throughout the body. It consists of two hormone ligands: insulin-like growth factor-1 (*IGF1*) and insulin-like growth factor-2 (*IGF2*) (Liu *et al*., 1993). The two *IGF* cell surface receptors are *IGF*-Type 1 receptor (*IGF1*R) and *IGF*-Type 2 receptor (*IGF2*R). The *IGF1*R has an increased affinity compared to *IGF2* and binds *IGF1* whereas *IGF2* is bound at an approximately 10-fold decreased affinity. *IGF*2R binds IGF2 with an increased affinity compared to *IGF1*. Insulin binds *IGF1*R at a decreased affinity than *IGF1* and is unable to bind to *IGF2*R.

The first QTL studies for *IGF2* was performed using a candidate gene approach based on an intercross between wild boar and Large White domestic pigs (Andersson-Eklund *et al*., 1998). A QTL on the short

arm of chromosome 2 with moderate effect on muscle mass was detected using a conventional Mendelian inheritance model. The presence of an imprinting effect and an imprinted QTL (paternally expressed) was also detected on the distal tip of chromosome 2 in pigs (SSC2) that has effects on lean meat content (Jeon *et al*., 1999). The QTL indicated 15.4% of F2 generation's phenotypic variance of longissimus muscle area, 14% of heart weight and 10.4% of backfat depth. The results indicated that the paternally expressed QTL locates at the same position as *IGF2*. This result and the fact that both the gene and the QTL were imprinted, made *IGF2* gene a possible candidate based on the QTL effect. The allele in the Large White breed found at the *IGF2* gene linked to the QTL was associated with larger muscle mass and reduced BFT, but that this QTL had no notable effect on abdominal fat. The *IGF2* linked QTL was also found in experimental crosses of Pietrain and Large White pig breeds where results indicated that the QTL at the end of SSC2 was imprinted and paternally expressed (Nezer *et al*., 1999). Therefore, *IGF2* gene was regarded as a potential candidate for the QTL at the distal end of SSC2. The effects on muscle mass and fat deposition were major and of the same magnitude as that reported for the *RYR1* gene. These two loci combined explained 50% of the Pietrain-Large White difference for muscularity and leanness. No evidence for interaction between the QTL at *IGF2* gene and *RYR1* gene locus was found. Sequence analysis (Nezer *et al*., 1999) found a single nucleotide mutation, G to A transition in *IGF2*, which increased lean yield by 2.7% (Meadus, 2000).

The QTL at *IGF2* and *FAT1* on chromosome 4 were the two QTL with the greatest effect on body composition and fatness, present in the wild boar-Large White cross (Andersson *et al*., 1994; Szyda *et al*., 2003). The QTL at *IGF2* controlled mainly muscle mass whereas *FAT1* had a major effect on fat deposition (Jeon *et al*., 1999). The two QTL loci explained 33% variance for lean meat content in ham, 31% for percentage of lean meat and 26% for the average depth of backfat. The *IGF2* microsatellite was also found to be highly polymorphic, with three alleles among wild boars founders and an additional two alleles among eight Large White founders (Jeon *et al*., 1999). It is important to have polymorphic markers due to more variation amongst the microsatellite markers.

IGF2 explained 25% of the phenotypic variation of leanness in a study using experimental crosses (Sheller *et al*., 2002). However, it did not influence daily weight gain and pH_u of meat. Previous studies have confirmed that the *IGF2* gene is an imprinting gene in pigs and constitutes an important QTL for muscle mass and fat deposition. The test reached the genome-wide threshold ($P<0.01$) for average BFT and loin-eye area. The favorable alleles showed in the Yorkshire breed, when transmitted through the sire, reduced average backfat by 0.1 cm and increased loin-eye area by 1.0 cm^2, when compared to alleles in the Berkshire breed (Lee *et al*., 2001; Aslan *et al*., 2012).

Regions with significant QTL for muscle fibre traits or significant QTL for meat quality were detected on several chromosomes (SSC1, 2, 3, 4, 5, 13, 14, 15 and 16). Loci controlling lean meat content

segregated on SSC6. The results presented in the study indicated that loci affecting meat colour and meat quality traits, such as related to water binding capacity, like pH value and conductivity, segregate in many populations including commercial breeds and are located on the p-arm of SSC3. Previous studies from Karlsson & Lundström (1992) and Rosenvold & Andersen (2003) have shown that stress, exercise or fighting results in higher muscle temperature and lactic acid content and faster pH decline. The proportion of slow and fast twitch fibres has been related to insulin resistance, as well as fat catabolism (Simoneau and Kelly, 1997). Any stress during pre-slaughter causes net glycogen depletion and higher ultimate pH (pH_u) (Terlouw *et al*., 2005). A QTL scan in a porcine experimental population based on Duroc and Berlin Miniature pigs confirmed the presence of QTL's for microstructural muscle properties as well as biophysical parameters of meat quality and traits related to body composition, i.e. pH and lean meat content (Wimmers *et al*., 2006). A QTL for meat colour was reported on SSC13 (Wimmers *et al*., 2006; van Wijk *et al*., 2007). A summary of QTL's for carcass traits is shown in Table 1.2.

Table 1.2 Summary of QTL mapping results by chromosome for carcass quality traits

Chromosome	QTL	Reference	GeneBank Id.
2	Drip loss, %	van Wijk *et al*., 2007; Cherel *et al*., 2011	407541
2	Ham colour	van Wijk *et al*., 2007; Cherel *et al*., 2011	100326151
2	Back fat	Rattink *et al*., 2000; Cherel *et al*., 2011	407269
3	Colour	van Wijk *et al*., 2007; Cherel *et al*., 2011	407338
4	pH ultimate	van Wijk *et al*., 2007; Cherel *et al*., 2011	449418
4	Fat	Evans *et al*., 2003; Tortereau *et al*., 2010	100325974
7	Backfat, IMF	De Koning *et al*., 1999; Tortereau *et al*., 2010	100324167
10	Lean meat	van Wijk *et al*., 2007; Cherel *et al*., 2011	407272
	Backfat	De Koning *et al*., 1999; Tortereau *et al*., 2010	119336596

1.3.3 Association between *IGF2* gene on meat and carcass traits

Pig breeds can be classified as genetically lean and genetically obese (Wood, 1984) with the two extreme examples being the Duroc (genetically obese) and Pietrain (genetically lean). Selection for a leaner

pig results in decreased ratio between fat deposition and lean deposition. Consequently, fat quality is negatively affected. Since the early 1990's, there has been an interest in the intramuscular fat (IMF) content of pigs since higher IMF levels are associated with improved eating quality of pork (Bejerholm and Barton-Gade, 1986; Jakobsen, 1992, de Vries *et* al., 1998; Faucitano *et al*., 2003; Serão *et al*., 2011). Intramuscular fat content is also determined as seen in some breeds such as the Duroc with higher IMF compared to other breeds (Barton-Gade, 1987; Visser *et al*., 2003; Burkett, 2009).

The imprinting inheritance mode of the *IGF2* gene was reported by several studies (Buys, 2003; van Laere *et al*. 2003; Van den Maagdenberg, 2007). Van Laere *et al*. (2003) reported that a G to A transition in *IGF2* gene is the causative quantitative trait nucleotide. This single nucleotide mutation adds approximately 3 to 4% more lean meat to pigs. The link of the mutation with the desired phenotype is 100%, regardless the origin of the pedigree (Buys, 2003). It allows for the selection of carcass leanness based directly on the functional nucleotide at the DNA level. The *IGF2* gene has an effect on the production of lean meat. Boars tested for *IGF2* can be used to either increase or decrease back fat. Boars with *IGF2* +/+ genotypes can be used to increase lean yield, while those with *IGF2* -/- genotype can be used to decrease lean yield. Actual breeding trials confirmed the use of *IGF2* gene where pigs from the selected boars were leaner with a reduced back fat and more uniform compared to those from unselected boars (Sheller *et al.*, 2002; Clark *et al*., 2014). The carcass leanness measures also showed differences between the *IGF2* genotypes and an association study revealed that the 'A' allele increased the weight of loin, weight of ham, carcass meat percentage, and decreased average backfat thickness (Liu, 2003). Highly significant effects of the *IGF2* mutation on body composition traits were observed, as well as significant effects on growth performance. The influence of the *IGF2* gene mutation on meatiness has further been confirmed by a number of studies in various pig populations (Estélle *et al.*, 2005; Van den Maagdenberg *et al*., 2008a; Gardan *et al*., 2008; Burgos *et al*., 2012; Oczkowicz *et al*., 2012; Clark *et al*., 2014).

Meat Quality Traits

A complex of biochemical processes are responsible for the conversion of muscle to meat. Carcass temperature and the rate and extent of pH decline are major determining factors of the muscle to meat biochemical processes. In this study, the carcass and meat quality as well as the fat and fatty acid analysis of the *IGF2* genotypes was studied. The meat quality traits as shown in the results section are introduced below.

pH

A contributory factor to pork colour is the extent and rate of pH decline in early *post-mortem*, an important factor in determining the quality of fresh pork. When glycogen metabolism is rapid, lactic acid,

is produced and hence the rapid decline in pH (Lonergan *et al.*, 2007). Good quality pork is associated with a gradual decline in pH, therefore with a rapid decline there is an increased chance that the combination of high temperatures and low pH can create conditions that favour the denaturation of proteins. An increasing paleness in meat is inversely proportional to pH therefore a decrease in pH results is associated with an increase in paleness. If the pH decline happens too rapidly after slaughter, resulting in a very low pH at a high temperature, it will result in very pale meat (Barbut *et al.*, 2008). If the pH_u is high (where glycogen depletion occurs pre-slaughter resulting in little or no lactic acid production) the meat will be dark and firm with a dry (DFD) surface (Andersson, 2000). DFD meat allows the growth of spoilage organisms which are inhibited at the usual pH_u of meat (Newton & Gill, 1981).

Fibre typing

Colour variations between muscles are due to differences in pigment content and muscle metabolism and therefore is a major determinant of meat colour. Red muscles (oxidative muscles) depend on an oxidative metabolism which requires large amount of myoglobin for oxygen supply and storage. Glycolytic muscles uses glycogen as an energy source, have a pale colour and are hence referred to as 'white muscles' (Jeong *et al.*, 2012). Colour differences between muscles with different metabolism, are possible to observe with the naked eye. Differences in muscle fibre type composition are greater between muscles than differences between animals from different genotypes or breed (Lefaucher & Gerrard, 1998). Maltin *et al*. (1997), examined muscle fibre characteristics of *Longissimus dorsi* from pigs of eight breeding stock companies and reported that variation in fibre size and type existed among the population of pigs. Those differences did however not contribute to differences in the sensory quality measurements, such as juiciness or pork flavour. In a recent study, Clark *et al.* (2014) indicated that muscle fibre cross-sectional area and intermediate fibre area (Type 11A) appeared to be reduced in IGF2 A^{pat} pigs as compared with G^{pat} pigs.

Meat colour

Meat colour is of utmost importance as it forms the basis for consumers for product selection (Dransfield, 2008) and is dependent upon myoglobin content and the amount of oxygen available for reacting (Hur *et al*., 2004). The colour of pork is determined by many factors including genotype, breed, gender, diet, muscle type and extrinsic factors such as pre-slaughter handling and slaughter procedure (Rosenvold & Andersen, 2003), which influences pH decline, furthermore, storage conditions and storage time (Faustmann & Cassens., 1990). Brewer *et al*. (2001) reported the stabilization of colour parameters were unaffected by bloom time. Variations in meat colour have been observed among pig breeds (Oliver *et al*., 1994; Blanchard *et al*., 1999). Recent literature where *IGF2* was studied (Van den Maagdenberg *et*

al., 2008a; Burgos *et al*., 2012; Clark *et al*., 2014) has shown that the colour of the *Longissimus dorsi* was affected by the A allele with higher colour values, and may result in paler meat.

Water holding capacity (WHC)

Drip loss is also an important factor for meat quality as it affects consumer perception and nutritive value of finished products (Muchenje & Ndou. 2010). In a study by Joo *et al*. (2000) where pork quality categories were investigated, highly marbled meat with less drip loss was observed in pork *Longissimus dorsi*. In the same study, the increased IMF appeared to affect the WHC of pork loin during cold storage. Protein characteristics are responsible for the water holding capacity in pork. Meat proteins have no net charge at pH 5.1. As the pH of meat reaches pH 5.1 (isoelectric point), the WHC drops drastically because of protein denaturation. An increased pH decline at high carcass temperature cause an increased drip loss in fresh pork chops and less WHC (Andersson, 2000). Product weight loss of between 1-3% drip loss represent an economic loss to both processors and retailers and can be as high as 10% in PSE products (Melody *et al*., 2004). Therefore, understanding the process of drip loss and preventing drip loss is important in the meat industry. Lean meat contains approximately 70% water and most of the water is held within the structure of the muscle and muscle cells. Loss of water may involve different mechanisms and may occur at different times during storage (Huff-Lonergan & Lonergan, 2005).

Shear Force

Consumers consider tenderness as an important attribute of meat. Tenderness and tenderisation is the result of complex interactions of factors such as, collagen content, cross-linking of collagen, sarcomere length, proteolysis, intramuscular fat content and variation in the rate of tenderisation (Wheeler & Koohmaraie, 1999; Van Laack *et al*., 2001; Purslow, 2005). The contribution of each of these factors is muscle dependent. The state of myofibrils in meat is related to the variation in tenderness. The myofibrillar protein fraction in muscle tissue contains the proteolytic enzymes which are responsible for tenderisation during the ageing process. Meat tenderness is determined by the structural myofibrillar proteins, namely, titin, nebulin, desmin, vinculin and troponin-T which are responsible for keeping the structural integrity of myofibrils.

Early pH decline plays a pivotal role in regulating the rate of *post mortem* tenderisation (Josell *et al*., 2003c). Clark *et al*. (2014) reported no differences in shear force between the homozygous *IGF2* alleles and this was also confirmed by Van den Maagdenberg *et al*. (2008a) where tenderness was unaffected.

Lipids

The total fat content of pig carcasses range between 24-29% (Realini *et al*., 2010), while the fat content of lean meat is approximately 1% (Wood *et al*., 2008). Lipid characteristics are important for the technological quality of pork (Hugo & Roodt, 2007). Good quality fat in pigs has been defined as firm and white, while poor quality fat is soft, oily, wet, grey and floppy (Wood, 1984). If the lipids in pork become too unsaturated, the pork would not be suitable for the processing industry and be liable to oxidative breakdown, increasing rancidity problems (Teye *et al*., 2006b). Increased polyunsaturated fatty acid (PUFA) levels are associated with an increased occurrence of oxidation and rancidity and the monounsaturated fatty acids (MUFA) are associated with the soft, greasy texture of the fat (Hadorn *et al*., 2008). Recently, the emphasis has shifted from fat quantity to fat quality in most livestock species, including pigs (Webb & O' Neill, 2008).

1.3.4 Association between ryanodine receptor (*RYR1*) and pork leanness

Muscle tissue is mainly composed of muscle fibres and the muscle growth potential in pigs is therefore dependent on the total number of fibres (Dwyer *et al.*, 1993; Stinckland & Goldspink., 1973), which is known to be fixed before birth. Hyperplasia and hypertrophy are responsible for the increase in total muscle mass and are the result of proliferation, differentiation and the fusion of myoblasts and satellite cells that are controlled by a number of regulatory factors including the insulin-like growth factor system (Velloso, 2008). The *IGF2* mutation on muscle growth and lean meat content may be associated with effects on biochemical and histochemical muscle fibre characteristics. The ryanodine receptor (*RYR1*) gene, also known as the "Halothane" gene (*HAL*) a major gene, located on chromosome 6 (Harbitz *et al*., 1990) affecting meat quality, and was the first practical manipulation of a major gene, in pig breeding using a molecular genetic approach (Otsu *et al.*, 1991). The primary defect resides in a single point mutation (Arg614Cys) in the porcine *RYR1* protein. The ryanodine receptor regulates Ca2+ transport across the cell membrane in muscle cells (Bjurström *et al*., 1995). In the past, a Halothane challenge test was done, in which pigs were subjected to inhalation of Halothane gas in order to identify carriers of the mutation (Basic *et al*., 1997). In the event of a reaction to the Halothane test, both parents of the animal were suspected of being carriers of the mutation. Pigs were then classified as Halothane positive or Halothane negative. This gene also results in, or is closely linked to, a gene or genes involved in determining muscling and leanness (Greaser, 1986). The results of many studies have demonstrated that the *HAL*-1843 gene accounts only for about 25-35% of pale, soft, exudative (PSE) meat observed in commercial abattoirs (Allison *et al*., 2006). A meat quality analysis conducted by MacLennan and Phillips (1992), showed that under intense stress conditions, a rapid glycogen disintegration leads to increase of lactic acid content in muscle cells of the mutated gene carriers. Consequently the level of muscle acidification increases. The affected animals are

a source of PSE pork. In other studies pigs heterozygous for the *RYR1* genotype demonstrated that they were characterized by 4-5% higher meat content and 14% lower fat content in carcass as compared to mutation-free pigs (Rejduch *et al*., 2005). Elimination of this gene from pig populations resulted in improvement of meat quality because PSE meat was simultaneously eliminated.

The recessive allele is associated with more lean meat. It is this association that has allowed the gene frequency to be increased initially through selection for increased muscularity. These studies indicate that the *RYR1* gene exerts an important influence on parameters of meat quality, that is, drip loss and pH, and percentage carcass lean meat and therefore is regarded as a gene with major effect on these two traits (Otto *et al*., 2007). Hamilton *et al*. (2000) investigated the effect of the *RYR1* and Rendement Napole (*RN*) genes on carcass and meat quality characteristics (pH, colour and water-holding capacity) of pigs and reported that carrier animals had shorter carcasses than normal animals. Fontanesi *et al*. (2008) also reported significant results for the effect of the *RN* gene on meat quality traits where pH values were low, contributing to PSE meat. On the other hand, a positive effect on lean muscle deposition was reported by Le Roy *et al*. (2000).

A study in South Africa in 1999 (Rhode & Harris., 1999), indicated a low frequency of the recessive (nn) homozygous genotype (associated with poor meat quality) and an increase in the frequency of the normal (NN) genotype. South African pig breeders became more aware of the incidence of the *RYR1* gene and realized the importance of eliminating the number of carriers and recessive animals from their breeding stock. In a survey conducted by Soma *et al*. (2005), to determine the *MH*-gene status of pigs in nucleus herds and AI stations in South Africa indicated a low number of carriers (Nn) in the population tested.

A study aimed to investigate the effect of the *IGF2* mutation on biochemical and histochemical muscle fibre characteristics in relation to the *RYR1* genotype (Nn vs. NN) found no effect of the *IGF2* or the *RYR1* genotype on muscle fibre type composition (Van den Maagdenberg *et al*., 2008a). However, a two-way interaction between *IGF2* and *RYR1* genotype or gender on fibre type composition was observed. In the same study, the mutation in the *RYR1* did not influence birth weight, average daily gain, lean meat content or average daily lean meat growth, although an increased percentage of carcass weight and *Longissimus dorsi* muscle cross-sectional area were found in Nn animals. Muscle *IGF2* expression does not appear to be the only factor explaining muscle growth, because normal (NN) animals had less muscle that their heterozygous counterparts, despite their higher *IGF2* mRNA expression. Further investigation is necessary to understand the interaction between the *IGF2* mutation and *RYR1* genotype. The effect of the *IGF2* paternal allele (A^{pat} and G^{pat} animals that inherited and the mutant and wild type paternal allele of interest) were evaluated on carcass and meat quality traits in Nn and NN genotypes and to rule out possible unfavourable correlated responses of the *IGF2* mutation and to examine the interaction between these two major genes (Van den Maagdenberg *et al.*, 2008b). Increased lean meat content and higher incidence of

PSE meat in nn or Nn pigs compared to Nn pigs was observed (De Smet *et al*., 1996; Monin *et al*., 1999). Fernandez *et al*. (2002) reported colour defects during cooking of PSE meat.

1.3.5 Fat Quality in Pigs

Food industries have laid complaints that pork quality is deteriorating despite its health benefits (Wood, 2001). Although fatty acid (FA) profiles may impose possible health effects (Aharoni *et al*., 1995; Fisher *et al*., 2000), they also contribute to the sensory characteristics of meat (Wood *et al*., 2003). The fat composition of pork varies between the muscle type and muscle fibre composition and is influenced by factors such as genotype, rearing and feed (Cardenia *et al*., 2011).

Fat quality is determined by several factors such as breed, gender and diet (Scheffler & Gerrard, 2007). It is known that both breed and management from rearing up to slaughter time will affect FA composition, which is closely related to IMF levels in meat (Hansen *et al*., 2000). The differences in FA composition between breeds subjected to various pre-slaughter conditions can be explained by differences in the proportion of IMF as the ratio of polyunsaturated FA to saturated fatty acid (PUFA/SFA) (Muchenje *et al*., 2009c). The ratio decreases with increasing fat level of pork. To date, limited data exists linking the *IGF2* gene with fat characteristics (Reina *et al*., 2012; Oczkowicz *et al*., 2012; Clark *et al*., 2014).

It was indicated that subcutaneous fatty acid composition of industrial pigs is 36% saturated fatty acid (SFA), 44% MUFA and 12% PUFA (Gandemer, 2002). Moisture percentage of the *Longissimus dorsi* muscle, as determined by proximate composition analysis was lower in *IGF2* A^{pat} pigs compared to G^{pat} pigs (Clark *et al*., 2014). Increased intramuscular fat in *Longissimus dorsi* muscle was reported by Burgos *et al*. (2012) and Oczkowicz *et al*. (2012). This suggested that *IGF2* may be a candidate gene for differential regulation of subcutaneous and intramuscular fat deposition. Lean breeds tend to have poor quality fat (Tor *et al*., 2001; Nguyen *et al*., 2004). Burgos *et al*. (2012), noted an increase in percentage intramuscular fat (%IMF) within the *Longissimus dorsi* muscle in pigs carrying the A genotype. Other authors reported either a decrease or no change in IMF due to the A genotype (Reina *et al*., 2012; Sanchez del Pulgar *et al*., 2013). PUFA content was reduced and SFA percentages were increased in dry-cured hams and shoulders of pigs with the *IGF2* A genotype compared with the G genotype (Reina *et al*., 2012). This can be an indication of differences in lipid metabolism between males and females (Högberg *et al*., 2003).

Breed comparisons have shown that the genotype and feeding regime determines the quality attributes of fat regarding content, composition and uniformity as well as oxidative stability (Rosenvold & Andersen, 2003). Pig diet is another important factor with an effect on intramuscular fatty acid composition (Högberg *et al*., 2002). Changes can be brought about by different feeding regimes, which is, feed with fat and oils with varying fatty acid compositions (Mas *et al*., 2011; Vicente *et al*., 2013).

Pig genetics accounts for at least 30% of the variation in meat quality (Anderson, 2000). The influence of genetics on pork quality comprises differences among breeds as well as differences amongst animals within the same breed. Wariss *et al.* (1990) indicated that the tendency of lean breeds to have poorer fat quality was due to their leanness and thinner subcutaneous fat layer instead of other inherent breed differences. Cameron and Enser, (1991) compared Duroc and RYR1 negative British Landrace pigs for intramuscular FA composition. The Duroc breed is known for a higher IMF content relative to the backfat content of other breeds. It was also noted that it remains unclear whether the Duroc would show different FA proportions at a similar fat level when compared to the Landrace breeds.

Gender also has an influence on fatty acid composition because of its effect on carcass fatness (Nűrnberg *et al*., 1998). Bruwer *et al.* (1991), indicated that castrate carcasses contained the most fat, gilts were intermediate and boars had the highest lean meat content. Several authors have indicated sex differences for pork FA composition (Hartmann *et al.*, 1997; Warnants *et al.*, 1996). Muscle phospholipid FA composition does not seem to differ between gilts and barrows, but higher PUFA concentrations have been found in total lipid or triacylglycerols for gilts, even after differences in fat content correction. Residual sex effects independent of fat content seems to exist for FA composition. Kouba and Bonneau (2009) found that in Large White X Landrace castrated males, kidney fat grew more rapidly than subcutaneous or IMF. The IMF content has a major influence on pork quality as it determines marbling of the muscle. Pork loin should have at least 2% fat in the lean meat else it appears too dry after cooking.

In South Africa, the carcass weights are mostly 50-55 kg (porkers) or 70 -75 kg (baconers), with a maximum of 100 kg (Vervoort, 1997; Pieterse *et al.*, 2000). A low slaughter weight of pigs in South Africa, results in low backfat thickness (Hugo *et al.*, 1999). It was reported by Wood *et al.* (1989) that pigs with heavier slaughter weights had physiologically more mature fat which is more saturated, compared to pigs with lighter slaughter weights. Nűrnberg *et al*. (1998) indicated that the effect of age on fatty acid profiles was also related to body fatness. During growth, the proportion of energy available for fat deposition in pigs increases, such that the rate of *de novo* fatty acid synthesis is also increased. This results in an increased synthesis of SFA's and a reduction in C18:2 and C18:3n-3 contents.

There are nine different fatty acid-binding proteins (FABP's) which are each tissue specific and has a role in the regulation of FA uptake and intracellular transport (Gerbens *et al*., 2001). The heart fatty acid-binding protein (HFABP) and adipocyte fatty acid-binding protein (AFABP) are responsible for the transport of metabolized FA's. HFABP and AFABP were responsible for an increase of fat content by 1% in Duroc and Meishan pig breeds (Gerbens *et al*., 2001; Katsumata, 2011). Polymorphisms in the adipocyte and HFABP genes, AFABP and HFABP, were found to be associated with genetic variation of IMF content in the Duroc pig population (Burkett, 2009). The proportion of individual fatty acids change as the fat

content of the animal and its meat increases from early life to slaughter (Wood *et al.*, 2008). Slaughter weight also has an effect on fatty acid composition.

1.4 Conclusions

The underlying genetic variation influences lean meat and fat content in pigs. The *IGF2* gene has been shown to be a useful candidate gene for selection of lean meat. In addition to genetic factors, it is worthy to note that there are other technological factors that also play a role in the quality of the final meat or meat product. Results of sequencing efforts are already available and are being used to select markers for improved growth and meat quality. Such opportunities using DNA technology can therefore increase the responses in the South African pig populations and can be used in selection decisions much earlier as compared to the collection of phenotypic information.

CHAPTER TWO: THE FREQUENCY OF TWO MAJOR GENES, *RYR1* AND *IGF2*, IN THE SOUTH AFRICAN PIG POPULATION

This chapter presents the first phase of this study where the frequency of the two major genes in the South African pig population was investigated. The chapter was prepared by combining the data from the three publications, as indicated below:

Scientific publications:

Peer-review Journals

Soma, P., van Marle-Koster, E. & Frylinck, L. (2014). Frequency of the malignant hyperthermia gene in the South African pig industry. *South African Journal of Animal Science.* 44 (4), 384-387.

Congresses:

International

Soma P., van Marle-Koster E. & Frylinck. L. (2012). Malignant Hyperthermia Gene Status of Pigs in South Africa. Proceedings of the 58th International Congress of Meat Science and Technology, Montreal, Canada, 12-17th August 2012.

Soma P., van Marle-Koster E. & Frylinck. L. (2013). Insulin-like Growth Factor 2 Gene Status in the South African Pig Population. Proceedings of the 59th International Congress of Meat Science and Technology, Izmir, Turkey, 18-23rd August 2013.

CHAPTER TWO: THE FREQUENCY OF TWO MAJOR GENES, *RYR1* AND *IGF2*, IN THE SOUTH AFRICAN PIG POPULATION

2.1 Introduction

There is a constant increase in consumer demand for high quality meat (Joo *et al*., 2013). The meat industry is therefore obligated to produce and supply meat of guaranteed quality. For the pig industry, the development and application of new technologies to improve efficiency of pig production, is vital. Two major genes, *RYR1* and *IGF2*, have already shown to have an effect on meat quality and lean muscle deposition in pigs (Fujii *et al*., 1991; Milan *et al*., 2000; Burgos *et al*., 2012; Clark *et al*., 2014). The first phase of this study therefore focused on determining the frequency of these two major genes in the South African pig population.

Malignant hyperthermia (MH) is a genetic disease that affects calcium regulation in muscle, and results in sudden death and/or pale, soft and exudative (PSE) meat (Tarrant *et al*., 1986). The presence of the mutation in pigs is certain to result in inferior meat (Goodwin, 1994; Monin *et al*., 1999; Correa *et al*., 2013). In the past, a Halothane challenge test was done, in which pigs were subjected to inhalation of Halothane gas in order to identify carriers of the mutation (Basic *et al*., 1997). In the event of a reaction to the halothane test, both parents of the animal were suspected of being carriers of the mutation. Pigs were then classified as halothane positive or halothane negative. Only during the 1990's *RYR1* gene was discovered that resulted in a DNA test for MH and was patented by the University of Toronto with accuracy approaching 100% (United States Patent 6001976). Genomic testing allowed for the identification of the MH genotypes, MH heterozygous (Nn), MH homozygous (nn) and non-carriers of the MH mutation (NN). The test provides the pork industry with a powerful tool to detect the *RYR1* gene in live pigs and eradicate it from the industry. The elimination of the MH mutation from breeding stock has a major benefit towards producing PSE-free meat (Goodwin, 1994; Monin *et al*., 1999; Wendt *et al*., 2000; Lahucky *et al*., 2002; Soma *et al*., 2014).

Currently in South Africa, an estimated 50% - 60% of all slaughtered offspring are sired through artificial insemination (AI) (South African Pork Producers Organization (SAPPO), 2014). The distribution of the *RYR1* gene through AI (especially the heterozygous alleles) may have an effect in commercial herds, and cause substantial financial losses further down the supply chain if not controlled. Data from the Agricultural Research Council database collected between 1992 and 1997 (Nel *et al*., 1993) indicated that the prevalence of NN homozygous (non-carriers) was low in the early 1990's, with more than 77% of the population being non carriers (NN). However, from 2000 to 2003, a total of 1194 pigs, both sows and boars, were tested for the *RYR1* gene. A decrease was found in the frequency of the NN homozygous genotypes and an increase in the Nn heterozygous genotypes (21% in 2000 to 30% in 2003) (ARC database). These figures stressed the importance of testing a wider sample of the pig population that included all breeds that

contribute to the commercial market. Soma *et al*. (2012) reported the frequency of the *RYR1* gene at the nucleus level and boars used for AI from samples collected over a period of two years where the frequency of normal (NN) genotype was high. It was also noted that transport over a substantial distance to abattoirs is a reality for many of the slaughter pigs in South Africa. The absence of the MH mutation does not imply resistance to adverse changes in pork meat.

However, the presence of the mutation in pigs is certain to result in inferior meat (Goodwin, 1994; Monin *et al*., 1999) and therefore the removal of the MH mutation from breeding stock should eliminate a major contributing factor to PSE meat in South Africa. By the turn of the century it was generally concluded world-wide that it is imperative to remove the MH mutation from pig populations as the market discriminates against pork of inferior quality (Wendt *et al*., 2000).

South African pig breeders are further faced with the challenge of breeding leaner pigs. There are approximately 350 commercial pork producers in South Africa. About 50% of the production is used for processing products such as sausages, bacon and other products. South Africa's agricultural statistics show that the consumption of pork has overtaken that of lamb/mutton (www.redmeatsa.co.za/industrystatistics). There is great scope in South Africa to increase pork production and consumption. The genetics of pig breeding is studied intensively in order to produce pork for human consumption that is mainly free of fat. The major gene, *IGF2*, has been shown to be associated with muscle growth in pigs (Buys, 2003; Estélle *et al*., 2005; Oczkowicz *et al*., 2009). The *IGF2* gene is located on porcine chromosome 2 and a SNP in intron 3 of this gene has been reported to have large effects on certain carcass quality traits such as lean meat content (Jeon *et al*., 1999; Nezer *et al*., 1999). The test for the *IGF2* gene, as detailed in Chapter 1, allows for knowing if the pig has the gene for leanness or fatness and accurately detects the genetic mutation associated with meat quality. Both genotypes, homozygous A/A (mutant lean) and G/G (fat allele), are useful in breeding programs, as the 'A' allele is useful in producing leaner pigs and the 'G' allele can be used in sow lines for enhanced sow productivity and fertility (Buys *et al*., 2006; Jafarikia *et al*., 2009). The *IGF2* gene has potential to be included in South African pig breeding programs.

The aim of Phase 1 of this project was to determine the frequency of the *RYR1* and *IGF2* genes in South African pig populations at nucleus and commercial levels.

2.2 Material and Methods

Sample collection

Ethical approval for this study was obtained from the Animal Use and Care Committee (AUCC), reference number EC014-12, in the Faculty of Natural and Agricultural Science at the University of Pretoria as well as the Animal Ethics committee of the Agricultural Research Council, Animal Production Institute (ARC, API). For both the *RYR1* and *IGF2* genotyping, hair with follicles were collected by plucking from the tail side of the body of the animal and stored in paper envelopes marked with the appropriate animal identification numbers.

DNA extraction, quantification and genotyping

RYR1 gene

A total of 439 boars were genotyped comprising of animals from 11 seed stock herds and three AI stations representative of the South African Landrace (SAL), Large White (LW), Duroc, Pietrain, Chester White and Kolbroek pig breeds. For the investigation on a commercial level a further number of 1500 hair samples of randomly selected commercial/slaughter pigs were collected from 15 major pork producers distributed throughout various geographical regions of South Africa. For *RYR1* genotyping, DNA was extracted from the hair roots using a modified Proteinase K digestion method (Higuchi & Bradley., 1998; Soma *et al*., 2013). The license for the MH test is held with the South African Pork Producers organization (SAPPO). The premix PCR solution consisted of the *RYR1* gene specific primers (20 µM), 100 µM each of dATP, dCTP, dGTP, and dTTP, Taq polymerase (SepSci) 0.3mM of MgCl2 buffer and deionized water. The *RYR1* gene-specific primers were 5'- GTTCCCTGTGTGTGTGCAATGGTG-3' (forward) and 5'-ATCTCTAGAGCCAGGGAGCAAGTTCTCAGTAAT-3' (reverse) (United States Patent 6001976). The PCR program used included a denaturing step at 95 ºC for 1 min, followed by annealing of the primers at 58 ºC for 2 min, with an extension step at 72 ºC for 2 min. Forty cycles of this 3-step procedure was performed in a GeneAmp 9700 PCR machine. The samples were run on an acrylamide gel, stained with ethidium bromide and visualization under ultra violet light. Controls with known genotypes as well as no template controls (NTC) were included in each run.

IGF2

Prior to commencement of the *IGF2* screening, a license agreement was secured between Gentec, a Belgian corporation, active in the field of developing and marketing of biotechnological tools for the pig breeding sector, and the ARC, finalized on 12 September 2011. Gentec, the Université de Liége (Belgium) and MELICA H.B. (Sweden) share the ownership of the Technology. The company has exclusive and world-wide license to market and exploit all type of agricultural applications of the *IGF2* gene technology, *IGF2* screening of boars was done at the nucleus level, which included AI stations and breeding companies. A total of 439 boars were genotyped. For phase 2 of the study, additional *IGF2* screening was focused on boars that were active in commercial breeding herds during the period between July and September 2013. A total of 100 boars representing the SAL, LW, Duroc and Chester White pig breeds, from two South African pig breeders who were interested in participating in the study, were further genotyped. Both breeders have all four breeds as part of their breeding herds. Similar systems are employed, that is, housing type and feeding regimes.

For the genotyping, DNA extraction was performed using 10-15 hair roots based on the protocol of Sambrook *et al*., (1989) and adapted as described by Some *et al*., 2014. The DNA was quantified using the Qubit® 2.0 fluorometer instrument (Invitrogen). The protocol for dsDNA BR Assay kit was used (Invitrogen) and the DNA concentrations ranged from 1.07 µg/mL to 71.0 µg/mL. Primer Express software version 3.0.1 (Applied Biosystems®, Foster City, California) for primer and probe design was used. The Taqman SNP assay was custom designed by Applied Biosystems. The primers used were *IGF2* F (AGCCAGGGACGAGCCT) AND *IGF2* R (GAGGCCCGCGGACTC) which amplified at 106 bp of the pig *IGF2* gene. ROX, an internal standard dye, is contained in the reaction mix. An allelic discrimination assay was performed using the ABI Prism 7500 Real Time PCR machine (Applied Biosystems®, Foster City, California) using the Taqman® Universal PCR Master Mix without AmpErase. Reactions were performed in a total volume of 25 µL and according to the Taqman® Universal PCR Master Mix protocol. The protocol included two initial steps: 95 °C for 10 min and 92 °C for 15 s (AmpliTaq Gold activation) and 40 cycles of 92 °C for 15 s (denaturation) and 1 min at 60 °C (annealing/extending). Samples with known genotypes (A/A, A/G and G/G) were used as positive controls and a no template control (NTC) was included in each run. The results were analyzed using the sequence detection software v. 2.0 (Applied Biosystems). See Table 2.1 for the interpretation of the three *IGF2* genotypes and Figure 2.1 for the allelic discrimination plot.

Table 2.1 Interpretation of the *IGF2* genotypes using the Custom TaqMan® SNP Genotyping Assay (Applied Biosystems®)

Genotype			**Interpretation**
A/A	*IGF2* +/+	Homozygous	More lean
G/G	*IGF2* -/-	Homozygous	More Fat
A/G	*IGF2* +/-	Heterozygous	Depends: If A from sire, more lean. If A from dam, more fat.

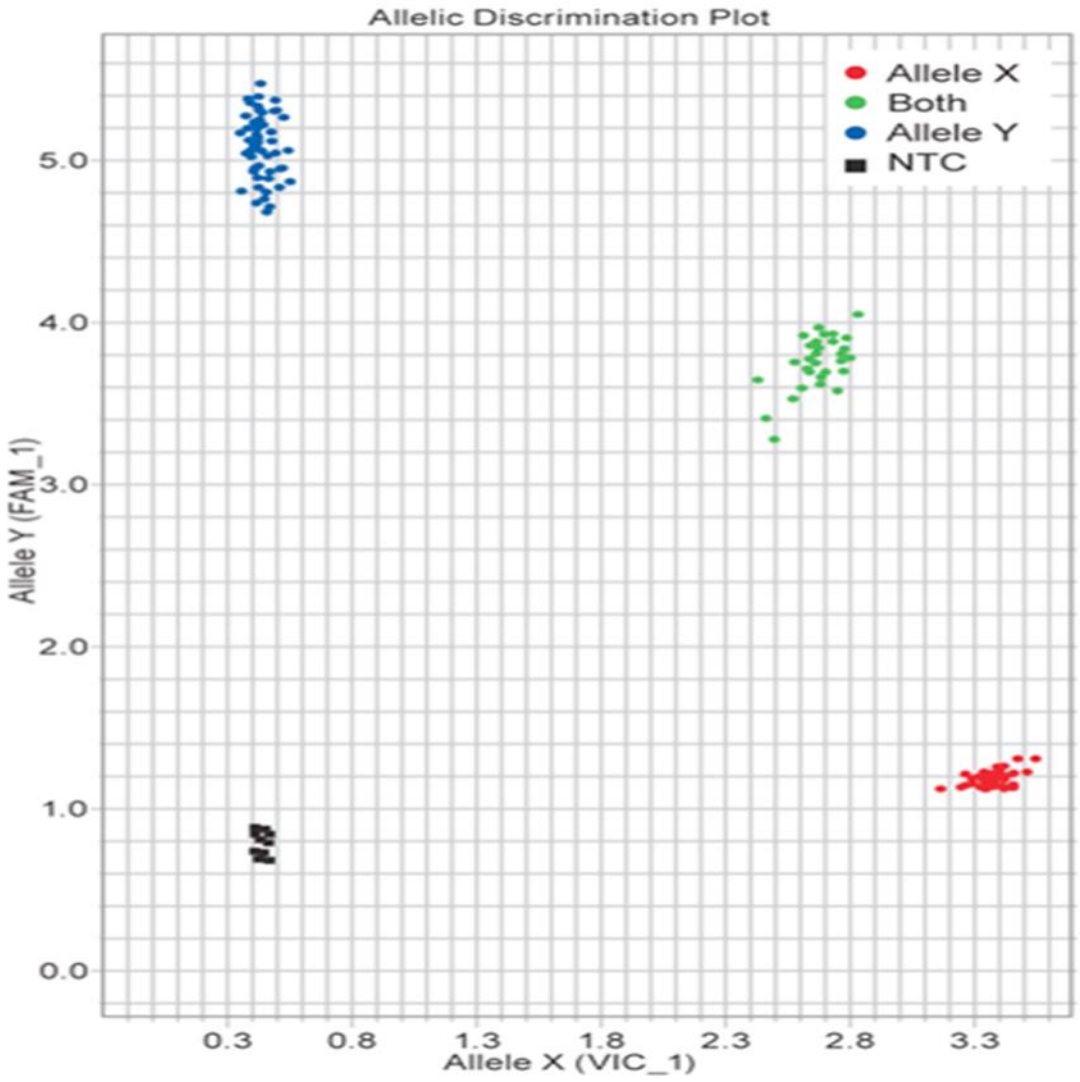

Figure 2.1 *IGF2*: Scatter plot of allelic discrimination assay

There was a request from the South African pig industry to further investigate the *IGF2* genotypes and its role on carcass and meat quality as well as fat and fatty acid characteristics, within the South African Large White and Landrace populations. The identification of the heterozygous A/G boars identified during the additional screening assisted in obtaining the daughters of these boars and the dams. The daughters of these boars were used in the growth trial for phase 2 of this study.

Statistical analyses

The *RYR1* and *IGF2* genotypic data was stored in an Excel database and analyzed with the test for the homogeneity of independent samples (Strasheim *et al*., 1999) using SAS version 9.3 (SAS, 2011) program.

2.3 Results

The percentage of genotypes for the *RYR1* gene observed in the seed stock boars and from AI stations is presented in Table 2.2. Carrier animals (Nn) were low for all breeds, with no carrier animals for the Chester and Kolbroek.

Table 2.2 *RYR1*- gene test results of the South African pig population (Soma *et al*., 2014)

Breed	**No. of Boars**	***RYR1* Test Results**					
		NN	**%**	**Nn**	**%**	**nn**	**%**
SA Landrace	90	85	94	5	6	-	
Large White	158	157	99	1	1	-	
Duroc	42	42	100	-		-	
Pietrain	4		-	3	75°	1	25°
Chester	3	3	100°	-		-	
Kolbroek	11	11	100°	-		-	
Synthetic lines	131	123	94	8	6	-	
Total	439	421		17		1	

°Percentage based on low sample size

The number of carriers and non-carriers of pigs obtained from abattoirs is shown in Table 2.3. Results from the pigs slaughtered at the various abattoirs indicated that 96.4% of the pigs tested did not carry the mutation. Fifty one (3.4%) of the pigs were carriers (Nn) and three animals (0.2%) were homozygous (nn), having inherited a copy of the mutation from both parents.

Table 2.3 Overall *RYR1* results from pigs sampled at abattoirs in South Africa

Genotype	Number of animals
NN (Normal)	1446
Nn	51
nn	3
Total	1500

Statistical analyses using the test for homogeneity of independent samples (Strasheim *et al*., 1999) indicated that there are no significant differences with regard to the prevalence of the *RYR1* gene in the Duroc and South African Landrace breeds. The Composite, Pietrain and Large White breeds showed that there are differences amongst the *RYR1* gene in these breeds compared to the *RYR1* gene over all the breeds (Table 2.4).

Table 2.4 Test for homogeneity of independent breeds from AI stations and nucleus herds, showing *P*-values (Strasheim *et al*., 1999)

Breed	P-value
Chester	1.0000
Composite	0.0271
Duroc	0.3798
Kolbroek	1.0000
Large White	0.0193
Pietrain	<0.0001
South African Landrace	0.7532

The *IGF2* representation in the South African pig population is presented in Table 2.5. Only 11.4% of the animals tested had the A/A genotype (mutant). About 65% of the animals tested carried the G/G genotype (wildtype). The Kolbroek, which is an indigenous breed to South Africa showed a higher frequency of the A/A genotype. The Pietrain and Duroc breeds showed 100% and 95% of the G/G genotype respectively.

Table 2.5 Breeds, number of animals tested, *IGF2*-gene status in total population

Breed	**No. of Boars**	***IGF2* Test Results**					
		A/A	**%**	**G/G**	**%**	**A/G**	**%**
SA Landrace	91	21	23	32	35	38	42
Large White	145	13	9	100	69	32	22
Duroc	38	-	-	36	95	2	5
Pietrain	24		-	24	100*	-	-
Chester	3	-	-	2	67*	1	33°
Kolbroek	11	9	82*	2	18*	-	
Synthetic lines/Composite	100	4	4	75	75	21	21
Total	412	47		271		94	

*Percentage based on low sample size

The representation of the A/G genotype from the additional 100 boars screened is shown in Figure 2.2. Twelve boars, six from each breeder, with the heterozygous A/G genotype were identified. The G allele frequency was found to be higher in the LW population compared to the SAL. The LW population also showed a larger number that inherited the A/G genotype.

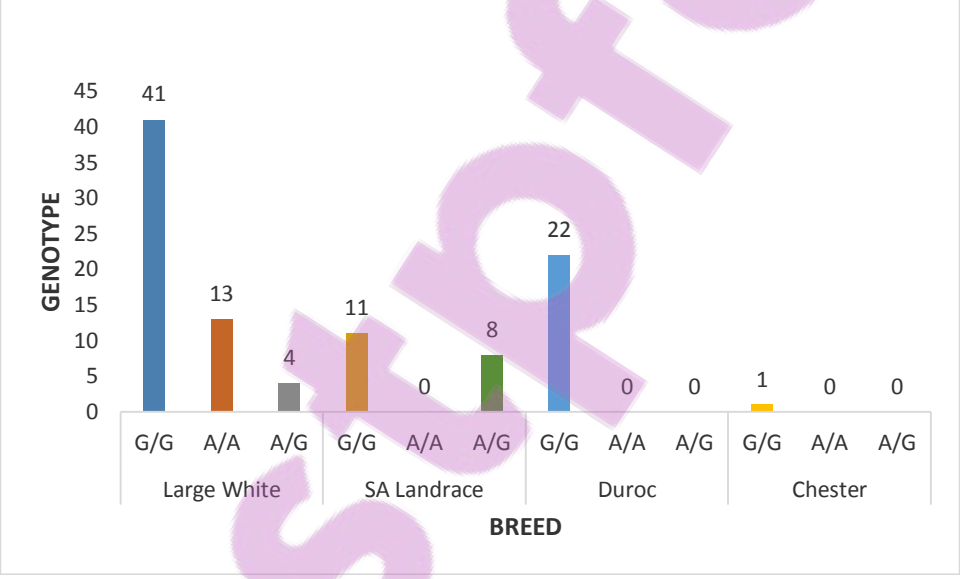

Figure 2.2 *IGF2* genotypes of 100 breeding boars from four breeders

The SAL and LW populations used in this study for the measurement of meat and fat quality traits from the 2 breeders are shown in Figure 2.3. Both homozygous *IGF2* genotypes had higher frequencies in animals received from breeder 1.

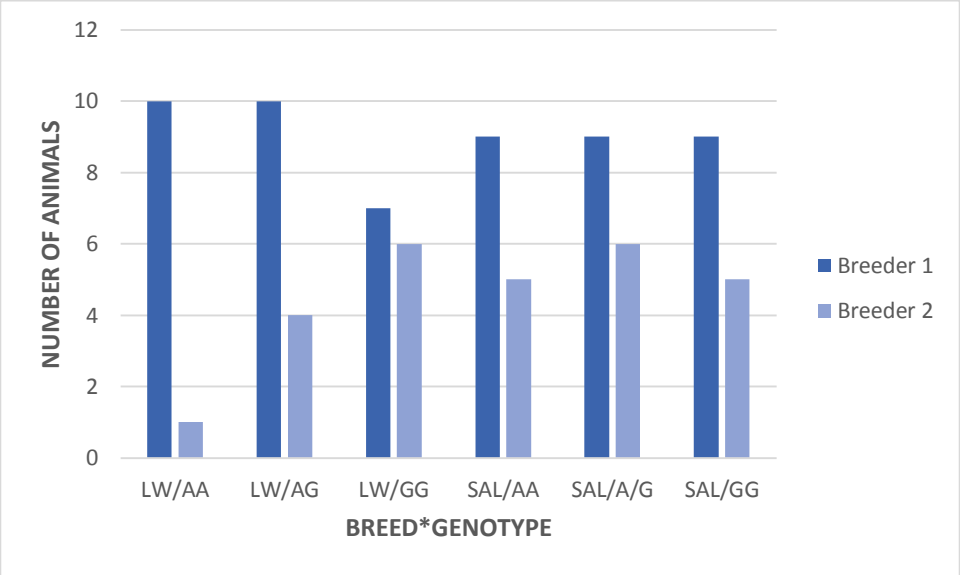

Figure 2.3 Representation of animals from the SAL and LW populations from the two stud herds

2.4 Discussion

The *RYR1* gene status, mutant allele (n), in the boars from the seed stock herds and AI stations was low. The Duroc, Chester and Kolbroek breeds tested were all homozygous (NN) animals. The number of carriers (Nn) was 6% in the SAL and only 1% in the LW population. The synthetic breeding lines had the highest percentage of carriers when compared to the other populations investigated, while only one recessive (nn) animal was identified in the Pietrain population. Some of the pig populations under review showed that there are breed effects, which in some cases are related to the presence or absence of the *RYR1* gene. Breeds such as the Pietrain with outstanding carcass characteristics tend to have a higher incidence of carriers (Monin *et al*., 1980). Most breeders are aware of the negative effect of the *RYR1* gene on meat quality and aim to avoid importation of carrier animals (SAPPO-Global Meat News.com). DNA testing is an essential tool for control of *RYR1* genes in the herd. There was a marked difference in the incidence of carriers of the mutation observed in samples from different producers, ranging from 0% to 12.7%. This, however, may reflect different approaches with regard to breeding policy, as the three Pietrain animals that inherited the mutation (Nn), did not originate from producers where the incidence of carriers was high (>10%). The adverse effects of pork obtained from non-carriers of the *RYR1* mutation (NN individuals) as a result of transport stress are well documented (Nyberg *et al*., 1998; Hambrecht *et al*., 2004). The use of

this information can contribute to future management and informed breeding programs to effectively reduce or eliminate the *RYR1* gene. The South African Department of Agriculture, Forestry and Fisheries (DAFF), has policies in place where the criteria amongst others, including *RYR1*-gene free, for the import of semen and embryos is regulated, and no imports are authorized without fulfilment of this requirement (SA Studbook; Pig Breeders Society, DAFF, 2014).

For the *IGF2* screening, the frequency of the homozygous G/G was high in the LW and Pietrain populations, with frequencies of 69% and 100% respectively. This indicates that the G allele has already been fixed in some of the breeding populations. Although genetic differences among breeds and lines can be important, there is also a large amount of genetic variability among individuals within a breed or line. Differences among individual sires within a population, for example, can often be much larger than differences between populations (Zhang & Plastow, 2011). The heterozygous A/G and A/A genotypes had the highest frequency in the SAL population, with frequencies of 42% and 23% respectively, within the AI and nucleus herds. From the Duroc boars tested in this study, 95% had the G/G genotype. This is in agreement with the general description of the Duroc breed, being recommended for use as a terminal sire due to its meat quality characteristics (Visser, 2003). The result of the Duroc pigs in this study were contrary to that reported by Ruan *et al*. (2013), where the favorable A allele increasing lean production was almost fixed. It was in agreement where the commercial Chinese Landrace and Large White had high frequencies of the A/A allele. The results are also in accordance with findings reported by Yang *et al*. (2006) and Ojeda *et al*. (2008). Jungerius *et al*. (2004) reported frequencies of over 80% for the A allele in three Large White lines but the allele was not fixed in either line. The contrast of the mutant allele and high allele frequencies in all three lines where the mutation was present suggests that once the mutant allele is present within a population, it reaches high allele frequencies due to the positive selection for lean growth.

In the 100 boars screened from the four breeders, the Chester was not a popular breed whereas the Duroc is often used in breeding programs (Visser, 2003). From the *IGF2* screening to produce progeny, it was apparent that breeders are already selecting for leaner pigs (A/A), as the frequency of the A/A genotype was higher from animals received from breeder 1 compared to breeder 2. Fontanesi *et al*. (2010), reported balanced *IGF2* allele frequencies in the Italian Large White pigs. A wide range of different *IGF2* allele frequencies were observed when comparing other Landrace populations (Ojeda *et al*., 2008). This may reflect different selection strategies and use of the paternal and maternal Landrace lines in crossbreeding programs.

2.5 Conclusions

From the results presented in this study, it is apparent that the prevalence of the *RYR1* gene, 'n' allele in the pig populations sampled is low. The differences observed between the two SAL and LW populations with the different *IGF2* genotypes may be a result of different selection strategies employed at the respective farms where the animals originated from. This chapter demonstrated the frequencies of only two major genes influencing lean meat growth in the South African pig population. As indicated in Chapter 1, additional genetic markers (*RN* , CAST) exist with effects on meat quality. The search for new genetic markers can identify other genes involved in muscle development. The usefulness of new markers for pig production must always be evaluated to prevent economic losses when meat quality is affected. Thus, with the identification of new markers, meat quality should be assessed. The increasing availability of commercial tests provides opportunities for producers to customise the use of genetics for their selection objectives and breeding programs.

CHAPTER THREE: THE EFFECT OF THE *IGF2* GENE ON MEAT AND FAT QUALITY TRAITS IN SOUTH AFRICAN LARGE WHITE AND LANDRACE PIG POPULATIONS

3.1 Introduction

One of the major objectives in animal production has been to increase meat yield of livestock species. This has been achieved by applying quantitative animal breeding methodology over the past 50 years (Dekkers, 2012). Selection based on phenotypic performance recording, pedigree and estimated breeding values have been most effective, but there are some limitations with regards to traits that are sex-limited, costly and difficult to measure (Wood *et al*., 2004; Rothschild *et al*., 2007). The availability of genomic tools however, holds the potential to further purposefully improve selection strategies.

Pig producers have interest in both growth and carcass traits with a focus on lean meat yield. A number of studies have reported Quantitative trait Loci (QTL) on almost all chromosomes of the pig for traits such as growth, carcass and meat quality (Hu *et al*., 2005; Meyers *et al*., 2007; Cherel *et al*., 2011). In particular, chromosome 2 of the pig has been widely investigated and Insulin-like growth factor 2 (*IGF2*) gene has been implicated to have an effect on carcass leanness and meat quality. Based on its physiological functions, the *IGF2* gene has been considered a candidate gene for a QTL affecting muscularity in pigs. This QTL has important practical implications for the South African pig industry since it is an imprinted gene and only the paternal allele is expressed in the progeny, regardless of the sow's genotype. The use of homozygous sires should be able to increase the lean-meat content in the progeny. A sow's lifetime reproduction has however been reported to be decreased as a result of selection for increased leanness and lowering fat deposition (Brisbane & Chesnais, 1996; Jafarikia *et al*., 2009; Luc *et al*., 2013; Ruan *et al*., 2013). The demand for leaner carcasses by the meat packers and consumers may be in conflict with longevity of sows and may have implications on the replacement costs of sows in breeding programs. The *IGF2* gene can provide a possible solution to this conflict, due to the imprinting mechanism of the gene where sows can be selected for the G^{pat} allele to enhance productivity and sow fertility. However, selection for lean growth are also associated with undesirable effects on meat quality. Thus, new genetic-based selection tools that aim to improve performance traits in pigs has to be accompanied by an evaluation of the correlated effect on pork quality.

In the past few years, pig breeding programs have focused on selection for faster growing pigs and lean meat production, which also resulted in negative effects on meat and carcass quality characteristics (Van den Maagdenberg et al., 2008a; Aarestrup, 2012). Mutations that improve carcass leanness are often associated with reduced meat quality such as the ryanodine receptor (*RYR1*) mutation and the Rendement Napole (*RN*) mutation that increase loin eye area and reduce back fat depth but reduce loin quality by reducing water holding capacity (WHC) affecting the juiciness of meat (Oliver *et al*., 1994; Rosenvold &

Andersen, 2003). The effects of the *RYR1*, commonly known as the *MH* gene, on carcass and meat quality traits have been widely described (Hamilton *et al*., 2000; Soma *et al*., 2014; Czyzak-Runowska *et al*., 2015). It has been associated with an increased lean meat content in pigs but also with the undesirable pale, soft and exudative (PSE) meat (higher drip loss). Besides mutations with negative effects, genes such as the *IGF2* has been identified with a positive impact on lean yield without the negative effects on meat quality. This single nucleotide polymorphism (SNP) within intron 3 of *IGF2* (van Laere *et al*., 2003) gene accounts for up to 30% of the variation in muscle mass and a reduced back fat deposition.

Meat quality can differ to some extent between breeds and genotypes. Therefore, the relationship between performance traits and carcass conformation and meat quality is a critical issue in animal production (Sellier, 1998). At heavier weights, the quality of pork could be better in terms of tenderness, flavour and juiciness due to an increased intramuscular fat content. However, increased slaughter weight is accompanied by decreased feed efficiency and reduced lean deposition (Latorre *et al*, 2004; Chu *et al*., 2012; Swantek *et al*., 2013). The lean to fat ratio is important in determining carcass quality. Other important carcass quality traits include dressing yield, carcass length and proportion of carcass cuts such as loin, ham, shoulder and belly. Since most of the fat on pork cuts sits on the outside, it can be easily removed as there is very little fat inside the muscle fibres (Wood *et al*., 2003; Alonso *et al*., 2012). Pork fat quality determines the product in which the fat can be used. The amount of fat in the carcass and muscle influences the fatty acid composition (Wood *et al*., 2008). It is estimated that around half of all South African pork is utilized by the meat processing industry to manufacture bacon, sausages, hams and other meat products. Fat is one of the most favorable raw materials in processed products and is important in the processing, textural and sensory characteristics of processed products (Leick *et al*., 2010). Consumer attitude towards pork are often influenced by sensory attributes such as flavor, tenderness and juiciness, in addition to the biochemical and physical parameters such as water holding capacity, shear force, pH_u and intramuscular fat content (Bonneau & Lebret, 2010). Good fat quality is thus imperative to the South African processing industry to ensure good quality finished pork products. The association of *IGF2* gene on pork quality in the South African pig populations are unknown and its effect on carcass and meat quality in the South African Large White (LW) and Landrace (SAL) pig breeds have not been studied. The effect of selecting for leaner or fatter carcasses requires improved understanding in order to improve performance traits without compromising meat and carcass quality. The aim of this paper was to evaluate the effect of the *IGF2* genotypes on pork meat quality and fatty acid composition from the South African Large White and Landrace pig breeds.

3.2 Material and methods

Acquisition of animals for IGF2 analyses of meat and fat quality

Twelve boars with the heterozygous A/G genotype identified from the first phase of the *IGF2* screening, were mated with homozygous A/A and G/G dams in order to produce progeny of all three genotypes. Therefore, the offspring that were heterozygous (A/G) for the *IGF2* mutation received and expressed the G^{pat} allele, while homozygous AA offspring expressed the A^{pat} allele. In this study, the *IGF2* allele (A or G) received from the sire, was of primary interest since the *IGF2* gene is paternally expressed. Fifty two pure bred SAL and fifty one LW gilts were selected from two populations. The proportion of animals from the two populations and the representative genotypes is shown in Table 3.1.

Table 3.1 Percentage distribution of SAL and LW, representing two populations, per genotype

Population	% SAL	% LW	% A/A genotype	% G/G genotype	% A/G genotype
1	47	53	35	31	35
2	52	48	14	36	50

Due to limited space availability, the growth of the pigs was conducted in two phases. In the first phase, there were 29 SAL and 30 LW gilts from population 1, with weights between 6 and 32 kg. In the second phase, there were 23 SAL and 21 LW, with higher weights ranging from 11 to 41 kg. All animals were grown out to reach a slaughter weight of about 100 kg during both phases.

Growth and housing

Ethical approval for the study was obtained from the Animal Use and Care Committee (AUCC), (reference number EC014-12), in the Faculty of Natural and Agricultural Science at the University of Pretoria as well as the Animal Ethics committee of the Agricultural Research Council, Animal Production Institute (ARC, API).

The growth phase was conducted at the pig facility of the ARC-API. The animals were housed in 5 x 3 m fully slatted pens with concrete floors, in enclosed and temperature controlled houses. The animals were monitored daily for good health and activity. These observations were recorded daily for the duration of the trial. The animals were fed a custom mix pellet (Grower 1) until they reached a weight of 50 kg, followed by a custom mix meal (Grower 2) until they reached slaughter weight of about 100 kg. Fat, fibre and lysine were a minimum of 2.5%, 5% and 1.1% respectively. Grower 2 protein, calcium and total phosphorous was lower with 16%, 0.65% and 0.5% respectively. The pigs were fed *ad libitum* to meet the

recommended nutrient requirements for growing pigs (NRC, 1998) and water was available throughout the trial. Animals from both breeders received the same feeding treatment. Animals were also weighed and recorded on a weekly basis. Seven animals were lost during the trial due to ill health.

One feed trough and one water nipple per pen was available. Feed intake per pen was monitored with bins weighed before feeding and feed weighed back weekly. Animals (~10 per pen) were weighed and recorded on a weekly basis. Each pen was covered with saw-dust and was changed on a regular basis and/or as soon as it became too wet. In addition, several plastic bottles with stones inside were placed in each pen, to avoid boredom. It was beyond the scope of this study to investigate growth and hence the animals were grown to slaughter weight of 100 kg for further analysis for the effect of the *IGF2* genotypes on meat and fat characteristics. The average starting and end weights per population per breed are summarised in Table 3.2.

Table 3.2 Average starting and end weights per population per breed

Population*Breed	Genotype	Average starting weight (kg)	Average end weight (kg)
Population 1, SAL	SAL/AA	20.0	101.0
	SAL/AG	24.8	102.6
	SAL/GG	19.4	103.2
Population 1, LW	LW/AA	22.3	99.8
	LW/AG	11.5	99.3
	LW/GG	11.4	97.7
Population 2, SAL	SAL/AA	30.9	103.1
	SAL/AG	31.9	89.7
	SAL/GG	23.8	101.4
Population 2, LW	LW/AA	14.8	95.0
	LW/AG	34.5	86.9
	LW/GG	34.5	96.0

Slaughtering and sampling

The animals were slaughtered over a period of two months. Eighty one animals representative of the two populations and two genotypes were used for carcass and meat analysis (Table 3.3). Due to the high cost of analysis, only 60 animals *Longissimus dorsi* (*LD*), backfat (BF) and belly fat were processed for fat and fatty acid analysis. These comprised 10 samples of each of the *IGF2* genotypes for both the SAL and LW breeds.

Table 3.3 Overall representation of animals used for carcass and meat analysis

***IGF2* Genotype**	**LW**	**SAL**
A/A	11	14
A/G	14	14
G/G	14	14

The animals were slaughtered at the ARC, API, Irene abattoir. The pigs were weighed before slaughter (live weight). Transportation of the pigs was done under conditions of minimal stress. Transport to the abattoir was approximately 5 minutes. The pigs were kept in lairage for approximately 1 hour prior to slaughter. Clean water was provided at all times. Animals were slaughtered by electrical stunning at 250 V for 7-10 seconds before exsanguination.

The warm carcass weights were recorded soon after slaughter before the carcasses were hung in a cold storage room for chilling at 4 °C, where they remained for 24 hours. Cold carcass weights were recorded the following day before the carcass was split along the midline. Temperature and pH measurements (Eutech Instruments, CyberScan pH II pH/mV/°C meter) of the *LD* were taken at 1 hour and a final measurement was taken at 24 hours *post mortem* and a sample for fibre typing was taken 1 hour *post mortem* at the *LD*. Samples were collected from belly fat, backfat, and *LD* on the day of slaughter or 24 hours *post mortem*, depending on the purpose of the sample.

Carcass fat and lean meat measurements were taken at the P2 site which is located approximately 59 mm from the midline of the carcass lateral to the head and perpendicular to the last rib, using the Hennessy Grading Probe (HGP) (Aus-Meat, 2000). The probe shaft was inserted fully through the carcass, 90° to the skin, with the plate flat against the surface. Three measurements were displayed, namely, fat depth (mm), lean muscle depth (mm) and lean meat percentage (%). Eye-muscle area (*LD)*, eye muscle lean meat depth and eye muscle fat thickness were measured between 9^{th} and 10^{th} rib by taking and calibrated image from a steak cut at the same P2 site as defined under the Hennessey method using external Olympus video photo adapter mounted on to an Olympus camera.

One hundred gram sample for analysing fibre typing and fibre area was removed from *LD* directly after exsanguination and frozen in liquid nitrogen**.** *LD* samples were collected 24 hours *post mortem* for the following purposes; instrumental colour, water holding capacity (WHC), drip loss on fresh or vacuum-aged samples (1 and 7 days *post mortem*; 2 ± 1°C), meat tenderness, measured by Warner Bratzler shear force (WBSF), and myofibril fragment length (MFL), thiobarbituric acid reactive substances (TBARS) and free thiol content on vacuum-aged samples (1 and 7 days *post mortem*; 2 ± 1 °C) and then frozen at −20 °C

until analyzed. Additional *LD*, backfat and belly fat samples were collected for proximate analyses, and fatty acid composition. Samples for fatty acid composition were sealed in vials under a blanket of nitrogen gas and stored at -20 °C until analyses.

Fibre typing and fibre areas

For histochemical demonstration of succinic dehydrogenase situated in mitochondria, the nitro-blue tetrazolium technique of Bourne and Malaty (1953) was used for determining fibre typing and fibre areas. Fibres were classified under 100 x magnification by means of a video image analyser (Soft Imaging System, Olympus, Japan) into red, intermediate and white fibres according to the intensity of the staining reaction. Figure 3.1 illustrates how fibres were counted and percentages calculated. Fibre cross-sectional areas were also determined with a VIA (Figure 3.2).

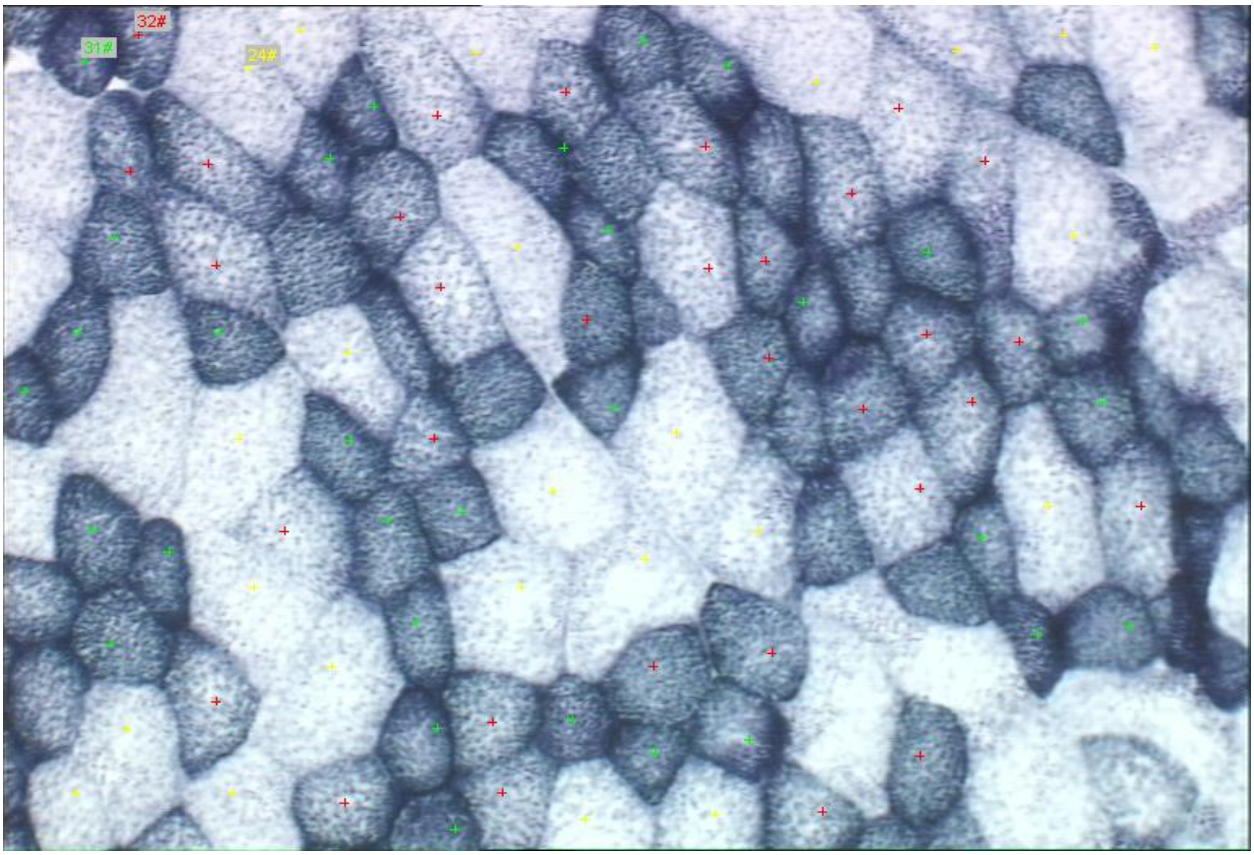

Figure 3.1 Illustration of how red, intermediate and white muscle fibres are counted, from which percentages are calculated (Snyman, 24 January 2014, ARC Meat Industry Centre)

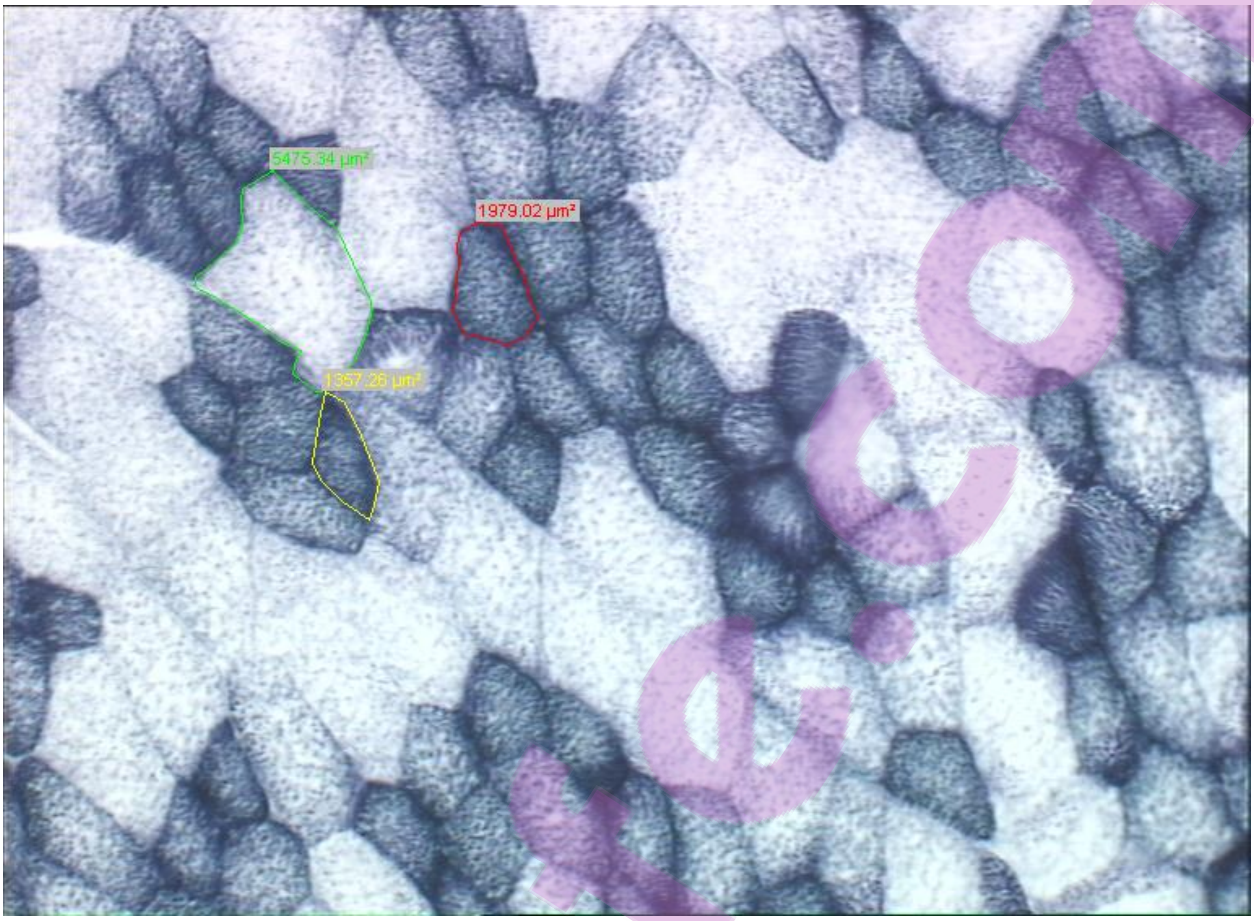

Figure 3.2 Illustration of how fibre areas were measured (Snyman, 24 January 2014, ARC Meat Industry Centre)

Drip loss, water holding capacity and colour

Two cubes of 10 x 10 x 20 mm^3 were cut from the remaining second fresh steaks to determine drip loss of fresh loin muscle. The cubes were suspended on a pin inside a sample bottle (200 mL) ensuring that the meat did not touch the sides of the bottle and stored for 3 days at 2 ± 1 °C. The amount of drip was measured as the difference between the sample mass before and after and was expressed as a percentage of the starting mass. Drip loss or purge for aged samples were determined by measuring the amount of purge remaining in the bag after removing the 30 mm *LD* steak for colour measurement. The steak was removed and lightly dried with tissue paper. Drip was expressed as a percentage of the combined mass of the steak and drip.

Water holding capacity (WHC) is described as the ability of defined sample to retain intrinsic or extrinsic fluids under specified conditions (Jaurequi, 1981). The WHC was determined by calculating the ratio of meat area and liquid area after pressing a 400 – 600 mg fresh meat sample on a filter paper (Whatman 4) sandwiched between two Perspex plates, and pressed at a constant pressure of 300 psi for 60 seconds according to the method described by Irie, *et al.* (1996). The areas were measured by video image analyses using Olympus video photo adapter at a magnification of two.

Instrumental pork colour measured with a Konica-Minolta 600d spectrophotometer which was used with the software package SpectraMagic NX Pro (Konica-Minolta) (CIE. 1978) and drip loss were determined on fresh samples (24 hours *post mortem*) and vacuum-packed aged samples (7 days *post mortem*). The samples were cut into two steaks of ~20 mm thickness and colour was measured on one steak directly after cut and then allowed to bloom for 60 minutes at room temperature (20 ºC) with its freshly cut surface facing upwards before repeating colour recordings. Recordings were done in triplicate at three positions on the steak surface. Colour measurements followed the CIE colour convention (CIE, 1978), where the three fundamental outputs were L*, a* and b*. L* is lightness on a scale of 0 (all light absorbed) to 100 (all light reflected); a* spans from +60 (red) to -60 (green) and b* spans from +60 (yellow) to -60 (blue). Saturation index, also known as Chroma (intensity of the red colour), was calculated as square root of a*2 + b*2 and hue angle (the meat discolouration), defined as tan-1 (b*/a*) that describes the fundamental colour of a substance (MacDougall, 1977). Hue is an indication of discolourisation or brownness – thus the development of met-myoglobin on the surface of the meat. Mean values were used for statistical analysis.

Warner Bratzler Shear Force Measurements

Warner Bratzler shear force (WBSF) samples were frozen at -20 ˚C and then processed into 30 mm steaks by means of a band saw. The frozen steaks were thawed at ± 1 °C for 24 hours and cooked using an oven-broiling (Mielé, model H217, Mielé & Cie, Gütersloh, Germany) method with direct radiant heat (American Meat Science Association (AMSA, 1995). The steaks were broiled at 260 °C (pre-set) to 70 °C internal temperature and cooled down to 18 °C. Six round cores (12.7 mm diameter) were removed from the steaks parallel to the muscle fibres (AMSA, 1995). Each core was sheared once through the centre, perpendicular to the fibre direction, by a Warner Bratzler shear device mounted on an Universal Instron apparatus (Model 4301, Instron Ltd, Buckinghamshire, England; cross head speed = 200 mm/min) and the mean value of the six recordings were used as a shear value.

Myofibril fragmentation

Myofibril fragment lengths (MFL) of *LD* on 1 day and aged 7 days *post mortem* were measured by means of video image analyses (VIA). Myofibrils were extracted according to Culler *et al*. (1978), as modified by Heinze and Bruggemann (1994). One hundred myofibril fragments per sample were examined and measured with an Olympus BX40 system microscope at a magnification of 400X. An illustration on how the MFL's are measured is illustrated in Figure 3.3. The mean values were used for statistical analysis.

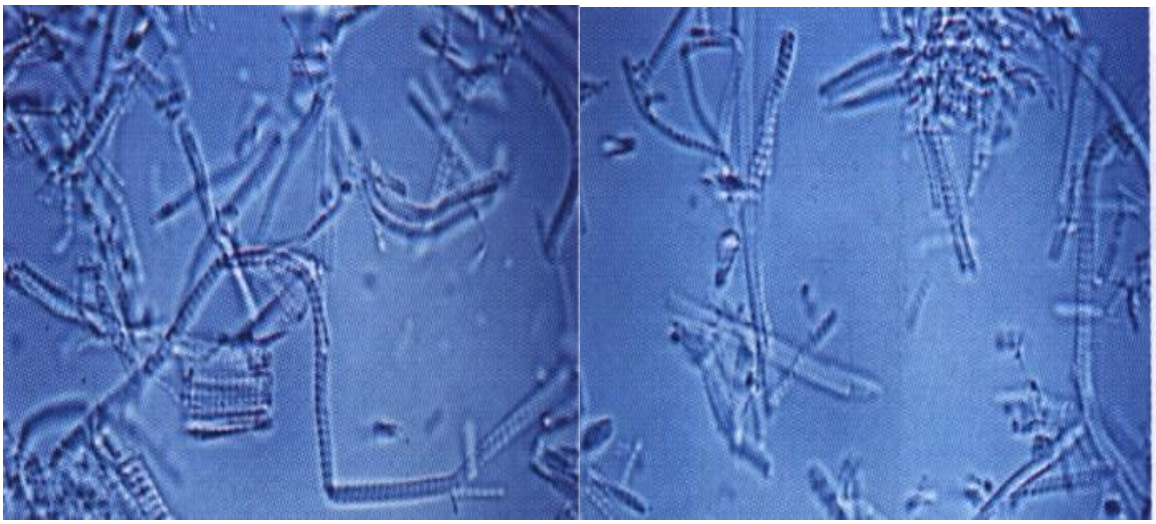

Figure 3.3 Illustration to show how myofibril fragment lengths are measured on 1 and 7 day *post mortem* (Snyman, ARC, Meat Industry Centre)

Free thiol group determination

Intramuscular protein oxidation was measured as the amount of free thiol groups in proteins determined as µM thiol per mg protein (high values indicate low oxidation). Homogenates were prepared by homogenizing 1.0 g of minced sample in 25 mL 5.0% sodium dodecylsulfate (SDS) in 0.10 M Tris-HCl buffer, pH 8.0 (tris(hydroxymethyl) aminomethane) using an Ultra Turrax (T25, IKA-Labortechnik, Janke & Kunkel, Staufen, Germany). The homogenates were then heated in an 80 °C water bath for 30 min and the supernatant was filtered through a paper filter (3HW; Munktell & Filtrak GmbH, Bärenstein, Germany). The protein concentration of the filtrate was determined by measuring absorbance at 280 nm using a standard curve prepared from 0 to 3 mg/mL bovine serum albumin. No absorbance was detected at wavelengths above 300 nm, hence, myoglobin did not interfere with the assay. Free thiol determination was carried out by diluting the filtrates to a concentration of 1.5 mg/mL with 5% SDS in Tris-HCl buffer (0.10 M, pH 8.0). Then the assay was prepared by mixing 0.50 mL diluted filtrate, 2.0 mL Tris-HCl buffer (0.10 M, pH 8.0) and 0.5 mL of 10 mMDTNB (5,5′-Dithiobis (2-nitrobenzoic acid)) in Tris-HCl buffer (0.10 M,

pH 8.0). After 30 min, the absorbance of the samples was measured at 412 nm against an aqueous reference solution of 0.50 mL 5% SDS in 2.0 mL mL Tris-HCl buffer (0.10 M, pH 8.0). Duplicate homogenates and triplicate measurements on each homogenate were made for each meat sample and the mean values used for statistical analysis.

Thiobarbituric acid reactive substances (TBARS)

Intramuscular lipid oxidation was evaluated using 2-Thiobarbituric acid (TBA; 4,6-dihydroxy-2-mercapto-pyrimidin) as described by Raharjo *et al*. (1992). Briefly, 4.0 g of meat was homogenized in 15 mL 5.0% (w/v) aqueous solution of trichloroacetic acid (TCA) for 1 min using an Ultra Turrax homogenizer (T25, IKA-Labortechnik, Janke & Kunkel, Staufen, Germany). The meat slurry was centrifuged at 10,000 g for 10 min and the supernatant was filtered through a paper filter (MN615; Macherey-Nagel, Düren, Germany). 2.0 mL of filtrate was mixed with 2.0 mL 40 mM TBA and incubated at 94±1 °C in a waterbath for 10 min. The absorbance of the red pigment formed was scanned from 400 to 600 nm (DU 7500 Beckman, Beckman Instruments Incorporated, Fullerton, California, USA). Results are expressed as 2-thiobarbituric acid reactive substances (TBARS) in mg malondialdehyde per kg meat using a standard curve prepared from 1,1,3,3-tetraethoxypropane (malonaldehyde). A 78% recovery value of malondialdehyde-TBA complex was used in the final calculations. Mean values of two independent determinations were used for statistical analysis.

Lipid extraction and fractionation (BF, belly fat and LD)

Extraction of total lipids from the muscle (approximately 5 g) and fat tissue (approximately 1 g) was performed quantitatively, according to Folch *et al*. (1957), using chloroform and methanol in a ratio of 2:1. Butylated hydroxytoluene (BHT) was added to the chloroform:methanol mixture as an antioxidant, at a concentration of 0.001%. The extracts were dried under vacuum in a rotary evaporator and further dried in a vacuum oven at 50 ºC for 3 h, with phosphorus pentoxide, as a moisture adsorbent. Total extractable fat content (EFC) was determined by weighing and expressed as % fat (w/w) per 100 g tissue. The fat free dry matter (FFDM) content was determined by weighing the residue on a pre-weighed filter paper, used for Folch extraction after drying. By determining the difference in weight, the FFDM could be expressed as % FFDM (w/w) per 100 g tissue. The moisture content of muscle and fat tissue was determined by subtraction (100% -% lipid - % FFDM) and expressed as % moisture (w/w) per 100 g of tissue.

Fatty acid analysis (BF, belly fat and LD)

Total lipid (± 10 mg) was methylated to prepare fatty acid methyl esters (FAME) for gas chromatographic (GC) analysis by using methanol-BF3 (Slover and Lanza, 1979). FAME were quantified, using a Varian 430 flame ionization gas chromatography (GC), with a fused silica capillary column (Chrompack CPSIL 88, 100 m length, 0.25 mm ID, 0.2 µm film thicknesses). Analysis was performed using an initial iso-thermic period (40 °C for 2 min.). Thereafter, the temperature was increased at a rate of 4 °C/min. to 230 °C. Finally, an iso-thermic period of 230 °C for 10 min. followed. FAME's, in n-hexane (1 µL), were injected into the column, using a Varian CP-8400 auto-sampler. The detector and injection port were both maintained at 250 °C. Hydrogen, at 45 psi, functioned as the carrier gas and nitrogen was used as the makeup gas. Galaxy Chromatography Data System Software recorded the chromatograms. Identification of sample FAME was made by comparing the relative retention times of FAME peaks from samples, with those of standards obtained from Supelco (Supelco 37 Component Fame Mix 47885-U, Sigma-Aldrich Aston Manor, Pretoria, South Africa). Nonadecanoic acid (C19:0) (SIGMA N553377 – 1G) was used as the internal standard to improve quantitative FAME estimation. FA data were used to calculate the following ratios of FA's: total SFA's total MUFA's; total PUFA's; PUFA/SFA; Δ9 desaturase index (C18:1c9/C18:0); total omega-6; total omega-3; the ratio of omega-6 to omega-3 (n-6)/(n-3) FAs. Atherogenicity index (AI) was calculated as: AI = (C12:0 + 4 x C14:0 + C16:0)/(MUFA + PUFA) (Chilliard *et al*., 2003). Double bond index (DBI) was calculated as: DBI = Σ % of UFA x number of double bonds of each UFA (Alam and Alam, 1986). Iodine value (IV) for fat was calculated from FA data according to Ham *et al*. (1998).

Statistical Analyses

The experimental design was a completely randomised design. The treatment design was a factorial with two factors i.e. 3 genotypes (A/A, G/G and A/G) and the two breeds (LW and SAL). When data was collected over time as repeated measurements the time factor was included as a sub-plot factor in the analysis of variance (ANOVA). All data collected were subjected to an appropriate analysis of variance. Fisher's protected t-LSD (Least Significant Difference) was calculated to compare treatment means of significant effects (Snedecor & Cochran, 1980). All the analyses were done using SAS v9.3 statistical software (SAS, 1999). The residuals were examined for deviations from normality, and outliers causing skewness were removed.

3.3 Results

Weight measurements, Dressing %, % Temperature loss, % Drip loss and Water holding capacity (WHC), (Breed, Genotype and Breed*Genotype interaction)

The breed effect on carcass weights, WHC, dressing percentage, percentage drip loss and percentage temperature loss are presented in Table 3.4. There were no significant differences ($P>0.05$) observed for live animal weight, warm and cold carcass weights and dressing percentage between the SAL and LW breeds. Percentage drip loss of the SAL was significantly different ($P<0.05$) to the LW with a percentage drip loss of 6.63% compared to 5.57%. The percentage temperature carcass loss was higher in LW, 2.07%, compared to 1.82 in SAL. There was significant breed differences observed between the LW and SAL for WHC ($P<0.05$), with a mean value of 0.30 for LW and 0.28 for SAL.

Table 3.4 Effects of breed on carcass weights, Dressing %, % Temperature loss, % Drip loss and Water holding capacity (WHC)

Trait	Breed		*P*-Value
	LW (Mean±Std Dev)	SAL (Mean±Std Dev)	
Live weight (kg)	99.16±6.93	100.90±5.94	0.231
Warm carcass weight (kg)	83.69±5.72	85.20±5.57	0.226
Cold carcass weight (kg)	81.96±5.70	83.69±5.54	0.167
Dressing %	82.68±1.85	82.73±2.15	0.912
% Temperature carcass loss	2.07±0.22[a]	1.82±0.36[b]	0.001
% Drip loss	5.57±2.34[b]	6.63±2.18[a]	0.044
Water holding capacity	0.30±0.05[a]	0.28±0.04[b]	0.031

[a],[b] = Different superscripts within the same row differ significantly ($P<0.05$)

In Table 3.5 the genotype effect on carcass weights, dressing percentage, percentage temperature carcass loss, percentage drip loss and water holding capacity are shown with no significant differences in live weight between the three genotypes. There were however, differences ($P<0.05$) between the warm and cold carcass weights with the A/A genotype having a lower warm carcass weight and lower cold carcass weight compared to the G/G genotype for both traits. There were no significant genotype differences for dressing percentages and WHC.

Table 3.5 Effect of genotypes on Carcass weights, Dressing %, % Temperature loss, % Drip loss and Water holding capacity (WHC)

Trait	Genotype			*P*-value
	A/A	A/G	G/G	
Live weight (kg)	98.8±5.0	99.3±6.2	102.2±7.7	0.117
Warm carcass weight (kg)	83.0±4.7^{b}	83.9±4.1^{ab}	86.6±6.7^{a}	0.052
Cold carcass weight (kg)	81.4±4.7^{b}	82.3±5.0^{ab}	84.1±6.7^{a}	0.049
Dressing %	82.4±2.1	82.6±1.8	83.2±2.1	0.321
% Temperature carcass loss	1.96±0.35	1.92±0.34	1.94±0.27	0.833
% Drip loss	6.44±2.40	6.11±2.27	5.76±2.30	0.666
Water holding capacity	0.29±0.04	0.29±0.05	0.29±0.04	0.980

^{a,b} = Different superscripts within the same row differ significantly (P<0.05)

The effect of breed*genotype combination is presented in Table 3.6. The SAL G/G animals were overall the heaviest with a live weight mean value of 104.00 kg and the LW A/A, live weight mean value of 98.18 kg, the lightest, at the time of slaughter. The SAL G/G consequently also displayed higher warm (88.52 kg) and cold (87.02 kg) weights. There was no significant differences (P>0.05) for WHC across the breed*genotype combinations. Dressing percentage was significant (P<0.05) for breed*genotype interaction with the LW A/G and the SAL A/G displaying mean values 83.35% and 81.78% respectively for dressing percentage.

Table 3.6 Breed*Genotype interaction on weight, WHC, Dressing %, % Drip loss and % Temperature loss

Trait	Breed*Genotype						*P*-value
	LW/AA	LW/AG	LW/GG	SAL/AA	SAL/AG	SAL/GG	
Live weight (kg)	98.2±5.05	98.7±7.02	100.5±8.39	99.3±5.04	99.9±5.49	104.0±6.69	0.758
Warm carcass weight (kg)	82.0±4.17	83.9±5.72	84.8±6.83	83.9±5.13	83.8±4.39	88.5±6.34	0.452
Cold carcass weight (kg)	80.3±4.15	82.2±5.70	83.1±6.82	82.3±5.01	82.3±4.44	87.0±6.31	0.450
Dressing %	81.8±1.09^{b}	83.34±1.97^{a}	82.8±2.02^{ab}	82.9±2.60^{ab}	81.8±1.17^{b}	83.7±2.16^{a}	0.022
% Temperature loss	2.12±0.25	2.06±0.22	2.04±0.20	1.83±0.37	1.80±0.40	1.83±0.31	0.894
% Drip loss	5.37±2.21	6.01±2.18	5.25±2.71	7.34±2.25	6.21±2.43	6.36±1.62	0.375
Water holding capacity	0.31±0.04	0.30±0.06	0.30±0.04	0.28±0.03	0.28±0.05	0.28±0.03	0.778

^{a,b} = Different superscripts within the same row differ significantly (P<0.05)

pH and Temperature (Breed, Genotype, Breed/Genotype interaction)

There was no significant breed difference observed for pH_u and temperature from 1 hour to 24 hours *post mortem* between the LW and SAL breeds (Table 3.7). The pH_u and temperature decreased steadily from 1 to 24 hours *post mortem*.

Table 3.7 Breed effects on pH_u and Temperature, 1 hour and 24 hours *post mortem*

	Trait	LW	SAL	*P*-value
pH_u	1 hour *post mortem*	6.11	6.11	0.801
	24 hours *post mortem*	5.29	5.28	
Temperature °C	1 hour *post mortem*	36.07	36.75	0.127
	24 hours *post mortem*	3.89	3.60	
pH / Temperature	1 hour *post mortem*	0.19	0.19	0.596
	24 hours *post mortem*	1.62	1.70	

There were no significant difference between the *IGF2* genotypes on pH_u and temperature (Table 3.8). Although not significant, there was a difference within the genotypes from 1 hour to 24 hours *post mortem* for the pH/Temperature ratio (Figure 3.4). The A/A genotype had a higher ratio than the other two *IGF2* genotypes at 24 hours *post mortem.*

Table 3.8 Genotype effects on pH_u and temperature, 1 and 24 hours *post mortem*

Trait		**Genotype**			***P*-value**
		A/A	**A/G**	**G/G**	
pH_u	1 hour *post mortem*	6.12	6.11	6.10	0.952
	24 hours *post mortem*	5.28	5.29	5.28	
Temperature °C	1 hour *post mortem*	36.45	36.48	36.36	0.531
	24 hours *post mortem*	3.22	3.93	4.04	
pH / Temperature	1 hour *post mortem*	0.19	0.19	0.20	0.212
	24 hours *post mortem*	1.85	1.57	1.57	

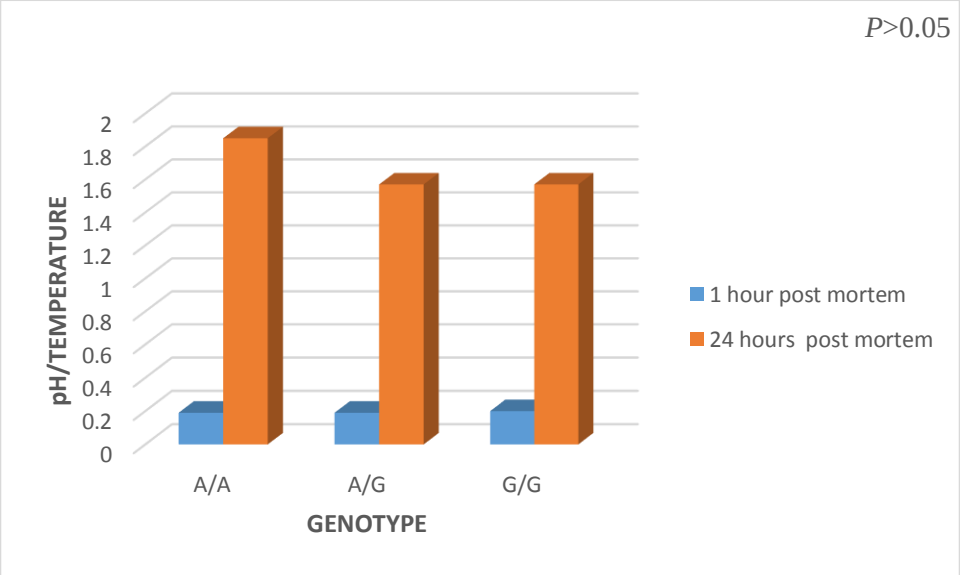

Figure 3.4 pH/Temperature ratio within *IGF2* genotypes

There was a difference ($P<0.05$) for the pH/temperature ratio for breed*genotype interaction (Table 3.9)

Table 3.9 Breed*Genotype interaction on pH_u and temperature

Trait		Breed*Genotype						P-value
		LW/AA	LW/AG	LW/GG	SAL/AA	SAL/AG	SAL G/G	
pH_u	1 hour *post mortem*	6.25	5.96	6.15	6.03	6.25	6.05	0.098
	24 hours *post mortem*	5.32	5.24	5.32	5.25	5.33	5.25	
Temperature °C	1 hour *post mortem*	35.73	36.51	35.89	37.01	36.45	36.82	0.302
	24 hours *post mortem*	2.68	4.06	4.75	3.64	3.81	3.33	
pH / Temperature	1 hour *post mortem*	0.20	0.18	0.20	0.18	0.19	0.20	0.008
	24 hours *post mortem*	2.16^{a}	1.54^{bc}	1.24^{c}	1.61^{b}	1.60^{b}	1.90^{ab}	

^{a,b,c}= Different superscripts within the same row differ significantly (P<0.05)

Eye Muscle (Breed, Genotype and Breed/genotype interaction)

Breed effect on eye muscle (VIA) and Hennessy Grading Probe (HGP) measurements is presented in Table 3.10. There were no significant differences for the eye muscle area (EMA) (P>0.05). The eye muscle fat (EMF) and the total area differed significantly (P<0.05) between the two breeds. The EMF and percentage fat was higher in the SAL breed. There were significant differences observed between the SAL and LW breeds for Hennessy meat percentage, Hennessy fat and percentage temperature loss with the SAL displaying higher mean values for these traits compared with the LW.

Table 3.10 Breed effects on Video Image Analyzer and Hennessy Grading Probe measurements

Trait	LW (Mean±Std Dev)	SAL (Mean±Std Dev)	*P*-Value
VIA			
EMA (mm^2)	5446±557	5350±733	0.506
EMF (mm^2)	1991±631^{b}	2627±514^{a}	<0.001
Total area (meat + fat) mm^2	7437±671^{b}	7977±778^{a}	0.002
% Fat	26.51±7.14^{b}	32.97±5.89^{a}	<0.001
Hennessy Grading Probe measurements			
Hennessy meat, mm	57.55±4.55	55.53±7.03	0.166
Hennessy meat, %	67.18±1.10^{a}	66.35±1.59^{b}	0.001
Hennessy fat, mm	16.38±4.01^{b}	19.71±3.26^{a}	<0.001

^{a,b} = Different superscripts within the same row differ significantly (P<0.05)

The G/G genotype displayed differences (P<0.05) with a mean value of 5658 mm² for EMA. This can be due to the G allele being associated with 'fatter' animals, hence a larger EMA. There were no differences observed for EMF, total area and percentage fat between the different *IGF2* genotypes. The HGP measurements displayed no significant differences (P>0.05) between the three genotypes for Hennessy meat and fat, as well as meat percentage (Table 3.11). Although not significant at P<0.05, there is a trend for Hennessy fat and meat percentage that the A/A genotype differed from the G/G genotype.

Table 3.11 Genotype effects on eye muscle and Hennessy Grading Probe measurements

Trait	**Genotype**			***P*-value**
	A/A	**A/G**	**G/G**	
VIA				
EMA (mm²)	5239±539[b]	5305±615[b]	5658±736[a]	0.052
EMF (mm²)	2378±741	2354±645	2227±582	0.759
Total area (meat + fat) mm²	7617±742	7659±618	7886±950	0.281
% Fat	31.0±7.90	31.0±7.58	28.08±6.01	0.354
Hennessy Grading Probe measurements				
Hennessy meat, mm	56.58±4.04	55.94±6.95	56.92±6.75	0.842
Hennessy meat, %	66.69±2.09	66.86±1.76	67.64±1.89	0.155
Hennessy fat, mm	18.80±4.23	18.59±3.93	16.87±3.62	0.135

[a],[b] = Different superscripts within the same row differ significantly (P<0.05)

Breed*Genotype effect on eye muscle (VIA) and HGP measurements is shown in Table 3.12. The SAL G/G had the highest EMA of 5754 mm² and the SAL A/A the lowest with a mean value of 5107 mm². The LW A/A had the lowest EMF with a mean value of 1783 mm² and the SAL A/A the highest with a value of 2846 mm². For total area, the SAL G/G had the highest mean value of 8237 mm² and the LW A/A the lowest with a mean value of 7191 mm². There was a large difference in total area observed between these two breed*genotype combinations. The LW A/A had the lowest percentage fat with a mean value of 24.6%. The SAL A/A had the highest percentage fat and Hennessy fat (mm). There were no differences observed between the breed*genotype combinations for Hennessy meat (mm). Hennessy meat percentage, Hennessy fat and dressing percentage were significant (P<0.05) for breed*genotype effects. The LW/AA had the highest Hennessy meat percentage of 68.41% compared to the SAL/AA with a percentage of

65.34%. For Hennessy fat, an inverse relationship was observed, where the SAL/AA had the higher measurement of 21.69 mm and the LW/AA the lowest measurement of 15.13 mm.

Table 3.12 Breed*Genotype interaction on eye muscle and Hennessy Grading Probe measurements

Trait	Breed*Genotype						*P*-values
	LW/AA	**LW/AG**	**LW/GG**	**SAL/AA**	**SAL/AG**	**SAL/GG**	
VIA							
EMA (mm²)	5408±549	5361±490	5570±646	5107±511	5253±727	5754±841	0.408
EMF (mm²)	1783±566	2155±702	1991±597	2846±482	2539±548	2483±460	0.077
Total area	7191±611	7516±547	7561±821	7952±674	7793±667	8237±988	0.425
% Fat	24.60±6.92	28.35±8.12	26.13±6.19	35.72±4.52	32.65±6.64	30.19±5.27	0.090
Hennessy Grading Probe measurements							
Hennessy meat, mm	59.29±2.60	55.97±97	57.99±5.15	54.71±3.84	55.92±8.544	55.93±8.06	0.442
Hennessy meat, %	68.41±1.66^{a}	66.92±2.19^{b}	68.31±1.80^{a}	65.34±1.21^{c}	66.81±1.32^{b}	66.97±1.79^{b}	0.008
Hennessy fat, mm	15.13±2.99^{c}	18.26±4.76^{b}	15.33±3.22^{c}	21.69±2.41^{a}	18.91±3.10^{b}	18.40±3.45^{b}	0.002

^{a}, ^{b}, ^{c} = Different superscripts within the same row differ significantly (P<0.05)

The G/G genotype for EMA was significantly (P<0.05) different from the A/G and A/A. Even with the breed/genotype the SAL G/G had a larger area for EMA compared to the LW genotype combinations. With the EMF, the G/G genotype had the higher value for the LW breed. The total area (meat and fat) was the highest for the SAL G/G and lowest for the LW A/A genotype. Accompanied by breed difference, the homozygous A/A genotype and G/G genotypes showed variation for EMA. For percentage fat, a similar pattern showed where the A/A, which is associated with leanness, had the lowest percentage for the LW breed. For the Hennessy meat percentage and Hennessy fat (mm), the *IGF2* genotypes did not have any effect. For breed genotype interaction, the SAL A/A had the highest Hennessy meat percentage which may be attributed to the characteristic 'A' allele. The SAL had a higher Hennessy fat (mm) measurement, compared to the LW.

Fibre Typing (Breed, Genotype and Breed*Genotype interaction)

There were no significant (P>0.05) breed differences for percentage intermediate, white, and red fibres. The genotype effect on fibre typing is presented in Table 3.13. The intermediate fibre areas (Type11A) and red fibre areas (P<0.05) measured in G/G genotype were larger than that of the A/A genotype. Although not significant the white fibre areas of the G/G genotype also tended to be larger than that of the A/A genotype. The G/G genotype had a higher percentage intermediate fibres than that of A/A (P>0.05).

Table 3.13 Genotype effects on fibre typing

Trait	**Genotype**			***P*-value**
	A/A	**A/G**	**G/G**	
Intermediate fibre area (Type11A), µm²	6102±1184[b]	6702±1386[ab]	7157±1847[a]	0.052
White fibre area (Type 11B), µm²	9536±1640	9765±1801	10393±2294	0.261
Red fibre (Type1) area, µm²	3641±663[b]	4004±757[ab]	4302±1031[a]	0.022
% red fibres (Type1)	25.0±5.1	24±4.0	23.0±4.0	0.216
% intermediate fibres (Type11A)	27.40±4.38	29.16±5.29	30.33±3.23	0.088
% white fibres (Type 11B)	47.52±4.54	46.43±5.86	49.62±5.13	0.777

[a], [b] = Different superscripts within the same row differ significantly (P<0.05)

Breed*Genotype effect on fibre typing is shown in Table 3.14. There was no significant difference between the genotypes for intermediate fibre (Type11A), white fibre (Type 11B) areas and percentage red and white fibres (P>0.05). The percentage intermediate fibres was the lowest for the LW/AA group. The red fibre area for both the LW/AA and SAL/AA were approximately the same size with mean values of 3628.09 µm² and 3654.64 µm² respectively. The percentage intermediate fibres was the highest in the LW/GG (30.94%) and the lowest for the LW/AA (26.89%).

Table 3.14 Breed*Genotype effects on fibre typing

Trait	Breed*Genotype						*P*-value
	LW/AA	**LW/AG**	**LW/GG**	**SAL/AA**	**SAL/AG**	**SAL/GG**	
Intermediate fibre area (Type11A), µm²	6119.09±1087.41	6513.00±1041.17	7139.54±1918.52	6089.07±1296.21	6879.33±1664.73	7177.92±1852.58	0.88
White fibre area (Type 11B), µm²	9566.45±1223.44	9542.43±2017.31	10118.23±211.35	9512.29±1953.01	9973.20±1618.24	10691.33±2534.75	0.84
Red fibre (Type1) area, µm²	3625.09±609.10	4192.00±867.90	4018.33±759.27	3654.64±725.20	3816.79±601.97	4586.08±1212.81	0.12
% red fibres (Type1)	26.04±6.67	25.39±4.40	23.35±4.31	24.33±4.57	23.49±3.26	22.64±3.11	0.88
% Intermediate fibres (Type11A)	26.89±.4.16	29.89±5.10	30.94±2.58	27.80±4.66	28.49±5.55	29.60±3.87	0.58
% white fibres (Type 11B)	47.07±4.67	44.73±5.33	45.71±5.23	47.87±4.57	48.02±6.05	47.70±5.03	0.68

Colour (Breed, Genotype and Breed*Genotype interaction)

Colour (24 hours post mortem)

The effect of breed on colour measured 24 hours *post mortem* directly after cut (no blooming) is presented in Table 3.15. L*, b* and Chroma displayed significant differences ($P<0.05$) between the SAL and LW. For all these measurements, the SAL had higher values (meat was lighter, showed more yellowness characteristics and the red colour was less intense).

Table 3.15 Breed effects on colour, 24 hours *post mortem*

Colour trait	LW (Mean±Std Dev)	SAL (Mean±Std Dev)	*P*-value
L*, 0 hour	50.68±3.08[b]	53.73±4.91[a]	0.001
a*, 0 hour	0.37±1.00	0.73±1.17	0.115
b*, 0 hour	9.52±1.25[b]	10.40±1.87[a]	0.008
Chroma, 0 hour	9.59±1.28[b]	10.49±1.91[a]	0.008
Hue, 0 hour	88.23±5.74	86.67±6.07	0.214

[a], [b] = Different superscripts within the same row differ significantly ($P<0.05$)

The *IGF2* genotypes had no effect on the colour measurements performed 24 hours *post mortem*, directly after cut (Figure 3.5).

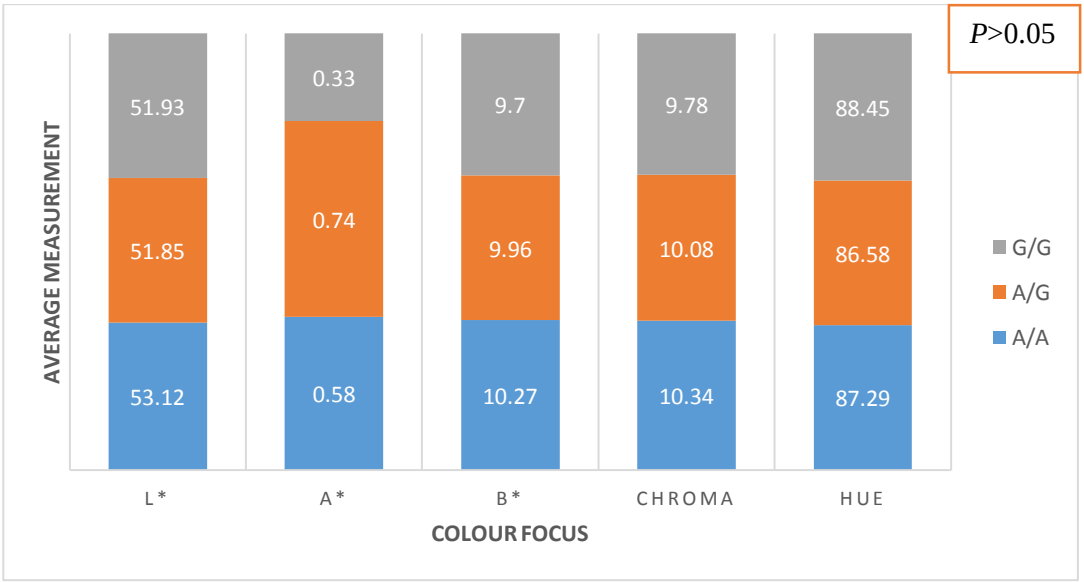

Figure 3.5 Genotype effects on colour parameters measured 24 hours *post mortem* directly after cut

The effect of breed*genotype interaction on colour parameters measured 24 hours *post mortem* directly after cut is presented in Table 3.16. There was no effect of breed*genotype interaction on a* and Hue. The SAL A/A had the highest L* value of 54.73 compared to the LW/AA. For b*, there was no difference between the *IGF2* genotypes within the LW breed. For b* and Chroma, the SAL A/A had the highest values of 10.89 and 10.97 respectively. The SAL A/A, A/G and G/G did not differ in terms of L* although they all were overall lighter compared to LW. LW was overall darker than SAL, but LW/GG was the darkest even significantly darker than LW/AA and LW/AG, which did do not differ from each other.

Table 3.16 Breed*Genotype interaction on Colour 24 hours *post mortem*

Colour trait	LW A/A	LW A/G	LW G/G	SAL A/A	SAL A/G	SAL G/G	*P*-value
L*, 0 hour	51.07±3.77	51.07±2.54	49.93±2.93	54.73±5.42	52.60±4.16	53.92±5.05	0.729
a*, 0 hour	0.34±0.86	0.43±1.22	0.33±0.88	0.77±1.02	1.05±1.32	0.32±1.07	0.735
b*, 0 hour	9.48±1.08	9.68±1.46	9.38±1.17	10.89±2.29	10.24±1.49	10.03±1.68	0.540
Chroma, 0 hour	9.54±1.09	9.76±1.53	9.45±1.16	10.97±2.33	10.38±1.58	10.11±1.69	0.547
Hue, 0 hour	88.25±5.20	88.32±6.57	88.10±5.42	86.54±4.90	84.90±6.71	88.80±6.01	0.629

Colour measurements for 1 day and 7 days post mortem, breed effect

The effect of colour on breed for 1 and 7 days *post mortem* is presented in Figure 3.6. All colour focus parameters were significant ($P<0.05$) for breed* 1 and 7 days interaction. L*, a*, b* and Chroma values were the highest on 7 days *post mortem* for both the SAL and LW breeds. There was an increase in these values from 1 to 7 days *post mortem*. Hue values decreased for both the SAL and LW breeds from 1 to 7 days *post mortem*. There was much variation between the two breeds on 1 and 7 days *post mortem* for the L* and a* measurements. For b* and Chroma, the SAL on 1 day *post mortem* and the LW 7 days *post mortem* were similar with mean values of 11.43 and 11.40 for b* and 11.57 and 11.66 for Chroma. The hue mean values for LW 1 day *post mortem* was 85.29 and 84.44 for the SAL 1 day *post mortem.*

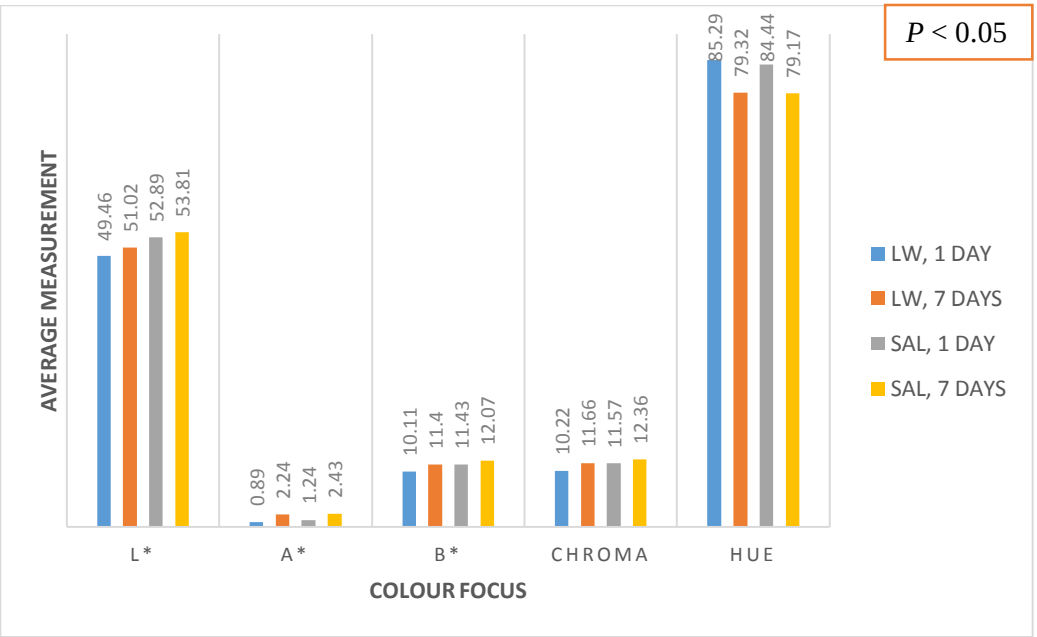

Figure 3.6 Colour measurements, 1 and 7 days *post mortem*, breed effects

The effect of genotype on colour measurements, 1 and 7 days *post mortem* are presented in Table 3.17. The A/A 7 days *post mortem* had the highest mean value of 53.43 for L* and showed the most variation from the A/G and G/G genotypes. The A/G genotype on 1 day *post mortem* had the lowest L* mean of 50.97. There was no variation between the A/A and G/G genotypes for a* on 1 day *post mortem*. These genotypes also had the lowest a* mean values of 1.01 and 0.88 respectively. The A/G and A/A 7 days *post mortem*, had the highest a* mean values of 2.57 and 2.43 respectively. For b*, the A/G and G/G, 1 day *post mortem* showed no variation with means of 10.75 and 10.65. The A/A 7 days *post mortem* had the highest b* mean value of 12.14. For Chroma, no variation was observed between the three different genotypes for 1 day *post mortem*. For 7 days *post mortem*, the A/A and G/G showed variation with Chroma mean values of 12.40 and 10.74 respectively. There was an increase in Chroma measurements from 1 to 7 days *post mortem*. For hue, measurements decreased from 1 to 7 days *post mortem*. The homozygous genotypes showed no variation, with hue means of 85.68 and 85.11 for 1 day *post mortem*. Both homozygous genotypes decreased on 7 days *post mortem*, with a hue mean value of 80.55 for G/G.

Table 3.17 Genotype effects on colour measurements, 1 and 7 days *post mortem*

Colour trait		**A/A**	**A/G**	**G/G**	***P*-value**
L*	1 day *post mortem*	51.36	50.97	51.49	0.101
	7 days *post mortem*	53.43	51.91	52.23	
a*	1 day *post mortem*	1.01	1.30	0.88	0.157
	7 days *post mortem*	2.43	2.57	2.01	
b*	1 day *post mortem*	11.03	10.75	10.65	0.467
	7 days *post mortem*	12.14	11.72	11.42	
Chroma	1 day *post mortem*	11.14	10.90	10.74	0.436
	7 days *post mortem*	12.40	12.04	11.66	
Hue	1 day *post mortem*	85.11	83.86	85.68	0.471
	7 days *post mortem*	79.01	78.27	80.55	

The breed*genotype interaction on colour 1 and 7 days *post mortem* is presented in Table 3.18. For L*, the LW/AA had the lowest mean value of 48.14 and the SAL A/A the highest mean value of 53.88 for 1 day *post mortem*. There was a slight increase in L* mean values from 1 to 7 days *post mortem* across all genotypes. The LW/AA genotype on 1 day *post mortem* had the lowest L* mean value of 0.64. The G/G genotype for both breeds showed no variation with a L* mean value of 2.01. There was an estimated two-fold increase in a* measurements from 1 to 7 days *post mortem* across all breed*genotype combinations. The measurements from 1 to 7 days *post mortem* increased across all breed*genotype combinations for b* and Chroma measurements. The SAL/AA 7 days *post mortem* had the highest mean value of 12.40 for b* and the LW A/A had the lowest mean value of 9.63. For Chroma, the SAL/AA 7 days *post mortem* had a mean value of 12.67 and the LW/AA 1 day *post mortem* the lowest mean value of 9.72. The mean values increased across breed*genotype combinations from 1 to 7 days *post mortem* for hue. The SAL/GG had the lowest mean value for both 1 and 7 days *post mortem*.

Table 3.18 Breed*Genotype interaction on colour, 1 and 7 days *post mortem*

Colour trait		LW/AA	LW/AG	LW/GG	SAL/AA	SAL/AG	SAL/GG	*P*-value
L*	1 day *post mortem*	48.14	50.25	49.72	53.88	51.64	53.26	0.100
	7 days *post mortem*	51.60	51.21	50.33	54.87	52.55	54.12	
a*	1 day *post mortem*	0.64	1.04	0.93	1.30	1.54	0.84	0.161
	7 days *post mortem*	2.36	2.37	2.01	2.49	2.75	2.01	
b*	1 day *post mortem*	9.63^{g}	10.46^{ef}	10.14^{fg}	12.13^{ab}	11.02^{de}	11.15^{d}	0.003
	7 days *post mortem*	11.80^{bc}	11.43^{cd}	11.02	12.40^{a}	11.99^{abc}	11.83^{abc}	
Chroma	1 day *post mortem*	9.72^{f}	10.57^{e}	10.25^{ef}	12.25^{abc}	11.21^{d}	11.24^{d}	0.004
	7 days *post mortem*	12.07^{bc}	11.72^{cd}	11.26^{d}	12.67^{a}	12.35^{ab}	12.05^{bc}	
Hue	1 day *post mortem*	85.87	84.96	85.15	84.52	82.83	86.22	0.519
	7 days *post mortem*	78.94	78.83	80.17	79.07	77.74	80.93	

^{a}, ^{b}, ^{c}, ^{d}, ^{e}, ^{f}, ^{g} = Different superscripts within the same row differ significantly (P<0.05)

Meat tenderness relatedness measurements in LD (WBSF and MFL) for 1 day and 7 days post mortem (Breed, Genotype, Breed*Genotype interaction)

WBSF was the only characteristic that showed differences (P<0.05) for the *IGF2* genotypes. The A/G genotype had a WBSF mean value of 5.39 kg and the G/G genotype a WBSF mean value of 4.92 kg. The MFL was significant (P<0.05) for breed. The results for breed effect on WBSF and MFL, 1 and 7 days *post mortem* is shown in Figure 3.7 and Table 3.19. For WBSF, both breeds displayed mean values of 5.34 kg for 1 day *post mortem*. For 7 days *post mortem*, the WBSF value decreased for both breeds. There were significant differences (P<0.05) differences between 1 and 7 days *post mortem* between the two breeds. For the MFL's, a similar pattern was shown where the MFL mean values for both breeds decreased from 1 to 7 days *post mortem*. The highest MFL mean value was demonstrated by the SAL breed on 1 day *post mortem*. The lowest MFL mean value was for the LW breed on 7 days *post mortem*.

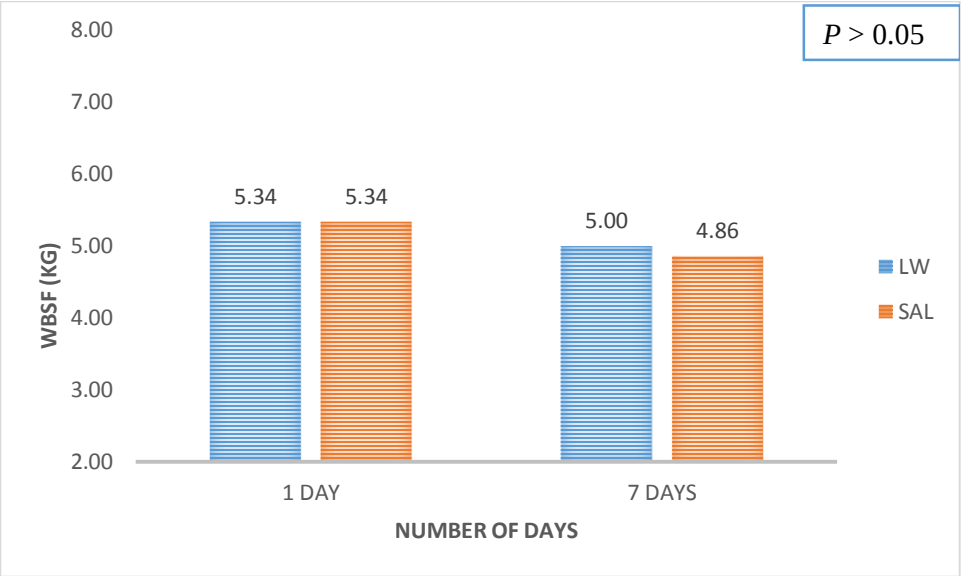

Figure 3.7 Breed effects on Warner Bratzler Shear force measurements, 1 and 7 days *post mortem*

Table 3.19 Breed effects on myofibril fragment length, 1 and 7 days *post mortem*

Population			***P*-value**
LW	1 day *post mortem*	50.43	<0.001
	7 days *post mortem*	42.26	
SAL	1 day *post mortem*	59.35	0.001
	7 days *post mortem*	48.01	

The genotype effect on WBSF and MFL 1 and 7 days *post mortem* is presented in Table 3.20. The WBSF mean value was the highest for the A/G genotype on 1 day *post mortem* as well as on 7 days *post mortem* with mean values of 5.62 kg and 5.16 kg respectively. The G/G 7 days *post mortem* for WBSF was the lowest with a mean value of 4.70 kg. There was not much variation between the A/A and G/G genotypes on 1 day *post mortem* but much variation demonstrated on 7 days *post mortem* for the same genotypes. For the MFL's, the highest mean value was on 1 day *post mortem* for the G/G (58.05 µm) genotype. The A/G, G/G and A/A genotypes did not display any variation on 7 days *post mortem*.

Table 3.20 Genotype effects on Warner Bratzler Shear force and myofibril fragment length, 1 and 7 days *post mortem*

Characteristic		A/A	A/G	G/G	*P*-value
WBSF	1 day *post mortem*	5.20	5.62	5.14	0.718
	7 days *post mortem*	4.91	5.16	4.70	
MFL	1 day *post mortem*	54.48	53.01	58.05	0.547
	7 days *post mortem*	44.97	44.40	46.56	

The effect of breed*genotype interaction on WBSF is shown in Figure 3.8. The SAL A/G on 1 day *post mortem* had the highest WBSF mean value of 5.92 kg. It also showed much variation with the other breed genotype combinations. The LW genotypes on 1 day *post mortem* showed no variation. The SAL G/G on 7 days *post mortem* had the lowest mean value. For the MFL's (Figure 3.9), the SAL A/A and SAL G/G 1 day *post mortem* were very similar with mean values of 62.29 µm and 64.40 µm respectively. The lowest mean value was observed within the LW A/A genotype 1 day *post mortem* with a value of 44.55 µm. A similar pattern was observed for 7 days *post mortem*. The SAL G/G had the highest MFL mean value of 50.93 µm on 7 days *post mortem*.

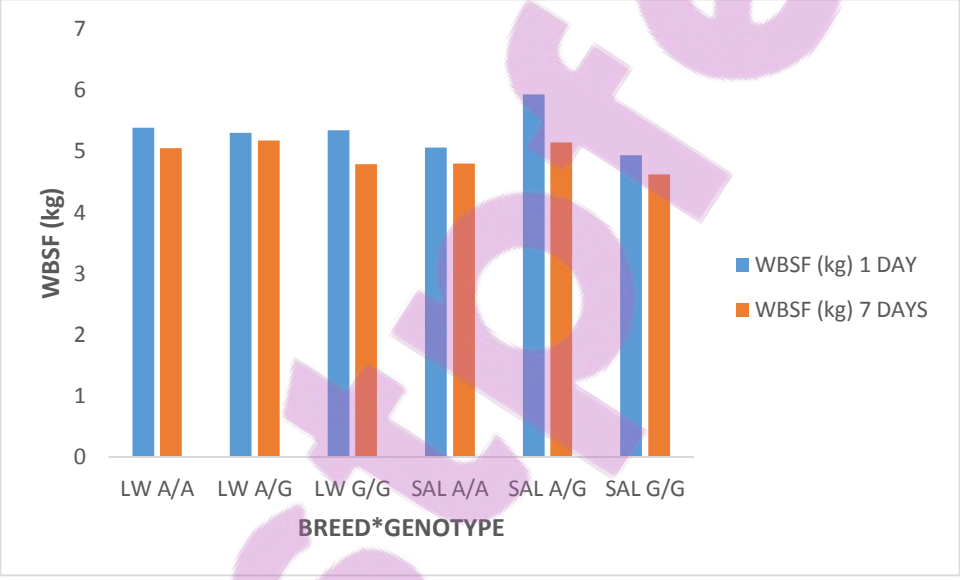

Figure 3.8 Breed*Genotype effects on Warner Bratzler Shear force, 1 and 7 days *post mortem* Average ageing effect differed significantly ($P<0.05$)

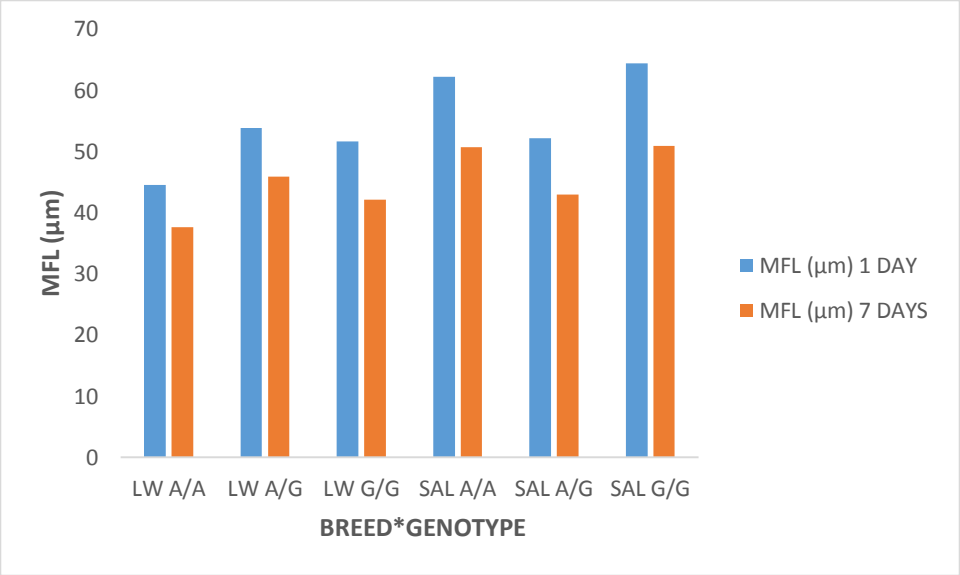

Figure 3.9 Breed*Genotype effects on myofibril fragment length, 1 and 7 days *post mortem*
Average ageing effect differed significantly ($P<0.05$)

Oxidative stability, 1 and 7 days post mortem (Breed, Genotype and Breed*Genotype interaction)

Thiols were only significant for day ($P<0.05$). The breed effect on protein oxidation is illustrated in Figure 3.10. There was no difference between the LW and SAL breeds for 1 day *post mortem*. The highest value (most stable) was on 1 day *post mortem*, mean value of 72.12 nmol/mg for LW and 72.05 nmol/mg for SAL. The lowest values (less stable) were on 7 days *post mortem*, with a mean value of 65.41 nmol/mg for LW and 66.33 nmol/mg for SAL.

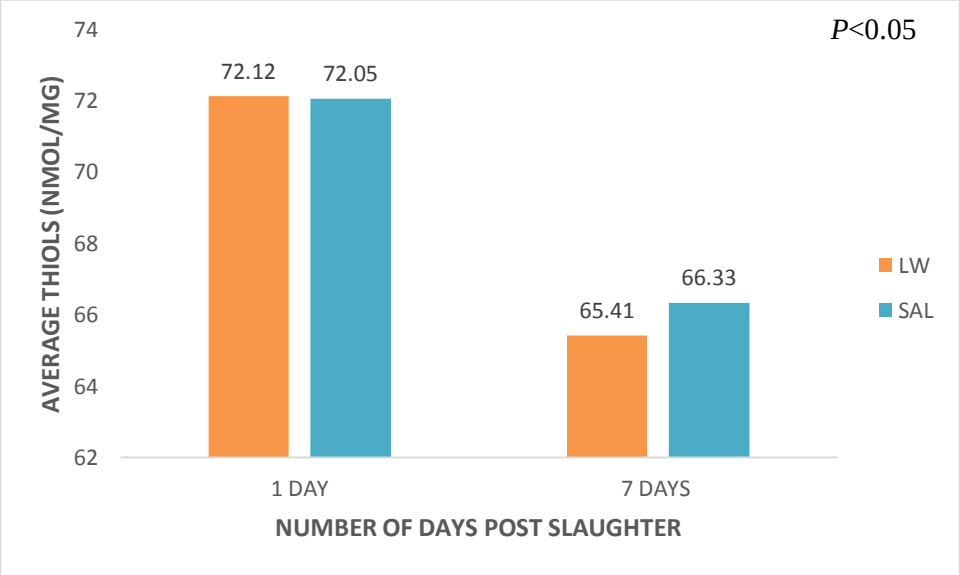

Figure 3.10 Breed effects on protein oxidation, 1 and 7 days *post mortem*

The *IGF2* genotype effect on protein oxidation, 1 and 7 days *post mortem* is illustrated in Figure 3.11. The highest thiols values were on 1 day *post mortem* with the highest value in the A/A genotype, followed by 71.89 nmol/mg in the G/G and 70.69 nmol/mg in the A/G genotype. There was no difference between the genotypes for 7 days *post mortem* with mean values of 66.26 nmol/mg for A/A, 65.90 nmol/mg for A/G and 65.54 nmol/mg for G/G.

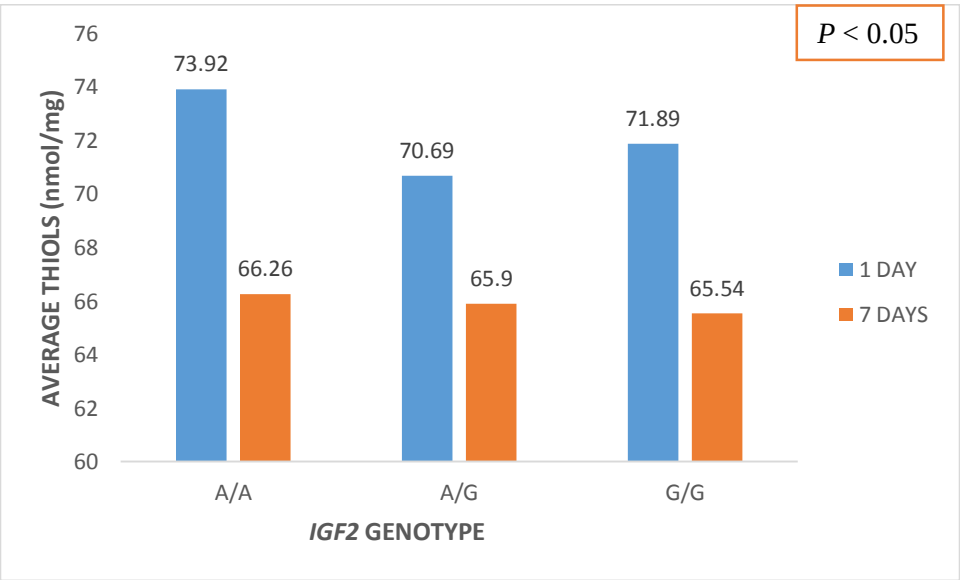

Figure 3.11 Genotype effects on protein oxidation, 1 and 7 days *post mortem*

The effect of breed*genotype interaction on protein oxidation is demonstrated in Figure 3.12. The SAL/AA and LW/AA had the highest mean values of 74.01 nmol/mg and 73.81 nmol/mg respectively for 1 day *post mortem*. The lowest mean value (64.60 nmol/mg) was for the LW/AA 7 days *post mortem*. This was significantly different to the other breed*genotypes reported here. SAL and LW A/G and G/G 1 day showed no variation. The mean values across all breed*genotype combinations decreased on 7 days *post mortem*. Within the SAL genotypes 7 days *post mortem*, no variation was observed. For both breeds, there was a difference for Thiols within breed*genotype and breed*genotype*day interaction.

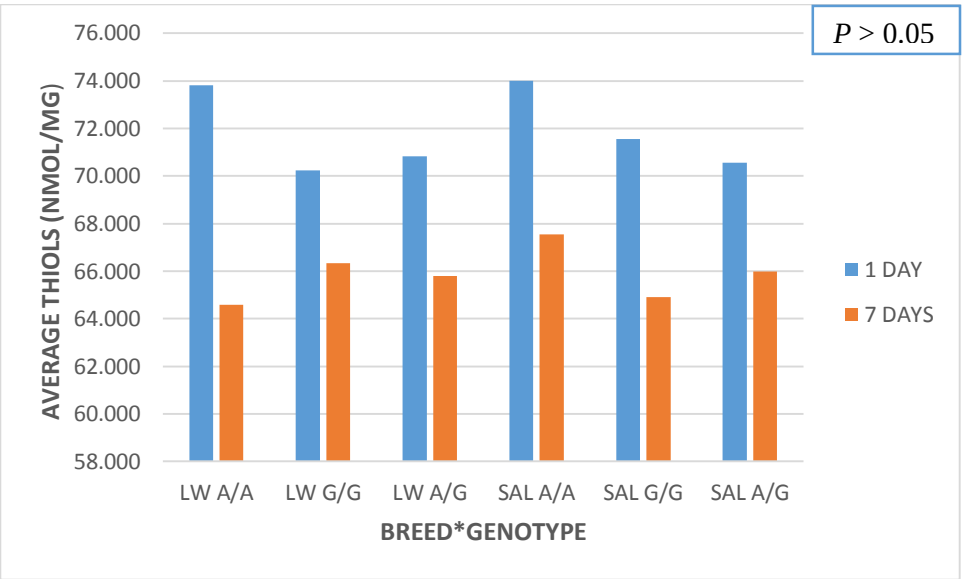

Figure 3.12 Breed*Genotype interaction on protein oxidation, 1 and 7 days *post mortem*

The effect of breed on lipid oxidation is presented in Figure 3.13. TBARS were significant for breed and day ($P<0.05$). The TBARS increased from 1 to 7 days *post mortem* for both the LW and SAL breeds. Both breeds were almost similar on 1 day *post mortem* with TBARS of 0.17 mg/kg and 0.18 mg/kg for the LW and SAL respectively. Within the LW breed, the mean values increased from 0.17 mg/kg to 0.21 mg/kg and in the SAL breed, the mean TBARS value increased from 0.18 mg/kg to 0.24 mg/kg. There was also a difference observed in the LW and the SAL 7 days *post mortem*.

Figure 3.13 Effects of breed on lipid oxidation, 1 and 7 days *post mortem*

The genotype effect on lipid oxidation is illustrated in Figure 3.14. There was no genotype difference observed for 1 day *post mortem* with TBARS of 0.18 mg/kg, 0.17 mg/kg and 0.18 mg/kg for A/A, A/G and G/G genotypes. There was a difference in 7 days *post mortem* TBARS for the A/A and A/G genotypes, with mean values of 0.24 mg/kg and 0.22 mg/kg respectively. There was also an increase in TBARS for the G/G 7 days *post mortem*, from 0.18 mg/kg on 1 day to 0.22 mg/kg on 7 days *post mortem*. This measure (TBARS), which is a product quality indicator, is important for the evaluation of the shelf-life of the meat (Warnants *et al*., 2001).

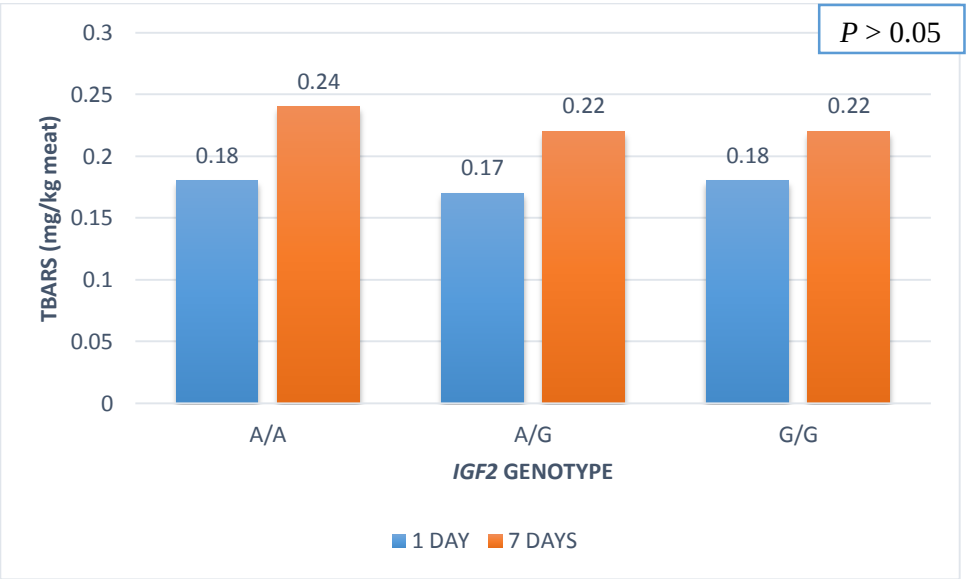

Figure 3.14 Effects of *IGF2* genotypes on lipid oxidation, 1 and 7 days *post mortem*

The effect of the breed*genotype interaction on lipid oxidation is presented in Figure 3.15. The TBARS for 1 day *post mortem* showed no significant differences for the SAL breed. The SAL/AA 7 days *post mortem* had the highest mean value of 0.25 mg/kg. The other breed*genotype combinations for 7 days *post mortem* showed no differences with mean values ranging between 0.21 mg/kg and 0.23 mg/kg.

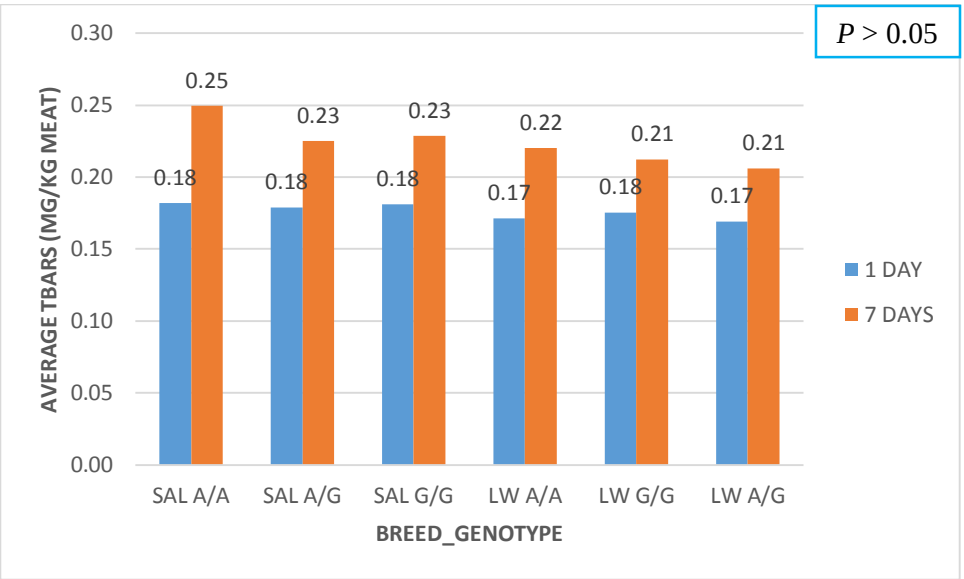

Figure 3.15 Breed*Genotype interaction on lipid oxidation, 1 and 7 days *post mortem*

Chemical composition

Fat composition (Breed, Genotype and Breed*Genotype interaction for belly fat, BF and LD))

The effect of breed on fat composition of belly fat, BF and *LD* are presented in Table 3.21. Extractable Fat in belly and back was significantly higher ($P<0.05$) in SAL compared to LW and the extractable fat in *LD* did not differ between SAL and LW. The opposite pattern was observed for moisture with significance ($P<0.05$) for belly fat and BF. Fat free dry matter (FFDM) was significantly lower ($P<0.05$) in SAL BF, in contrast to belly fat where no difference is detected between SAL and LW. FFDM did not differ significantly ($P>0.05$) between LW and SAL belly fat.

Table 3.21 Breed effects on proximate composition, % (Belly fat, Backfat and *Longissimus dorsi* muscle)

Treatment	LW	SAL	*P*-value
Belly fat			
Fat	66.78±7.41[b]	71.08±4.81[a]	0.007
FFDM	8.86±1.48	8.53±1.25	0.333
Moisture	24.36±6.40[a]	20.39±4.21[b]	0.005
BF			
Fat	77.54±4.83[b]	80.97±2.52[a]	0.001
FFDM	8.81±1.98[a]	7.91±1.22[b]	0.047
Moisture	13.64±4.16[a]	11.12±2.05[b]	0.022
LD			
Fat	1.87±0.55	1.66±0.47	0.121
FFDM	24.29±0.78	24.61±0.78	0.107
Moisture	73.84±0.94	73.73±0.90	0.631

[a], [b] = Different superscripts within the same row differ significantly ($P<0.05$)

FFDM of belly fat was the only significant (P<0.05) measurement with a genotype effect (Table 3.22). Although not significant, the G/G genotype had a higher mean value than the A/A genotype for fat %, in *LD*. Fat % in LD also represents intramuscular fat (IMF) percentage.

Table 3.22 Genotype effects on proximate composition (Belly fat, Backfat and *Longissimus dorsi* muscle)

Treatment	Genotype			*P*-value
	A/A	A/G	G/G	
Belly fat				
Fat	68.03±6.87	71.09±5.68	67.68±6.86	0.149
FFDM	9.18±1.43[a]	8.15±1.41[b]	8.76±1.11[ab]	0.050
Moisture	22.79±5.91	20.76±4.97	23.56±6.16	0.228
BF				
Fat	78.62±5.65	79.83±3.26	79.24±3.37	0.582
FFDM	8.18±1.51	8.69±2.00	8.23±1.58	0.570
Moisture	13.20±4.57	11.49±2.87	12.53±2.69	0.205
LD				
Fat	1.76±0.56	1.67±0.39	1.87±0.58	0.469
FFDM	24.48±0.92	24.67±0.53	24.20±0.83	0.162
Moisture	73.75±0.97	73.67±0.66	73.93±1.10	0.655

[a], [b] = Different superscripts within the same row differ significantly (P<0.05)

Fat and moisture of BF were significant (P<0.05) for breed*genotype interaction. In belly fat and BF, the extractable fat content of SAL/AA was higher than that of LW/AA. This can be explained by animals that were representative of the LW breed whereby the A allele may possibly already be fixed in sire breeds or lines in the LW. Breeders may already be selecting for leaner pigs without considering the effect on fat quality. The LW/AA had the highest mean value of 26.88%, 16.37% and 74.03% for moisture in belly fat, BF and *LD* respectively (Table 3.23).

Table 3.23 Breed*Genotype effects on proximate composition (Belly fat, Backfat and *Longissimus dorsi* muscle)

Treatment	Breed*Genotype						*P*-value
	LW/AA	**LW/AG**	**LW/GG**	**SAL/AA**	**SAL/AG**	**SAL/GG**	
Belly fat							
Fat	63.31±6.21	70.05±7.30	66.99±7.74	72.75±3.37	72.13±3.52	68.37±6.19	0.070
Fat Free Dry Matter	9.81±1.35	8.00±1.25	8.78±1.34	8.55±1.26	8.30±1.60	8.74±0.88	0.149
Moisture	26.88±5.43	21.95±6.53	24.24±6.79	18.69±2.64	19.58±2.52	22.89±5.74	0.093
BF							
Fat	74.86±5.68^{c}	79.19±3.92^{ab}	78.59±3.92^{b}	82.38±1.96^{a}	80.46±2.47^{ab}	79.97±2.67^{ab}	0.013
Fat Free Dry Matter	8.77±1.43	8.89±2.68	8.76±1.86	7.59±1.42	8.49±1.09	7.63±0.98	0.717
Moisture	16.37±4.56^{a}	11.92±3.57^{b}	12.64±3.08^{b}	10.03±0.99^{b}	11.05±2.06^{b}	12.40±2.35^{b}	0.004
LD							
Fat	1.83±0.62	1.67±0.31	2.11±0.61	1.70±0.52	1.67±0.48	1.62±0.44	0.294
Fat Free Dry Matter	24.14±0.71	24.69±0.57	24.03±0.93	24.82±1.02	24.65±0.53	24.37±0.72	0.340
Moisture	74.03±0.87	73.65±0.66	73.85±1.26	73.48±1.03	73.69±0.69	74.02±0.96	0.440

^{a}, ^{b}, ^{c} = Different superscripts within the same row differ significantly ($P<0.05$)

Percentage fatty acids and fatty acid ratios ((Breed, Genotype and Breed*Genotype interaction)

The breed effect of significant percentage fatty acids in *LD*, belly fat and backfat is shown in Table 3.24. The ratio of n-6 and n-3 fatty acids was also significant ($P<0.05$) between the LW and SAL breeds. C20:0 was the only significant % fatty acid for belly and *LD*. See Addendum A, B and C for complete breed effect on fatty acid percentages and calculated ratios. The amount of C20:0 in belly was slightly higher for the SAL than that in LW. Breed was a significant source of variation for the percentages of the fatty acids which contributes to the differences in % total FA between the SAL and LW breeds. There were differences observed between the SAL and LW for the different fatty acids. The total SFA and MUFA ratios were higher in the SAL population compared to the LW with ratios of 34.36 and 42.16 respectively. The double bond index and iodine values were higher in LW than the SAL.

Table 3.24 Significant breed effects on % total FA and FA ratios: Backfat

Treatment	**LW**	**SAL**	***P*-value**
% total fatty acids:			
LD			
C20:0	0.12±0.02[b]	0.14±0.03[a]	0.017
Belly fat			
C20:0	0.12±0.02[b]	0.14±0.03[a]	0.017
BF			
C18:0	11.24±1.07[b]	12.00±1.21[a]	0.014
C18:1c9	34.79±2.61[b]	36.33±1.97[a]	0.009
C18:2c9,12 (n-6)	23.07±4.47[a]	20.43±2.47[b]	0.003
C20:0	0.18±0.02[b]	0.21±0.03[a]	0.001
C20:2c11,14 (n-6)	0.81±0.13[a]	0.74±0.10[b]	0.016
C20:3c11,14,17 (n-3)	0.18±0.03[a]	0.16±0.03[b]	0.036
C20:5c5,8,11,14,17 (n-3)	0.02±0.02[b]	0.04±0.02[a]	0.037
Fatty acid ratios:			
Total SFA	33.18±2.18[b]	34.36±1.97[a]	0.026
Total MUFA	40.52±3.18[b]	42.16±2.38[a]	0.021
Total PUFA	26.30±5.02[a]	23.47±2.89[b]	0.005
Total n-6	24.13±4.62[a]	21.42±2.57[b]	0.004
PUFA:SFA	0.80±0.20[a]	0.69±0.12[b]	0.004
n-6/n-3	11.15±0.81[a]	10.53±0.77[b]	0.004
C18:0/C18:2	0.51±0.16[b]	0.60±0.10[a]	0.015
Double Bond Index (DBI)	96.27±7.61[a]	92.14±4.86[b]	0.009
Iodine value (IV)	82.84±6.54[a]	79.28±4.14[b]	0.009

[a], [b]= Different superscripts within the same row differ significantly ($P<0.05$)

The significant *IGF2* genotype effect on *LD*, belly fat and backfat is shown in Table 3.25. The A/A genotype had significantly higher percentages for total C15:0, C17:0, C18:2c9,12 (n-6), C20:2c11,14 (n-6) and C20:3c11,14,17 (n-3) in *LD* (Addendum F). For C16:0 and C18:1c7, the A/G and G/G genotypes displayed higher total percentages. There was no significant differences ($P>0.05$) for the fatty acid ratios in *LD*. For percentage fatty acids in *LD*, the A/A genotype was different compared to the other two *IGF2* genotypes. There were significant genotype differences for the SFA's (C15:0, C16:0, C17:0 and C20:0) in belly fat (Table 3.25). C15:0, C17:0 and C20:2c11,14 (n-6), was higher in A/A compared to A/G and G/G genotypes. C16:0 and C20:0 was lower in A/A than in A/G and G/G genotypes. Refer to Addendum D for genotype effect in belly fat. The genotype effect on % total FA and FA ratios in BF is shown in Addendum E. There was a genotype effect on the % SFA's (C15:0, C17:0 andC20:0). The A/G and G/G had lower amounts of C15:0 and C17:0 in contrast to the A/A genotype in BF. C20:0 was the lowest percentage in the A/A genotype. For C20:2c11,14 (n-6), the A/A genotype had a higher mean value of 0.82 in BF. There was no significant differences ($P>0.05$) for the genotype effect on fatty acid ratios in BF.

Table 3.25 Significant (P<0.05) genotype effects on % total FA and FA ratios: Belly fat, Backfat and *Longissimus dorsi* muscle

Treatment	Genotype			*P*-value
	A/A	A/G	G/G	
% total fatty acids				
LD				
C15:0	0.02±0.02[a]	0.00±0.01[b]	0.01±0.02[b]	0.005
C16:0	21.54±1.00[b]	22.41±0.88[a]	22.61±1.17[a]	0.004
C17:0	0.20±0.05[a]	0.16±0.03[b]	0.16±0.05[b]	0.004
C18:1c7	5.25±0.59[b]	5.52±0.51[ab]	5.71±0.68[a]	0.058
C18:2c9,12 (n-6)	17.52±3.31[a]	14.97±3.23[b]	14.57±4.23[b]	0.029
C18:3c9,12,15 (n-3)	0.74±0.36[a]	0.53±0.16[b]	0.52±0.17[b]	0.010
C20:2c11,14 (n-6)	0.45±0.12[a]	0.36±0.05[b]	0.36±0.06[b]	0.002
C20:3c11,14,17 (n-3)	0.08±0.04[a]	0.06±0.02[b]	0.06±0.03[b]	0.031
Belly fat				
C15:0	0.02±0.02[a]	0.01±0.01[b]	0.01±0.01[b]	0.016
C16:0	22.41±0.82[b]	23.17±1.03[a]	22.69±1.10[ab]	0.040
C17:0	0.22±0.07[a]	0.17±0.04[b]	0.18±0.05[b]	0.017
C20:0	0.14±0.02[b]	0.16±0.03[a]	0.16±0.02[a]	0.007
C20:2c11,14 (n-6)	0.62±0.09[a]	0.54±0.07[b]	0.57±0.09[b]	0.006
BF				
C10:0	0.00±0.00[b]	0.00±0.00[b]	0.00±0.01[a]	0.015
C15:0	0.03±0.02[a]	0.02±0.01[b]	0.02±0.02[b]	0.056
C17:0	0.30±0.09[a]	0.24±0.05[b]	0.26±0.07[ab]	0.031
C20:0	0.18±0.03[b]	0.20±0.03[a]	0.20±0.03[a]	0.058
C20:5c5,8,11,14,17 (n-3)	0.02±0.02[b]	0.03±0.03[ab]	0.04±0.01[a]	0.032
Fatty acid ratios:				
LD				
Total SFA	34.68±1.67[b]	35.81±1.40[a]	36.06±1.70[a]	0.023
PUFA:SFA	0.67±0.15[a]	0.56±0.15[ab]	0.55±0.19[b]	0.049
Atherogenicity Index (AI)	0.39±0.03[b]	0.41±0.03[a]	0.41±0.04[a]	0.022
C18:0/C18:2	0.70±0.16[b]	0.86±0.24[ab]	0.91±0.31[a]	0.036

[a], [b] = Different superscripts within the same row differ significantly (P<0.05)

The significant breed*genotype effect on belly fat and backfat is shown in Table 3.26. The individual % total FA and FA ratios were not significant (P>0.05) for breed*genotype interaction in *LD* (Addendum I).

Table 3.26 Significant (P<0.05) breed*genotype effects on belly fat and backfat

Treatment	Breed*Genotype						*P*-value
	LW/AA	**LW/AG**	**LW/GG**	**SAL/AA**	**SAL/AG**	**SAL/GG**	
% total fatty acids:							
Belly fat							
C16:0	21.99±0.67^{b}	23.69±0.79^{a}	22.58±1.07^{b}	22.82±0.76^{b}	22.65±1.01^{b}	22.79±1.18^{b}	0.008
C20:2c11,14 (n-6)	0.68±0.07^{a}	0.52±0.07^{b}	0.57±0.09^{b}	0.57±0.07^{b}	0.56±0.07^{b}	0.56±0.09^{b}	0.010
C20:3c11,14,17 (n-3)	0.16±0.02^{a}	0.11±0.02^{b}	0.12±0.03^{b}	0.12±0.03^{b}	0.12±0.02^{b}	0.12±0.03^{b}	0.014
C22:5c7,10,13,16,19 (n-3)	0.18±0.03^{a}	0.14±0.02^{b}	0.16±0.04^{ab}	0.14±0.02^{b}	0.14±0.03^{b}	0.15±0.03^{b}	0.038
BF							
C15:0	0.04±0.02^{a}	0.02±0.02^{b}	0.02±0.01^{b}	0.02±0.02^{b}	0.02±0.01^{b}	0.02±0.02^{b}	0.040
C16:0	19.65±1.12^{c}	21.72±1.52^{a}	20.26±0.86^{bc}	21.24±0.93^{ab}	20.79±1.23^{ab}	20.89±1.48^{ab}	0.007
C16:1c9	1.15±0.29^{b}	1.47±0.18^{a}	1.25±0.19^{ab}	1.36±0.37^{ab}	1.21±0.15^{b}	1.29±0.23^{ab}	0.013
C17:0	0.34±0.06^{a}	0.23±0.06^{b}	0.24±0.02^{b}	0.26±0.10^{b}	0.25±0.06^{b}	0.27±0.11^{ab}	0.045
C18:1c9	32.95±2.68^{b}	36.11±1.80^{a}	35.30±2.35^{a}	36.60±2.40^{a}	36.37±1.80^{a}	36.00±1.81^{a}	0.035
C18:2c9,12 (n-6)	26.15±4.24^{a}	20.45±4.10^{b}	22.61±3.37^{b}	19.68±2.34^{b}	20.77±2.71^{b}	20.89±2.40^{b}	0.006
C18:3c9,12,15 (n-3)	1.99±0.33^{a}	1.52±0.30^{b}	1.73±0.29^{ab}	1.57±0.27^{b}	1.71±0.27^{b}	1.66±0.24^{b}	0.006
C20:2c11,14 (n-6)	0.91±0.10^{a}	0.73±0.11^{b}	0.79±0.09^{b}	0.72±0.09^{b}	0.75±0.08^{b}	0.76±0.13^{b}	0.003
C20:3c11,14,17 (n-3)	0.20±0.03^{a}	0.16±0.03^{b}	0.17±0.03^{b}	0.15±0.03^{b}	0.17±0.03^{b}	0.16±0.04^{b}	0.006
C22:5c7,10,13,16,19 (n-3)	0.17±0.04^{a}	0.14±0.02^{b}	0.17±0.03^{a}	0.13±0.03^{b}	0.16±0.05^{ab}	0.15±0.03^{ab}	0.040
Fatty acid ratios:							
Belly fat							
Total SFA	33.90±1.77^{b}	36.32±1.57^{a}	35.22±2.27^{ab}	35.78±1.85^{a}	35.15±1.43^{ab}	35.07±1.68^{ab}	0.029
Total n-3	2.12±0.23^{a}	1.65±0.36^{b}	1.83±0.45^{b}	1.78±0.31^{b}	1.84±0.29^{ab}	1.81±0.25^{b}	0.042
PUFA:SFA	0.68±0.10^{a}	0.51±0.12^{b}	0.57±0.14^{b}	0.55±0.09^{b}	0.55±0.09^{b}	0.55±0.09^{b}	0.053
Atherogenicity Index	0.40±0.02^{b}	0.44±0.02^{a}	0.42±0.04^{ab}	0.42±0.02^{ab}	0.41±0.03^{b}	0.42±0.04^{ab}	0.016
Double Bond Index (DBI)	92.31±4.26^{a}	84.83±5.18^{b}	87.90±6.07^{b}	86.39±4.34^{b}	86.97±3.92^{b}	87.15±3.96^{b}	0.029
Iodine value (IV)	79.41±3.65^{a}	73.03±4.47^{b}	75.63±5.21^{b}	74.37±3.75^{b}	74.82±3.35^{b}	74.99±3.40^{b}	0.031
BF							
Total SFA	31.85±1.47^{b}	34.46±2.55^{a}	33.24±1.67^{ab}	34.79±2.04^{a}	34.10±2.00^{a}	34.18±2.01^{a}	0.037
Total MUFA	38.39±3.50^{b}	42.26±2.13^{a}	40.92±2.67^{a}	42.63±3.09^{a}	41.98±2.12^{a}	41.84±1.92^{a}	0.026
Total PUFA	29.77±4.73^{a}	23.28±4.57^{b}	25.85±3.76^{b}	22.57±2.77^{b}	23.92±3.15^{b}	23.98±2.82^{b}	0.005
Total n-6	27.33±4.36^{a}	21.41±4.24^{b}	23.65±3.45^{b}	20.64±2.43^{b}	21.77±2.81^{b}	21.90±2.52^{b}	0.006
Total n-3	2.44±0.43^{a}	1.88±0.36^{b}	2.19±0.32^{ab}	1.93±0.36^{b}	2.15±0.36^{ab}	2.08±0.33^{b}	0.005
PUFA:SFA	0.94±0.19^{a}	0.69±0.18^{b}	0.78±0.14^{b}	0.65±0.09^{b}	0.71±0.14^{b}	0.71±0.12^{b}	0.005

^{a}, ^{b}, ^{c} = Different superscripts within the same row differ significantly (P<0.05)

Breed*Genotype interaction on belly fat is presented in Addendum G. C16:0 and C20:2c11,14 (n-6) (P<0.05) were significant for breed*genotype interaction in belly fat. The SFA's, total n-6, PUFA:SFA and n-6/n-3 were significant (P<0.05). The AI was significant in belly fat (P<0.05) with the LW/AG displaying the highest mean value of 0.44. The DBI and IV were also significant (P<0.05) where the LW/AA was significantly higher for both DBI and IV in contrast to the other breed*genotype combinations. Although not significant for the SAL/AA, the IV values were high when compared to the LW/AA. The SFA's (C15:0, C16:0 and C17:0) were significant for breed*genotype interaction in BF (Addendum H). With regard to MUFA's, C16:1c9 and C18:1c9 were also significant (P<0.05) in BF. PUFA's, C18:2c9,12 (n-6), C18:3c9,12,15 (n-3), C20:2c11,14 (n-6), C20:3c11,14,17 (n-3) and C22:5c7,10,13,16,19 (n-3) were significant (P<0.05) for breed*genotype interaction in BF. Fatty acid ratios were significant (P<0.05) for all measurements (total SFA, MUFA and PUFA) except n-6/n-3 and desaturase index.

3.4 Discussion

The effect of the *IGF2* gene on meat and carcass characteristics as well as the fatty acid composition of the SAL and LW populations were evaluated. From this study, it was apparent that the *IGF2* did not have a negative impact on meat and carcass characteristics. There was significant genotype effects on eye muscle area, fibre area (intermediate and red), colour on 7 days *post mortem*, WBSF (meat tenderness), Thiols on 1 day *post mortem* and TBARS on 7 days *post mortem* (oxidative stability). For fat and calculated fatty acid ratios, there were significant breed, genotype and breed*genotype interactions.

While few studies have investigated how the *IGF2* gene may have altered meat, carcass and fat quality, the limited available data suggest that the mutation has little impact on fresh meat and carcass quality characteristics. The results obtained in this study on meat and carcass quality confirm the results obtained by other authors (Estelle *et al*., 2005; Van den Maagdenberg *et al*., 2007; Van den Maagdenberg *et al*., 2008a; Oczkowicz *et al*., 2009; Fontanesi *et al*., 2010). Clark *et al*. (2014) reported that there was no effect (P>0.01) on standardised chilled side weights from either *IGF2* genotype as well as whole loin weights and whole loin percentage of chilled side weights were not different (P >0.01) between genotypes. For dressing percentage and drip loss, Clark *et al*. (2014) reported no differences (P>0.01) between pigs with the A^{pat} and G^{pat} alleles. Other studies (Van Den Maagdenberg *et al*., 2008a; Burgos *et al*., 2012) reported similar results which are in agreement with the findings in the current study where dressing percentage and drip loss did not differ between genotypes (P>0.05). On the other hand, breed effects were found for % temperature carcass loss, % drip loss and water holding capacity (Table 3.4) although the average live weights and carcass weights did not differ.

It has been shown that the rate at which pH falls as well as the pH_u of the meat has an important effect on WHC whereby during the conversion of muscle to meat, WHC will be reduced. (Lonergan, 2007).

The higher the rate of pH fall, the lower the WHC will be. The pH_u values in this study were in the acceptable range at 1 hour *post mortem* of 5.9 – 6.20. In this study, there was a normal decline in pH and temperature which is a good indication of a good quality pork product, tender and juicier pork (Lonergan *et al*., 2007). There were also no breed differences or differences between the *IGF2* genotypes found for pH and temperature. An increased rate in pH decline, as well as an increased rate in pH decline at high temperatures, will result in both a loss of WHC (Offer, 1991) and higher drip-loss. *Post-mortem* pH and temperature influences the rate and extent of protein denaturation, oxidation and proteolysis, lipid oxidation, colour characteristics, WHC and sensory attributes of meat (Rosenvold & Andersen, 2003). For the pH/temperature ratio at 24 hours *post mortem,* there was no significant difference (P>0.05) between the *IGF2* genotypes, but the pH/temperature decline slopes differed within the A/A genotype. This is indicative of leaner carcasses chilling more rapidly which also coincides with the result obtained for EMA of the A/A genotype.

In studies investigating factors affecting pork quality, Nold *et al*. (1999), reported that boars had a higher drip loss when slaughtered at 100 kg, while the drip loss was similar at 110 kg, implying that weight at slaughter plays an important role. In contrast, no differences *were* observed between gilts and boars for drip loss percentages of L*ongissimus dorsi* samples (Beattie *et al*., (1999). Dube *et al*. (2011) reported that increasing the total test period, feed intake was associated with decreasing (P <0.001) lean percentage, drip-free lean percentage, drip loss and dressing percentage. The findings in the current study are in accordance with findings reported by Dube *et al* (2011) where a general decline in carcass yield was observed, depicted by a decreased carcass length in the Landrace breed and a reduced eye muscle area in the Large White breed. The eye muscle area of the Large White population in this study was 5446 mm², although not significant (P>0.05) for the breed effect.

The population of pigs in the current study showed a greater difference in eye muscle area compared with previous reports. In the current study, the G/G genotype displayed a significant (P<0.05) larger eye muscle area of 5658 mm² and the A/A genotype an eye muscle area of 5239 mm². This may be attributed to the differences in international genetics and management practices. Previous studies (Jeon *et al*., 1999; van den Maagdenberg *et al*., 2008a) reported loin eye area was 4-5% greater in A^{pat} pigs compared with G^{pat} pigs. The larger eye muscle area for the SAL G/G with an area value of 5658 mm² compared to 5239 mm² for the A/A genotype can be attributed to the genotype SAL/GG associated with more fat.

Colour measurements for 24 hours *post mortem* (normal colour measurements), were not different between the *IGF2* genotypes (P>0.05). Similar findings were reported by Clark *et al*. (2014) whereby colour scores were not different (P≥0.25) in A^{pat} pigs and G^{pat} pigs. Colour of the *Longissimus dorsi* as measured by L* (increasing values indicate a whiter colour) indicate that paternal A allele may result in paler meat (Van Den Maagdenberg *et al*., 2008a; Burgos *et al*., 2012). However, the average difference

between the two studies is less than 1 L* unit, and therefore it is unlikely to be meaningful to consumers. Meat colour is of utmost importance as it forms the basis for the consumer for product selection. It is influenced by a variety of factors such as species, breed, genotype as well as pre-slaughter handling, slaughter procedure and pH. An increasing paleness in meat is inversely proportional to pH meaning that a decrease in pH results in an increase in paleness. There was no significant difference (P>0.05) between the *IGF2* genotypes for meat colour. Most of the differences observed in meat colour was due to breed differences. Burgos *et al.* (2012) and Van Den Maagdenberg *et al*. (2008a) reported colour of the L*ongissimus dorsi* as measured by L* (increasing values indicate a whiter colour) indicating that the *IGF2* A allele may result in paler meat. Similar findings were reported by Clark *et al*. (2014) whereby colour scores were not different (P≥0.25) in A^{pat} pigs and G^{pat} pigs. Greater challenges with colour could become apparent in breeding programs and genetic lines susceptible to quality issues.

One of the most important meat quality traits is tenderness. In pork, the tenderisation process, which has the fastest rate when compared to beef or lamb, is related to the lowest ratio of calpastatin to calpain in the porcine muscle (Ilian *et al*., 2001; Koohmaraie, 1992). Shear force is known to be well correlated with the sensory perception of pork tenderness (Hovenier *et al*., 1993). The WBSF measures the amount of force (N = 9.81kg) required to cut through the muscle fibres. Higher WBSF values indicate tougher meat and lower values more tender meat. Muscles with a larger fibre size, especially type IIB fibre, exhibit tougher meat than muscles of smaller fibre size in cattle (Renand *et al*., 2001) and in pig (Karlsson *et al*., 1993). Previous studies have reported that intensive selection for lean growth in pigs may cause a large genetic change in fibre type composition (Weiler *et al*., 1995).

Calpain activity and calpastatin levels play a key role in *post mortem* proteolysis. The calpastatin (*CAST)* gene has been reported by a number of authors to have an effect on pork tenderness, firmness, juiciness, pH and meat colour (Lindholm-Perry *et al*., 2009; Gandolfi *et al*., 2011). The changes in WHC and drip loss, calpain/calpastatin system affects majority of the processes of *post mortem* tenderisation and the conversion of muscle to meat (Kemp *et al*., 2010). The WBSF values decreased from 1 to 7 days *post mortem*, for both populations and all three genotypes. WBSF in the range of 5 to 5.5 kg is a relative "tender" starting point at 1 day *post mortem*, although there was not much further tenderisation up to 7 days *post mortem* (4.8 to 5.2 kg). The meat samples showed continuous improvement in WBSF values throughout the storage period of 7 days with an average shear force value of 4.93 kg. Overall, the meat from the carcasses in this study, would be considered relatively tender. Van den Maagdenberg *et al*. (2008a) reported that tenderness (33.82N for A/A and 33.58N for G/G; 1N = 9.81 kg) was not affected by the *IGF2* genotype. It was also reported that chops from gilt carcasses were not different (P>0.18) for chops from barrow carcasses. In a study by Clark *et al*. (2014), the WBSF was less than 3.0 kg in 86% of the chops evaluated

at 21 days of ageing. One could deliberate that it would be of interest to study longer ageing periods such as 14 days *post mortem* provided that food safety is taken into account.

Myofibrillar breakdown measures the lengths of muscle fibre fragments that can indirectly be related to the amount of structural breakdown of muscle through proteolysis (Olson *et al*., 1976; Doŝler *et al*., 2007). Shorter fibre fractions signal more structural breakdown and possibly more tender meat. Although no significant differences between the genotypes, the two populations displayed much reduction in MFL's, an indication of tender pork. Van den Maagdenberg *et al*., (2008a) reported no significant differences in MFL's between the homozygous *IGF2* genotypes. The proteolytic enzymes were significantly more active in LW than in SAL (MFL is overall significantly shorter in the LW than in SAL at 7 days *post mortem*) the proteolytic activity was not large enough to manifest itself in the WBSF differences and more tender meat. So, if the normal calpain proteolytic ageing process did not influence the tenderness result, other mechanisms must have played a greater role in influencing the tenderness result. It seems that the background tenderness in pork is lower overall and that the connective tissue characteristics might be worth while studying.

Protein oxidative stability was significant ($P<0.05$) for the *IGF2* genotypes. Free thiols describing protein oxidative stability were generally lower after 7 days *post mortem*, therefore showing less stability (lower free thiols means more oxidation). The free thiol content of the LW/AA genotype, decreased the most (64.60 units), giving the poorest stability towards protein oxidation after 7 days ageing. The lack of variation can possibly be explained by the muscle fat levels of the *IGF2* genotypes were relatively low resulting in lower availability of polyunsaturated fatty acids that could have been prone to oxidation as well as the likeliness of higher levels of anti-oxidants. Another possibility may be the vacuum-packaging that may have limited exposure to oxygen and therefore have inhibited oxidation in general. Between the two populations, genotypes and breed*genotype interaction, the TBARS values increased. Higher readings were recorded on 7 day *post mortem*. This showed a clear tendency towards increased lipid oxidation in the meat from the SAL and LW. In this study, the total SFA and PUFA:SFA ratios were significant ($P<0.05$) for the genotype effect in *LD*. Higher PUFA and MUFA content in *LD* could lead to less oxidative stability and higher percentage of IMF could also result in less oxidative stability. The reduced susceptibility to oxidation could possibly be attributed to the higher percentages of SFA's in *LD*. Joo *et al*, (2002) reported that lipid oxidation of loins increased rapidly with storage of 7 days.

In a study by Burgos *et al*. (2012), the only significant difference in IMF content was the higher fat content of *Longissimus dorsi* (*LD*) IMF in A/A pigs. It was noted an increase in intramuscular fat percentage within the *Longissimus dorsi* muscle in pigs that carry the paternal A allele. The results suggest that the accumulation of fat in subcutaneous deposits appears to be regulated independently from fat deposition within muscles. This raises possibilities to search for genes or metabolic pathways that could

increase IMF but not subcutaneous fat, which has advantage for pork quality and economic advantage for pork production. However, other studies report either a decrease or no change in intramuscular fat deposition in the ham or shoulder due to the paternal A allele (Reina *et al*., 2012; Sánchez del Pulgar *et al*., 2013).

In pig breeding, the reduction of fat is measured by a selection for decreased backfat thickness, which creates a reduction in IMF (Grindflek et al., 2001). Backfat is genetically positively correlated with both growth and IMF. The higher the IMF values, the more backfat a pig is likely to have. A higher content of PUFA in organically produced pigs may not only be a result of different feed, but also caused by higher lean meat percentage (Hansen *et al*., 2006). This is in accordance with our findings whereby, the LW/AA animals were significantly different with a higher total PUFA content in BF. The results in this study confirm previous work of other authors (Van den Maagdenberg *et al*., 2008a; Clark *et al*., 2014). The *IGF2* 'G' allele has potential to increase the fat content of the carcass (BF and belly fat) and decrease in protein rich cuts such as the loin.

While reports on fresh belly characteristics of pigs with different *IGF2* alleles is limited, Reina *et al*. (2012) reported greater SFA percentages and reduced PUFA in dry-cured hams and dry-cured shoulders from A^{pat} pigs compared with G^{pat} pigs. It has been well established that lighter and thinner bellies with greater unsaturated fats are more difficult to slice than heavier and thicker bellies with greater saturated fats (Seman *et al*., 2013). The belly contains a greater amount of fat, therefore, the SAL/AA in this study would have decreased fat accumulation and decreased belly weights, ultimately decreasing lean yield. The *IGF2* A/A genotype had an effect on the saturated fatty acids (SFA's) with higher C15:0 and C17:0 percentages observed in the *LD*, belly fat and backfat and lower C20:0 percentages in belly fat and backfat and C16:0 in *LD* compared to that of A/G and G/G. Only one MUFA namely C18:1c7 showed a genotypic effect in *LD* where AA had the lowest percentage followed by A/G and G/G having the highest percentage. The PUFA's were significantly (P<0.05) higher in the A/A genotype in *LD*. The G/G genotype had the highest percentage for total SFA's in *LD* compared to the A/G and A/A genotype. This is in agreement with literature that the 'G' allele is associated with more fat.

For the breed*genotype interaction in backfat, the LW/AA displayed higher mean values for the fatty acid ratios calculated and the lowest mean values for the total SFA and MUFA. This implies the differences observed are due to the breed effect, whereby the LW shows a trend of higher SFA's and PUFA's and decreased MUFA percentages. For C16:0 in belly fat for the A/G genotype, a similar pattern was observed whereby the LW/AG showed the highest percentage amongst the breed*genotype combinations.

The extractable fat content was higher for the G/G genotype in BF and *LD*. Although slightly lower in belly fat, the G/G genotype still displays advantage in the South African pork processing industry. In

theory, this increase in fat content should contribute to improved quality of processed pork products (Wood *et al*., 2003). The economic gain for producers is beneficial regarding carcass acceptance although the initial growth cost may also be increased. However, the use of the 'G' allele will depend on the market for which the meat is produced as processors prefer more fat for the processed hams and sausages. The trend of the A/A genotype displaying higher extractable fat content for belly fat with a fat percentage of 68%, offers an advantage for the South African meat industry where the population under investigation in this study demonstrated typical *IGF2* 'G' allele characteristics. This pattern implies that the LW/AA population in this study, has both *IGF2* alleles in significant proportions. It is also an indication of selection pressure towards producing leaner meat that the incidence of the A/A genotype has reduced the effect of the G allele on fat content.

Person *et al*. (2005) suggested that bacon from thin bellies (48% moisture and 36% fat) and average bellies (41% moisture and 4 % fat) had greater consumer sensory attributes compared with thick bellies (40% moisture and 46% fat). Therefore from the results reported in this study, the bacon from the SAL/AA pigs (73% fat and 19% moisture) would likely have similar sensory attributes compared to bacon from the SAL/GG pigs (68% fat and 23% moisture).

The iodine value is a measurement to estimate the amount of unsaturation in the fatty acids. Due to the fact that unsaturated fatty acids are 'softer' or less firm, the iodine value serves as an indicator of the overall carcass fat firmness. Although, Reina *et al*. (2012) did not report iodine value, using the AOCS (1998) equation and the average percentages reported, the iodine value of dry cure hams from the A^{pat} pigs was 1.47 units greater than the G^{pat} pigs. Additionally, the iodine value of dry-cure shoulders was 2.60 units greater in A^{pat} pigs compared with G^{pat} pigs. The mean IV values obtained in the current study are contradictory to that proposed by Barton-Gade (1987) indicating that a maximum IV value of 70 would produce firm fat. It was also reported by the same author that thinner BF corresponds to a lower percentage of extractable fat content, higher water and protein content, higher IV and more unsaturated fatty acids. Clark *et al*. (2014) reported that iodine values appeared to be greater (P>0.09) in bellies from A^{pat} pigs compared with bellies from G^{pat} pigs.

Previous studies have reported less extractable fat in gilts compared to barrows (Cisneros *et al*., 1996; Fernandez-Duenas *et al*., 2008). Additionally, the moisture content reported was also greater in gilts compared to barrows. The ratio between n-6 and n-3 fatty acids plays an important role in reducing the risk of coronary heart disease (American Heart Association, 2008; Wu *et al*., 2014). The atherogenicity index is a measure of the quality assessment and comparison of different foods and is also related to the risk of cardiovascular disease (German & Dillard, 2004). In this study, the atherogenicity index of the A/A genotype in *LD* was significantly (P<0.05) lower than the A/G and G/G genotypes which is in accordance that leaner animals are expected to have lower double bond index and atherogenicity index. The benefits

for the health conscious consumer is thus increased with the A/A genotype. A higher atherogenicity index implies a lower PUFA:SFA ratio which makes the meat unhealthy for consumers.

The abundance of oleic, palmitic and linoleic acids, varying between 14% and 36%, in the current study, is in accordance with the study by Clop *et al*., (2003) who reported similar results and attributed this to previous evidence of differences between the parental lines for the percentages of oleic acid. Ntawubizi *et al*. (2010) reported results where oleic and linoleic acids were also most abundant. In a study by Ntawubizi *et al*. (2009), the effect of gender on intramuscular fatty acid composition was reported. It was concluded that the differences in fatty acid composition between the sexes was partly the indirect result of differences in the fat content. It was reported that the IMF of Duroc pigs had higher concentrations of PUFA than Landrace (Cameron & Enser., 1991). Similarly, Zhang *et al*. (2007) analyzed *LD* muscle from eight different pig breeds and reported that the total SFA was the highest in Duroc pigs; Poland China pigs had a greater MUFA content than other breeds except the Spotted pig breed. The PUFA content was the highest in the Hampshire, Landrace and Yorkshire pigs as compared to the other breeds. MUFA and SFA have a positive influence on the sensory characteristics of pork such as firmness and flavor (Carrapiso *et al*., 2003). In pigs, as growth progresses, the amount of energy required for protein deposition decreases, increasing the amount of energy available for fat deposition (Enser *et al*., 1996). The direct incorporation of C18:2n-6 fatty acids into body tissues decreases and the conversion of excess energy to SFA's and MUFA's fat by *de novo* lipogenesis increases, as growth in pigs progresses (Raj *et al*., 2010). The higher percentages of SFA's obtained in backfat in the current study may be attributed to the final slaughter weight at 100 kg and not at a decreased slaughter weight of 70 kg (baconers), reaffirming that final weight at slaughter plays an important role in fatty acid composition. The fatty acid composition of the IMF is influenced by several factors such as breed, sex and genotype, and environmental factors of which diet plays an important role (Nürnberg *et al*., 1998; De Smet *et al*., 2004).

3.5 Conclusions

Meat quality is a compilation of different traits which are influenced by different physiological processes *post mortem*. This implies the control of a wide variety of factors not only during the conversion of muscle to meat but also in breeding programs where meat quality has to be included. From the results presented in this chapter, it was shown that the *IGF2* genotypes can lead to increased leanness in the SAL and LW pig populations. For the majority of the meat quality traits measured, the selection of the IGF2 genotypes holds no major negative effect. The A/A genotype has potential to benefit the South African pig industry displaying leaner carcasses. With the recent focus on health conscious consumers, meat from the A/A carcasses can be considered. The PUFA content also showed to be beneficial to the pork processing

industry. The findings from this study offers a possible solution to the inverse relationship between healthy consumers and the technological aspects of meat quality.

CRITICAL REVIEW AND RECOMMENDATIONS

The South African pig industry is a relatively small industry contributing about 0.3% of the world pig population and in context of the South African agricultural sector, it contributes less than 5% to the primary agricultural sector. South Africa is ranked third in Africa, in comparison to the largest pig population found in China, with the United States of America second. Pork is produced throughout South Africa across all nine provinces. Piggeries with highly regulated housing systems are employed that are comparable with international pig farming systems.

The production of pork in South Africa super cedes its consumption. Consumers have become more health conscious than before. Pork was considered to contain excess fat and was avoided by many consumers. The global meat industry responded to consumer demands and have adopted new technologies to produce leaner pigs.

High through-put techniques such as DNA arrays and proteomics, present opportunities to identify multiple genes and further understand the genetic basis of meat quality. The availability of the Porcine SNP60K is already being utilized to identify genes affecting meat quality and other traits. Many countries abroad are already involved in high through-put genotyping. South Africa is currently not engaged in the setting up of reference populations. With TOPIGS SA, delivering healthy pigs with advanced Dutch and Canadian genetics, and Kanhym Estate (under the license of PIC) also a supplier of improved swine genetics at the forefront, South Africa stands to continue to produce pigs with excellent genetics.

With the advances made in the availability of genetic technologies, the identification, technological and economic implications of the *RYR1* gene has been well researched in South Africa. The testing for the *RYR1* gene commenced in 1992 in the South African pig industry. Rhode & Harris., (1999), indicated a low frequency of the recessive (nn) homozygous genotype (associated with poor meat quality) and an increase in the frequency of the normal (NN) genotype. In a survey conducted by Soma *et al*. (2005), to determine the *RYR1*-gene status of pigs in nucleus herds and AI stations in South Africa indicated a low number of carriers (Nn) in the population tested. Soma *et al*., (2012), reported the incidence of the *RYR1* gene to be low in seed stock herds. It was also reported in the same study, that 96.4% of the animals tested from abattoirs, did not carry the mutation. Selection programs now rely immensely on the DNA test for the *RYR1* gene mutation to select animals with desirable and economic important traits.

In the late 1990's, the *IGF2* gene was identified to assist in selecting animals with the lean or fat allele. Literature has indicated that there are other genes involved in carcass leanness but the test for the *IGF2* gene is a leading, novel, diagnostic test already available to assist in selection programs. The genotyping cost has decreased thus making the *IGF2* gene test an affordable tool to be offered to the South African pig industry.

This study was the first attempt to genotype the SAL and LW pig populations in South Africa to determine the *IGF2* gene status and to evaluate the effect of the *IGF2* genotypes on meat and carcass characteristics. As the demand for pork continues to increase, increased selection pressure for more efficient, higher yielding pigs will remain. Therefore, selection may provide a means to meet the market demand for lean meat and bacon. Selection for fast growth or lean meat is often accompanied with a negative impact on meat quality characteristics.

In Chapter 2, the results have shown that 'AA' and 'GG' genotypes for *IGF2* are relatively well distributed throughout the SAL (42%) and LW (22%) breeds. No AA genotypes were observed in the Duroc, Pietrain and Chester populations tested in this study, but it should be noted that the samples sizes were relatively small as these breeds are not widely used by the South African stud breeders. The use of the RT-PCR assay presented in this study, will allow a rapid identification of the *IGF2* genotypes in South African pig populations. The ease of its use at low cost (approximately 15 US$ or R210) and the ability to genotype large populations will make the assay on *IGF2* an invaluable tool for South African pig breeders and meat scientists. The genotyping of South African pig populations for *IGF2* could be an important part of breeding programs in the future.

Chapter 3 of this study has demonstrated that selection pressure to produce leaner pigs has resulted in the 'A' allele to become nearly fixed in the SAL and LW population of one of the breeders tested. Due to the imprinting effect of the *IGF2* gene, differential selection may provide a means to meet market demands for 'leaner' or 'fatter' pigs in the South African pig industry. Pigs with the 'A' allele may be bred to improve lean meat yield with minimal implications upon carcass quality, while pigs with the 'G' allele may be selected for superior fat quality and meat processing. The selection of the fat allele in sow lines will not influence the carcass quality of the offspring. It was found in this study that the *IGF2* genotypes had no negative effect on meat and fat quality traits. From a nutritional point of view, the fatty acid composition from the animals of the different *IGF2* genotypes, is likely to have a positive effect on consumer perception of the pork product.

Genetic differences between breeds and specific genotypes do exist for the synthesis of individual fatty acids, which require more research attention at the molecular and biochemical levels. Producers may be indirectly selecting for a number of biological factors, some of which also affect meat and carcass quality. For example, SAL and LW are comparable in total backfat, but the fat distribution between the belly and *LD* is different. By selecting the particular *IGF2* allele might have beneficial effects in certain muscles and not in others. Fat is important in the overall acceptability of meat, to both meat technologists and the consumer. Fat quality forms an important part of meat quality.

Findings from this research indicate that both the *IGF2* 'A' and 'G' alleles can benefit the South African pig industry where pigs can be included in selection programs with additional *IGF2* genotypic

information. It was also evident that gene-assisted selection cannot be performed considering only the effect of a single gene but requires the incorporation of more complex phenomena such as the interaction between genes.

In order to cope with an unpredictable future, genetic reserves capable of readily responding to directional forces imposed by a broad spectrum of factors must be maintained. In addition, with increasing global human population pressures, the quantity of protein-based, staple foods and other products will have to increase. Future research studies should focus on extending the *IGF2* genotyping across all major South African pig breeds. This could provide insight to the potential of individual animals and breeds in breeding programs. Due to the high cost of analysis, this study was limited to only two populations and a small sample size. Follow-up studies can include enzyme studies such as calpains that also affect meat quality. Additional genetic markers need to be studied in relation to meat quality to fully assess the effects on carcass, meat and fat quality.

Next generation genome sequencing is becoming more affordable and is already being applied to livestock species (Rubin *et al*., 2010). The availability of the sequenced porcine genome and large-scale SNP chips, Porcine SNP60K, low cost genome scans will allow the identification and mapping of functional allelic variants affecting meat quality in South African commercial pig populations. The increasing value of genomics and the potential of genomics to increase the control of qualitative characteristics of meat and other economically important physiological functions, it is expected to further contribute to improve meat and carcass quality in pigs. This technology presents an opportunity to genotype South African pig breeds at the genome level that will be relevant to future pig breeding programs in South Africa.

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ADDENDUM

Addendum – Chapter 3

Addendum A Breed effects on percentage total fatty acids and fatty acid ratios: Belly fat

Treatment	LW	SAL	*P*-value
% total fatty acids:			
C10:0	0.02±0.01	0.02±0.01	0.642
C12:0	0.03±0.01	0.03±0.01	0.127
C14:0	1.12±0.08	1.10±0.11	0.430
C15:0	0.01±0.02	0.01±0.01	0.078
C16:0	22.75±1.10	22.75±0.97	0.999
C16:1c9	1.97±0.35	1.93±0.39	0.749
C17:0	0.20±0.06	0.19±0.06	0.803
C17:1c10	0.02±0.05	0.04±0.06	0.142
C18:0	10.86±1.27	11.10±1.12	0.441
C18:1t9	0.02±0.01	0.02±0.01	0.505
C18:1c9	37.07±3.00	38.02±1.90	0.154
C18:1c7	4.73±0.64	4.78±0.53	0.750
C18:2c9,12 (n-6)	17.77±3.67	16.64±2.30	0.146
C20:0	0.15±0.02[b]	0.17±0.03[a]	0.005
C18:3c6,9,12 (n-3)	0.00±0.00	0.00±0.00	0.308
C20:1c11	0.57±0.10	0.58±0.10	0.694
C18:3c9,12,15 (n-3)	1.45±0.32	1.41±0.22	0.598
C20:2c11,14 (n-6)	0.59±0.10	0.56±0.07	0.147
C20:3c8,11,14 (n-6)	0.07±0.02	0.07±0.01	0.385
C20:3c11,14,17 (n-3)	0.13±0.03	0.12±0.02	0.102
C20:4c5,8,11,14 (n-6)	0.19±0.04	0.19±0.03	0.528
C20:5c5,8,11,14,17 (n-3)	0.03±0.02	0.04±0.02	0.232
C22:5c7,10,13,16,19 (n-3).	0.16±0.04[a]	0.14±0.03[b]	0.058
C22:6c4,7,10,13,16,19 (n-3)	0.10±0.04	0.09±0.04	0.590
Fatty acid ratios			
Total SFA	35.15±2.09	35.33±1.64	0.685
Total MUFA	44.36±3.83	45.39±2.51	0.232
Total PUFA	20.49±4.16	19.28±2.62	0.166
Total n-6	18.63±3.78	17.47±2.37	0.148
Total n-3	1.87±0.40	1.81±0.27	0.475
PUFA:SFA	0.59±0.14	0.55±0.09	0.157
n-6/n-3	10.03±0.68[a]	9.70±0.59[b]	0.046
Atherogenicity Index (AI)	0.42±0.03	0.42±0.03	0.965
Desaturase Index	3.46±0.48	3.46±0.43	0.974
C18:0/C18:2	0.65±0.21	0.68±0.11	0.454
Double Bond Index (DBI)	88.34±5.93	86.84±3.95	0.218
Iodine value (IV)	76.03±5.09	74.73±3.39	0.218

[a], [b] = Different superscripts within the same row differ significantly (P<0.05)

Addendum B Breed effects on percentage total fatty acids and fatty acid ratios: Backfat

Treatment	LW	SAL	*P*-value
% total fatty acids:			
C10:0	0.00±0.00	0.00±0.01	0.845
C12:0	0.01±0.01	0.01±0.01	0.658
C14:0	0.91±0.08	0.90±0.09	0.487
C15:0	0.02±0.02	0.02±0.02	0.064
C16:0	20.54±1.46	20.97±1.20	0.177
C16:1c9	1.29±0.26	1.29±0.27	0.953
C17:0	0.27±0.07	0.26±0.09	0.551
C17:1c10	0.05±0.07	0.07±0.09	0.395
C18:0	11.24±1.07^{b}	12.00±1.21^{a}	0.014
C18:1t9	0.01±0.01	0.01±0.01	0.248
C18:1c9	34.79±2.61^{b}	36.33±1.97^{a}	0.009
C18:1c7	3.81±0.44	3.88±0.36	0.494
C18:2c9,12 (n-6)	23.07±4.47^{a}	20.43±2.47^{b}	0.003
C20:0	0.18±0.02^{b}	0.21±0.03^{a}	0.001
C18:3c6,9,12 (n-3)	0.00±0.01	0.00±0.01	0.646
C20:1c11	0.57±0.08	0.58±0.06	0.726
C18:3c9,12,15 (n-3)	1.74±0.35	1.64±0.26	0.186
C20:2c11,14 (n-6)	0.81±0.13^{a}	0.74±0.10^{b}	0.016
C20:3c8,11,14 (n-6)	0.08±0.02	0.08±0.01	0.599
C20:3c11,14,17 (n-3)	0.18±0.03^{a}	0.16±0.03^{b}	0.036
C20:4c5,8,11,14 (n-6)	0.17±0.03	0.17±0.03	0.836
C20:5c5,8,11,14,17 (n-3)	0.02±0.02^{b}	0.04±0.02^{a}	0.037
C22:5c7,10,13,16,19 (n-3)	0.16±0.03	0.15±0.04	0.154
C22:6c4,7,10,13,16,19 (n-3)	0.07±0.04	0.07±0.04	0.969
Fatty acid ratios:			
Total SFA	33.18±2.18^{b}	34.36±1.97^{a}	0.026
Total MUFA	40.52±3.18^{b}	42.16±2.38^{a}	0.021
Total PUFA	26.30±5.02^{a}	23.47±2.89^{b}	0.005
Total n-6	24.13±4.62^{a}	21.42±2.57^{b}	0.004
Total n-3	2.17±0.43	2.05±0.35	0.211
PUFA:SFA	0.80±0.20^{a}	0.69±0.12^{b}	0.004
n-6/n-3	11.15±0.81^{a}	10.53±0.77^{b}	0.004
Atherogenicity Index (AI)	0.36±0.04	0.37±0.03	0.154
Desaturase Index	3.11±0.23	3.06±0.41	0.637
C18:0/C18:2	0.51±0.16^{b}	0.60±0.10^{a}	0.015
Double Bond Index (DBI)	96.27±7.61^{a}	92.14±4.86^{b}	0.009
Iodine value (IV)	82.84±6.54^{a}	79.28±4.14^{b}	0.009

^{a}, ^{b} = Different superscripts within the same row differ significantly (P<0.05)

Addendum C Breed effects percentage total fatty acids: *Longissimus dorsi* muscle

Treatment	LW	SAL	*P*-value
% total fatty acids:			
C10:0	0.02±0.02	0.02±0.02	0.728
C12:0	0.01±0.01	0.01±0.01	0.487
C14:0	0.95±0.12	0.88±0.19	0.123
C15:0	0.01±0.02	0.01±0.02	0.153
C16:0	22.27±1.03	22.10±1.20	0.526
C16:1c9	2.35±0.39	2.26±0.50	0.420
C17:0	0.17±0.04	0.18±0.05	0.295
C17:1c10	0.33±0.20	0.35±0.23	0.752
C18:0	12.00±0.89	12.10±0.02	0.632
C18:1t9	0.02±0.02	0.02±0.01	0.490
C18:1c9	35.46±3.30	34.72±3.77	0.412
C18:1c7	5.57±0.63	5.42±0.60	0.347
C18:2c9,12 (n-6)	15.37±3.58	16.00±4.03	0.510
C20:0	0.12±0.02[b]	0.14±0.04[a]	0.017
C18:3c6,9,12 (n-3)	0.06±0.03	0.06±0.04	0.886
C20:1c11	0.45±0.07	0.46±0.07	0.956
C18:3c9,12,15 (n-3)	0.62±0.28	0.58±0.24	0.539
C20:2c11,14 (n-6)	0.40±0.10	0.38±0.08	0.442
C20:3c8,11,14 (n-6)	0.32±0.12	0.35±0.16	0.401
C20:3c11,14,17 (n-3)	0.07±0.03	0.06±0.03	0.373
C20:4c5,8,11,14 (n-6)	2.52±1.18	2.92±1.38	0.232
C20:5c5,8,11,14,17 (n-3)	0.21±0.08	0.22±0.08	0.658
C22:5c7,10,13,16,19 (n-3)	0.47±0.18	0.53±0.22	0.231
C22:6c4,7,10,13,16,19 (n-3)	0.22±0.10	0.23±0.10	0.716
Fatty acid ratios:			
Total SFA	35.56±1.68	35.46±1.72	0.819
Total MUFA	44.19±3.97	43.15±4.63	0.351
Total PUFA	20.25±4.84	21.39±5.78	0.404
Total n-6	18.61±4.45	19.71±5.40	0.385
Total n-3	1.65±0.42	1.68±0.42	0.740
PUFA:SFA	0.58±0.16	0.61±0.18	0.441
n-6/n-3	11.40±1.26	11.71±1.31	0.376
Atherogenicity Index (AI)	0.41±0.03	0.40±0.04	0.447
Desaturase Index	2.97±0.33	2.87±0.33	0.286
C18:0/C18:2	0.83±0.27	0.81±0.25	0.722
Double Bond Index (DBI)	93.71±8.91	96.09±10.82	0.354
Iodine value (IV)	79.77±7.29	81.66±8.82	0.367

[a], [b] = Different superscripts within the same row differ significantly (P<0.05)

Addendum D Genotype effects percentage total fatty acids and fatty acid ratios: Belly fat

Treatment	Genotype			*P*-value
	A/A	A/G	G/G	
% total fatty acids				
C10:0	0.02±0.01	0.02±0.01	0.03±0.01	0.249
C12:0	0.03±0.01	0.03±0.01	0.03±0.01	0.800
C14:0	1.09±0.09	1.12±0.09	1.13±0.11	0.453
C15:0	0.02±0.02[a]	0.01±0.01[b]	0.01±0.01[b]	0.016
C16:0	22.41±0.82[b]	23.17±1.03[a]	22.69±1.10[ab]	0.040
C16:1c9	1.90±0.39	1.99±0.34	1.96±0.38	0.772
C17:0	0.22±0.07[a]	0.17±0.04[b]	0.18±0.05[b]	0.017
C17:1c10	0.05±0.07[a]	0.01±0.04[b]	0.02±0.05[ab]	0.115
C18:0	10.91±1.51	11.12±0.90	10.91±1.15	0.821
C18:1t9	0.02±0.01	0.02±0.01	0.02±0.01	0.696
C18:1c9	36.90±2.34	37.91±2.23	37.83±2.96	0.387
C18:1c7	4.61±0.54	4.88±0.61	4.77±0.59	0.362
C18:2c9,12 (n-6)	18.28±3.01[a]	16.30±2.85[b]	17.03±3.23[ab]	0.113
C20:0	0.14±0.02[b]	0.16±0.03[a]	0.16±0.02[a]	0.007
C18:3c6,9,12 (n-3)	0.00±0.01	0.00±0.00	0.00±0.00	0.768
C20:1c11	0.56±0.09	0.58±0.11	0.58±0.11	0.844
C18:3c9,12,15 (n-3)	1.53±0.24	1.36±0.27	1.41±0.29	0.132
C20:2c11,14 (n-6)	0.62±0.09[a]	0.54±0.07[b]	0.57±0.09[b]	0.006
C20:3c8,11,14 (n-6)	0.08±0.01[a]	0.07±0.02[b]	0.07±0.01[ab]	0.088
C20:3c11,14,17 (n-3)	0.14±0.03[a]	0.12±0.02[b]	0.12±0.03[b]	0.013
C20:4c5,8,11,14 (n-6)	0.19±0.03[ab]	0.18±0.03[b]	0.20±0.03[a]	0.075
C20:5c5,8,11,14,17 (n-3)	0.03±0.12	0.03±0.02	0.04±0.02	0.264
C22:5c7,10,13,16,19 (n-3)	0.16±0.03	0.14±0.03	0.16±0.04	0.182
C22:6c4,7,10,13,16,19 (n-3)	0.09±0.04	0.09±0.04	0.09±0.04	0.918
Fatty acid ratios:				
Total SFA	34.84±2.01	35.74±1.58	35.14±1.95	0.277
Total MUFA	44.04±3.03	45.42±2.96	45.17±3.70	0.376
Total PUFA	21.12±3.39[a]	18.85±3.24[b]	19.69±3.65[ab]	0.105
Total n-6	19.17±3.10[a]	17.10±2.93[b]	17.87±3.31[ab]	0.104
Total n-3	1.95±0.32[a]	1.74±0.33[b]	1.82±0.35[ab]	0.136
PUFA:SFA	0.61±0.12[a]	0.53±0.10[b]	0.56±0.12[ab]	0.064
n-6/n-3	9.86±0.67	9.86±0.61	9.87±0.69	0.998
Atherogenicity Index (AI)	0.41±0.03	0.43±0.03	0.42±0.04	0.146
Desaturase Index	3.44±0.50	3.44±0.38	3.51±0.48	0.861
C18:0/C18:2	0.62±0.15	0.70±0.14	0.67±0.20	0.223
Double Bond Index (DBI)	89.35±5.17[a]	85.90±4.60[b]	87.52±5.00[ab]	0.076
Iodine value (IV)	76.89±4.44[a]	73.93±3.95[b]	75.31±4.29[ab]	0.076

[a], [b] = Different superscripts within the same row differ significantly (P<0.05)

Addendum E Genotype effects percentage total fatty acids and fatty acid ratios: Backfat

Treatment	Genotype			*P*-value
	A/A	A/G	G/G	
% total fatty acids:				
C10:0	0.00±0.00^{b}	0.00±0.00^{b}	0.00±0.01^{a}	0.015
C12:0	0.01±0.01	0.01±0.01	0.01±0.01	0.467
C14:0	0.89±0.08	0.92±0.09	0.91±0.09	0.494
C15:0	0.03±0.02^{a}	0.02±0.01^{b}	0.02±0.02^{b}	0.056
C16:0	20.44±1.29^{b}	21.25±1.43^{a}	20.56±1.21^{ab}	0.087
C16:1c9	1.26±0.34	1.34±0.21	1.27±0.20	0.537
C17:0	0.30±0.09^{a}	0.24±0.05^{b}	0.26±0.07^{ab}	0.031
C17:1c10	0.07±0.08	0.04±0.07	0.06±0.09	0.474
C18:0	11.48±1.45	11.66±1.10	11.72±1.03	0.768
C18:1t9	0.01±0.01	0.01±0.01	0.01±0.01	0.918
C18:1c9	34.78±3.11^{b}	36.24±1.76^{a}	35.63±2.08^{b}	0.110
C18:1c7	3.82±0.54	3.89±0.32	3.82±0.32	0.809
C18:2c9,12 (n-6)	22.92±4.71^{a}	20.61±3.39^{b}	21.80±3.00^{ab}	0.097
C20:0	0.18±0.03^{b}	0.20±0.03^{a}	0.20±0.03^{a}	0.058
C18:3c6,9,12 (n-3)	0.00±0.00	0.00±0.01	0.00±0.01	0.550
C20:1c11	0.57±0.05	0.59±0.08	0.56±0.07	0.470
C18:3c9,12,15 (n-3)	1.78±0.36	1.61±0.29	1.70±0.78	0.211
C20:2c11,14 (n-6)	0.82±0.14^{a}	0.74±0.10^{b}	0.78±0.11^{ab}	0.068
C20:3c8,11,14 (n-6)	0.08±0.01	0.07±0.02	0.08±0.01	0.151
C20:3c11,14,17 (n-3)	0.18±0.04	0.16±0.03	0.17±0.03	0.278
C20:4c5,8,11,14 (n-6)	0.17±0.02	0.16±0.03	0.17±0.03	0.473
C20:5c5,8,11,14,17 (n-3)	0.02±0.02^{b}	0.03±0.03^{ab}	0.04±0.01^{a}	0.032
C22:5c7,10,13,16,19 (n-3)	0.15±0.04	0.15±0.04	0.16±0.03	0.630
C22:6c4,7,10,13,16,19 (n-3)	0.06±0.04	0.06±0.05	0.08±0.04	0.211
Fatty acid ratios:				
Total SFA	33.32±2.30	34.29±2.24	33.68±1.85	0.313
Total MUFA	40.51±3.88	42.12±2.07	41.35±2.33	0.167
Total PUFA	26.17±5.28^{a}	23.60±3.83^{b}	24.96±3.40^{ab}	0.104
Total n-6	23.99±4.85^{a}	21.59±3.51^{b}	22.82±3.10^{ab}	0.094
Total n-3	2.19±0.47	2.02±0.38	2.14±0.32	0.312
PUFA:SFA	0.80±0.21^{a}	0.70±0.16^{b}	0.75±0.13^{ab}	0.111
n-6/n-3	11.03±0.88	10.80±0.97	10.71±0.66	0.380
Atherogenicity Index (AI)	0.36±0.03	0.38±0.04	0.37±0.03	0.155
Desaturase Index	3.07±0.44	3.13±0.26	3.05±0.24	0.753
C18:0/C18:2	0.53±0.14	0.59±0.15	0.55±0.12	0.312
Double Bond Index (DBI)	95.98±7.74	92.27±6.45	94.47±5.38	0.143
Iodine value (IV)	82.60±6.65	79.40±5.54	81.27±4.62	0.140

^{a}, ^{b} = Different superscripts within the same row differ significantly (P<0.05)

Addendum F Genotype effects on percentage total fatty acids and fatty acid ratios: *Longissimus dorsi* muscle

Treatment	Genotype			*P*-value
	A/A	A/G	G/G	
% total fatty acids:				
C10:0	0.02±0.02	0.02±0.02	0.03±0.02	0.403
C12:0	0.01±0.01	0.01±0.01	0.01±0.02	0.394
C14:0	0.89±0.17	0.91±0.12	0.95±0.19	0.481
C15:0	0.02±0.02^{a}	0.00±0.01^{b}	0.01±0.02^{b}	0.005
C16:0	21.54±1.00^{b}	22.41±0.88^{a}	22.61±1.17^{a}	0.004
C16:1c9	2.15±0.40	2.34±0.38	2.42±0.52	0.166
C17:0	0.20±0.05^{a}	0.16±0.03^{b}	0.16±0.05^{b}	0.004
C17:1c10	0.34±0.25	0.38±0.18	0.30±0.21	0.514
C18:0	11.88±0.78	12.12±0.77	12.16±0.74	0.472
C18:1t9	0.02±0.02	0.02±0.02	0.02±0.01	0.810
C18:1c9	34.17±3.00	35.50±3.27	35.61±4.20	0.360
C18:1c7	5.25±0.59^{b}	5.52±0.51^{ab}	5.71±0.68^{a}	0.058
C18:2c9,12 (n-6)	17.52±3.31^{a}	14.97±3.23^{b}	14.57±4.23^{b}	0.029
C20:0	0.12±0.02^{b}	0.14±0.03^{a}	0.14±0.03^{ab}	0.110
C18:3c6,9,12 (n-3)	0.06±0.04	0.07±0.03	0.07±0.04	0.546
C20:1c11	0.45±0.06	0.45±0.07	0.46±0.07	0.834
C18:3c9,12,15 (n-3)	0.74±0.36^{a}	0.53±0.16^{b}	0.52±0.17^{b}	0.010
C20:2c11,14 (n-6)	0.45±0.12^{a}	0.36±0.05^{b}	0.36±0.06^{b}	0.002
C20:3c8,11,14 (n-6)	0.33±0.12	0.35±0.12	0.33±0.18	0.955
C20:3c11,14,17 (n-3)	0.08±0.04^{a}	0.06±0.02^{b}	0.06±0.03^{b}	0.031
C20:4c5,8,11,14 (n-6)	2.08±1.44	2.75±0.94	2.61±1.48	0.889
C20:5c5,8,11,14,17 (n-3)	0.22±0.10	0.22±0.07	0.21±0.07	0.844
C22:5c7,10,13,16,19 (n-3)	0.52±0.21	0.50±0.17	0.48±0.22	0.776
C22:6c4,7,10,13,16,19 (n-3)	0.22±0.12	0.23±0.09	0.23±0.09	0.947
Fatty acid ratios:				
Total SFA	34.68±1.67	35.81±1.40	36.06±1.70	0.023
Total MUFA	42.38±3.59	44.16±3.81	44.52±5.20	0.251
Total PUFA	22.94±4.51^{a}	20.03±4.67^{ab}	19.42±6.13^{b}	0.085
Total n-6	21.10±4.17^{a}	18.44±4.30^{ab}	17.87±5.75^{b}	0.090
Total n-3	1.84±0.39^{a}	1.60±0.38^{ab}	1.55±0.43^{b}	0.062
PUFA:SFA	0.67±0.15^{a}	0.56±0.15^{ab}	0.55±0.19^{b}	0.049
n-6/n-3	11.56±1.27	11.57±0.76	11.53±1.70	0.993
Atherogenicity Index (AI)	0.39±0.03^{b}	0.41±0.03^{a}	0.41±0.04^{a}	0.022
Desaturase Index	2.89±0.31	2.94±0.30	2.94±0.39	0.868
C18:0/C18:2	0.70±0.16^{b}	0.86±0.24^{ab}	0.91±0.31^{a}	0.036
Double Bond Index (DBI)	98.19±8.96	93.88±8.66	92.52±11.33	0.174
Iodine value (IV)	83.52±7.26	79.82±7.09	78.71±9.22	0.149

^{a}, ^{b} = Different superscripts within the same row differ significantly (P<0.05)

Addendum G Breed*Genotype effects on percentage total fatty acids and fatty acid ratios: Belly fat

Treatment	Breed*Genotype						*P*-value
	LW/AA	**LW/AG**	**LW/GG**	**SAL/AA**	**SAL/AG**	**SAL/GG**	
% total fatty acids:							
C10:0	0.02±0.01	0.02±0.02	0.03±0.01	0.02±0.02	0.02±0.01	0.03±0.02	0.653
C12:0	0.03±0.01	0.03±0.01	0.03±0.01	0.03±0.01	0.03±0.01	0.03±0.01	0.908
C14:0	1.09±0.06	1.16±0.08	1.12±0.10	1.09±0.11	1.08±0.08	1.14±0.13	0.267
C15:0	0.03±0.02^{a}	0.01±0.01^{b}	0.01±0.01^{b}	0.01±0.02^{b}	0.00±0.01^{b}	0.01±0.02^{b}	0.154
C16:0	21.99±0.67^{b}	23.69±0.79^{a}	22.58±1.07^{b}	22.82±0.76^{b}	22.65±1.01^{b}	22.79±1.18^{b}	0.008
C16:1c9	1.89±0.31	2.12±0.38	1.89±0.33	1.92±0.47	1.85±0.24	2.03±0.44	0.203
C17:0	0.25±0.07^{a}	0.17±0.05^{b}	0.17±0.03^{b}	0.20±0.07^{b}	0.18±0.04^{b}	0.19±0.06^{b}	0.189
C17:1c10	0.02±0.06^{b}	0.00±0.00^{b}	0.03±0.06^{ab}	0.07±0.08^{a}	0.02±0.05^{b}	0.02±0.05^{ab}	0.293
C18:0	10.36±1.30^{b}	11.10±1.13^{ab}	11.12±1.35^{ab}	11.46±1.56^{a}	11.13±0.67^{ab}	10.71±0.93^{ab}	0.131
C18:1t9	0.02±0.01	0.02±0.01	0.02±0.01	0.02±0.00	0.02±0.01	0.02±0.01	0.360
C18:1c9	36.14±2.35	37.51±2.62	37.57±3.88	37.66±2.18	38.31±1.82	38.09±1.82	0.815
C18:1c7	4.62±0.50	4.95±0.73	4.61±0.66	4.60±0.60	4.80±0.51	4.92±0.48	0.442
C18:2c9,12 (n-6)	19.77±2.75^{a}	16.09±3.46^{b}	17.45±4.03^{ab}	16.79±2.56^{b}	16.51±2.25^{b}	16.61±2.33^{b}	0.199
C20:0	0.14±0.01^{c}	0.15±0.02^{c}	0.16±0.02^{bc}	0.15±0.03^{c}	0.18±0.03^{a}	0.17±0.02^{ab}	0.451
C18:3c6,9,12 (n-3)	0.00±0.00	0.00±0.00	0.00±0.01	0.00±0.01	0.00±0.01	0.00±0.00	0.167
C20:1c11	0.57±0.09	0.58±0.12	0.56±0.11	0.56±0.10	0.58±0.10	0.59±0.12	0.770
C18:3c9,12,15 (n-3)	1.64±0.17^{a}	1.30±0.31^{b}	1.41±0.37^{ab}	1.41±0.25^{ab}	1.42±0.22^{ab}	1.40±0.22^{ab}	0.113
C20:2c11,14 (n-6)	0.68±0.07^{a}	0.52±0.07^{b}	0.57±0.09^{b}	0.57±0.07^{b}	0.56±0.07^{b}	0.56±0.09^{b}	0.010
C20:3c8,11,14 (n-6)	0.08±0.02^{a}	0.07±0.02^{b}	0.07±0.01^{ab}	0.07±0.01^{ab}	0.07±0.01^{b}	0.07±0.01^{ab}	0.502
C20:3c11,14,17 (n-3)	0.16±0.02^{a}	0.11±0.02^{b}	0.12±0.03^{b}	0.12±0.03^{b}	0.12±0.02^{b}	0.12±0.03^{b}	0.014
C20:4c5,8,11,14 (n-6)	0.20±0.03	0.18±0.04	0.20±0.03	0.18±0.03	0.18±0.02	0.20±0.04	0.560
C20:5c5,8,11,14,17 (n-3)	0.04±0.02^{ab}	0.02±0.02^{b}	0.04±0.03^{a}	0.03±0.02^{ab}	0.04±0.02^{a}	0.04±0.02^{a}	0.182
C22:5c7,10,13,16,19 (n-3)	0.18±0.03^{a}	0.14±0.02^{b}	0.16±0.04^{ab}	0.14±0.02^{b}	0.14±0.03^{b}	0.15±0.03^{b}	0.038
C22:6c4,7,10,13,16,19 (n-3)	0.11±0.04	0.09±0.04	0.09±0.05	0.07±0.03	0.10±0.04	0.10±0.05	0.178
Fatty acid ratios:							
Total SFA	33.90±1.77^{b}	36.32±1.57^{a}	35.22±2.27^{ab}	35.78±1.85^{a}	35.15±1.43^{ab}	35.07±1.68^{ab}	0.029
Total MUFA	43.26±3.10	45.17±3.61	44.66±4.73	44.83±2.91	45.66±2.31	45.68±2.43	0.873
Total PUFA	22.85±3.02^{a}	18.51±3.91^{b}	20.13±4.55^{ab}	19.39±2.92^{b}	19.19±2.58^{b}	19.25±2.63^{b}	0.152
Total n-6	20.73±2.82^{a}	16.85±3.56^{b}	18.30±4.13^{ab}	17.62±2.63^{b}	17.36±2.31^{b}	17.44±2.39^{b}	0.176
Total n-3	2.12±0.23^{a}	1.65±0.36^{b}	1.83±0.45^{b}	1.78±0.31^{b}	1.84±0.29^{ab}	1.81±0.25^{b}	0.042
PUFA:SFA	0.68±0.10^{a}	0.51±0.12^{b}	0.57±0.14^{b}	0.55±0.09^{b}	0.55±0.09^{b}	0.55±0.09^{b}	0.053
n-6/n-3	9.76±0.65^{abc}	10.22±0.48^{a}	10.10±0.83^{ab}	9.97±0.71^{abc}	9.50±0.53^{c}	9.64±0.42^{bc}	0.056
Atherogenicity Index	0.40±0.02^{b}	0.44±0.02^{a}	0.42±0.04^{ab}	0.42±0.02^{ab}	0.41±0.03^{b}	0.42±0.04^{ab}	0.016
Desaturase Index	3.54±0.48	3.42±0.46	3.43±0.55	3.35±0.54	3.45±0.29	3.59±0.43	0.490
C18:0/C18:2	0.53±0.11^{b}	0.72±0.18^{a}	0.69±0.26^{a}	0.70±0.14^{a}	0.69±0.10^{a}	0.66±0.11^{ab}	0.091
Double Bond Index (DBI)	92.31±4.26^{a}	84.83±5.18^{b}	87.90±6.07^{b}	86.39±4.34^{b}	86.97±3.92^{b}	87.15±3.96^{b}	0.029
Iodine value (IV)	79.41±3.65^{a}	73.03±4.47^{b}	75.63±5.21^{b}	74.37±3.75^{b}	74.82±3.35^{b}	74.99±3.40^{b}	0.031

^{a}, ^{b}= Different superscripts within the same row differ significantly (P<0.05)

Addendum H Breed*Genotype effects on percentage total fatty acids and fatty acid ratios: Backfat

Treatment	Breed*Genotype						*P*-value
	LW/AA	LW/AG	LW/GG	SAL/AA	SAL/AG	SAL/GG	
% total fatty acids:							
C10:0	0.00±0.00	0.00±0.00	0.01±0.01	0.00±0.00	0.00±0.00	0.00±0.01	0.984
C12:0	0.01±0.01	0.01±0.01	0.01±0.01	0.01±0.01	0.01±0.01	0.01±0.01	0.681
C14:0	0.87±0.07^{b}	0.96±0.08^{a}	0.90±0.08^{ab}	0.90±0.10^{ab}	0.87±0.08^{b}	0.92±0.10^{ab}	0.081
C15:0	0.04±0.02^{a}	0.02±0.02^{b}	0.02±0.01^{b}	0.02±0.02^{b}	0.02±0.01^{b}	0.02±0.02^{b}	0.040
C16:0	19.65±1.12^{c}	21.72±1.52^{a}	20.26±0.86^{bc}	21.24±0.93^{ab}	20.79±1.23^{ab}	20.89±1.48^{ab}	0.007
C16:1c9	1.15±0.29^{b}	1.47±0.18^{a}	1.25±0.19^{ab}	1.36±0.37^{ab}	1.21±0.15^{b}	1.29±0.23^{ab}	0.013
C17:0	0.34±0.06^{a}	0.23±0.06^{b}	0.24±0.02^{b}	0.26±0.10^{b}	0.25±0.06^{b}	0.27±0.11^{ab}	0.045
C17:1c10	0.06±0.08	0.04±0.07	0.04±0.07	0.08±0.09	0.04±0.08	0.08±0.11	0.711
C18:0	10.78±0.68^{b}	11.34±1.12^{ab}	11.61±1.24^{ab}	12.18±1.70^{a}	11.97±1.03^{a}	11.84±0.80^{a}	0.275
C18:1t9	0.01±0.01	0.01±0.01	0.01±0.01	0.01±0.01	0.01±0.01	0.01±0.02	0.968
C18:1c9	32.95±2.68^{b}	36.11±1.80^{a}	35.30±2.35^{a}	36.60±2.40^{a}	36.37±1.80^{a}	36.00±1.81^{a}	0.035
C18:1c7	3.65±0.62^{b}	4.02±0.32^{a}	3.76±0.27^{ab}	3.99±0.43^{ab}	3.77±0.28^{ab}	3.88±0.37^{ab}	0.076
C18:2c9,12 (n-6)	26.15±4.24^{a}	20.45±4.10^{b}	22.61±3.37^{b}	19.68±2.34^{b}	20.77±2.71^{b}	20.89±2.40^{b}	0.006
C20:0	0.17±0.02^{c}	0.19±0.02^{bc}	0.19±0.02^{bc}	0.20±0.03^{ab}	0.21±0.03^{a}	0.22±0.04^{a}	0.948
C18:3c6,9,12 (n-3)	0.00±0.00	0.00±0.01	0.00±0.01	0.00±0.00	0.00±0.01	0.00±0.01	0.974
C20:1c11	0.56±0.05	0.60±0.09	0.55±0.08	0.58±0.05	0.58±0.07	0.57±0.06	0.376
C18:3c9,12,15 (n-3)	1.99±0.33^{a}	1.52±0.30^{b}	1.73±0.29^{ab}	1.57±0.27^{b}	1.71±0.27^{b}	1.66±0.24^{b}	0.006
C20:2c11,14 (n-6)	0.91±0.10^{a}	0.73±0.11^{b}	0.79±0.09^{b}	0.72±0.09^{b}	0.75±0.08^{b}	0.76±0.13^{b}	0.003
C20:3c8,11,14 (n-6)	0.09±0.02	0.07±0.02	0.08±0.01	0.08±0.01	0.07±0.02	0.08±0.02	0.689
C20:3c11,14,17 (n-3)	0.20±0.03^{a}	0.16±0.03^{b}	0.17±0.03^{b}	0.15±0.03^{b}	0.17±0.03^{b}	0.16±0.04^{b}	0.006
C20:4c5,8,11,14 (n-6)	0.17±0.03	0.15±0.03	0.18±0.03	0.17±0.02	0.17±0.03	0.17±0.03	0.333
C20:5c5,8,11,14,17 (n-3)	0.02±0.02^{b}	0.02±0.02^{b}	0.04±0.01^{a}	0.03±0.02^{ab}	0.04±0.03^{a}	0.04±0.02^{a}	0.111
C22:5c7,10,13,16,19 (n-3)	0.17±0.04^{a}	0.14±0.02^{b}	0.17±0.03^{a}	0.13±0.03^{b}	0.16±0.05^{ab}	0.15±0.03^{ab}	0.040
C22:6c4,7,10,13,16,19 (n-3)	0.07±0.04^{ab}	0.05±0.04^{b}	0.09±0.03^{a}	0.05±0.04^{b}	0.08±0.05^{ab}	0.07±0.04^{ab}	0.072
Fatty acid ratios:							
Total SFA	31.85±1.47^{b}	34.46±2.55^{a}	33.24±1.67^{ab}	34.79±2.04^{a}	34.10±2.00^{a}	34.18±2.01^{a}	0.037
Total MUFA	38.39±3.50^{b}	42.26±2.13^{a}	40.92±2.67^{a}	42.63±3.09^{a}	41.98±2.12^{a}	41.84±1.92^{a}	0.026
Total PUFA	29.77±4.73^{a}	23.28±4.57^{b}	25.85±3.76^{b}	22.57±2.77^{b}	23.92±3.15^{b}	23.98±2.82^{b}	0.005
Total n-6	27.33±4.36^{a}	21.41±4.24^{b}	23.65±3.45^{b}	20.64±2.43^{b}	21.77±2.81^{b}	21.90±2.52^{b}	0.006
Total n-3	2.44±0.43^{a}	1.88±0.36^{b}	2.19±0.32^{ab}	1.93±0.36^{b}	2.15±0.36^{ab}	2.08±0.33^{b}	0.005
PUFA:SFA	0.94±0.19^{a}	0.69±0.18^{b}	0.78±0.14^{b}	0.65±0.09^{b}	0.71±0.14^{b}	0.71±0.12^{b}	0.005
n-6/n-3	11.26±1.00^{ab}	11.41±0.82^{a}	10.79±0.47^{abc}	10.80±0.73^{abc}	10.19±0.69^{c}	10.62±0.84^{bc}	0.101
Atherogenicity Index	0.34±0.03^{c}	0.39±0.04^{a}	0.36±0.02^{bc}	0.38±0.03^{ab}	0.37±0.03^{abc}	0.38±0.04^{ab}	0.015
Desaturase Index	3.06±0.25	3.20±0.19	3.06±0.24	3.08±0.60	3.06±0.31	3.05±0.26	0.741
C18:0/C18:2	0.42±0.09^{b}	0.59±0.18^{a}	0.53±0.15^{ab}	0.63±0.11^{a}	0.59±0.11^{a}	0.57±0.09^{a}	0.040
Double Bond Index (DBI)	101.37±6.67^{a}	91.52±7.54^{bc}	95.91±5.59^{b}	90.604.20^{c}	93.02±5.46^{bc}	92.87±4.96^{bc}	0.006
Iodine value (IV)	87.23±5.72^{a}	78.79±6.50^{b}	82.51±4.82^{b}	77.97±3.59^{b}	80.01±4.66^{b}	79.91±4.23^{b}	0.006

^{a}, ^{b}= Different superscripts within the same row differ significantly (P<0.05)

Addendum I Breed*Genotype effects on percentage total fatty acids and fatty acid ratios: *Longissimus dorsi* muscle

Treatment	Breed*Genotype						*P*-value
	LW/AA	LW/AG	LW/GG	SAL/AA	SAL/AG	SAL/GG	
% total fatty acids:							
C10:0	0.02±0.02	0.02±0.02	0.03±0.02	0.02±0.02	0.02±0.02	0.02±0.02	0.454
C12:0	0.01±0.01	0.01±0.02	0.01±0.02	0.01±0.01	0.01±0.01	0.01±0.02	0.808
C14:0	0.90±0.16	0.96±0.11	0.98±0.10	0.87±0.18	0.86±0.11	0.92±0.26	0.811
C15:0	0.02±0.02	0.00±0.01	0.00±0.01	0.02±0.02	0.00±0.01	0.02±0.02	0.551
C16:0	21.44±0.97	22.69±0.85	22.69±0.76	21.65±1.06	22.14±0.87	22.52±1.52	0.517
C16:1c9	2.16±0.37	2.45±0.35	2.44±0.42	2.14±0.45	2.24±0.40	2.39±0.64	0.759
C17:0	0.21±0.04	0.16±0.02	0.14±0.03	0.20±0.06	0.16±0.03	0.18±0.06	0.269
C17:1c10	0.34±0.27	0.38±0.16	0.27±0.15	0.34±0.25	0.38±0.20	0.33±0.26	0.882
C18:0	11.82±0.77	12.08±1.04	12.11±0.89	11.93±0.82	12.16±0.41	12.21±0.59	0.999
C18:1t9	0.02±0.02	0.02±0.02	0.02±0.01	0.01±0.01	0.02±0.01	0.02±0.01	0.237
C18:1c9	34.39±3.37	34.93±2.86	37.08±3.31	33.96±2.75	36.06±3.70	34.14±4.64	0.188
C18:1c7	5.29±0.65	5.53±0.44	5.88±0.69^{a}	5.20±0.55^{b}	5.52±0.60^{ab}	5.54±0.66^{ab}	0.666
C18:2c9,12 (n-6)	17.31±3.74^{a}	15.14±2.89^{ab}	13.66±3.39^{b}	17.73±3.01^{a}	14.79±3.69^{ab}	15.48±4.95^{ab}	0.640
C20:0	0.13±0.02^{b}	0.12±0.02^{b}	0.13±0.02^{b}	0.12±0.02^{b}	0.16±0.04^{a}	0.15±0.04^{ab}	0.072
C18:3c6,9,12 (n-3)	0.05±0.04	0.08±0.02	0.06±0.02	0.06±0.03	0.06±0.03	0.07±0.06	0.178
C20:1c11	0.46±0.07	0.43±0.07	0.47±0.06	0.44±0.05	0.47±0.07	0.45±0.09	0.286
C18:3c9,12,15 (n-3)	0.75±0.41^{a}	0.54±0.14^{abc}	0.56±0.20^{abc}	0.73±0.32^{ab}	0.53±0.18^{bc}	0.47±0.12^{c}	0.854
C20:2c11,14 (n-6)	0.47±0.13^{a}	0.36±0.05^{b}	0.36±0.07^{b}	0.43±0.11^{ab}	0.36±0.05^{b}	0.36±0.06^{b}	0.721
C20:3c8,11,14 (n-6)	0.33±0.13	0.36±0.11	0.28±0.09	0.33±0.12	0.34±0.13	0.39±0.22	0.262
C20:3c11,14,17 (n-3)	0.08±0.05^{a}	0.06±0.01^{ab}	0.06±0.02^{ab}	0.08±0.03^{ab}	0.06±0.02^{ab}	0.05±0.03^{b}	0.936
C20:4c5,8,11,14 (n-6)	2.79±1.66^{ab}	2.77±0.77^{ab}	1.99±0.83^{b}	2.81±1.27^{ab}	2.72±1.12^{ab}	3.23±1.76^{a}	0.215
C20:5c5,8,11,14,17 (n-3)	0.23±0.12	0.22±0.05	0.19±0.05	0.22±0.08	0.22±0.09	0.23±0.07	0.454
C22:5c7,10,13,16,19 (n-3)	0.53±0.23^{ab}	0.49±0.13^{ab}	0.38±0.12^{b}	0.51±0.19^{ab}	0.50±0.20^{ab}	0.57±0.26^{a}	0.189
C22:6c4,7,10,13,16,19 (n-3)	0.24±0.14	0.21±0.06	0.21±0.10	0.19±0.10	0.24±0.12	0.25±0.07	0.264
Fatty acid ratios:							
Total SFA	34.54±1.67	36.04±1.58	36.10±1.44	34.81±1.74	35.56±1.20	36.02±2.00	0.773
Total MUFA	42.67±4.05	43.74±3.24	46.16±4.09	42.09±3.25	44.62±4.51	42.87±5.87	0.306
Total PUFA	22.79±4.96	20.23±3.95	17.74±4.58	23.09±4.28	19.82±5.6	21.11±7.21	0.485
Total n-6	20.90±4.57	18.63±3.68	16.29±4.21	21.30±3.96	18.23±5.14	19.46±6.82	0.482
Total n-3	1.89±0.43	1.60±0.29	1.46±0.43	1.79±0.37	1.60±0.49	1.65±0.42	0.512
PUFA:SFA	0.67±0.17	0.57±0.13	0.50±0.13	0.67±0.15	0.56±0.17	0.59±0.22	0.551
n-6/n-3	11.11±1.15	11.66±0.83	11.44±1.71	12.00±1.28	11.48±0.70	11.63±1.77	0.433
Atherogenicity Index	0.38±0.03	0.42±0.02	0.42±0.02	0.39±0.03	0.40±0.03	0.41±0.05	0.675
Desaturase Index	2.92±0.35	2.91±0.33	3.08±0.33	2.86±0.28	2.97±0.28	2.81±0.42	0.316
C18:0/C18:2	0.72±0.18	0.83±0.21	0.95±0.35	0.69±0.15	0.88±0.27	0.86±0.28	0.669
Double Bond Index (DBI)	98.31±9.72	93.73±7.27	89.09±7.83	98.06±8.65	94.06±10.46	95.95±13.55	0.451
Iodine value (IV)	83.60±7.85	79.72±6.00	75.99±6.44	83.44±7.03	79.94±8.53	84.44±11.02	0.470