Fat content and fatty acid composition of South African Wagyu beef

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Declaration

I, Twanette Duvenage, hereby declare that this dissertation, submitted for the MSc (Agric) Animal Science: Animal Physiology degree at the University of Pretoria, is my own work and has not previously been submitted by me for a degree at any other university.

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Abstract

The number of Wagyu breeders is growing and the availability of Wagyu beef has increased in South Africa over the past few years. Virtually no research has been done on local Wagyu beef. Diets, feeding and cattle-rearing strategies of Wagyu cattle in South Africa differ from those in Japan. Most Wagyu's for slaughter are of the F1 generation. This may affect the fat content and fatty acid (FA) composition of South African Wagyu beef. The fat content and the FA composition are influenced by many factors. Wagyu cattle are known to be genetically predisposed to produce vast amounts of marbling or intramuscular fat in the beef cuts. A common misconception among consumers is that all fat in red meat is saturated and will have a negative effect on health, especially non-communicable diseases. On the contrary, red meat has many benefits for human health, and is a source of essential FA, of fat-soluble vitamins and minerals, as well as of protein and energy. In this study South African Wagyu beef and beef from composite feedlot cattle were compared in respect of fat content and FA composition.

Samples were collected from different fat depots in carcasses of 13 randomly selected Wagyu and 13 composite feedlot cattle at a commercial abattoir. Intramuscular (IM), subcutaneous fat (SCF) and perirenal fat (PRF) samples were collected from each carcass. All samples were collected on the left side of each carcass. FA analysis was done on 78 samples to determine the FA composition of each anatomical location for both Wagyu and typical composite feedlot cattle. Ether extracts were done on all the IM Wagyu samples to determine the actual fat percentage and to compare that to the estimated fat percentage given by the marbling score. One would assume that the higher amount of fat in Wagyu beef would be unhealthy because it is the same FA in the same ratio as in beef from composite feedlot cattle in South Africa.

The results of this study show that there is a difference in the FA composition between Wagyu and composite feedlot cattle. The same FAs were detected in both; however the amount of each FA differs. More IMF is found in Wagyu than in composite feedlot cattle. The ratio of the main FA groups was also calculated and compared. The ratio of n-6/n-3 is significantly lower in Wagyu beef than in composite beef, which is more favourable when comparing this ratio to the recommended daily intake.

The measures used to determine marbling score is the Australian marbling score and the MIJ camera marbling score. For each marbling score these measurements gave an estimated fat percentage. These estimates were compared to the actual fat percentage. These two measurements were significantly correlated. The MIJ camera marbling scores were better correlated to the actual fat percentages.

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List of Abbreviations

Abbreviation	Name
C10:0	Capric acid
C12:0	Lauric acid
C13:0	Tridecylic acid
C14:0	Myristic acid
C14:1	Myristoleic
C16:0	Palmitic acid
C16:1	Palmitoleic acid
C17:0	Margaric acid
C18:0	Stearic acid
C18:1	Oleic acid
C18:1n9c	Oleic acid
C18:1n9t	Elaidic
C18:2	Linoleic acid
C18:2n6c	Linoleic acid
C18:2n6t	Linolelaidic
C18:3n3	α-Linolenic
C18:3n6	γ-Linolenic
C20:0	Arachidic
C20:1	Gondoic acid
C20:2	Eicosadienoic acid
C20:3n6	Homo-γ-Linolenic
C20:4n6	Arachidonic
C21:0	Heneicosylic acid
C22:0	Behenic
C22:1n9	Erucic
C22:2n9	Docosadienoic
C23:0	Tricosylic acid
EFAs	Essential fatty acids
FA	Fatty acids
IM	Intramuscular
IMF	Intramuscular fat
MUFA	Mono-unsaturated fatty acids

PR	Perirenal
PRF	Perirenal fat
PUFA	Polyunsaturated fatty acids
SC	Subcutaneous
SCF	Subcutaneous fat
SFA	Saturated fatty acids
UFA	Unsaturated fatty acids

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Chapter 1: Introduction and Motivation

1.1 Introduction

There is a demand from Wagyu beef cattle breeders and abattoirs in South Africa for research on the effects of local production systems and practices to determine the meat quality of this breed, because virtually no research has been done on local South African Wagyu cattle to date (Wagyu.org.za, 2018). The cattle feeding systems and feed ingredients used in South Africa differ from those used in Japan. This may affect the rate and extent of fattening of South African Wagyu cattle, with subsequent effects on the fat content and fatty acid (FA) composition. Most of the local Wagyu cattle that are fattened and slaughtered are F1 Wagyu crosses, which differ from those fed in Japan (Wagyu.org.za, 2018).

The FA content of beef has an effect on human health; for example, polyunsaturated FAs (PUFAs) play an anti-carcinogenic and cardioprotective role in human health (Suksombat *et al.*, 2016). Oleic acid is one of the most abundant FAs in bovine tissue (Smith & Smith, 2014). Oleic acid (18:1 n-9) intake reduces the risk of metabolic diseases in humans (Gotoh & Joo, 2017). The oleic acid concentration in subcutaneous adipose tissue in Wagyu cattle is 52.9%, which is high in comparison to other cattle (Smith *et al.*, 2006). Intramuscular fat (IMF) is the main contributor to oleic acid found in meat cuts, thus Wagyu meat will have a high proportion of oleic acid and consequently a high proportion of mono-unsaturated fatty acids (MUFA) (Gotoh & Joo, 2017).

The fat content of beef has an effect on the beef quality in respect of marbling score, which is highly regarded, and eating experience, which is what Wagyu cattle are famous for. Marbling contributes to the tenderness of beef. The IMF is located between muscle fibres in such a way that it causes a dilution or disorganisation of perimysium connective tissue, resulting in beef that is perceived to be more tender (Gotoh & Joo, 2017). The higher concentration of unsaturated FA (UFA) results in a unique mouth feel because UFA has a lower melting point. This means that fat is liquefied in the mouth during consumption of Wagyu beef (Roh *et al.*, 2006; Gotoh *et al.*, 2018; Piao *et al.*, 2018).

1.2 Aim

The aim of this research was to determine the differences in fat content and FA composition between local Wagyu cattle and composite feedlot cattle, in terms of the predominant anatomical adipose tissue locations, namely IMF, subcutaneous fat (SCF), and perirenal fat (PRF).
The first objective of this study was to compare the FA composition of Wagyu cattle to that of composite feedlot cattle. In theory the FA composition should be very similar between Wagyu cattle and composite feedlot cattle, owing to the extensive biohydrogenation of FAs in the rumen (Webb & O'Neill, 2008).

The second objective was to determine the actual fat percentage in the intramuscular Wagyu samples, in order to determine the correlation between the actual fat percentage and the estimated fat percentage given by the marbling score.

Chapter 2: Literature review

2.1 Introduction

Wagyu cattle are genetically predisposed to produce marbling in beef. Marbling, also known as IMF, is seen as white flecks of fat deposited between the muscle fibre bundles in the skeletal muscles (Frank *et al.*, 2016; Bermingham *et al.*, 2018).

In the current study two types of cattle will be referred to: composite feedlot cattle and South African Wagyu cattle. Reference will be made to anatomical adipose tissue location, especially in the statistical approach, involving IMF, SCF, and PRF. The location refers to the anatomical locations that were sampled in this study.

2.2 Brief history of the establishment of Wagyu cattle

Originally Japanese cattle were used as labour/draught animals (Motoyama *et al.*, 2016). These native Japanese cattle were well adapted to the unique climate and environment in Japan. In the 1860s the Japanese culture adopted a more Westernised lifestyle (*Gotoh et al., 2018*). This meant that the consumption of beef increased. To cope with this new demand for beef, Japan started improving the genetics of native Japanese cattle breeds that had previously only been used for labour (Motoyama *et al.*, 2016). In the 1900s, Japan started crossing its indigenous breeds with imported breeds such as Shorthorn, Braunvieh, Holstein, Simmental, Ayrshire and Devon cattle (Gotoh *et al.*, 2018). At first none of the crossbreds seemed successful because of their inferior draught ability (Motoyama *et al.*, 2016). From these crosses, breeders soon realised an excessive increase in the body size and meat yield of the animals, at the expense of meat quality (Gotoh *et al.*, 2018). All crossbreeding stopped in 1910 (Motoyama *et al.*, 2016). The Japanese started intra-breeding cattle, which led to the establishment of the Japanese Black breed in 1944 (Gotoh *et al.*, 2018). Since then this breed has been used to improve meat production (Gotoh *et al.*, 2018; Motoyama *et al.*, 2016).

Japanese beef differs from that in the rest of the world because the breeders worked on increasing the quality of the beef rather than the quantity (Gotoh *et al.*, 2018). They had the opportunity to focus on beef quality because unlike other countries, the Japanese consumer still makes use of rice as the main dish, while beef is served as a side dish (Gotoh *et al.*, 2018).

Four breeds are commonly referred to as Wagyu. These include Black, Brown (Akaushi), Shorthorn and Polled (Gotoh *et al.*, 2018). Among these Wagyu breeds Japanese Black cattle have the ability to accumulate most marbling or IMF. Values as high as 50% IMF in the ribeye area of Japanese Black cattle have been reported (Motoyama *et al.*, 2016). It is believed that the reason for this high IMF content is the way these animals have adapted to Japanese winters (Motoyama *et al.*, 2016). In winter there is little to no green forage available, which has led to cattle suffering from

a vitamin A deficiency (Hirooka, 2014). Vitamin A is fat-soluble, which means that it is stored in the adipose tissue of the body (Hirooka, 2014). It is believed that this phenomenon caused natural and artificial selection of cattle with more “storage space” for vitamin A (Hirooka, 2014), in other words, cattle with inherently larger amounts of IMF.

In Japan prerequisites have to be met for beef to be licensed to the public as Wagyu beef. These prerequisites for being certified as legitimate Wagyu beef are a calf registration system by which cattle can be verified and a beef traceability system (Motoyama *et al.*, 2016).

2.3 Origin of Wagyu Cattle in South Africa

In South Africa the two main Wagyu breeds are Japanese Brown and Japanese Black. Brian Angus introduced Wagyu cattle to South Africa for the first time in 1999 (Woodview.co.za, 2018). Angus imported Wagyu genetics directly from Shogo Takeda, a renowned Wagyu breeder in Japan (Woodview.co.za, 2018). Angus used Wagyu embryos from the USA and Woodview Wagyu implanted them in local surrogate cows in the Free State (Bennett, 2013). Later Angus imported embryos from Australia and bought donor cows for embryo flushing (Bennett, 2013). Subsequently many other breeders have entered the South African Wagyu industry/market (Bennett, 2013; Woodview.co.za, 2018).

2.4 The Wagyu cattle industry in South Africa

The development of the Wagyu industry has stimulated the establishment of a Wagyu breeder society and the number of members of this society is growing. By April 2018 membership had doubled in one year from 50 to 100 members (Wagyu.org.za, 2018). By January 2019 membership had increased to 131 (Wagyu.org.za, 2019). An estimated 20 000 Wagyu calves were expected to go through the feedlot in that year and the next (Wagyu.org.za, 2018). The demand for Wagyu products is still strong. Projections show that the South African industry will absorb at least 60 000 to 100 000 carcasses before alternative markets need to be considered (Wagyu.org.za, 2018). The import of embryos and the sale of local Wagyu embryos continue (Wagyu.org.za, 2019). This shows interest and growth in the Wagyu industry of South Africa.

The goal is to produce premium quality beef to satisfy the market demand. Wagyu beef is a niche market and comes with a premium price tag (Wagyu.org.za, 2018). The Wagyu breeder society has set out to be one of the first completely traceable ones throughout the beef value chain in South Africa with a certified Wagyu beef (CWB) protocol and tag bundle system (Wagyu.org.za, 2018). This will ensure the purity of the product available for consumer purchase. This has been achieved by constantly educating the breeders on how the system works and why it is important (Wagyu.org.za, 2018)

The Wagyu Society of South Africa (WSA) is recognised by the South African Animal Improvement Act of 1998. Wagyu beef in South Africa is defined as any animal that is sired by a WSA registered fullblood or purebred sire and must have a minimum breeding level of crossbred Wagyu F1, at least 50% Wagyu breed content with a maximum variation of plus or minus 5% (Wagyu.org.za, 2018). There is a distinct difference between fullblood and purebred Wagyu cattle. A fullblood Wagyu pedigree can be traced back to an ancestor in Japan, in other words a fullblood animal has 100% Wagyu genetics, originating from an ovum and sperm from Wagyu animals (Armstrong, 2018). A purebred animal was bred pure, usually by using artificial insemination (AI) (with Wagyu semen) on cows from a different breed (Bradfield & Hunlun, 2018). The purebred process starts with a F1 that is only 50% Wagyu and this increases as breeding (AI) continues. For example, if an F1 Wagyu is bred with a fullblood or purebred Wagyu, the calves will be F2, which means 75% Wagyu crossbred, an F3 will be 87% Wagyu crossbred and an F4 >93% Wagyu, which is called purebred (Armstrong, 2018).

Local producers such as Woodview make use of a grading system to classify Wagyu beef (Woodview.co.za, 2018). In contrast to the composite classification system, a classification system considers marbling. This is done by making use of a marbling score. As the marbling score increases, so does the price per kilogram of Wagyu beef (Woodview.co.za, 2018). The CWB programme requires the marbling score not to be any lower than 3 (Wagyu.org.za, 2018). In South Africa the CWB programme makes use of the MIJ camera to measure marbling of the ribeye area (Wagyu Meat – Absolute Wagyu, 2019).

2.5 Composite feedlot cattle

In the current study reference will be made to composite feedlot cattle, meaning the typical beef cattle that are finished in South African feedlots.

The South African feedlot industry started in the1960s when a few entrepreneur cattle farmers were forced to “overwinter” their cattle on grain and/or potato by-products owing to lack of grazing. At first the facilities and procedure were primitive and unreliable. Later these farmers started implementing US technologies to improve the industry. Professionals in animal nutrition and health later contributed to the industry’s progress. It was only in the early 1970s that the different competitors in the feedlot industry came together to establish the South African Feedlot Association (SAFA). Today the South African feedlot industry is flourishing and produces approximately 75% of all beef in the country. According to the Red Meat Abattoir Association, South African grain-fed beef is lean, young and tender. Cattle are grain-fed to achieve consistent quality and maintain health standards.

Feedlots prefer to buy male weaner calves between eight and ten months of age that are beef breeds or beef breed crosses with the potential to produce economically to a final carcass weight

of 450-470 kg in the A class. In South Africa the feedlot feeding period is at least 120 days to produce A class carcasses at these weight parameters (SAFA, 2020).

According to SAFA the production parameters for feedlot cattle are as shown in Table 2.5.1.

Table 2.5.1 Production parameters of typical composite feedlot cattle in South Africa

Entry mass	230 kg
Exit mass	460 kg
Carcass mass	268 kg
Average daily gain	1,65 kg
Feed intake	12,5 kg/day
Water intake	55-60 L in summer
	40-45 L in winter

(South African Feedlot Association, 2020)

Feedlots rely not only on these parameters, but also consider the price margin, feed margin and other expenses when buying cattle (Kzndard, 2017). These measures lead to a feedlot using other methods to ensure it produces lean and tender beef that is still profitable. This may include using hormones to increase lean meat production and to improve feed efficiency (South African Feedlot Association, 2020) or limiting energy intake during the growing or finishing period or slaughtering finished cattle at an earlier age (Owens *et al.*, 1995)

2.5.1 Feeding period

The average time spent in a feedlot, in South Africa, is 133 days (Oosthuizen, 2018). According to the abattoir used in this study the composite feedlot cattle were on feed for 138 days and the Wagyu cattle were on feed for 690 days. The live weight and carcass weight for both Wagyu and composite feedlot cattle are shown in Table 2.5.2. The live weight of Wagyu cattle is higher than that of composite cattle. This difference is due to the duration of the feeding period; Wagyu cattle were on feed for 552 days more than composite cattle.

Table 2.5.2 Growth during feeding period

Live mass (kg)		Cold mass (kg)	
Comp	Wagyu	Comp	Wagyu
557	687	308,3	429
450	662	287,5	421
472	705	295,3	416
470	685	293,3	414
504	701	312,1	402
421	623	263,3	384
490	567	316	401
503	771	314,3	446
477	695	294,3	440
508	711	311	435
516	638	322,8	385
413	664	246,2	409
470	657	291,8	401
Days fed			
138	690	138	690

Comp = composite feedlot cattle

2.5.2 Nutritional composition

The aim in composite feedlot cattle, as described above, is to produce lean beef as quickly as possible while still being ethical towards live animals. The aim remains to produce composite feedlot cattle economically. In Table 2.5.3 the feed commodities are shown. These feed commodities are typically used in South African feedlot systems. Hominy chop, maize silage and milled maize are energy sources in the feedlot ration (Evans & Johnson, 2019). Maize silage is also a source of effective fibre. Effective fibre is necessary for proper rumen fermentation to take place (Banakar *et al.*, 2018). Molasses is a poor energy source but its main function is to act as a binder in the ration and also to improve palatability (Evans &Johnson, 2019). Sunflower oil cake is a source of protein (Evans & Johnson, 2019). Milled wheat straw and eragrostis hay are added to the ration as a source of roughage. Roughage is necessary in a ruminant diet to stimulate chewing. During chewing, saliva that contains buffers is produced (Morrison, 1959). These buffers help to keep the acidity in the rumen in a range that promotes the environment for fibre-digesting microbes (Morrison, 1959). Limestone is used as a calcium-containing mineral supplement (McDonald et al., 2011). A high-protein concentrate (HPC) refers to a commercially available blend including protein,

minerals and salt (Evans &Johnson, 2019). Zilpaterol is a beta-agonist used as growth-promoting molecule to improve average daily gain and red meat yield in the last few days before slaughter (Montgomery *et al.*, 2009). The nutrient composition shown in Table 2.5.3 is in line with the recommended nutrient composition of the NRC 2016.

Table 2.5.3 Nutritional composition of feed for composite feedlot cattle

Composite feedlot cattle Feed commodities		
Starter	**Grower**	**Finisher**
Hominy chop Milled maize Molasses Maize silage Sunflower oil cake (38%) Milled wheat straw/ Eragrostis Limestone HPC - Wheaten bran, vit/mineral premix, salt, urea, Availa-Zn, monensin, virginiamycin,	Hominy chop Milled maize Molasses Maize silage Sunflower oil cake (38%) Milled wheat straw/ Eragrostis Limestone HPC - Wheaten bran, vit/mineral premix, salt, urea, Availa-Zn, monensin, virginiamycin,	Hominy chop Milled maize Molasses Maize silage Sunflower oil cake (38%) Milled wheat straw/ Eragrostis Limestone HPC - Wheaten bran, vit/mineral premix, salt, urea, Availa-Zn, monensin, virginiamycin, (zilpaterol)
Nutrient composition		
ME Energy - 11,86 MJ/kg TDN - 76,67% NEm - 1,90 NEg - 1,26 CP - 13,45% Roughage value - 15,96	ME Energy - 12,25 MJ/kg TDN - 79,17% NEm - 1,98 NEg - 1,47 CP - 12,54% Roughage value - 12,7	ME Energy - 12,30 MJ/kg TDN - 79,48% NEm - 1,99 NEg - 1,48 CP - 12,48% Roughage value - 12,08
Days on feed		
10 to 20	10 to 20	70 to 100 and 30 to 35 (zilpaterol) + 4 days withdrawal (finisher)

2.5.3 Feeding Wagyu cattle

When producing Wagyu cattle, the aim is completely different from that of producing composite feedlot cattle. To achieve a significant amount of marbling, Wagyu cattle need do reach older ages before slaughter (Lunt *et al.*, 2005). In Japan Wagyu cattle are raised in group-fed pens (Gotoh *et al.*, 2009). Wagyu cattle are fed a highly concentrated diet from 11-30 months of age to ensure accumulation of IMF (Gotoh *et al.*, 2018). Roughage fed consists of beer bran, hay and rice straw (Gotoh *et al.*, 2018). From 11 to 18 months of age the diet's concentration level increases from 36.8% to 86.4% over time and the roughage decreases. In the final stage, 18 months of age until slaughter, the ration consists of 84.2%-86.4% concentrate and 13.6%-15.8% roughage (Gotoh *et al.*, 2018). During the finishing period cattle are provided with as much concentrate as possible and rice straw *ad libitu*m (Gotoh *et al.*, 2018). Cattle are provided with a constant source of water and blocks consisting of minerals, salt and a diuretic. More than 90% of the concentrate used to fatten Wagyu cattle in Japan is imported (Gotoh *et al.*, 2018). Japanese farmers have considered manipulating the vitamin A levels to produce a higher marbling score without increasing SCF (Gotoh *et al.*, 2018). This has only been effective in cattle breeds genetically predisposed to produce marbling. Currently, Japanese farmers keep the vitamin A levels low in the middle fattening period and increase the vitamin A levels during the finishing period to prevent hepatic disease and swelling (Oka *et al.*, 1998; Kawachi, 2006).

Lunt *et al.* (2005) studied Wagyu and Angus cattle at US feedlot endpoints (525 kg) and Japanese endpoints (650 kg). In the above-mentioned study Lunt's results confirmed that Wagyu cattle must be raised to a greater physiological age before their IMF content differs from that of Angus cattle. IMF continued to increase to above 20% in Wagyu cattle, while the IMF content plateaued by 16 months of age in Angus cattle. The weaning weight of the Wagyu cattle (169 kg) was lower than that of Angus cattle (211 kg). The ADG in Lunt's study was also lower for the Wagyu. This can confirm that Wagyu cattle have a poorer growth rate than the typical US breed types (Lunt *et al.*, 2005). Lunt concluded that breed, diet and slaughter endpoint all contribute to the observed adipose tissue compositional difference between Wagyu and typical US cattle breed types.

In a study done by Lawrence *et al.* (2007), biotin supplementation of Wagyu X Black Angus F1 cross cattle were evaluated to test whether or not biotin supplementation had an effect on marbling. Lawrence *et al.* (2007) concluded that biotin supplements had no significant effects on marbling and that genetics played an important role in the expression of marbling.
South African Wagyu cattle are kept in camps where grazing is available and are fed the grower diet shown in Table 2.5.4. This is done because of how much longer the Wagyu cattle have to be fed to produce the expected marbling, as explained above. The roughage value is much higher in the Wagyu ration (Table 2.5.4) in comparison to the roughage in composite feedlot cattle (Table

2.5.4). Because grazing is available, maize silage as a source of effective fibre can be left out of the ration. A feed additive, Xtract X60-7065, is used to promote growth to achieve the best possible ADG for Wagyu cattle. This notion is supported by Lunt *et al.'s* study mentioned above.

Table 2.5.4 Nutritional composition of feed for Wagyu cattle

Wagyu Feed commodities	
Grower	**Finisher**
Hominy chop Milled maize Molasses Sunflower oil cake (38%) Milled wheat straw/ Eragrostis Vitamin E, mineral premix, Xtract X60-7065 ruminant, acid buff, salt and limestone	Hominy chop Milled maize Molasses Sunflower oil cake (38%) Milled wheat straw/ Eragrostis Vitamin A and E, mineral premix, Xtract X60-7065 ruminant, acid buff, salt and limestone
Nutrient composition	
ME energy - 12,10 MJ/kg TDN - 80,55% NEm - 1,95 NEg - 1,33 CP - 13,86% Roughage value - 18,04	ME energy - 12,13 MJ/kg TDN - 80,59% NEm - 1,98 NEg - 1,37 CP - 13,91% Roughage value - 17,48
Days on feed	
Weight-dependent - feed to about 500 kg LW	Weight-dependent - feed from > 500 kg

2.6 Nature of fatty acids

FAs form part of frequently occurring lipids. FAs are amphipathic compounds. Amphipathic molecules have a polar end and a non-polar group on the other end (Campbell & Farrell, 2015). In this case, FAs have a carboxyl group at the polar end and a hydrocarbon chain at the nonpolar tail. The carboxyl group is hydrophilic while the hydrocarbon chain is hydrophobic (Campbell & Farrell, 2015). Naturally occurring FAs contain an even number of carbon atoms and are generally unbranched (Smith, 2014). FAs containing double bonds between carbon atoms are unsaturated and FAs that only contain single bonds between carbon atoms are saturated (Smith, 2014). In unsaturated FAs (UFAs) the stereochemistry/configuration of the double bond is usually cis (Campbell & Farrell, 2015). The difference between *cis* and *trans* FAs is important for the FAs' overall shape. The notation used to describe FAs is as follows: C18:0 shows an 18-carbon saturated FA (SFA), C18:1 describes an 18-carbon FA with one double bond, making it unsaturated (Campbell & Farrell, 2015). This nomenclature can represent many double bonds, where one double bond is known as MUFAs and more than one double bond represents PUFAs (Example: C18:3) (Campbell & Farrell, 2015). The position of the double bond is determined by the way FAs are synthesised in an organism. UFAs have lower melting points, resulting in oils instead of fat (Campbell & Farrell, 2015). Hydrogenation is the proses of converting oil to fat, or UFAs to SFAs. This process is used commercially, for example producing margarine from plant oils. The specific melting point depends on the level of unsaturation (Campbell & Farrell, 2015).
Fat content refers to the mass of fat accumulated in the fat depots. FA composition refers to the specific FAs of which the fat, IMF, SCF and PRF are made up.

2.7 Fatty acids in ruminants

The sources of lipids in meat are muscle fibres, subcutaneous adipose tissue (SCF), intermuscular (seam) and intramuscular adipose tissue (IMF) (Smith & Smith, 2014). After the trim fat has been removed from beef cuts, the main contributor to lipid content of meat is intramuscular adipose tissue. Lean meat contains approximately 1% extractable lipid (Smith & Smith, 2014). It has been found that trimmed beef of the Japanese Black cattle can contain more than 35% extractable lipid (Smith & Smith, 2014). Therefore the IMF content is significantly higher in Wagyu cattle. The FA in bovine adipose tissue comes from two pathways; *de novo* FA synthesis and FAs derived from desaturation (Smith & Smith, 2014). Palmitic, stearic, oleic and linoleic acids are the most abundant FAs in IMF of beef and pork (Smith & Smith, 2014). The first three FAs mentioned are from endogenous synthesis and linoleic acid is derived from plant material included in the diet (Smith & Smith, 2014).

The FA composition and amount of fat in meat affect the shelf-life, palatability and nutritive value of meat (Kazala *et al.*, 1999). The fat content and the FA composition of beef are influenced

by both environmental and genetic factors, the main ones being age, diet and breed type (Turk & Smith, 2009).

2.8 Factors that influence the fat content and FA composition of meat

2.8.1 Maturity

The effect of aging, or degree of maturity, changes the body composition (Owens *et al.*, 1995). Age has an effect on the amount of fat produced; both the number and size of adipocytes increase with the growth of the animal (Motoyama *et al.*, 2016). Fat mass increases quadratically with age and protein mass increases more linearly with age (Owens *et al.*, 1995). Cafe *et al.* (2006) studied the effect that slow or rapid growth to weaning would have on carcass characteristics. No adverse effects on carcass composition were found in cattle grown slowly to weaning compared to those grown rapidly to weaning. Greenwood *et al.* (2006) conducted a similar study and both studies found that restricted growth to weaning did not have any deleterious effects on marbling at slaughter. These results support the notion that fat mass increases with age (Greenwood *et al.*, 2006).

2.8.2 Breed

As mentioned previously, Wagyu are known for their excessive marbling and high MUFA content. It has been proposed that this is due to elevated activity of delta 9 desaturase activity (Sturdivant *et al.,* 1992). However, in a study done by Cameron et al. (1994) stearoyl-CoA activity and mRNA concentration were measured in subcutaneous samples from American Wagyu and Angus cattle. No significant difference was found between the two. There is insignificant evidence to suggest that there is a difference in FA concentration between beef breeds. However, Wagyu cattle have been reported to be genetically predisposed to produce a higher MUFA concentration than other beef breeds (De Smet *et al.*, 2004). The Wagyu breed is also known for excessive marbling and lower external fat (De Smet *et al.*, 2004).

Breed type is an important factor when looking at Wagyu. As has been established, Wagyu cattle have an inherent genetic ability to produce more fat as IMF (Gotoh *et al.*, 2018).

2.8.3 Diet and feeding

Beef IMF composition is largely influenced by genetic factors and diet has a smaller effect (Lunt *et al.*, 2005; Aldai *et al.*, 2007; Schmutz *et al.*, 2014). Feeding systems and/or fattening of cattle influences the growth and carcass characteristics, whether using concentrated feeding or pasture feeding (Webb & O'Neill, 2008). Changing the FA composition to a more desirable one by using feeding systems has been attempted in cattle (Webb & O'Neill, 2008). The FA composition of meat is influenced more strongly by dietary factors than genetic factors (De Smet *et al.*, 2004). However, in ruminants it is difficult to change the FA compostion owing to biohydrogenation, which will be discussed under the next subheading.

Research has concluded that Wagyu produce marbling at a higher rate in feedlot/grain-fed systems than on pasture systems (Smith *et al.*, 2009; Motoyama *et al.*, 2016). The objective of Suksombat *et al.'s* (2016) study was to determine the effect of palm and/or linseed oil supplementation on carcass quality, sensory evaluation and the FA profile of beef from crossbred Wagyu beef steers. Suksombat concluded that supplementation of linseed oil rich in C18:3n-3 did not influence feed intake, live weight changes, carcass and muscle characteristics, sensory and physical properties. Linseed oil supplementation increased C18:3n-3, C22:6n-3 and n-3 PUFA, while it decreased C181t-11, C18:2n-6, *cis*-9, *trans*-11, and *trans*-10, *cis*-12 conjugated linoleic acids, n-6 PUFA and n-6:n-3 ratio in the *Longissimus dorsi* and *semimembranosus* muscles.

Oxidation of FA proceeds at a higher rate in ruminants than in pigs, dispite the lower PUFA proportions (Wood *et al.*, 2008). Vitamin E is vital to enhance the nutritional value of meat, particularly in ruminants where higher concentrations of vitamin E from grass feeding prevent FA oxidation and extend the shelf life of meat colour (Wood *et al.*, 2008).

2.8.4 Species differences

Species is the major source of variation in meat FA composition (De Smet *et al.,* 2004; Jiang *et al.,* 2010). Diet plays an insignificant role in ruminant FA composition. Typically all ruminants have similar composition of FAs because of the biohydrogenation taking place in the rumen (Wood *et al.*, 2008). FA deposition varies among different species owing to the digestive process. Feeding strategies have been shown to change the FA composition in monogastric animals (Turk & Smith, 2009). Dietary FAs undergo fewer transformations in monogastric animals, therefore the FA composition of the tissue mimics the FA composition of the diet (De Smet & Vossen, 2016). Thus the FA composition of pork can be modified by dietary means to be higher in PUFA (Wood *et al.*, 2008). Normally beef has a lower PUFA/SFA ratio compared to monogastrics because of biohydrogenation of UFAs in the rumen (De Smet *et al.,* 2004; Jiang *et al.,* 2010). Beef FA concentration is relatively resistant to dietary modifications (Wood *et al.*, 2008). As a result of

lipolysis and biohydrogenation taking place during rumination, ruminants' tissue is much higher in SFAs and lower in PUFA compared to that of monogastrics (De Smet & Vossen, 2016; Wood and Enser,1997). However, this causes a more favourable ratio of n-6/n-3 in red meat.

Dietary UFA is partially biohydrogenated to *trans* UFA or completely biohydrogenated to SFA in the rumen (Suksombat *et al.*, 2016). In ruminants all PUFA are biohydrogenated to SFA (Nürnberg *et al.*, 1998) After biohydrogenation, only small proportions of linoleic acid are available for incorporation into tissue lipids (Wood *et al.*, 2008). A potential solution is to increase the PUFA in the ruminant diet or to use a "protected" PUFA source in the ruminant diet (Dance *et al.,* 2009). However, this is solution is costly and might not increase the PUFA significantly (Dance *et al.,* 2009).

Later, in the adipose tissue (Sturdivant *et al.,* 1992) the SFAs, the main ones being palmitic acid and stearic acid, go through a delta 9 desaturation process catalysed by the enzyme stearoyl-coenzyme A desaturase (SCD) to produce UFA, palmitoleic acid (C16:1n-7) and oleic acid (C18:1n-9) (Smith & Smith, 2014).The greatest SCD activity is located in the adipose tissue (Smith & Smith, 2014). This points to the importance of SCD in determining FA composition (Smith & Smith, 2014).

2.8.5 Anatomical location and fat deposition

The FA composition of different fat deposition sites differs; for example, a difference was found in double-muscled cows of the Belgian Blue cattle breed (Webb et al., 1998). FA composition depends on the amount of fat in the carcass and muscle (Wood *et al.*, 2008). Adipose tissue has a much higher FA content than muscle, but the FA composition of the two is more or less similar (Webb & O'Neill, 2008; Wood *et al.*, 2008).

In ruminants PUFA are conserved in the muscle, whereas in pigs PUFA concentrations are higher in the adipose tissue (Wood *et al.*, 2008; De Smet & Vossen, 2016). Of the two major PUFA, 18:2n-6 is more rapidly taken up into muscle than 18:3n-3 (Wood *et al.*, 2008). Therefore 18:2n-6 reaches high levels in the muscle (Wood *et al.*, 2008).

FA composition differs between anatomical adipose tissue locations, including intra- and intermuscular, abdominal and subcutaneous adipose tissue (Aldai *et al.*, 2007; Turk & Smith, 2009). The level of saturation increases with increasing distance from the exterior of the animal, e.g. SCF will be less saturated than abdominal fat (Tume, 2004). SCF has the highest proportion of MUFA and IMF has the highest proportion of PUFA and greater ratios of n-6/n-3 PUFAs (Aldai *et al.*, 2007; Liu et al., 2015). In a study done by Liu *et al.* (2015), it was confirmed that the FA composition of PRF and IMF differs (Liu *et al.* 2015). The proportions of C18:1n9, C18:2n6, C18:3n3 and n-6 FAs are greater in the *m. Longissimus dorsi* than in PRF (Liu et al., 2015). PRF contains the greatest concentration of total FAs (Webb et al., 1998). Additional variation still exists

in FA composition in bovine carcasses, e.g. MUFA is highest in the brisket and SFA is highest in the flank (Turk & Smith, 2009). It seems that anatomical location and fat deposition site has an influence on the FA composition (Gotoh et al., 2018).

2.8.6 Temperature

Temperature can have an effect on the activity of stearoyl-coenzyme A (Kouba *et al.*, 1999). Seasonal and climate variation can have a direct and indirect effect on the FA composition of beef. The feed type and feed quality are affected indirectly by seasonal and climate changes. However this will only be of importance in pasture-based systems. Climate change will have a direct effect only on the SFA composition (Tume, 2004) owing to temperature homeostasis in the deeper tissues. Therefore the level of saturation increases with increasing distance from the exterior of the animal, e.g. SCF will be less saturated than abdominal fat (Tume, 2004). FA composition changes with the aim of maintained lipid fluidity for normal metabolic function. This regulation is done by delta 9 desaturation, with activity being greater in cooler tissues (Tume, 2004).

2.8.7 Sex

Sex has an influence on FA composition, e.g. it has been found that bulls have higher levels of linoleic acid and lower levels of oleic acid in subcutaneous and intramuscular fat in comparison to steers (Sturdivant *et al.,* 1992).

Marbling levels are higher in females than in immunocastrated bulls (Carvalho *et al.*, 2015). Males have greater SCF thickness and ribeye area (Carvalho *et al.*, 2015).

In a study by Zembayashi *et al.* (1995), they concluded that the differences in FA composition that they observed were due to sex. This is important in Wagyu cattle, since they can potentially be used to improve the FA composition of other cattle genetically.

Level of fatness also has an effect on the FA composition and the ratio of PUFA/SFA. As the fat content increases, the level of SFA and MUFA will increase at a faster rate than PUFA (De Smet *et al.*, 2004).

The lipid composition and FA profile/composition of marbled beef/IMF in beef can be manipulated based on the genotype, time on feed and finishing diet (Wood *et al*., 2008).

2.9 Essential fatty acid

Essential FAs (EFAs) are FAs that cannot be synthesised by the human body, but have to be obtained through the diet (Campbell & Farrell, 2015). EFAs consist of two groups, the omega-6 (n-6) group and omega-3 (n-3) group (Das, 2006; Di Pasquale, 2009). The EFAs are PUFAs, as they contain two or more double bonds (Das, 2006). In the omega-3 family alpha linolenic acid is the

main FA and in the Omega-6 family is linoleic acid the primary FA. During metabolism in the human digestive system alpha linolenic acid is transformed to eicosapentaenoic acid and docosahexaenoic acid (Di Pasquale, 2009). The omega-6 linoleic acid is converted to gamma linoleic acid.

Humans have evolved from a hunter-gatherer diet with a small but equal amount of omega 3 and omega-6 FAs to a diet high in SFAs (Simopoulos, 2002). In a modern society the substitution of dietary SFAs for n-6 has been encouraged (Simopoulos, 2003). Today the intake of n-6 is much higher than the original ratio of 1-2:1 of n-6: n-3 (Simopoulos, 2003). Today's ratio ranges from 20-30:1 for n-6:n-3 .This is due to increased consumption of vegetable oils containing omega-6 (National Research Council US and Assembly of Life Sciences US,1976 ; Simopoulos, 2003). Intake of n-3 is much lower today owing to a decrease in fish consumption and the production of animal feed rich in n-6, leading to the production of meat poor in n-3. Modern agriculture has decreased the n-3 content in many foods (Simopoulos, 2002; 2003).

EFAs are responsible for normal metabolism and overall good health (Di Pasquale, 2009). In fact, the balance of omega-6 and omega-3 is very important for homeostasis and normal development (Simopoulos, 2002). The recommended ratio varies from 1:1 to 4:1, depending on the type of disease that is under consideration (Erasmus, 1996; Simopoulos, 2002).

2.10 Specific fatty acids

The differentiation of preadipocytes is set apart by the expression of genes, such as stearoyl-coenzyme A desaturase (SCD) and enzymes that support de novo FA biosynthesis (Smith and Smith, 2014). In the early development of preadipocytes, they typically contain high concentrations of SFA palmitic acid and stearic acid and low concentrations of their delta 9 desaturase product, palmitoleic acid and oleic acid (Smith and Smith, 2014). Palmitic acid is produced by both FA synthase and acetyl-coenzyme A carboxylase (Smith and Smith, 2014). Stearic acid is produced by adding two carbons to palmitic acid. The products of delta 9 desaturation of palmitic and stearic acid are palmitoleic acid and oleic acid (Smith and Smith, 2014). This process is catalysed by SCD. The concentration of each of these FAs is determined by the activity of SCD and the availability of UFA in the diet (Smith and Smith, 2014). In a livestock diet oleic acid, linoleic acid and α-linolenic acid are typically consumed (Smith and Smith, 2014). Oleic acid is the most abundant FA in cattle adipocytes and the predominant FA in beef (Smith *et al*., 2006).

Wagyu cattle with high levels of marbling have a higher proportion of MUFA due to a high concentration of oleic acid (Gotoh & Joo, 2017). MUFA has little effect on cholesterol. MUFA are heart-healthy fats, because they can lower low-density lipoprotein cholesterol while increasing

high-density lipoprotein cholesterol (Gotoh & Joo, 2017). There is no scientific evidence to indicate that beef with high levels of oleic acid will increase the risk of disease (Smith *et al.*, 2006). The concentration of oleic acid in beef is positively correlated to the palatability of beef (Smith *et al.*, 2006). This may be due to the mouth feel experienced by the consumer due to lower melting points of oleic acid (Smith *et al.*, 2006).

Stearic acid is one of the main FAs that affect fat hardness (Smith *et al.*, 2006)

The *trans* FAs associated with beef are vaccenic acid, rumenic acid and conjugated linoleic acid (CLA) isomers (Webb intechOpen, 2021). CLA is not part of the EFAs, although CLA has a significantly positive effect on human health (Pasquale, 2009). CLA is found in dairy products and meat (Pasquale, 2009).

2.11 Health aspects

A common misconception among consumers is that all SFAs have a negative effect on human health in respect of cholesterol and cardiovascular disease (Troy *et al.,* 2016). Fat has been proven to have fewer adverse effects on health than carbohydrates (Sondike *et al.*, 2003). Dietary fat plays an important role in both the health and functioning of the human body. Dietary fat is defined as triacylglycerides, phospholipids and sterols. Nutrients related to dietary fat, such as fat-soluble vitamins, are crucial to health (Lichtenstein *et al.,* 1998). Food containing high-quality protein is also desirable for good health (Johnston *et al.*, 2004) Beef provides high-quality protein of high biological value, as well as other micronutrients (Upmann *et al.*, 2014) and essential amino acids for body maintenance and growth. The B vitamins and minerals such as zinc, iron, selenium, phosphate, magnesium, copper and potassium are found in beef and are more bioavailable to humans. Beef contains high levels of lipids, which provide energy and EFAs (Troy *et al.,* 2016). Fat acts as a vehicle for fat-soluble vitamins (Frank *et al.,* 2016). Beef can be a good source of oleic acid as well as short and long chain omega-3 FAs, with proven health benefits (Frank *et al.*, 2016). CLA has a range of benefits: enhancement of the immune system, anti-atherosclerotic, anti-carcinogenic (Troy *et al.,* 2016) and anti-diabetic properties. The trans-11 vaccenic acids and their isomers CLA cis-9, trans-11, have demonstrated anti-carcinogenic properties (Turpeinen, 2002; Tricon, 2005; Lichtenstein *et al.,* 1998). UFA has been shown to increase satiety significantly compared to saturated fats (Frank *et al.*, 2016).

Some studies suggest that when eating SFAs in their “natural” form, such as cheese and meat, they are less atherogenic (Bermingham *et al.,* 2018). A meta-analysis conducted by O’Connor *et al.* (2017) show that meat consumption has very little impact on cholesterol levels. In a

study published in the IARC Monographs, consumption of processed meat was classified as carcinogenic to humans owing to a positive association with consumption of processed meat and colorectal cancer (Bouvard *et al.,* 2015). The same study classified red meat as probably carcinogenic to humans owing to the positive association between consumption of red meat and colorectal cancer (Bouvard *et al.,* 2015). Cohort groups were used to determine the carcinogenicity of red and processed meat (Bouvard *et al.,* 2015). This might have led to bias in the study (Troy *et al.,* 2016). Epidemiological studies have proposed an association between the consumption of red meat (SFA contribution to the diet) and the development of cardiovascular disease and colon cancer (Bouvard *et al.,* 2015). However, these studies suffer from limitations and no direct link between red meat and the cause of these diseases has been proven (Troy *et al.,* 2016; Godfray *et al.,* 2018). In such studies the lifestyle of the subjects, inability to measure the exact intake and identify the proposed causative agent limits the reliability of the conclusions (Troy *et al.,* 2016; Godfray *et al.,* 2018).

Genetics and diet influence the degree and type of lipid deposited in the beef/carcass. Omega-3 derived from red meat can make a significant contribution to the daily requirements for human health (Bermingham *et al.,* 2018). Grass-fed beef generates more PUFA (omegas) but this can lead to undesirable flavours for the consumer (Wood and Enser, 1997; Bermingham *et al.,* 2018); thus, if Wagyu beef contains more PUFA than composite beef (both grain-fed/feedlot), then the potential is there to market the product as such, emphasising the already existing flavour profile. It is possible that high-quality Wagyu beef can deliver the amount of omega-3 required by the consumer (Bermingham *et al.,* 2018).

2.12 Consumption of beef

Traditionally consumers valued fatty meat cuts, which were associated with rich flavour and superior palatability (Lichtenstein *et al.,* 1998; Frank *et al.,* 2016). However, the demand for healthier food has increased because of health concerns the consumer is faced with. Consumers' view of animal fat has been significantly negative in the USA, Australia and other English-speaking countries in the past few decades (Lichtenstein *et al.,* 1998; Frank *et al.,* 2016; Gotoh *et al.*, 2018). The consumer is essentially the last step in the meat production chain. Thus, meeting consumers' expectation is important in influencing their shopping habits and consumption (Font-i-Furnols & Guerrero, 2014). Red meat is a popular choice among South Africans; 51% of the population ate red meat three or more times a week, according to a study conducted by Radder and Le Roux in 2005. The study highlighted the complexity of food choice and the factors affecting meat choice (Radder & Le Roux, 2005). The income of the consumer is pivotal, since the consumption of beef by the middle class is increasing in many countries, among others Korea, Japan and Brazil (Troy *et al.,* 2016). Culture and religion play a large role in determining whether a consumer will consider

beef. A negative perception exists about the health aspects related to beef. A high incidence of coronary heart disease in South Africa may indicate that South Africans are not concerned about human health factors associated with red meat consumption (Radder & Le Roux, 2005). This, however, is uncertain, since there is a lack of resent studies done on South African meat consumption.
Considering all these factors, beef remains a popular meat product among large sections of society (Troy *et al.,* 2016).

2.13 Eating quality of meat

The eating quality of meat is greatly improved by fat in meat, yet many consumers avoid visible fat on meat, because of health concerns (Frank *et al.*, 2016; Troy *et al.*, 2016). Fat in meat plays an important role in overall meat palatability (Frank *et al.*, 2016).
Considering flavour as a meat quality factor, a change in the flavour of red meat may be due to a change in the diet of cattle, which will alter the tissue FA composition. For example, a change in the ratio of n-6/n-3, due to the grass that is fed, can lead to off flavours (Wood and Enser, 1997). IMF acts as a substrate and reservoir of flavour (Frank *et al.*, 2016).

Beef palatability is strongly influenced by three traits – tenderness, juiciness and flavour (Frank *et al.*, 2016). In Texas two studies involving a taste panel were conducted by O'Quinn *et al.* (2012) and Corbin (2014). In 2012 the study was conducted to determine the effect of the level of fat in beef strips on the palatability traits and overall acceptability (O'Quinn *et al.*, 2012). The palatability traits included tenderness, juiciness and flavour. Overall, the palatability traits increased with an increase in fat level (O'Quinn *et al.*, 2012). Juiciness increased as the fat level increased and tenderness was rated higher for samples containing a greater fat content. In the results of this study, Wagyu were scored lower for flavour and overall acceptability (O'Quinn *et al.*, 2012). It is possible that the upper limit for a fat level acceptable to consumers has been reached. In this study it was concluded that flavour was highly correlated with overall beef acceptability and fat contributed most to consumers liking beef raised in the USA (O'Quinn *et al.*, 2012).

In 2014, attempts were made to eliminate the halo effect caused by tenderness to evaluate the effect of marbling on palatability traits more accurately. In other words, tenderness was kept constant in all the treatments by making use of Warner-Bratzler shear force values of < 33.34N (Corbin *et al.*, 2014). In the results of this study it was unclear whether or not consumers were able to distinguish between high tenderness and superior juiciness and flavour (Corbin *et al.,* 2014). This may be due to the high fat/marbling level; fat in meat decreases the bulk density, meaning less muscle fibre and collagen per unit of meat consumed (Frank *et al.*, 2016). This phenomenon is held responsible for consumer difficulty to distinguish between meat quality factors (Frank *et al.*,

2016). However, this study indicates that fat plays a role in all three palatability factors. IMF increases tenderness, juiciness and flavour (Muchenje *et al.,* 2009). Thus, trying to evaluate a single palatability trait without the influence of the others is difficult because of the relationship among tenderness, juiciness and flavour (Corbin *et al.*, 2014; O'Quinn *et al.*, 2017). The consumers' flavour score increased with increased fat percentage. This did not hold up for grass-finished steaks. Grass-fed beef has an increased level of PUFA, which can cause off flavours (Corbin *et al.*, 2014; Wood & Enser, 1997). Thus, producing beef without undesirable flavours is equally important as increasing palatability traits.

For instance, if beef steak is lacking in one or more palatability traits, it may be deemed unsatisfactory by consumers (O'Quinn *et al.*, 2017). Conversely, a steak can be deemed acceptable in response to one outstanding palatability trait (O'Quinn *et al.*, 2017). This concept was studied by O`Quinn *et al*. The study focused on the interactions between palatability factors – tenderness, juiciness and flavour - and how these affect the overall eating quality. Relative data from the previous studies mentioned here and others were used to evaluate the odds that one failing palatability trait can render beef unacceptable to consumers and to develop a model to determine how much each of the palatability traits contribute to eating quality (O'Quinn *et al.*, 2017). The study also looked at marbling as a factor determining eating quality. It was concluded that using a marbling score to determine eating quality remains a challenge because of the vast number of other factors that influence eating quality (O'Quinn *et al.*, 2017).

Japan switched to Wagyu heifers and Wagyu steers in its intensive feeding systems (Gotoh *et al.*, 2018). This switch and the genetic ability of Wagyu cattle to produce marbling has resulted in greater fat deposition in Japanese Wagyu cattle in comparison to European beef breeds (Gotoh *et al.*, 2018). The IMF improves carcass characteristics such as juiciness, flavour and tenderness (Frank *et al.*, 2016; Gotoh *et al.*, 2018). Therefore IMF is used as a good indicator of quality and is used by graders in Japanese abattoirs to grade Wagyu beef (Gotoh *et al.*, 2018).

2.14 Conclusion

The Wagyu industry in South Africa is developing and growing at a steady pace. Producing South African Wagyu cattle differs from the traditional methods used in Japan. Feed is imported to Japan, so the feed ingredients will be relatively similar; the difference comes in when looking at the feeding system and duration of feeding. The majority of Wagyu carcasses slaughtered in South Africa are F1 (50% Wagyu) carcasses. Japan raises and slaughters fullblood Wagyu carcasses. Many studies have been done on the FA composition of Japanese Wagyu. From the literature the many factors that influence meat's FA composition come across and also the difficulty in altering ruminant FA composition in a significant way. FA in meat is important for human health by providing essential and non-essential FAs that are important for normal body functions. The quality of meat, in respect of tenderness, juiciness and flavour, is also influenced by fat in meat. These qualities are largely driven by consumer preference for meat. Wagyu beef is said to have a higher ratio of UFA, which can be beneficial to health aspects. The shelf life of meat is influenced by the fat content and FA composition. The purpose of this study is to determine the FA composition of South African Wagyu cattle and the amount of fat in Wagyu meat and how that compares to the marbling scores awarded to Wagyu meat.

Chapter 3: Materials and Method

3.1 Sample collection

Samples were taken from 13 Wagyu carcasses consisting of the Wagyu breed type currently available in South Africa. Samples were also taken from 13 composite feedlot cattle carcasses comprised of the hybrid cattle breed type typically seen in South African feedlots. To preserve the confidentiality agreement the abattoir and source of above mentioned cattle breed types will not be stated. From each carcass an Intramuscular (IM), subcutaneous fat (SCF) and perirenal fat (PRF) sample were collected. Consequently there was a total of 78 samples. For efficiency and ease, only eight samples were put through the laboratory analysis at a time. The FA extraction process took two days for every batch of eight samples.

All samples were collected on the left side of each carcass. All the carcasses were quartered between the sixth and seventh rib to allow for marbling scores to be captured with the MIJ camera. The IM samples were taken from the *m. Longissimus dorsi* muscle at the cross-section through the m. *Longissimus dorsi* muscle at the point where each carcass was quartered. The SCF samples were taken from the same position, but on the outside of the carcass. PRF samples were taken from the left hindquarter in approximately the same position on each carcass. All the samples were stored and sealed in polyethylene bags and frozen (-20°C) until FA analysis could commence. Carcass data available at the abattoir were collected along with the photos captured by the MIJ camera used by the abattoir to determine marbling scores.

The research commenced from a post-mortem state. All laboratory procedures commenced at the Nutrilab facilities at the University of Pretoria. Intramuscular meat samples were freeze-dried to determine dry matter and to conduct ether extracts. Ether extract analyses were conducted on all the IM samples to determine the percentage of IMF in the *m. Longissimus dorsi* muscle, at the exact anatomical position where the MIJ camera took the photo to determine the marbling score.

3.2 Sample preparation

To prepare for freeze-drying, the IM samples were milled and 25 g weighed out for freezing at -39°C for two to three days. Then the 25g milled samples were moved into the freeze-dryer for four to five days at -45°C. In the freeze-drier aluminum foil trays were used to separate the individual samples according to carcass number. After freeze-drying the samples were ground into finer particles to allow for proper ether extracts and dry matter analysis to be done. The remainder of the freeze-dried samples were stored in plastic zip lock bags until the FA analysis could take place. Lipid extraction was done by means of an adaptation of AOAC 996.06 (2000), Chapter 41, pp. 20-24 (oils and fat).

3.3 Materials used

The following list of apparatus was used to conduct the FA analysis:

- Gas chromatograph – Shimadzu GC-2010 Plus at UP facility will be used.
- Capillary column
- Test tubes with sealable caps
- 150 ml beakers
- Weighing balance
- Freeze-dryer
- Foil containers
- Scalpel blades and tweezers
- Spatula
- Gloves
- Measuring cylinders
- Boiling granules
- Rotamax mixer
- Water bath with test tube tray
- Vortex mixer
- 100°C oven
- Mechanical pipette with tips
- Aspirator
- Nitrogen blowing unit

Reagents used during FA analysis:

- Pyrogallic acid
- Hydrochloric acid 32%
- Ethanol
- Diethyl ether
- Petroleum ether
- Chloroform
- Nitrogen stream
- Sulfuric acid 2%
- Toluene
- Hexane
- H_2O
- Na_2SO_4
- C15 internal standard: 1 mg/ml pentadecanoic acid in hexane

- Preparation: 100 mg pentadecanoic acid with 100 ml hexane in 100 m^3 flask with stopper.
- External standard: Supelco 37 component FAME mix std.

3.4 Glassware preparation

All glassware was washed with hot water and detergent before commencing with analysis. Glassware items that came into contact with the samples were also rinsed with ethanol. After rinsing, all the glassware items were placed in a drying oven at 20°C to dry.

3.5 Digestion of samples

The method used is an adaptation of that described in AOAC 996.06 (2000), Chapter 41, pp. 20-24 (oils and fat). From each freeze-dried intramuscular sample, 1 g was weighed into a screw-cap test tube. The SCF and PRF samples are fat dens, therefor only 0.1 g of each sample was weighed out into test tubes. To facilitate the mixing of the reagents with the sample, two glass mixing beads were added to each test tube. Pyrogallic acid (100 mg) was added to each test tube using a laboratory spatula. Pyrogallic acid was included as antioxidant and acted as a preservative during lipid extraction. Internal standards (2 ml) were added to each test tube. The internal standard (C15:0 pentadecanoic acid) was prepared beforehand as 1 mg/ml pentadecanoic acid in hexane and kept in a volumetric flask with a stopper in the reagent fridge. A pipette was used to add 2.0 ml absolute ethanol and 10 ml 32% HCL. Each test tube was mixed after the addition of a new reagent by using a vortex mixer. The test tubes were sealed with caps and placed in a water bath at 75°C with a moderate agitation speed of 25 n for 40 min. Every 10 min the test tubes were removed from the water bath, one by one, and mixed on the vortex mixer. After digestion, all the test tubes were removed from the water bath and placed in the fume hood to cool to room temperature.

3.6 Extraction of fatty acids

Once the test tubes had cooled to room temperature 20 ml of diethyl ether was added to the test tubes, which were then hand-shaken for 5 min. The content of the test tube was then poured into a 150 ml glass beaker and placed on the rotomixer. Then 20 ml of petroleum ether was added to each glass beaker and mixed with a glass rod while on the rotomixer. After thorough mixing the glass beakers were placed in the fume hood and left for two hours or until the layers had separated. The fume hood was switched off during this time to prevent the top layer from evaporating completely. When the layers could be distinguished clearly, the top layer was separated into a clean glass beaker and left overnight in the fume hood to dry. For the drying process, the fume hood was switched on.

3.7 Methylation of fatty acids to produce fatty acid methyl esters (FAMEs)

The extracted fat residue was dissolved in 3 ml chloroform and 3 ml diethyl ether. By using a glass pipette the content of the glass beakers was transferred to clean test tubes. The test tubes were placed under a steady nitrogen stream until all the liquid had dried, after which 2 ml of 2% sulfuric acid in methanol and 1 ml toluene were added to the test tubes. The test tubes were then placed in an oven at 100°C for 45 min. After the first 10 min, each test tube was taken out of the oven and shaken to ensure dispersal. After the test tubes had cooled to room temperature, 5 ml of distilled water was added to each test tube, then 4 ml of hexane and approximately 1 g of anhydrous sodium sulphate were added to each test tube. The test tubes were sealed with a polyethylene lined cap and mixed for one minute. The layers were allowed to separate and the top layer was transferred to a 2 ml vial. The top layer contained the FAMEs.

3.8 Gas chromatography analysis

The GC provided by the University of Pretoria was a Shimadzu GC-2010 Plus with an FID detector. An autosampler was used to inject each sample from the 2 ml vials into the GC. Table 3.1 shows the GC parameters used.

Table 3.1 GC Parameters

Items	Value	Units
SPL 1 temperature	250	°C
SPL 1 pressure	100	kPa
Total flow	50	ml/min
Purge flow	3	ml/min
Primary pressure		kPa
Column Temperature	125	°C
Column ID		
FID1 temperature	260	°C
FID1 makeup flow	30	ml/min
FID1 H2 flow	40	ml/min
FID1 air flow	400	
FID1 detector		
FID1 flame		

3.9 Oven temperature program

The GC oven was set to increase the temperature to 125°C and hold it for 1.5 minutes, then increase by 4°C/min to reach a temperature of 204°C and hold that for 0 minutes before increasing by 1.5°C/min to reach a temperature of 226.3°C and holding that for 3 min and finally increasing by 2°C/min to 240°C and holding that for 10 min. The total run time was 55.97 min per injected sample.

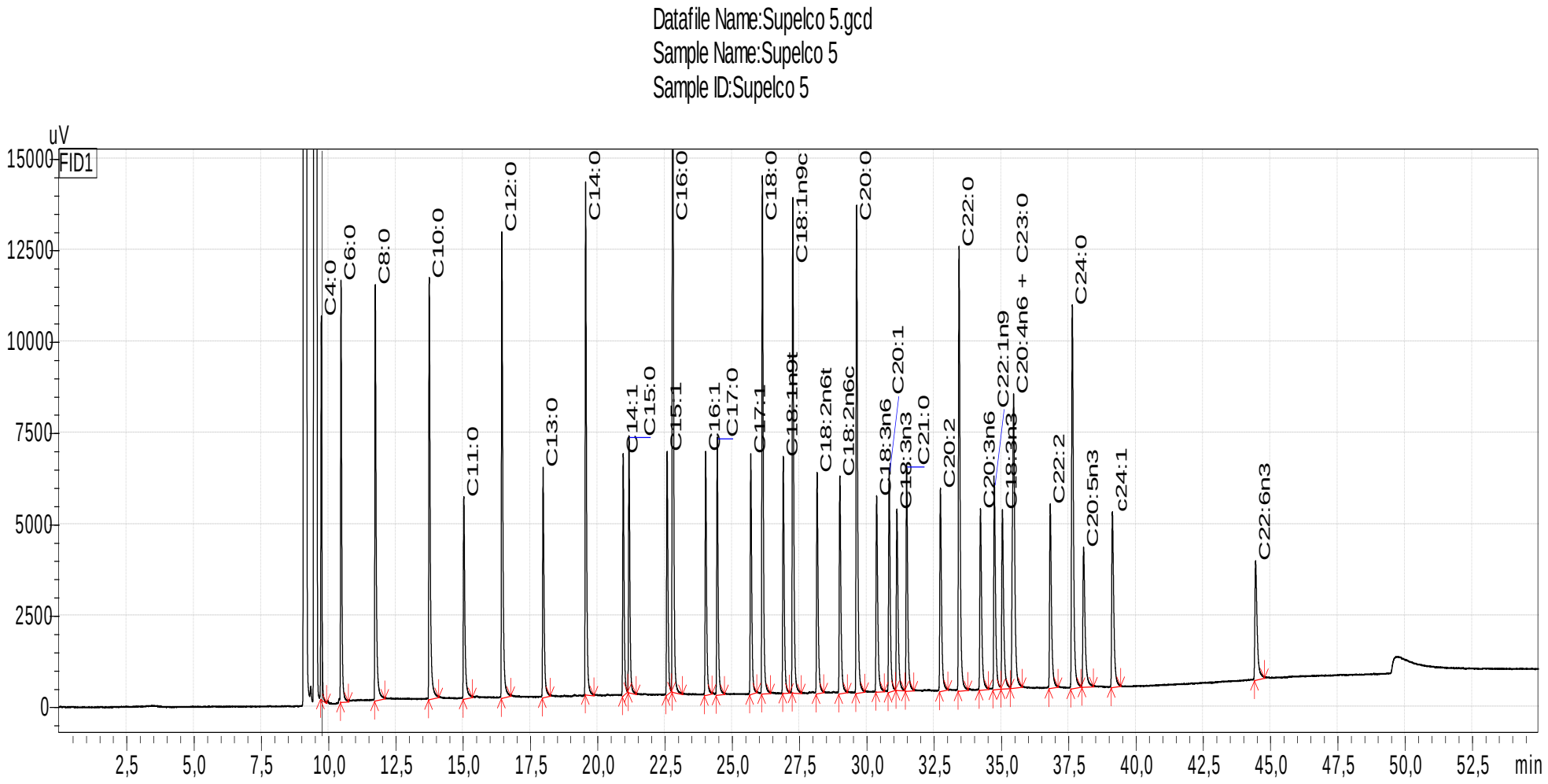
3.10 Standards used

An internal standard C15:0 pentadecanoic acid was used and an external standard Supelco-37 was used to identify FAs (Figure 3.1).

3.11 Statistical analysis

Peak areas were obtained from gas chromatography laboratory solutions software and recorded in Excel spreadsheets where the gravimetric (mg/g) and molar percentage was calculated. All statistical analysis was done using IBM SPSS Statistics 26. Multivariate analysis of variance was used to determine differences in FAs in all locations (IM, PR and SCF) and types (Wagyu and composite). Post-hoc analysis was done using Bonferroni's test and the Scheffe test.

Figure 3.1 Supelco-37 used as external standard



Chapter 4: Results and discussion

4.1 Fatty acid composition

Summary statistics of the FA composition of the Intramuscular (IM), subcutaneous fat (SCF) and perirenal fat (PRF) samples from Wagyu and composite feedlot cattle are presented in Table 4.1. The FA profiles are expressed in both molar proportion (molar percentage) and as gravimetric concentration (mg/g). Although molar proportions are generally reported in literature, the gravimetric concentration (mg/g) is useful from a nutritional perspective to indicate the actual content of FA in beef relative to suggested dietary FA requirements made by health professionals. The predominant FAs identified in the samples were oleic acid (C18:1n9c), palmitic acid (C16:0), stearic acid (C18:0) and myristic acid (C14:0) respectively, which agree with previous reports (Sturdivant *et al.*, 1992; Wood & Enser, 1997; Yang *et al.*, 1999; Wood *et al.*, 2008; Gotoh, *et al.*, 2009; Turk & Smith, 2009; Hu *et al*., 2010; Smith, 2014).

Table 4.1: Mean (± SD) FA composition of IM, PR and SCF samples obtained from Wagyu cattle and composite feedlot cattle as percentage of total FA identified (molar percentage) and gravimetric concentrations (mg/g; mean ± SD)

Fatty acid	Molar % (w/w%; n=78)	Gravimetric concentration (mg/g meat; n=78)
C10:0	0,043±0,011	0,114±0,062
C12:0	0,066±0,021	0,175±0,102
C13:0	0,006±0,010	0,010±0,025
C14:0	3,117±0,482	8,527±4,667
C14:1	0,582±0,448	1,683±1,770
C16:0	26,796±2,463	74,059±37,563
C16:1	2,827±1,806	7,903±7,272
C17:0	0,978±0,179	2,723±1,530
C18:0	21,539±9,544	61,380±47,706
C18:1n9t	2,779±1,631	7,800±6,606
C18:1n9c	37,109±9,281	103,791±58,919
C18:2n6t	0±0,003	0,001±0,012
C18:2n6c	3,187±1,969	7,097±3,745

C20:0	0,099±0,086	0,264±0,327
C18:3n6	0,013±0,014	0,025±0,036
C20:1	0,127±0,088	0,367±0,364
C18:3n3	0,134±0,034	0,341±0,169
C21:0	0,243±0,085	0,675±0,425
C20:2	0,033±0,024	0,072±0,068
C22:0	0,027±0,037	0,038±0,043
C20:3n6	0,075±0,081	0,117±0,086
C22:1n9	0,002±0,006	0±0,003
C20:4n6+C23:0	0,219±0,363	0,192±0,174

4.2 Fatty acid composition of IMF, PRF and SCF in Wagyu and composite cattle

Table 4.2 shows the mean (± SD) FA content of Wagyu and composite cattle in the different anatomical locations included in this investigation. Table 4.2 indicates that some FAs were detected in all three anatomical locations while other FAs are only detected in two or fewer anatomical locations. For example, in Table 4.2 it is indicated that tridecylic acid (C13:0), linolelaidic acid (C18:2n6t), arachidic acid (C20:0), eicosadienoic acid (C20:2), behenic acid (C22:0) and docosadienoic acid (C22:1n9) were not detected in all the anatomical adipose tissue locations. Linolelaidic acid (C18:2n6t) was detected only in the PRF of composite feedlot cattle. This may be due to PRF being the fat deposition site that contained the greatest concentration of total FAs (Webb et al., 1998). The FA composition differs in different anatomical adipose tissue locations as has been proven in numerous studies (Webb et al., 1998; Webb & O'Neill, 2008; Wood *et al.*, 2008; De Smet & Vossen, 2016; Aldai *et al.*, 2007; Turk & Smith, 2009). Refer also to Tables 4.3 and 4.4, which show the effect of anatomical location of FA composition. Table 4.3 is given in molar proportion and shows the effect of anatomical location on the FA composition. Table 4.4 shows the same effect of anatomical location on FA composition but in gravimetric concentration. Gravimetric concentration is given to improve understanding of the nutritional value.

Table 4.2: Comparison of mean (± SD) FA content of IMF, PRF and SCF in both composite cattle and Wagyu cattle as gravimetric concentration (mg/g; mean ± SD)

Fatty acid name	Composite (mg/g)		Wagyu (mg/g)	
C10:0	IM	0,027±0,011	IM	0,060±0,010
	PR	0,194±0,043	PR	0,162±0,025
	SCF	0,127±0,031	SCF	0,113±0,021
C12:0	IM	0,046±0,014	IM	0,073±0,014
	PR	0,314±0,089	PR	0,214±0,033
	SCF	0,199±0,044	SCF	0,201±0,036
C13:0	IM	0,008±0,006	IM	0,013±0,002
	PR	0,039±0,052	PR	0
	SCF	0	SCF	0
C14:0	IM	1,711±0,483	IM	3,597±0,535
	PR	12,043±3,711	PR	12,668±1,762
	SCF	10,286±1,408	SCF	10,838±1,620
C14:1	IM	0,176±0,093	IM	0,848±0,245
	PR	0,658±0,454	PR	1,224±0,197
	SCF	2,010±0,900	SCF	5,182±1,015
C16:0	IM	13,837±3,264	IM	36,429±3,002
	PR	95,927±9,228	PR	114,146±13,856
	SCF	82,659±6,545	SCF	101,358±10,239
C16:1	IM	1,003±0,406	IM	4,698±1,093
	PR	3,723±1,279	PR	7,050±1,100
	SCF	8,930±2,678	SCF	22,017±5,342
C17:0	IM	0,572±0,159	IM	1,086±0,103
	PR	4,338±0,547	PR	4,323±0,365
	SCF	3,106±0,820	SCF	2,911±0,402
C18:0	IM	14,237±4,625	IM	16,923±2,869
	PR	140,862±16,601	PR	100,745±13,736

	SCF	62,084±12,376	SCF	33,432±6,172
C18:1n9t	IM PR SCF	2,092±0,643 18,832±2,387 13,513±2,918	IM PR SCF	1,155±0,333 6,534±1,097 4,675±1,208
C18:1n9c	IM PR SCF	16,169±4,770 91,134±19,329 128,540±18,849	IM PR SCF	54,739±6,332 148,087±16,602 184,079±18,367
C18:2n6t	IM PR SCF	0 0,007±0,026 0	IM PR SCF	0 0 0
C18:2n6c	IM PR SCF	3,690±0,314 13,339±1,561 9,754±1,163	IM PR SCF	2,739±0,278 6,830±1,001 6,231±1,057
C20:0	IM PR SCF	0,093±0,033 0,802±0,203 0	IM PR SCF	0,080±0,023 0,589±0,088 0,021±0,041
C18:3n6	IM PR SCF	0,014±0,007 0,052±0,059 0,032±0,043	IM PR SCF	0,028±0,002 0,006±0,021 0,018±0,035
C20:1	IM PR SCF	0,044±0,020 0,220±0,099 0,421±0,183	IM PR SCF	0,188±0,065 0,369±0,119 0,958±0,483
C18:3n3	IM PR SCF	0,095±0,024 0,463±0,065 0,338±0,052	IM PR SCF	0,188±0,029 0,497±0,110 0,462±0,112
C21:0	IM PR SCF	0,131±0,079 0,748±0,275 0,906±0,320	IM PR SCF	0,292±0,061 0,755±0,127 1,218±0,295
C20:2	IM PR	0,370±0,009 0,140±0,092	IM PR	0,050±0,008 0,145±0,016

	SCF	0	SCF	0,060±0,512
C22:0	IM PR SCF	0,053±0,007 0,057±0,068 0	IM PR SCF	0,032±0,014 0,085±0,029 0
C20:3n6	IM PR SCF	0,106±0,028 0,033±0,052 0,012±0,030	IM PR SCF	0,160±0,017 0,152±0,032 0,240±0,048
C22:1n9	IM PR SCF	0,005±0,006 0 0	IM PR SCF	0 0 0
C20:4n6+C23:0	IM PR SCF	0,477±0,094 0,068±0,048 0,030±0,040	IM PR SCF	0,354±0,050 0,087±0,031 0,135±0,031

Table 4.3 Effect of anatomical location of adipose tissue (IM, PR and SCF) on the molar proportions of FAs (w/w %; mean ± SD)

Fatty acid name	SCF (w/w %; mean ± SD)	PRF (w/w %; mean ± SD)	IMF (w/w %; mean ± SD)
C10:0	0,035±0,008^{a}	0,045±0,010^{b}	0,049±0,009^{b}
C12:0	0,058±0,014^{a}	0,069±0,024^{ab}	0,072±0,021^{b}
C13:0	0^{a}	0,005±0,011^{a}	0,013±0,009^{b}
C14:0	3,054±0,481	3,278±0,493	3,017±0,421
C14:1	1,005±0,473^{a}	0,236±0,108^{b}	0,506±0,256^{c}
C16:0	26,391±2,146	26,545±2,619	27,452±2,558
C16:1	4,330±1,942^{a}	1,352±0,479^{b}	2,797±1,194^{c}
C17:0	0,872±0,204^{a}	1,100±0,110^{b}	0,963±0,134^{a}
C18:0	14,092±5,886^{a}	30,761±6,932^{b}	19,763±6,960^{c}
C18:1n9t	2,714±1,602^{a}	3,255±1,711^{b}	2,37±1,512^{c}
C18:1n9c	44,416±5,799^{a}	30,109±7,652^{b}	36,802±8,156^{c}
C18:2n6t	0	0,001±0,005	0
C18:2n6c	2,348±0,762^{a}	2,575±0,938^{a}	4,638±2,677^{b}
C20:0	0,003±0,086^{a}	0,177±0,053^{b}	0,117±0,061^{c}

C18:3n6	0,007±0,011[a]	0,007±0,012[a]	0,240±0,010[b]
C20:1	0,191±0,114[a]	0,074±0,031[a]	0,116±0,051[b]
C18:3n3	0,115±0,026[a]	0,122±0,023[a]	0,164±0,029[b]
C21:0	0,303±0,086[a]	0,192±0,056[b]	0,234±0,072[b]
C20:2	0,008±0,013[a]	0,036±0,016[b]	0,0544±0,017[c]
C22:0	0[a]	0,0179±0,014[b]	0,063±0,042[c]
C20:3n6	0,034±0,032[a]	0,023±0,019[a]	0,169±0,070[b]
C22:1n9	0[a]	0[a]	0,005±0,010[b]
C20:4n6+C23:0	0,010±0,013[a]	0,018±0,013[a]	0,937±0,333[b]

[abc] Means with different superscript letters in the same row differ (P<0,05)

[ABC] Means with different superscript letters in the same row differ (P<0, 01)

Table 4.4 Effect of anatomical adipose tissue location (IM, PR and SCF) on FAs (mean ± SD) in gravimetric concentration (mg/g).

Fatty acid name	SCF (mg/g; Mean ± SD)	PRF (mg/g; Mean ± SD)	IMF (mg/g; Mean ± SD)
C10:0	0,120±0,063[a]	0,178±0,038[b]	0,044±0,020[c]
C12:0	0,200±0,040[a]	0,264±0,084[b]	0,060±0,019[c]
C13:0	0[a]	0,019±0,041[b]	0,011±0,005[ab]
C14:0	10,562±1,514[a]	12,355±2,864[b]	2,654±1,084[c]
C14:1	3,596±1,871[a]	0,941±0,448[b]	0,512±0,388[c]
C16:0	92,008±12,720[a]	105,037±14,810[b]	25,133±11,922[c]
C16:1	15,474±7,853[a]	5,387±2,061[b]	2,850±2,050[c]
C17:0	3,009±0,641[a]	4,331±0,456[b]	0,829±0,293[c]
C18:0	47,758±17,718[a]	120,803±25,324[b]	15,580±4,012[c]
C18:1n9t	9,094±5,010[a]	12,683±6,530[b]	1,624±0,693[c]
C18:1n9c	156,310±33,681[a]	119,610±33,985[b]	35,454±20,420[c]
C18:2n6t	0	0,004±0,0185	0
C18:2n6c	7,992±2,101[a]	10,085±3,559[b]	3,215±0,565[c]
C20:0	0,011±0,031[a]	0,695±0,188[b]	0,086±0,029[c]
C18:3n6	0,025±0,039	0,029±0,049	0,021±0,010
C20:1	0,690±0,451[a]	0,294±0,132[b]	0,116±0,087[c]
C18:3n3	0,400±0,107[a]	0,480±0,090[b]	0,141±0,054[c]
C21:0	1,062±0,341[a]	0,752±0,210[b]	0,212±0,107[c]

C20:2	0,030±0,047[a]	0,142±0,065[b]	0,043±0,011[a]
C22:0	0[a]	0,071±0,054[b]	0,042±0,015[c]
C20:3n6	0,126±0,123[b]	0,092±0,074[a]	0,133±0,036[b]
C22:1n9	0[a]	0[a]	0,002±0,004[b]
C20:4n6+C23:0	0,082±0,064[a]	0,078±0,041[a]	0,415±0,097[b]

[abc] Means with different superscript letters in the same row differ (P<0,05)

[ABC] Means with different superscript letters in the same row differ (P<0, 01)

This study deals with two groups of cattle, F1 generation Wagyu cattle and composite feedlot cattle, which are different breeds. In Table 4.2, 4.5 and 4.6 the excessive IMF in Wagyu is clearly seen. Table 4.5 shows the effect of the two cattle groups on the FA composition. Looking at the p-values given in Table 4.5, it is evident that most of the FAs differ significantly between the two cattle groups, except for C18:2n6t, C18:3n3, C21:0, C20:0 and C20:3n6. The following FAs had higher proportions in composite feedlot cattle: C18:1n9t, C18:2n6t, C182n6c, C20:0, C20:2, C22:0, C22:1n9 and C20:4n6+C23:0 (Table 4.2). Significant differences in FAs between these two cattle group types are due to genetics, leading to the ability of Wagyu cattle to produce large amounts of IMF, as discussed in the literature review. Long chain (C20-22) PUFA are found in adipose tissue and muscle neutral lipid in pigs and sheep, but not in cattle (Wood *et al.*, 2008), which could explain why these FAs were detected in such small amounts, or not at all. In ruminants the ratio of C18:0/C18:2n6 decreases during fattening (Wood *et al.*, 2008). Wagyu cattle are fattened over a longer time frame than composite feedlot cattle. This explains why C18:2n6 is lower in Wagyu IMF. According to Wood *et al.* (2008), there is a decline in the proportion of PUFA in ruminant muscles during fattening due to low levels of neutral lipids in ruminants. Of the two major PUFA, C18:2n6 is taken up into the muscle more rapidly than C18:3n3 (Wood *et al.*, 2008). Table 4.6 shows the same effect of breed type but the data are represented in gravimetric concentration (mg/g). This is done to gain better understanding of the nutritional value of these two cattle groups. In Wagyu cattle C18:2n9t was not detected. This may indicate that Wagyu cattle have less *trans* FAs than composite feedlot cattle. However, this difference is insignificant, as shown in Table 4.5.

Table 4.5 Summary statistics (means ± SD) of the pooled fatty acid data for the effect of cattle breed type (composite and Wagyu) on the molar proportions of FAs (w/w %; mean ± SD)

Fatty acid name	Composite (w/w %; mean ± SD)	Wagyu (w/w %; mean ± SD)	p-value
C10:0	0,046±0,011^{A}	0,034±0,009^{B}	0,001
C12:0	0,077±0,024^{A}	0,055±0,009^{B}	0,000
C13:0	0,008±0,012^{A}	0,004±0,005^{B}	0,008
C14:0	3,251±0,523^{a}	2,982±0,399^{b}	0,012
C14:1	0,371±0,266^{A}	0,794±0,494^{B}	0,000
C16:0	25,336±1,703^{A}	28,256±2,241^{B}	0,000
C16:1	1,844±0,925^{A}	3,810±1,940^{B}	0,000
C17:0	1,045±0,178^{A}	0,91±0,156^{B}	0,000
C18:0	27,264±8,260^{A}	15,813±7,004^{B}	0,000
C18:1n9t	4,289±0,746^{A}	1,268±0,394^{B}	0,000
C18:1n9c	30,877±7,631^{A}	43,340±6,049^{B}	0,000
C18:2n6t	0,001±0,004	0	0,321
C18:2n6c	4,517±2,027^{A}	1,857±0,339^{B}	0,000
C20:0	0,126±0,100^{A}	0,072±0,060^{B}	0,000
C18:3n6	0,016±0,015^{a}	0,020±0,011^{b}	0,01
C20:1	0,089±0,047^{A}	0,165±0,103^{B}	0,000
C18:3n3	0,134±0,038	0,133±0,029	0,814
C21:0	0,236±0,094	0,249±0,074	0,432
C20:2	0,035±0,032	0,031±0,013	0,147
C22:0	0,039±0,048^{A}	0,016±0,014^{B}	0,000
C20:3n6	0,073±0,107	0,077±0,040	0,634
C22:1n9	0,003±0,008^{A}	0^{B}	0,003
C20:4n6+C23:0	0,321±0,479^{A}	0,116±0,129^{B}	0,000

^{abc} Means with different superscript letters in the same row differ (P<0,05)

^{ABC} Means with different superscript letters in the same row differ (P<0, 01)

Table 4.6 Summary statistics (means ± SD) of the effect of cattle breed type (composite and Wagyu) on gravimetric FA concentration (mg/g; mean ± SD)

Fatty acid name	Composite (mg/g; mean ± SD)	Wagyu (mg/g; mean ± SD)	P-value
C10:0	0,116±0,076	0,112±0,046	0,471
C12:0	0,187±0,125^{a}	0,163±0,070^{b}	0,022
C13:0	0,016±0,034^{a}	0,004±0,007^{b}	0,025
C14:0	8,013±5,095^{a}	9,034±4,200^{b}	0,021
C14:1	0,948±0,971^{A}	2,418±2,074^{B}	0,000
C16:0	64,141±37,047^{A}	83,977±35,843^{B}	0,000
C16:1	4,552±3,733^{A}	11,255±8,376^{B}	0,000
C17:0	2,672±1,684	2,773±1,378	0,339
C18:0	72,394±54,216^{A}	50,366±37,725^{B}	0,000
C18:1n9t	11,4791±7,394^{A}	4,121±2,446^{B}	0,000
C18:1n9c	78,614±49,777^{A}	128,968±57,055^{B}	0,000
C18:2n6t	0,002±0,0151	0	0,321
C18:2n6c	8,928±4,184^{A}	5,267±2,008^{B}	0,000
C20:0	0,298±0,381^{A}	0,230±0,264^{B}	0,002
C18:3n6	0,033±0,044^{a}	0,173±0,025^{b}	0,049
C20:1	0,228±0,196^{A}	0,505±0,436^{B}	0,000
C18:3n3	0,299±0,162^{A}	0,383±0,166^{B}	0,000
C21:0	0,565±0,416^{A}	0,755±0,425^{B}	0,002
C20:2	0,059±0,079^{a}	0,085±0,053^{b}	0,011
C22:0	0,036±0,047	0,039±0,040	0,691
C20:3n6	0,050±0,055^{a}	0,184±0,052^{b}	0,000
C22:1n9	0,002±0,004^{A}	0^{B}	0,002
C20:4n6+C23:0	0,191±0,215	0,192±0,123	0,955

^{abc} Means with different superscript letters in the same row differ (P<0,05)

^{ABC} Means with different superscript letters in the same row differ (P<0, 01)

4.3 Main categories of fatty acids

In this section the main categories of FAs referred to are SFA and UFA. The latter is divided into MUFA and PUFA. In Table 4.7 the mean (±SD) for the main categories of FAs can be seen. SFAs are higher in composite feedlot cattle (57,429±8,2555) than in Wagyu cattle (48,399±7,9014). UFA are higher in Wagyu cattle (51,601±7,9014) than in composite cattle (42,570±8,2556). MUFA are higher in Wagyu but the proportion of PUFA is lower in Wagyu compared to composite cattle. This is due to n-6 being lower in Wagyu. In Table 4.9 it is shown that these differences are significant. In Table 4.7 the EFAs are also shown; n-3 is very similar between Wagyu and composite cattle. In Table 4.9 it is clear that n-3 does not differ significantly between Wagyu and composite feedlot beef. The ratio of n-6/n-3 is very high, as indicated in all the tables below, but there is a significant difference between Wagyu and composite feedlot cattle for this ratio. This difference is due to the n-6 FA being significantly lower in Wagyu cattle. This results in a lower n-6/n-3 ratio in Wagyu cattle. The recommended daily intake ratio of n-6/n-3 varies between 1:1 and 1:4. This ratio is higher in both cattle groups, but the ratio of n-6/n-3 in Wagyu cattle is closer to the recommended daily intake than that of composite feedlot cattle.

Table 4.7 The total mean (±SD) for the main categories of FAs and their ratios

Fatty acid groups	Composite (mean±SD)	Wagyu (mean±SD)
SFA	57,429±8,256	48,399±7,901
UFA	42,570±8,256	51,601±7,901
MUFA	37,473±8,482	49,377±7,919
PUFA	5,097±2,657	2,223±0,515
n-3	0,134±0,038	0,133±0,029
n-6	4,928±2,601	2,060±0,487
n-9	35,167±7,441	44,609±5,837
UFA/SFA	0,776±0,252	1,120±0,345
MUFA/SFA	0,686±0,246	1,073±0,339
PUFA/SFA	0,091±0,050	0,047±0,012
n-6/n-3	35,65±12,01	15,64±2,42
n-3/n-9	0,004±0,002	0,003±0,001
n-6/n-9	0,148±0,086	0,047±0,0124
(n-3+n-6)/n-9	15,28±8,70	5,0±1,30

Table 4.8 Comparison of means (±SD) of the composition of main categories of FAs in IM, PR SCF in the different cattle breed types (Wagyu and composite)

Fatty acid group	Location	Composite (mean ±SD)	Location	Wagyu (mean±SD)
SFA	IM	56,1±4,20	IM	47,4±3,02
	PR	66,7±4,50	PR	57,7±3,57
	SC	49,5±3,70	SC	40,1±2,88
UFA	IM	43,9±4,20	IM	52,6±3,02
	PR	33,3±4,50	PR	42,3±3,57
	SC	50,5±3,70	SC	59,9±2,88
MUFA	IM	35,4±4,01	IM	49,8±3,12
	PR	29,6±4,39	PR	40,4±3,69
	SC	47,4±3,86	SC	57,9±2,99
PUFA	IM	8,5±1,86	IM	2,9±0,24
	PR	3,7±0,29	PR	1,9±0,26
	SC	3,2±0,39	SC	1,9±0,26
n-3	IM	0,1±0,03	IM	0,2±0,02
	PR	0,1±0,01	PR	0,1±0,03
	SC	0,1±0,02	SC	0,1±0,03
n-6	IM	8,2±1,85	IM	2,7±0,22
	PR	3,5±0,28	PR	1,8±0,24
	SC	3,1±0,38	SC	1,8±0,24
n-9	IM	33,2±3,56	IM	45,1±2,65
	PR	28,4±4,05	PR	38,3±3,54
	SC	43,9±3,36	SC	50,4±2,92
UFA/SFA	IM	0,8±0,14	IM	1,1±0,13
	PR	0,5±0,11	PR	0,7±0,11
	SC	1,0±0,14	SC	1,5±0,35
MUFA/SFA	IM	0,6±0,12	IM	1,1±0,13
	PR	0,5±0,10	PR	0,7±0,11
	SC	1,0±0,14	SC	1,5±0,18
PUFA/SFA	IM	0,2±0,04	IM	0,1±0,00
	PR	0,1±0,01	PR	0,0±0,00
	SC	0,1±0,01	SC	0,0±0,01

n-6/n-3	IM PR SC	47,5±12,24 29,9±7,58 29,5±4,53	IM PR SC	17,7±1,94 14,53±1,70 14,69±2,26
n-3/n-9	IM PR SC	0,005±0,0013 0,004±0,0009 0,002±0,0005	IM PR SC	0,003±0,0005 0,003±0,0009 0,002±0,0006
n-6/n-9	IM PR SC	0,2±0,06 0,1±0,01 0,07±0,012	IM PR SC	0,05±0,007 0,04±0,009 0,03±0,005
(n-3+n-6)/n-9	IM PR SC	25,6±6,60 12,9±1,81 7,2±1,29	IM PR SC	6,2±0,77 4,9±0,99 3,7±0,63

In the two cattle groups the ratio of UFA/SFA and MUFA/SFA is higher in Wagyu cattle. The ratio of PUFA/SFA is higher in composite feedlot cattle, for the same reasons as mentioned above.

Table 4.9 Summary statistics (means ± SD) of the effect of cattle breed type (composite and Wagyu) on main category of FAs and FA ratios

Fatty acid	Composite (mean±SD)	Wagyu (mean±SD)	P-value
SFA	57,4±8,26	48,4±7,90	0,00
UFA	42,6±8,26	51,6±7,90	0,00
MUFA	37,5±8,48	49,4±7,92	0,00
PUFA	5,1±2,66	2,2±0,52	0,00
n-3	0,1±0,04	0,1±0,03	0,814
n-6	4,9±2,60	2,1±0,49	0,00
n-9	35,2±7,44	44,6±5,84	0,00
UFA/SFA	0,8±0,25	1,1±0,34	0,00
MUFA/SFA	0,7±0,25	1,1±0,33	0,00
PUFA/SFA	0,1±0,05	0,0±0,01	0,00
n-6/n-3	35,66±12,01	15,64±2,42	0,00
n-3/n-9	0,004±0,0015	0,003±0,0008	0,00
n-6/n-9	0,1±0,08	0,04±0,012	0,00
(n-3+n-6)/n-9	15,2±8,70	5,0±1,30	0,00

Table 4.10 Effect of anatomical adipose tissue location (IM, PR and SCF) on the main categories of FAs and FA ratios (mean ± SD)

Fatty acid	IMF	PRF	SCF
SFA	51,7±5,70^{A}	62,2±6,10^{B}	44,8±5,75^{C}
UFA	48,3±5,70^{A}	37,8±6,10^{B}	55,2±5,76^{C}
MUFA	42,6±8,11^{A}	35,0±6,80^{B}	52,7±6,37^{C}
PUFA	5,7±3,15^{A}	2,8±0,93^{B}	2,5±0,72^{C}
n-3	0,2±0,03^{A}	0,1±0,02^{B}	0,1±0,03^{B}
n-6	5,4±3,12^{A}	2,6±0,93^{B}	2,4±0,73^{B}
n-9	39,2±6,80^{A}	33,4±6,26^{B}	47,1±4,55^{C}
UFA/SFA	1,0±0,21^{A}	0,6±0,16^{B}	1,3±0,29^{C}
MUFA/SFA	0,8±0,25^{A}	0,6±0,17^{B}	1,2±0,29^{C}
PUFA/SFA	0,1±0,05^{A}	0,0±0,01^{B}	0,1±0,01^{B}
n-6/n-3	32,6±17,46^{A}	22,2±9,52^{B}	22,1±8,32^{B}
n-3/n-9	0,004±0,0014^{A}	0,003±0,0010^{A}	0,002±0,0005^{B}
n-6/n-9	0,1±0,10^{A}	0,08±0,042^{B}	0,05±0,020^{C}
(n-3+n-6)/n-9	15,9±10,89^{A}	8,9±4,31^{B}	5,5±2,04^{C}
^{ABC} Means with different superscript letters in the same row differ (P<0,05)			

4.4 Marbling score and actual fat content

The marbling score and actual fat content (percentage) of the Wagyu carcasses were further investigated. The abattoir uses the MIJ camera fat percentage estimates to determine the marbling score of Wagyu carcasses. The abattoir takes photos of the eye muscle at the fifth rib by using the MIJ camera. These photos are then sent to Japan to assess the marbling scores. Each marbling score (varying from 1 to 9) has a predetermined IMF percentage; in other words, it indicates the amount of fat to be expected in Wagyu meat of that specific marbling score. The aim of this part of the research was to compare the measures used to determine the marbling score with the actual IMF content in Longissimus muscle samples. These measures included the Australian marbling scores fat percentage estimates and the MIJ camera fat percentage estimates. These measures were then compared to the actual fat percentage found by ether extracts. The marbling score given to the Wagyu carcasses was also compared to these two measures and the actual fat percentage.

Figure 4.1 shows that there is a correlation between the Australian marbling score and MIJ camera with R square values of 0.997, 0.970 and 0.970 for quadratic, exponential and logistic values respectively. The quadratic model fits the data best; this is confirmed by a P-value < 0.05 (p-value = 0.00). The high R square values indicate that the correlation between the variables is high. The quadratic regression equation is $y = 17.880 - 0.466\,x + 0.45\,x^2$ for Figure 4.1 where 17.880 is the intercept. Y is the dependent variable and x is the independent variable and this continues throughout this section.

Figure 4.1 Correlation between AUS marbling fat percentage and MIJ camera fat percentage

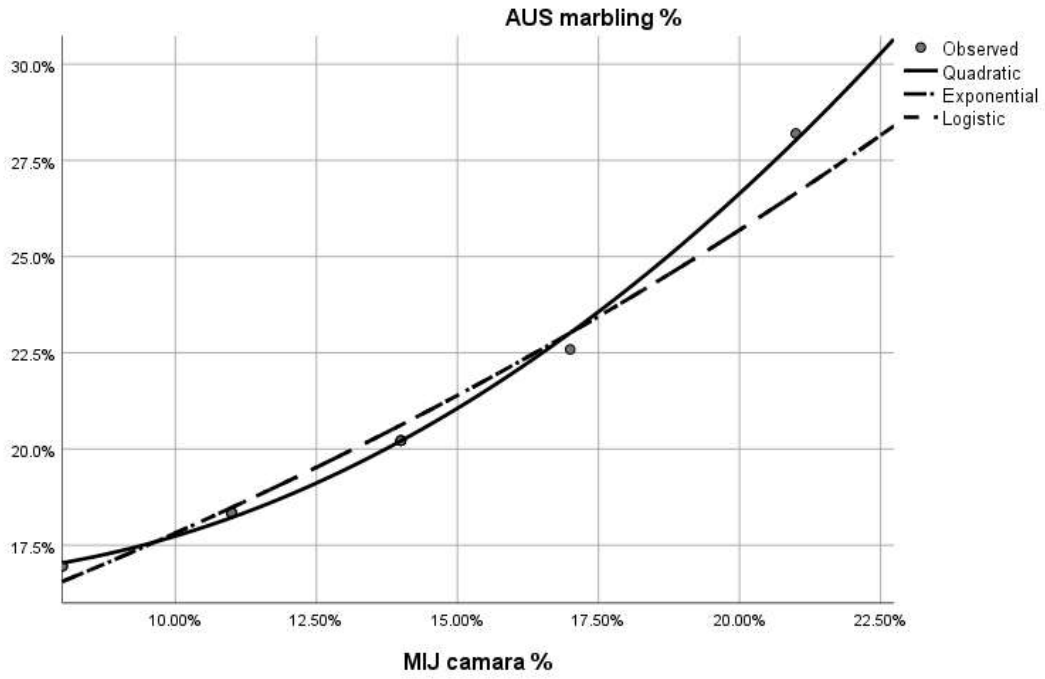


Figure 4.2 shows the correlation between the actual fat percentage and the AUS marbling percentage. The R square value is 0.333, which is moderate, with a p-value of 0.039 (p-value < 0.05). This indicates the strength of the relationship between the dependent and independent variables, in this case fat percentage and AUS marbling score respectively. The inverse regression equation of Figure 4.2 is $y = 85.271 + \frac{(-664.130)}{x}$, where -664.130 is the slope and 85.271 is the intercept.

Figure 4.2 Correlation between actual fat percentage and AUS marbling percentage

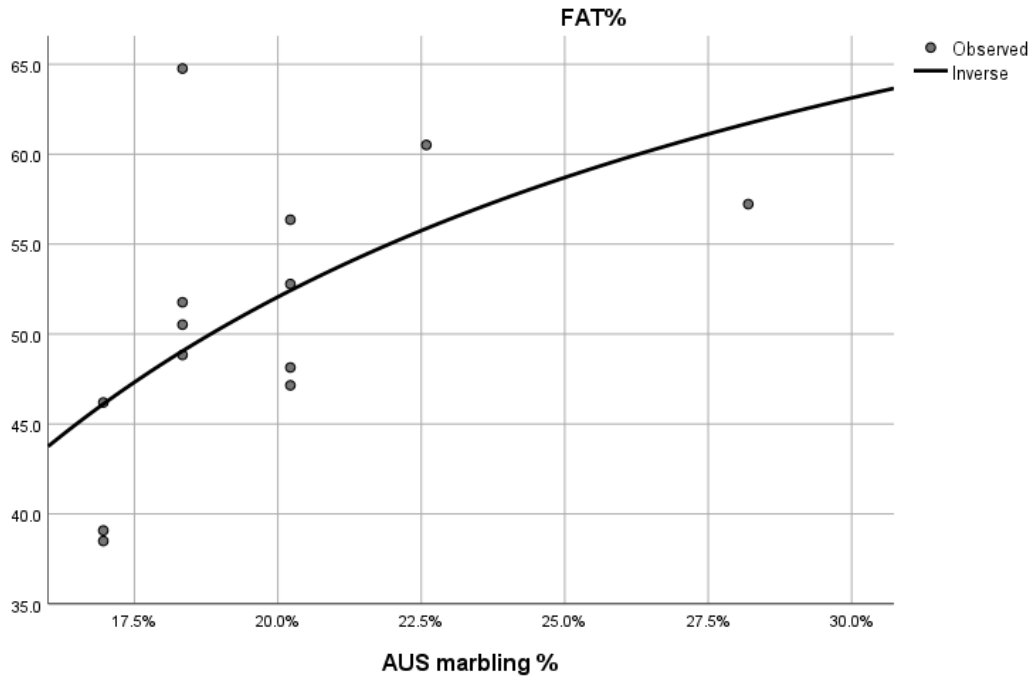
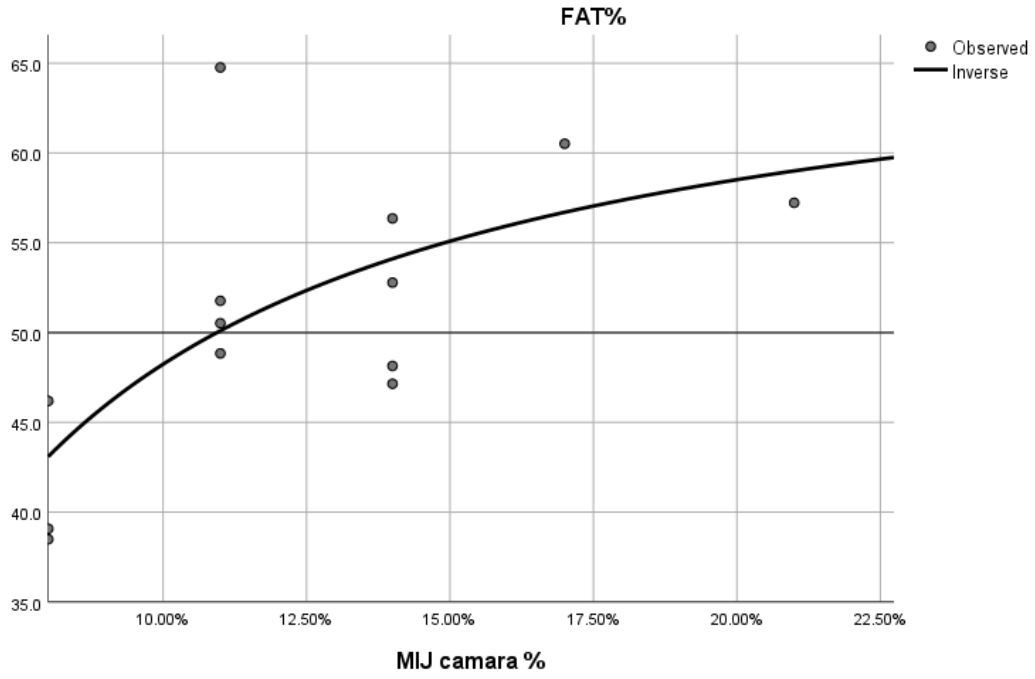


Figure 4.3 Correlation between actual fat percentage and MIJ camera percentage

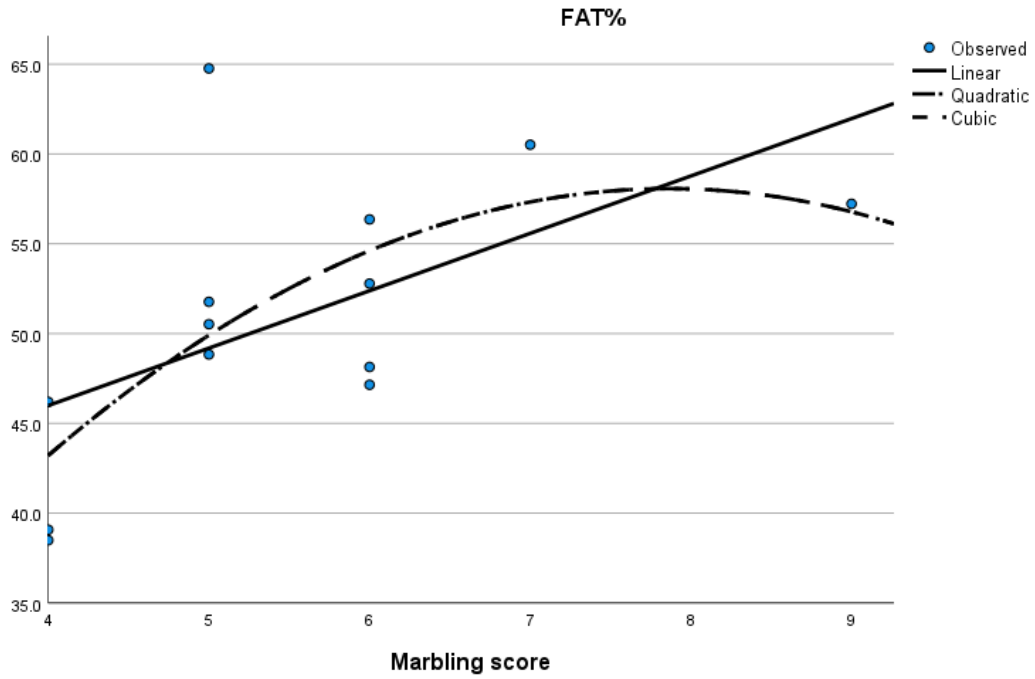


The R square value of Figure 4.3 is 0.465, which is higher than the R square value of Figure 4.2. This indicates a stronger correlation between the actual fat percentage and MIJ camera estimated fat percentage. This correlation is supported by a p-value = 0.010 (p-value <

0.05). The inverse regression equation of Figure 4.3 is $y = 68.794 + \frac{(-205.573)}{x}$, where -205.573 is the slope and 68.794 is the intercept.

Figure 4.4 shows the correlation between actual fat percentage and marbling score with R square values of 0.339, 0.446 and 0.446 for linear, quadratic and cubic scores respectively. The p-values of the linear and quadratic regression model are 0.037 and 0.05 respectively. The lower R square value for the linear regression is because the biological process studied does not follow a linear model, even though the p-value is lower than 0.05. The p-value of the quadratic regression model just makes the cut-off point. The quadratic regression model does describe the data better, hence the higher R square value. The quadratic regression equation is $y = -3.499 + 15.662\,x - 0.996\,x^2$, where -3.499 is the intercept.
The correlation between the fat percentage and the marbling score is described best by the quadratic model.

Figure 4.4 Correlation between actual fat percentage and marbling scores



In Figures 4.5 and 4.6 the linear model fits the data very well with R squared values of 0.99 and 0.97 respectively. The linear regression equation of Figure 4.5 is $= (-2.503) + 2.702\,x$, where 2.702 is the slope and -2.503 is the intercept. The linear regression equation of figure 4.6 is $y = 7.721 + 2.160\,x$, where 2.160 is the slope and 7.721 is the intercept. The correlation between the MIJ camera and the marbling scores (figure 4.5) is higher than the correlation in Figure 4.6. This is because the abattoir used MIJ camera results to estimate the marbling scores.

The actual fat percentage is more similar to the MIJ camera fat percentage than the AUS marbling fat percentage. This is also true for the marbling scores. However, there are still some inconsistencies between marbling score and actual fat percentage. In some of the samples the actual fat percentage for a high marbling score was lower than the actual fat percentage of a sample with a lower marbling score. For example, a marbling score of 9 had a fat percentage of 57%, while a marbling score of 7 had a fat percentage of 60%. This inconsistency may be due to a number of factors. There is a human factor involved when samples are taken and when ether extracts are performed. The way in which marbling scores are given is still subjective and very dependent on appraisal of the fineness of the fat, not necessarily the amount of fat. The fineness of the marbling is very important in the Japanese culture and is referred to as frosting. Performing ether extracts on the entire meat cut, not just the *m. Longissimus dorsi,* might also be more representative, considering that the MIJ camera photos are of the entire meat section. In Addendum A the photos taken by the MIJ camera are shown.

Figure 4.5 Correlation between the MIJ camera percentage and marbling score

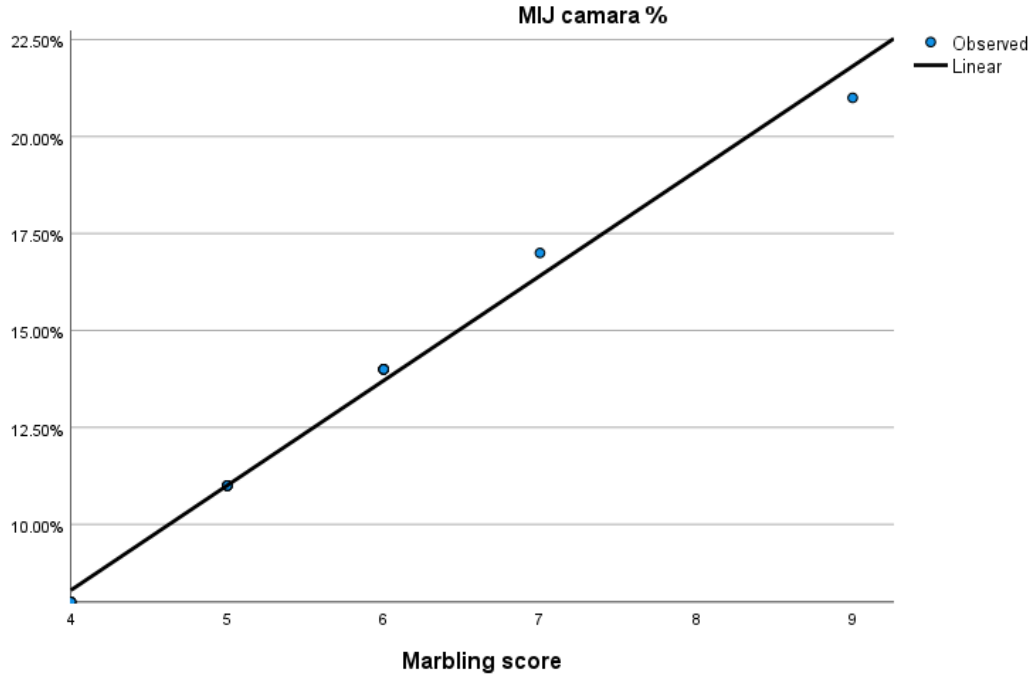
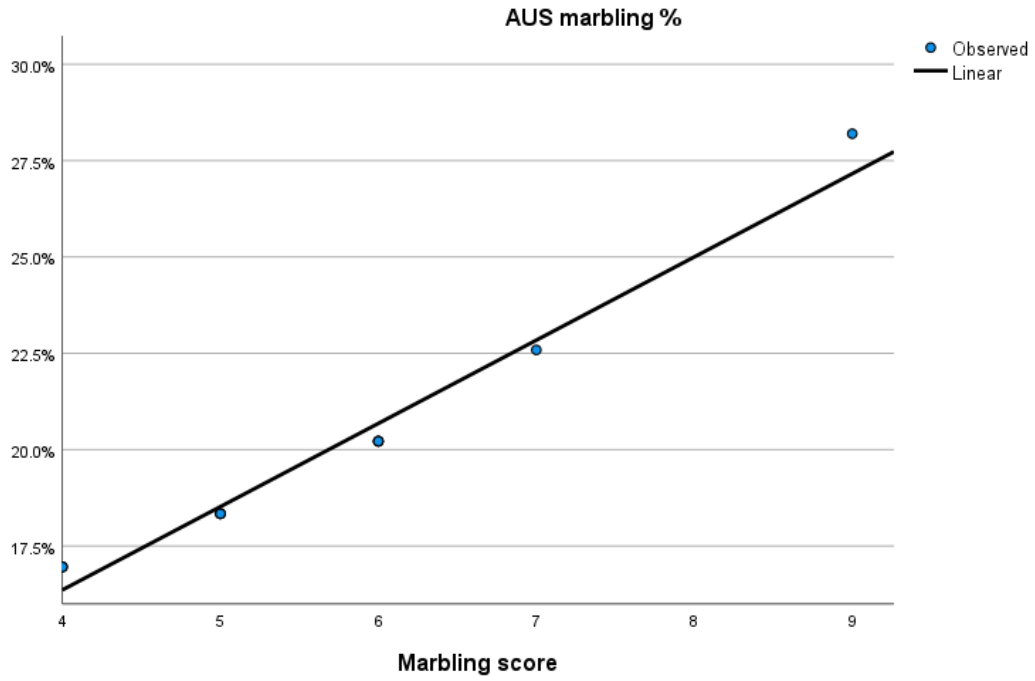


Figure 4.6 Correlation between the AUS marbling percentage and marbling score



Chapter 5: Conclusions

The fat content and FA composition of loin meat samples and other fat deposits in South African Wagyu cattle were determined and compared to those in composite feedlot cattle. In theory the FA composition should be very similar between these two groups. It was found that the FA composition was similar between the groups; the major differences were in the amount of each FA present. From the results it is clear that there is a significant difference in FA content between Wagyu and composite feedlot cattle. This is due to Wagyu beef having large amounts of IMF.

It was found that the ratios of the main FA groups differ significantly between Wagyu and composite feedlot cattle. In this study the ratios of UFA/SFA and MUFA/SFA were higher in Wagyu beef, which corresponds to what was previously reported in the literature. The ratio of n-6/n-3 is lower in Wagyu and compares more favourably with the recommended daily intake of n-6/n-3, compared to composite cattle. The ratios of these EFAs are important for normal development and homeostasis in the human body. The recommended ratios of these EFAs are 1:1 or 1:4. This study proves that Wagyu beef may be beneficial for human health when considering the proportions and content of EFAs. Wagyu beef has a more favourable ratio of EFAs and is higher in MUFAs. In this study Wagyu beef contained less PUFA, contrary to previous reports in the literature that the amount of PUFA was lower in Wagyu because of the lower concentrations of n-6 FAs. The effect of these differences on consumer health will require more studies. These differences can also affect the marketing of products which can lead to an increase or decrease in consumption, depending on the effect this information has on the public.

Two different measures were used to assign marbling to Wagyu beef scores. The actual fat percentage found in Wagyu beef corresponded well with the MIJ camera measurement to estimate the muscle fat percentage and hence the level of marbling. Some inconsistencies were observed in terms of marbling scores, which did not compare well with the actual fat percentage. Further investigation revealed that the marbling score is also affected by the fineness of the marbling, especially in Japan. A higher marbling score could indicate finer marbling; however, the actual fat percentage present in that meat cut was lower than in a meat cut with a lower marbling score.

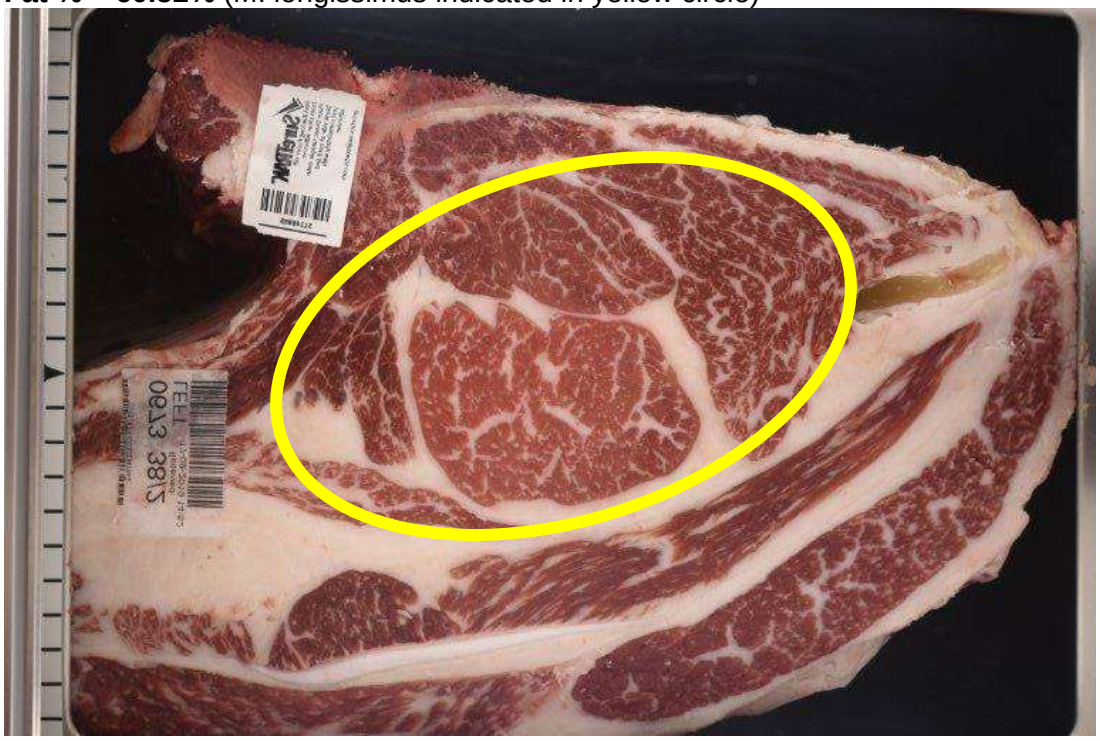
South African Wagyu beef is a new source of red meat and more studies are required to understand the effects of production factors on the marbling scores and fat content of this beef.

Addendum A

In the photos below the *M. Longissimus dorsi* is indicated by a circle.

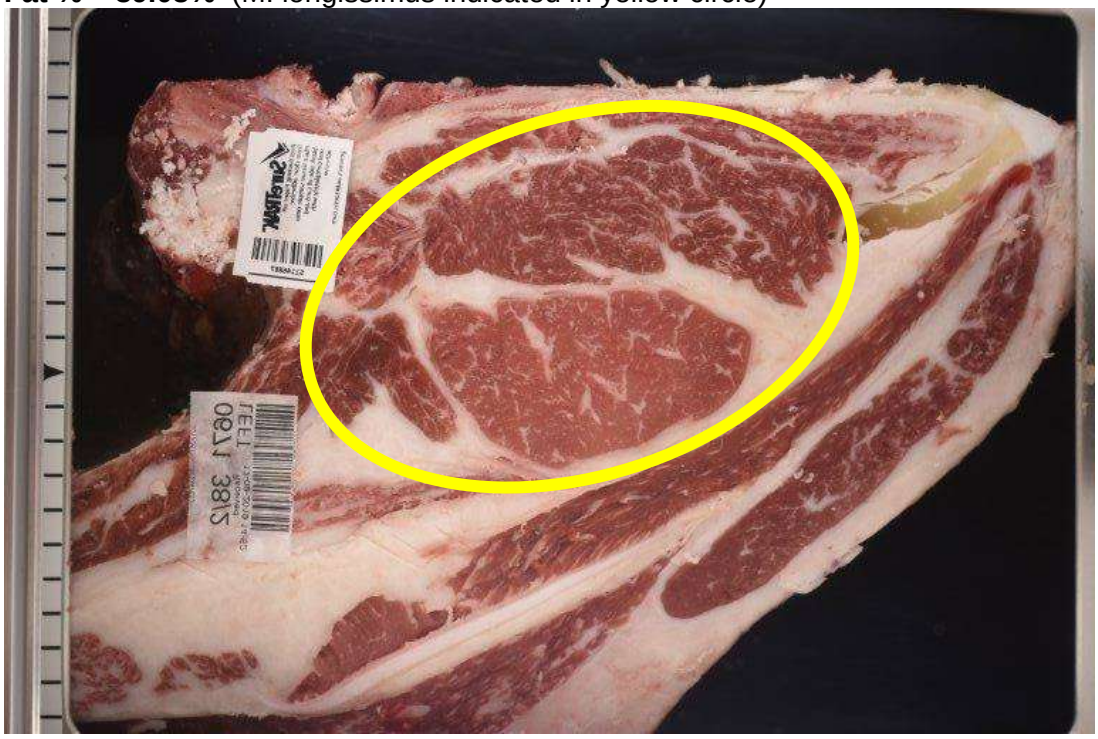
MIJ Camera

Marbling score = 7
Carcass weight = 401 kg
Fat % = 60.52% (M. longissimus indicated in yellow circle)



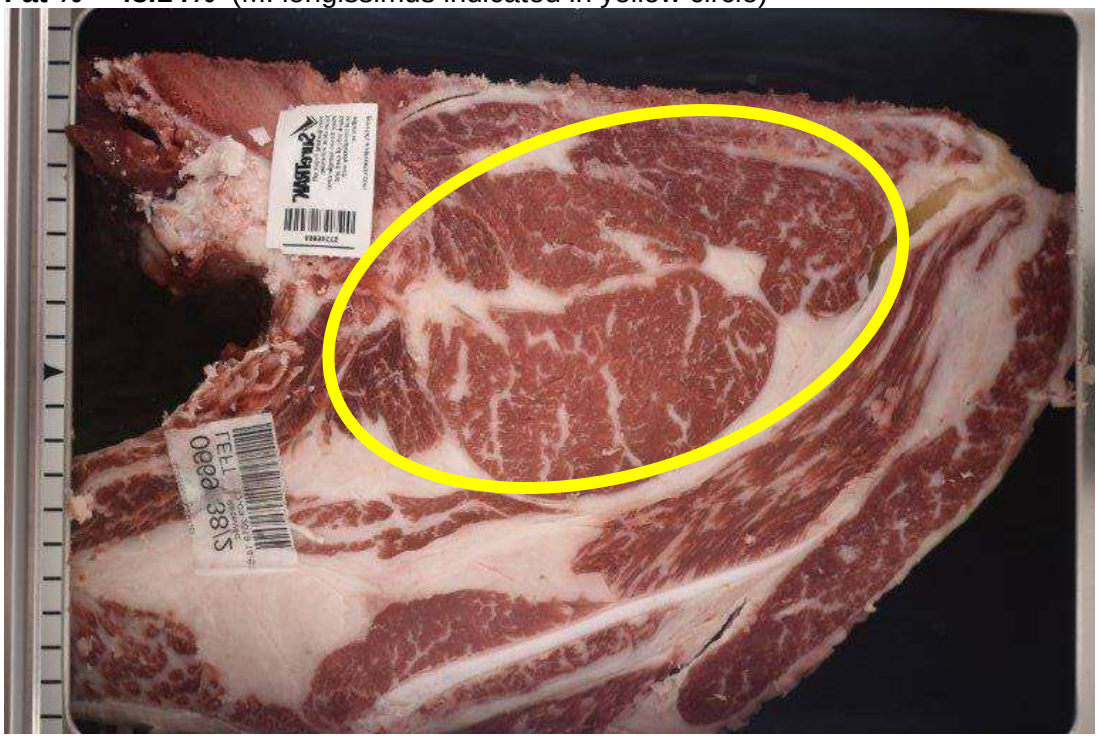
Marbling score = 5
Carcass weight = 409 kg
Fat % = 48.84% (M. longissimus indicated in yellow circle)

Marbling score = 4
Carcass weight = 385 kg
Fat % = 39.08% (M. longissimus indicated in yellow circle)

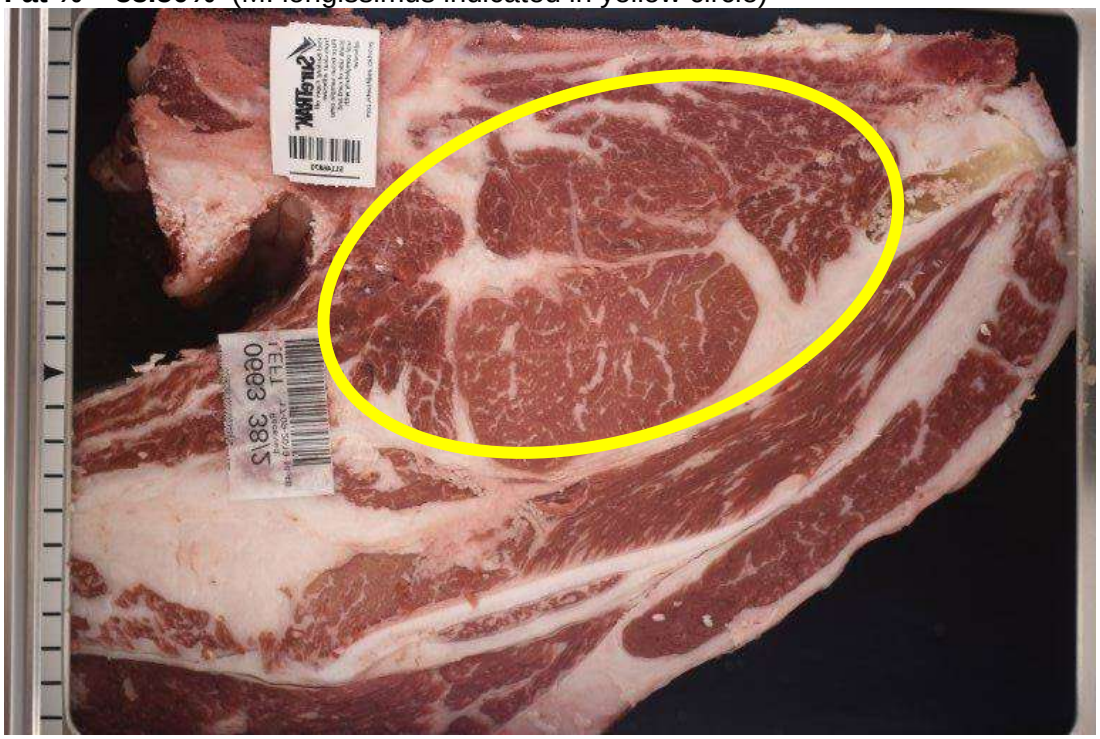


Marbling score = 5
Carcass weight = 435 kg
Fat % = 50.52% (M. longissimus indicated in yellow circle)

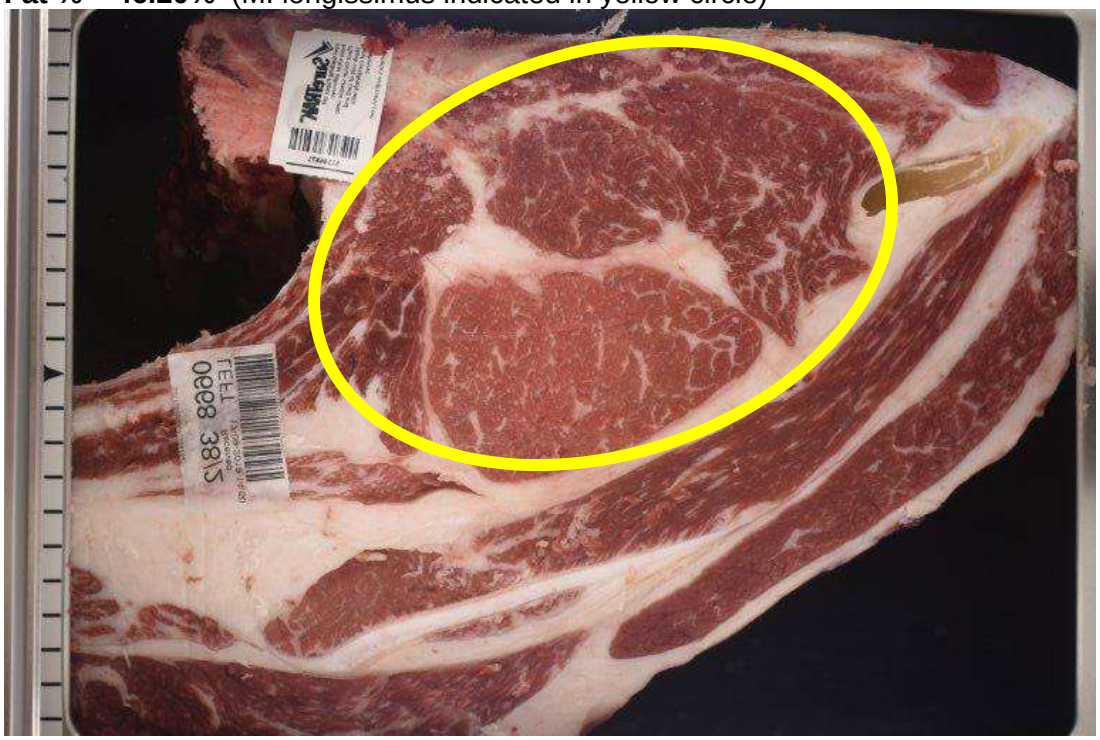
Marbling score = 6
Carcass weight = 440 kg
Fat % = 48.14% (M. longissimus indicated in yellow circle)



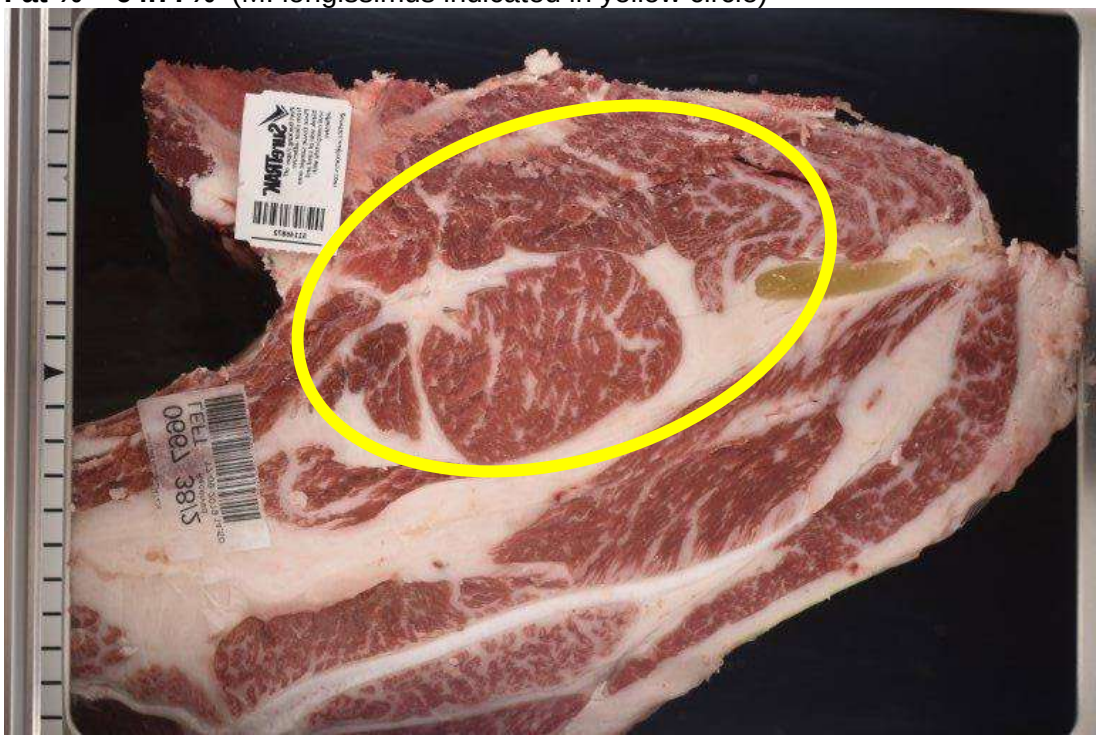
Marbling score = 4
Carcass weight = 416 kg
Fat % = 38.50% (M. longissimus indicated in yellow circle)



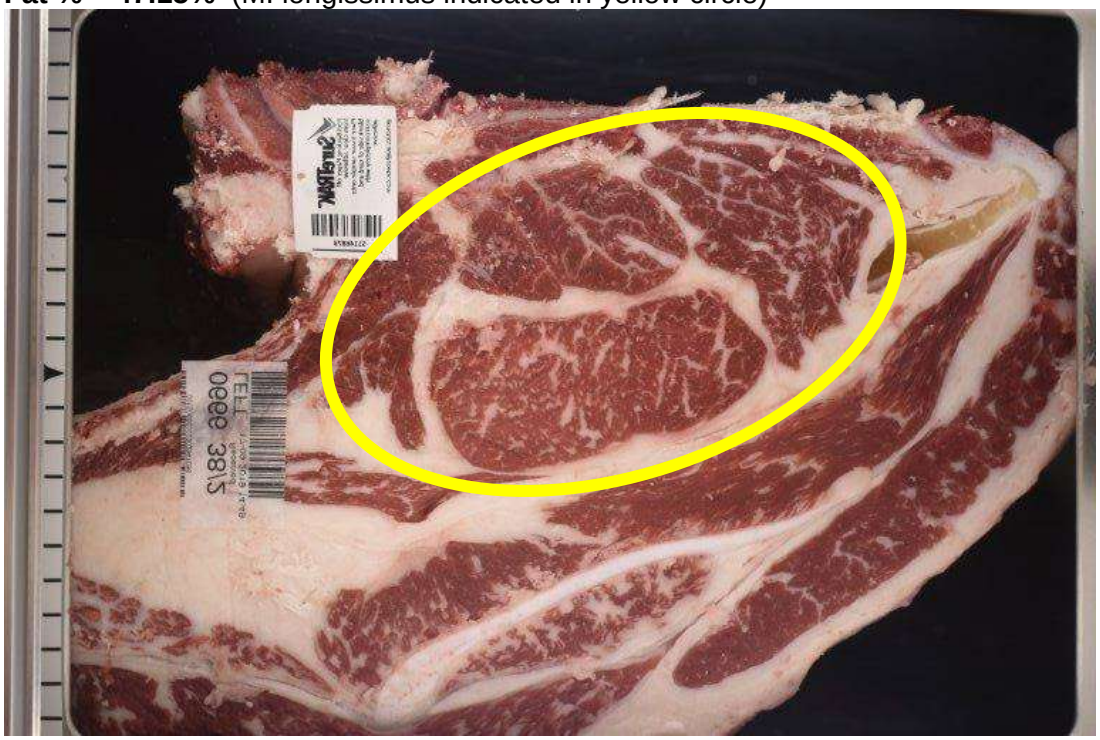
Marbling score = 4
Carcass weight = 446 kg
Fat % = 46.20% (M. longissimus indicated in yellow circle)



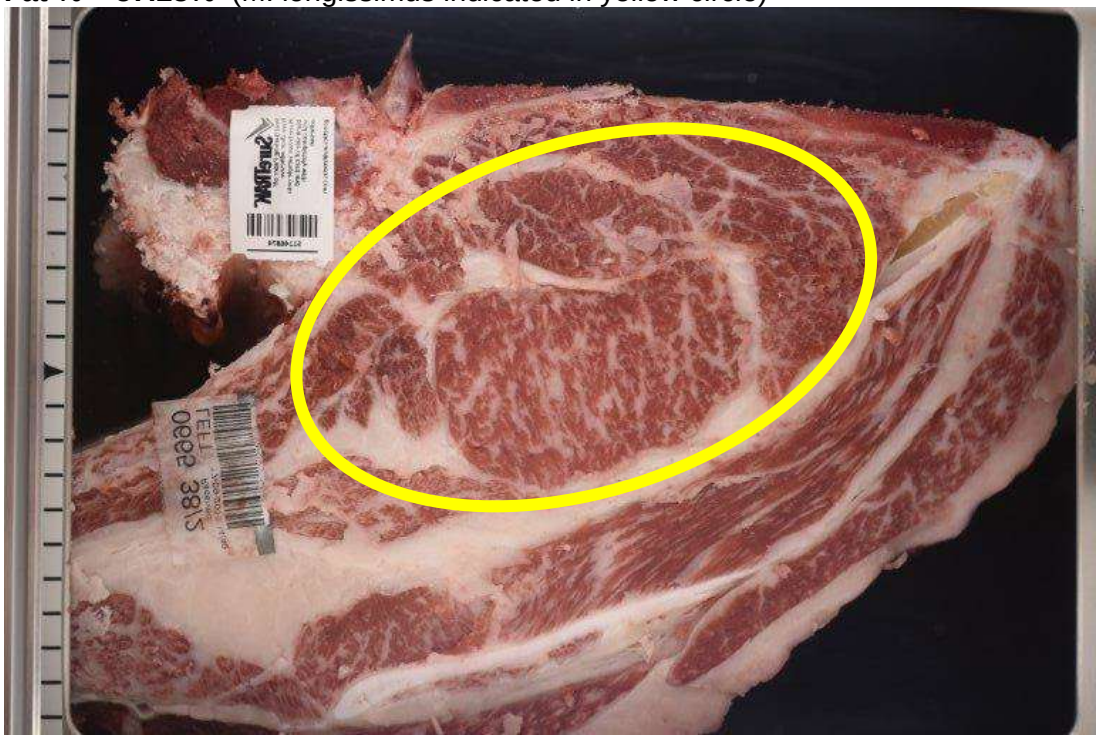
Marbling score = 5
Carcass weight = 401 kg
Fat % = 64.77% (M. longissimus indicated in yellow circle)



Marbling score = 6
Carcass weight = 384 kg
Fat % = 47.15% (M. longissimus indicated in yellow circle)

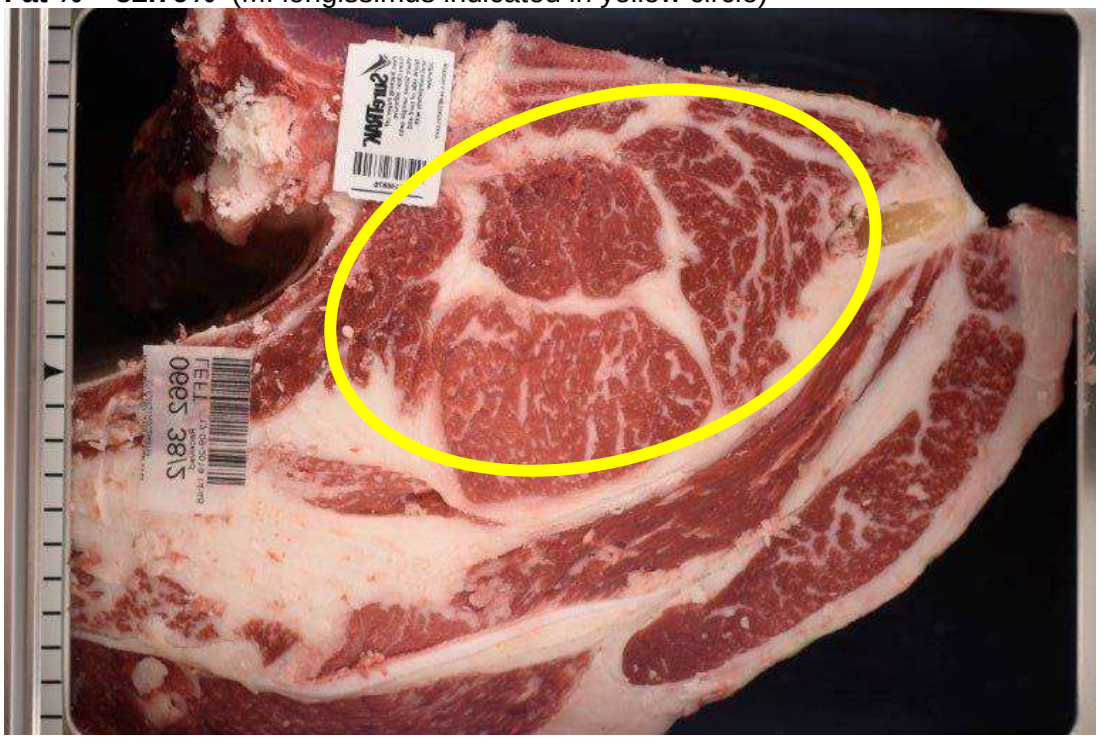


Marbling score = 9
Carcass weight = 402 kg
Fat % = 57.23% (M. longissimus indicated in yellow circle)



Marbling score = 5
Carcass weight = 414 kg
Fat % = 51.77% (M. longissimus indicated in yellow circle)

Marbling score = 6
Carcass weight = 421 kg
Fat % = 52.79% (M. longissimus indicated in yellow circle)



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