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Analysis of the trans fatty acid content of beef from major retailers in Gauteng province.

by

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Summary

The harmful health effects related to TFA intake are specifically those associated with coronary heart disease (CHD), cardiovascular disease (CVD) and related diseases (EFSA, 2010; Judd *et al*., 1994; Mensink & Katan, 1990). Intake of TFA that exceeds 5 grams per portion is associated with increased risk of CHD (Wagner *et al*., 2008). TFA originates from three different sources, the first being the partial hydrogenation of vegetable oils, and to a lesser extent fish oils, heat treatments such as deep-fat frying cooking techniques used in the fast food industry, and lastly there are the natural forms of TFA that originates as by-products from the metabolism of poly-unsaturated fatty acids (PUFA) by anaerobic bacteria in the rumen (Ratnayake & Zehaluk, 2005; Richter *et al*., 2009; Stender *et al*., 2008).

Industrial TFA content within products has been found to be as high as 60% of total fatty acid content and sometimes even higher (Stender *et al*., 2008). In comparison the ruminant derived TFA found in ruminant fat has been reported to be as high as 6% with TFA in milk fat ranging from 4% to 6% of the total fatty acid profile (Stender *et al*., 2008). Ruminant fat may contain up to 20% of the TFA content as the C16:1 *trans* isomer range, which is not found in industrial TFA profile (Fritsche & Steinhart, 1997). The most common TFA isomer from industrial origin is elaidic acid (Stender *et al*., 2008; Weggemans *et al*., 2004).

Analysis of *trans* fatty acid composition would benefit the South African red meat industry, especially at the hand of the proposed Regulations Relating to *Trans* fat in the Foodstuffs, Cosmetics and Disinfectants Act, Act 54 Of 1972 (Government Gazette, 2011), and the absence of these values for South African red meat. The aim of this study was to quantitatively determine the TFA content of South African beef at a regional level. The data obtained shows that the *trans* fatty acid content of South African beef varies between 0.2 milligram fatty acid per gram beef for C18:3t-9,t-12,t-15 and 0.17 milligram fatty acid per gram beef for C18:3c-9,t-12,c-15. Although the statistical significance was proven ($P < 0.05$), on a practical level these concentrations are with reasonable certainty practically negligible. The *trans* fatty acids that showed statistical significance have concentration values lower than the recommended benchmark values for human consumption and health. It was estimated that

CVD is the number one cause of death globally and consumption of TFA that exceeds five grams per portion is associated with increased risk of CHD (Wagner *et al*., 2008; WHO, 2008).

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TABLE OF CONTENTS

CHAPTER4: RESULTS AND DISCUSSION

CHAPTER 1: INTRODUCTION

1.1 PROJECT THEME

Meat science focusing on trans fatty acids in beef from retailers in Gauteng province.

1.2 PROJECT TITLE

Analysis of the trans fatty acid content of beef from major retailers in Gauteng province.

1.3 AIMS

The aims of this project were to:

1. Analyse and quantify the trans fatty acid content of South African beef at a regional level in the Gauteng region
2. Provide guidelines for *trans* fatty acid content values for South African beef.

1.4 MOTIVATION

Trans fatty acids (TFA) are the sum of all unsaturated fatty acids that contain one or more isolated, non-conjugated double bonds in the *trans* geometric configuration (Albuquerquea *et al*., 2011). According to the European Food Safety Authority (EFSA), TFA do not serve any vital functions in the human body owing to the fact that TFA are neither synthesised nor required by the human body. Health professionals worldwide recommend a reduction in overall consumption of saturated fatty acids (SFA), TFA and cholesterol, while increasing intake of n-3 polyunsaturated fatty acids (PUFAs) (Griel & Kris-Etherton, 2006; Kris-Etherton & Innis, 2007).

The harmful health effects related to TFA intake are specifically those associated with Coronary Heart Disease (CHD), Cardiovascular Disease (CVD) and related diseases (EFSA, 2010; Judd *et al*., 1994; Mensink & Katan, 1990). Intake of TFA that exceeds 5 grams per portion is associated with increased risk of CHD (Wagner *et al*., 2008). TFA originates from three different sources, the first being the partial hydrogenation of vegetable oils; and to a lesser extent fish oils, heat treatments such as deep-fat frying cooking techniques used in the fast food industry, and lastly there are the natural forms of TFA that originates as by-

products from the metabolism of Poly-unsaturated fatty acids (PUFA) by anaerobic bacteria in the rumen (Ratnayake & Zehaluk, 2005; Richter *et al*., 2009; Stender *et al*., 2008).

Industrial TFA content within products has been found to be as high as 60% of total fatty acid content and sometimes even higher (Stender *et al*., 2008). In comparison the ruminant derived TFA found in ruminant fat has been found to be as high as 6% with TFA in milk fat ranging from 4% to 6% of the total fatty acid profile (Stender *et al*., 2008). Ruminant fat may contain up to 20% of the TFA content as the C16:1 *trans* isomer range, which is not found in industrial TFA profile (Fritsche & Steinhart, 1997). The most common isomer being of industrial TFA origin is elaidic acid also known as C18:1, vaccenic acid C18:1t-11, rumenic acid C18:2 (Stender *et al*., 2008; Weggemans *et al*., 2004).

Vaccenic acid and rumenic acid are the *trans* isomers that are specific to ruminant derived fats (Albuquerquea *et al*., 2011). It has been postulated that ruminant derived *trans* vaccenic acid may not be associated with an increased risk of CHD or CVD. This is due the conversion of *trans* vaccenic acid to the CLA isomers which is known for numerous health benefits (Huth, P.J., 2007; Turpeinen *et al*., 2002). *Trans* unsaturated fatty acids when consumed in excessively large amounts cause elevated plasma LDL cholesterol and a reduced HDL Cholesterol state which is associated with CVD, CHD and Diabetes (Katan, 2000; Zock & Mensink, 1996). Little information is available on *trans* fatty acid content in South Africanbeef as well as the factors that influence the *trans* fatty acids levels in beef under South African conditions.

Analysis of *trans* fatty acid composition of beef would benefit the South African meat industry, especially at the hand of the proposed Regulations Relating to *Trans* fat in the Foodstuffs, Cosmetics and Disinfectants Act, Act 54 Of 1972 (Government Gazette, 2011), and the absence of these values for South African red meat. The aim of this study was to quantitatively demonstrate the TFA content of South African beef at a regional level. The data obtained will be used to provide guidelines for South African beef.

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CHAPTER 2: LITERATURE REVIEW

2.1 Introduction

Trans fatty acids (TFA) are the sum of all unsaturated fatty acids that contain one or more isolated, non-conjugated double bonds in the *trans* geometric configuration (Albuquerquea *et al*., 2011). According to the European Food Safety Authority (EFSA), TFA do not serve any vital functions in the human body owing to the fact that TFA are neither synthesised nor required by the human body. Health professionals worldwide recommend a reduction in overall consumption of saturated fatty acids (SFA), TFA and cholesterol, while increasing intake of *n-3* polyunsaturated fatty acids (PUFAs) (Griel & Kris-Etherton, 2006; Kris-Etherton & Innis, 2007). Thus no Population Reference Intake (PRI), Average Requirement (AR) or Adequate Intake (AI) value have been established for TFA (EFSA, 2010). The damaging health effects related to TFA intake are specifically those associated with Coronary Heart Disease (CHD), Cardiovascular Disease (CVD) and related diseases (Mensink & Katan, 1990; Judd *et al*., 1994; EFSA, 2010). It was estimated that CVD is the number one cause of death globally and consumption of TFA that exceeds five grams per portion is associated with increased risk of CHD (Wagner *et al*., 2008; WHO, 2008).

There is relatively poor information on TFA in livestock as well as the specific factors that influence these TFA levels in beef under South African conditions. The factors of most concern are species, feeding systems, and hormonal growth promoters and feed additives. We do know that there are differences among these factors; yet the extent to which these factors play a role remains an area of uncertainty. It is speculated that of these factors, feeding systems in terms of intensive feedlot versus extensive grazing production systems play the most important role in *trans* fat content (Webb & Erasmus, 2013).

There is a lot of debate about effects of different production systems on the final product in terms of meat quality characteristic (Webb & Erasmus, 2013). Analysis of TFA composition would benefit the South African red meat industry, especially at the hand of the proposed Regulations Relating to *Trans* fat in the Foodstuffs, Cosmetics and Disinfectants Act, Act 54 Of 1972 (Government Gazette, 2011). This review explores the origins, values and importance of distinguishing between the sources of TFA isomers and what it means for human health and heart disease. Furthermore legislation and labelling of TFA content and declaration on food labels are

discussed. Factors such as feeding systems, hormonal growth promoters, feed additives as well as species, and their effect on TFA are also reviewed.

2.2 TFA isomers and origins

The most important *trans* isomers in human biology are the mono-unsaturated and di-unsaturated fatty acids which contain sixteen and eighteen carbon atoms. That is C16:1 *trans*, C18:1 *trans* and C18:2 *trans* fatty acids (Koletzko & Decsi, 1997). C18:1 *trans* isomers encompass 80-90% of total TFA content in foods (Steinhart *et al*., 2003b). The importance of different tertiary structures relay to the different crystalline packing which ultimately result in differing melting points of these fatty acids (Koletzko & Decsi, 1997). The *trans* isomeric fatty acids have much greater melting points than their corresponding *cis* isomeric fatty acids. The melting point of Oleic acid (C18:1 *cis*) is 10-11°C whereas the corresponding *trans* isomer, Elaidic acid (C18:1 *trans*) has a melting point of 44.5-45.5°C (Koletzko & Decsi, 1997).

2.2.1 Conjugated Linoleic Acid (CLA)

Conjugated Linoleic Acid (CLA) is not included in the definition of TFA as it has conjugated double bonds (EFSA, 2010). CLA is a collective term used for all conjugated geometric and positional isomers of C18:2 (Linoleic acid) (Yurawecza *et al*., 1998; Schmid *et al*., 2006). CLA is abundant in meat and milk derived from ruminants which have antioxidant and anti-carcinogenic properties (McDonald, 2000). These CLA isomers are formed during bacterial isomerisation and biohydrogenation of 18 carbon PUFAs in the rumen; primarily Linoleic acid and alpha-Linolenic acid, as well as the desaturation of *trans* fatty acids within the adipose tissue and mammary gland tissue of ruminants (Lock & Bauman, 2004).

Conjugated Linoleic Acid (CLA) became of interest when it was found to inhibit the initiation of mouse epidermal tumours and was identified as conjugated linoleic acid (Ha *et al*., 1987). CLA has also been associated with affirmative effects against diseases such as cancers, cardiovascular diseases, diabetes, body composition, immune system and bone health (Pariza, 2004). Although much of the data available on CLA are from trials involving animal subjects and not humans, the findings thus far are encouraging (Schmid *et al*., 2006). The major isomer of the CLA group is C18:2 *c9,t11* also known as Rumenic acid and comprises about 90% of the total CLA isomer range

(Yurawecza *et al*., 1998; Steinhart *et al*., 2003a). Other isomers include *c10,t12/ t10,c12/ c11,c13/ c9/c11/ c10,c12/t9,t11/ t10,t12* (Ha *et al*., 1989). A new isomer (C18:2 *t7,c9*) has been detected in milk, cheese, beef, human milk as well as human adipose tissue (Yurawecza *et al*., 1998).

TFA, including CLA, act as metabolic modifiers of lipid metabolism and it was found that moderately obese humans who consumed more than 3.4 grams CLA per day had a significant decrease in body fat mass (Blankson *et al*., 2000). These results have also been found in experiments with pigs (Ostrowska *et al*., 1999), hamsters (Gavino *et al*., 2000) and cows (Griinari *et al*., 1998). The *c9,t11* and *t10,c12* CLA isomers are dominant in synthetic preparations of CLA and are often found to be in a 1:1 ratio (Larsen *et al*., 2003). This is not the same for naturally occurring CLA isomers found in beef and dairy products where about 80% of the isomers are in the *c9,t11* isomer conformation (Fritsche & Steinhardt, 1998). Ruminant derived meat such as beef and lamb has much higher concentrations of CLA relative to non-ruminant derived meat such as pork and chicken. The highest concentrations have been found in lamb at 19.0 mg per g lipid (Knight *et al*., 2004) and beef at 10.0 mg per g lipid (Raes *et al*., 2004).

2.2.2 Origin of trans fatty acids in ruminants

TFA originates from three different sources, the first being the partial hydrogenation of vegetable oils; and to a lesser extent fish oils, heat treatments such as deep-fat frying cooking techniques used in the fast food industry, and lastly there are the natural forms of TFA that originates as by-products from the metabolism of polyunsaturated fatty acids (PUFAs) by anaerobic bacteria in the rumen (Ratnayake & Zehaluk, 2005; Stender *et al*., 2008; Richter *et al*., 2009). Unsaturated fats have a natural tendency to become oxidised over time by the absorption of oxygen, thus leading to the production of hydroperoxides which makes the product rancid (Albuquerquea *et al*., 2011).

2.2.3 Industrial trans fatty acids

Partial hydrogenation of vegetable oils gained popularity during the 1950's in the United States of America due to the process allowing for the creation of cheaper margarines and shortenings with longer shelf lives as well as improved organoleptic properties, texture and firmness (De Roos *et al*., 2001 ; Tarrago-Trani *et al*., 2006). Partial hydrogenation of vegetable oils

involves a process where hydrogen gas is bubbled through the oil at high temperatures in the presence of a metal catalyst such as Nickel (Priego-Capote *et al*., 2007). This partial saturation of unsaturated fatty acids allows for a reduction in the amount of double bonds occurring in the fatty acid chain and thus preventing and significantly slowing down rancidity from occurring (Albuquerquea *et al*., 2011). The reaction parameters can be manipulated in such a manner as to reduce the degree of hydrogenation and as a result the amount of *trans* double bonds that will ultimately develop (De Roos *et al*., 2001). These parameters include the pressure of the hydrogen gas, the temperature and time at which the processes occur and the concentration of the metal catalyst (Albuquerquea *et al*., 2011).

2.3 Industrial derived trans fatty acids opposed to ruminant derived trans fatty acids

Bacterial biohydrogenation and industrial hydrogenation results in a multiplicity of geometrical and positional TFA isomers, the C18:1 *trans* isomers predominating (Weggemans *et al*., 2004). Low levels of C18:2 *trans* and C18:3 *trans* are also present (Ovesen *et al*., 1996; Precht & Molkentin, 2000). Ruminant and industrial fats contain exactly the same C18:1 *trans* isomers but in different proportions (Weggemans *et al*., 2004). The profile of either sources of TFA depend largely on the hydrogenation conditions such as rumen pH and diet type for ruminant biohydrogenation or catalyst type and temperature in the case of industrial hydrogenation (Weggemans *et al*., 2004).

2.3.1 The origin and purpose of industrial produced trans fatty acids

Industrial TFA are produced during partial hydrogenation of vegetable fats and fish oils and are common to products such as margarines, spreads and shortenings, baked goods and deep fried foods such as those in fast food outlets (Ascherio *et al*., 1999; Stender *et al*., 2008). According to the Foodstuffs, Cosmetics and Disinfectants Act, Act 54 of 1972 (Government Gazette, 2011), industrially produced TFA are all the geometrical isomers of monounsaturated fatty acids (MUFAs) with one *trans* double bond, i.e. C16:1, C18:1, C20:1, C22:1 and PUFAs with one or more *trans* double bonds, i.e. C18:2, C18:3, C20:2 and C22:2 having non-conjugated carbon-carbon double bonds in the trans configuration and that are interrupted by at least one methylene group (Government Gazette, 2011). This definition excludes natural TFA.

Vegetable shortenings are used mainly in ready-to-eat foods and in deep-fat frying procedures (Katan, 2000). Industrial hydrogenation of vegetable oils is performed under relatively high pressures in the presence of a metal catalyst with the temperature being as high as 400°C (Stender *et al*., 2008). Industrial TFA content within products has been found to be as high as 60% of total fatty acid content and sometimes even higher (Stender *et al*., 2008). In comparison, the ruminant derived TFA found in ruminant fat has been found to be as high as 6% with TFA in milk fat ranging from 4% to 6% of the total fatty acid profile (Stender *et al*., 2008). Ruminant fat may contain up to 20% of the TFA content as the C16:1 *trans* isomer range, which is not found in industrial TFA profile (Fritsche & Steinhart, 1997). Thus C16:1 *trans* as well as butyric acid can be used as markers for ruminant derived TFA when analysing mixtures of ruminant and industrial TFA (Stender *et al*., 2008). It should be noted that in some instances C16:1 *trans* isomers are present in partial hydrogenated fish oils (Stender *et al*., 2008). The concentrations of TFA in ruminant products are subject to change due to changes in the animals' feed as well as seasonal changes (Jakobsen *et al*., 2006). This variation on a gram per serving is significantly lower relative to the variations observed for TFA from industrial sources (Jakobsen *et al*., 2006). The most common *trans* isomer being of industrial origin is Elaidic acid (C18:1*t-9*), meaning the *trans* double bond is at the ninth carbon (Weggemans *et al*., 2004; Stender *et al*., 2008).

2.3.2 Ruminant derived trans fatty acids and the impact on human health

Vaccenic acid and Rumenic acid are the *trans* isomers that are specific to ruminant and dairy fat (Albuquerquea *et al*., 2011). Vaccenic acid (C18:1*t-11*) accounts for 50-80% of the total unsaturated fatty acids found in ruminant derived fats and is the product of biohydrogenation of C18:2*n-6* (Lock & Bauman, 2004; Weggemans *et al*., 2004; Stender *et al*., 2008; Wood *et al*., 2008). Humans and rodents have the ability to desaturate vaccenic acid to the *c-9,t-11* CLA isomer (Turpeinen *et al*., 2002).

It has been postulated that ruminant derived *trans* vaccenic acid may not be associated with an increased risk of CHD or CVD. This is due the conversion of *trans* vaccenic acid to the CLA isomers which is known for many health benefits (Turpeinen *et al*., 2002; Huth, 2007). Within adipose tissues vaccenic acid is converted to CLA by the Stearoyl-CoA desaturase enzyme (Wood *et al*., 2008). This enzyme is also responsible for converting oleic acid (C18:1*c-9*) to stearic acid

(C18:0). It is interesting to note that oleic acid, vaccenic acid as well as CLA are found in much higher concentrations in neutral lipids and adipose tissue relative to phospholipids and muscle tissues (Wood *et al*., 2008). It was believed that oleic acid is converted directly to stearic acid but it was found that oleic acid is in fact converted to a multitude of *trans* C18:1 positional isomers. *Trans* C18:1 isomers serve as precursors for the synthesis of SFA within the rumen as well as CLA synthesis at tissues level such as in the mammary tissues (Mosley *et al*., 2002). Bioactivity of vaccenic acid *per se* and how it could potentially affect chronic diseases in humans are not clear (Gebauer *et al*., 2011).

TFA from ruminants are believed to have no effect on heart health as with those found from industrially produced TFA. Motard-Bélanger *et al*. (2008) conducted a study where the effects of ruminant TFA were compared to industrial TFA on a gram for gram basis and how it influenced plasma LDL concentrations as well as other cardiovascular disease risk factors in healthy subjects. It should be noted that the 38 participants were aged 32 to 47 and were healthy non-smokers. The study design was a controlled double blind randomized crossover design with three diets representing a high TFA intake (10.2 grams per 2500kcal), moderate TFA intake (4.2 g per 2500kcal) and a control diet (2.2 g per 2500kcal). The results of this study concluded that high dietary intakes of TFA from ruminant origin may adversely affect cholesterol homeostasis whilst moderate intakes of ruminant derived TFA that are well above the upper limit of human consumption have neutral effects on cholesterol homeostasis.

Results from four epidemiological studies suggest that ruminant derived TFA have less detrimental effects on heart health (Willett *et al*., 1993; Ascherio *et al*., 1994; Kuhlsen *et al*., 2005; Sun *et al*., 2007;) while opposing results from two epidemiological studies showed ruminant TFA have the same negative health effects as those caused by industrial TFA (Oomen *et al*., 2001; Clifton *et al*., 2004). It should be noted that the study designs as well as experimental approaches from these opposing studies are different.

2.4 The nutrient composition of red meat

Red meat has long been identified erroneously as being high in fat, especially SFA which can be associated with many human diseases, but in truth lean meat only contains 2-3% fat (Williams,

2000; Nuernberg *et al*., 2005). The predominant SFA are C14:0 (myristic acid), C16:0 (palmitic acid) and C18:0 (stearic acid) (Scollan *et al*., 2006). It is also important to take note of the importance of animal fat in terms of being a concentrated source of energy, provides flavour, aroma and texture in meat, serves as a carrier of fat soluble vitamins A, D, E and K as well as essential fatty acids, it is also important for growth and maintenance of vital body functions (Nuernberg *et al*., 2005). The lean component of red meat is considered to be a relatively high biological value protein source which is highly digestible (Williams, 2007). The digestibility values of red meat, beans and whole wheat are 94%, 78% and 86% respectively (Williams, 2007). Red meat is relatively low in fat and sodium, high in antioxidants and contains many bioactive substances such as taurine, carnitine, carnosine, ubiquinone, glutathione and creatine (Williams, 2007).

Lean red meat is an excellent source of long chain omega-3 PUFAs, vitamins B12 and B6, vitamin D, niacin, riboflavin, pantothenic acid, selenium, iron, zinc and phosphorus. A 100 gram sample of lean red meat provides more than 25% of the recommended daily intake (RDI) value of all the above mentioned nutrients (National Health and Medical Research Council, 2006). Mutton is extremely nutrient dense and is the richest source of thiamin, vitamins B6 and B12, phosphorus, iron and zinc (Williams, 2007). There are no limiting amino acids in the profile of red meat and it provides lysine, threonine, methionine, phenylalanine, tryptophan, leucine, isoleucine and valine (Williams, 2007). The *trans* fatty acid content in beef ranges from 0.01-0.21 gram per serving which is 5% less than the content in beef fat (Ratnayake & Zehaluk, 2005). Grass finished beef have relatively greater proportions of cholesterol-neutral C18:0 fatty acids and lesser proportions of cholesterol-lowering SFA such as C14:0 and C16:0 (Daley *et al*., 2010).

Droulez *et al*. (2006), put forward a nutrient profile of Australian meat. The following red meat fatty acid composition data from Australia is based on predominantly grass fed animals which has less SFA and more n-3 PUFAs relative to grain fed animals (Marmer *et al*., 1984). The objective was to determine the fatty acid content of red meat available for purchase in Australia. Samples of beef, lamb, veal and mutton cuts were purchased at random from ten retail outlets. The sample size of ten purchases per cut is consistent with previous studies conducted with red meat (Sadler *et al*., 1993). The results obtained indicated that the lean component of all the

respective meat samples has less than five grams total fatty acids per 100 gram edible portion with veal containing less than 1.5 grams total fatty acids per 100 gram edible portion.

The SFA content comprised approximately 40% and 48% of total fatty acids in the lean component and fat component respectively. Overall the SFA content was found to be less than two grams per 100 gram edible portion in the lean component of raw red meat. The PUFA in the lean components ranged from 14-24% of the total fatty acid profile. It was also found that the CLA content as well as TFA content is mainly concentrated in the fat component of red meat while the lean component contains less than 50 milligrams per 100 gram edible portions. The C18:1 *trans* fatty acid content of the lean component of red meat cuts were found to be less than 3% of the total fatty acid profile. Lean meat provides a heart healthy protein source with possible healthy TFA profiles (Williams, 2007; Motard-Bélanger *et al*., 2008).

2.5 The health implications of trans fatty acids in humans

Nearly all lipid containing foods in the Western diets contribute to n-6 PUFA and alpha-Linolenic acid intake while only meat, eggs and seafood contribute to beneficial n-3 PUFA intake in the human diet (Meyer *et al*., 2003). Results of published epidemiological studies are conflicting with respect to TFA and its effect on blood cholesterol homeostasis (Willett *et al*., 1993; Ascherio *et al*., 1994; Aro, 2001; Oomen *et al*., 2001; Clifton *et al*., 2004; Kuhlsen *et al*., 2005; Sun *et al*., 2007). Industrial TFA are usually used in dietary trials and the assumptions are that *trans* isomers have similar metabolic effects regardless of the origins. Yet the isomer profile of industrial TFA and ruminant TFA are in fact very different. *Trans* vaccenic acid (C18:1*t-11*) is specific to the ruminant TFA profile while elaidic acid (C18:1*t-9*) is specific to the industrial TFA profiles (Parodi, 1976; Aro *et al*., 1998). *Trans* vaccenic acid is desaturated to *c-9,t-11* CLA by delta-9 desaturase enzyme (Turpeinen *et al*., 2002). This is not true for neither t*rans* elaidic acid nor C18:0,*t10* (Salminen *et al*., 1998; Griinari *et al*., 2000).

2.5.1 Epidemiological studies on the effects of trans fatty acids

Trans unsaturated fatty acids when consumed in excessively large amounts cause elevated plasma LDL cholesterol and a reduced HDL cholesterol state which is associated with CVD, CHD and diabetes (Zock & Mensink, 1996; Katan, 2000). Most dietary intervention studies that have

shown the negative relation between TFA consumption and heart disease made use of industrial TFA sources such as enriched margarines to analyse the effects of TFA on cholesterol homeostasis and not a ruminant derived TFA product (Allison *et al*., 1999; Wagner *et al*., 2008). In an eighteen year follow-up study which consisted of 3686 Danes aged 30-71 years it was found that there was no association between absolute or energy adjusted ruminant TFA intakes and increased risk of CHD (Jakobsen *et al*., 2008). The participants were at baseline with no records of previous CHD incidences and it was also found that in female participants an inverse association of ruminant TFA consumption and risk of CHD existed, thus indicating a protective mechanism. The conclusions reached in this study stated that ruminant TFA intake is not associated with higher risk of CHD and that TFA intake from dairy and read meat products is an issue of no concern to public health (Jakobsen *et al*., 2008).

It is commendable to note that ruminant TFA cannot be removed from ruminant fat prior to marketing and the fact that it has been part of the human diet for millennia makes it certain to say that TFA from red meat and related products are of no concern to human health when consumed in moderation (Stender *et al*., 2008). This is in agreement with results from other studies (Enser *et al*., 1998; Weggemans *et al*., 2004). In the Nurses' health study it was showed that an inverse relationship existed between ruminant TFA intake and CHD risk but the results were statistically non-significant. It was however showed with significance that a positive relation existed between industrial TFA intake and increased CHD risk (Willett *et al*., 1993).

While reducing TFA intake levels it is likely that an inverse increase in SFA will occurs and thus it is recommended that people do not consume high fat processed foods on a daily basis (Harrington, 1994). The effects of consuming TFA are less favourable when compared to the effects of consuming SFA (Ratnayake & Zehaluk, 2005). De Roos *et al*. (2001), investigated cholesterol homeostasis when replacing a margarine rich in TFA with one rich in SFA. Thirty two non-smoking individuals were used with a mean age of 30 years. Two controlled diets were used for four weeks each in a randomized crossover design. Both diets consisted of the same conventional food items as the background diets with the only differences being the supplemental margarines. One of the margarines was rich in TFA while the other was rich in the saturated lauric acid (C12:0). The results showed that the TFA rich diet produced an LDL/HDL ratio of 2.2 while that

of the SFA rich diet was 1.8. It must be stressed that the authors do not encourage the consumption of either TFA or SFA rich diets but suggest that when products require solid fats for texture or firmness, the replacement of TFA with solid tropical fats rich in lauric acid appears to be more favourable. It has also been recommended that when replacing partial hydrogenated vegetable oil in processed foods that instead coconut oil and other palm kernel oils high in SFA are used (Harrington, 1994).

2.5.2 Guidelines for trans fatty acid consumption

Intake of TFA that exceeds five grams per portion is associated with increased risk of CHD (Wagner *et al*., 2008). One large portion of French fries together with a hamburger, each weighing 200 grams, can provide from 0.2 grams up to more than five grams of TFA in a single serving, which is more than the current RDA values which recommend an intake value of less than 1% of total energy i.e. 2.5-3 grams per day (Wagner *et al*., 2008). Additional factors that collectively contribute to CHD risk are smoking, hypercholesterolemia, hypertension, physical inactivity and obesity (Stender *et al*., 2008).

2.6 Recommendations and First world country developments

Recommendations from health professionals vary from eliminating all fats from the diet to a moderate consumption of fats due to importance it has in the human body (Webb & O'Neill, 2008). Currently health professionals worldwide recommend a reduction in overall consumption of SFA, TFA and cholesterol, while increasing intake of n-3 PUFAs and CLA and beef is an excellent source of these fatty acids (Griel & Kris-Etherton, 2006; Kris-Etherton & Innis, 2007; Warren *et al*., 2008). A low n-6/n-3 PUFA ratio is desirable in terms of reducing the risks associated with many chronic diseases (Nuernberg *et al*., 2005). Results from epidemiological studies and controlled clinical trials demonstrate that by replacing SFA and TFA with UFA, especially n-3 PUFAs, the risk of CHD can effectively be reduced. This strategy is more effective than simply reducing total fat consumption (Hu & Willet, 2002; Renaud & Lanzmann-Petithory, 2002; Sanders, 2003).

It is recommended that people maintain their TFA intake to a minimum and if possible none at all (National Heart Foundation Australia, 2009). There are no nutritional benefits from TFA in the human diet (Willett & Ascherio, 1994). Various health institutions and organisations have

given dietary recommendations for TFA intake, most of which recommend the intake of TFA remain less than 1% of total energy intake or as low as possible (Hunter, 2006).

2.6.1 The Danish TRANSFAIR study

During the 1995 and 1996 TRANSFAIR study it was established that high levels of TFA were found in certain industrial fats and processed foods such as French fries, pastries, biscuits, wafers, fried meats, fast foods, microwave popcorn and soup powders (EFSA, 2004). Unsaturated vegetable oils do not provide the desired textures in Danish pastries, croissants and wafers neither does it provide stability of fats used in biscuits and repeated deep frying (Harrington, 1994). The Danish population had a TFA Daily Intake value (%DV) of more than five grams per day prior to 2003 (Stender *et al*., 2006). This level of intake raised concerns amongst health professionals and as a result parliament was pressured to pass legislation. In March 2003 the Danish government banned the sale of all food containing more than 2% industrially produced TFA (Stender & Dyerberg, 2003; Stender *et al*., 2006). Producers of table margarines were the most responsive to the pressure of passed legislation. Consequently intake of industrial TFA was virtually eliminated in Denmark, without any nutritional or economic side effects (Leth *et al*., 1998).

2.6.2 The Canadian example in trans fatty acid regulations

In January 2003 the Canadian government mandated labelling of TFA on food labels (Friesen & Innis, 2006). Industrially produced TFA in human milk raised concerns due to possible adverse effects on infant growth and development. Since then, the total TFA in human milk significantly decreased from approximately 7.1 grams per 100 grams fatty acids in 1998 to approximately 4.6 grams per 100 grams fatty acids in 2006 (Friesen & Innis, 2006).

2.6.3 Proposed regulations on TFA food labelling by the FDA

During 1999, the FDA proposed a ruling for TFA labelling on food and dietary supplements only when the TFA content equated to or more than 0.5 grams per serving (DHHS/FDA, 1999). July 2003 the final ruling was passed for mandatory declaration of TFA content on food labels by January 2006 (DHHS/FDA, 2003). It was ruled that the TFA content should appear on the food item label on a separate line right underneath the Saturated fat declaration (DHHS/FDA, 2003). Declaration of TFA should be expressed in grams per serving to the nearest half gram increment

below five grams as well as to the nearest gram increment above five grams (Mossaba *et al*., 2009). No Daily Value (%DV) for TFA have been established due to no scientific basis being established as of yet (Moss, 2006). The %DV is a reference value which aids the consumer in using food label information to plan a healthy diet (Mossaba *et al*., 2009).

In the Foodstuffs, Cosmetics and Disinfectants Act, Act 54 of 1972 it is stated that foods containing more than 2 g per 100 g of oil or fat are prohibited and "*trans* fat free" claims are for foods that contain less than (or equal to) 0.01 g per 100 g of total fat or oil in the final product foodstuff (Government Gazette, 2011).

2.6.4 Industry response to proposed trans fatty acids regulations

Since TFA labelling regulations were issued it has been found that the food industry has been attempting to reduce dietary TFA as well as reformulating food products in an attempt to reduce or eliminate the TFA levels. There is an increased demand for validated quantification methods of total TFA in foods and dietary supplements which are rapid, accurate, reproducible and sensitive (Mossaba *et al*., 2009). Methods involved in TFA analysis is beyond the scope of this paper but it should be mentioned that current official GC methods for TFA determination requires 100m fused capillary columns, coated with 100% cyanopropyl polysiloxane stationary phases using hydrogen gas as the carrier gas (Mossaba *et al*., 2009). Currently 100m SP 2560, 100m CPSil 88 and 100m Rtx 2560 are the only columns that meet the requirements for maximum TFA separation (Mossaba *et al*., 2009). According to Mossaba *et al*. (2009), the Attenuated Total reflection- Fourier transform infrared spectroscopy (ATR-FTIR) method, with respect to food labelling, is viable, rapid, and a complementary method to gas chromatography and can be used to quickly determine total TFA for food labelling purposes.

2.7 The labelling of food products and the consumer's perceptions

For the purpose of nutrition labelling, *trans* fats are defined as the sum of all unsaturated fatty acids with one or more isolated double bonds in the *trans* configuration. According to the FDA this definition excludes CLA (Mossaba *et al*., 2009). This definition defines TFA by their chemical structure regardless of origin (Mossaba *et al*., 2009). The policy put forward by the FDA is one that states that the nutritive values for labelling be based on product composition as

determined by laboratory analysis of each unit. But the FDA does not specify the required methodology to be used when analysing the TFA content in foods (Mossaba *et al*., 2009).

2.7.1 The challenges and perceptions of food labels

The concerned and modern consumer is mostly one belonging to the developed first world countries and concerns himself with human health, modern production systems, transport and slaughter methods as well as animal welfare (Harrington, 1994). The "Western diet" is one that has been characterised by too high fat, especially saturated fats, sugar and salt while being too low in fresh fruit, vegetables and complex carbohydrates (Harrington, 1994). It has been found that consumers have substantial difficulties in forming clear meat-quality expectations (Bernue´s *et al*., 2003). The lack of consumer orientated information with respect to meat labelling as well as the type of information requested by the consumer remains a challenge (Bernue´s *et al*., 2003). Meat labelling is a practice that can restore consumer confidence in red meat and related products (Corcoran *et al*., 2001). Some challenges that are presented with red meat labelling involves the fact that red meat is naturally variable, difficulties in delivery of consistent quality, meat is usually sold in small quantities at retail level as well as pre-packing practices by the retailer or butchers (Verbeke & Viaene, 1999). Another challenge that faces labelling of red meat is one that lies with the consumer. It was established that consumers do not know how to use food labels or lack of understanding food labels (Capps, 1992).

2.7.2 Research demonstrating the consumers label prefernces

Bernue´s *et al*. (2003) conducted a study with the aim of understanding the labelling information demanded by European red meat consumers. It was found that mandatory data requested by the average consumer was most importantly information on the "Consume by" date and the origin of the meat. The least important information requested was the brand name and cooking instructions. Amongst Italian and French consumers the most important information requested was the system of production, traceability, system of quality assurance and the meat cut name. These were requested above information such as nutrition data as well as time of maturation of the meat. Scottish and English consumers expressed lower interests in labelling information and were more concerned with origin of beef. Spanish consumers requested maturation period, nutritive information, origin as well as consume by data. The authors

concluded that in order of importance the information requested by the average European consumer are origin of meat, consume by date, system of production, traceability of animals and products and last but not least quality assurance systems in place. The authors recommended that there is a need to integrate meat labelling and marketing to produce a consistent product image.

2.8 Factors that influence the *trans* fatty acid profile in red meat

2.8.1 Nutritional and physiological background on muscle nutrition

Much consideration has been given to meat quality obtainable from feedlot fattened opposed to grass fattened livestock and it is worthy to note that much of this debate is encouraged by the industries involved, which make use of the opportunity to promote the concept of branded meat products (Webb & Erasmus, 2013). As a result of the fatty acid imbalance in human diets, dietary strategies have been employed to enhance the nutritional and health value of the intramuscular fat of cattle (Alfaia *et al*., 2009).

Nutritional value is determined primarily by the ratio of PUFA/SFA; taken as (C18:2 + C18:3)/(C14:0 + C16:0 + C18:0), as well as the *n-6/n-3* ratio in meat and the ideal would be a reduced SFA content and increased PUFA and CLA content (Scollan *et al*., 2001; Warren *et al*., 2008) The PUFA/SFA (P:S) as well as the *n-6/n-3* fatty acid ratios are primary values used to measure the degree of nutritional quality of meat and are ratios used for human nutrition (Warren *et al*., 2008). In human nutrition these values have benchmark values to indicate a healthy lifestyle that contests various CHD and associated diseases. These recommended benchmark values are > 0.7 for the P:S ratio and < 5 for the *n-6/n-3* ratio (Raes *et al*., 2004).

Neutral lipids also known as triglycerides are the major lipid class in adipose tissue while phospholipids are the major lipid class in muscle tissue. Phospholipids contain much higher levels of PUFA and lower SFA content for the subsequent function as a constituent of cellular membranes (Webb *et al*., 1998; Wood *et al*., 2008). C18:1*c-9* (Oleic acid) is formed from C18:0 (Stearic acid) by Stearoyl CoA desaturase and is a major component of neutral lipids (Wood *et al*., 2008). In ruminant animals, lipids accumulate mainly as triglycerides within adipocytes which are located in subcutaneous, intermuscular and intramuscular adipose tissue as well as abdominal fat depots such as omental and perirenal adipose (Zervas & Tsiplakou, 2011). Dietary *n-6* and *n-3*

PUFA is incorporated into adipose and muscle tissues despite rumen biohydrogenation. Ruminant animals preferentially incorporate essential fatty acids into muscle tissues rather than into adipose tissue (Wood *et al*., 2008).

Intramuscular fat consists predominantly of triacylglycerols and phospholipids, and it is the triacylglycerols that are deposited in the adipocytes that serve as a concentrated energy source for the body (Scollan *et al*., 2006). In ruminant animals the fatty acids obtained in the triacylglycerols are influenced by the diet while the PUFAs located in the phospholipids are much higher than that located in triacylglycerols while being less influenced by diet. The PUFAs in phospholipids consist of essential fatty acids such as C18:2*n-6* and C18:3*n-3* as well as their long chain derivatives C20:5*n-3* Ecosapentaenoic acid (EPA) and C22:6*n-3* Decosahexaenoic acid (DHA) (Scollan *et al*., 2006). The fatty acid composition determines the firmness and oiliness of adipose tissue as well as the oxidative stability of muscle tissues, which influences the flavour and colour of the meat product (Wood *et al*., 2008).

2.8.2 The effect of production systems on trans fatty acid content in beef

The production system or feeding systems used to raise livestock has a marked influence on the animal's growth rate, age at slaughter, carcass weight, carcass fat and fatty acid content, dressing percentage, muscle to fat ratio, lipid profile, oxidative stability, fat thickness and organoleptic properties such as meat and fat colour and flavour which ultimately affects meat quality (Carrasco *et al*., 2009; Frylinck *et al*., 2013). Different combinations of pasture-based systems together with intensive systems are used to raise livestock and the degree to which these are used largely rely on resources available as well as climatological conditions (Webb & Erasmus, 2013). In South Africa, production systems are largely dependent on the environmental and economic conditions and subsequent changes from extensive to intensive finishing are employed prior to marketing. Feedlot systems have the beneficial advantage of achieving high growth rates which yield acceptable carcasses at a younger age than those from extensive production systems and this is mainly due to feed resources being used with great efficiency following meticulous formulating and mixing (Webb & Erasmus, 2013).

2.8.3 Feedlot finished cattle and the beef nutrient profile

Due to the definite need for increased *n-3* fatty acids in the human diet, it has become of interest to increase the alpha-linolenic acid (C18:3*n-3*) content in meat with the feeding of grass and pasture (Warren *et al*., 2008). This fatty acid is converted by the desaturase and elongase enzymes into the desired long chain *n-3* PUFAs such as EPA and DHA (Warren *et al*., 2008). This is not true for concentrate based diets (grains and protein supplements) which are high in linoleic acid (C18:2*n-6*), the precursor of the *n-6* PUFAs (Warren *et al*., 2008). Plants have the unique ability to synthesise *de novo n-3* PUFAs, which serve as the building blocks of the *n-3* series of essential fatty acids (Scollan *et al*., 2006). Cattle are frequently fattened on concentrate diets and as a result produce meat with unfavourable *n-6/n-3* ratios due to concentrates being high in C18:2*n-6* (Nuernberg *et al*., 2005). The opposite is true for forages that are high in C18:3*n-3* which enhances the *n-6/n-3* ratio in meat (Scollan *et al*., 2001). Meat from cattle finished on concentrate feed typically has higher intramuscular fat than that from pasture finished cattle and as a result also has higher levels of triacylglycerols relative to phospholipids and lower levels of PUFA together with higher levels of SFA and MUFA (Wood *et al*., 2008).

2.8.4 Pasture finished cattle and the beef nutrient profile

Pasture feeding has become favourable with regard to the positive effects it has on the fatty acid profile on subsequent meat produced even though it is at the expense of increased growth rate (Webb & O'Neill, 2008). A lower *n-6/n-3* ratio is obtained when grass and pasture feeding is employed (Wood et al., 2008). Grass fed animals produce meat products that exhibit better oxidative stability due to higher concentrations of natural antioxidants such as vitamin E, which stabilises the PUFAs (Gatellier *et al*., 2004; Wood *et al*., 2008).

2.8.5 Specific fatty acids from production systems and their role in trans fatty acid beef content

2.8.5.1 Alpha-linolenic acid, the dominant fatty acid in pasture

C18:3*n-3* (alpha-linolenic acid) is the major fatty acid found in grasses. A higher level of this fatty acid is found within muscle tissue compared to adipose tissues. It is an essential fatty acid but does not compete well for insertion into phospholipid when compared to C18:2*n-6*. A combination of greater biohydrogenation together with a long rumen transit time for forages limits the amount of this fatty acid available for tissue uptake relative to C18:2*n-6* (Wood *et al*., 2008). CLA concentrations in meat are determined by factors such as season, animal genetics and production

system while the most important factor being diet. This is due to the substrates the diet provides for CLA formation (Schmid *et al*., 2006). Higher CLA concentrations found in muscle are associated with higher intramuscular fat content (Raes *et al*., 2004). To increase the concentrations of CLA in meat, strategies such as feeding pasture based diets are used as well as using various oilseeds high in PUFA, such as sunflower seeds, linseeds and safflower seeds. The increased CLA in meat from pasture grazed animals are attributed to the high *n-3* PUFA (especially C18:3*n-3*) content characteristic of grass (De La Torre *et al*., 2006; Schmid *et al*., 2006).

2.8.5.2 Linoleic acid, the dominant fatty acid in concentrate feed

C18:2*n-6* (Linoleic acid) is a major fatty acid in concentrate feeds (grains and oilseeds) of all species. It is degraded into MUFAs and SFA within the rumen. Its incorporation into adipose and muscle tissue in relation to quantity in the diet is relatively greater than for any other fatty acids. It is deposited into muscle phospholipids at a higher rate (relative to adipose tissue) where it and its long chain products (C20:4*n-6* arachidonic acid) compete well for insertion into the phospholipid molecules (Wood *et al*., 2008).

2.8.6 Research into the effects of production systems and the effect on trans fatty acid content in beef

Priolo *et al*. (2002), used lambs at 37 days age to evaluate the effects of grazing natural pasture in France and concentrate-based diets on meat quality. Two groups were allowed to graze natural pasture each at a different growth rate (high and low). The other two groups were raised in stalls and fed grain based concentrates to achieve the same growth rates as from the grass groups. Animals were slaughtered at 35 kg where the high growth rate groups and the slow growth rate groups were 129 days and 163 days of age at slaughter respectively. Stall fed lambs produced heavier carcasses with better muscular conformation scores and they were also fattier than those from grass fed lambs. The meat from stall fed lambs were scored as being superior in juiciness as well as in tenderness relative to those from grazing natural pastures. The subcutaneous fat from grass fed lambs was yellower and harder and the meat darker (up to 24 hours of display) compared to grain fed lamb.

Aurousseau *et al*. (2004), used 32 Ile-de-France lambs in a study to analyse the effects of feeding systems and growth rates on triglyceride and phospholipid and their respective fatty acid content in the *M. longissimus thoracis.* A factorial 2x2 design was used where the feeding systems were either grass outdoor or indoor/stall concentrate and hay feeding. Two growth rates were analysed, that being high and low growth rates. It was found that growth rate had no effect on lipid, triglyceride or phospholipid contents in the *M. longissimus thoracis.* It was established that relative to indoor/stall concentrate and hay feeding, the grass outdoor system resulted in lower triglyceride and higher phospholipid content. The triglyceride fraction contained higher levels of C18:0, C18:3*n-3*, CLA and other *trans* MUFAs while at the same time had lower proportions of C16:0, MUFAs, C18:2*n-6* and other *n-6* PUFAs. The phospholipids had lower MUFAs, C18:2*n-6* and other *n-6* PUFAs and higher levels of C18:3*n-3*. The key findings of this study demonstrate that meat lipids from grass fed lambs showed a composition potentially beneficial for human health.

Pordomingo *et al*. (2012), evaluated the effects of feedlot backgrounding strategies versus complete pasture grazing on moisture, protein, total lipids and lipid profiles of pasture finished Angus heifers. The feedlot backgrounding scenarios were 40, 70 or 100% alfalfa hay diets followed with pasture finishing. At the end of the backgrounding stage, it was found that the concentration of omega-3 fatty acids was highest in the pasture group at the end of the backgrounding and after pasture finishing. This group also had the lowest *n-6/n-3* ratio. Backgrounding diets based on 70 and 100% hay or pasture grazing had greater CLA concentrations in the lipid fraction relative to that of the 40% hay diets. These results suggest that residual effects of backgrounding strategies are detectable within intramuscular fat of pasture finished heifers.

Realini *et al*. (2004), conducted a study with the aim of evaluating the effect of pasture versus concentrate feeding with or without antioxidant supplementation on carcass characteristics, fatty acid composition and quality of beef. Thirty Hereford steers were used and half of the steers finished on concentrate were supplemented with a 1000 I.U. of Vitamin E per head per day for 100 days. It was established that steers finished on a concentrate diet had heavier carcass weights, greater conformation scores, greater degree of finish, greater fat depth and rib eye areas relative to steers finished on pasture. Pasture fed cattle do not have the same degree of finish as grain fed cattle due to lower energy available in forages (Muir *et al*., 1998).The

meat was also lighter in colour and the fat was whiter. Pasture finished steers had darker meat with fat that was more yellow in colour. The fatty acid content was twofold greater in concentrate finished steers and greater percentages of C14:0, C16:0 and C18:1 was found in the intramuscular fat. Pasture finished steers had greater concentrations of CLA and CLA isomers as well as greater concentrations of C18:0, C18:2, C18:3, C20:4, C20:5 and C22:5. It was concluded that finishing cattle on pasture enhances the unsaturated fatty acid profile together with CLA and omega-3 fatty acids of intramuscular fat in beef.

These results are in agreement with those obtained by Nuernberg *et al*. (2005), where German Simmental and Holstein bulls were used to examine the effects of breed and feeding systems on the content of *n-3* PUFA and CLA in muscle tissues. An indoor concentrate based feeding system resulted in *n-6/n-3* ratios of 8.3 and 6.5 for the respective breeds whereas the ratios for grass based systems were 2.0 and 1.9 respectively. The grass based system consisted of summer pasture feeding followed by a winter indoor period of grass silage and linseed containing concentrate feed. The grass based feeding system resulted in significantly higher levels of *n-3* PUFAs in the *longissimus* muscle lipids as well as higher levels of C18:1*trans* fatty acid isomers and CLA*c-9,t-11* in both breeds. The meat was also more stable in terms of oxidation due to the naturally high levels of Vitamin E in the forage. The grass fed bulls however had significantly lower daily gains and they were significantly older at slaughter. The muscle colour was darker and the meat tougher relative to the concentrate fed cattle. Bulls slaughtered from the grass based system were 4-6 months older than the concentrate fed bulls and thus explains the tougher meat from older animals. Due to the higher levels of *n-3* PUFAs in the meat from grass fed bulls, a higher score was given for fishy flavour. When an energy dense concentrate diet is substituted with a lower energy dense grass based diet the results are carcasses with a beneficially lower intramuscular fat content but at the expense of flavour and tenderness. The conclusions reached in this study stated that feeding grass and linseed had positive effects on the fatty acid profile of the meat produced as well as increasing the levels of *n-3* PUFA and CLA. Although a healthier product is produced, slower growth rates and darker tougher meat is of consequence.

Keane & Allen (1998), conducted a study in Ireland to evaluate the effects of three production systems on animal performance, carcass composition and meat quality. They

compared an intensive system which consisted of finishing young bulls on silage and concentrates and then slaughtered at 19 months, a conventional system which consisted of finishing young bulls on silage and concentrates and then slaughtered at 24 months, and an extensive system which consisted of finishing young bulls on pasture and then slaughtered at 29 months. Two slaughter weights were used; light being 640 kg and heavy being 720 kg. Daily gains from 7 months age were 1.18, 0.83 and 0.70 kg for Intensive, Conventional and Extensive grouped animals respectively. The carcass composition was superior (better conformation and lowest fat scores) in the Intensive group while the Conventional as well as the Extensive group were similar in this regard. The conventional group had greater carcass and muscle fatness scores with a twofold higher carcass output per hectare. The Intensive group had a threefold higher carcass output per hectare. The Extensive group had relatively poorer muscle colour but the gross margin per animal and the gross margin per hectare was highest in this group.

Alfaia *et al*. (2009), showed that diet has a major impact on the fatty acid composition on beef, where 27 of the 36 fatty acids and 10 of the 14 CLA isomers analysed were affected. Beef fat from pasture fed bulls had a superior nutritional quality compared to that from concentrate fed bulls. They also showed that meat fatty acid composition was an effective parameter to discriminate between ruminant feeding systems, as well as the various finishing periods on concentrate feeds. Meat from pasture fed animals and from bulls with a two month concentrate finishing period had PUFA/SFA and *n-6/n-3* ratios in the intramuscular fat that complied with recommended values for human health. This was not the case for meat from animals with a longer finishing period on concentrate. Concentrate feeding significantly decreased the concentration of the *c9, t11* CLA isomer relative to the pasture fed bulls.

2.8.7 The South African beef production system

South African beef production systems are dictated by the availability and type of natural resources, local consumer demands and lastly by commercial viability (Frylinck *et al*., 2013). According to SA Beef Carcass Classification System (SABCCS) the average slaughter age is 12 to 16 months before the eruption of permanent incisors (A-age class) or just after the eruption of permanent incisors (AB-age class). The B-age class is pasture reared cattle with eruption of 3 to 6 permanent incisors. B-age class cattle are seldom produced from feedlot systems (Frylinck *et al*.,

2013). South African beef and lamb are generally produced under extensive farming environments (Webb & Erasmus, 2013). Grass feeding normally results in slower growth rates due to the fact that often there is a lack in hormonal growth promoters as well as concentrate feeding strategies and instead feed supplements are limited to strategic mineral, energy and protein licks (Webb & Erasmus, 2013).

2.9 Hormonal feed additives and growth promoters

Red meat producers have been using growth promoting agents for over 50 years to improve muscle leanness, increase average daily gain, stimulate feed intake, and enhance the feed efficiency of animals (Johnson *et al*., 2013). Hormonal growth implants and feed additives such as anabolic steroids and beta-adrenergic agonists are used in the beef industry to obtain improved growth rates and feed efficiency during the fattening phase, and subsequently superior carcass composition and quality (Webb & Erasmus, 2013). The use of hormonal growth implants and feed additives has allowed producers to comply with the demands of consumers by being proficient in managing the extent of carcass fat accumulation and thus producing a leaner product (Webb & Erasmus, 2013). The changes in performance result in economic benefits to beef producers and influence the relative price competitiveness of beef relative to other dietary protein sources (Johnson *et al*., 2013).

2.9.1 Anabolic steroids used in beef production and their effects

Anabolic steroids used in cattle and sheep undergo rigorous testing and inspection before being declared as safe with no health (livestock and humans) or environmental risks (Webb & Erasmus, 2013). In South Africa the use of growth promoting chemicals are under strict control with sound withdrawal periods to ensure the safety of both animal and the consumer (Webb & Erasmus, 2013). Commonly used steroid compounds include estrogens (estradiol-17beta (E2) and zeranol), androgens (testosterone and trenbolone acetate) and progestins (progesterone and melengestrol acetate). The synthetic compounds used in beef cattle production are zeranol, trenbolone acetate, and melegestrol acetate while E2, testosterone and progesterone are naturally occurring (Johnson *et al*., 2013). Melegestrol acetate is orally active and thus administered in such a way but the rest of the anabolic steroids are administered as a compressed pellet implant with various inert carrier compounds in the back of the middle of the ear of cattle

(Johnson *et al*., 2013). These growth promoting implants increase circulating as well as locally produced Insulin-like growth factor-I (IGF-I) which is a potent stimulator of skeletal muscle growth and differentiation. IGF-I stimulate skeletal muscle protein synthesis by enhancing satellite cell activity and consequently increase skeletal muscle growth while reducing skeletal muscle proteolysis (Lee *et al*., 1990; Johnson *et al*., 2013).

2.9.2 Beta-adrenergic agonist used in beef production systems

Beta-adrenergic agonist are compounds similar to naturally occurring endogenous catecholamines (norepinephrine and epinephrine) which are used as feed additives to improve feed efficiency at the end of the fattening phase by placing focus on more protein synthesis rather than fat accretion (Mersmann, 1998; Johnson *et al*., 2013). These synthetic products such as Ractopamine hydrochloric acid and Zilpaterol hydrochloric acid provide comparable production benefits as steroid implants, but differ in the application as well as mode of action (Johnson *et al*., 2013). The results of such treatments during the final 20-25 to 30-42 days of the fattening period are leaner carcasses with improved conformation (Webb & O'Neill, 2008; Johnson *et al*., 2013).

Modification of growth is a physiological response produced when a beta-adrenergic agonist such as norepinephrine and epinephrine binds to a beta-adrenergic receptor, such that increased skeletal muscle accretion and decreased fat accretion is achieved (Mersmann, 1998). This is particularly useful when synthetic beta-adrenergic agonists are administered orally (They are orally active in the parts per million (ppm) concentration) to increase muscle mass whilst reducing carcass fat mass in pigs, sheep and cattle (Mersmann, 1998).

2.9.3 Research into the effects of steroid hormones in beef production

Moloney *et al*. (1990) evaluated the effects of a beta-agonist (L-644,969) on growth performance as well as carcass composition of Friesian steers. They used four groups containing 18 steers, averaging 380 kg body weight, and were individually given ad libitum access to a pelleted concentrate. The diet contained 0.00, 0.25, 1.0 or 4.0 ppm L-644,969 and was distributed in the final 12 weeks of the finishing period. They found that live weight gain was not affected but feed consumption was linearly reduced and feed conversion efficiency was linearly increased relative to controls. L-644,969 quadratically increased carcass weight, dressing percentage and

lean meat yield. The beta-adrenergic agonist altered the distribution of lean meat such that a greater proportion of the total lean meat was in the hind portion of carcasses of subsequent treated animals.

Webb & Casey (1995) showed that the fat content as well as the fatty acid profile of meat from subsequent beta-adrenergic agonists treated cattle may be more acceptable from a health perspective. C18:1 (oleic acid) was deposited at greater concentrations in the subcutaneous fat of steers supplemented with a beta-adrenergic agonists relative to those treated with either trenbolone acetate in combination with oestradiol-17beta or those treated with the beta-agonist in combination with trenbolone acetate and oestradiol-17beta. They also concluded that the beta-agonist or beta-agonist in combination with trenbolone acetate and oestradiol-17beta resulted in a shift toward the deposition of SFA in muscle adipose tissue.

2.10 The effect of species on beef trans fatty acid profiles

The major difference in beef and lamb fatty acid profiles are that ovine adipose and muscle tissue contain the full range of PUFAs. This is not true for cattle as they rather conserve long chain PUFAs in muscle phospholipids (Wood *et al*., 2008). There are also differences in specific fatty acids and the levels at which they are present in the respective meats.

Enser *et al*. (1996) determined the fatty acid content and composition of retail samples of meat. They also assessed them with respect to UK dietary recommendations. They randomly purchased fifty beef sirloin steaks, pork chops and lamb chops from four supermarkets. The percentage of muscle in the samples was 84.4 ± 4.3, 69.8 ± 7.7 and 78.9 ± 7.1 for beef, lamb and pork, respectively, with fatty acid contents of 3.84 ± 1.3, 4.73 ± 1.66 and 2.26 ± 0.7 grams per 100 grams muscle, respectively. They determined that the adipose tissue fatty acid contents were 70.0 ± 8.2, 70.6 ± 8.6 and 65.3 ± 9.4 grams per 100 grams of tissue. A range of C20:0 and C22:0 PUFAs were present in the muscle of all three species as well as in the pork adipose tissue. The concentrations in lamb and beef adipose tissue were too low to measure. The mean PUFA/SFA ratios for beef, lamb and pork muscle tissue were 0.11, 0.15 and 0.58 respectively, while the *n−6/n−3* ratios were 2.1, 1.3 and 7.2 respectively. The mean PUFA/SFA ratios for beef, lamb and pork adipose tissue were 0.05, 0.09 and 0.61 respectively and the *n-6/n-3* ratios were 2.3, 1.4 and

7.6 respectively. The conclusions reached in this study stated that the meat of ruminant species is a valuable source of PUFAs, particularly the C20:0 and C22:0*n−3* fatty acids, in the human diet. When considered as part of a balanced diet, the low PUFA/SFA ratio of the ruminant meat, the relatively high *n-6/n-3* ratio of pork as well as the total fatty acid content does not differ significantly from the nutritional value of lean meat.

Leth *et al*. (1998) evaluated the fatty acid composition of meat from ruminants (Denmark) with special emphasis on some *trans* fatty acids. They used 39 samples of beef, 20 samples of veal and 34 samples of lamb. They found that lamb had relatively the highest content of SFA with a value of 52.8 ± 1.8 grams per 100 grams fatty acids. Beef and veal had SFA values of 45.3 ± 3.1 and 45.4 ± 0.8 grams per 100 grams fatty acids respectively. When looking at the *cis* MUFA content beef, veal and lamb had 49.2, 44.9, and 37.7 grams per 100 grams fatty acid respectively. The PUFA values ranged from 3.3, 5.8 and 5.0 grams per 100 grams fatty acids for beef, veal and lamb. Looking at the *trans* fatty acids found in beef, veal and lamb the *trans* C18:1 was attained at 2.1, 4.0 and 4.5 grams per 100 grams fatty acid respectively. The values of *trans* C16:1 were 0.24, 0.14 and 0.79 grams per 100 grams fatty acid respectively for beef, veal and lamb.

2.11 Conclusion

TFA are neither synthesised nor required by the human body. Health professionals worldwide recommend a reduction in overall consumption of SFA, TFA and cholesterol, while increasing intake of n-3 PUFAs. TFA originates from partial hydrogenation of vegetable oils and fish oils, heat treatments and biohydrogenation of PUFA by rumen microbes Intake of TFA that exceeds 5 grams per portion is associated with increased risk of CHD. Additional factors that collectively contribute to CHD risk are smoking, hypercholesterolemia, hypertension, physical inactivity and obesity. CLA is an abundant isomer in meat and milk derived from ruminants which have antioxidant and anti-carcinogenic properties and is not considered in the TFA definition. The most common industrial produced *trans* isomer is elaidic acid (C18:1 *t-9*) while vaccenic acid and rumenic acid are the *trans* isomers specific to ruminant and dairy fat. TFA from ruminants are believed to have no effect on heart health as with those found from industrially produced TFA.

Vaccenic acid as well as CLA are found in much higher concentrations in the fat portion of red meats relative to that which is found in the lean components. The lean component of red meat has a high biological value as a protein source which is highly digestible. Lean meat provides a heart health protein source with potential healthy TFA profiles. While reducing TFA intake levels it is likely that an inverse increase in SFA will occurs and thus it is recommended that people do not consume excessively high fat processed foods on a daily basis. Health professionals worldwide recommend a reduction in overall consumption of SFA, TFA and cholesterol, while increasing intake of n-3 PUFAs. Since TFA labelling regulations were issued in first world countries it has been found that the food industry has been making effort to reduce dietary TFA as well as reformulating food products in an attempt to reduce or eliminate the TFA levels.

The “Western diet” is one that has been characterised by too high fat, especially saturated fats and now *trans* fats, sugar and salt while being too low in fresh fruit, vegetables and complex carbohydrates. Fried meats and convenience foods have become popular together with a sedentary life style. Red meat is a source of abundant health beneficial compounds and is invaluable as a protein source. It is for that reason that consumers should be educated on the nutritive quality red meat has to offer, and this could be achieved by product labelling practices. Meat labelling is a practice that can restore consumer confidence in red meat and related products with respect to quality and nutritive value. Challenges that face labelling of red meat lie with the consumer as well as the product itself. It was established that consumers do not know how to use food labels or do not understand the information provided on food labels.

There is relatively poor information on *trans* fatty acids in livestock as well as specific factors that influence the *trans* fatty acids levels in red meat under South African conditions. We do know that there are differences among these factors; however the extent to which these factors play a role remains an area of uncertainty. It is speculated that feeding systems play the most significant role in *trans* fat content.

Pasture feeding has become favourable with regard to the positive effects it has on the fatty acid profile on subsequent meat produced even though it is at the expense of increased growth rate. A lower *n-6/n-3* ratio is obtained when grass and pasture feeding is employed. The use of

hormonal growth implants and feed additives has allowed producers to comply with the demands of consumers by being proficient in managing the extent of carcass fat accumulation and thus producing a leaner product. The changes in performance result in economic benefits to beef producers and influence the relative price competitiveness of beef relative to other dietary protein sources. Analysis of *trans* fatty acid composition would benefit the South African meat industry, especially at the hand of the proposed Regulations Relating to *Trans* fat in Foodstuffs, and the absence of these values for South African beef.

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Chapter 3: Materials and Methods

3.1 Sample preparations

Fifty beef sirloin samples were purchased from major outlets on the same day in the following Gauteng cities with their respective regions; Johannesburg and Pretoria north, south, east, and west. The meat samples were placed in a portable Campmaster 45Lt Fridge/Freezer 12V/220V set at -20°C during the purchasing journey of 8 hours across the Gauteng regions for preservation and keeping quality. Meat samples were vacuum sealed that evening using a Genesis vacuum sealer until further analysis at UP NutriLab, University of Pretoria. The meat samples were taken and all visible fat was cut off using a scalpel prior to blending each individual sample into a homogenous mixture using a BUCHI Mixer B-400. The visible fat was cut off in order to analyse the intramuscular fat content within the muscle tissue and the nutritional contribution it has to the human diet. The dissecting equipment (glass cutting board, scalpel and mixer beaker and blades) were cleaned with soapy warm water after each sample was processed. The blended samples were stored overnight in aluminium containers in the fridge for further preparations. Approximately 10grams wet matter were weighed out to analyse the meat samples for initial dry matter analysis (AOAC, 1990), which consisted of placing the wet samples in an oven set at 105°C over night. The dry samples were weighed back and disregarded at the end of dry matter analyses recording. The remainder of the samples were freeze dried and then milled for approximately 10 seconds into a fine powder form using a Hamilton Beach Commercial blender. The milled samples were placed into large plastic containers with screw caps and labelled.

3.2 Ether Extraction

Determination of ether extract was performed using the Soxtec method using the Tecator Soxtec System 1040 Extraction unit (AOAC, 1990). The temperature setting of the service unit was set to 80°C. Approximately 2 grams of dried, milled meat samples were weighed out onto Whatman 125mm diameter filter paper which were folded and placed into an extraction thimble and stoppered with a fat-free cotton wool plug. Each sample was done in duplicate. The thimbles were placed accordingly into the extraction unit. The fat flasks used to collect the ether extract were heated in an oven at 105°C for 10 minutes and

then cooled in a desiccator for 5 minutes. The fat flasks were weighed and filled with 50ml petroleum ether. The extraction unit was set onto “Boiling” for 1hour after which the unit was placed into “Rinse” mode for an additional 30 minutes. At the end of the rinsing period the extraction system was closed and the ether was collected for 20 minutes. The extraction cup were removed and dried in an oven for 30 minutes at 105°C. The thimbles were removed and content discarded while the remaining petroleum ether was collected and recycled for subsequent use to clean the extraction cups at the end of the process. The extraction cups were placed in an oven to dry for 5 minutes at 105°C and subsequently weighed. The calculations used for ether extract were as follows:

$$\%\text{Ether Extract (fat)} = \frac{(\text{Cup + Ether extract}) - \text{Cup mass only}}{\text{Sample mass}} \times 100$$

3.3 Medium and long chain fatty acid analyses

Fatty acid analyses were performed by Nutrilab at The University of Pretoria.
The lipids were extracted from freeze dried samples according to the chloroform: methanol (2:1, v/v) method by Folch *et al*. (1957), with modifications as described and practiced by Webb & Casey (1995). Fatty acids were obtained by means of a saponification procedure as put forward by Christi (1982) with modifications. The methyl esters of the respective fatty acids were prepared by the addition of 14%$BF3/CH_3OH$. Fatty acids were separated and identified using a Varian 3300 FID chromatograph with a 100meter WCOT fused silica capillary column. Prior to running the samples in the GC, a standards solution containing methyl esters of fatty acids known to occur in meat and in known concentrations was prepared and injected in order to determine and check the retention times of the different fatty acids. Standards for the fatty acid methyl esters and their isomers were also obtained from Nu Chek Prep.Inc., Elysim, Minesota (USA).

3.4 Samples preparation for Long chain fatty acid analyses

A 30ml test tube was filled with 10 ml phosphate buffered saline solution and 1 gram milled meat sample. A 1000µl internal standard C15:0 was added to the test tube and

homogenised using a mechanical homogeniser set at low speed for approximately 30 seconds, followed by high speed for 30 seconds. The homogenised mixture was then poured into glass tubes which were then rinsed with 2ml PBS. The mixtures were centrifuged for 15minutes using a bench top centrifuge set at 3000rpm. Subsequently 5ml of supernatant was removed into a clean 10ml test tube and labelled accordingly. To this supernatant 2ml of Chloroform with 1ml 0.1 NHCL was added and mixed by means of a vortex. The phases are rested until separated and centrifuged once more for 15 minutes at 3000rpm. The chloroform was then transferred into a clean 10ml test tube and closed with a Teflon lined screw cap. The extraction method was repeated once more with 2ml aliquot chloroform and then combined with the aforementioned chloroform extract. The chloroform was then evaporated under a stream of nitrogen until no visible liquid was observed.

3.5 Derivatisation

To the aforementioned test tube, 1ml methanolic KOH was added and then heated for 20 minutes at 60°C. Once the test tube was cooled down, 1ml BF3 in methanol was added and the tube mixed by means of a vortex. The mixture was then heated once more for 30 minutes at 60°C. Once the test tubes cooled down 1ml saturated NaCl in water was added together with 1ml hexane. The mixture was mixed using the vortex and centrifuged for 3 minutes at 3000rpm. The tubes were rested until the phases were separated. The upper hexane layer was transferred into a clean labelled 1ml auto-sampler vial containing approximately 100mg anhydrous Na_2SO4. These mixtures were allowed to stand for 15 minutes. The samples are able to keep for several weeks in the freezer. The samples were manually injected into the Varian 3300 gas chromatograph and data recorded. Fatty acid concentrations were determined using a Varian 3300 gas chromatograph equipped with a FID detector and WCOT fused silica coating CP-Sil 88, 100m x 0.25mm DF 0.2µm. The carrier gas used was Helium set at a flow rate of 50ml/min. The gas chromatograph program was programed as outlined by Simela (2005):

Initial column temperature: 150°C

Initial holding time: 2 minutes

Final column temperature: 200°C

Rate:	5°C per minute
Holding time:	13 minutes
Injector temperature:	230°C
Detector temperature:	240°C
Range:	11

Fatty acids were identified by comparisons with the retention times of fatty acid methyl ester peaks of standard solutions containing methyl esters of known fatty acids and of known concentrations obtained from Sigma. Fatty acids were expressed as a proportion of total long chain fatty acids (w/w, %) and in gravimetric concentrations (mg.g^{-1} of tissue sample) (Webb *et al.*, 1998).

The concentration of each fatty acid in the sample was determined by the following formulae as outlined by Simela (2005):

$$\text{Fatty acid concentration (µg/g lipid)} = \frac{PAsa}{PAst} \times [FA]st \text{ µg/ml} \times \frac{1}{[Lipid]} \text{ g/ml}$$

$$\text{Fatty acid in meat (µg/g)} = \text{[lipid] g/ml} \times \frac{extract}{sampleweight} \times \text{[fatty acid] µg/g lipid}$$

Where PAsa = peak area in sample; PAst = peak area in standard; [FA]st = fatty acid concentration in standard; extract = volume of lipid extract (ml); sample weight = weight of meat sample (g) from which lipids were extracted.

3.6 Statistical Analyses

The raw data was recorded in an excel spread sheet and all statistical procedures were carried out with the IBM SPSS Statistics Windows software package, version 2.1 (SPSS Inc., Chicago, IL, USA). Differences among the fatty acid percentages in each of the regions were determined using a multivariate analysis of variance (MANOVA) utilising the GLM procedure of SAS (1992). Post-hoc analyses were done with Bonferonni's range test ($P < 0.05$). Quantitative analysis of the beef samples from the respective regions will be

conducted as well as the extent to which they influence the *trans* fatty acid content in South African beef.

CHAPTER 4: Results and Discussion

4.1 Fatty acid composition of sirloin steak samples

Summary statistics of the composition of medium and long-chain fatty acids in freeze dried bovine *m. longissimus dorsi* samples from the Gauteng region are presented in Table 1. The fatty acid profiles are expressed in both molar proportion of total fatty acids (w/w%) and as gravimetric concentrations (mg/g). The predominant fatty acids identified in samples from beef sirloins were oleic acid (C18:1c-9), palmitic acid (C16:0) and stearic acid (C18:0) respectively, which is in agreement with previous reports (Enser *et al*., 1998; Webb *et al*., 1998; Webb & Casey, 1995; Warren *et al*., 2008; Wood *et al*., 2008 & Muchenje *et al*., 2009).

Table 1: Mean (± SD) fatty acid composition of bovine *m. longissimus dorsi* (*LD, sirloin*) samples obtained from the Gauteng region expressed as percentage of total fatty acids identified (Molar %) and gravimetric concentrations (milligrams fatty acid per gram sirloin)

Fatty acid	**Molar %** (w/w%; n=48)	**Gravimetric concentration** (mg FA/g beef; n=48)
C14:0	nd	nd
C16:0	29.18 ± 3.664	58.04 ± 24.600
C18:0	20.44 ± 5.589	40.01 ± 17.285
C18:1 (C18:1t-9 + C18:1c-9 (*n-9*))	46.53 ± 7.480	94.21 ± 47.621
C18:1t-9	6.82 ± 2.755	13.19 ± 6.953
C18:1c-9 (*n-9*)	39.71 ± 8.793	81.02 ± 44.641
C18:2 (*n-6*)	3.02 ± 0.955	5.98 ± 3.057
t-9,t-12	0.14 ± 0.134	0.29 ± 0.206
c-9,t-12	0.12 ± 0.097	0.27 ± 0.270
t-9,c-12	0.01 ± 0.029	0.03 ± 0.109
c-9,c-12	2.75 ± 0.923	5.39 ± 2.724
C18:3 (*n-3*)	0.39 ± 0.183	0.86 ± 0.594
t-9,t-12,t-15	0.09 ± 0.098	0.20 ± 0.159
t-9,t-12,c-15	nd	nd
t-9,c-12,t-15	nd	nd
c-9,t-12,t-15	nd	nd
c-9,c-12,t-15	nd	nd
c-9,t-12,c-15	0.07 ± 0.059	0.17 ± 0.179
t-9,c-12,c-15	0.12 ± 0.094	0.27 ± 0.241

c-9,c-12,c-15	0.09 ± 0.064	0.22 ± 0.153
CLA (*n-6*)	0.43 ± 0.234	0.90 ± 0.738
TFA	7.19	13.99
SFA	49.61	98.05
UFA	50.38	101.95
SFA/UFA	0.98	0.96
MUFA	46.96	95.11
PUFA	3.41	6.84
PUFA:SFA	0.07	0.07
n-3	0.39	0.86
n-6	3.45	6.88
n-6/n-3	8.85	8.00

CLA , conjugated linoleic acid (C18:2c-9,t-11);

FA~Fatty acid;

MUFA, monounsaturated fatty acids (C18:1t-11 + C18:1c-9);

nd ~ not detected;

PUFA, polyunsaturated fatty acids (sum of C18:2 isomers + sum of C18:3 isomers);

SFA, saturated fatty acids (C16:0 + C18:0);

TFA, trans fatty acids;

UFA, unsaturated fatty acids.

4.2 Specific fatty acid content of longissimus dorsi muscle samples

4.2.2 Oleic acid content of beef samples

The C18:1 fatty acid pool (comprising the different C18:1 isomers) was the most abundant MUFA in bovine *m. longissimus dorsi* samples with a 46.53 ± 7.48% (94.21 ± 47.62 mg/g *LD*) contribution to the total fatty acid profile. These values are typical of values for feedlot grain fed cattle observed for oleic acid as noted by Webb and Casey (1995). Leheska *et al*. (2008) reported that oleic acid constituted the greatest concentration of MUFA in both grass and grain fed beef samples, but it was the grain fed beef samples that contained the highest concentrations of oleic acid.

It is imperative to take note that few studies treat oleic acid (C18:1c-9) and elaidic acid (C18:1t-9) as separate isomers in the analyses, which often result in higher final values for C18:1. Oleic acid (C18:1c-9, $P < 0.05$) dominated the C18:1 isomer range and constituted 39.71 ± 8.79% (81.02 ± 44.64 mg/g *LD*) of the C18:1 fatty acids in beef, while the *trans* isomer, elaidic acid (C18:1t-9, $P < 0.05$), made up an additional 6.82 ± 2.75% (13.19 ± 6.95 mg/g *LD*). The elaidic acid content of *m. longissimus dorsi* samples obtained in the present study is almost double that reported by Droulez *et al*. (2006), who reported a value of less than 3% of total fatty acid content. This finding may be explained by typical production and breed differences as examined by Webb & Casey (1995), who found that breed differences

in sheep influenced the proportions of myristic acid (C14:0), heptadecenoic acid (C17:1) and oleic acid (C18:1*cis*).

Enser *et al*. (1998) reported values for elaidic acid and oleic acid in cattle fed concentrates versus grass diets. They found that oleic acid concentrations were respectively 34.7% and 29.8% in grass versus concentrate fed cattle. Values for elaidic acid were found to vary between 2.50% and 2.69% in grass and concentrate diets respectively.

4.2.3 Palmitic acid and stearic acid content of beef samples

The molar proportions of palmitic acid (C16:0) and stearic acid (C18:0) were 29.17 ± 3.66% and 20.43 ± 5.58% respectively and thus giving a total SFA% of 49.61% (98.05 mg/g *m. longissimus dorsi*). Subsequently the quantitative contributions made by palmitic acid (C16:0; $P < 0.05$) and stearic acid (C18:0; $P < 0.05$) were 58.04 ± 24.6 mg/g *LD* and 40.01 ± 17.28 mg/g *LD* respectively. Palmitic acid (C16:0) is the principal end product of *de novo* fatty acid synthesis. It can be subjected to further elongation resulting in the formation of stearic acid (C18:0). Both these fatty acids can be converted to mono-unsaturated fatty acids (MUFAs) by Δ-9 desaturase enzyme to form palmitoleic acid (C16:1*n-9*) and oleic acids (C18:1c-9) (Mapiye *et al.,* 2012).

4.2.4 Essential fatty acid content in beef samples

The molar proportion of the essential fatty acid (EFA) linoleic acid (C18:2; $P < 0.05$) was 3.01 ± 0.95% (.98 ± 3.06 mg/g *LD*) with the C18:2 c-9,c-12 isomer contributing 2.74 ± 0.92% (5.39 ± 2.72 mg/g *LD*). Linolenic acid (C18:3) is another EFA and was reported to be 0.40 ± 0.2%. These values are negligible in terms of practical significance as the contributions made are diminutive when looked at in gravimetric concentrations. Feeding system plays the most significant role in the fatty acid content of meat; grazing systems (grass/ forages) result in higher *n-3* PUFA content due to grass being naturally high in the *n-3* precursor fatty acid linolenic acid (C18:3). Grain based (concentrate) systems result in higher *n-6* PUFA content since grains are higher in the *n-6* precursor fatty acid linoleic (C18:2) (Mitchell *et al*., 1991)

4.2.5 CLA content of beef samples

The major isomer of the CLA group is rumenic acid (C18:2 *c-9,t-11*; $P < 0.05$) which contributes 0.43 ± 0.23% (0.90 ± 0.74mg/g *LD*) (Table 1) to the mean total fatty acid content of longissimus dorsi muscle samples. CLA concentrations in meat are determined by factors such as season, animal genetics and production system with the most important factor being diet, which directly supplies substrates for subsequent CLA formation (Schmid *et al*., 2006). Warren *et al*. (2008) reported CLA values ranging from 0.57% for cattle fed a concentrate diet relative to 0.23% CLA for those fed grass silage. These values confirm the assumption made in the present study that the beef sirloin steak (*m. longissimus dorsi*) samples from the Gauteng region are primarily from feedlot cattle fed concentrate diets.

Warren *et al*. (2008) reasoned that the results of their study was conflicting with those of other studies (French *et al*., 2000; Aurousseau *et al*., 2004) where CLA production was higher in grass grazed cattle. This was due to the fact that the major source of PUFA in those other studies were primarily from forage intake only and in the study conducted by Warren *et al*. (2008) the total PUFA intake was similar in both concentrate and grass silage diets. The concentrate diet was high in full fat soya, thus high in linoleic acid (C18:2 *n-6*), the precursor of the *n-6* PUFAs (Warren *et al*., 2008). C18:2*n-6* (linoleic acid) is a major fatty acid in concentrate feeds (grains and oilseeds) of all species. Linoleic acid is degraded into MUFAs and SFA within the rumen. Its incorporation into adipose and muscle tissue in relation to quantity within the diet is relatively greater than for any other fatty acids.

Linoleic acid is deposited into muscle phospholipids at a higher rate (relative to adipose tissue) where it and its long chain products (C20:4*n-6;* Arachidonic acid) compete well for insertion into the phospholipid molecules (Wood *et al*., 2008). Table 2 represents data collected by previous studies, as presented by Raes *et al*. (2004), on the effects of diet/feeding system on the intramuscular CLA content in beef.

The data presented in Table 2 indicates that when the diet richly supplies C18:3*n-3* (alpha-linolenic acid) in the form of either grass or linseed, increased proportions of CLA are observed. Diets that are high in C18:2*n-6* (Linoleic acid) such as concentrate diets and fish oil supplements produced similar results. The highest value observed in this meta-analysiswas 1.08 g CLA/100 g of total fatty acids when feeding 22 kilograms of grass DM (French *et al*., 2000). It is of value to take note that regardless of varying feeding systems and diet supplementation strategies used in ruminants, the level of CLA content in beef are always much lower relative to the content found in the milk of ruminants (Raes *et al*., 2004).

Table 2: Effect of nutrition on the CLA (C18:2 c-9,t-11) content in beef (g/100 g of total fatty acids) as presented by Raes *et al*., 2004.

Diet	CLA (C18:2 c-9,t-11)	Reference
High corn concentrate	0.32	Beaulieu *et al*., 2002
High corn concentrate + 5% soybean oil	0.36	Beaulieu *et al*., 2002
Concentrate (megalac) + grass silage	0.32	Enser *et al*., 1999
Concentrate (linseed) + grass silage	0.80	Enser *et al*., 1999
Concentrate (fish oil) + grass silage	0.57	Enser *et al*., 1999
Concentrate (linseed + fish oil) + grass silage	0.73	Enser *et al*., 1999
Barley based concentrate + grass silage ad lib	0.47	French *et al*., 2000
Concentrate + straw	0.37	French *et al*., 2000
5 kg concentrate + 6 kg grass DM	0.54	French *et al*., 2000
2.5 kg concentrate + 12 kg grass DM	0.66	French *et al*., 2000
22 kg grass DM	1.08	French *et al*., 2000
Concentrate + maize silage	0.86	Fritsche & Fritsche 1998

Concentrate + maize silage	0.76	Fritsche & Fritsche 1998
Grain finished	0.36	Laborde *et al*., 2001
Grain finished	0.35	Laborde *et al*., (2001)
Concentrate	0.29	Lorenz *et al*., 2002
Pasture	0.32	Lorenz *et al*., 2002
Concentrate	0.66	Madron *et al*., 2002
Concentrate + 127 g extruded full-fat soybeans/kg DM	0.69	Madron *et al*., 2002
Concentrate + 256 g extruded full-fat soybeans/kg DM	0.77	Madron *et al*., 2002
Barley-based feedlot (800 g/kg)	0.17	Mir *et al*., 2000a
Barley-based feedlot (800 g/kg)	0.18	Mir *et al*., 2000a
Concentrate (n-6 rich)	0.52	Nürnberg *et al*., 2002
Pasture	0.55	Nürnberg *et al*., 2002
Concentrate (n-6 rich)	0.56	Nürnberg *et al*., 2002
Pasture	0.60	Nürnberg *et al*., 2002
Concentrate (n-6 rich)	0.34	Raes *et al*., 2003b
Concentrate (linseed based) + grass silage	0.59	Raes *et al*., 2003b
Concentrate (linseed based) + maize silage	0.50	Raes *et al*., 2003b
Feedlot fed	0.26	Rule *et al*., 2002
Range/pasture fed	0.41	Rule *et al*., 2002
Finished on grass	0.55	Scollan *et al*., 2002a
Finished on grass + red clover	0.60	Scollan *et al*., 2002a
Finished on grass + white clover	0.63	Scollan *et al*., 2002a
Concentrate (+ ground linseed) + maize silage	0.29	Strzetelski *et al*., 2001
Concentrate (+ rapeseed oil cake) + maize silage	0.31	Strzetelski *et al*., 2001
Concentrate (+ soybean oil meal) + maize silage	0.21	Strzetelski *et al*., 2001
Grain finished concentrates	0.06	Yang *et al*., 2002
Pasture finished	0.22	Yang *et al*., 2002

4.2.6 Nutritional implications of fatty acids in meat

Nutritional value of meat is predominantly verified by the ratio between SFAs and PUFAs as well as the balance between the n-6 and n-3 fatty acids (Warren *et al*., 2008). Cattle are frequently fattened on concentrate diets and as a result produce meat with unfavourable *n-6/n-3* ratios due to concentrates being high in C18:2*n-6* (Nuernberg *et al*., 2005). C18:3*n-3* (alpha-linolenic acid) is the major fatty acid found in grasses. Higher concentrations of C18:3*n-3* are found within muscle tissue compared to adipose tissues. It is an essential fatty acid but does not compete well for insertion into phospholipid when compared to C18:2*n-6*. A combination of greater biohydrogenation together with a long rumen transit time for forages limits the amount of this fatty acid available for tissue uptake relative to C18:2*n-6* (Wood *et al*., 2008).

The opposite is true for forages that are high in C18:3*n-3* which enhances the *n-6/n-3* ratio in meat (Scollan *et al*., 2001). Meat from cattle finished on concentrate feed typically has higher intramuscular fat than that from pasture finished cattle and as a result also has higher levels of triacylglycerols relative to phospholipids and lower levels of PUFA together with higher levels of SFA and MUFA (Wood *et al*., 2008). Pasture feeding has become favourable with regard to the positive effects it has on the fatty acid profile on subsequent meat produced even though it is at the expense of increased growth rate (Webb & O'Neill, 2008). A lower *n-6/n-3* ratio is obtained when grass and pasture feeding is employed (Wood *et al*., 2008). In a study conducted by Enser *et al*. (1998), it was reported that the P:S ratio was 2-3 times higher in animals fed the concentrate diet. It was related to the fact that a high concentration of C18:2 were present in the concentrate diet while animals fed the grass based diet had higher levels of C18:0. They furthermore reported that the n-6:n-3 ratios differed significantly between the production systems, being approximately nine times higher in the concentrate fed group.

4.2.7 *Trans* fatty acids and saturated fatty acids

The total mean TFA content of *m. longissimus dorsi* samples was 7.19% (13.99 mg TFA/g *LD*) while the SFA constituted 49.61% (98.05 mg/g *LD*) and UFA made up 50.38% (101.95 mg/g *LD).* The relative proportions of SFA and USFA resulted in a SFA/UFA ratio of 0.98 (Table 1). Ruminant animals consume diets, fresh grass or concentrate, that are innately low in fat but high in PUFA which is subject to microbial biohydrogenation within the rumen environment. The results of such biochemical reactions lead to the production of SFA which are absorbed and deposited within various tissues (Warren *et al*., 2008). It was noted by Enser *et al*. (1998) that no effect of production system was observed on the percentage values of the major *trans* fatty acid, C18:1 *trans.* Granting that the trans fatty acid content of meat is generally low and therefore practically negligible, it was reported by Casey & Webb (1995) that concentrate feeding may result in a small increase (3.1 – 3.9%) in the proportion of trans fatty acids in sheep.

The molar proportions for TFA reported by Stender *et al*. (2008) were found to be as high as 6% and thus the values reported for the current study are 1.2% higher. Warren *et al*. (2008) reported an average value of 3.3% for TFA in cattle fed concentrate diets, this being 3.89% lower than the values of the current study. The SFA value obtained in the present study closely agrees with the SFA values described by Droulez *et al*. (2006), who reported a SFA value of 48%. Vaccenic acid and Rumenic acid are the *trans* isomers that are specific to ruminant and dairy fat (Albuquerquea *et al*., 2011). Vaccenic acid (C18:1*t-11*) accounts for 50-80% of the total unsaturated fatty acids found in ruminant derived fats and is the product of biohydrogenation of C18:2*n-6* (Lock & Bauman, 2004; Weggemans *et al*., 2004; Stender *et al*., 2008; Wood *et al*., 2008).

Humans and rodents have the ability to desaturate vaccenic acid to the *c-9,t-11* CLA isomer (Turpeinen *et al*., 2002). It has been postulated that ruminant derived *trans* vaccenic acid may not be associated with an increased risk of CHD or CVD. This is due the conversion of *trans* vaccenic acid to the CLA isomers which is known for many health benefits (Turpeinen *et al*., 2002; Huth, 2007). Within adipose tissues vaccenic acid is converted to CLA by the Stearoyl-CoA desaturase enzyme (Wood *et al*., 2008). This enzyme is also responsible for converting oleic acid (C18:1*c-9*) to stearic acid (C18:0). It is interesting to note that oleic acid, vaccenic acid as well as CLA are found in much higher concentrations in neutral lipids and adipose tissue relative to phospholipids and muscle tissues (Wood *et al*., 2008). It was believed that oleic acid is converted directly to stearic acid but it was found that oleic acid is in fact converted to a multitude of *trans* C18:1 positional isomers. *Trans* C18:1 isomers serve as precursors for the synthesis of SFA within the rumen as well as CLA synthesis at tissues level such as in the mammary tissues (Mosley *et al*., 2002).

TFA from ruminants are believed to have no effect on heart health as with those found from industrially produced TFA. Motard-Bélanger *et al*. (2008) conducted a study where the effects of ruminant TFA were compared to industrial TFA on a gram for gram basis and how it influenced plasma LDL concentrations as well as other cardiovascular disease risk factors in healthy subjects. It should be noted that the 38 participants were aged 32 to 47 and were healthy non-smokers. The study design was a controlled double blind randomized crossover design with three diets representing a high TFA intake (10.2 grams per 2500kcal), moderate TFA intake (4.2 g per 2500kcal) and a control diet (2.2 g per 2500kcal). The results of this study concluded that high dietary intakes of TFA from ruminant origin may adversely affect cholesterol homeostasis whilst moderate intakes of ruminant derived TFA that are well above the upper limit of human consumption have neutral effects on cholesterol homeostasis.

4.2.8 P:S and *n-6/n-3* ratio in beef samples

The PUFA/SFA (P:S) as well as the *n-6/n-3* fatty acid ratios are primary values used to measure the degree of nutritional quality of meat and are ratios used for human nutrition (Warren *et al*., 2008). In human nutrition these values have benchmark values to indicate a healthy lifestyle that contests various CHD and associated diseases. These recommended benchmark values are > 0.7 for the P:S ratio and < 5 for the *n-6/n-3* ratio (Raes *et al*., 2004).

The values obtained from the current study differ somewhat from the benchmark values presented by Raes *et al*., (2004). The P:S ratio from the present study was 0.63 units lower than recommended with a value of only 0.07. The *n-6/n-3* value for the present study is 8.0, thus being 3 units above the benchmark value. Reas *et al*. (2004) calculated the P:S ratio as (C18:2n-6 + C18:3n-3)/(C14:0 + C16:0 + C18:0), while the *n-6/n-3* ratio was calculated as (C18:2n-6+ C20:3n-6+ C20:4n-6+ C22:4n-6)/(C18:3n-3 + C20:5n-3 + C22:5n-3 + C22:6n-3). It should be noted that in the present study that C14:0 was not detected and

none of the longer chain (C20–22) *n-6* and *n-3* PUFAs (C20:3*n-6*, C20:4*n-6*, C22:4*n-6*, C20:4*n-3*, C20:5*n-3*, C22:5*n-3*, C22:6*n-3*) were detected. This is due to very low concentrations of these subsequent fatty acids that are not detected and hence not measurable.

The *m. longissimus* is also a relatively pale muscle and thus low in phospholipids (Enser *et al*., 1996). On the word of De Smet *et al*. (2004) the P:S ratio is influenced primarily by genetics and the overall fat level of the animal and not so much by nutrition as it is in the case of monogastric animals. Nutrition plays a much smaller part on this ratio due to the natural biohydrogenation that occurs within the rumen, where dietary UFAs are hydrogenated to SFAs.

De Smet *et al*. (2004) reported that the C18:0, C18:1*n-9*, C18:3 and all subsequent *n-3* PUFAs were significantly higher in the grass fed group while C18:2 and all subsequent *n-6* PUFAs were higher in the concentrate fed group. The *n-6/n-3* ratio on the other hand is very much so influenced by the dietary fatty acid composition. By supplying high *n-3* precursors in the diet, the total *n-3* content of the meat is increased with a parallel decrease in the *n-6* content due to lower dietary *n-6* supply.

It is therefore favourable to finish ruminants on pastures due to a decrease of the *n-6/n-3* ratio (Raes *et al*., 2004).

In the present study it is safe to assume that the animals were finished on concentrates by reason of the *n-6* value (6.88 mg/g LD) being much higher than the *n-3* value (0.86mg/g LD) giving an unfavourable ratio of 8.0. Enser *et al*. (1998) reported that the P:S ratio was 2-3 times higher in those animals fed a concentrate diet relative to those fed grass. This is explained by the fact that concentrates contain higher concentrations of C18:2 while grass contains higher concentrations of C18:0. They also reported that the C18:2 to C18:3 and *n-6/n-3* ratios differed significantly between the different production systems, being 9 times higher in the concentrate fed group. The supply of dietary fatty acids as well as the species of animal has an effect on the *n-6/n-3* fatty acid ratio.

Monogastric animals generally produce meat which has a relatively higher *n-6/n-3* fatty acid ratio than meat from ruminants due to monogastric animals being fed concentrate diets that are higher in linoleic acid (Raes *et al*., 2004). It is possible to achieve a favourable intramuscular *n-6/n-3* fatty acid ratio in ruminant meat products by supplying diets rich in specific ingredients such as fish oil, fish meal, and linseed oil as well as providing forages rich in *n-3* fatty acid. This will innately lead to a decrease in *n-6* fatty acid production with little or no effect on the P:S ratio(Raes *et al*., 2004).

4.3 Fatty acid composition of sirloin steak samples from Gauteng cities

Table 3: Comparison of mean (± SD) fatty acid content of *m. longissimus dorsi* in respective city regions expressed as molar proportions (mol%)

Fatty acid name	Johannesburg City and Region	Mean (mol%)	Pretoria City and Region	Mean (mol%)
C16:0	N	28.27 ± 3.367	N	31.37 ± 7.540
	S	29.19 ± 5.354	S	27.71 ± 3.440
	E	29.53 ± 1.603	E	28.21 ± 2.760
	W	29.45 ± 2.178	W	30.08 ± 1.091
C18:0	N	17.70 ± 3.163	N	26.13 ± 8.346
	S	23.76 ± 6.208	S	19.00 ± 3.107
	E	18.28 ± 7.254	E	22.73 ± 4.010
	W	16.87 ± 2.190	W	21.20 ± 4.442
C18:1	N	50.16 ± 5.423	N	38.18 ± 15.983
	S	43.28 ± 5.876	S	49.55 ± 2.882
	E	48.38 ± 8.183	E	45.09 ± 2.454
	W	50.12 ± 3.275	W	44.80 ± 4.911
C18:1t-9	N	5.64 ± 1.086	N	8.29 ± 2.493
	S	6.34 ± 2.310	S	7.23 ± 2.655
	E	5.34 ± 3.036	E	7.13 ± 2.361
	W	6.99 ± 3.569	W	7.81 ± 3.766
C18:1c-9	N	44.52 ± 5.817	N	29.89 ± 16.845
	S	36.94 ± 6.247	S	42.33 ± 3.773
	E	43.04 ± 10.374	E	37.96 ± 4.442
	W	43.12 ± 6.156	W	36.98 ± 7.511
C18:2	N	2.92 ± 0.493	N	3.88 ± 1.962
	S	2.96 ± 1.021	S	2.96 ± 0.566
	E	2.78 ± 0.780	E	3.06 ± 0.326
	W	2.52 ± 1.091	W	3.33 ± 0.630
t-9,t-12	N	0.17 ± 0.035	N	0.25 ± 0.387
	S	0.11 ± 0.049	S	0.13 ± 0.064
	E	0.15 ± 0.080	E	0.14 ± 0.042
	W	0.09 ± 0.096	W	0.13 ± 0.070
c-9,t-12	N	0.14 ± 0.112	N	0.08 ± 0.114
	S	0.13 ± 0.080	S	0.11 ± 0.101
	E	0.16 ± 0.086	E	0.15 ± 0.094
	W	0.08 ± 0.109	W	0.12 ± 0.105
t-9,c-12	N	0.02 ± 0.053	N	nd
	S	0.01 ± 0.024	S	nd
	E	0.03 ± 0.049	E	nd
	W	nd	W	0.02 ± 0.036
c-9,c-12	N	2.59 ± 0.411	N	3.55 ± 1.838
	S	2.71 ± 1.071	S	2.73 ± 0.528
	E	2.44 ± 0.839	E	2.80 ± 0.367
	W	2.34 ± 0.989	W	3.05 ± 0.737
C18:3	N	0.52 ± 0.086	N	0.17 ± 0.192
	S	0.45 ± 0.048	S	0.41 ± 0.225
	E	0.43 ± 0.209	E	0.44 ± 0.145
	W	0.46 ± 0.129	W	0.25 ± 0.176
t-9,t-12,t-15	N	0.11 ± 0.019	N	0.04 ±0.069
	S	0.14 ± 0.037	S	0.08 ± 0.042
	E	0.08 ± 0.096	E	0.11 ± 0.029
	W	0.18 ± 0.204	W	0.04 ± 0.066
t-9,t-12,c-15		nd		nd
t-9,c-12,t-15		nd		nd
c-9,t-12,t-15		nd		nd
c-9,c-12,t-15		nd		nd
c-9,t-12,c-15	N	0.11 ± 0.026	N	0.04 ± 0.055
	S	0.08 ± 0.031	S	0.09 ± 0.043
	E	0.14 ± 0.090	E	0.06 ± 0.059
	W	0.05 ± 0.049	W	0.02 ± 0.035
t-9,c-12,c-15	N	0.20 ± 0.091	N	0.03 ± 0.056
	S	0.12 ± 0.032	S	0.17 ± 0.118
	E	0.10 ± 0.083	E	0.14 ± 0.061
	W	0.13 ± 0.126	W	0.08 ± 0.072
c-9,c-12,c-15	N	0.11 ± 0.035	N	0.06 ± 0.088
	S	0.10 ± 0.037	S	0.08 ± 0.063

	E	0.11 ± 0.057	E	0.13 ± 0.038
	W	0.09 ± 0.113	W	0.11 ± 0.055
CLA	N	0.45 ± 0.235	N	0.28 ± 0.211
	S	0.37 ± 0.161	S	0.38 ± 0.103
	E	0.56 ± 0.273	E	0.47 ± 0.176
	W	0.58 ± 0.500	W	0.35 ± 0.212

CLA , conjugated linoleic acid (C18:2c-9,t-11);

nd ~ not detected.

The mean content of fatty acids of *m. longissimus dorsi* in respective city regions are expressed as molar proportions (mol%) and it can be observed that the two regions differ slightly in either their production systems or source of animals. Previous values confirmed the assumption made that the beef samples (*m. longissimus dorsi*) from the Gauteng region are primarily from feedlot cattle fed concentrate diets where the C18:1 content is greater than the C18:0 content (Table 3).

4.3.1 Palmitic acid content of beef samples from respective cities

The C16:0 fatty acid content in Table 3 (27.71 – 31.37%) in the present study is greater than the values reported by Muchenje *et al*. (2009) who had values ranging from 21.72 – 22.32%. Warren *et* al. (2008) reported values for C16:0 in concentrates and grass based diets that averaged 27.52% and 29.20% for the concentrate and grass silage diets respectively. The high C16:0 values observed in the present study when compared to those obtained by Muchenje *et* al. (2009) is concerning due to the fact that C16:0 is said to be responsible for raising low-density lipoprotein (LDL) serum cholesterol and thus not desirable (Muchenje *et al*., 2009). This statement is in disagreement with Daley *et al*. (2010) who has referred to C16:0 as a cholesterol-lowering SFA. The scope of the present study is to focus on quantifying the TFA content of beef in a typical urban area at retail level and thus the role of C16:0 in human health needs to be explored in further studies.

4.3.2 Stearic acid content of beef samples from respective cities

The C18:0 content observed in Johannesburg South (23.76 % ± 6.208) differ from the other Johannesburg regions and this may be due to the main supplier or feedlot of that region implementing a different feeding/finishing strategy as to the rest of the Johannesburg regions (Table 3). C18:0 is mainly affected by variables such as age, fat content and feeding system. With the increase in chronological age the fat content of the animal increases and so too does the fatty acid composition of total lipid. This is due to the triacylglycerols, which increases with fatness, are less unsaturated than the phospholipids which are more constant and saturated within muscle membranes (Enser *et al*., 1998; Barton *et al*., 2007).

C18:0 and C18:1 add up to more than 60% of total fatty acids within all anatomical locations of livestock and these two fatty acids occur mainly within the triacylglycerol fraction (Webb *et al*., 1998). The statement is in agreement with Webb & Casey (1995), who reported that, the concentration of particular fatty acids such as C14:0, C16:0, C16:1, C18:0

and C18:1 increased ($P < 0.05$) with increasing live weight in sheep. Webb & Casey (1995) also stated that while slaughter weight could not be said to be an important contributor to observed differences in molar proportions of these fatty acids in the subcutaneous adipose tissue, it did however significantly affect the gravimetric fatty acid content in the respective fatty acids. Greater concentrations of the above fatty acids were detected in subcutaneous adipose tissue of wethers slaughtered at 43 kg live weight as opposed to those slaughtered at 37 kg live weight. Consequently, subcutaneous adipose tissue of heavier wethers contained greater concentrations of total fatty acids, which can be explained by a greater displacement of water molecules in the adipocytes by both saturated as well as unsaturated fatty acids.

4.3.3 Oleic acid content of beef samples from respective cities

The C18:1c-9 fatty acid varies from 36.94 to 44.52% in Johannesburg and 29.89 to 42.33% in Pretoria (Table 3). These values for oleic acid are typical of ruminants fed grain/concentrate diets in feedlots as noted by the results obtained by Webb & Casey, (1995). Leheska *et al*. (2008) reported that oleic acid constituted the greatest concentration of MUFA in both grass and grain fed beef samples, but it was the grain fed beef samples that contained the highest concentrations of oleic acid. Enser *et al*. (1998) found that oleic acid concentrations were 34.7% and 29.8% in grass and concentrate diets respectively. The values obtained for meat samples in Pretoria north are typical of concentrate fed cattle while the rest of the values for both Pretoria and Johannesburg reflect grass fed values. It is of importance to realise that oleic acid has been found to reduce human LDL-cholesterol while simultaneously increasing HDL-cholesterol concentrations in blood and thus lower risk factor to coronary problems (Katan *et al*., 1994).

4.3.4 The effect of diet on beef fatty acid content

Diet has by far the greatest effect on fatty acid composition as was reported by Warren *et al.* (2008), who observed that a grass silage diet produced greater concentrations of SFAs and MUFAs as well as an increase in the concentrations of all *n-3* fatty acids at the expense of *n-6* PUFAs. They reported that a concentrate based diet produced higher concentrations of total *n-6* PUFAs, predominantly regarding the C18:2*n-6* content. The concentrate based diet also produced greater concentrations of C18:1*trans* as well as CLA, especially within the neutral lipid content. The values reported by Warren *et al*. (2008) for CLA were in disagreement with those reported by French *et al*. (2000) and Aurousseau *et al*. (2004), who had the opposite effect where the grass based diets had produced greater CLA concentrations. This could be due to, as explained by Warren *et al.* (2008), the fact that the forage diets in the other two studies were the major source of PUFA intake, as opposed to in the above mentioned study the intake of total PUFA was similar in both silage grass and concentrate diet.

4.3.4.1 The effect of grain feeding on beef fatty acid content

Grain based diets produce greater proportions of C18:1*trans* as previously mentioned. Values reported in the present meta-analysis are well above those reported for TFA (< 3%) by Droulez *et al*. (2006) but are closer to those values reported by Stender *et al*. (2008), who reported molar proportions as high as 6% . In Table 3 it can be seen that the Pretoria regions have greater ($P < 0.05$) C18:1*t-9* values (average 7.62%) relative to the Johannesburg regions (average 6.08%). The assumption that the Pretoria regions are supplied by feedlots that finish the cattle on relatively higher grain based diets than those supplying the Johannesburg regions are compelling. Although these differences are small, they are statistically significant ($P < 0.05$).

4.3.4.2 The effect of pasture feeding on beef fatty acid content

Pasture feeding or finishing livestock on pasture positively effects the fatty acid composition by reducing the *n-6/n-3* ratio but at the expense of growth performance (Webb & O'Neil, 2008). It was reported by Enser *et al*. (1998) that production system has no effect on the molar proportions of the major TFA C18:1*trans*. Enser *et al*. (1998) reported values for C18:2 and C18:3 in animals fed grass based diets and concentrate based diets. It was found that grass based diets produced C18:2 fatty acid values of 2.5% while concentrate based diets produced fatty acid values of 8.3%. The values reported for C18:2 and C18:3 in the present study are in disagreement with values reported by Enser *et al*. (1998) as they are in the lower range. This is regarded as a positive fact due to a lower *n-6* intake from these beef samples. The C18:3 values reported for the grass based diet and the concentrate diet are 1.2% and 0.5% respectively (Table 3). The values in the present study range from 0.17% to 0.52% with the greater values being from meat supplied to the Johannesburg regions. Consumers are there for being supplied with a meat product with beneficially higher *n-3* fatty acid content.

4.4 Effects of City and Region on fatty acid composition of beef

Table 4: Summary statistics (LSMeans ± SD) of effect (P < 0.05) of city and region on *m. longissimus dorsi* fatty acid profiles expressed as molar proportions (ANOVA with Type III sum of squares)

	Factors studied	
Fatty acid	City (P=F) (Molar% FA)	Region (P=F) (Molar% FA)
C16:0	0.836 ± SD	0.762
C18:0	0.042[a]	0.529
C18:1	0.084	0.701
C18:1t-9	0.068	0.789
C18:1c-9	0.039[b]	0.786
C18:2	0.069	0.577
t-9,t-12	0.413	0.317
c-9,t-12	0.645	0.626
t-9,c-12	0.260	0.861
c-9,c-12	0.067	0.665
C18:3	0.004[c]	0.408
t-9,t-12,t-15	0.042[d]	0.789
t-9,t-12,c-15	nd	Nd
t-9,c-12,t-15	nd	Nd
c-9,t-12,t-15	nd	Nd
c-9,c-12,t-15	nd	Nd
c-9,t-12,c-15	0.007[e]	0.029[f]
t-9,c-12,c-15	0.187	0.720
c-9,c-12,c-15	0.614	0.618
CLA	0.078	0.312
Totals	0.327	0.433

[a, b, c, d, e, f] Means with different superscripts differ significantly at P < 0.05

CLA, conjugated linoleic acid (C18:2c-9,t-11);

FA, Fatty acid;

nd ~ not detected.

The effect of city proves to be much more significant on the fatty acid percentage in beef samples relative to the effect of region (Table 4). City had a significant (P < 0.05) effect on C18:0, C18:1c-9, C18:3, C18:3 t-9,t-12,t-15 and C18:3 c-9,t-12,c-15. The data in table 4 suggest that the different cities have different beef suppliers but the regions have the same abattoirs. The effect of region was only significant for C18:3 c-9,t-12,c-15. The *trans* fatty acids that were significantly (P < 0.05) affected by city were the C18:3 *trans* isomers t-9,t-12,t-15 and c-9,t-12,c-15.

The focus of the present study is on the *trans* fatty acids in beef at retail level. From the data in table 1 it is observed that the concentrations of the above mentioned fatty acids are very small and are consequently considered practically negligible. Concentrations for C18:0 were 40.01milligram fatty acid per gram of beef and for C18:1c-9 it was recorded as 81.02 milligram fatty acid per gram of beef.

The *trans* fatty acids that were significantly affected by city were C18:3t-9,t-12,t-15 and c-9,t-12,c-15. Although the statistical significance is proven ($P < 0.05$), on a practical level the concentrations are 0.2 milligram fatty acid per gram beef and 0.17 milligram fatty acid per gram beef and are with reasonable certainty practically negligible.

The *trans* fatty acids that showed statistical significance have concentration values lower than the recommended benchmark values for human consumption and health. It was estimated that CVD is the number one cause of death globally and consumption of TFA that exceeds five grams per portion is associated with increased risk of CHD (Wagner *et al*., 2008; WHO, 2008).

4.5 Effects of metropolitan region on fatty acid composition of beef

Table 5: Effect of metropolitan region (Johannesburg vs. Pretoria) on the mean (± SD) fatty acid composition (expressed as molar proportions) of *m. longissimus dorsi* samples (ANOVA with Type III sum of squares)

Fatty acid (mol%)	Johannesburg	Pretoria
C16:0	29.13 ± 3.211	29.23 ± 4.174
C18:0	19.06 ± 5.459[a]	21.93 ± 5.453[b]
C18:1	48.07 ± 6.167	44.87 ± 8.513
C18:1t-9	6.12 ± 2.628	7.59 ± 2.739
C18:1c-9	41.95 ± 7.463[a]	37.28 ± 9.619[b]
C18:2	2.78 ± 0.852	3.28 ± 1.011
t-9,t-12	0.13 ± 0.073	0.16 ± 0.179
c-9,t-12	0.13 ± 0.097	0.14 ± 0.098
t-9,c-12	0.02 ± 0.037	0.01 ± 0.201
c-9,c-12	2.51 ± 0.829	3.00 ± 0.970
C18:3	0.46 ± 0.128[a]	0.32 ± 0.208[b]
t-9,t-12,t-15	0.13 ± 0.119[a]	0.07 ± 0.057[b]
t-9,t-12,c-15	nd	nd
t-9,c-12,t-15	nd	nd
c-9,t-12,t-15	nd	nd
c-9,c-12,t-15	nd	nd
c-9,t-12,c-15	0.09 ± 0.061[a]	0.05 ± 0.052[b]
t-9,c-12,c-15	0.14 ± 0.093	0.11 ± 0.096
c-9,c-12,c-15	0.11 ± 0.067	0.09 ± 0.064
CLA	0.49 ± 0.266	0.37 ± 0.176
Totals	99.99 ± 0.463	100.00 ± 0.000

[a & b] Means with different superscripts are significantly different at P<0.05

nd ~ not detected

The Gauteng region is a good representation of modern South African beef consumption. The data in the present study shows that the bulk of beef originated from feedlots where animals are finished on concentrate based diets. Statistical significance was observed for various fatty acids as can be seen in table 5. The molar proportions obtained for C18:0 in beef samples from Johannesburg and Pretoria were 19.06 ± 5.459 and 21.93 ± 5.453 respectively. This shows that differences in fatty acid content do indeed exist by 2.9% between the cities but the variation (5.4%) between the cities are very small and could be considered to be of practical insignificance.

C18:1 had molar proportions of 48.07 ± 6.167 and 44.87 ± 8.513 in Johannesburg and Pretoria respectively. Oleic acid is the predominant fatty acid in beef as previously mentioned. The difference in molar proportions is a mere 3.2% difference and the variation between the cities is so small that they can be considered negligible.

The molar proportions obtained for C18:1c-9 isomer in Johannesburg and Pretoria were 41.95 ± 7.463 and 37.28 ± 9.619 respectively. A difference of 4.7% with existing but

practically negligible variation was obtained for oleic acid in the present study. These values for oleic acid are typical of ruminants fed grain/concentrate diets in feedlots as noted by the results obtained by Webb & Casey, (1995). It is of importance to realise that oleic acid has been found to reduce human LDL-cholesterol while simultaneously increasing HDL-cholesterol concentrations in blood and thus lower risk factor to coronary problems (Katan *et al*., 1994).

The *trans* isomers of C18:3 that showed statistically significant differences between Johannesburg and Pretoria were t-9,t-12,t-15 and c-9,t-12,c-15 with molar proportions of 0.13 ± 0.119 and 0.07 ± 0.057 and 0.09 ± 0.061and 0.05 ± 0.052 respectively. The *trans* fatty acid content in the beef samples are practically small and degree of variation that exist between the cities are of practical consideration insignificant.

The small differences observed in above mentioned fatty acids are possible due to differences in feed sources such as grazing and concentrate feeds of cattle from different areas. Thus carcasses sold in one part of the city may originate from different abattoirs, which each receive cattle from different locations.

The *trans* fatty acids that showed statistical significance ($P < 0.05$) between the cities have concentration values lower than the recommended benchmark values for human consumption (five grams per portion) and health. The values for Gauteng are as low as 0.07% and as high as 0.13% and it is safe to say with reasonable certainty that beef does not pose a threat in terms of TFA content for human consumption.

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CHAPTER 5: CONCLUSION AND RECOMMENDATIONS

The Gauteng region is a good representation of modern South African beef consumption. The data in the present study shows that the bulk of beef originated from feedlots where animals are finished on concentrate based diets. Statistical significance was observed for various fatty acids as can be seen in table 5. The molar proportions obtained for C18:0 in beef samples from Johannesburg and Pretoria were 19.06 ± 5.459 and 21.93 ± 5.453 respectively. This shows that differences in fatty acid content do indeed exist by 2.9% between the cities but the variation (5.4%) between the cities are very small and could be considered to be of practical insignificance.

C18:1 had molar proportions of 48.07 ± 6.167 and 44.87 ± 8.513 in Johannesburg and Pretoria respectively. Oleic acid is the predominant fatty acid in beef as previously mentioned. The difference in molar proportions is a mere 3.2% difference and the variation between the cities is so small that they can be considered negligible.

The molar proportions obtained for C18:1c-9 isomer in Johannesburg and Pretoria were 41.95 ± 7.463 and 37.28 ± 9.619 respectively. A difference of 4.7% with existing but practically negligible variation was obtained for oleic acid in the present study. These values for oleic acid are typical of ruminants fed grain/concentrate diets in feedlots as noted by the results obtained by Webb & Casey, (1995). It is of importance to realise that oleic acid has been found to reduce human LDL-cholesterol while simultaneously increasing HDL-cholesterol concentrations in blood and thus lower risk factor to coronary problems (Katan *et al*., 1994).

The *trans* isomers of C18:3 that showed statistically significant differences between Johannesburg and Pretoria were t-9,t-12,t-15 and c-9,t-12,c-15 with molar proportions of 0.13 ± 0.119 and 0.07 ± 0.057 and 0.09 ± 0.061and 0.05 ± 0.052 respectively. The *trans* fatty acid content in the beef samples are practically small and degree of variation that exist between the cities are of practical consideration insignificant.

The *trans* fatty acids that showed statistical significance ($P < 0.05$) between the cities have concentration values lower than the recommended benchmark values for human consumption (five grams per portion) and health. The values for Gauteng are as low as

0.07% and as high as 0.13% and it is safe to say with reasonable certainty that beef does not pose a threat in terms of TFA content for human consumption.